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Efficacy of Cameroonian *Ocimum canum* Sims (Lamiaceae) leaf extract fractions against *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae), infesting Bambara groundnut

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Abstract
Botanicals could be eco-friendly alternatives to chemical pesticides in stored product protection. *Ocimum canum* leaf powder was sequentially extracted in hexane, acetone and methanol, and the fractions were tested for their potential in protecting stored Bambara groundnut seeds against the infestation of *Callosobruchus maculatus*. Bambara groundnut seeds (50 g) were treated with 1 and 5 g/kg of extract to evaluate the repellence, mortality, F_1 progeny production, seed damage of the beetle as well the persistence of the test substances. The hexane fraction was more repellent than the other two, recording repellence class V at 1 g/kg. Hexane fraction also showed superior toxicity, causing 100% mortality of *C. maculatus* at 5 g/kg within 7 days of exposure. All the fractions, at 5 g/kg, somewhat completely inhibited progeny emergence and seed damage. Hexane fraction had sufficient efficacy to be a component of storage pest management package of *C. maculatus*.

Keywords: *Ocimum canum*, fractions, *Callosobruchus maculatus*, repellency, toxicity

1. Introduction
The storage of harvested grains remains one of the best solutions to tackle food insecurity in sub-Sahara African countries, such as Cameroon, where the dry season could be longer than the wet season. The growing of crops is done only during the wet season, whereas the harvested products are needed year round, rendering storage unavoidable. Unfortunately, stored products suffer from heavy damage caused by pests, especially insects. The pulse beetle, *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae, Bruchinae), is a major pest of economically and nutritionally important leguminous grains, such as Bambara groundnut, *Vigna subterranea* [1, 2]. As in other countries of sub-Saharan Africa, *C. maculatus* was reported to be a leading pest of Bambara groundnut in Cameroon [3], where the grain legume is cultivated as a subsistence crop by resource poor farmers. Infestation of *V. subterranea* by *C. maculatus* leads to a significant reduction in the viability of planting seeds, which could be more than 50 %, after the emergence of only the F_1 progeny [2].
The use of synthetic residual chemicals is the dominant control option for stored product insects in Africa, although these insecticides degrade the environment. Therefore, there is an urgent need to develop safer methods for the protection of stored grains against insect infestation. Kitch and Ntoukam [4] proposed wood ash for the control of *C. maculatus* in Cameroon, but this method was not efficient in controlling the immature stages of the pest. Botanical insecticides, that is, products based on powders, extracts or purified substances of plant origin could be more efficient and safe in stored product protection. Therefore, in the present study, we investigated the effect of hexane, acetone and methanol extracts from the leaves of *Ocimum canum*, a culinary plant widely available in Northern Cameroon, as a toxicant against *C. maculatus*. The ability of the extracts to inhibit progeny production, reduce damage and suppress population increase, as well as the persistence of their insecticidal activity, was examined. No previous study has reported on leaf extracts of *O. canum* against *C. maculatus*.

2. Material and methods

2.1 Insects

Callosobruchus maculatus were reared on cowpea (variety Vya Moutourwa) in the laboratory under ambient conditions. Adult *C. maculatus* were obtained from infested cowpea grains at Mokolo market, Far-North region, Cameroon in November 2013. The Bambara groundnut seeds (variety Kodek) were collected from farmers at Mokolo during harvest time. The seeds were disinfested by keeping them in a freezer at -18 ºC for three weeks prior to the bioassays. The seeds were then kept under experimental conditions for at least two weeks before use. The initial moisture content of the seeds was 10.66 ± 1.15%, determined by the method of AFNOR [5].

2.2 Collection and processing of plant materials

The green leaves of *Ocimum canum* were collected in November 2013 around Mogode in the Mayo-Tsanaga Division, Far-North region of Cameroon (latitude 10°36.25"N and longitude 13°34.46"E, altitude 1005 meter above sea level (m.a.s.l)). The identity of the plant was confirmed at the Cameroon National Herbarium in Yaounde, where voucher specimen (Serial number: 26811/SRFCam) was kept. The Far-North region is in the Sudano-Sahelian agro-ecological zone of Cameroon [6]. This agro-ecology is characterized by two seasons: wet (June to September) and dry (October to May). Annual rainfall ranges between 800 and 1000 mm. Annual mean temperature is 29 °C, with a maximum of 39 °C in March and minimum of 17 °C in January. Average annual R.H. stands at 67%.

The leaves were dried in a room at Mokolo under ambient conditions for 72 h and then crushed in a mortar until the powder passed through a 0.4 mm mesh sieve. Powders were stored in a deep-freezer at the temperature of -18 °C until needed for extraction.

Two thousand three hundred grams of *O. canum* powder were mixed with 7.5 L of hexane and stirred for 30 min and allowed to stand for 24 hours in the laboratory at Yaounde, Cameroon, and then re-stirred again. After 48 hours the mixture was then filtered with a filter paper (Whattman no. 1). The residue obtained after filtration was put through the process above again and the filtrate was admixed with the one obtained initially. After the hexane extraction was done, the paste left was dried for 10 h at room temperature in the laboratory and then used for Acetone extraction and followed by methanol extraction. The filtrates obtained with hexane, acetone and methanol were then separately concentrated in a Rotavapor at 70 ºC, 60 ºC and 65 ºC, respectively at 120 rpm. The extract yield of 6.393%, 8.663% and 6.873% were obtained by using hexane, acetone and methanol, respectively. Extracts were stored in a refrigerator at 4 ºC until needed for bioassay.

Azadirachta indica seed oil was used as a positive control in this study. The ripe seeds (de-pulped by birds) were collected on the ground under *A. indica* trees at Maroua (latitude 10°33.16' N, longitude 14°815.04' E and altitude of 356 m.a.s.l.), Far-North region, Cameroon in August of 2013. The collected seeds were dried on jute bags placed on the ground under shade. The dried seeds were dehusked, and the kernels obtained were used for oil extraction [7] using the traditional kneading method. Kernels were pounded in a mortar, and the powders obtained were moistened with warm water (0.25 L for 2.6 kg of powder) in a plastic container until a dough-like material was formed. The paste obtained was kneaded for several minutes and then pressed firmly with the hand. Kneading and pressing were alternated to collect maximum oil from the *A. indica* seed cake. To avoid degradation, the oil obtained was stored in a refrigerator at 4 ºC until needed for bioassay.

2.3 Phytochemical screening of *Ocimum canum* extracts

The plant extracts were phytochemically screened using standard procedures for the detection of sterols, saponins, cardiac glycosides, tannins, flavonoids, terpenoids and alkaloids as described in Adeniyi [8].

2.4 Repellency test

Repellency test was conducted using the olfactometer method [9]. A linear olfactometer made of 30 cm plastic tube, having 2 cm diameter with a hole at its middle was used. At each end, a small container was placed with 10 g of Bambara groundnut grains. One container contained grains treated with plant materials and the other the positive control (*A. indica* oil) or the negative control containing seeds treated with the respective solvent used to dilute each of the extract: hexane for hexane extract, acetone for acetone extract and methanol for methanol extract. Two treatment concentrations (1 and 5 g/kg grains) were used. Twenty insects (≤ 48 h old) of mixed sex were separately introduced in the hole at the middle of the olfactometer. The choice of insects was observed for a period of two hours. Only the insect within the seeds in either end was considered to have made a choice. For each trial, five replications were made. Repellence was evaluated according to the formula used by Talukder and Howse [10] and Liu and Ho [11].

PR = 2 × (C – 50); C = percentage of insects choosing the control end. When PR > 0 the extract was repellent and when PR < 0 the extract was attractive.

The average values were then categorized according to the scale in Table 1 [12].

Table 1: Repellency scale from the least to the most repellent, 0 to V

Class	Repellence rate (%)	Interpretation
0	> 0.01 to < 0.1	Non repellent
I	0.1 to 20	Very weakly repellent
II	20.1 to 40	Moderately repellent
III	40.1 to 60	Averagely repellent
IV	60.1 to 80	Fairly repellent
V	80.1 to 100	Very repellent

2.5 Adult toxicity test and progeny production

Two different masses (0.05 and 0.25 g) each of the hexane, acetone and methanol extracts and *A. indica* seed oil (positive control) were separately mixed with 50 g grains in glass jars, which corresponded to the concentrations of 1 and 5 g/kg, respectively. The negative controls consisted of grains with solvent. The experimental design was a 1 insecticidal plant × 3 solvent extracts × 2 concentrations × 7 exposure periods factorial, arranged in a Complete Randomized Design, with each treatment replicated 4 times. The content of each jar were hand-shaken properly to ensure complete coating of the grains with the extracts or *A. indica* seed oil. The grains were then air-dried for two hours to evaporate the solvent [13]; the time allowed for complete evaporation of solvent in the previous

study (one hour) was modified in the present investigation (two hours) because of differences in environmental factors among the sites of the studies. A group of 20 adults of *C. maculatus* of mixed sex, aged 1-2 days, was added separately into the jars containing the treated grains. These jars were covered with a muslin cloth and a perforated metal lid to facilitate proper aeration and prevent entry and exit of insects. Adult mortality was recorded 1, 2, 3, 4, 5, 6 and 7 days after treatment. Insects were considered dead when no movement was observed after touching them carefully with entomological forceps. After the 7-day mortality recordings, all the live and dead insects were separated from the grains and discarded. The grains were left inside the bottles on laboratory shelves and all the F_1 progeny were counted.

2.6 Damage bioassay

The experimental units from the adult toxicity test and progeny production above were used in the bioassay to assess population increase and damage. After the F_1 progeny recordings, all the insects, dead and live, as well as the grains from each jar were left in their respective jars on laboratory shelves for a total period of four months starting from infestation. The undamaged grains and damaged grains (with characteristic hole) were counted and weighed for each jar. The final moisture content of the grains was also determined. The final grain weight was considered as the weight of sample without insects at the end of the experiment minus the weight of additional moisture and amount of insecticidal material.

- *The weight of additional moisture content was expressed as follow:*

Sample initial weight × [final moisture content (%) – initial moisture content (%)]/ [100 – final moisture content (%)]

- *Percent weight loss was determined as follows:*

[(initial weight – final weight)/initial weight] × 100

2.7 Persistence test

Twenty five gram grain samples were treated with the extracts of *A. indica* oil at 5 g/kg and then stored for 0, 7, 14, 30 and 60 days. Negative control treatments consisted of grains treated with solvents alone. After this, the treated grains, positive controls and negative controls were infested with 20 adult insects of mixed sex, aged 1-2 days. All treatments were replicated four times. At each persistence time assessment, the experiment was similar to that described for the adult toxicity test above, but for the fact that mortality recordings were done only five days post-infestation.

2.8 Data analysis

Abbott's formula [15] was used to correct for control mortality. Data on % cumulative corrected mortality, % reduction in progeny production, % grain damage, % weight loss and PR were arcsine-transformed [square root(x/100)] and the number of F_1 progeny produced was log-transformed (x + 1). The transformed data were subjected to the ANOVA procedure of the statistical analysis system [16, 17]. Tukey's (HSD) multiple range test (P = 0.05) was applied for mean separation.

3. Results

3.1 Phytochemical constituents of *Ocimum canum* fractions

The results of the chemical analysis of the three fractions from *O. canum*, using hexane (apolar), acetone (intermediate) and methanol (polar) are presented in Table 2. Phenolic compounds, saponins, flavonoids, alkaloids and steroids were present in the methanolic and acetone fractions. Only two chemical groups, saponins and steroids, were found in the hexane fraction. Tannic acid was exclusively present in the methanol fraction.

Table 2: Chemical composition of the hexane, acetone and methanol extracts of *Ocimum canum* leaves

	Extracts		
Compounds	**Hexane**	**Acetone**	**Methanol**
Phenolic compounds	–	+	+
Alkaloids	–	+	+
Saponins	+	+	+
Tannins	–	–	+
Flavonoids	–	+	+
Steroids	+	+	+
Triterpenoids	–	–	–
Cardiac glycosides	–	–	–

– absent, + present

3.2 Repellency test

Azadirachta indica seed oil and the extracts from *O. canum* were repellent to *C. maculatus,* although with different intensities (Table 3). The two concentration levels showed similar repellency for the *O. canum* extracts, but for *A. indica* oil, repellency increased with concentration level. The hexane fraction was very repellent, while the other two fractions (acetone and methanol) were moderately repellent. The repellent action of *A. indica* seed oil was comparable to those of the methanol and acetone fractions, but inferior to that of the hexane fraction of *O. canum*.

Table 3: Mean percent repellency values for three fractions of *Ocimum canun* leaves and *Azadirachta indica* seed oil against *Callosobruchus maculatus*

Extract/Concentration (g/kg)		
	Mean percent repellency	**Class**
I*ndica* oil		
1	27.75 ± 6.63	II
5	73.56 ± 6.43	IV
Hexane		
1	91.57 ± 3.47	V
5	83.61 ± 8.41	V
Acetone		
1	47.33 ± 6.23	III
5	57.79 ± 9.31	III
Methanol		
1	50.87 ± 9.32	III
5	59.80 ± 5.37	III

3.3 Toxicity

As expected, compared to the control, the *A. indica* seed oil and the three *O. canum* leaf extracts generally caused significant mortality to *C. maculatus* which was dose-dependent (Table 4). The beetle was more susceptible to the *A. indica* oil at the higher tested concentration (5 g/kg) than the extracts. At the higher concentration (5 g/kg), complete mortality (100%) of *C. maculatus* was achieved within two and seven days for *A. indica* seed oil and the hexane fraction of *O. canum*, respectively, while the methanol and acetone fractions attained maximum mortality of 66.20% and 61.79%

respectively, seven days post-treatment. In the contrary, the extracts were more potent towards *C. maculatus* compared with the oil at the lower tested concentration of 1 g/kg. Within four days after treatment, maximum mortality caused to the beetle by the *O. canum* extracts ranged from 30 – 37% and by *A. indica* oil 12.01%. Even at seven days post-treatment, the extracts (49 – 55%) caused higher mortality to *C. maculatus* than the oil (30.76%). Among the three extracts, the hexane fraction was superior to the methanol and acetone fractions, causing 100% mortality to the beetle at the 5 g/kg level seven days post-treatment, while the methanol (66.20%) and acetone (61.75%) caused less than 70% mortality within the same time-point and concentration level.

Table 4: Corrected cumulative mortality of *Callosobruchus maculatus* exposed in grains treated with three fractions of *Ocimum canum* leaves and *Azadirachta indica* seed oil

Product	Conc. g/kg	Days after treatment / % Mortality (mean ± SE) †							
		1	2	3	4	5	6	7	*F* value‡
Indica oil	0	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	
	1	3.75 ± 1.25Cb	3.88 ± 1.29^{Cb}	7.83 ± 1.56^{BCb}	12.01 ± 2.61^{BCb}	12.14 ± 2.58^{BCb}	20.95 ± 5.44^{ABb}	30.76 ± 4.09^{Ab}	10.27***
	5	91.12 ± 2.42^{Ba}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}	48.59***
	F value‡	290.10***	5742.47***	3813.81***	1314.49***	1344.54***	281.98***	470.72***	
Hexane fraction	0	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	
	1	7.76 ± 4.58^{Cb}	24.84 ± 4.71^{BCb}	28.99 ± 2.68^{Bb}	30.19 ± 2.92^{Bb}	33.38 ± 3.24^{ABb}	39.59 ± 3.67^{ABb}	49.01 ± 5.58^{Ab}	10.11***
	5	29.61 ± 3.52^{Da}	57.70 ± 3.74^{Ca}	81.68 ± 4.77^{Ba}	91.81 ± 3.55^{ABa}	97.14 ± 1.65^{Aa}	98.53 ± 1.47^{Aa}	100.00 ± 0.00^{Aa}	73.77***
	F value‡	22.56***	127.83***	208.26***	310.75***	551.22***	470.76***	583.09***	
Acetone fraction	0	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	
	1	3.75 ± 2.39^{Da}	14.28 ± 1.26^{Da}	31.25 ± 2.42^{Ca}	37.54 ± 4.38^{Ca}	40.65 ± 3.82^{BCa}	51.31 ± 1.82^{ABa}	55.89 ± 1.21^{Ab}	48.39***
	5	6.32 ± 3.14^{Da}	15.53 ± 1.94^{Da}	36.38 ± 2.20^{Ca}	40.11 ± 3.81^{Ca}	46.05 ± 3.87^{BCa}	52.78 ± 1.14^{ABa}	61.79 ± 1.24^{Aa}	54.25***
	F value‡	2.64^{ns}	141.27***	317.51***	128.17***	169.72***	1410.53***	2475.27***	
Methanol fraction	0	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	
	1	3.88 ± 2.51^{Ea}	15.79 ± 2.15^{DEa}	24.27 ± 1.18^{CDb}	30.56 ± 1.60^{BCb}	38.16 ± 3.96^{ABb}	41.59 ± 4.13^{ABb}	49.99 ± 3.61^{Ab}	29.11***
	5	12.63 ± 5.18^{Ca}	21.05 ± 2.15^{BCa}	41.74 ± 4.06^{Ba}	51.39 ± 2.66^{ABa}	52.21 ± 3.16^{ABa}	55.80 ± 3.16^{ABa}	66.20 ± 3.50^{Aa}	30.19***
	F value‡	3.81^{ns}	123.74***	209.82***	506.38***	205.13***	218.59***	279.65***	

†Means within the column and line followed respectively by the same small and capital letter do not differ significantly at the 5% level according to Tukey's test

‡ ns P > 0.05; *** P < 0.001

3.4 F1 progeny production

The two dosages of the A. indica oil and the three fractions of O. canum reduced F1 progeny emergence in C. maculatus by at least 60% (Table 5). At the higher 5 g/kg concentration, the A. indica oil completely suppressed F1 progeny emergence in the beetle, which was comparable to the almost complete suppression (97.57 – 99.22%) by the three leaf extracts. For the lower concentration 1 g/kg, the A. indica oil (96.92%) was superior to the three O. canum extracts (63.32 – 74.35%) vis-à-vis progeny suppression.

Table 5: Progeny production of Callosobruchus maculatus in grains treated with two dosages of Azadirachta indica seed oil and extracts from Ocimum canum

Product/concentration (g/kg)	Number (mean ± SE) of F_1 progeny†	Percentage (mean ± SE) reduction in F_1 progeny emergence relative to control†
A. indica oil		
0	31.50 ± 0.65^{a}	0.00 ± 0.00^{b}
1	1.00 ± 0.71^{b}	96.92 ± 2.14^{a}
5	0.00 ± 0.00^{b}	100.00 ± 0.00^{a}
F-value‡	1049.18***	2112.26***
Hexane extract		
0	32.25 ± 1.31^{a}	0.00 ± 0.00^{c}
1	8.25 ± 0.55^{b}	74.35 ± 2.79^{b}
5	0.25 ± 0.25^{c}	99.22 ± 0.78^{a}
F-value‡	349.05***	952.03***
Acetone extract		
0	31.50 ± 0.65^{a}	0.00 ± 0.00^{c}
1	11.50 ± 0.87^{b}	63.32 ± 3.44^{b}
5	0.75 ± 0.48^{c}	97.57 ± 1.55^{a}
F-value‡	523.39***	516.55***
Methanol extract		
0	30.50 ± 0.29^{a}	0.00 ± 0.00^{c}
1	11.90 ± 2.50^{b}	63.90 ± 2.50^{b}
5	0.25 ± 0.25^{c}	99.17 ± 0.83^{a}
F-value‡	1092.29***	1090.83***

† Means within the same column for each extract or oil followed by the same letter do not differ significantly at the 5% level according to Tukey's test.

‡ *** $P < 0.001$

3.5 Damage and weight loss

In general, the damage and weight loss of Bambara groundnut grains that were treated with A. indica seed oil and leaf extracts of O. canum, infested and stored for four months were statistically lower than those of the control (P = 0.0001) (Table 6). At the dosage level of 5 g/kg, the treated grains recorded very little or no damaged grains and weight loss, which was comparable to the almost no grain damage and weight loss for treatments with the A. indica seed oil at 1 g/kg. The grains treated with 1 g/kg of the leaf fractions of O. canum suffered high grain damage and much weight loss, although these were lower than for the control.

Table 6: Grain damage and weight loss caused by Callosobruchus maculatus in Bambara groundnut grains treated with Azadirachta indica oil and extracts of Ocimum canum and then stored for four months

Product/Concentration (g/kg)	Grain damage (%)	Grain weight loss (%)
Neem oil		
0	100.00 ± 0.00^{a}	21.39 ± 0.92^{a}
1	1.53 ± 1.17^{b}	0.72 ± 0.71^{b}
5	0.27 ± 1.10^{b}	0.00 ± 0.00^{b}
F-value	6805.52***	327.07***
Hexane extract		
0	100.00 ± 0.00^{a}	18.79 ± 0.89^{a}
1	69.82 ± 14.16^{b}	8.18 ± 1.04^{b}
5	0.33 ± 0.33^{c}	0.00 ± 0.00^{c}
F-value	67.96***	142.27***
Acetone extract		
0	100.00 ± 0.00^{a}	21.39 ± 0.92^{a}
1	76.48 ± 9.42^{b}	10.27 ± 2.01^{b}
5	3.92 ± 3.47^{c}	0.04 ± 0.04^{c}
F-value	74.72***	54.63***
Methanol extract		
0	100.00 ± 0.00^{a}	19.96 ± 1.95^{a}
1	96.92 ± 3.08^{a}	12.88 ± 2.15^{b}
5	0.36 ± 0.36^{b}	0.01 ± 0.01^{c}
F-value	1000.33***	36.35***

Means within the same column followed by the same letters for each product do not differ significantly at the 5% level according to Tukey's test.

***: $P < 0.001$

3.6 Persistence on grains

The persistence of the A. indica seed oil and leaf extracts from O. canum on treated Bambara groundnut grains (5 g/kg) showed that their potency decreased significantly with storage time, although not always linearly (Table 7). The mortality caused to C. maculatus by A. indica seed oil did not vary among days 0, 7, 14, and 30, but were higher than for day 60. For the hexane leaf extract of O. canum, the insect mortality was similar among days 7, 14, 30 and 60, although lower than for day 0. The acetone and methanol extracts of the plant were more potent against C. maculatus on day 0 compared to all the other days.

Table 7: Corrected cumulative mortality of Callosobruchus maculatus exposed in Bambara groundnut grains treated with of A. indica seed oil and extracts from Ocimum canum at 5 g/kg dosage rate after different periods of storage

Infestation Period (days)	Products/Mortality				*F*-value
	Neem oil	Hexane	Acetone	Methanol	
0	98.61 ± 1.39^{Aa}	57.27 ± 3.08^{Ba}	44.12 ± 1.70^{Ca}	50.82 ± 2.97^{BCa}	104.16^{***}
7	100.00 ± 0.00^{Aa}	36.76 ± 2.54^{Bb}	18.30 ± 2.63^{Cb}	15.22 ± 1.17^{Cbc}	533.56^{***}
14	94.36 ± 2.41^{Aa}	26.20 ± 2.99^{Bb}	7.27 ± 1.50^{Cc}	21.25 ± 4.61^{Bbc}	158.36^{***}
30	87.79 ± 5.57^{Aa}	29.50 ± 2.23^{Bb}	16.16 ± 2.04^{BCb}	13.69 ± 1.50^{Cc}	113.79^{***}
60	48.02 ± 1.63^{Ab}	27.67 ± 1.64^{Bb}	12.02 ± 1.31^{Cbc}	26.61 ± 1.94^{Bb}	80.95^{***}
F-value	56.57^{***}	25.34^{***}	57.57^{***}	30.20^{***}	

Means within the column followed by similar lower cast letter and within the same line followed by the same lower cast letter do not differ significantly at the 5% level according to Tukey's test.

*** $P < 0.001$

4. Discussion

Phytochemical products can increase income of rural farmers and promote safety and quality of food and life in general [18]. Indeed, many plant species contain secondary metabolites that are potent against several pest species. Different bioactive agents in plant such as total phenolic compounds, alkaloids, saponins, tannins, flavonoids, steroids and triterpenoids among others were reported to have insecticidal properties [19-24] and these compounds may act as repellents, toxicants, deterrents and reproduction inhibitors [25].

The repellence activity of O. canum tested in this study may be attributed to these compounds. The higher repellence activity of hexane extract fraction which had less compounds than acetone and methanol extract fractions suggested that some of those compounds may have either antagonistic effects or may be attractant as found previously by Ngamo *et al*. [8]

The extracts from the leaves of O. canum possess good insecticidal potential because of their phytochemical constituents. Hexane extract with less chemical groups than acetone and methanol extracts was most toxic. Similar results were reported by Gopalakrishnan and Martin Rathi [26] where benzene chloroform extract containing only steroids was more active than water extract containing phenolic compounds, tannins, saponins, reducing sugars and aromatic acids. The secondary compounds of plants are a vast repository of compounds with a wide range of biological activities. This may partially explain these results. Furthermore, hexane extract had gluey aspect which acetone and methanol extracts lacked. Another way for this extract to kill insect might be by gluing the insect's wing cover and legs, which hinders mobility. The insect thus lost energy by trying to get loose and when combined with the toxic effects of compounds in the leaf extracts, could lead to the death of the insect. Since the entomotoxic effects of the hexane fraction of O. canum was comparable to that of A. indica seed oil, this extract at 5 g/kg could be applied in stored grains to protect them against the infestation of C. maculatus and may be other stored product beetles. Moreover, the grains treated with O. canum extracts did not taste bitter like A. indica oil used in medicine [27].

In addition to causing adult mortality, the different O. canum extracts and A. indica seed oil either completely hindered or significantly reduced progeny emergence of C. maculatus. No difference in F1 progeny emergence was observed among the two concentrations of A. indica oil. There is a possible presence of oviposition deterrence that could be overlooked; the correlation between F1 progeny emergence and mortality observed on treated grains as reported by Udo [28] was not significant in the case of extracts from O. canum. When the grains are coated with the extracts, their surfaces may deter egg-laying. The extract itself may also coat eggs and kill them by asphyxiation. The assertion by Jacobson [25] on the chemosterilant properties of many plants may also support partially the results of our investigations which indicated inhibition of progeny production of C. maculatus in treated grains.

In seeking to make improvements to pulse grain supply, an important element to consider is postharvest losses and major donors have recently been focusing on loss reduction strategies [29, 30]. Percentage damage up to 40.6 was recorded in the Far North region of Cameroon within only 40 days of storage [3]. In this study up to 100 % untreated grains were damaged and O. canum extracts at 5 g/kg, completely or almost suppressed grain damage. Damage includes reduction in kernel weight, caused by the burrowing larvae as they feed, and this diminish their market value due to the presence of insects inside the kernels.

For a long storage period insecticidal products should be persistent under fluctuating environmental conditions. The persistence of insecticidal products is influenced by the nature of its insecticidal chemical compounds. The insecticidal activity of essential oils was reported to decrease rapidly with time because of the high volatility of their chemical compounds [31], whereas A. indica products were reported to be more persistent because of the low volatility of their main insecticidal compound, Azadirachtin [32, 33]. The extracts of O. canum tested in the present study showed satisfactory insecticidal potency up to 30 days, and thus could adequately protect grains against the infestation of C. maculatus for one month.

5. Conclusion

From the present findings, it could be concluded that O. canum extracts, especially when the solvent is hexane, are potent insecticides against C. maculatus. At 5 g/kg, the efficacy of hexane extract was similar to that of A. indica oil, although the hexane extract was more repellent. At this content level, all the tested insecticidal products somewhat completely inhibited progeny production and suppressed grain damage as well as weight loss. Due to the above virtues combined with satisfactory persistence, these O. canum extracts could be of value to farmers for the protection of their grains against the infestation of C. maculatus.

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7. References

1. Nyamador SW. Influence des traitements à base d'huiles essentielles sur les capacités de reproduction de *Callosobruchus subinnotatus* Pic. et de *Callosobruchus maculatus* F. (Coleoptera: Bruchidae): mecanisme d'action de l'huile essentielle de *Cymbopogon giganteus* chiov. Thèse de Doctorat, Université de Lomé, Togo, 2009, 177.
2. Magagula CN, Maina YT. Activity of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) on selected bambara groundnut (*Vigna subterranea* L. Verdc.) landraces and breeding lines. Journal of Biology, Agriculture and Healthcare. 2012; 2(3):84-91.
3. Kouninki H, Sobda G, Nukenine NE. Screening of Bambara groundnut (*Vigna subterranea*) lines for *Callosobruchus maculatus* resistance in the Far North Region of Cameroon. Journal of Renewable Agriculture. 2014; 2(1):18-22.
4. Kitch LW, Ntoukam G. Le stockage du niébé dans la cendre, Bulletin Technique 1. Institut de la Recherche Agronomique du Cameroun (IRA), Centre de Recherche Agronomique de Maroua, CRSP Projet de Stockage, niébé, Maroua, Cameroun, 1994, 14.
5. AFNOR (Association Française de Normalisation). Recueil des normes françaises des produits dérivés des fruits et légumes. Jus de fruits. 1ère édition, Paris (France), 1982, 27.
6. IRAD. Les caractéristiques des différentes zones agro-écologiques. Revue scientifique 2007 de l'IRAD. Yaoundé, 2007, 84.
7. Nukenine EN, Tchiegang C, Mekouo AAT, Tofel KH, Adarkwah C, Obeng-Ofori D *et al*. Efficacy of Calneem derived from Ghanaian neem seeds and seed oils from two locations in Cameroon against *Sitophilus zeamais* (Coleoptera: Curculionidae) on maize. International Journal of Tropical Insect Science. 2011; 31(4):225-234.
8. Adeniyi SA, Orjiekwe CL, Ehiagbonare JE, Arimah BD. Preliminary phytochemical analysis and insecticidal activity of ethanolic extracts of four tropical plants (*Vernonia amygdalina, Sida acuta, Ocimum gratissimum* and *Telfaria occidentalis*) against beans weevil (*Acanthscelides obtectus*). International Journal of the Physical Sciences. 2010; 5(6):753-762.
9. Ngamo TSL, Noudjou WF, Ngassoum MB, Mapongmestsem PM, Aminatou Boubakary AB, Malaisse F *et al*. Investigation on both chemical composition and insecticidal activities of essential oils of *Vepris heterophylla* (Rutaceae) from two localities of Northern Cameroon towards *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Research Journal of Biological Sciences. 2007; 2(1):57-61.
10. Talukder FA, Howse PE. Deterrent and insecticidal effects of extracts Pithraj, *Aphanamixis polystachya* (Meliaceae), against *Tribolium castaneum* in storage. Journal of Chemical Ecology. 1993; 19:2463-2471.
11. Liu ZL, Ho SH. Bioactivity of essential oil extracted from *Evodia rutaecarpa* Hook F. and Thomas against the grain storage insects, *Sitophilus zeamais* Motsch and *Tribolium castaneum* (Herbst), Journal of stored Product Research. 1999; 35(4):317-328.
12. Juliana G, Su HCF. Laboratory studies on several plant materials as insect repellents for protection of cereal grains. Journal of Economy and Entomology. 1983; 76:154-157.
13. Talukder FA, Howse PE. Repellent, toxic and food protectant effects of pithraj, *Aphanamixis polystachya* extracts against the pulse beetle, *Callosobruchus chinensis* in storage. Journal of Chemical Ecology. 1994, 20(4).
14. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology. 1925; 18:265-267.
15. Zar JH. Biostatistical Analysis, 4th edn. Prentice Hall, Inc., Upper Saddle River, NJ, 1999, 662.
16. SAS. The SAS System Version 9.1 for Windows. SAS Institute, Cary, NC, 2003.
17. Obeng-Ofori D. The use of botanicals by resource poor farmers in Africa and Asia for the protection of stored agricultural products. Stewart Postharvest Review 2007; 3(6):10.
18. Kabaru JM, Gichia L. Insecticidal activity of extracts derived from different parts of the mangrove tree *Rhizophora mucronata* (rhizophoraceae) Lam. against three anthropoids. African Journal of Science and Technology. 2001; 2(2):44-49.
19. Stevenson PC, Dayarathna T, Belmain SR, Veitch NC. Bisdesmosidic saponins from *Securidaca longepedunculata* (Polygalaceae) with deterrent and toxic properties to Coleapteran storage pests. Journal of Agricultural Food Chemistry. 2009; 57:8860-8867.
20. Ntalli NG, Menkissoglu-Spiroudi Urania. Pesticides of Botanical Origin: a Promising Tool in Plant Protection, Pesticides - Formulations, Effects, Fate, Prof. Margarita Stoytcheva (Ed.), 2011, ISBN: 978-953-307-532-7.
21. Prashith Kekuda TR, Karthik S, Nandini KC, Vinayaka KS, Mukunda S. Total phenol content, Insecticidal and Amylase inhibitory efficacy of *Heterodermia leucomela* (L). Annals of Biological Research. 2011; 2(4):38-43.
22. Kituta R, Mukondwa N, Bahananga M, Mashimango B, Sanvura B. Evaluation *in vitro* de l'activité insecticide des alcaloïdes, saponines, terpenoïdes et steroïdes extraits de *Capscicum frutescens* L. (solanaceae) contre *Antestiopsis orbitalis ghesquierei*, insectes ravageurs des caféiers.

International Journal of Innovation and Applied Studies. 2014; 8(3):1231-1243.
23. Tofel HK, Nukenine EN, Stähler M, Adler C. Bio-efficacy of *Azadirachta indica* A. Juss oil extracted from sun- and shade-dried seeds against two stored-products beetles. International Journal of Biosciences. 2015; 7(2):135-151.
24. Jacobson M. Plants, insects, and man-their interrelationships. Economic Botany 1982; 36(3):346-354.
25. Gopalakrishnan S, Martin Rathi J. Insecticidal activity of aerial parts of *Synedrella nodiflora* Gaertn (Compositae) on *Spodoptera litura* (FAB.). Journal of Central European Agriculture. 2005; 6(3):223-228.
26. Ngassoum MB, Ousmaila H, Ngamo LT, Maponmetsem PM, Jirovetz L, Buchbauer G. Aroma compounds of essential oils of two varieties of the spice plant *Ocimum canum* Sims from northern Cameroon. Journal of Food Compost Anal. 2004; 17(2):197-204.
27. Udo IO. Evaluation of the potential of some local spices as stored grain protectants against the maize weevil *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae). Journal of Applied Sciences and Environmental Management. 2005; 9:165-168.
28. FAO/AfDB. Framework paper on postharvest loss reduction in Africa. UN Food and Agriculture Organisation (AGST), Rome, Italy, 2009, 56.
29. World Bank. Missing Food: The case of postharvest grain losses in Sub-Saharan Africa. The World Bank, Washington DC, USA, 2010, 115.
30. Ngamo TSL, Ngatanko I, Ngassoum MB, Mapongmestsem PM, Hance T. Persistence of insecticidal activities of crude essential oils of three aromatic plants towards four major stored product insect pests. African Journal of Agricultural Research. 2007; 2(4):173-177.
31. Tamgno BR, Ngamo TSL. Utilisation des produits dérivés du neem *Azadirachta indica* A. Juss comme alternatifs aux insecticides synthétiques pour la protection des semences de maïs et de sorgho dans la Vallée du Logone. Sciences, Technologies et Développement 2014; 15:1-8.
32. Tofel HK, Nukenine EN, Stähler M, Adler C. Insecticidal efficacy of *Azadirachta indica* powders from sun- and shade-dried seeds against *Sitophilus zeamais* and *Callosobruchus maculatus.* Journal of Entomology and Zoology studies. 2015; 3(1):100-108.