academicJournals

Vol. 16(9), pp. 388-399, 1 March, 2017
DOI: 10.5897/AJB2017.15881
Article Number: 22E331862997
ISSN 1684-5315
Copyright © 2017
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Effect of the essential oils of *Thymus vulgaris*, *Cinnamomum zeylanicum* and *Mentha piperita* on fungal growth and morphology

Wilson Agwanande Ambindei[1]*, Pierre Michel Dongmo Jazet[2], Leopold Ngoune Tatsadjieu[3], Priya P[4], Manilal VB[4], Krishnakumar B[4] and Paul Henri Amvam Zollo[5]

[1]ENSAI, University of Ngaoundere, P.O. box 455, Ngaoundere, Cameroon.
[2]Faculty of Science, University of Douala, P.O. box 24157, Douala, Cameroon.
[3]IUT, University of Ngaoundere, P.O. box 455, Ngaoundere, Cameroon.
[4]Environmental Technology Division, CSIR-NIIST, 695019 Thiruvananthapuram, India.
[5]Faculty of Science, University of Ngaoundere, P.O. box 455, Ngaoundere, Cameroon.

Received 9 January, 2017; Accepted 27 January, 2017

This study aimed to evaluate the antifungal activity of essential oils of *Thymus vulgaris* L., *Cinnamomum zeylanicum* B. and *Mentha piperita* L. on some saprophytic fungi. Essential oils were extracted by hydro-distillation, and chemical composition was analysed by gas chromatography coupled with mass spectrometry (GC-MS). *T. vulgaris* had as major components, Thymol (35.12%), *p*-cymene (25.36%) and γ–terpinene (12.48%). E-B-Caryophyllane (21.82%), E-Cinnamaldehyde (13.03%) and eugenol (12.15%) were primary in *C. zeylanicum*. Menthol (33.59%), menthone (18.47%) and α-pinene (8.21%) were primary in *M. piperita*. Applying the micro-atmospheric method, essential oils were tested against *Rhizopus oryzae* Went & Prins, *Rhizopus stolonifer* Ehrenb, *Aspergillus tamarii* Taka, *Aspergillus parasiticus* Speare, *Aspergillus flavus* Link and *Talaromyces purpureogenus* purpureogenum. The minimum inhibitory concentrations were 3 to 8, 5 to 16 and 13 to 23 µL/75mL air space for *T. vulgaris, C. zeylanicum* and *M. piperita*, respectively. Means of percentage inhibition were compared through one-way ANOVA by the Tukey test. Scanning electron microscopy revealed fungal cell wall deformation after exposure to essential oil vapour. These essential oils can be exploited as alternatives to synthetic food preservatives.

Key words: Essential oil, *Aspergillus*, *Rhizopus*, *Talaromyces,* fungal morphology, food preservation.

INTRODUCTION

Food spoilage initiated by fungi infestation dates back to the time when primitive man began to cultivate crops and store food (Pitt and Hocking, 2009). Research into fungal food spoilage and its prevention, therefore, had clearly

*Corresponding author. E-mail: agwanande@gmail.com. Tel: +237-675229178 / 697196598.

Author(s) agree that this article remains permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

been that of urgent necessity: lacking in spectacular appeal, it is, however, often neglected. Synthetic fungicidal powders often used include Mancozeb, Thirame, methyl Thiophanate, etc. However, the use of these chemicals as food preservatives poses several problems particularly, environmental pollution, toxicity to living organisms, persistence as residues in food products, resistance of pathogenic agents (Etter et al., 2003).

It is well established that certain plants and their metabolites possess antimicrobial properties (Nguefack et al., 2004). These properties are significantly due to their volatile fraction, that is, essential oils (Hulin et al., 1998) and the presence of other bioactive substances such as phenols. Essential oils have a very broad spectrum of action, since they inhibit the growth of microorganisms (Ambindei et al., 2014) as well as insects (Tatsadjieu et al. 2007).

Thymus, Mentha and Cinnamum species are essential oil producing plants, and are being exploited for different properties. In native medicine, flowering parts and leaves of *Thymus* species have been extensively used as herbal tea, tonic, carminative, antitussive and antiseptic, as well as for treating colds (Maksimovic et al., 2008; Rota et al., 2008). Several studies have revealed the anti- oxidant, viral, inflammatory and microbial potential of the essential oil of *Thymus vulgaris* (Nickavar et al., 2005; Sessou et al., 2012).

Essential oils of *Mentha* species are generally used to flavor liquors, breads, salads, soups and cheese, as well as in cosmetics (Yadegarinia et al., 2006). Herbalists consider peppermint (*Mentha piperita*) as an astringent, antipruritic, antispasmodic, antiemetic, carminative, analgesic, antimicrobial and a stimulant (Hoffman, 1996; Jiofack et al., 2010).

Cinnamon can serve as a blood and digestive tonic, as a natural food preservative, and also exhibits antibacterial as well as antifungal properties (Kalemba and Kunicka, 2003; Jazet et al., 2007).

The main objective of this study was to evaluate the antifungal activity of the essential oils of *T. vulgaris, Cinnamomum zeylanicum* and *M. piperita* against some food spoilage fungi. This is based on the hypothesis that essential oils of these plants can inhibit fungal growth and development. Specifically, the chemical composition of the essential oils, the percentage inhibition, the nature of inhibition, minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC) and effect of essential oils on fungal morphology were evaluated.

MATERIALS AND METHODS

Whole mature plant of *T. vulgaris* L., was harvested in Dschang (geographical coordinates: 5°27' North and 10°04' East); leaves of *C. zeylanicum* B. and whole plant of *M.a piperita* L. were harvested in Mbouda (5°38' North and 10°15' East) in the West Region of Cameroon. These plants were harvested in September 2013 and were identified in the National Herbarium in Yaounde - Cameroon where voucher specimens were kept.

Fungal species

Fungal species were isolated from contaminated stored grains from Ngaoundere-Cameroon, their DNA were extracted, amplified and sequenced. The corresponding DNA sequence was blasted in the NCBI gene bank (Ambindei et al., 2016). The six fungal strains used in this study were a strain each of *Rhizopus oryzae, Aspergillus tamarii, Aspergillus parasiticus, Rhizopus stolonifer, Aspergillus flavus* and *Talaromyces purpureogenus.*

Extraction and chemical composition analyses of essential oils

Essential oils from the different plants were extracted by hydrodistillation with the help of a Clevenger apparatus. Essential oils obtained were analysed by gas chromatography and gas chromatography coupled with mass spectrometry (GC/MS) (Jazet et al., 2010).

Gas chromatography was done in a Varian CP-3380 GC with flame ionization detector (FID) fitted with a fused silica capillary column (30 m x 0.25 mm coated with DB5, film thickness 0.25 µm). The operating conditions were: Injection temperature: 200°C; detection temperature: 200°C; temperature program 50 – 200°C at 5°C/min; carrier gas nitrogen, with a flow rate of 1 mL/min.

GC-MS analyses were performed using a Hewlett-Packard apparatus equipped with an HP1 fused silica column (30 m x 0.25 mm, film thickness 0.25 m) and interfaced with a quadrupole detector (GC quadrupole MS system, model 5970). Column temperature was programmed from 70 – 200°C at 10°C/min; injector temperature was 200°C. Helium was used as carrier gas at a flow rate of 0.6 mL/min. The mass spectrometer was operated at 70 eV. The operating conditions were: temperature programming: 70 to 200°C at 10°C/min; Injection temperature: 200°C; Flow rate of vector gas (helium): 0.6 mL/min, injection volume: 0.1 µL of essential oil solution diluted at 10% in hexane.

Compounds were identified by comparing the calculated retention indices and the mass spectrum with those given in literature (Adams, 2007).

Antifungal activity

The micro-atmosphere method described by Sulaiman (2013) was used at varying amount of essential oils. The inhibiting action of the essential oil vapour is highlighted. The inoculum is placed at the center of a solidified medium in a Petri dish. A given quantity of essential oil is placed on the lid of the petri dish and the dish incubated in reverse position. The solution to be tested evaporates and the volatile phase carries on an inhibiting activity on the germ tested. The essential oil has no direct contact with the inoculum, only the essential oil vapour.

Preparation of culture medium

A potato dextrose agar w/chloramphenicol HIMEDIA brand culture medium was used. According to the manufacturer's instructions, 39.05 g of medium was suspended in 1000 mL distilled water. The suspension was heated to boil so as to enable complete dissolution of medium. It was then sterilised by autoclaving at 121°C at a pressure of 1 bar for 15 min.

Inoculation and application of essential oil

20 mL of culture medium was poured in a Petri dish of diameter 90 mm and height 10 mm. The resultant air space volume in the Petri dish was calculated to be 75 ml. After solidification, a 6 mm well

was created at the centre of the medium and a mycelial disc from a two-day old pure culture of the respective fungus was deposited into the well. Pure essential oil at varying volumes of 5, 10, 15, 20 and 25 µL were placed on the lid of the Petri dish with the help of a micropipette, giving corresponding concentrations of 5, 10, 15, 20 and 25 µL/75 ml air space. Placed in a reverse position, the Petri dishes were sealed with parafilm so as to prevent cross contamination. Each concentration was repeated thrice. A negative control was carried out with no essential oil. No reference volatile antifungal substance was available, hence the absence of a positive control. The Petri plates were then incubated at 28±2°C. The incubation duration was dependent on the growth rate of the specific fungus. Mycelial growth diameter (in mm) was recorded after every 24 h until the control plates were completely covered.

Expression of results

Percentage inhibition

The percentage inhibition of fungal growth was calculated as compared to the control without essential oil by the formula:

$$\text{Inhibition (\%)} = \frac{D_c - D_t}{D_c} \times 100$$

Where D_c (mm) = diameter of fungal growth in the control dish; D_t (mm) = diameter of fungal growth in the test dish

Nature of inhibition

The fungistatic or fungicidal nature of the active essential oil was determined by transferring the inoculated discs from the Petri dishes which had 100% inhibition to a new plate with fresh PDA/chloramphenicol agar medium without essential oil. The absence of growth signifies death of inoculum hence the essential oil was fungicidal; the presence of growth signifies that essential oil was fungistatic.

For each fungal strain, a 3 x 6 factorial design (three essential oils, six concentrations) was used for data analyses. Means of percentage inhibition were compared by the Tukey test through one-way ANOVA using OriginPro 8.0 software. Graphs were plotted using Microsoft Excel 2016.

Effect of essential oil on fungal morphology

The effect of essential oil vapour on fungal morphology was studied by observation of exposed and non-exposed fungi species with a scanning electron microscope (Hafedh et al., 2010). At the end of incubation, fungal mycelia bearing distinct features were harvested with the help of sterile forceps and air-dried. This was to eliminate water from fungal hyphae and to preserve surface structure and prevent collapse of the cells when exposed to the SEM's high vacuum. Before viewing, dried samples were mounted in a JEOL JSC 1200 model coater and coated with a thin layer of gold to prevent the build-up of an electrical charge on the surface and to give a better image. The SEM used was a JEOL scanning electron microscope, JSM 5600 LV model.

RESULTS AND DISCUSSION

Extraction yield of essential oils

M. piperita had the highest extraction yield (4.20%), followed by *T. vulgaris* (2.93%) and *C. zeylanicum* (1.44%). These differences in yield might be due to the different metabolic rates and specific intrinsic properties of the plants. *M. piperita* and *T. vulgaris* from Pančevo, Serbia, (Soković et al., 2007) had yields of 3.2 and 3%, respectively. *C. zeylanicum* from Cocotomey-Atlantique, Southern Benin had a yield of 1.1% from dried leaves (Yehouenou et al., 2012). Differences among same plants might be as a result of differences in harvest time, the agro-ecological zones, postharvest treatments and processing conditions.

Chemical composition of essential oils

The compounds and their respective percentages present in the different essential oils as analyzed by GC/MS are shown in Table 1. All three essential oils were each composed of more than thirty compounds, most of which were monoterpenes, especially oxygenated monoterpenes.

T. vulgaris had 46.91% of oxygenated monoterpenes with major ones being thymol (35.12%), linalool (4.72%), camphor (2.38%) and carvacrol (2.01%), against 45.00% monoterpene hydrocarbons. Sesquiterpenes hydrocarbon made up 6.68% while oxygenated sesquiterpenes were only 1.25%. As for monoterpene hydrocarbons, the major constituents were *p*-cymene (25.36%), γ–terpinene (12.48%), myrcene (1.38%) and α-terpinene (1.09%). The main sesquiterpene was E-B-caryophyllane (4.72%), with the others below 1%.

The essential oil of the leaves of *C. zeylanicum* had 35 constituents. The dominant groups of the compounds were oxygenated monoterpenes (50.96%) and sesquiterpene hydrocarbons (27.91%). Monoterpene hydrocarbons constituted 17.74% while oxygenated sesquiterpene were only 2.18%. For individual constituents, E-cinnamaldehyde, eugenol and 2-hexyl-(Z)- cinnamalehyde were the dominant oxygenated monoterpenes with 13.03, 12.15 and 10.66%, respectively. Other oxygenated monoterpenes were eugenyl acetate (4.3%), E-cinnamic acid (3.26%) and linalool (2.8%). The major sesquiterpene hydrocarbons were E-B-caryophyllane (21.82%) and β-cubebene (4.22%). As for monoterpene hydrocarbons, the majority were α–pinene (6.31%), camphene (2.76%), limonene (2.5%) and β–pinene (2.24%). There were only two constituents of oxygenated sesquiterpenes: caryophellene oxide (1.94%) and guaiol (0.23%).

With respect to *M. piperita* essential oil, of the 34 identified compounds, oxygenated monoterpenes constituted 68.46%, monoterpene hydrocarbons constituted 26.30% while sesquiterpene hydrocarbons had 2.47%. Unlike *T. vulgaris* and *C. zeylanicum,* there was no oxygenated sesquiterpene. The major oxygenated monoterpenes were menthol (33.59%), menthone (18.47%), piperitone (4.66%), menthyl acetate (3.73%) and isoborneol (3.5%). Limonene (13.36%), α- pinene

Table 1. Chemical composition of essential oils of the studied plants.

S/N	RI	Compounds (in order of elution)	Relative percentage		
			T. vulgaris	*C. zeylanicum*	*M. piperita*
		Non-terpenes	**0.14**	**1.06**	**0.18**
1	-	Heptanal*	0.08	0.15	-
2	989	3 – Octan-2-one	0.06	-	0.18
3	956	Benzaldehyde	-	1.06	-
		MTH	**45.00**	**17.74**	**26.30**
4	922	α – Thujene	0.94	0.17	0.23
5	930	α – Pinene	0.89	6.31	8.21
6	945	Camphene	0.99	2.76	0.32
7	971	Sabinene	0.52	-	-
8	974	β – Pinene	0.19	2.24	0.69
9	985	Myrcene	1.38	0.42	1.24
10	1002	α – Phellandrene	0.11	1.25	-
11	1008	δ – 3 – Carene	-	0.16	0.90
12	1014	α-Terpinene	1.09	-	0.21
13	1021	*p* – Cymene	25.36	1.75	0.28
14	1025	Limonene	0.46	2.50	13.36
15	1031	(Z)-β-ocimene	-	-	0.09
16	1056	γ – Terpinene	12.48	-	0.34
17	1063	p – menthe-3,8-diene	0.57	-	-
18	1085	Terpinolene	-	0.19	0.44
		MTO	**46.91**	**50.96**	**68.458**
19	1028	1,8-Cineole	0.12	0.56	1.28
20	1095	Linalool	4.72	2.80	0.40
21	1122	(Z)-Epoxy-ocimene	-	-	0.44
22	1137	E-pinocarveol	-	-	0.22
23	1143	Camphor	2.38	-	0.23
24	1151	Nerol oxide	-	0.50	-
25	1154	Menthone	-	-	18.47
26	1159	Z-Isocitral	-	0.80	-
27	1163	Isoborneol	-	-	3.50
28	1169	Borneol	1.14	0.95	-
29	1174	Terpinen-4-ol	0.97	0.23	-
30	1176	Menthol	-	-	33.59
31	1177	Isomenthol	-	-	0.60
32	1187	α – Terpineol	-	0.49	0.55
33	1195	Myrtenol	-	0.19	-
34	1226	Cis-Carveol	-	0.31	-
35	1249	Geraniol	-	0.28	-
36	1253	Piperitone	-	0.29	4.66
37	1270	E - Cinnamaldehyde	-	13.03	-
38	1287	Safrole	-	0.21	-
39	1291	Menthyl acetate	-	-	3.73
40	1293	Thymol	35.12	-	-
41	1294	Carvacrol ethyl, ether	0.30	-	-
42	1299	Carvacrol	2.01	-	-
43	1355	Eugenol	0.16	12.15	-
44	1446	E-Isoeugenol	-	-	0.80
45	1456	E-Cinnamic acid	-	3.26	-

Table 1. Contd.

46	1525	Eugenyl Acetate	-	4.30	-
47	1765	2-hexyl-(Z)- Cinnamalehyde	-	10.66	-
		STH	**6.68**	**27.91**	**2.44**
48	1346	α-Cubebene	-	-	0.21
49	1371	α – Copaene	-	0.93	-
50	1378	β – Cubebene	-	4.22	-
51	1385	β – Bourbonene	0.21	-	-
52	1408	Z-Caryophyllene	-	-	0.30
53	1420	β-Cedrene	-	-	0.31
54	1423	E-B-Caryophyllane	4.72	21.82	-
55	1464	(E)- 9-epi-Caryophyllene	-	-	0.76
56	1477	D – Germacrene	0.21	-	0.24
57	1483	α – Murolene	0.78	-	-
58	1498	α – selinene	-	0.95	0.26
59	1515	γ – Cadinene	0.27	-	-
60	1524	δ – Cadinene	0.49	-	0.35
		STO	**1.25**	**2.18**	**0.00**
61	1587	Caryophellene oxide	0.98	1.94	-
62	1613	Guaiol	-	0.23	-
63	1621	Iso caryophyllene	0.03	-	-
64	1763	14-oxy-α-Muurolene	0.23	-	-

* Identified tentatively; - = absent; MTH = monoterpenes hydrocarbons; MTO = oxygenated monoterpenes; STH = sesquiterpenes hydrocarbons; STO = Oxygenated sesquiterpenes.

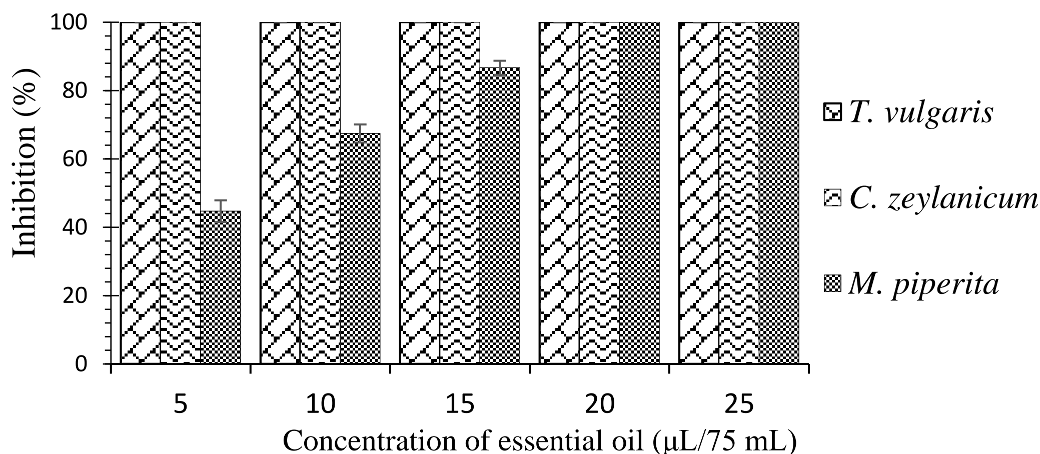

Figure 1. Percentage inhibition of essential oils on *R. oryzae* after 5 incubation days.

and myrcene (1.24%) were the major monoterpene hydrocarbons. All identified sesquiterpenes were below 1%.

In vitro inhibitory effect of essential oil vapour on fungal growth

Not all essential oil concentrations showed total inhibition; growth rate was different based on the essential oil type and the concentration. Incubation duration for *R. oryzae* lasted for 5 days as the control petri dish was completely covered. The percentage inhibition of mycelial growth of *R. oryzae* by different concentrations of essential oil vapour is as shown on Figure 1. Inhibition increased with increase in essential oil concentration. After five incubation days, all the essential oils showed total

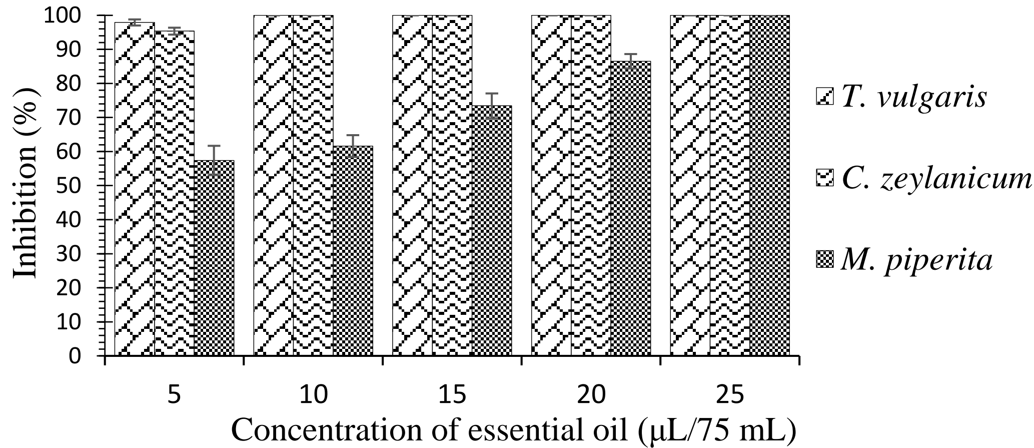

Figure 2. Percentage inhibition of essential oils on *A. tamarii* after 9 incubation days.

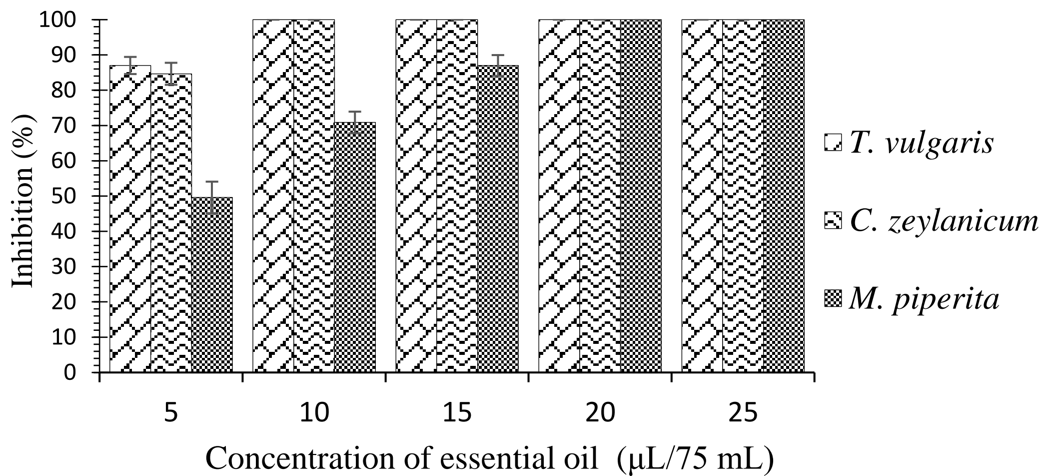

Figure 3. Percentage inhibition of essential oils on *A. parasiticus* after 8 incubation days.

inhibition at 5 µL for *T. vulgaris* and *C. zeylanicum*, and at 20 µL for *M. piperita*. According to the post-hoc Tukey test, there was no significant statistical difference (p>0.05) per concentration of *T. vulgaris* and *C. zeylanicum,* whereas *M. piperita* showed significant statistical difference (p<0.05) in inhibition rate at volumes up to 15 µL. Specifically, the percentage inhibition of *M. piperita* varied from 44.71 ± 3.21 (for 5 µL) to 100% (20 µL).

As illustrated in Figure 2, the higher the amount of essential oil, the greater the percentage inhibition, with 100% inhibition being attained with 10 µL essential oil of *T. vulgaris* and *C. zeylanicum*, and 25 µL for *M. piperita*. For *M. piperita*, there was a positive correlation between percentage inhibition and amount of essential oil. After nine incubation days, the Petri plates with 5 µL essential oil had the least percentage inhibition (57.38±4.32% for *M. piperita*). Differences in percentage inhibitions of *T. vulgaris* and *C. zeylanicum* were not significant statistically (p>0.05) at all amount of essential oils within the incubation period.

Figure 3 is an illustration of the inhibition of growth of *A. parasiticus* by different volumes of essential oils after eight days of incubation. As in the other cases, the least percentage inhibition was obtained at 5 µL with values of 49.61±4.50, 84.65±3.12 and 87.01±2.42% for *M. piperita, C. zeylanicum* and *T. vulgaris*, respectively. Differences in percentage inhibitions at all amounts of essential oils were not significant statistically for *T. vulgaris* and *C. zeylanicum*, meanwhile *M. piperita* showed statistically different (p<0.05) results per amount of essential oil up to 15 µL. The result of the percentage inhibition of essential oils on *R. stolonifer* after five incubation days is as illustrated in Figure 4.

T. vulgaris and *C. zeylanicum* exhibited total inhibition at 10 µL, while *M. piperita* showed total inhibition at 15

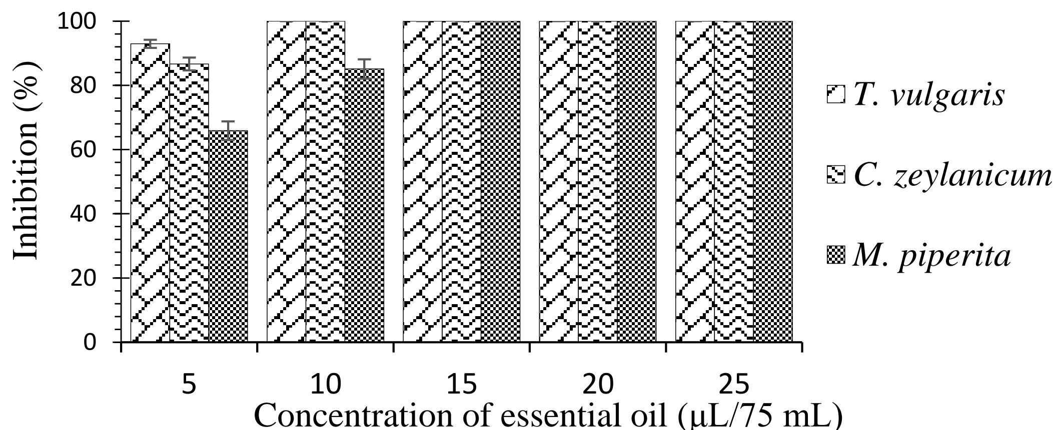

Figure 4. Percentage inhibition of essential oils on *R. stolonifer* after 5 incubation days.

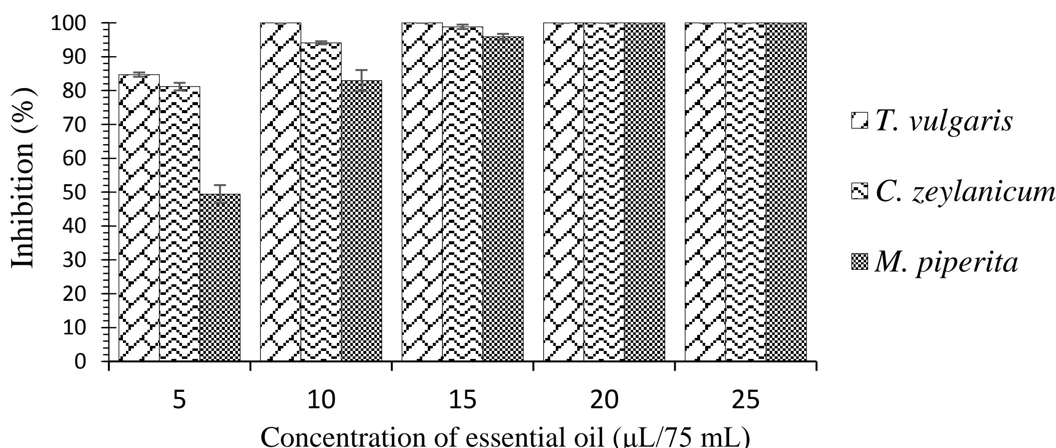

Figure 5. Percentage inhibition of essential oils on *A. flavus* after 8 incubation days.

µL. The minimum percentage inhibitions were 65.88±2.88, 86.67±2.02 and 92.94±1.24% for *M. piperita*, *C. zeylanicum* and *T. vulgaris*, respectively, values significantly different from each other (p<0.05). As in the other cases, especially as demonstrated by *M. piperita*, an increase in the amount of essential oil increased the inhibition rate. As illustrated on Figure 5, percentage inhibition of *A. flavus* increased with increasing amount of essential oils. Incubation lasted for eight days. Total inhibition was exhibited at 10 µL for *T. vulgaris* and 20 µL for *C. zeylanicum* and *M. piperita*. In contrast to the other fungal species, at volumes of 5 and 10 µL, all essential oils showed significantly different percentage inhibition from each other (p<0.05). The least percentage inhibition per essential oil was 49.41±2.66, 81.18±1.12 and 84.71±0.64 for *M. piperita, C. zeylanicum* and *T. vulgaris,* respectively. Incubation of *T. purpureogenus* lasted for nine days, and the percentage inhibition is as reported in Figure 6.

Essential oils of *T. vulgaris* and *C. zeylanicum* exhibited total inhibition at 5 µL, while *M. piperita* exhibited total inhibition at 20 µL. A minimum inhibition of 60.71±2.34% was recorded by *M. piperita* with a volume of 5 µL. There was no significant difference (p>0.05) in the activity of *T. vulgaris* and *C. zeylanicum* as both showed total inhibition at all volumes applied. However, at amounts of 15 µL and lesser, *M. piperita* showed statistically significant (p<0.05) results from the other essential oils, and at higher volumes, all three essential oils had similar outcomes. After noting the volume of essential oil for which total inhibition was exhibited from the preliminary tests, the minimum amount of each essential oil required for total inhibition was determined and is shown in Table 2.

Except for *A. tamarii* and *A. flavus,* there was no significant statistical difference between the MIC values for *T. vulgaris* and *C. zeylanicum.* Meanwhile, MIC values for *M. piperita* were statistically different from the other essential oils for all fungal species except for *A. flavus* were results with *C. zeylanicum* were statistically the

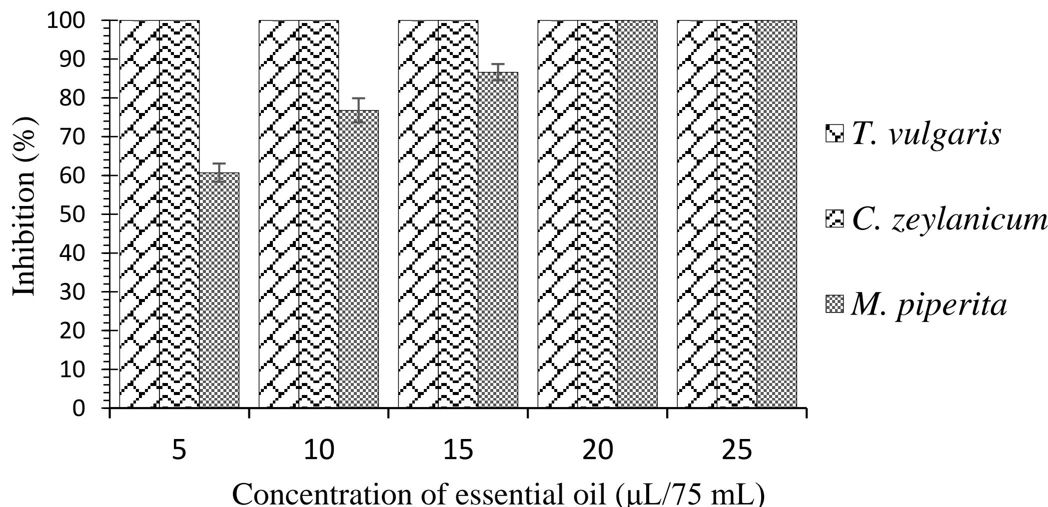

Figure 6. Percentage inhibition of essential oils on *T. purpureogenus* after 9 days.

Table 2. Minimum inhibitory concentration **(**MIC**)** of essential oils.

	MIC per respective fungus (µL/75 mL air space)					
	R. oryzae	***A. tamarii***	***A. parasiticus***	***R. stolonifer***	***A. flavus***	***T. purpureogenus***
T. vulgaris	3.7±0.6[a]	6.0±0[a]	9.0±1[a]	7.3±0.6[a]	8.3±0.6[a]	4.3±0.6[a]
C. zeylanicum	4.7±0.6[a]	9.0±1[b]	8.7±0.6[a]	8.0±0[a]	16.3±0.6[b]	5.3±0.6[a]
M. piperita	18.0±0[b]	23.0±1[c]	18.0±1[b]	12.7±0.6[b]	17.3±0.6[b]	17.7±0.6[b]

Values bearing same letter on the same column are not statistically different ($p<0.05$) according to the Tukey test.

same ($p>0.05$). On a general note, *T. vulgaris* was the most active, followed by *C. zeylanicum* then *M. piperita*.

The antifungal activity of these essential oils is a function of their individual chemical compositions. Essential oil components either exhibit synergism, additive, antagonistic or can portray individual properties (Burt, 2004). The presence of phenolic compounds in the different essential oils renders them good antifungal agents. Thymol, linalool, carvacrol and eugenol are indications of an outstanding antifungal potential (Hyldgaard et al., 2012) as in the case of *T. vulgaris* essential oil. The relative high percentage of cinnamaldehyde and its derivatives in *C. zeylanicum* essential could be responsible for its antifungal activity (Carmo et al., 2008). In addition, the presence of other components such as caryophyllane (21.82%), α-pinene (6.31%), eugenol acetate (4.3%) could also confer antifungal activity.

The carvacrol precursor *p*-cymene, on its own, is not an excellent antifungal agent (Aligiannis et al., 2001; Bagamboula et al., 2004), but will boost the activities of components with functional side groups (Ultee et al., 2000; Rattanachaikunsopon and Phumkhachorn, 2010). The presence of *p*-cymene therefore in the essential oils, especially in *T. vulgaris* and *C. zeylanicum* is a strong indication of increased antifungal activity. Menthol and menthone components of essential oils are very good antifungal compounds (Sulaiman, 2013). These two components, in addition to α-pinene, limonene may be responsible for the antifungal activity of *M. piperita.* The presence of phenolic compounds like carvacrol, thymol, eugenol and menthol increase the antimicrobial activity of essential oils. This is attributed to the presence of an aromatic nucleus and a phenolic -OH group known to be reactive and to form hydrogen bonds with active sites of target enzymes (Velluti et al., 2003).

Nature of inhibition

Upon re-inoculation of mycelial discs of Petri dishes that showed total inhibition in freshly prepared culture medium, the three essential oils showed different nature of inhibitions, depending on their concentration. Even at the maximum tested concentration of 25 µl/75 ml air space, the action of *M. piperita* essential oil was fungistatic on all tested fungi. *C. zeylanicum* was fungicidal at 25 µl/75 ml air space to *R. oryzae* and *T. purpureogenus*, and fungistatic to the other tested fungi species. *T. vulgaris* was fungicidal to *T. purpureogenus* at

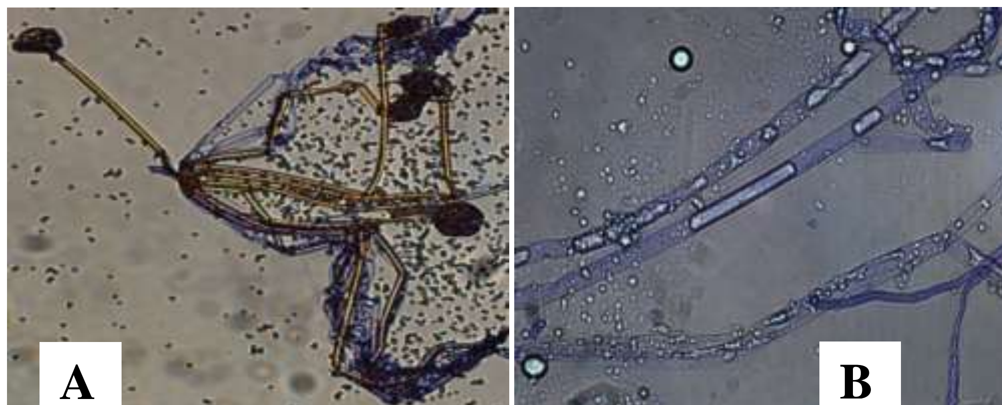

Figure 7. Unexposed (A) and exposed (B) *R. oryzae* to *C. zeylanicum* essential oil vapour as viewed with an optical microscope at 20 and 40x magnification, respectively after staining with lactophenol cotton blue.

20 µl/75 mL air space, and at a concentration of 25 µl/75 mL air space, was fungicidal to *R. oryzae, A. tamari* and *A. flavus.* Its action on *R. stolonifer* and *A. parasiticus* was only fungistatic.

Morphological change of fungi exposed to essential oil vapour

Morphological change due to exposure to essential oil vapour was studied in order to determine the site of action. Because of their relatively rigid vegetative and distinct features (Sporangiophores (specialised aerial hyphae), sporangia and spores), *R. oryzae* and *R. stolonifer* were used to elaborate the change in fungal morphology after exposure to essential oil vapour. Figure 7 shows images of *R. oryzae* unexposed and exposed to *C. zeylanicum* vapour as taken by an optical microscope.

R. oryzae not exposed to essential oil vapour displayed characteristic rigid hyphae bearing sporangia and spores. This rigid vegetative body was evident by the lactophenol cotton blue that stains the chitin portion of the fungal cell wall, giving it a good contrast. Upon exposure to essential oil vapour, the vegetative bodies of the fungi lost their rigidity, leading to poor or no development of sporangia. Since these spore forming bodies were absent, it justified the complete absence of fungal spores. At the level of the hyphae, treated samples showed poor colouration when stained with lactophenol cotton blue dye, because a higher microscopic magnification (40×) was used. The major component of the fungal cell wall, chitin, was therefore not well developed.

The 3-dimensional views of *R. oryzae* and *R. stolonifer* unexposed and exposed to *C. zeylanicum* essential oil vapour as viewed with a scanning electron microscope (SEM) are as shown in Figures 8 to 11. In the absence of essential oil (Figures 8 and 10), *R. oryzae* and *R. stolonifer* showed distinct fungal structures: rigid hyphae, bearing sporangia with spores. The presence of essential oil vapour led to abnormal fungal growth. As a result, hyphae were not well developed, lost fungal rigidity, had no sporangium formation and hence, complete absence of spores. The lost in rigidity implies poorly developed cell wall, leading to a change in morphology. These findings are complementary with that of other researchers who put to evidence the poor development of *A. niger* by *C. zeylanicum* essential oil (Carmo et al., 2008). This disruption of the cell wall will definitely lead to leakage of cytoplasmic content of cell. These modifications in the cytological structure may be related to the interference of the essential oil with enzymes responsible for cell wall synthesis (Shukla et al., 2000).

The antifungal property of essential oils could involve inhibition of extracellular enzymes synthesis and the disruption of the cell wall structure resulting in lack of cytoplasm, damage of integrity and ultimately mycelial death. Cytoplasm granulation, cytoplasm membrane rupturing, cytoplasm hyperacidity and break down of the electron transport chain are some structural and metabolic events possibly related to the antifungal property of essential oils (Lopez-Diaz et al., 2002; Hyldgaard et al., 2012). It is also reported that essential oils are able to interfere with the mitochondrial membrane system by a membrane-disruptive activity closely associated with the enzymatic reactions, such as respiratory electron transport, protein transport and coupled phosphorylation (Atanda et al., 2006; Rasooli et al., 2006).

Conclusion

The antifungal activity of the vapour phase of the essential oils of *T. vulgaris*, *C. zeylanicum* and *M. piperita* could be attributed to major components present in the respective essential oils. The high concentration of

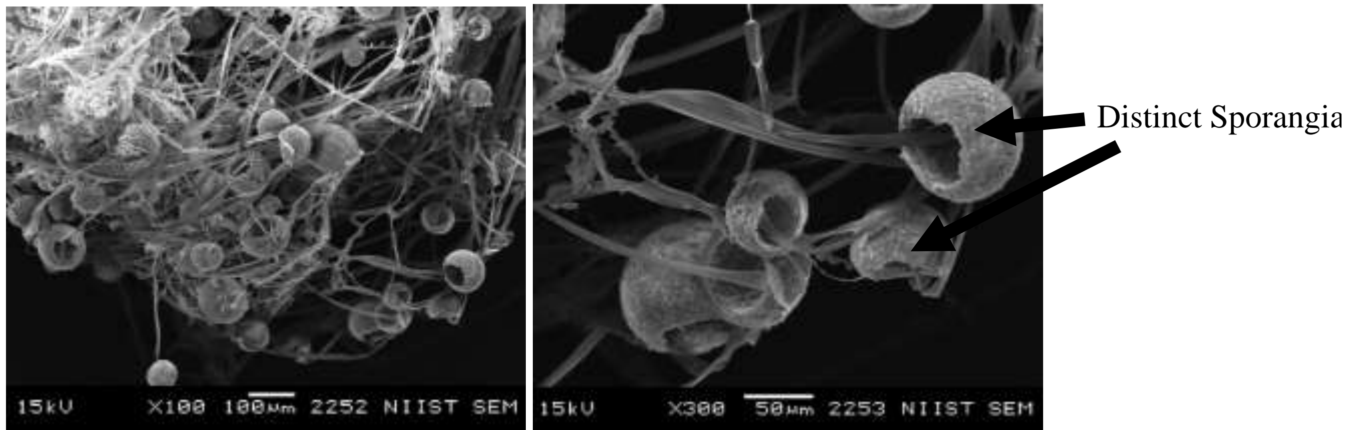

Figure 8. *R. oryzae* unexposed to essential oil vapour as viewed with a SEM.

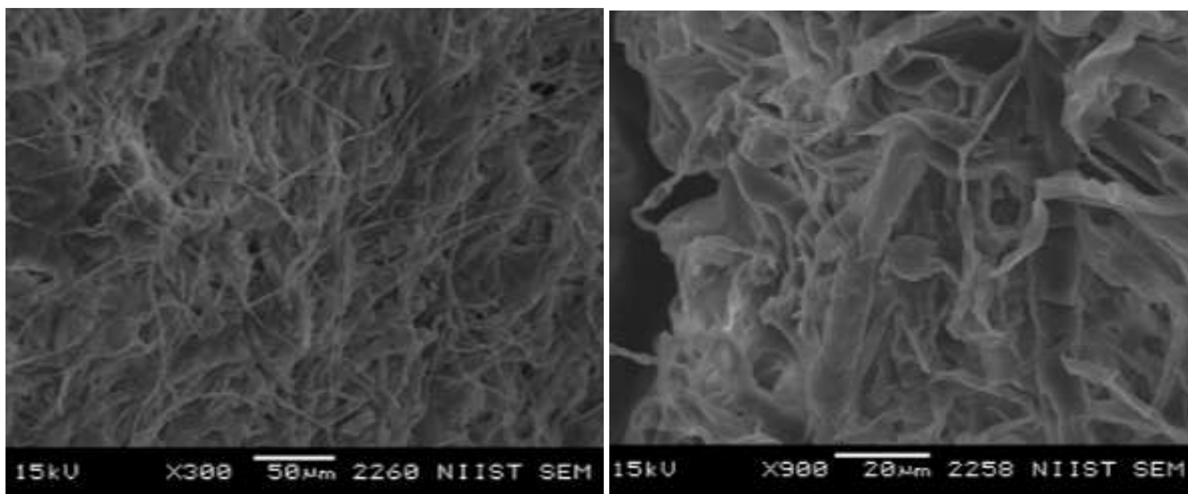

Figure 9. *R. oryzae* exposed to *C. zeylanicum* essential oil vapour as viewed with a SEM.

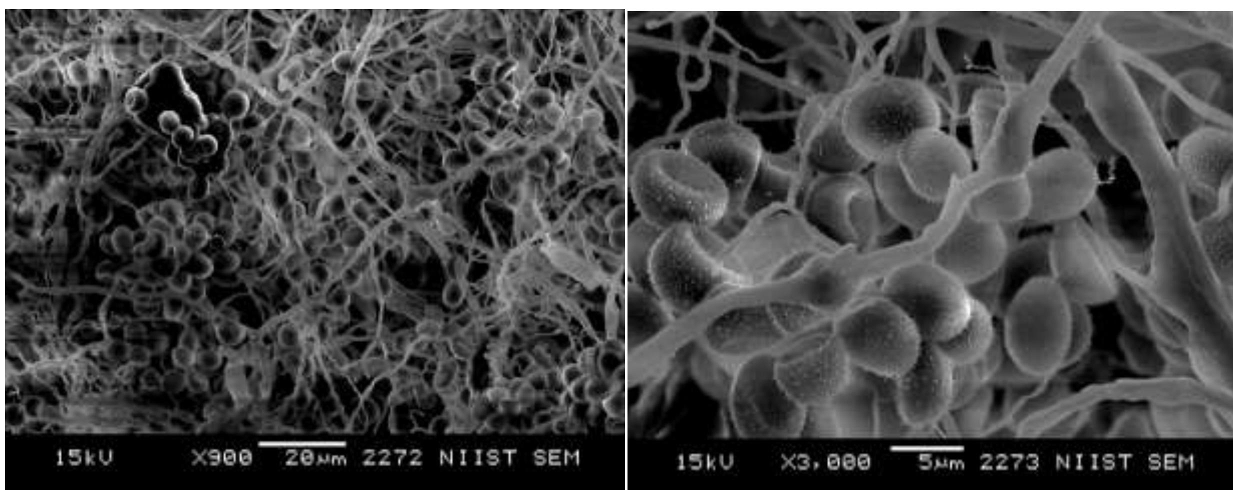

Figure 10. *R. stolonifer* non-exposed to essential oil vapour as viewed with a SEM.

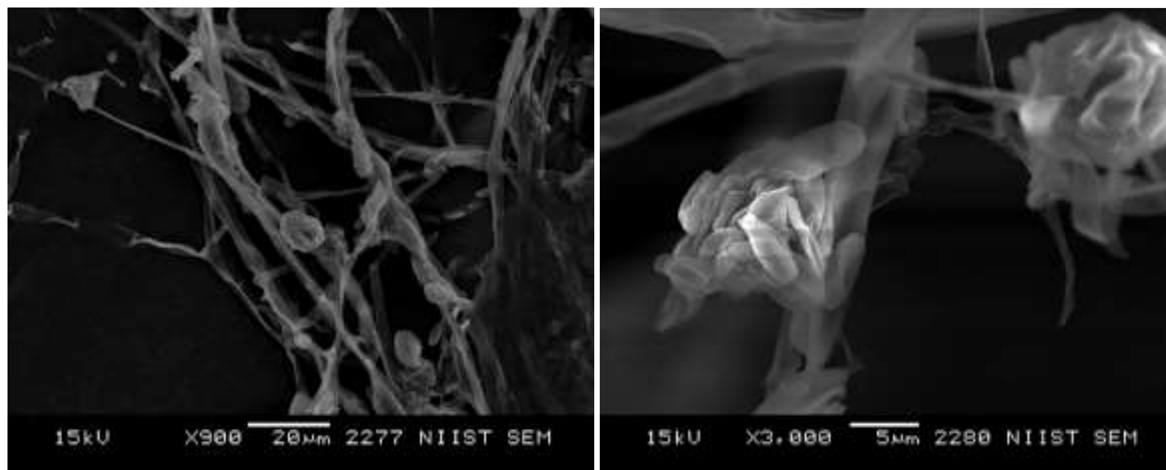

Figure 11. *R. stolonifer* exposed to *C. zeylanicum* essential oil vapour as viewed with a SEM.

oxygenated monoterpene contributed to the increased antifungal activity of the essential oil against tested fungi. Improper development of fungi in the presence of essential oils portrays the fungicidal potential of these essential oils. The fact that essential oils of *T. vulgaris*, *C. zeylanicum* and *M. piperita* are generally recognised as safe gives more virtue for these natural products to be applied conveniently, especially in the vapour phase, as a food preservative instead of synthetic fungicides with multiple site effects.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The authors sincerely acknowledge The World Academy of Science (TWAS) and the Council of Scientific and Industrial Research (CSIR) India for financial support; the National Institute for Interdisciplinary Science and Technology (CSIR-NIIST), particularly, the Division of Process Engineering and Environmental Technology (PEET) for enabling them carry out this work in their laboratory; and ENSAI of the University of Ngaoundere for laboratory analysis and supervision.

REFERENCES

Adams RP (2007). Identification of Essential Oil Components by Gas Chromatography / Quadrupole Mass Spectrometry. Allured: Carol Stream, USA. 804p.

Aligiannis N, Kalpoutzakis E, Mitaku S, Chinou IB (2001). Composition and antimicrobial activity of the essential oils of two *Origanum* species. J. Agric. Food Chem. 49:4168-4170.

Ambindei WA, Tatsadjieu NL, Jazet DPM, Priya P, Anie M, Manilal VB, Krishnakumar B, Amvam Zollo PH (2016). Isolation and Molecular Identification of Fungi in Stored Maize (*Zea mays* L) and Groundnuts (*Arachis hypogaea* L) in Ngaoundere, Cameroon. Am. J. Microbiol. Res. 4(3):85-89.

Ambindei WA, Tatsadjieu NL, Sameza ML, Sonwa TE, Nguimatsia F, Jazet PMD (2014). Antifungal Activities against some *Aspergillus species* of the Essential oils of *Canarium schweinfurthii* and *Aucoumea klaineana* growing in Cameroon. Int. J. Curr .Microbiol. Appl. Sci. 3(5):691-701.

Atanda OO, Akpan I, Oluwafemi F (2006). The potential of some essential oils in the control of *A. parasiticus* CFR 223 and aflatoxin production. Food Control 18:601-607.

Bagamboula CF, Uyttendaele M, Debevere J (2004). Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. Food Microbiol. 21:33-42.

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

Carmo ES, Oliveira EL, Souza EL Sousa FB (2008). Effect of *Cinnamomum zeylanicum* Blume essential oil on the growth and morphogenesis of some potentially pathogenic aspergillus species. Braz. J. Microbiol. 39:91-97.

Etter A, De Putter H, van Bilsen JGPM (2003). Film coating the seed of cabbage (*Brassica oleracea* L. convar. Capitata L.) and Cauliflower (*Brassica oleracea* L. var. Botryti L.) with inidacloprid and spinosad to control insect pests. Crop Prod. 22:761-768.

Hafedh H, Fethi BA, Mejdi S, Emira N, Amina B (2010). Effect of *Mentha longifolia* L. ssp *longifolia* essential oil on the morphology of four pathogenic bacteria visualized by atomic force microscopy. Afr. J. Microbiol. Res. 4:1122–1127.

Hoffman D (1996). The complete illustrated holistic herbal. Rockport, MA: Element Books Inc.

Hulin V, Mathot AG, Mafart P, Dufosse L (1998). Les proprieties anti-microbiennes des huiles essentielles et composes d'arômes (Antimicrobial properties of essential oils and flavour compounds). Sci. Aliments.18(6):563-582.

Hyldgaard M, Mygind T, Meyer RL (2012). Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front. Microbiol. 3:12

Jazet DPM, Tatsadjieu LN, Tchoumbougnang F, Sameza ML, Dongmo NB, Amzam ZPH Menut C (2007). Chemical Composition, Antiradical and Antifungal Activities of Essential Oil of the Leaves of *Cinnamomum zeylanicum* Blume from Cameroon. Nat. Prod.Com. 2(12):1287-1290.

Jazet DPM, Tchoumbougnang F, Ndongson DB, Agwanande W, Amvam ZPH, Menut C (2010). Chemical characterization, antiradical, antioxidant and anti-inflammatory potential of the essential oils of *Canarium schweinfurthii* and *Aucoumea klaineana* (Burseraceae)

growing in Cameroon. Agric. Biol. J. N. Am. 1(4):606-611.
Jiofack T, Fokunang C, Guedje N, Kemeuze V, Fongnzossie E, Nkongmeneck BA, Mapongmetsem PM, Tsabang N (2010). Ethnobotanical uses of medicinal plants of two ethnoecological regions of Cameroon. Int. J. Med. Med. Sci. 2(3):60-79.
Kalemba D, Kunicka A (2003). Antibacterial and antifungal properties of essential oils. Curr. Med. Chem. 10(10):813-829.
Lopez-Díaz TML, González CJ, Moreno B, Otero A (2002). Effect of temperature, water activity, pH and some antimicrobials on the growth of *Penicillium oslonii* isolated from the surface of Spanish fermented meat sausage. Food Microbiol. 19:1-7.
Maksimovic Z, Stojanovic D, Sostaric I, Dajic Z, Ristic M (2008). Composition and radical-scavenging activity of *Thymus glabrescens* Willd. (*Lamiaceae*) essential oil. J. Sci. F. Agric. 88:2036-2041.
Nguefack J, Leth V, Mathur SB, Amvam ZPH (2004). Evaluation of five essential oils from aromatic plants of Cameroon for controlling food spoilage and mycotoxin producing fungi. Int. J. Food Microbiol. 94(3):329-340.
Nickavar B, Mojab F, Dolat-Abadi R (2005). Analysis of the essential oils of two *Thymus* species from Iran. Food Chem. 90:609-611.
Pitt JI, Hocking AD (2009). Fungi and Food Spoilage, 3rd edn. London: Blackie Academic and Professional.
Rasooli I, Rezaei MB, Allameh A (2006). Growth inhibition and morphological alterations of *Aspergillus niger* by essential oils from *Thymus eriocalyx* and *Thymus x-porlock*. Food Control 17:359-364.
Rattanachaikunsopon P, Phumkhachorn P (2010). Assessment of factors influencing antimicrobial activity of carvacrol and cymene against *Vibrio cholerae* in food. J. Biosci. Bioeng. 110:614-619.
Rota MC, Herrera A, Martinez RM, Sotomayor JA, Jordan MJ (2008). Antimicrobial activity and chemical composition of *Thymus vulgaris, Thymus zygis* and *Thymus hyemalis* essential oils. Food Control 19:681-687.
Sessou P, Farougou S, Sohounhloué D (2012). Major component and potential applications of plant essential oils as natural food preservatives: a short review research results. Int. J. Biosci. 2(8):45-57.
Shukla AC, Shahi SK, Dixit A (2000). Epicarp of *Citrus sinensis*: a potential source if natural pesticides. Indian Phytopathol. 53:468-471.
Soković M, Petar DM, Dejan B van Griensven LJLD (2007). Chemical Composition and Antibacterial Activity of Essential Oils of Ten Aromatic Plants against Human Pathogenic Bacteria. Food 1(2):220-226.
Sulaiman AAY (2013). Antifungal Activity of Volatiles from Lemongrass (Cymbopogon citratus) and Peppermint (Mentha piperita) Oils against Some Respiratory Pathogenic Species of Aspergillus Int. J. Curr. Microbiol. Appl. Sci. 2(6):261-272.
Tatsadjieu NL, Ngassoum MB, Nukenine NE, Mbawala A, Aoudou Y (2007). Antifungal an Anti-insect Activities of Three Essential Oils on *Aspergillus flavus* Link and *Sitophilus zeamais* Motsch. Nat. Prod. Commun. 2(12):1291-1294.
Ultee A, Bennik MHJ, Moezelaar R (2000). The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Appl. Environ. Microbiol. 68:1561-1568.
Velluti A, Sanchis V, Ramos AJ, Marı'n SS (2003). Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B1 production by *Fusarium proliferatum* in maize grain. Int. J. Food Microbiol. 89:145-154.
Yadegarinia D, Gachkar L, Rezaei MB, Taghizadeh M, Astaneh SA, Rasooli I (2006). Biochemical activities of Iranian *Mentha* piperita L. and Myrtus communis L. essential oils. Phytochemistry 67(12):49-55.
Yehouenou B, Sessou P, Houinsou RL, Noudogbessi JP, Alitonou GA, Toukourou F Sohounhloue D (2012). Chemical composition and Antimicrobial activities of *Cinnamomum zeylanicum* Blume dry Leaves essential oil against Food-borne Pathogens and Adulterated Microorganisms. Res. J. Biol. Sci. 1(6):18-25.