Shukla, R., P. Singh, B. Prakash, A. Kumar, P.K. Mishra and N.K. Dubey, 2011. Efficacy of essential oils of *Lippia alba* (Mill.) N.E. Brown and *Callistemon lanceolatus* (Sm.) Sweet and their major constituents on mortality, oviposition and feeding behaviour of pulse beetle, *Callosobruchus chinensis* L. Journal of Science Food and Agriculture **91**, 2277–2283.

Southgate, B.J., 1979. Biology of Bruchidae. Annual Review of Entomology **24**, 449-473.

Thakur, D. R. and B. Devi, 2016. Biopesticidal efficacy of *Berberis lycium* and *Cannabis sativa* against *Callosobruchus chinensis* Linnaeus. Journal of Insect Science **29**, 227-232.

Waterworth, P.D., 1986. Internal seed infesting insects. Part I & II. Beltsville: USDA, 1-136 pp.

Effectiveness of Essential Oils from Ngaoundere, against Post-Harvest Insect and Fungal Pests of Maize

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Abstract

Successful storage of harvest is a matter of utmost importance in the Sudano-Guinean agro-ecological zone where intense cultivation takes place only once a year. Poor and rudimentary drying/storage methods, high relative humidity as well as inaccessibility to the chemical pesticides leave stored maize at the mercy of insect and fungal attack. Insect attack favours secondary attack by fungi; both leading to a fall in the nutritional, sanitary and organoleptic qualities of the stored maize. Thus, poor peasant farmers are left with the choice of locally available botanicals as alternatives to chemical pesticides. It is against this backdrop that this study seeks to determine the insecticidal efficacy of essential oils from the leaves of *Chenopodium ambrosioides* and *Cupressus sempervirens* together with their 50/50 binary combination against the maize weevil, *Sitophilus zeamais,* and the fungi: *Rhizopus stolonifer* and *Aspergillus flavus* on stored maize. Insect mortality and progeny inhibition and the inhibition of fungal invasion were evaluated. Pesticidal activities of both essential oils increased with ascending dose of application. 200 µL/kg of the binary combination caused 100% mortality within 14 days and it completely inhibited progeny production in the weevil. The mixture of the two oils showed additive effects against the weevils and fungi. The two essential oils in isolation significantly inhibited fungal spore invasion in 21 days of storage although *A. flavus* was less susceptible than *R. stolonifer*. Therefore both plants could provide active botanical pesticides against *S. zeamais* and fungal pests in stored maize.

Key words: botanical, essential oil, fungal spore, stored maize pests, food security

1. Introduction

Sub-Saharan Africa is the most vulnerable region in the world with the average amount of food available per person per day being 1,300 calories compared to the world wide average of 2,700 calories (FAO, 2013). In 2012, maize had a yield of 70,076,591 tons in Africa (FAOSTAT, 2015). It is grown in diverse agro-ecological zones and farming systems, and consumed by people with varying food preferences and socio-economic background (Langsi *et al.,* 2017a). Cameroon with agriculture as its backbone has about 70% of its active population involved in agriculture, which contributes to about 25% of the GDP (FAO, 2008). Stored maize, especially in regions of high humidity is highly prone to attack by insect and fungal pests. High humidity and water content favour fungal growth (Pitt and Hocking, 2009). The most prolific insect is *Sitophilus zeamais* which bores holes and creates hotspots suitable for fungal growth. The fungi now produce mycotoxins thereby lowering the quality and also rendering it hazardous for consumption (Rashad *et al.*, 2013).

Plants which make excellent leads for new pesticide development (Napoleao *et al.,* 2013) could be used. Essential oils from plants generally contain chemicals which have both curative and protective potentials on stored products (Hamdani *et al.,* 2015). *Chenopodium ambrosioides* L. (Amaranthaceae) and *Cupressus sempervirens* L. (Cupressaceae) locally used as botanicals were chosen for this work. *Ch. ambrosioides* L. (Amaranthaceae) is a plant whose powders have been studied against *Sitophilus*

zeamais Motschulsky for Toxicity, oviposition suppression, ovicidal and larvicidal effects (Abiodoun *et al.,* 2010; Ntonifor *et al.,* 2011; Tapondjou *et al.,* 2002). Tapondjou *et al.* (2002) did *in-vitro* toxicity and progeny control effects using the essential oils while with *Cu. sempervirens* L. (Cupressaceae), mortality, progeny and Repellency effects have also been studied (Achiri *et al.,* 2015, Tapondjou *et al.,* 2005) on *S. zeamais* Motschulsky. Essential oils have also been proven to have antimicrobial and positive food technological potentials such as: *in-vivo* effectiveness and anti-oxidant properties of *Ocimum grattisimum*, *Lippia rugosa* and *Xylopia aethiopica* on *Aspergillus flavus* on maize fungi (Tatsadjieu *et al.,* 2010), antibacterial property of methanol, ethanol and hexane extracts (Sati *et al.,* 2015), significant antifungal activity of *Cu. Sempervirens* (Amri *et al.,* 2013).

The main objective of this work was the use of essential oils of *Ch. ambrosioides* L. and *Cu. sempervirens* L. available locally to control maize weevils (*S. zeamais* Motschulsky) and fungi (*Rhizopus stolonifer* and *Aspergillus flavus).* And specifically to evaluate their effectiveness on insect: Mortality, progeny production and fungal invasion *in-vivo* on treated maize grains.

2. Materials and methods

Insects and maize substrate

2.1. Test maize

The Acid Tolerant Population (ATP) variety of maize was collected from farmers in Big Babanki (North West Region, Cameroon) and identified in the cereals unit of the Institute of Agricultural Research for Development, IRAD, Bambui. The moisture content of maize used in bioassays was determined to be 12.669% (AFNOR, 1982). Weevils were obtained from laboratory stock cultures from the Crop Protection Laboratory of the Institute of Agricultural research for Development, IRAD, Bambui.

2.2. Collection of plant material and extraction of essential oils

Fresh green leaves of *Ch. ambrosioides* and *Cu. sempervirens* were collected from the University of Ngaoundere between December 2015 and February 2016, shade dried on laboratory benches (17.3–28.8°C), and hand crushed to get powder. The unseived powder was then packaged in black polythene bags and used for essential oil extraction.The essential oils were extracted by hydrodistillation with the help of a Clevenger apparatus and dried over anhydrous Sodium Sulphate. It yielded 0.812% (wt.wt) for *Ch. Ambrosioides* and 0.697% (wt/wt) for *Cu. Sempervirens*. Essential oils were analysed for component identification using an Agilent Technologies 6850 gas chromatograph coupled with a mass detector 5973 and a 7683B Series Injector autosampler. The essential oil was diluted with hexane which was injected in splitless mode at 200 °C. Components were separated in the oven following a temperature gradient starting from 50 °C and kept for 7 min; then raised to 300 °C (10 °C/min) and kept at this temperature for 4 min. Helium was used as carrier gas and retention indices were calculated according to Kovats, for alkanes C9-C24 compared with those reported by Adams (2007).

2.3. Bioassays

All bioassays were carried at a temperature of: 17.3–28.8°C and relative humidity: 56.3–97.8% from May to September 2016. Twenty five g of maize grains were placed in 500 mL glass jars. Aliquots of both essential oils and their 50/50 binary combination were applied to the maize grains at the following dosages 0 µL (control), 25 µL/kg, 50 µL/kg; 100 µL/kg, 200 µL/kg (diluted in 1mL acetone). All treatments were replicated 4 times. The maize-essential oil-acetone mixture was then hand shaken to permit complete coating of the maize by the essential oils and later left open for about 45 minutes on laboratory shelves to permit complete evaporation of the solvent. Afterwards, 20 adult (less than 7 days old) *S. zeamais* of mixed sex were separately added into each jar and kept on laboratory shelves. Insect mortality was recorded 1, 3, 7 and 14 days post treatment and percentage

insect mortality was corrected using the Abbott (1925) formula. All tests were carried at temperature: 17.3–28.8°C and relative humidity: 56.3–97.8%

On the 14th day post-infestation, the remaining live insects were removed and the different jars containing grains were kept under the same experimental conditions. The recording of F1 progeny was done once a week for 5 weeks commencing 6weeks post-infestation (Nukenine *et al.,* 2007). Percentage reduction in adult emergence or inhibition rate (% IR) was calculated as:

$$\%\boldsymbol{IR} = \frac{\mathbf{(Cn - Tn)x100}}{\mathbf{Cn}}$$

Where C_n is the number of newly emerged insects in the untreated (Control) jar and T_n is the number of insects in the treated jar.

2.4. Fungal invasion tests

Visibly contaminated stored maize samples surface sterilized using 2% $NaOCl_{(aq)}$ (Hocking *et al.,* 2006). Whole grains were placed directly on solidified PDA (potatoes dextrose agar: 200 g potato infusion, 20 g glucose, 15 g agar, pH of 5.6 with HCl) supplemented with Chloramphenicol (60 µg/mL) medium. The fungi were identified with the help of Domsch *et al.* (1980) manual.This involved treatment of self-contaminated maize grains with essential oils at varying concentrations and stored for a given duration. The method by Aoudou *et al*., (2012) was used. In order to homogenise the water content, the maize grains were soaked in sterile distilled water for 24 hours then dried in an oven at 45°C for 24 hours. Twenty five grams of maize grains were measured and put in 300mL glass jars on whose lids had been attached with the use of plaiting thread, 12 cm^2 Whatman no. 1 filter papers. The sealed containers containing the grains were sterilised by autoclaving at 1 atmosphere, 121°C for 15 minutes. Isolates of *Rhizopus stolonifer* and *Aspergillus flavus* were cultured on a PDA/chloramphenicol medium until the development of mature spores. With the aid of a haemocytometer, a 10^5 spores/mL suspension was prepared. In aseptic conditions, the sterile grains were inoculated with 2 mL of spore suspension (10^5spores/mL) of each fungus species. The containers were sealed and allowed at ambient conditions for three days to allow spore invasion. The filter papers were then soaked with different concentrations of essential oil (0, 20, 40, 60 and 80 µL/Kg) and sealed. Storage was maintained at ambient conditions for 30 days while growth inhibition was accessed every 7 days beginning from day 1 (Chatterjee, 1990). The percentage of contaminated grains was calculated using the formula:

$$\text{Percentage of contaminated grains } (\%\text{CG}) = \frac{\text{NCG}}{\text{TNG}} \times 100$$

Where: NCG = number of contaminated grains; TNG = total number of grains.

2.5. Statistical Analysis

Adult mortality was corrected relative to natural mortality in the controls using Abbott (1925) formula. Data on mortality and progeny production was transformed by using √(x + 0.5), then later ANOVA was done using statistical package for social sciences (SPSS) software. Tukey test (HSD) was used for mean separation for both weevil and fungal data. The dose–mortality response was analyzed by probit analysis (Finney 1971) using the maximum likelihood estimation. The co-toxicity coefficient per *Ch. ambrosioides* and *Cu. sempervirens* mixture was determined using the formula by Sun & Johnson (1960).

3. Results and Discussion

In both oils, monoterpenes hydrocarbons were predominant (Table 1). Monoterpene hydrocarbons constituted 69.2% in *Cu. sempervirens* and 79.87% in *Ch. ambrosioides*. The greatest in *Ch. ambrosioides* being 4-carene (46.32%). Only monoterpenes were found. However, in *Cu. sempervirens* monoterpene hydrocarbons predominated followed by the hydrogenated monoterpenes, the oxygenated sisqueterpenes and last by the oxygenated sisqueterpenes. Of all

the compounds identified α -pinene was the most concentrated (20.10 %) followed by β -phellandrene (6.94%). All sisqueterpenes were below 1% in concentration.

Tapondjou *et al.* (2002) instead found more elevated proportions of Cymol (50%) and terpinene (37.6%) in *Ch. ambrosioides* harvested from Mbouda in the West Region, Cameroon. We also noted the presence of lower proportions of ascaridole (0.86%) same as Rafaela *et al.* (2014) found in Brazil (0.87%) but lower than that found in samples by Ali *et al*. (2016) (Yemen) and Tapondjou *et al.* (2002).

With *Cu. Sempervirens,* Tapondjou *et al.* (2005) instead noticed the absence of cymol, the *Cupressus* essential oil also showed an elevated presence of hydrogenated monoterpenes. A similar chemical composition rich in α-pinene (27.5 to 35.8%), α-cedrol (7.7 to 19.3%), δ-3-carene (5.8 to 13.2%) was found by Amri *et al.* (2013) in Tunisia. While Mazari *et al.* (2010) in Algeria also found that the majority of the compounds found are hudrocarbon monoterpenes. Tapondjou *et al.* (2005) also found α –pinene to be the most abundant component.

The essential oils and their binary combination caused significant the mortality of adult *S. zeamais* relative to the control (Table 2). Mortality rates were generally higher for *Ch. ambrosioides* than for *Cu. Sempervirens* and the binary combination at the dosages of 50 and 100 uL/kg, irrespective of the exposure period. However, at the highest dosage level of 200 uL/kg, *Ch. ambrosioides* essential oil and the binary combination caused greater mortalities than *Cu. Sempervirens* within the first 24 hours of exposure. Only the binary combination achieved 100% mortality with 14 days of exposure at the 200 ul/kg dosage level although this was similar to the 95.83% mortality caused by *Ch. ambrosioides* essential oil. Langsi *et al.* (2017b) found 100% mortality with *Ch. Ambrosioides* and the 50:50 binary combination of *Chenopodium ambrosioides* and *Cupressus sempervirens* within 72hours of exposure.

Table 1: Chemical compositions of *Cupressus sempervirens* and *Chenopodium ambrosioides* essential oils

N°	KI	Name	Percentage (%)	
			Cu. sempervirens	*Ch. ambrosioides*
Monoterpene hydrocarbons			69.2	79.87
	935	α –Pinene	17 .59	/
	1008	3-carene	25 .91	/
	1012	4-carene	/	46.32
	1017	p-cymene	/	32 .62
Oxygenated Monoterpenes			16.95	1.55
	1260	Ascaridole	/	0.86
Sisqueterpene hydrocarbons			4.21	0.00
Oxygenated Siqueterpenes			0.7	0.00
Total			91 .34	81.42

Table 2: Corrected cumulative mortality (Mean ± S.E) of *Sitophilus zeamais* due to treatment of maize grains with essential oils of *Chenopodium ambrosioides, Cupressus sempervirens* from Ngaoundere and their 50:50 binary combination

Exposure Period (days)	Content (µL/kg)	Mortality *Ch. ambrosioides*	*Cu. sempervirens*	50 :50 Combination	$F_{(2, 9)}$
	00	0.00 ± 0.00A	0.00 ± 0.00A	0.00 ± 0.00A	/
	25	0.00±0.00A	0.00 ± 0.00A	0.00 ± 0.00A	/
1	50	5.00 ±0.00Aa	7.50±1.44ABb	2.50± 1.44Aa	54.000***
	100	61.25 ±3.15Bb	13.75 ±2.39Ba	20.00 ±2.04Ba	456.000***
	200	73.75±4.73Cb	33.75±2.39Ca	72.50 ±4.79Cb	634.776***

$F_{(4, 15)}$		204.992***	64.403***	165.321***	
	00	0.00 ± 0.00A	0.00 ± 0.00A	0.00 ± 0.00A	/
	25	0.00±0.00A	0.00 ± 0.00A	0.00 ± 0.00A	/
3	50	15.00 ±2.04Bb	10.63 ±0.47Ba	7.70 1.49Aa	140.522***
	100	72.50±4.79Cc	17.75 ±1.32Ba	33.36 ±3.41Bb	348.703***
	200	80.00±3.54Cb	69.61 ±3.56Ca	82.17±7.71Cb	455.209***
$F_{(4, 15)}$		198.000***	286.881***	83.283 ***	
	00	0.00 ± 0.00A	0.00 ± 0.00A	0.00 ± 0.00A	/
	25	0.00±0.00A	0.00 ± 0.00A	0.00 ± 0.00A	/
7	50	17.14 ±2.89Bc	20.75 ±1.95Bb	17.83±4.22Ba	123.536***
	100	82.65±2.55Cc	21.10 ±3.88Ba	43.62 ±1.62Cb	900.446***
	200	92.02±1.50Db	74.61±6.25Ca	98.75 ±1.25Db	1640.870***
$F_{(4, 15)}$		602.138***	80.529***	388.999***	
	00	0.00 ± 0.00A	0.00 ± 0.00A	0.00 ± 0.00A	/
	25	0.00 ± 0.00Aa	1.32 ±1.32Aab	2.63 ±1.52Ab	3.857*
14	50	19.08±3.31Ba	24.02 ±1.82Ba	40.20±3.65Bb	197.281***
	100	93.20±2.59Cc	25.88 ±4.30Ba	48.03 ±2.25Bb	923.394***
	200	95.83±1.39Cb	80.70±5.40Ca	100.00±0.00Cb	2646.476***
$F_{(4, 15)}$		611.088***	101.609***	401.344***	

P = 0.05 (Chi-square test). (P<0.01); ***: very highly significant (P<0.001).

The co-toxicity indices indicated that the combination of the two essential oils resulted in additive effect vis-a-vis *S. zeamais* mortality on the treated maize grains for all time-points (Table 3). With additive effects, it has been proven that combinations of insecticidal materials have the advantages to increase the efficacy by complementing the bio-efficacy of the individual products and simultaneously lowering their use on the one hand and broadening the spectrum of activity and overcoming pest resistance to individual pesticide on the other hand (Das, 2014).

Table 3: Lethal contents and co-toxicity coefficients of binary combinations on the mortality of *Sitophilus zeamais* due to treatment of maize grains with essential oils of *Chenopodium ambrosioides, Cupressus sempervirens* from Ngaoundere

Period of exposure	Product	LC_{50} (µL/kg)	Co-toxicity index	Interpretation
	Ch. ambrosioides	114.48		
1 Day	*Ch. sempervirens*	244.58	103.19	Additive
	50:50 combination	151.14		
	Ch. ambrosioides	91.15		
3 Days	*Ch. sempervirens*	167.15	97.09	Additive
	50:50 combination	121.51		
	Ch. ambrosioides	76.31		
7 Days	*Ch. sempervirens*	138.18	106.47	Additive
	50:50 combination	92.35		
	Ch. ambrosioides	67.60		
14 Days	*Ch. sempervirens*	121.05	116.60	Additive
	50:50 combination	74.40		

All the essential oils and their binary combination significantly inhibited progeny production. These inhibitions all increased with dose of administration (Table 4). Apart from *Cu. sempervirens* with 43%, even the lowest concentrations of all the oils inhibited progeny production by more than 50% relative to the control. The 50/50 binary combination at 200 µL/kg, completely supressed progeny production. This was followed by *Ch. ambrosioides* with 99% while *Cupressus* had 95% inhibition to progeny production.

Table 4: F_1 progeny (Mean ± S.E) of *Sitophilus zeamais* due to treatment of maize seeds with essential oils of *Chenopodium ambrosioides, Cupressus sempervirens* from Ngaoundere and their binary combination

Product Content (µL/kg)	*Ch. ambrosioides* F_1	% reduction of F_1	*Cu. sempervirens* F_1	% reduction of F_1	50/50 binary combination F_1	% reduction of F_1

00	27 ± 1.29d	0.00 ± 0.00a	27±1.29d	0.00 ± 0.00a	27±1.29d	0.00 ± 0.00a
25	13±0.41c	51.31±3.82b	17±1.29c	36.04±7.53b	8.75±0.48c	67.46±1.92b
50	11±0.41b	58.90±3.02b	11±0.96b	56.61±5.71c	4.25±0.48b	84.10±2.08c
100	7±0.41b	74.01±0.52c	9.50±0.65b	64.48±3.48c	2.75±0.48ab	89.78±1.82c
200	0.5 ± 0.29a	98.00±1.16d	1.75±0.48a	93.23±2.16d	0.00 ±0.00a	100±0.00d
$F_{(4, 15)}$	213.222***	259.538***	89.987***	56.791***	247.261***	704.133***

Means ± S.E. in the same column for the same category of insecticide, followed by the same letter do not differ significantly at $P = 0.05$ (Tukey test). Each datum represents the mean of four replicates of 20 insects each. ***: very highly significant ($P<0.001$).

Percentage inhibition generally increased with dose administered. They are shown in Figures 1 (a, b and c). The 80 µL/L dose of *Ch. ambrosioides* gave 88% inhibition of *Rhizopus* growth in the first week. In all the doses administered, inhibition decreased from a maximum in 7 days to less than 10% in 21 days (Figure 1a). From Figure 1.b, it is noticed that *Cu. sempervirens* at 80 µL/L gave 55% inhibition of *Rhizopus'* spore germination and all the different concentrations dropped to zero within 21 days. The 50:50 binary combination however was more efficient than both plants used separately. Its 80 and 60 µL/L doses gave respectively 96 and 90% inhibition to *R. stolonifer* spore germination. Essential oils are very well known for their bactericidal, bacteriastatic, virucidal, fungicidal activity due to their medicinal properties against the wide range of pathogenic microorganisms (Akthar *et al.* 2014, Ambindei *et al.,* 2017). However, the spectrum of antimicrobial activity is dependent on the tested pathogens, measurement conditions and the source of the antimicrobial compounds (Turgis *et al.,* 2009).

Figures 2 (a, b and c) show the efficiency of essential oils from Ngaoundere on *Aspergillus flavus.* From the results, *Aspergillus* was more sensitive to the essential oils of both plants and their binary combination than *Rhizopus.* In figure 2.a, the 80 µL/L content gave 100% inhibition in seven days of exposure while the lower doses all gave inhibitions more than 60%. With *Cu. Sempervirens* percentage inhibition did not go beyond 65% for all the doses administered on *Aspergillus*. And all the percentage inhibitions dropped to zero within 21 days of exposure (Figure 2.b) all these results on *Aspergillus* are in agreement with those of Mahmood *et al.* (2013). With the 50:50 binary combination, percentage inhibition of 100 was gotten after 7 days with the 80 and 60 µL/L essential oil concentrations on *Aspergillus*. The percentage inhibitions dropped to zero within 21 days of exposure. This was followed by the 40 and 20 µL/L with respectively 90% and 80%. However, all inhibitions fell to zero after 21 days of exposure (Figure 2.c). 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, Ambindei *et al.,* 2016, 2017)

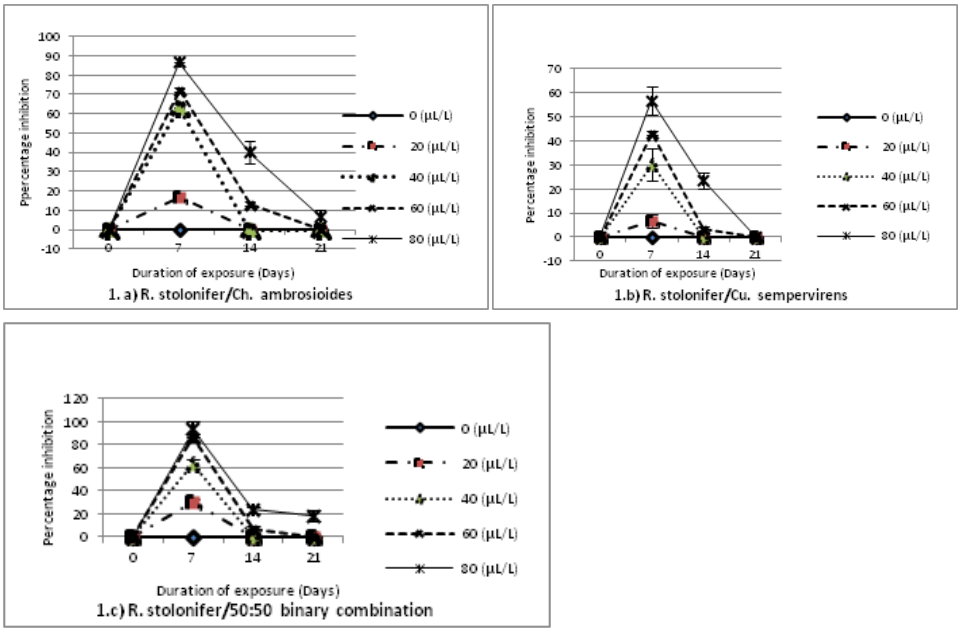

Figure 1: *In vivo* percentage inhibition of *Rhizopus stolonifer* growth on contaminated maize as a result of treatment with essential oils of *Chenopodium ambrosioides, Cupressus sempervirens* and their 50/50 binary combination from Ngaoundere.

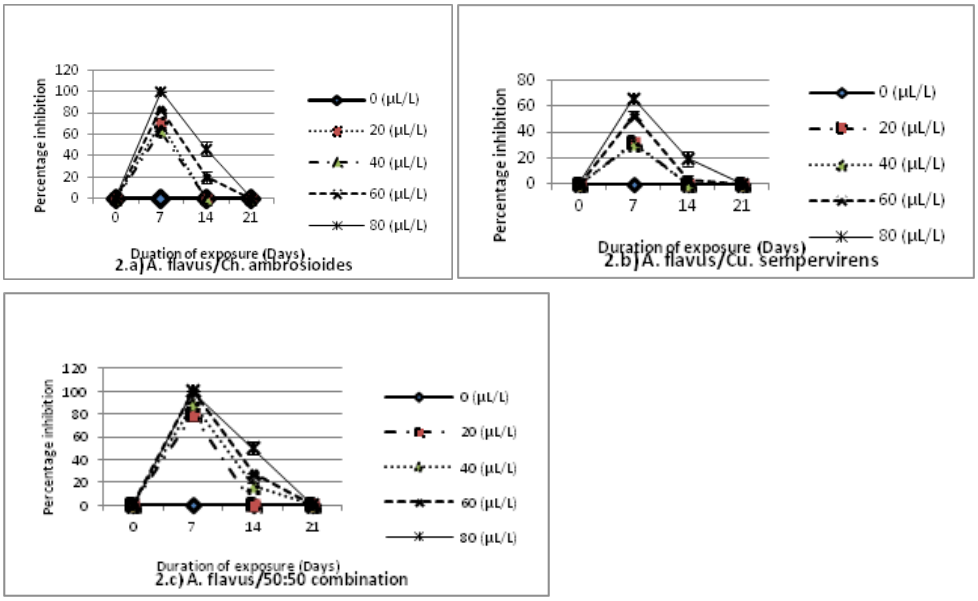

Figure 2: *In vivo* percentage inhibition of *Aspergillus flavus* growth on contaminated maize as a result of treatment with essential oils of *Chenopodium ambrosioides, Cupressus sempervirens* from Ngaoundere and their 50/50 binary combination

Both essential oils were found to be rich in α –pinene and cymol, while *Chenopodium* contains ascaridole; which are all reference fungicides and insecticides (Mazari *et al.,* 2010; Yang *et al,* 2007; Demirci *et al.,* 2007). P-cymene and p-cymeene found in both oils, on their own, are not an excellent

antifungal agent (Bagamboula *et al.,* 2004), but will boost the activities of components with functional side groups (Rattanachaikunsopon and Phumkhachorn, 2010).

Essential oils of *Ch. ambrosioides* and *Cu. sempervirens* available locally presented additive insecticidal and fungicidal efficacy against both weevils and fungi. The ability to control the proliferation of *S. zeamais, A. flavus* and *R. stolonifer* in stored maize by these essential oils is dose dependent and increases with period of exposure to the pesticide. Therefore both pesticides stand highly recommended due to their insecticidal and progeny control effects as well as their ability to inhibit fungal spore germination on stored maize.

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References

ABBOTT, W.S., 1925: A method of computing the effectiveness of an insecticide. -Journal of Economic Entomology. **18**: 266-267.

ABIODUN, A., DENLOYE, WINIFRED, A., MAKANJUOLA, OLUWAKEMI, K., TESLIM *et al.,* 2010: Toxicity of *Chenopodium ambrosioides* L. (Chenopodiaceae) products from Nigeria against three storage insects. Journal of Plant Protection Research. **50**: 3

ACHIRI, D.T. AND M.A. NJWENG, 2015: Bioactivity of cypress leaf powder (*Cupressus macrocarpa*) on cowpea weevil (*Callosobruchus maculatus* Fabr. Coleoptera: Bruchidae) and maize weevil (*Sitophilus zeamais* Motschulsky, Coleoptera: Curculionidae) in stored maize grains in Cameroon. International Journal of Interdisciplinary and Multidisciplinary Studies. **4**, 1-10

ADAMS, R. P., 2007: Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Corporation. **469** pp. Carol Stream, IL, 60188, USA

AKTHAR, M.S., DEGAGA, B., AZAM, T. Antimicrobial activity of essential oils extracted from medicinal plants against the pathogenic microorganisms, 2014. A review. Issues in Biological Sciences and Pharmaceutical Research. **2,** 1-7.

ALI, G.A-k., REBECCA, A.C., ANNIKA, D., ANDREA, P., OTHMAN, S.S. AL-H., NASSER A.A.A., WILLIAM N.S., LUDGER, W. 2016. Chemical composition and biological activity of essential oil of *Chenopodium ambrosioides* from Yemen. American Journal of Essential Oils and Natural Products; **4**, 20-22

AMBINDEI, A.W., JAZET, P.D.M., TATSADJIEU, L.N., PRIYA, P., MANILAL, V., KRISHNAKUMAR, and Z.P.H. AMVAM, 2017: Effect of the essential oils of *Thymus vulgaris, Cinnamomum zeylanicum* and *Mentha piperita* on fungal growth and morphology. African Journal of Biotechnology. **16,** 388-399

AMBINDEI, A.W., TATSADJIEU, L.N., JAZET, P.D.M., PRIYA, P., ANIE, M., MANILAL, V., KRISHNAKUMAR, B. and Z.P.H. AMVAM, 2017. Isolation and Molecular Identification of Fungi in Stored Maize (*Zea mays* L) and Groundnuts (*Arachis hypogaea* L) in Ngaoundere, American Journal of Microbiological Research. **4,** 85-89

AMRI, I., HAMROUNI, L., HANANA, M., GARGOURI, S. and B. JAMOUSSI, 2013. Chemical composition, bio-herbicidal and antifungal activities of essential oils isolated from Tunisian common cypress (*Cupressus sempervirens* L.) Journal of Medicinal Plants Research **7,** 1070-1080

AOUDOU. Y., TATSADJIEU, N.L., JAZET, D.P.M., and C.M. MBOFUNG, 2012. Inhibition of fungal development in maize grains under storage condition by essential oils. International Journal of Biosciences **2**, 41-48

BAGAMBOULA, C.F., UYTTENDAELE M., and J. DEBEVERE, 2004. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. Food Microbiology **21**, 33-42.

DAS, S.K., 2014. Scope and relevance of using pesticide mixtures in crop protection: a critical review. International Journal of Environmental Sciences and Toxicity Research **2,** 119-123.

DEMIRCI, B., KOSAR, M., DEMIRCI, F., DINE M, and K. BASER, 2007. Antimicrobial and anti oxidant activities of essential oils of *Chaerophyllum libanoticum* Boiss. et Kotschy, Food Chemistry **105**, 1512-1517

FAO, 2008. "An Introduction to the Basic Concepts of Food Security: Food Security Information for Action. Practical Guides" FAO. Rome

FAO, 2013. "FAO's Response to the 2012 Sahel Crisis". FAO, Rome,

FAOSTAT, 2015. FAO Statistics Division 2015 | 04 October

FINNEY, D.J., 1971. Probit analysis. Cambridge University. Press. London.

HAMDANI, F.Z., ALLEM, R., MEZIANE, M., SETTI, B., ALI, A.S. and M. BOURAI, 2015. Chemical composition and antifungal activity of essential oils of Algerian citrus. African journal of biotechnology. **14**, 1048-1055

HOCKING, A.D., PITT, J.I., SAMSON, R.A., and U. THRANE, 2006. Advances in food mycology. Springer, New York

HYLDGAARD, M., MYGIND, T., and R.L. MEYER, 2012. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Frontlines of Microbiology **3,** 12

LANGSI, D.J., NUKENINE, E.N., FOKUNANG, C.N., SUH, C., W.J. GOUDOUNGOU, 2017b. Potentials of essential oils of *Chenopodium ambrosioides* L. and *Cupressus sempervirens* L. against stored maize pest, *Sitophilus zeamais* Motschulsky. **5,** 309-313.

LANGSI D.J., FOKUNANG C.N., E.N NUKENINE, 2017a. Potentials of Fractionated Extracts of plants to control maize weevils. Lap Lambert Academic Publishing. 17 Meldrum Streed, Beau Bassin 71504, Mauritius

MAHMOUD, Z., ISHTAQ, M., MUHAMMED, U.Q., and A.S. MUNIR, 2013. Investigation of Phytochemical composition and antimicrobial activities of essential oil extracted from Lignin-containing *Cupressus sempervirens.* Bioresources **8,** 1625-1633

MANU, I.N., TARLA, D.N., CHEFOR, G-F., NDEH, E.E., and I. CHIA, 2015. Socio-economic Analysis and Adoption of Improved Maize (*Zea mays* L.) Varieties by Farmers in the North West Region of Cameroon. Asian Journal of Agricultural Extension, Economics & Sociology. **4,** 58-66

MAZARI, K., NASSIMA; B., CHAHRAZED, B., and F. XAVIER, 2010. Chemical composition and antimicrobial activity of essential oils isolated from Algerian *Juniperus phoenicea* L. and *Cupressus sempervirens* L. Journal of Medicinal Plants Research **4,** 959-964

NAPOLEAO, T.H., BELMONTE B.D.R., PONTUAL, E.V., ALBUQUERQUE, DE L.P., SA R, PAIVA L.M., *et al.,* 2013. Deleterious effects of *Myracrodruon urundeuva* leaf extract and lectin on maize weevil, *Sitophilus zeamais* (Coleopera: Curculionidae). Journal of Stored Product Research **54,** 26-33.

NTONIFOR, N.N., FORBANKA, D.N., AND J.V. MBUH, 2011. Potency of *Chenopodium ambrosioides* powders and its combinations with wood ash on *Sitophilus zeamais* in stored maize. Journal of Entomology. **8**, 375-383

NUKENINE, E.N., ADLER, C. AND C. REICHMUTH, 2007. Efficacy evaluation of plant powders from Cameroon as post-harvest grain protectants against the infestation of *Sitophilus zeamais* Motchulsky (Coleoptera: Curculionidae). Journal of Plant Diseases Protection **114**, 30-36.

PITT, J.I. AND A.D. HOCKING, 2009. Fungi and Food Spoilage. Springer. **230** pages

RAFAELA, D.S., MARCOS, A.M.G., MAGDA, R.A., FERREIRA, LUIZ, A.L.S. and P.R. KARINA, 2014. Chemical composition of the essential oil from leaves of *Chenopodium ambrosioides* L. grown in Recife-PE, Brazil. Artigo Original / Research. 12 pages

RASHAD, A., SULEIMAN, K., ROSENTRATER, C. and J. BERN, 2013. Effects of Deterioration Parameters on Storage of Maize, Agricultural and Biosystems Engineering Conference Proceedings and Presentations, Agricultural and Biosystems Engineering. **52** pages

RATTANACHAIKUNSOPON, P. and P. PHUMKHACHORN, 2010. Assessment of factors influencing antimicrobial activity of carvacrol and cymene against *Vibrio cholerae* in food. Journal of Biosciences and Bioengineering **110**, 614-619

TAPONDJOU, A.L., ADLER, C., FONTEM, D.A., BOUDA, H. and C. REICHMUTH, 2005. Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* du Val. Journal of Stored Products. Research **41**, 91-102 ·

TAPONDJOU, L.A., ADLER, C., BOUDA, H. and D.A. FONTEM, 2002. Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six stored product beetles. Journal of Stored Products Research **38**, 395–402

TURGIS, M., HAN, J., CAILLET, S. and M. LACROIX, 2009. Antimicrobial activity of mustard essential oil against *Escherichia coli* O157:H7 and *Salmonella typhi.* Food Control. **20**, 1073–1079.

YANG, J.K., CHOI, M.S., SEO. W.T., RINKERD, L., HAN, S.W. AND G.W CHEONG, 2007. Chemical composition and antimicrobial activity of *Chamaecyparis obtusa* leaf essential oil, Fiterapia **78**, 149-152

Insecticidal contact toxicity of several essential oils against stored product pests

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Abstract

Results of laboratory bioassays in Petri dishes on evaluation of contact toxicity of *Illicium verum*, *Artemisia absinthium* and *Abies sibirica* essential oils (EOs) against larvae of khapra beetle, *Trogoderma granarium* Ev., adults of grain weevil, *Sitophilus granarius* L., and rice weevil, *Sitophilus oryzae* L., and confused flour beetle, *Tribolium confusum* Duv., and larvae and adults of the lesser mealworm, *Tenebrio molitor* L., are presented. EOs commercial samples from retail pharmacy were tested at doses 0.01, 0.25, 0.50, 0.75 and 1.00 µl/cm^2. A treated Petri dish surface treted with acetone was used as a control. The experiment was carried out in triplicate. Mortality of insects was assessed after 1, 3, 6 and 24 hours post exposure. After exposure insects were placed into untreated Petri dishes for 3 days. The main components of the *A. absinthium* EOs are thujil alcohol (19.65%), phellandrene (16.71%), borneol (12.1%) and thujone (11.55%) was found. The major component of *I. verum* EOs was anethole (98.64%). Isobornyl acetate (57.25%), α-pinene (13.55%) and limonene (10.62%) were found as the main components of *A. sibirica* EOs. *S. oryzae* and *S. granarius* were most sensitive to each EO. *I. verum* EOs was the most effective and caused 100% mortality of each insect at the dose 0.25 µl/cm^2.

Keywords: essential oils, stored product pests, contact toxicity, *Illicium verum*, *Artemisia absinthium*, *Abies sibirica*