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(RESEARCH ARTICLE)

Insecticidal activity of essential oils of *Chenopodium ambrosioides* and *Cupressus sempervirens* and their binary combinations on *Sitophilus zeamais*

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Abstract

Maize is cultivated worldwide and used as food and for fuel production. It is usually attacked and destroyed during storage by *Sitophilus zeamais*. With inaccessibility to synthetic pesticides, farmers are left with the choice of using locally available plant based pesticides. For this reason, we tested the insecticidal potentials of essential oils (EOs) of *Chenopodium ambrosioides* and *Cupressus sempervirens* and their binary combinations against *S. zeamais* on stored maize. Mortality, progeny inhibition, repellence and damage were tested. Pesticide characteristics of both essential oils were dose-dependent, 200 µL/kg of all the combinations caused at least 80% mortality within 14 days of storage while the 50:50 combination completely inhibited progeny production. Moreover, 8 µL of all the EO were repellent to the weevils. The 50:50 binary combination was the most active in all the tests carried out. Pesticidal interactions between the oils in combination were mostly additive and synergistic. There was also a good control of insect population increase and grain damage after six months of storage. Therefore both EOs can be recommended for the control of *S. zeamais*.

Keywords: Botanical pesticides; Essential oil; Grain damage; Maize insect pests

1. Introduction

Food security and poverty reduction are priorities we need to tackle in the Sub-Saharan region with the average amount of food available per person per day of 1,300 calories compared to the worldwide average of 2,700 calories [1]. Food safety crisis in the Sahel, driven by chronic poverty, high food prices, drought and low agricultural production, affect 18.7 million people across the region in 2013 [2]. Agricultural products are on-farm consumed while generating income. Cereals are a major source of food and contribute to about 50% of the total dietary energy supplies for this region [3]. Maize is the most widely-grown staple food crop in Sub-Sahara Africa (SSA), occupying more than 33 million ha each year with a yield of 70 million tonnes [4]. Importantly, maize is a staple food crop grown in diverse agro-ecological zones and farming systems, and consumed by people with varying food preferences and socio-economic backgrounds in SSA. Its central role as a staple food is comparable to that of rice or wheat in Asia, with consumption rates being the highest in eastern and southern Africa [5]. Cameroon is a country with a strong agricultural economy. Almost 70% of the active population is involved in agriculture, which contributes to about 25% of the Gross Domestic Product [3], with 55% of its rural population involved in agricultural activity, living in extremely poor conditions [6]. Moreover, the practice of agriculture is rendered difficult by the absence of farming

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tools, fertilizers, illiteracy, farm to market roads, pest problems, drying and storage facilities [7]. To ensure food security for the whole year, farmers store more than 75% of their harvested maize and cowpea and they have to protect them from weevil attacks [8]. Therefore, plants with pesticidal characterstics could be used [9]. *Chenopodium ambrosioides* L. (Amaranthaceae) and *Cupressu ssempervirens* L. (Cupressaceae) are plants used for insecticidal purposes by local populations in the North-West Region of Cameroon. *Ch. ambrosioides*is a plant whose extracts have been studied against *S. zeamais* Motschulsky for its oviposition suppression, ovicidal and larvicidal effects [10-12]. Tapondjou et al., (2002) evaluated *in-vitro* toxicity and progeny control effects of both EOs while with *Cu. Sempervirens* mortality, progeny and repellence effects have been studied on *S. zeamais* Motschulsky [13-14]. The main objectives of this work were to evaluate the efficacy of EOs of *Ch. Ambrosioides* and *Cu. sempervirens* and their binary combinations in the control of *S. zeamais.* We also evaluated their efficacy on insect mortality, progeny production inhibition, repellence and grain damage.

2. Materials and methods

2.1. Plant material

2.1.1. 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 Institute of Agricultural Research for Development (IRAD) Bambui. The water content of maize used in bioassays was evaluated by the [15] method and found to be 12.67 ± 0.34%. The weevils were obtained from stock cultures from the crop protection laboratory of IRAD, Bambui. Fresh leaves of *Ch. ambrosioides* and *Cu. sempervirens* were collected from IRAD Bambui from December 2015 to February 2016, shade dried, and hand crushed to get powder.

2.2. Extraction of essential oils by hydrodistillation

Essential oils were extracted by hydrodistillation of the shade dried powders using a Clevenger apparatus at the Laboratory of Industrial Chemistry and Bio-resources of the National Advanced School of Agro-Industrial Sciences (ENSAI, Ngaoundere). The oils collected were dried on $Na_2SO_{4\,(s)}$, weighed and stored in the dark at 4^{o}C in opaque bottles. All bioassays were carried out from May to September 2016.

2.3. Analysis of chemical composition by GC/MS

Essential oils were analyzed for component identification using an Agilent Technologies 6850 gas chromatograph coupled with a mass detector 5973 and a 7683B Series Injector autos ampler. The EOs were diluted by adding 1 mL of hexane to 1 µL of oil and 1 µL of the sample was injected in splitless mode. The resulting data was elaborated using MSD ChemStation and the NIST deconvolution software AMDIS. The column was 5% phenylmethylpolysyloxane (30 m x 0.25 mm; film thickness 0.25 µm). Injector temperature was kept 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 with a flow of 1.1 mL/ min. The mass detector settings were as follows: ionization voltage, 70eV; scan rate, 2.91 scan/s; mass range, 50-500; transfer line, 230 °C. Essential oil components were identified by:

(a) Comparison of their relative retention times and mass fragmentation with those of authentic standards and

(b) Computer matching against NIST98 library and Golm Metabolome Database (GMD), as well as retention indices as calculated according to Kovats, for alkanes C9-C24 compared with those reported by Adams [16].

2.4. Insect mortality and progeny control

Twenty five grams of maize was placed in 500 mL glass jars. Aliquots of the *Ch. ambrosioides* (AA)*, Cu. sempervirens* (AB) as binary combinations (75:25, 50:50 and 25:75) were applied at the following concentrations 0 µL/kg (control), 25, 50; 100, and 200 µL/kg (each concentration diluted in 1mL acetone to permit distribution on grains). All treatments were replicated 4 times. The maize-essential oil-acetone mixture was then manually shaken. Then, the jars were left open for 45 min to allow complete evaporation of the solvent. Afterwards, 20 unsexed adults, less than 7 days old, were added into each jar and kept on laboratory shelves. Insect mortalities were recorded at 1, 3, 7 and 14 d after treatment. All tests were carried out at the following conditions: temperature: 17.3–28.8 °C and relative humidity: 56.3–97.8%.

2.5. Progeny production inhibition assessment

On the 14th day post-infestation, all the insects left were discarded and the different jars containing grains were kept under the same experimental conditions. The recording of F_1 progeny was done once a week for 5 weeks commencing from six weeks after infestation [17].

Percentage reduction in adult emergence (% IR) was calculated as:

$$\%IR = \frac{(\mathrm{Cn} - \mathrm{Tn})\mathrm{x}100}{\mathrm{Cn}}$$

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

2.6. Repellency test

The repellence test used was adopted from several authors [18] [19]. Four solutions of 1, 2, 4 and 8 µL of essential oils were dissolved in 1 mL of acetone. Whatman nº1 filter papers were cut into two equal halves and placed inside petri dishes (110 mm diameter). One half of each filter paper was treated with essential oil solution by using a micro pipette. The other half of the filter paper was treated with acetone only. The essential oil treated and acetone treated filter papers halves were air-dried to evaporate the solvent completely. EO treated and acetone treated half-dishes were then attached lengthwise, edge-to-edge with adhesive tape and placed at the bottom in glass petri dish (height 15 mm × radius 55 mm). Ten adults of insects were released at the centre of the petri dishes and then the petri dishes were covered and kept in the dark. Four replicates were set for each concentration of essential oils. Number of the insects on both treated and untreated halves was recorded every hour for four hours in mild light. The average was then calculated. The percentage repellence (PR) was calculated using the formula by [19] given by:

$$PR = 2*(C-50)$$

Where: C is the percentage of insects in the negative control half. The results were interpreted following the scale by [18] (Table 1)

Table 1 Interpretation of percentage repellence [18]

Class	Repellence rate (%)	Interpretation
X	>0.01 to < 0.1	Non repellent
I	0.1 to 20	Very low repellence
II	20.1 to 40	Low repellence
III	40.1 to 60	Moderately repellent
IV	60.1 to 80	Repellent
V	80.1 to 100	Very repellent

2.7. Damage bioassay

The mixture essential oils of *Ch. ambrosioides* and *Cu. sempervirens* of ratios (75:25, 50:50 and 25:75) used in the study. Two doses of these essential oil mixtures, 25 and 200 µL/kg on 100 g of grain in 1 L jars were prepared as described earlier. A group of 50 (< 7 d old) adult insects of mixed sexes was introduced into each jar containing treated or untreated grain (control in 1 mL acetone). Each treatment was repeated 3 times. After 6 months, the number of living and dead insects was determined for each jar. Damage assessment was carried out by determining the weight of the grains without powder (final weight) as well as the proportion of grains with holes in 50 randomly selected grains.

Percentage weight loss was determined as follows:

[(initial weight - final weight)/ initial weight] × 100.

Grain damage was determined as follows: 100 grains were randomly selected from each jar and the number of damaged (grains with holes) and undamaged grains were counted.

2.8. Statistical analysis

Adult mortality was corrected relative to natural mortality in the controls using Abbott's formula [20]. Data on mortality and progeny production was transformed by using $\sqrt{(x + 0.5)}$, then later ANOVA was done using statistical package for social sciences (SPSS) version 20 software. Tukey test (HSD) was used for mean separation. Probit analysis was used to calculate the lethal doses that cause 50% mortality (LD_{50}) after 1, 3, 7 and 14 days after treatment.

3. Results

3.1. Chemical composition

As seen in table 2, with *Chenopodium ambrosioides,* all compounds identified were hydrogenated monoterpenes with the highest being 4-carene (53%). With, *Cupressus sempervirens,* 46 compounds were identified and the hydrogenated monoterpenes (69.2%) were dominant followed by the oxygenated monoterpenes (16.95%), the hydrogenated sesquiterpenes (4.21%) and last by the oxygenated sesquiterpenes (0.7%). Of all the compounds identified, 3-carene was the most concentrated (25.91%) followed by α-pinene (17.59%). Of the oxygenated monoterpenes, terpinen-4-ol was the most concentrated (4.73%). All sesquiterpenes were below 1% in concentration. Others were present as trace amounts (less than 0.1%). With chemical composition, found more elevated proportions of Cymol (50%) and terpinene (37.6%) [12]. It is also noted the absence of even trace amounts of ascaridole earlier found in samples [21-22, 12]. This variation in content of volatiles could be explained by geographical locations of the plant. The result of present analysis of *Cu. sempervirens* essential oil also confirmed the absence of cymol which was shown in previous work of Tapondjou et al., (2005) whereas their study revealed an elevated presence of hydrogenated monoterpenes [14]. 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 Ismail et al., (2013) [23]. While Mazari et al., (2003) also found that the majority of the compounds found were hydrocarbon monoterpenes [24].

Table 2 Chemical composition of *Ch. ambrosioides* essential oil

KI*	Compound Name	Percentage (%)
1013	4-carene	52 .88
1017	p-cymene (Cymol)	29 .03
1035	τ-terpinene	1 .23
1227	3-carene	2 .12
Total		85 .26

*KI: Kovats Index

3.2. Mortality

The mortality of *S. zeamais* by contact upon treatment with essential oils of *Ch. ambrosioides* and *Cu. sempervirens* are shown in Table 4. Mortality increased with dose administered and time of exposure. There were also significant differences between the same concentrations of essential oils of both plants in the different periods of exposure. The highest dose of *Ch. ambrosioides* killed over 90% of the weevils after 24 h of exposure against 54% with *Cu. sempervirens.* Tapondjou et al., (2005) reported 5% mortality on 0.2 mL/cm^2 of filter paper *in vitro* after 24 h with *S. zeamais* being the least susceptible of all the tested insects to *Ch. ambrosioides* essential oil [14]. They also reported that *Eucalyptus saligna* was more active than *Cu. sempervirens*. Furthermore, Ntonifor et al., (2011) working on powders of *Ch. ambrosioides,* recorded 100% mortality of *S. zeamais* at a dose of 20 g/kg [11]. By the 14th d, both insecticides showed appreciable toxicity with more than 80% of mortality. When applied alone *Ch. ambrosioides* showed the most efficient insecticidal activity from day 1 to day 14 after infestation. In fact, mortality was at 100% after 72 h of treatment. Tapondjou et al., (2005), [14] registered 100% mortality of *S. zeamais* after 48 h *in-vitro* by using *Ch. ambrosioides* at a dose of 6.4%. Terpinen-4-ol is reported as a potent miticide [26]. The toxicity of the volatile oil from *Ch. ambrosioides* is generally attributed to ascaridole, cymol and a-terpinene [14]. Surprisingly, we did not detect ascaridol in our sample. The toxicity of *Ch. ambrosioides* on *S. zeamais* was also reported both as contact powder and as fumigant ethanolic extract of essential oil causing 100% mortality within 48 h [10].

Table 3 Chemical composition of *Cupressus sempervirens* essential oil

KI*	Name	Percentage (%)
	Hydrogenated Monoterpenes	69.2
926	α-thujene	0 .34
935	α –Pinene	17 .59
977	Sabinene	9 .42
1008	3-carene	25 .91
1016	o-cymene	1 .26
1019	limonene	10 .62
1037	τ-terpinene	1 .12
1047	α-terpinene	0 .19
1050	Terpinolene	1 .99
1052	p-cymenene	0 .76
	Oxygenated Monoterpenes	16.95
1057	Linalol	0 .98
1069	trans-p-menth-2-en-1-ol	0 .37
1077	cis-p-menth-2-en-1-ol	0 .41
1083	Eucarvone	0 .22
1090	Umbellulone	3 .28
1095	terpinen-4-ol	4 .73
1201	p-menth-1-en-8-ol	1 .46
1209	cis-piperitol	0 .14
1217	6-Octen-1-ol, 3,7-dimethyl	0 .21
1218	thymol methyl ether	0 .36
1231	Verbenone	0 .27
1233	5-decen-1-ol	0 .49
1235	Piperitone	0 .57
1249	isobornyl acetate	0 .14
1263	propanoic acid, 2-octyl ester	0 .27
1281	α-terpineolacetate	2 .97
1421	borneol, butyrate	0 .08
	Hydrogenated Sesquiterpenes	4.21
1428	d-longifolene	0 .08
1444	epi-bicyclosesquiphellandrene	0 .21
1453	δ-cadinene	0 .13
1456	epi-bicyclosesquiphellandrene	1 .32
1464	Curcumene	tr
1469	α-farnesene	0 .08
1476	α-elemane	0 .46
1488	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene	0 .11
1490	δ-cadinene	0 .32
1493	calamenene	0 .45
1903	(5,9α-, 10β)-kaur-15-ene	0 .86
2042	Abietatriene	tr
1886	kaur-15-ene	0 .19
	Oxygenated Sesquiterpenes	0.7
1621	Spathulenol	0 .06
1624	caryophyllene oxide	0 .25
1636	Cubenol	0 .15
1652	α-cadinol	0 .24
1867	Rimuene	tr
2032	Phyllocladen	tr
	Total	91 .34

tr (<0.1 %); *KI: Kovats Index

Table 4 Mortality (Mean ± S.E) of *S. zeamais* on treated ATP maize grains with binary combinations of essential oils of *Ch. ambrosioides*and *Cu. sempervirens*.

Exposure Period	Concentration(µL/kg)	Ratio of *Ch. ambrosioides* to *Cu. Sempervirens* in essential oil			
		50:50	75:25	25:75	F*(2, 9)
		Adult mortality (Mean ± S.E) (%)			
1 day	00	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	/
	25	60.00± 4.56^{Bc}	30.00± 2.04^{Bb}	1.25± 1.25^{Aa}	93.868***
	50	58.75 ±2.39^{Bc}	23.75 ±1.25^{Bb}	5.00 ±0.00^{Aa}	255.477***
	100	70.00 ±2.04^{Bb}	73.75 ±4.27^{Cb}	13.75 ±2.39^{Ba}	86.605***
	200	88.75 ± 6.58^{Cb}	86.25 ± 6.88^{Cb}	16.25 ± 3.15^{Ba}	41.078***
F(4, 15)		74.820***	91.172***	15.773***	
3 days	00	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	/
	25	74.01 ±3.74^{Bb}	23.95 ±9.81^{Aba}	5.07 ±2.04^{Aba}	31.498***
	50	57.24 ± 4.07^{Bc}	34.14 ±3.64^{Bbb}	10.13 ± 3.47^{Ba}	53.289***
	100	63.42 ± 6.66^{Bb}	73.22 ±5.24^{Cb}	21.84± 1.51^{Ca}	22.173***
	200	93.68 ± 4.73^{Cb}	91.18 ±7.17^{Db}	26.97 ± 1.57^{Ca}	60.668***
F(4, 15)		63.257***	36.584***	30.596***	
7 days	00	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	/
	25	79.61 ±2.89^{Bc}	39.46±2.34^{Bb}	6.58 ±2.52^{Aa}	113.048***
	50	72.95 ± 2.19^{Bc}	53.43 ±3.11^{Bb}	13.23 ±3.34^{Aba}	70.614***
	100	76.97 ±4.59^{Bb}	88.81±5.98^{Cb}	21.35 ±4.30^{Ba}	40.876***
	200	97.50 ±2.50^{Cb}	97.22 ±2.78^{Cb}	58.55 ±4.35^{Ca}	25.948***
F(4, 15)		176.145***	132.171***	48.078***	
14 days	00	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	/
	25	83.23 ±2.56^{Bb}	39.34±2.63^{Ba}	29.82 ±2.95^{Ba}	60.672***
	50	77.74 ±2.32^{Bc}	59.01±3.24^{Cb}	39.18±2.51^{BCa}	37.001***
	100	87.56±4.67^{BCb}	95.50 ±2.83^{Db}	47.22±2.98^{Ca}	36.888***
	200	97.22± 2.78^{Cb}	96.88± 3.12^{Db}	76.90±3.69^{Da}	12.633***
F(4, 15)	416.000***	186.086***	235.631***	103.587***	

Means ± S.E. in the same column/row for the same category of insecticide, followed by the same letter do not differ significantly at $P = 0.05$ (Tukey's test). Each value represents the mean of four replicates of 20 insects. *: significant (P<0,01) ; ***: very highly significant (P<0,001). Chen: *Ch. ambrosioides*, Cupr: *Cu. sempervirens*, 50:50: mixture of equal quantities of *Ch. ambrosioides* and *Cu. sempervirens* essential oils. $F_{(df1, df2)}$

3.3. Pesticidal interactions

Insecticidal activity of EOs and their combinations are reported in Table 5. Synergistic effects were observed for binary combination (25:75). *Cupressus*, being a low toxicity product had its toxic power enhanced by combining it with just 25% *Chenopodium* (Table 5). Additive effects were observed on the 7th day post exposure between the 50:50 combination and 75% *Chenopodium*. With synergistic and 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 [25].

Table 5 Pesticidal interactions between binary combinations of Essential Oils of *Ch. ambrosioides* and *Cu. sempervirens* on *S. zeamais*

Ratio of *Ch. ambrosioides* to *Cu. sempervirens* in essential oil	LC_{50}	LC_{95}	*Co-tox. Ind	Significance	General X^2
1 Day					
50:50	42.96	777.79	22.34	Antagonistic	1922.463***
75:25	29.99	62.64	33.21	Antagonistic	706.252***
25:75	4.70	14.98	197.09	Synergistic	1058.937***
3 Days					
50:50	44.21	800.33	27.83	Antagonistic	
75:25	34.95	73.01	32.48	Antagonistic	
25:75	8.27	26.38	162.31	Synergistic	
7 Days					
50:50	52.64	953.04	86.46	Additive	
75:25	49.32	103.02	88.43	Additive	
25:75	9.98	31.82	75.36	Antagonistic	
14 Days					
50:50	56.90	1030.08	73.91	Antagonistic	
75:25	52.33	114.61	79.43	Antagonistic	
25:75	32.14	102.51	132.39	Synergistic	

P = 0.05 (Chi-square test). (P<0,01) ; ***: very highly significant (P<0,001). Chen: *Ch. ambrosioides*, Cupr: *Cu. sempervirens*. *: cotoxicity index

3.4. Progeny inhibition

Data on the progeny emergence experiments is reported on Table 6. The highest doses of both essential oils gave >90% inhibition. There were very high significant differences in the percentage reduction in progeny production between all the doses administered for both plants. All products were good progeny production inhibitors with very high significant differences. *C. ambrosioides* gave 100% progeny production inhibition at its highest dose**.** These results are in agreement with those of Tapondjou et al., (2002; 2005) also found good progeny control properties of *Cu. sempervirens* [12] [14].

Table 6 Percent reduction of progeny (Mean ± S.E) of *Sitophilus zeamais* on maize grains treated with mixture of essential oils of *Chenopodium ambrosioides* and *Cupressus sempervirens.*

Treatment	Ratio of *Ch. ambrosioides* to *Cu. Sempervirensin* essential oil					
	50/50		75:25		25:75	
Concentration (µL/kg)	Progeny	% reduction of Progeny	Progeny	% reduction of Progeny	Progeny	% reduction of Progeny
00	42.00 ± 1.41^{C}	0.00 ± 0.00^{A}	42.25 ± 1.31^{C}	0.0 ± 0.00^{A}	40.00 ± 0.41^{E}	0.00 ± 0.00^{A}
25	13.25 ± 1.11^{B}	68.37 ± 2.86^{B}	22.7 ± 1.38^{B}	45.85 ± 4.25^{B}	17.75 ± 0.63^{D}	55.64 ± 1.34^{B}
50	10.00 ± 0.41^{B}	76.16 ± 0.84^{C}	12.25 ± 0.62^{A}	70.84 ± 2.11^{C}	9.25 ± 0.75^{C}	76.90 ± 1.76^{C}
100	0.75 ± 0.48^{A}	98.21 ± 1.19^{D}	8.75 ± 0.75^{A}	79.29 ± 1.72^{C}	5.75 ± 0.48^{B}	85.59 ± 1.34^{D}
200	0.00 ± 0.00^{A}	100 ± 0.00^{D}	8.00 ± 0.71^{A}	81.06 ± 1.66^{C}	0.75 ± 0.25^{A}	98.11 ± 0.63^{E}
$F_{(4, 15)}$	403.078***	806.257***	203.127***	204.814***	841.588***	1050.092***

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's test). Each value represents the mean of four replicates of 20 insects. ***: very highly significant (P<0,001). Chen: *Ch. ambrosioides*, Cupr: *Cu. sempervirens*. $F_{(df1, df2)}$

3.5. Repellence

Table 7 shows the *in vitro* repellence EO activity in the various treatments. Generally, the 8 µL/kg dose was the most repellent with repellence indices greater than 60%. *Ch. ambrosioides* however was more repellent than *Cu. sempervirens* at all the doses administered. Appreciable ovicidal and lavicidal effects of powders of *Ch. ambrosioides* and *Cu. sempervirens* against *S. zeamais* have also been proven [11, 14]. Earlier literature showed a repellence activity of *Cu. sempervirens* greater than cymol [14].

Table 7 *In-vitro* repellency (Mean ± S.E) of *Sitophilus zeamais* on filter paper due to treatment with essential oils of *Ch. ambrosioides* and *Cu. sempervirens*

Ratio of *C. ambrosioides* to *C. sempervirens* in essential oil	Concentration (µL/kg)	% Repellence	Class	Interpretation
50:50	0	0.00 ± 0.00^{A}	/	/
	1	5.00 ± 9.57^{A}	I	Very low repellence
	2	20.00 ± 8.26^{A}	II	Low repellence
	4	40.00 ± 8.17^{AB}	III	Moderately Repellent
	8	83.19 ± 5.69^{B}	V	Very Repellent
$F_{(4, 15)}$		10.824^{***}		
75:25	0	0.00 ± 0.00^{A}	/	/
	1	28.57 ± 3.35^{AB}	II	Low repellence
	2	40.00 ± 8.16^{B}	III	Moderately Repellent
	4	55.00 ± 5.00^{B}	III	Moderately Repellent
	8	58.75 ± 7.18^{B}	IV	Repellent
$F_{(4, 15)}$		8.72^{**}		
25:75	0	0.00 ± 0.00	/	/
	1	20.00±14.14	II	Low repellence
	2	40.00±18.26	III	Moderately Repellent
	4	43.89±11.56	III	Moderately Repellent
	8	50.00±6.78	III	Moderately Repellent
$F_{(4, 15)}$		2.227^{ns}		

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's test). Each value represents the mean of four replicates of 10 insects each. **: very significant (P<0.01).). Chen: *Ch. ambrosioides*, Cupr: *and Cu. sempervirens* essential oils.$F_{(df1, df2)}$

3.6. Population increase and damage control

Population increase was evaluated counting the number of new insects that emerged from the initial 50 that were introduced into the treated jars. For dead insects, after 6 months of storage, the highest doses gave a non-significantly different average value of 50 with all the EOs while the lower doses also gave about 40 dead insects (Table 8). The notable difference came with the number of living insects present. With high significant differences, the 25 µL/kg content of 75% *Ch. ambrosioides* was the least toxic (more than 600) while with the 200 µL/kg content, 75% *Cu. sempervirens* was the least toxic. The best results were observed for the 50:50 binary combinations of EOs.

It was noted significant differences between the different fractions of the different essential oils at all the contents administered but very significantly different between the different contents of the same essential oil. With grain weight loss, the 50:50 binary combination was the best with 2% followed by 100% *Ch. ambrosioides* with 3%. With percentage of grains with holes, the 50:50 binary combination gave 2% also followed by the 75% *Ch. ambrosioides* and least by the 100% *Cu. sempervirens.* We noticed visible positive effects between the combinations of both oils: especially with respect to *Cu. Sempervirens.*

Table 8 *In-vivo* damage control (Mean ± S.E) of *Sitophilus zeamais* on maize due to treatment with essential oils of *Chenopodium ambrosioides* and *Cupressus sempervirens* and their binary combinations

Content (µL/kg)	Ratio of *Ch. ambrosioides* to *Cu. sempervirens* in essential oil					$F_{(4, 10)}$
	100:0	0:100	50:50	75:25	25:75	
	Number of dead insects					
0	18.33±2.96A	18.33±2.96^{A}	18.33±2.96^{A}	18.33±2.96^{A}	18.33±2.96	
25	39.33±1.76 Bab	45.33±0.88 ^{Bb}	36.67±2.03 ^{Bab}	34±2.08 ^{Ba}	35±2.89 ^{Ba}	4.978*
200	49.67±0.88C	49.67±0.33^{B}	50.67±0.33^{C}	50±0.00^{C}	53.33±1.76^{C}	2.608^{ns}
$F_{(2,6)}$	60.377***	91.966***	60.675***	57.364***	45.467***	
	Number of live insects					
0	780±25.98C	780±25.98C	780±25.98^{C}	780±25.98^{C}	780±25.98^{C}	
25	449.67±43.59 ^{Bab}	603.33±3.53 ^{Bb}	416.33±67.78 ^{Bab}	583.33±58.33 ^{Bb}	340±47.84 ^{Ba}	5.17*
200	61.67±7.27 ^{Aab}	78.33±1.67 ^{Aab}	38.67±10.73 ^{Aa}	60±11.55 ^{Aab}	100.67±0.67^{Ab}	6.11**
$F_{(2,6)}$	147.854***	485.196***	76.572***	98.662***	120.153***	
	% Grain Weight Loss					
0	29.39±1.78^{B}	29.39±1.78^{B}	29.39±1.78^{B}	29.39±1.78^{B}	29.39±1.78^{B}	
25	6.01±0 ^{Aa}	10.17±0.58 ^{Bb}	6.69±0.88 ^{Aab}	6.01±0.96 ^{Aa}	8.47±0.98 ^{Aab}	5.491*
200	3.01±0.58^{A}	4.67±0.88^{A}	2.02±0.59^{A}	4.67±0.33^{A}	4.34±0.89^{A}	2.964*
$F_{(2,6)}$	177.818***	117.604***	149.577***	137.448***	109.633***	
	% Grains with holes					
0	98±1.16^{C}	98±1.16^{C}	98±1.16^{C}	98±1.16^{C}	98±1.16^{C}	
25	85.33±3.53^{B}	86.67±3.53^{B}	87.33±0.67^{C}	74.67±4.01^{B}	85.33±1.76^{B}	3.030*
200	20±2.31 ^{Abc}	26.00±5.03 ^{Ac}	2±0 ^{Aa}	5.33±1.33 ^{Aa}	10.67±2.67 ^{Aab}	12.736*
$F_{(2,6)}$	275.047***	114.966***	4672.000***	356.386***	578.192***	

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's test). Each datum represents the mean of four replicates of 20 insects each. ***: very highly significant (P<0,001).

4. Conclusion

The binary combinations of the essential oils of *C. ambrosioides* and *Cu. sempervirens* tested on maize were very efficient insecticides against the maize weevils. These potentials to control the proliferation of *S. zeamais* in stored maize was also dose dependent and increased with period of exposure. Therefore both EOs can be recommended for their insecticidal, progeny control effects, high repellence and ability to prevent grain from damage caused by maize weevils and can be easily used in an integrated pest management practice.

Compliance with ethical standards

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Disclosure of conflict of interest

The author of this work and all co-authors attest to the fact that no part of this work is a product of plagiarism. All previously authored works used in this paper are backed with references. Hence there exists no conflict of interest.

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