



# **Impact of Environment on *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) Response to Acetone Extract of *Gnidia kaussiana* Meisn (Thymeleaceae) and *Ocimum canum* Sims (Lamiaceae) Botanical Insecticides**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors DK and ENN designed the study and wrote the protocol. Authors DK and KHT performed the statistical analysis. Authors DK, JWG, DGL, MA, JPA, BD and HMTN managed the analyses of the study. Author DK wrote the first draft of the manuscript. All authors read and approved the final manuscript.*

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## **ABSTRACT**

The response of pests to the effects of a botanical insecticide can vary spatially and temporally. To test whether efficacy of botanicals differed spatially, the insecticidal efficacy of *Gnidia kaussiana* and *Ocimum canum* against *Callosobruchus maculatus* was investigated in two different agro-ecological zones of Cameroon, i.e. Maroua and Ngaoundéré (sudano-sahelian and sudano-

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guinean zones, respectively). Experiments were, therefore, conducted to determine the insect mortality, progeny production, grain damage and weight loss in cowpea. *G. kaussiana* was more effective against *C. maculatus* in Maroua (LD<sub>50</sub> = 0.12 g/kg at 6<sup>th</sup> day of exposure) than in Ngaoundéré (LD<sub>50</sub> = 4.35 g/kg at 6<sup>th</sup> day of exposure). Moreover, it was more toxic than *O. canum* extract and neem seed oil (reference), irrespective to the agro-ecological zones. Overall, the performance of *O. canum* did not vary significantly between the two zones, and was slightly more active (LD<sub>50</sub> = 4.66 g/kg) than the reference insecticide neem seed oil (LD<sub>50</sub> = 4.89 g/kg) in Ngaoundéré in contrast to the results recoded in Maroua (LD<sub>50</sub> = 1.44 g/kg and 2.60 g/kg, respectively for neem seed oil and *O. canum* at 6 days post exposure). In view of the above, there were some discrepancies in extract performance between Maroua and Ngaoundéré. Thus, the establishment of dose rates of insecticidal products formulated from *G. kaussiana* must be specific to an environment in contrast to those from *O. canum*.

**Keywords:** *Callosobruchus maculatus*; *Gnidia kaussiana*; *Ocimum canum*; pest control; agro-ecological zones.

## 1. INTRODUCTION

In low-income countries, food loss and waste account for approximately 44 % of global loss and waste [1] and occurs mainly during post-harvest [2]. Postharvest losses vary greatly among commodities, production areas and seasons. Legumes such as beans, chickpeas, cowpea, and Bambara groundnut which are staple foods in parts of Africa are subjected to heavy losses due to insect pests during storage [3–7]. Eight out of ten cowpea genotypes screened in Cameroon [7] were susceptible in sudano-guinean and highly susceptible in sudano-sahelian zones to *Callosobruchus maculatus* during storage (dry season). This vulnerability, added to the instability of prices on the market, has led cowpea producers who do not have adequate tools of protection against *C. maculatus* to abandon this legume, known in developing countries as the “meat of poor people”.

To encourage producers to boost cowpea production and preservation; the safe, effective and accessible pest management techniques should be explored in order to control this voracious insect pest. Control measures applied against *C. maculatus* in northern Cameroon are the extensive use of residual chemical insecticides such as malathion and dichlorvos, an organophosphate locally known as “piapia”. However, these have harmful effects on the environment and promote the development of resistant strains. The growing awareness of the environmental cost of this practice and the risk that pesticide residues can constitute for human health are giving rise to a growing interest in alternative control strategies such as botanical insecticides which are more biodegradable, safer

to use and provide residue-free food [8–12]. *Gnidia kaussiana* and *Ocimum canum* were reported to be effective insecticides against *C. maculatus* infesting Bambara groundnut and cowpea in sudano-guinean agro-ecological zone of Cameroon [6,13]. These products may be extensively used against storage insect pests throughout the country and beyond. However, the response of pests to the effects of a botanical insecticide can vary spatially due to the difference in environmental conditions, especially temperature and relative humidity [14]. Ngaoundéré (sudano-guinean zone of Cameroon) is characterized by a dry season lasting 5 to 6 months in the year with annual rainfall average of about 1000 mm and with average temperatures of 22°C and relative humidity of 63%. Maroua (sudano sahelian zone of Cameroon) is also characterized by a dry season lasting 7 to 9 months annually with less abundant rainfall ranging from 300 to 900 mm/year and with average temperature of 28°C and relative humidity of 46% [15]. Hence, a study was undertaken to investigate the influence of environment on the insecticidal potential of *G. kaussiana* and *O. canum* against *C. maculatus* infesting stored cowpea in sudano sahelian and sudano-guinean zones of Cameroon.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Conditions

Experiments were carried out under ambient conditions in the Laboratory of Applied Zoology of the Department of Biological Sciences, University of Ngaoundéré (sudano-guinean zone) and in the Laboratory of Entomology in Maroua (sudano-sahelian zone). The temperature and

relative humidity were recorded hourly using RH / TEMP DATA LOGGER (EL-USB-2+), manufactured by LASCAR (China). The average temperature and humidity for each experiment is given in Table 1.

## 2.2 Test Cowpea Seeds and Insect Rearing

Cowpea seeds (variety Vya Moutourwa) were collected from farmers at Rhumzou, Mogodé sub-division, Mayo-Tsanaga division in far north region of Cameroon during harvest time. The seeds were kept in a freezer at -4°C during 3 weeks to eliminate eggs, larvae and pupae of *Callosobruchus maculatus* and parasitoids. The moisture content determined by the method of AFNOR [16] prior to the bioassays was 10.87 ± 0.13%.

Insects used were originally collected in Mokolo market, Mayo-Tsanaga division in far north Cameroon and subsequently cultured in the laboratory at each locality of the study. One hundred parent stocks of *C. maculatus* collected from untreated infested cowpea stocks were introduced into 500 g of sterilized cowpea in rearing medium and kept in the laboratory. The emerging F1 adults were used for the different assays.

## 2.3 Collection and Processing of Plant Materials

The roots of *Gnidia kaussiana* and fresh leaves of *Ocimum canum* were collected in the wild in November 2013 around Mogode (latitude 10°36.25' N and longitude 13°34.46' E, 1,005 m a.s.l.). The collected plant parts were air-dried in a room for 3 days and 7 days, respectively for *O. canum* and *G. kaussiana*, and then crushed in a wooden locally made mortar to get 0.4 mm fine

powder. The powdered materials were kept at 4°C until needed for extraction. Each plant was identified by the Cameroon National Herbarium, where voucher specimens (Serial numbers: 38259/HNC and 26811/SRFCam, respectively for *G. kaussiana* and *O. canum*) were deposited.

The organic extracts were obtained by cold maceration using acetone preceded by hexane (3:1, v/w), with the aim to fractionate the different compounds of botanicals according to their polarity. After the hexane extractions were done, the pastes were dried for 10 h at room temperature in the laboratory and then used for acetone extractions. The mixtures of paste and acetone were stirred for 30 min and allowed to stand for 24 hours and re-stirred again two times. The mixtures were filtered using Whatman filter paper (no. 1) after 48 hours. The residues obtained were put through the same process and the filtrates were respectively admixed with those obtained initially and then concentrated in vacuum at 60° C and 120 rpm using rotary evaporator. Extracts were stored in a refrigerator at 4° C until needed for bioassays.

*Azadirachta indica* Juss. seed oil was used as a positive control. The ripe seeds were collected on the ground under *A. indica* trees at Maroua (latitude 10°33.16' N, longitude 14°815.04' E, 356 m. a.s.l.), in August 2013. Seeds processing and oil extraction were done as described in previous study [17].

## 2.4 Phytochemical Screening of Extracts

The plant extracts were phytochemically screened using standard techniques [18] for the detection of sterols, saponins, cardiac glycosides, tannins, flavonoids, terpenoids and alkaloids.

**Table 1. Experimental conditions (mean temperature and humidity ± S.E., and range in parenthesis) at Maroua and Ngaoundéré**

Bioassays	Temperature (°C)		Relative humidity (%)	
	Maroua	Ngaoundéré	Maroua	Ngaoundéré
Toxicity	28.53 ± 2.09 (24.50 – 33)	24.66 ± 1.42 (22.50 – 29.50)	66.37 ± 4.12 (53.50 – 73.50)	76.41 ± 2.29 (68.00 – 80.50)
PPI	26.23 ± 2.34 (21.00 – 33.00)	23.58 ± 1.23 (21.00 – 27.50)	74.16 ± 6.01 (53.50 – 86.00)	78.96 ± 2.16 (71.00 – 83.50)
GD/GWL	26.14 ± 2.60 (17.50 – 45.50)	23.81 ± 1.35 (20.50 – 34.50)	71.21 ± 10.80 (17.00 – 86.50)	78.67 ± 3.77 (37.50 – 87.50)

PPI: progeny production inhibition, GD/GWL: grain damage/grain weight loss

## 2.5 Adult Toxicity and Progeny Production

Extracts were separately dissolved in acetone to get 250 mg/ml solutions. Four volumes, 0.1, 0.2, 0.4 and 0.8 ml corresponding to 0.025, 0.05, 0.1 and 0.2 g of each extract and neem seed oil (NSO: check) at the same weights were separately mixed with 50 g grains in glass jars which correspond to 0.5, 1, 2 and 4 g/kg grain, respectively. The volumes 0.9, 0.8, 0.6 and 0.2 ml of acetone were respectively added to jars containing 0.1, 0.2, 0.4 and 0.8 ml of extract to bring up each solution added to 1 ml. The control consisted of seeds treated with the solvent alone. The content of each jars was shaken to ensure proper coating of the seeds with the extract or NSO and the solvents allowed to evaporate [17]. Twenty unsexed weevils aged 1–2 days were introduced into each of the treatments and covered with muslin cloth and perforated metal lid to ensure proper aeration and prevent entry and exit of insects. Each treatment was replicated four times. Adult mortality of *C. maculatus* was observed at 24 hours interval during seven days. After the 7-day mortality recordings, insects were separated from the grains and discarded. The grains were left inside the bottles on laboratory shelves and all the F1 progeny subsequently emerging were daily counted until the end.

## 2.6 Damage and Weight Loss Assessment

The experimental units from the adult toxicity test and progeny production above were used to assess seed damage and weight loss. After the F<sub>1</sub> progeny recordings, all the insects, dead and alive, as well as the seeds from each jar were left in their respective jars on laboratory shelves for a total period of 4 months starting from infestation. At the end of the 4-month storage period, the extent of weevil damage was assessed using the exit-hole counted as a measure of damage to seeds. Percent weight loss was calculated as described in the previous study [13].

## 2.7 Data Analysis

Abbott's formula [19] was used to correct collected data with respect to the control mortality. Data on % cumulative corrected mortality, % reduction in progeny production, % grain damage and % weight loss were arcsine-transformed [ $\sqrt{x/100}$ ] and the number of F1 progeny produced was log-transformed ( $x$

+ 1). The transformed data were subjected to the ANOVA procedure of the statistical analysis system [20]. Tukey (Honest Significant Difference) multiple range test ( $P = 0.05$ ) was applied for mean separation. Probit analysis [20,21] was conducted to determine lethal dose of acetone extracts of both botanicals causing 50% (LD<sub>50</sub>) mortality of *C. maculatus* and to compute the effective contents (EC<sub>50</sub>) values of plant extracts required to reduce F1 progeny by 50%, relative to controls.

## 3. RESULTS

### 3.1 Chemical Constituents of Acetone Extracts of *Ocimum canum* and *Gnidia kaussiana*

Five and six allelochemicals groups were detected in acetone extracts of *Ocimum canum* and *Gnidia kaussiana*, respectively (Table 2). Saponins and steroids were only extracted from *O. canum* while tannins, terpenoids and cardiac glycoside were specific to *G. kaussiana*. The major component irrespective to the botanicals was polyphenolic compounds. Alkaloids, saponins and steroids were moderately abundant in *O. canum*, while flavonoids were present but not abundant. Alkaloids, flavonoids and cardiac glycosides were moderately abundant in *G. kaussiana*, while tannins and terpenoids were detected in trace.

### 3.2 Adulticidal Activity of *Ocimum canum* and *Gnidia kaussiana* against *Callosobruchus maculatus*

The effectiveness of acetone extract of *O. canum* varied from one environment to another, depending on dosages and periods of exposure (Fig. 1). At the content of 0.5 g/kg grains, the effectiveness of *O. canum* was significantly and positively correlated to temperature and negatively to relative humidity (Table 3). The lethal dose (LD<sub>50</sub>) highlighted that products were in general more toxic under conditions of sudano-sahelian (SS) zone (LD<sub>50</sub>: 1.07-6.92 g/kg grains for neem seed oil (NSO) and 2.13-25.57 g/kg grains for *O. canum*) than under conditions of sudano-guinean (SG) zone (LD<sub>50</sub>: 3.41-184.08 g/kg grains for NSO and 2.98-31.92 g/kg grains for *O. canum*), (Tables 4 and 5). However, since the  $X^2$  values were high ( $P < 0.05$ ), there was an inadequate fit of the model to the data recorded. Moreover, *O. canum* as well as neem seed oil (NSO) was slightly effective against *C. maculatus* either in the SS or in the SG zone, at 0.5 g/kg

grains. However, at the content of 1 g/kg grains and at long exposure periods, especially at 7<sup>th</sup> day, *O. canum* was more effective than NSO in the SG zone ( $t = 2.85$ ;  $P = 0.029$ ) in contrast to that observed in the SS zone ( $t = 3.74$ ;  $P = 0.010$ ). Also, at the contents of 2 and 4 g/kg grains and depending on the period of exposure,

NSO was more effective than *O. canum* in the SS zone and inversely in the SG zone. At 4 g/kg grains and 7 days post-exposure, *O. canum* was less toxic (65.4% insect mortality) than NSO (92.6% insect mortality) in the SS zone, while in the SG zone both products had the same efficacy and caused about 55% insect mortality.

**Table 2. Phytochemical analyses of *Ocimum canum* and *Gnidia kaussiana* extracts by acetone**

Plant	Compound							
	TPC	Alk.	Sap.	Tan.	Flav.	Ster.	Terp.	C. glyc.
<i>O. canum</i>	+++	++	++	—	+	++	—	—
<i>G. kaussiana</i>	+++	++	—	+	++	—	+	++

TPC = total phenolic compounds; Alk. = alkaloids; Sap. = saponins; Tan. = tannins; Flav. = flavonoids; Ster. = steroids; Terp. = terpenoids; C. glyc. = cardiac glycosides  
 — = absent; + = present but not abundant; ++ = moderately abundant; +++ = abundant

**Table 3. Correlation of mortality to temperature and relative humidity of sudano-sahelian (Maroua) and sudano-guinean (Ngaoundéré) zones of Cameroon**

Product	Temperature				Relative humidity			
	0.5 g/kg	1 g/kg	2 g/kg	4 g/kg	0.5 g/kg	1 g/kg	2 g/kg	4 g/kg
NSO	0.185 <sup>ns</sup>	0.481 <sup>ns</sup>	0.589	0.562	-0.185 <sup>ns</sup>	-0.481 <sup>ns</sup>	-0.589	-0.562
<i>O. canum</i>	0.534*	0.302 <sup>ns</sup>	0.269 <sup>ns</sup>	0.203 <sup>ns</sup>	-0.534*	-0.302 <sup>ns</sup>	-0.269 <sup>ns</sup>	-0.203 <sup>ns</sup>
<i>G. kaussiana</i>	0.585	0.640*	0.745*	0.739*	-0.585	-0.640*	-0.745*	-0.739*

NSO, neem seed oil  
 ns, not significant; \*\*,  $P < 0.001$ ; \*,  $P < 0.05$

**Table 4. Lethal dose toxicity (LD<sub>50</sub>) of acetone extract of *Ocimum canum* and *Gnidia kaussiana* on *Callosobruchus maculatus* in treated cowpea under sudano-sahelian (Maroua) zone**

Extract	DAI	n	Slope ± SE	LD <sub>50</sub>	95 % Fiducial limits		X <sup>2</sup>
					Lower	Upper	
<i>O. canum</i>	1	4	0.62 ± 0.26	194.45	ñ	ñ	5.49
	2	4	0.80 ± 0.23	25.57	8.97	963.59	11.99
	3	4	0.80 ± 0.22	19.39	7.61	396.44	12.97
	4	4	0.78 ± 0.21	12.52	5.69	138.32	13.70
	5	4	0.90 ± 0.20	4.96	3.14	13.60	20.14
	6	4	1.13 ± 0.20	2.60	1.97	3.97	32.70
	7	4	1.12 ± 0.20	2.13	1.63	3.06	32.75
<i>G. kaussiana</i>	1	4	1.17 ± 0.24	10.25	5.85	34.63	24.39
	2	4	1.10 ± 0.20	2.61	1.97	4.04	35.15
	3	4	1.35 ± 0.20	1.06	0.81	1.32	45.88
	4	4	1.14 ± 0.21	0.33	0.14	0.50	28.01
	5	4	1.33 ± 0.26	0.19	0.06	0.33	26.29
	6	4	1.58 ± 0.37	0.12	0.02	0.23	17.96
	7	4	2.0 ± 0.53	0.13	0.02	0.25	13.93
NSO	1	4	1.94 ± 0.67	10.08	ñ	ñ	8.25
	2	4	1.78 ± 0.29	6.92	4.90	12.76	38.17
	3	4	1.82 ± 0.27	5.56	4.16	8.37	45.51
	4	4	1.56 ± 0.21	3.06	2.45	4.23	52.77
	5	4	2.0 ± 0.22	1.89	1.62	2.24	84.94
	6	4	2.47 ± 0.23	1.44	1.26	2.24	116.82
	7	4	2.56 ± 0.24	1.07	0.93	1.21	114.97

LD<sub>50</sub> values considered significantly different when 95% fiducial limits do not overlap.

DAI: Day after infestation, NSO: neem seed oil, ñ: fiducial limits estimation was not possible or too large due to inadequate mortality

**Table 5. Lethal dose toxicity (LD<sub>50</sub>) of acetone extract of *Ocimum canum* and *Gnidia kaussiana* on *Callosobruchus maculatus* in treated cowpea under sudano-guinean (Ngaoundéré) zones**

Extract	DAI	n	Slope ± SE	LD <sub>50</sub>	95 % Fiducial limits		X <sup>2</sup>
					Lower	Upper	
<i>O. canum</i>	1	4	1.27 ± 0.47	55.12	13.90	204570	7.41
	2	4	0.97 ± 0.27	31.92	10.99	1085	12.80
	3	4	1.05 ± 0.26	19.54	8.54	189.02	16.54
	4	4	1.13 ± 0.23	9.89	5.64	34.21	23.50
	5	4	1.36 ± 0.22	5.12	3.64	9.28	36.94
	6	4	1.13 ± 0.21	4.66	3.21	89.31	29.48
	7	4	1.28 ± 0.20	2.98	2.29	4.47	39.32
<i>G. kaussiana</i>	1	4	1.67 ± 0.66	33.90	10.75	204193	6.42
	2	4	0.43 ± 0.30	4652	ñ	ñ	2.16
	3	4	0.41 ± 0.22	362.37	ñ	ñ	3.34
	4	4	0.39 ± 0.21	129.41	ñ	ñ	3.14
	5	4	0.44 ± 0.19	15.74	4.73	30422218	5.21
	6	4	0.54 ± 0.19	4.35	2.33	59.31	8.08
	7	4	0.54 ± 0.19	1.93	1.11	5.61	8.32
NSO	1	4	11.15 ± 6.71	5.01	ñ	ñ	0.00
	2	4	0.88 ± 0.38	184.08	ñ	ñ	5.25
	3	4	0.94 ± 0.29	51.27	13.89	9275	10.40
	4	4	1.05 ± 0.26	19.29	8.50	176.37	16.92
	5	4	1.08 ± 0.24	14.53	7.18	83.27	19.39
	6	4	1.38 ± 0.22	4.89	3.53	8.57	38.65
	7	4	1.44 ± 0.21	3.41	2.64	5.03	46.26

LD<sub>50</sub> values considered significantly different when 95% fiducial limits do not overlap.

DAI: Day after infestation, NSO: neem seed oil, ñ: fiducial limits estimation was not possible or too large due do inadequate mortality

*G. kaussiana* was more toxic than the standard insecticide NSO in both sites irrespective to the period of exposure and dosages (Fig. 1). At 2 g/kg grains in SS zone, *G. kaussiana* achieved 100% insect mortality, while the standard commercial insecticide NSO caused only 72.7%. In the SG zone, these products caused at the same dosage point 45.8% and 35.3% mortality, respectively. Moreover, it was more effective in the SS (LD<sub>50</sub> ≈ 0.13-2.61 g/kg grains) than in the SG zone (LD<sub>50</sub> ≈ 1.93-4652 g/kg grains), irrespective to dosages and periods of exposure (P < 0.001 and ≈ 0.001-0.026), (Tables 4 and 5). In fact, mortality was significantly and positively correlated with temperature and inversely with relative humidity at all tested contents of *G. kaussiana* (Table 3).

### 3.3 Progeny Production Inhibition of *Callosobruchus maculatus* Caused by *Ocimum canum* and *Gnidia kaussiana*

The number of emerged adults in the untreated grains under SS (117.00) and under SG conditions (52.25) was significantly higher than those recorded in treated grains (Table 6). The reduction rates were dose-dependent (P <

0.001). At 1 and 2 g/kg grains, the commercial standard insecticide NSO completely suppressed insect emergence from treated cowpea, i.e. 100% progeny production inhibition. The effective content (EC<sub>50</sub>) highlighted that NSO was more effective (EC<sub>50</sub> = 0.001 and 0.120 g/kg grains, respectively at Ngaoundéré and Maroua) than *O. canum* (EC<sub>50</sub> = 0.785 and 0.822 g/kg grains, respectively at Ngaoundéré and Maroua), in the both sites. Nevertheless, treatments with *O. canum* and *G. kaussiana* hindered the progeny production significantly. At 4 g/kg grains, a high reduction was recorded either at Maroua (4.5) or at Ngaoundéré (4.8), with the respective progeny production inhibition rates of 96.1% and 90.8%. Overall, correlation between insecticidal properties tested in terms of progeny production inhibition and climatic parameters of the 2 study locations were not significant, irrespective of botanicals (Table 8).

Except at 0.5 g/kg grains, the number of insects emerged from grains treated with *G. kaussiana* did not vary significantly from one environment to another. Moreover, the number of emerged insects as well as the progeny production inhibition rate recorded at Ngaoundéré from the

grains treated at 4 g/kg were not significantly different to those recorded for NSO in the same locality ( $P = 0.085$ ). But in general, NSO was more effective in inhibiting the production of progeny by *C. maculatus* than *G. kaussiana* ( $EC_{50} = 0.099$  and  $0.225$  g/kg grains, respectively at Ngaoundéré and Maroua).

### 3.4 Damage and Weight Loss

Four months after storage, 87.0 and 94.3% untreated grain damage corresponding respectively to 28.9 and 34.4 % weight losses were recorded under SG and SS zone,

respectively. Significant reduction of grain damage ( $F = 209.66-274.39$  at Maroua and  $28.47-159.43$  at Ngaoundéré;  $df = 4, 15$ ;  $P < 0.001$ ) as well as grain weight loss ( $F = 150.35-203.48$  at Maroua and  $80.22-68.24$  at Ngaoundéré;  $df = 4, 15$ ;  $P < 0.001$ ) was recorded in treated grains (Table 7). At dosages above 0.5 g/kg, the standard commercial insecticide NSO which was more effective averted cowpea weevil damage and no weight was lost. *O. canum* was more effective under conditions of SS zone than under those of SG zone to protect cowpea from damage ( $P \approx 0.001-0.050$ ) and weight loss ( $P < 0.001$  and  $\approx 0.004-0.045$ ). At 4 g/kg grains,

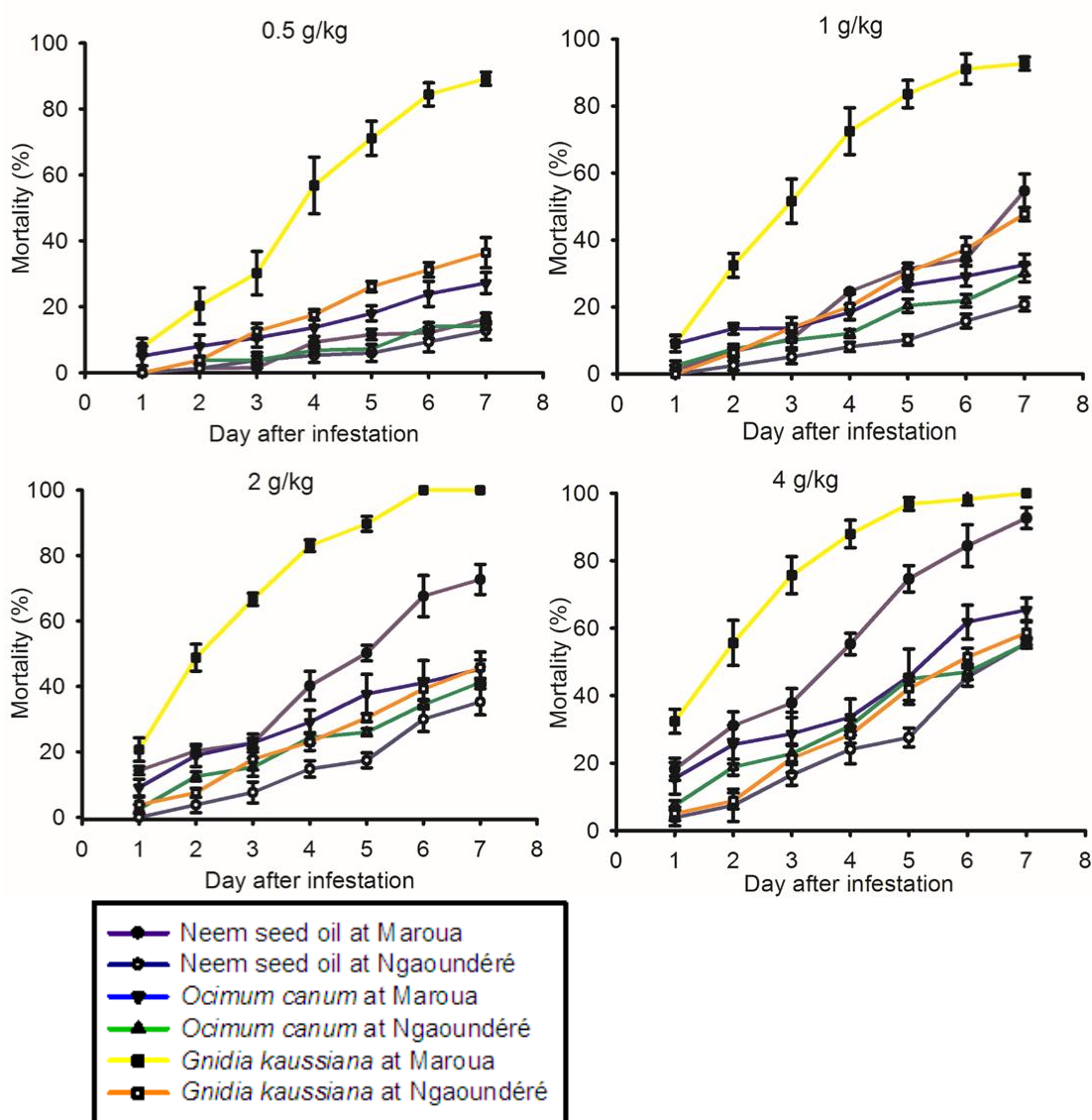


Fig. 1. Cumulative mortality (Means  $\pm$  SE) of *Callosobruchus maculatus* caused by acetone extracts of *Ocimum canum* and *Gnidia kaussiana* at 0.5, 1, 2 and 4 g/kg grain in sudano-sahelian (Maroua) and sudano- guinean (Ngaoundéré) zones

**Table 6. F<sub>1</sub> progeny production (mean ± S.E.) and inhibition rate (%) of *Callosobruchus maculatus* in treated cowpea with extracts of *Ocimum canum* and *Gnidia kaussiana*, under sudano-sahelian (Maroua) and sudano-guinean (Ngaoundéré) zones of Cameroon**

Product/ concentration (g/kg)	Emerged adults		Student t-test	Progeny production inhibition (%)		Student t-test
	Maroua	Ngaoundéré		Maroua	Ngaoundéré	
<b>NSO</b>						
0	117.0 ± 7.3 <sup>a</sup>	52.3 ± 5.9 <sup>a</sup>	6.880 <sup>***</sup>	0.0 ± 0.0 <sup>c</sup>	0.0 ± 0.0 <sup>b</sup>	-
0.5	12.5 ± 2.4 <sup>b</sup>	1.0 ± 0.6 <sup>b</sup>	4.663 <sup>**</sup>	89.2 ± 2.2 <sup>b</sup>	97.9 ± 1.2 <sup>a</sup>	3.490 <sup>**</sup>
1	0.0 ± 0.0 <sup>c</sup>	0.3 ± 0.3 <sup>b</sup>	1.000 <sup>ns</sup>	100.0 ± 0.0 <sup>a</sup>	99.6 ± 0.4 <sup>a</sup>	1.000 <sup>ns</sup>
2	0.0 ± 0.0 <sup>c</sup>	0.0 ± 0.0 <sup>b</sup>	-	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	-
4	0.8 ± 0.8 <sup>c</sup>	0.3 ± 0.3 <sup>b</sup>	0.632 <sup>ns</sup>	99.2 ± 0.8 <sup>a</sup>	99.4 ± 0.6 <sup>a</sup>	0.207 <sup>ns</sup>
F <sub>(4,15)</sub>	300.24 <sup>***</sup>	160.94 <sup>***</sup>		1780.28 <sup>***</sup>	5068.36 <sup>***</sup>	
EC <sub>50</sub>				0.120	0.001	
<b><i>O. canum</i></b>						
0	117.0 ± 7.3 <sup>a</sup>	52.3 ± 5.9 <sup>a</sup>	6.880 <sup>***</sup>	0.0 ± 0.0 <sup>d</sup>	0.0 ± 0.0 <sup>e</sup>	-
0.5	85.3 ± 7.6 <sup>b</sup>	35.3 ± 4.2 <sup>ab</sup>	5.750 <sup>***</sup>	27.2 ± 4.1 <sup>c</sup>	32.7 ± 2.0 <sup>d</sup>	1.189 <sup>ns</sup>
1	46.8 ± 69.2 <sup>c</sup>	22.0 ± 2.5 <sup>b</sup>	3.708 <sup>**</sup>	60.3 ± 4.0 <sup>b</sup>	57.5 ± 3.6 <sup>c</sup>	0.532 <sup>ns</sup>
2	6.5 ± 3.0 <sup>d</sup>	10.8 ± 1.0 <sup>c</sup>	1.357 <sup>*</sup>	93.9 ± 2.9 <sup>a</sup>	78.5 ± 3.4 <sup>b</sup>	3.475 <sup>**</sup>
4	4.5 ± 1.7 <sup>d</sup>	4.8 ± 1.1 <sup>c</sup>	0.123 <sup>ns</sup>	96.1 ± 1.5 <sup>a</sup>	90.8 ± 2.0 <sup>a</sup>	1.230 <sup>ns</sup>
F <sub>(4,15)</sub>	74.98 <sup>***</sup>	45.66 <sup>***</sup>		198.78 <sup>***</sup>	242.76 <sup>***</sup>	
EC <sub>50</sub>				0.785	0.822	
<b><i>G. kaussiana</i></b>						
0	117.0 ± 7.3 <sup>a</sup>	52.3 ± 5.9 <sup>a</sup>	6.880 <sup>***</sup>	0.0 ± 0.0 <sup>c</sup>	0.0 ± 0.0 <sup>c</sup>	-
0.5	37.0 ± 5.2 <sup>b</sup>	12.0 ± 1.8 <sup>b</sup>	4.539 <sup>**</sup>	68.6 ± 3.7 <sup>b</sup>	75.8 ± 4.7 <sup>b</sup>	1.192 <sup>ns</sup>
1	14.0 ± 2.8 <sup>c</sup>	7.8 ± 0.6 <sup>b</sup>	2.157 <sup>ns</sup>	87.9 ± 2.7 <sup>a</sup>	84.8 ± 1.7 <sup>b</sup>	0.958 <sup>ns</sup>
2	8.3 ± 2.2 <sup>c</sup>	7.3 ± 0.9 <sup>b</sup>	0.428 <sup>ns</sup>	93.0 ± 1.9 <sup>a</sup>	86.0 ± 1.5 <sup>b</sup>	3.080 <sup>*</sup>
4	3.8 ± 2.4 <sup>c</sup>	2.0 ± 0.7 <sup>c</sup>	0.701 <sup>ns</sup>	97.0 ± 1.9 <sup>a</sup>	96.0 ± 1.4 <sup>a</sup>	1.054 <sup>ns</sup>
F <sub>(4,15)</sub>	111.49 <sup>***</sup>	76.52 <sup>***</sup>		290.17 <sup>***</sup>	263.40 <sup>***</sup>	
EC <sub>50</sub>				0.225	0.099	

ns: P > 0.05, \*: P < 0.05, \*\*: P < 0.01, \*\*\*: P < 0.001

Means ± S.E. in the same column followed by the same letter do not differ significantly at P < 0.05 (Tukey's test)

NSO: neem seed oil

*O. canum* reduced grain damage by 9-fold under SS conditions and by 3.8-fold under SG agro-ecological zone compared to untreated grains. In the same order and at the same dosage point, weight loss reduction of about 21.8-fold and 4.8-fold were recorded.

Compared to the standard commercial insecticide NSO, *G. kaussiana* was in general less effective at protecting cowpea from insect attack and grain weight loss. However, that product at the content of 4 g/kg grains reduced weight loss at the same level like NSO under SS conditions (P = 0.317). Both products, *G. kaussiana* and *O. canum* had the same efficacy (P ≈ 0.080-0.957) to protect cowpea from weight loss under SG conditions, while under SS *O. canum* was more effective at the contents of 1 and 2 g/kg (P = 0.015). Variation between localities in term of climatic parameters did not impact significantly the ability of tested products

to protect cowpea from damage and weight loss (Table 8).

#### 4. DISCUSSION

Botanicals have been documented to confer repellent, feeding deterrent/antifeedant, toxicity, growth retardant, chemosterilant, and attractant activities on insects [22]. Our investigations showed that *Ocimum canum* and *Gnidia kaussiana* possess some of these activities against *Callosobruchus maculatus*.

The insecticidal activities of total phenolic compounds and flavonoids, alkaloids, saponins and steroids, detected in acetone extracts of *O. canum* and *G. kaussiana* of our study have been previously reported [23–26]. In the present investigations, the effectiveness of biological activities of toxic components from the tested products on *C. maculatus* depended on variables



**Table 7. Damage and weight loss caused by *Callosobruchus maculatus* on cowpea seeds treated with acetone extract of *Ocimum canum* and *Gnidia kaussiana* after four months of storage at Ngaoundéré and Maroua**

Product/ Dosage (g/kg)	Grain damage (%)		Student t-test	Grain weight loss		Student t-test
	Maroua	Ngaoundéré		Maroua	Ngaoundéré	
<b>NSO</b>						
0	94.2 ± 1.1 <sup>a</sup>	87.0 ± 3.4 <sup>a</sup>	2.00 <sup>ns</sup>	34.9 ± 1.5 <sup>a</sup>	28.9 ± 1.4 <sup>a</sup>	2.98 <sup>*</sup>
0.5	13.4 ± 1.9 <sup>b</sup>	1.1 ± 0.7 <sup>b</sup>	6.04 <sup>***</sup>	2.0 ± 0.6 <sup>b</sup>	0.1 ± 0.1 <sup>b</sup>	3.43 <sup>*</sup>
1	0.8 ± 0.8 <sup>c</sup>	0.1 ± 0.1 <sup>b</sup>	0.04 <sup>ns</sup>	0.0 ± 0.0 <sup>c</sup>	0.0 ± 0.0 <sup>b</sup>	0.45 <sup>ns</sup>
2	0.0 ± 0.0 <sup>c</sup>	0.0 ± 0.0 <sup>b</sup>	–	0.0 ± 0.0 <sup>c</sup>	0.0 ± 0.0 <sup>b</sup>	–
4	0.2 ± 0.2 <sup>c</sup>	0.0 ± 0.0 <sup>b</sup>	1.00 <sup>ns</sup>	0.0 ± 0.0 <sup>c</sup>	0.0 ± 0.0 <sup>b</sup>	1.00 <sup>ns</sup>
<i>F</i> <sub>(4,15)</sub>	1702.85 <sup>***</sup>	617.36 <sup>***</sup>		480.71 <sup>***</sup>	437.23 <sup>***</sup>	
<b><i>O. canum</i></b>						
0	94.2 ± 1.1 <sup>a</sup>	87.0 ± 3.4 <sup>a</sup>	2.00 <sup>ns</sup>	34.9 ± 1.5 <sup>a</sup>	28.9 ± 1.4 <sup>a</sup>	2.98 <sup>*</sup>
0.5	86.6 ± 1.7 <sup>a</sup>	45.6 ± 8.9 <sup>b</sup>	4.51 <sup>**</sup>	24.6 ± 1.3 <sup>b</sup>	10.6 ± 1.0 <sup>b</sup>	8.24 <sup>***</sup>
1	21.6 ± 1.6 <sup>b</sup>	31.3 ± 0.6 <sup>bc</sup>	5.60 <sup>***</sup>	4.9 ± 1.1 <sup>c</sup>	8.2 ± 0.7 <sup>b</sup>	2.62 <sup>**</sup>
2	9.1 ± 3.2 <sup>c</sup>	37.4 ± 4.1 <sup>bc</sup>	5.44 <sup>**</sup>	1.5 ± 0.5 <sup>c</sup>	7.9 ± 1.1 <sup>b</sup>	5.40 <sup>**</sup>
4	10.5 ± 4.0 <sup>bc</sup>	22.8 ± 1.0 <sup>c</sup>	3.01 <sup>*</sup>	1.6 ± 0.7 <sup>c</sup>	6.0 ± 1.4 <sup>b</sup>	2.86 <sup>*</sup>
<i>F</i> <sub>(4,15)</sub>	274.39 <sup>***</sup>	28.47 <sup>***</sup>		203.48 <sup>***</sup>	68.24 <sup>***</sup>	
<b><i>G. kaussiana</i></b>						
0	94.2 ± 1.1 <sup>a</sup>	87.0 ± 3.4 <sup>a</sup>	2.00 <sup>ns</sup>	34.9 ± 1.5 <sup>a</sup>	28.9 ± 1.4 <sup>a</sup>	2.98 <sup>*</sup>
0.5	77.5 ± 4.2 <sup>b</sup>	34.3 ± 0.5 <sup>b</sup>	10.11 <sup>***</sup>	26.3 ± 1.5 <sup>b</sup>	10.0 ± 1.1 <sup>b</sup>	8.67 <sup>***</sup>
1	29.7 ± 2.1 <sup>c</sup>	33.0 ± 2.9 <sup>b</sup>	0.95 <sup>ns</sup>	9.9 ± 1.0 <sup>c</sup>	8.31 ± 1.00 <sup>b</sup>	1.12 <sup>ns</sup>
2	27.5 ± 1.5 <sup>c</sup>	22.2 ± 1.1 <sup>c</sup>	2.81 <sup>*</sup>	6.1 ± 1.0 <sup>cd</sup>	5.1 ± 1.3 <sup>bc</sup>	0.59 <sup>ns</sup>
4	4.8 ± 2.8 <sup>d</sup>	14.5 ± 2.0 <sup>c</sup>	2.86 <sup>*</sup>	0.8 ± 0.5 <sup>d</sup>	2.4 ± 1.0 <sup>c</sup>	1.49 <sup>ns</sup>
<i>F</i> <sub>(4,15)</sub>	209.66 <sup>***</sup>	159.43 <sup>***</sup>		150.35 <sup>***</sup>	80.22 <sup>***</sup>	

ns:  $P > 0.05$ , \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$

Means ± S.E. in the same column followed by the same letter do not differ significantly at  $P < 0.05$  (Tukey test), NSO: neem seed oil

**Table 8. Correlation between insecticidal properties and climatic parameters of sudano-sahelian (Maroua) and sudano-guinean (Ngaoundéré) zones of Cameroon**

Product	Progeny production inhibition		Grain damage		Grain weight loss	
	Temp.	RH	Temp.	RH	Temp.	RH
Neem seed oil	-0.308 <sup>ns</sup>	0.308 <sup>ns</sup>	0.380 <sup>ns</sup>	-0.380 <sup>ns</sup>	0.000 <sup>ns</sup>	0.000 <sup>ns</sup>
<i>O. canum</i>	0.089 <sup>ns</sup>	-0.089 <sup>ns</sup>	-0.050 <sup>ns</sup>	0.050 <sup>ns</sup>	-0.002 <sup>ns</sup>	0.002 <sup>ns</sup>
<i>G. kaussiana</i>	0.053 <sup>ns</sup>	-0.053 <sup>ns</sup>	0.221 <sup>ns</sup>	-0.221 <sup>ns</sup>	0.293 <sup>ns</sup>	-0.293 <sup>ns</sup>

Temp., temperature; RH, relative humidity.  
ns, not significant

such as dosage, period of insect exposure to the product and experimental conditions. The toxic action of *O. canum* which recorded 55.4 and 65.4 % mortality, respectively under SS and SG zones after 7 days at 4 g/kg content was slow. Kosini et al. [17] found a similar trend on Bambara groundnuts with 61.79 % mortality under conditions of SG zone. Nevertheless, this product with mortality increasing linearly depending on dosage could be of value in the storage of cowpea against *C. maculatus* at higher content. Acetone extract of *G. kaussiana* which was more effective than *O. canum* contained the toxic components such as tannins,

triterpenoids and cardiac glycosides in addition to total phenolic, alkaloids and flavonoids compounds found in acetone extract of *O. canum*. This higher activity might perhaps attribute to synergistic effect of these components or especially to one or all of these specific components of *G. kaussiana*. In this regard, further studies are required to isolate and identify the active principle component. At low concentrations, acetone extracts of *G. kaussiana* also showed higher insecticidal activity than NSO. This suggest that active compounds are either more concentrated in *G. kaussiana* extract or *C. maculatus* adults are more sensitive to

active compounds from that plant than NSO. The probable mechanisms that caused the death of *C. maculatus*, as reported by previous researchers, could include alteration of voltage-gated sodium ion channels by phytochemicals [27], DNA intercalation, and interference of protein bio-synthesis and disruption of membrane stability in the insect by the plant's allelochemicals [28] or even disruption of insect's anti-oxidant system by the secondary metabolites [29]. The effectiveness of these mechanisms varied between environments due to difference in temperature and relative humidity. It increased with temperature and with the decrease of relative humidity. Similar result was reported [30] concerning the toxicity of spinosad against *Sitophilus oryzae*. In fact, interaction between chemicals and temperature during the process of chemical transfer in organism is more important than with relative humidity [31], because the rates of cuticle penetration, insecticide/target-site-interactions or insecticide detoxification enzyme activity were reported to be impacted by temperature [32]. At high temperature, the insecticide is easily and longer bound to its target site, and biological activities of esterases, oxydases, or glutathione S-transferases which are the detoxification enzymes [33,34] might decrease.

Also, tested products including the standard insecticide have exerted promising inhibition activities against F1 progeny emergence, highlighting the presence of ovicidal and larvicidal constituents in these products. Correspondingly, Kosini et al. [6] found that the acetone extract of *O. canum* was toxic to each immature stage of *C. maculatus* developing in treated cowpea. In fact, some secondary metabolites of plant extracts like terpenoids, alkaloids, and flavonoids found in the tested extracts have been reported to deter oviposition and to disrupt the endocrine system leading to the inhibition of moulting of larvae and biosynthesis of the ribonucleic acid that controls protein synthesis in the cell [12, 35–38]. Even environmental conditions affect *C. maculatus* susceptibility to acetone extract of *O. canum* and *G. kaussiana*, the ability of these insecticides regardless of the content, except 2 g/kg, to inhibit progeny production of cowpea weevils, in the current study, was the same under SS and SG agro-ecological zones of Cameroon. Adult mortality was then not correlated with the inhibition of progeny production. In addition to the ability of the products to reduce considerably the number of progenies produced, significant

reduction of grain damage and weight loss was recorded. The impact of environment on the ability of products to prevent grain damage and weight loss varied with insecticide origin. These parameters were strongly environment-dependent for treated cowpea with *O. canum* and did not for *G. kaussiana* and NSO treatments. *G. kaussiana* and NSO could therefore have a diversity of mode of action against *C. maculatus* compared to *O. canum*

## 5. CONCLUSION

Acetone extracts of *O. canum* and *G. kaussiana* were toxic to *C. maculatus*, effective at inhibiting progeny production and protecting grain weight loss, with some discrepancies in extract performance between Maroua (SS zone) and Ngaoundéré (SG zone). Thus, in storage structures of developing countries like Cameroon with a diversity of agro-ecological zones, insecticidal products from acetone extract of *O. canum* and especially of *G. kaussiana* could be exploited for the development of novel molecules more specific to the environment and with precise targets for sustainable insect pest management.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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