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Synergism and Antagonism of Essential Oil Fractions of *Cymbopogon citratus, Ocimum gratissimum* and *Thymus vulgaris* Against *Penicillium expansum*

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ABSTRACT

The prevailing spread of food-poisoning orchestrated by mycotoxin producing *Penicillium expansum* strains and others on post-harvested crops and food is a preoccupying issue in tropical countries especially Cameroon. The search for antifungal bio-preservatives to curb this health-threatening issue has become adamant. A study was carried out to evaluate the antifungal potential, constituents of *Cymbopogon citratus* (CC), *Ocimum gratissimum* (OG) and *Thymus vulgaris* (TV) *Essential oil* (EO) fractions, synergism and antagonism between active and non-active fractions against *P. expansum* predominant strains in Cameroon. The antifungal potency was determined by supplemented broth dilution technique which revealed EO fractions from OG was significantly active against *P. expansum* strains than those from CC and TV in that sequence at 1000 ppm. Gaseous phase chromatography coupled with mass spectrometry (GC/MS) illustrated fractions rich with oxygenated terpenes exhibited high antifungal potentials than their whole EO counterparts. Conversely, rich hydrocarbon terpenes fractions displayed a far lesser antifungal potency than their whole EO. Inter-blending active subfractions (CC_{1C}/OG_{1C} and CC_{1C}/TV_{1B}) for the three EO at 1000 ppm depicted a stronger antagonistic effect; whereas non active subfractions (CC_{1A}/OG_{1A} and CC_{1A}/TV_{1A}) inter-blends exhibited strong synergistic effects against the two strains of *P. expansum*. This pathfinder work urges the exploitation of this synergistic effects of active fractions as a probable optimized bio-preservative agent against *P. expansum*; but recommending the non usage of active fractions blend exhibiting antagonistic effect's for the control of the latter.

Key words: *Cymbopogon citratus, Ocimum gratissimum, Thymus vulgaris, Penicillium expansum*, bio-preservative, food-poisoning, antifungal potential

INTRODUCTION

Post-harvest decay of cereals and subsequent food poisoning of recent had become common in Cameroon. One of the catalysing biotic factor to somewhat behind these ills is *P. expansum*. This phytopathogen is a post-harvest fungus responsible for the deterioration of fruits, cereals and

vegetables in general, causing blue-mould-rot disease. This fungus synthesizes roquefortine C, citrinin, chaetoglobosins and the mycotoxin patulin, responsible for Human and animal's food poisoning (Andersen *et al*., 2004; Fung and Clark, 2004).

Studies reveals approximately 25% of marketed food commodities are contaminated by mycotoxins and this constitutes a health-threatening issue and economy loss for most of the tropical countries where these contaminated goods are pulled out of the market for security reasons (FAO, 2003). Restrictions imposed by food industries, trade zones and regulatory agencies on the use of some synthetic food additives (Matthew *et al*., 2005) had led to a retrospection and the eventual quest for alternatives; essentially biodegradable, eco and health-friendly of plant origin. Essential oils have been shown to be active against the above-mention phytopathogenic agents responsible for the deterioration of stored and conserved food crops (Hammer *et al*., 2003; Nguefack *et al*., 2007).

Importantly, recent studies shows essential oils of *O. gratissimum* (Bengyella *et al*., 2010) *C. citratus* and *T. vulgaris* (Nguefack *et al*., 2009) possess high antifungal potential against an array of phytofungi and are readily available and accessible for local farmers and wholesalers. Moreover, the bottom-line with these plants is the presence of several bioactive compounds including geranial, neral, thymol, terpinen-4-ol, linalool, carvacrol (Nielsen and Rios, 2000). It's been shown bio-guided fractionation of EO enhances the antimicrobial activity of fractions, rendering them more active than their complete or whole oil (Nguefack *et al*., 2009).

However, none of these studies had actually determined the constituents of each fraction nor determine the antifungal potential of these fractions or subfractions on *P. expansum* strains. Interestingly, little is known about the synergism or antagonism between active or non-active fractions of these plants EO. In this work, we evaluated the level of synergism and antagonism between some active and non-active bio-guided fractions of EO obtained from *O. gratissimum*, *C. citratus* and *T. vulgaris* against two strains of *P. expansum*; then correlates their activities with their chemical composition and their retention indexes.

MATERIALS AND METHODS

Plant materials were harvested at Obala (for *O. gratissimum* and *C. citratus*) and Bafoussam (for *T. vulgaris*) all in Cameroon. All bioassays and chemical analysis were carried out within the time frame of 2007-to-2009. Each specimen was confirmed by the Cameroon National Herbarium in Yaoundé according to the deposited Voucher specimens (N° Dang 18628/SRF/Cam-1968, Letouzey 5817/SRF/Cam-1966 and Westphal 42851/HNC-1978, respectively). Essential oils were obtained from air-dried leaves by hydrodistillation using the Clevenger apparatus as described by Lamaty *et al*. (1987). Recovered oils were dried over anhydrous sodium sulphate and stored in darkness at 4°C. Fungal strains (MRC 6935 and MRC 6939) of *P. expansum* were obtained from the Medical Research Council, PROMEC Unit, Cape Town, South Africa and culture on Potato Dextrose Agar (PDA) medium at 25°C, 12/12 h alternate day and night.

Forty milligrams of each essential oil was fractionated in packed 40 g silica gel 60-200 mesh, 30×750 mm chromatographic column under increasing gradient elution of ethylacetate (EA)-hexane (5%: 95%) to 100% Hexane (H) solely. The composition of each fraction was examined on aluminium backed silica gel thin layer chromatography (TLC) plates with hexane/ethyl acetate (3: 1 v/v) as the developing solvent. Spots were observed under UV light and reveal in an iodine vapour chamber and fractions showing similar patterns were pulled together. The antifungal

potential were evaluated for complete essential oils and their fractions using the supplemented broth dilution method (Benjilali *et al.*, 1984) accompanied with colony counts. Complete EO or their fractions were blended at different proportions of (100, 75/25, 50/50, 25/75% v/v) making-up 1000 ppm as final concentration; supplementing PDA broth Difco-conidial mixed of 9: 1 v/v in Eppendorfs. Each use conidia suspension was adjusted to approximately 10^7 conidia/mL/strain using a Bürker-Türk counting chamber. The Eppendorfs mixtures were incubated and vortexed at interval of 1 h for 3 h. Prior to incubation, three 10 fold serial dilution was made and 50 µL aliquots from each dilution were plated on PDA in petri-dishes of 90 mm. After 72 h of incubation under 12/12 h, 24±1°C alternate light and darkness; number of colony counts (N) were performed and expressed as number of Colony Forming Units (CFU) per ml per plate, calculated as follows:

$$N = N_{CFU} \times 20 \times \text{dilution factor}$$

The fungicidal activity was determined and expressed as number of decimal reduction of colony forming units per ml (NDR_{CFU}) and calculated using the following formula:

$$NDR_{CFU} = -\text{Log}\,[N^{+}/N_{o}]$$

N^{+}: No. of colony forming units per ml on supplemented media with EO.
N_{o}: No. of colony forming units per ml on non-supplemented media.

Fractions with antifungal potentials superior or equal to the whole EO or which represented a high proportion of the whole EO were pulled for synergism and antagonism assessment. Synergisms between EO fractions were assessed by comparing the NDR_{CFU} obtained with inter-blended fractions to those obtained with individual fractions. Each blend activity was qualified as synergistic or antagonistic when the NDR_{CFU} obtained were respectively superior or inferior to the arithmetic sum of NDR_{CFU} obtained with individualized fraction at the same concentration of 1000 ppm (Pandey *et al.*, 1983).

The samples were characterized using gas chromatography coupled to mass spectrometry (GC/MS) using Hewlett-Packard GC 6890A equipped with a HP-5MS (cross-linked methyl siloxane) fused column (30 m ×0.25 mM, film thickness 0.25 µm) and interfaced with a quadrupole detector (Model 5973). The apparatus was programmed as follows: Injector temperature 220°C, transfer line temperature at 280°C, carrier gas helium flow rate at mL min^{-1}, 70 ev ionization voltage and 1400 ev electron multiplier for mass ranging from 33-500. Comparing their retention time indices and their mass spectrum with standard markers identified compounds. Collected data were analyzed for variance using the One Way ANOVA package and the parametric student-t-test-Newman-Keuls at 95% confidence level.

RESULTS

From the three plants, three whole essential oils, twenty-six fractions were obtained in total and their antifungal potential evaluated. Some physical characteristics, extraction parameters, yields, activities of these oils and their individual fractions are equally displayed in Table 1.

Evaluating antifungal potential of *C. citratus* at 1000 ppm reveals maximum activity was exhibited by subfraction CC_{1C} with NDR_{CFU} values of 3.34 and 5.02 against MRC6935 and MRC6939, respectively. *C. citratus* strongest intra-blend antifungal potential was observed with

Table 1: Whole and fractions of EO characteristics and activity against *P. expansum* strains

					P. expansum strains NDR_{CFU} mL^{-1} at 1000 ppm	
EO fractions	Solvent system	Colour	Aspect	Percentage	MRC 6935	MRC 6939
CC_{1A}	H (100)	Light yellow	Fluid	17.41	0.22±0.02^{b}	0.37±0.06^{cd}
CC_{1B}	H (100)	Yellow	Fluid	16.47	0.78±0.06^{g}	0.89±0.30^{f}
CC_{1C}	H (100)	Pale yellow	Fluid	47.76	3.34±2.31^{i}	5.02±1.70^{h}
CC_{2}	H/EA (95/5)	Pale yellow	Fluid	10.82	0.51±0.04^{e}	0.79±0.16^{e}
CC_{3}	H/EA (85/15)	Orange yellow	Fluid	3.76	0.19±0.04^{b}	0.21±0.03^{b}
CC_{4}	H/EA (75/25)	Orange yellow	Viscous	1.55	0.12±0.05^{a}	0.15±0.03^{ab}
CC_{5}	H/EA (50/50)	Orange	Viscous	1.55	0.19±0.04^{b}	0.24±0.02^{b}
CC_{6}	H/EA (25/75)	Orange	Viscous	0.47	0.11±0.02^{a}	0.11±0.03^{a}
CC_{7}	EA (100)	Orange	Viscous	0.47	0.11±0.03^{a}	0.12±0.02^{a}
CC_{8}	Water	Pale yellow	Fluid	100.00	0.36±0.04^{c}	0.48±0.03^{d}
OG_{1A}	H (100)	Light yellow	Fluid	43.71	0.22±0.04^{b}	0.33±0.22^{c}
OG_{1B}	H (100)	Clear yellow	Fluid	8.95	0.91±0.14^{h}	0.82±0.04^{ef}
OG_{1C}	H (100)	Clear yellow	Fluid	29.42	>6.00±0.00^{j}	>6.00±0.00^{i}
OG_{2}	H/EA (95/5)	Orange yellow	Fluid	7.25	0.94±0.06^{h}	1.21±0.14^{g}
OG_{3}	H/EA (85/15)	Orange yellow	Fluid	4.26	0.16±0.03^{ab}	0.19±0.04^{b}
OG_{4}	H/EA (75/25)	Orange yellow	Fluid	4.90	0.14±0.02^{ab}	0.16±0.02^{ab}
OG_{5}	H/EA (50/50)	Clear yellow	Viscous	0.42	0.10±0.02^{a}	0.13±0.04^{ab}
OG_{6}	H/EA (25/75)	Clear yellow	Viscous	0.21	0.07±0.02^{a}	0.09±0.02^{a}
OG_{7}	EA (100)	Dark yellow	Viscous	0.85	0.06±0.02^{a}	0.09±0.04^{a}
OG_{8}	Water	Yellow	Fluid	100.00	0.47±0.04^{d}	0.80±0.03^{e}
TV_{1A}	H (100)	Light yellow	Fluid	54.09	0.13±0.04^{a}	0.15±0.05^{ab}
TV_{1B}	H (100)	Yellow	Fluid	31.36	>6.00±0.00^{j}	>6.00±0.00^{i}
TV_{2}	H/EA (95/5)	Dark orange	Fluid	7.95	0.62±0.16^{f}	0.42±0.07^{cd}
TV_{3}	H/EA (85/15)	Dark orange	Fluid	1.36	0.11±0.06^{a}	0.14±0.04^{ab}
TV_{4}	H/EA (75/25)	Dark orange	Fluid	3.63	0.10±0.04^{a}	0.12±0.07^{a}
TV_{5}	H/EA (50/50)	Dark orange	Fluid	0.68	0.09±0.02	0.11±0.04^{a}
TV_{6}	H/EA (25/75)	Orange yellow	Viscous	0.45	0.07±0.03^{a}	0.09±0.05^{a}
TV_{7}	EA (100)	Dark orange	Viscous	0.45	0.07±0.02^{a}	0.08±0.04^{a}
TV_{8}	Water	Orange yellow	Fluid	100.00	0.16±0.02^{ab}	0.18±0.03^{b}

Data are the Mean±SD of 3 repetitions with $p<0.05$. Fractions of *C. citratus* (CC) are: C_{1A}, CC_{1B}, CC_{1C}, CC_{2} to CC_{8}. Those of *O. gratissimum* (OG) are OG_{1A}, OG_{1B}, OG_{1C}, OG_{2} to OG_{8}. Fractions of *T. vulgaris* (TV): TV_{1A}, TV_{1B}, TV_{2} to TV_{8}. Subscript $_{1A}$, $_{1B}$, $_{1C}$ attached to OG, CC and TV represents subfraction OG1, CC1 and TV1 for each plant EO fractionated with 100% hexane which showed different profiles on TLC plates. Fractionating solvent are hexane (H), water and ethylacetate (EA). The remaining fractions were designated in function of the proportional mixed of their fractioning solvent as displayed on the table. Different superscipts within coloumn show significant differeance at $p<0.05$

CC_{1A}/CC_{1C} (25:75 v/v) with NDR_{CFU} at 0.81 and 1.07 against MRC6935 and MRC6939, respectively. However, an impressive activity was observed with a trio intra-blend of $CC_{1B}/CC_{1C}/CC_{2}$ (1:1:1 v/v/v) with NDR_{CFU} above unity as shown in Table 2.

Profiling antifungal potential of *O. gratissimum* at 1000 ppm unveiled OG_{1C} was the most active with NDR_{CFU} values above 6.00 for both pathogenic strains. A maximum antifungal effect was noted with intra-blends of OG_{1B}/OG_{2} (25:75 v/v) and OG_{1C}/OG_{2} (75:25 v/v) exhibiting an NDR_{CFU} value above 6.00 against both strains as depicted in Table 3.

Table 2: Activities of blended fractions of *C. citratus* (CC) EO at 1000 ppm against *P. expansum* strains

		P. expansum strains NDR_{CFU} mL^{-1} at 1000 ppm	
Fractions blend	Proportions (%) v/v	MRC 6935	MRC 6939
CC	100	0.36 ± 0.04^{b}	0.48 ± 0.03^{bc}
CC_{1A}	100	0.22 ± 0.02^{a}	0.39 ± 0.06^{b}
CC_{1B}	100	0.78 ± 0.06^{d}	0.89 ± 0.31^{d}
CC_{1C}	100	3.34 ± 2.31^{fg}	5.02 ± 1.70^{h}
CC_2	100	0.51 ± 0.04^{c}	0.79 ± 0.16^{d}
CC_{1A}/CC_{1B}	75/25	0.75 ± 0.07^{d}	0.86 ± 0.07^{d}
	50/50	0.91 ± 0.03^{e}	1.05 ± 0.10^{e}
	25/75	0.88 ± 0.03^{e}	0.89 ± 0.03^{d}
CC_{1A}/CC_{1C}	75/25	0.31 ± 0.02^{ab}	0.88 ± 0.11^{d}
	50/50	0.59 ± 0.01^{c}	0.93 ± 0.10^{de}
	25/75	0.81 ± 0.07^{de}	1.07 ± 0.15^{e}
CC_{1A}/CC_2	75/25	0.22 ± 0.04^{a}	0.56 ± 0.06^{c}
	50/50	0.27 ± 0.04^{ab}	0.63 ± 0.04^{cd}
	25/75	0.35 ± 0.02^{b}	0.67 ± 0.05^{cd}
CC_{1B}/CC_{1C}	75/25	0.70 ± 0.08^{d}	1.43 ± 0.29^{efg}
	50/50	1.61 ± 0.17^{f}	1.43 ± 0.31^{efg}
	25/75	1.62 ± 0.17^{f}	1.56 ± 0.25^{fg}
CC_{1B}/CC_2	75/25	0.59 ± 0.10^{c}	0.80 ± 0.08^{d}
	50/50	0.17 ± 0.05^{a}	0.40 ± 0.03^{b}
	25/75	0.21 ± 0.05^{a}	0.54 ± 0.05^{c}
CC_{1C}/CC_2	75/25	1.63 ± 0.19^{f}	0.89 ± 0.13^{d}
	50/50	0.53 ± 0.04^{c}	0.26 ± 0.01^{a}
	25/75	0.53 ± 0.09^{c}	0.24 ± 0.02^{a}
$CC_{1B}/CC_{1C}/CC_2$	1/1/1	1.56 ± 0.25^{f}	1.62 ± 0.17^{fg}

Data are the Mean±SD of 3 repetitions with $p<0.05$. CC is the whole or non fractionated EO of *C. citratus* (CC). Subfractions CC_{1A}, CC_{1B} and CC_{1C} represent *C. citratus* (CC) EO fractionated with 100% hexane which showed different profiles on TLC plates. CC_2 fraction is obtained with H/EA 95/5%v/v fractionating solvent. Different superscipts within coloumn show significant differeance at $p<0.05$

Assaying antifungal potency at 1000 ppm for *T. vulgaris* revealed best individualized activity was associated with fraction TV_{1B} with NDR_{CFU} valuing 6.00 for both pathogenic strains. Strongest intra-blend antifungal activity was observed with TV1A/TV1B (25:75 v/v) with NDR_{CFU} of 0.6 and 0.74 for strains MRC6935 and MRC6939, respectively as depicted in Table 4.

Inter-blending *C. citratus* and *T. vulgaris* fractions revealed a strong synergistic effect with active fractions CC_{1A}/TV_{1A} (50:50 v/v) with NDR_{CFU} values of 0.58 and 0.79 for strain MRC6935 and MRC6939, respectively. Besides this synergism, a very strong antagonism was observed with inter-blends of CC_{1C}/TV_{1B} (75:25 v/v); CC_{1C}/TV_{1B} (50:50 v/v) and CC_{1C}/TV_{1B} (25:75 v/v) as depicted in Table 5.

Best synergistic effect from inter-blending active fractions of *C. citratus* and *O. gratissimum* was noted with CC_{1A}/OG_{1A} (50:50 v/v) with NDR_{CFU} values of 0.81 and 0.68 for strains MRC6935 and MRC6939, respectively. Equally, all inter-blending of of CC and OG were generally more active than their individual fractions. Generally, inter-blending *C. citratus* and *O. gratissimum* generally expressed impressive synergism than those of *C. citratus* and *T. vulgaris* as depicted in Table 6. Antagonism with active fractions was observed inter-blending CC_{1C}/OG_{1C} (75:25 v/v) and CC_{1C}/OG_{1C} (50:50 v/v); hence, enhancing *P. expansum* viability to somewhat compared to their individual fractions as depicted in Table 6.

Table 3: Activities of mixed fractions of *O. gratissimum* (OG) EO at 1000 ppm against *P. expansum* strains

		P. expansum strains NDR_{CFU} mL^{-1} at 1000 ppm	
Fractions blend	Proportions (%) v/v	MRC 6935	MRC 6939
OG	100	0.47±0.04^{bc}	0.80±0.03^{de}
OG_{1A}	100	0.22±0.04^{a}	0.33±0.02^{b}
OG_{1B}	100	0.91±0.14^{de}	0.82±0.04^{de}
OG_{1C}	100	>6.00±0.00^{i}	>6.00±0.00^{h}
OG_2	100	0.94±0.06^{de}	1.21±0.14^{f}
OG_{1A}/OG_{1B}	75/25	0.51±0.07^{bc}	0.56±0.05^{c}
	50/50	0.77±0.10^{d}	0.67±0.06^{cd}
	25/75	0.85±0.14^{de}	0.75±0.07^{d}
OG_{1A}/OG_{1C}	75/25	1.03±0.07^{e}	0.70±0.08^{d}
	50/50	1.31±0.08^{f}	1.22±0.19^{f}
	25/75	0.40±0.04^{b}	0.33±0.06^{b}
$OG1A/OG_2$	75/25	0.29±0.04^{ab}	0.21±0.02^{a}
	50/50	0.31±0.05^{ab}	0.26±0.03^{a}
	25/75	0.40±0.04^{b}	0.33±0.06^{b}
OG_{1B}/OG_{1C}	75/25	1.16±0.14^{ef}	0.84±0.05^{de}
	50/50	1.67±0.19^{g}	1.49±0.30^{fg}
	25/75	>6.00±0.00^{i}	>6.00±0.00^{h}
OG_{1B}/OG_2	75/25	0.59±0.12^{cd}	0.51±0.06^{c}
	50/50	0.63±0.13^{cd}	0.72±0.03^{d}
	25/75	0.64±0.10^{cd}	0.80±0.06^{de}
OG_{1C}/OG_2	75/25	>6.00±0.00^{i}	>6.00±0.00^{h}
	50/50	1.33±0.35^{fg}	1.48±0.22^{fg}
	25/75	0.94±0.08^{de}	0.94±0.11^{ef}

Data are the Mean±SD of 3 repetitions with $p<0.05$. OG is the whole EO, while OG_2 was obtained with fractionating solvent H/EA 95/5%v/v. Subfractions OG_{1A}, OG_{1B} and OG_{1C} were obtained with 100% hexane and exhibited different profiles on TLC plates. Different superscipts within coloumn show significant differeance at $p<0.05$

Table 4: Activities of mixed fractions of *T. vulgaris* (TV) EO at 1000 ppm against *P. expansum* strains

		P. expansum strains NDR_{CFU} mL^{-1} at 1000 ppm	
Fractions blend	Proportions (%) v/v	MRC 6935	MRC 6939
TV	100	0.16±0.02^{a}	0.18±0.03^{ab}
TV_{1A}	100	0.13±0.04^{a}	0.15±0.05^{ab}
TV_{1B}	100	>6.00±0.00^{d}	>6.00±0.00^{e}
TV_2	100	0.62±0.16^{c}	0.42±0.07^{b}
TV_{1A}/TV_{1B}	75/25	0.44±0.18^{bc}	0.37±0.04^{b}
	50/50	0.51±0.04^{c}	0.41±0.05^{b}
	25/75	0.60±0.04^{c}	0.74±0.06^{c}
TV_{1A}/TV2	75/25	0.20±0.03^{ab}	0.17±0.03^{ab}
	50/50	0.30±0.01^{bc}	0.20±0.03^{ab}
	25/75	0.38±0.06^{bc}	0.30±0.03^{b}
$TV1B/TV_2$	75/25	0.24±0.04^{b}	1.11±0.18^{d}
	50/50	0.14±0.04^{a}	0.66±0.05^{c}
	25/75	0.13±0.02^{a}	0.13±0.02^{a}

Data are the Mean±SD of 3 repetitions with $p<0.05$. TV is the whole EO, while TV_2 was obtained with fractionating solvent H/EA 95/5%v/v. Subfractions TV_{1A} and TV_{1B} were obtained with 100% hexane and exhibited different profiles on TLC plates. Different superscipts within coloumn show significant differeance at $p<0.05$

Table 5: Activities EO blended Fractions of *T. vulgaris* (TV) *and C. citratus* (CC) at 1000 ppm against *P. expansum* strains

		P. expansum strains NDR_{CFU} mL^{-1} at 1000 ppm	
Fractions blends	Proportions (%) v/v	MRC 6935	MRC 6939
CC_{1A}	100	0.22 ± 0.02^{b}	0.39 ± 0.06^{c}
CC_{1C}	100	3.34 ± 2.31^{h}	5.02 ± 1.70^{f}
TV_{1A}	100	0.13 ± 0.04^{a}	0.15 ± 0.05^{a}
TV_{1B}	100	$>6.00\pm0.00^{i}$	$>6.00\pm0.00^{g}$
TV_{2}	100	0.62 ± 0.16^{ef}	0.40 ± 0.09^{c}
CC_{1A}/TV_{1A}	75/25	0.47 ± 0.05^{d}	0.56 ± 0.04^{d}
	50/50	0.58 ± 0.06^{ef}	0.79 ± 0.13^{e}
	25/75	0.31 ± 0.04^{bc}	0.44 ± 0.13^{cd}
CC_{1C}/TV_{1B}	75/25	0.70 ± 0.10^{f}	0.31 ± 0.08^{bc}
	50/50	0.70 ± 0.10^{f}	0.50 ± 0.12^{d}
	25/75	0.96 ± 0.14^{g}	0.56 ± 0.10^{d}
CC_{1C}/TV_{2}	75/25	0.68 ± 0.02^{f}	0.26 ± 0.04^{bc}
	50/50	0.34 ± 0.03^{c}	0.24 ± 0.19^{abc}
	25/75	0.21 ± 0.04^{ab}	0.22 ± 0.06^{a}

[b]Data are the Mean±SD of 3 repetitions with $p<0.05$. Subfractions CC_{1A}, CC_{1C}, TV_{1A} and TV_{1B} were obtained with 100% hexane and exhibited different profiles on TLC plates, while TV_{2} was obtained with fractionating solvent H/EA 95/5%v/v. Different superscipts within coloumn show significant differeance at $p<0.05$

Table 6: Activities EO blended Fractions of *C. citratus* (CC) *and O. gratissimum* (OG) at 1000 ppm against *P. expansum* strains

		P. expansum strains NDR_{CFU} mL^{-1} at 1000 ppm	
Fractions blends	Proportions (%) v/v	MRC 6935	MRC 6939
CC_{1A}	100	0.22 ± 0.02^{a}	0.39 ± 0.06^{b}
CC_{1C}	100	3.34 ± 2.30^{fg}	5.02 ± 1.70^{h}
OG_{1A}	100	0.22 ± 0.04^{a}	0.33 ± 0.02^{b}
OG_{1B}	100	0.91 ± 0.14^{d}	0.82 ± 0.04^{de}
OG_{1C}	100	$>6.00^{h}$	$>6.00^{i}$
OG_{2}	100	0.94 ± 0.06^{de}	1.21 ± 0.14^{fg}
CC_{1A}/OG_{1A}	75/25	0.60 ± 0.09^{b}	0.64 ± 0.06^{cd}
	50/50	0.83 ± 0.11^{d}	0.68 ± 0.09^{cd}
	25/75	0.55 ± 0.13^{bc}	0.56 ± 0.04^{c}
CC_{1C}/OG_{1B}	75/25	0.95 ± 0.15^{de}	0.70 ± 0.06^{d}
	50/50	0.60 ± 0.12^{c}	0.53 ± 0.03^{c}
	25/75	0.44 ± 0.13^{bc}	0.29 ± 0.04^{ab}
CC_{1C}/OG_{1C}	75/25	1.07 ± 0.18^{de}	0.90 ± 0.02^{e}
	50/50	1.27 ± 0.15^{ef}	0.92 ± 0.12^{e}
	25/75	5.00 ± 0.44^{g}	1.50 ± 0.22^{g}
CC_{1C}/OG_{2}	75/25	1.40 ± 0.03^{f}	1.05 ± 0.22^{ef}
	50/50	0.34 ± 0.06^{b}	0.19 ± 0.06^{a}
	25/75	0.36 ± 0.06^{b}	0.22 ± 0.05^{a}

Data are the Mean±SD of 3 repetitions with $p<0.05$. Subfractions of *C. citratus* (CC_{1A}, CC_{1C}) and subfractions of *O. gratissimum* (OG_{1A}, OG_{1B}, OG_{1C}) were obtained with 100% hexane showing distinctive profiles on TLC plates. $OG_{2}$is a fraction of *O.* gratissimum obtained with 95/5% v/v H/EA. Different superscipts within coloumn show significant differeance at $p<0.05$

GC-MS showed whole EO from CC was mainly oxygenated monoterpenes (OMT) (64.03 %), among which 58.94% was citrals. Very active subfraction CC_{1C} (100% hexane fractionation)

Table 7: Chemical composition (%) of the EO from *C. citratus* (CC) and some of its fractions (CC_{1A}, CC_{1B}, CC_{1C}, CC_2)

Compounds	R I	CC	CC_{1A}	CC_{1B}	CC_{1C}	CC_2
α-Pinene	934	0.12	0.68	-	0.13	0.21
Camphene	943	0.10	1.89	-	-	-
Myrcene	984	2.53	-	2.08	-	-
p-Cymene	1016	0.50	0.73	0.75	-	-
Limonene	1024	0.23	-	0.88	-	0.41
Linalol	1090	1.13	-	-	0.98	0.46
Camphor	1120	0.16	-	0.12	-	1.50
Borneol	1154	0.22	-	-	-	2.05
Terpinen-4-ol	1169	0.89	-	-	3.14	2.98
Z-Citral/Neral	1225	21.21	-	33.26	42.23	29.93
Geraniol	1238	0.52	-	0.54	0.74	0.38
E-Citral/Geranial	1252	37.73	-	30.26	45.16	28.17
Bornyl acetate	1300	0.74	-	0.31	-	1.42
Neryl acetate	1330	0.98	-	1.81	0.97	4.70
Geranyl acetate	1359	0.45	-	0.86	1.52	3.21
α-Copaene	1381	1.09	-	2.95	-	-
γ-Selinene	1403	0.68	3.77	0.59	-	-
β-Caryophyllene	1427	1.77	2.92	0.93	0.20	0.56
α-Humulene	1455	1.23	12.62	0.51	-	-
(E)α-sBisabolene	1465	0.70	4.42	0.93	-	-
α-Amorphene	1477	0.23	2.15	-	-	-
σ-Cadinene	1481	0.29	4.08	2.93	-	-
α-Selinene	1484	1.18	6.29	-	-	-
Germacrene D	1486	1.34	23.05	1.15	-	-
β-Selinene	1491	0.90	6.91	1.67	-	-
Δ-Cadinene	1518	0.97	4.69	0.88	-	-
α-Cadinene	1536	0.30	3.93	-	-	-
α-Caryophyllene	1558	1.56	-	2.90	-	-
Selina-6-en-4-ol	1578	8.87	-	3.80	0.10	7.24
Sesquiterpene alcohol	1597	1.66	-	0.98	-	2.27
Total		90.28	90.23	91.09	95.17	85.54
MTH		3.48	3.30	3.71	0.13	0.62
OMT		64.03	-	67.16	94.74	74.85
STH		12.24	86.93	15.44	0.20	0.56
OST		10.53	-	4.78	0.10	9.51

Monoterpene Hydrocarbons (MTH); Oxygenated Monoterpenes (OMT); Sesquiterpene Hydrocarbons (STH); Oxygenated sesquiterpens (OST); Retention Index (RI); Whole EO of *C. citratus* (CC); Subfractions CC_{1A}, CC_{1B} and CC_{1C} obtained with 100% hexane exhibiting different profiles on TLC plates; CC_2 fraction was obtained at 95/5% v/v H/EA fractionating solvent

possessed 45.16% citrals. Subfraction CC_{1A} was remarkable rich with 86.93% STH, while subfraction CC_{1B} and fraction CC_2 had 67.16% and 75.21% of OMT, respectively as depicted in Table 7.

GC-MS revealed essential oil from OG contain approximately 41.79% MTH and 44.61% OMT, predominantly thymol (40.61%) and p-Cymene (23.47%) displayed in Table 8. Active subfraction OG_{1C} and fraction OG_2 were 96.46 and 79.13% thymol rich, respectively. Complete EO *T. vulgaris* was predominantly made-up of 30.87 and 28.11% of p-cymene and thymol, respectively as active molecules as depicted in Table 8. Active TV_{1B} subfraction contained approximately 68.2% thymol, while less active sub-fraction TV_{1A} had approximately 71.11% p-Cymene.

Table 8: Chemical composition (%) of *O. gratissimum* (OG)and *T. vulgaris* (TV) EO's and some of their fractions (OG_{1A}, OG_{1B}, OG_{1C}, OG_{2}, TV_{1A}, TV_{1B}, TV_{2})

Compounds	RI	OG	OG_{1A}	OG_{1B}	OG_{1C}	OG_{2}	TV	TV_{1A}	TV_{1B}	TV_{2}
α-Thujene	924	3.59	5.16	0.21	-	0.45	0.91	2.12	-	-
α-Pinene	93	21.19	2.05	-	-	0.26	0.93	2.28	-	-
Camphene	943	0.13	0.24	-	-	0.11	0.88	2.23	-	-
β-Pinene	974	0.36	0.75	-	-	0.16	0.73	0.47	0.35	-
Myrcene	984	2.52	3.53	-	-	0.86	0.99	1.70	-	-
α-Terpinene	1012	2.09	-	0.31	-	0.41	1.20	-	-	-
p-Cymene	1016	23.47	61.82	9.89	0.57	2.61	30.87	71.11	0.31	0.12
Limonene	1024	1.51	2.36	-	0.17	0.72	0.89	1.57	0.27	-
γ-Terpinene	1048	6.93	0.97	0.24	-	0.97	5.88	4.80	-	-
Trans-Sabinene hydrate	1062	0.68	-	5.11	0.21	2.66	0.67	-	0.34	-
MMB	1087	0.78	1.49	-	-	0.68	-	-	-	-
Linalool	1090	0.54	-	-	0.21	2.58	3.19	-	5.34	8.21
Camphor	1120	-	-	3.91	-	2.65	1.64	-	4.10	5.03
Bornéol	1157	0.24	-	3.87	-	1.78	2.10	-	1.47	13.28
Terpinen-4-ol	116	92.28	-	8.75	3.17	12.68	1.25	-	2.98	4.47
Thymol	1278	40.61	-	49.36	92.01	55.11	28.11	-	68.20	38.96
Carvacrol	1290	0.26		5.08	0.86	1.30	2.60	-	2.30	8.07
Bornyl acetate	1300	-	-	-	-	-	3.46	-	5.90	4.32
α-Copaene	1381	1.11	1.61	-	-	0.53	0.48	0.56	0.48	-
B-Caryophyllene	1427	3.64	0.49	2.38	-	-	4.58	1.86	-	0.91
α-Amorphene	1477	-	-	-	-	-	0.32	0.50	-	-
β-Selinene	1491	1.95	4.25	-	-	-	-	0.24	-	-
α-Selinene	1484	0.75	1.13	-	-	0.25	0.18	-	-	-
Δ-Cadinene	1518	0.71	0.71	-	-	0.27	0.72	0.87	-	-
α-Caryophyllene	1558	0.84	5.49	-	0.50	0.35	0.73	5.17	1.93	0.11
Sesquiterpene alcohol	1597	-	-	-	-	1.37	-	-	-	-
Total		96.18	92.05	89.11	97.70	87.65	93.31	95.48	93.97	83.48
MTH		41.79	76.88	10.65	0.74	6.44	43.28	86.28	0.93	0.12
OMT		44.61	-	76.08	96.46	77.76	43.02	-	90.63	82.34
STH		9.00	13.68	2.38	0.50	1.40	7.01	9.20	2.41	1.02
OST		-	-	-	-	1.37	-	-	-	-
AC		0.78	1.49	-	-	0.68	-	-	-	-

Fractions of *Ocimum gratissimum* (OG) essential oil are OG_{1A}, OG_{1B} and OG_{1C} obtained with 100% hexane showing distinctive profiles on TLC plates, while OG_{2} fraction was obtained at 95/5% v/v H/EA fractionating solvent. Fractions of *Thymus vulgaris* (TV) essential oil are TV_{1A} and TV_{1B} obtained with 100% hexane showing distinctive profile on TLC plates, while TV_{2}fraction was obtained at 95/5% v/v H/EA fractionating solvent. Monoterpene Hydrocarbons (MTH); Oxygenated Monoterpenes (OMT); Sesquiterpene Hydrocarbons (STH); Oxygenated sesquiterpens (OST); Aromatic Compound (AC), 1-methyl-4(1-methylethenyl) benzene (MMB). Retention Index (RI)

DISCUSSION

The fungicidal activity displayed by *C. citratus, O. gratissimum* and *T. vulgaris* oils can be assigned to their high content in OMT 64.03, 44.61 and 43.02%, respectively as reported by Nguefack *et al.* (2007), this correlated with our findings. The low antifungal activities against the two strains of *P. expansum* observed with subfractions CC_{1A}, OG_{1A} and TV_{1A} rich in hydrocarbon terpenes compared to their complete EO affirmed OMT in these plants largely contributes for their antifungal potency. This observation further verifies the truism that oxygenated terpenes are overwhelmingly antimicrobial than their hydrocarbon counterparts as proposed by Hammer *et al.* (2003) and Dorman and Deans (2000).

It has been suggested that, borneol and bornyl-acetate lower antimicrobial activities of EO (Chalchat *et al*., 1987). This fall in line with our observation and can possibly explain the low antifungal activity of fractions and some subfractions such as CC_2, OG_2, TV2 and CC_{1B}, OG_{1B}, TV_{1B}, respectively containing the latter. Moreover, low antifungal activity of subfraction OG_{1B} (49.36% thymol; 5.08% carvacrol) compared to subfraction OG_{1C} (92.01% thymol and 0.86% carvacrol) could also be explained by the net decrease in thymol content due to its high isomer-carvacrol content and possibility of racemisation.

Overall, *O. gratissimum* whole EO exhibited the highest antifungal activity in NDR_{CFU} comparatively to complete EO of *C. citratus* and *T. vulgaris*; probably due to the high content of thymol (40.61% in OG, 28.11% in TV and absent in CC). The high antimicrobial activity of thymol had been proposed to be associated to the electron delocalisation system and the hydroxyl group in its structure (Ultee *et al*., 2002); this possibly explains observed high antifungal activity of *O. gratissimum*. It's been proposed structural configuration, functional groups, antagonistic and synergistic interactions between components of oils determines the intrinsic antimicrobial potential of EO (Dorman and Deans, 2000; Delaquis *et al*., 2002) supporting these factors harmonized well in *O. gratissimum* than in *T. vulgaris* and *C.citratus*.

It's been shown thymol, citrals, carvacrol and p-cymene play an important role in membrane swelling, causing membrane permeability (Dorman and Dean, 2000; Ultee *et al*., 2002), but with p-Cymene playing a more dominant role. This permeabilizing activity p-cymene probably enables the influx carvacrol into the cell so that a synergistic effect is achieved when the two are present. This supportively, explains the synergism observed with fractions blend of CC_{1A}/CC_{1B} and OG_{1A}/OG_{1B} due to the combined effect of the different components brought into the cocktail. However, synergism varied with the proportion of fractions blended and was important at 50/50% v/v compared to 25/75 and 75/25% v/v. This may imply 50/50% v/v blend contains sufficient quantity of p-cymene required to puncture *P. expansum* membrane. Hence, facilitating trans-membrane transportation of citrals, thymol and carvacrol into the cytosol. The synergistic effect observed with non-active fractions blend CC_{1A}/OG_{1A} and CC_{1A}/TV_{1A} could be explain by the increase concentration of oxygenated terpenes present in traces in each of the individual fractions.

The antagonistic effect observed with blended fractions CC_{1B}/CC_{1C}, CC_{1B}/CC_2, CC_{1C}/CC_2, OG_{1B}/OG_{1C}, OG_{1B}/OG_2, OG_{1C}/OG_2 and TV_{1B}/TV_2 could be related to the presence of borneol or bornyl-acetate; since they lower the activity of their individual fraction and the cocktail. Proportionate reduction of fractions containing the latter substantially increases the antifungal activity of the mixtures, affirming they are antagonist enhancers. Furthermore, fraction rich blend of borneol exhibited the highest antagonistic effect. Amazingly, blends of active fractions CC_{1C}/OG_{1C} and CC_{1C}/TV_{1B} revealed strong antagonism, probably due to neutralizing interactive effects between the active components of the fractions. Ultee *et al*. (2000) showed antimicrobial activity of carvacrol methyl ether was lower than carvacrol, due to blockage of the hydroxyl group with a methyl group. This implies such neutralizing interactive effect may block active groups of fractional components and enhance antagonism as observed with the latter blends. Reduction in oxygenated terpenes content or increases in borneol and it derivatives such as in fractions blend of CC_{1A}/CC_{1C}, OG_{1A}/OG_{1C}, OG_{1A}/OG_2, TV_{1A}/TV_{1B} and TV_{1A}/TV_2 possibly explain their antagonism.

CONCLUSION

These results pave way for the possible use of essential oils of these plants as food preservatives; but require further assessment of the pH variance on the synergistic and antagonistic effects.

Moreover, this study reveals very small and precise amount of essential oils can be used to produce a differentia antimicrobial effect with a probable minimal alteration in food taste. A comparative assessment of the degree of synergy of these essential oils on other phytopathogens and the ultimate evaluation of their toxicity to Humans and animals, can lead to their formulation as bio-preservatives against mycotoxins biotic agents.

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