Effects of bioactive extracts of *Ocimum canum* (Lamiaceae) on *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) in stored cowpea and soybean

Daniel KOSINI[1,*], Elias Nchiwan NUKENINE[2], Clément SAIDOU[3], Alembert Tiabou TCHINDA[4], Gabriel Agbor AGBOR[4]

(1. School of Health Sciences, Catholic University Institute of Saint Jérôme, Douala, Cameroon; 2. Department of Biological Sciences, Faculty of Science, University of Ngaoundere, Cameroon; 3. Department of Chemical Engineering and Environment, UIT of the University of Ngaoundere, Cameroon; 4. Medical Research Institute and Studies of Medicinal Plants (IMPM), Yaoundé, Cameroon)

Abstract: 【Aim】 The present study was carried out to evaluate the insecticidal, protectant and repellent potentials of hexane, acetone and methanol extracts of leaves of *Ocimum canum* Sims (Lamiaceae) against *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae). 【Methods】 Cowpea and soybean were coated with extracts from *O. canum* at dosages of 1 and 5 g/kg grains and commercial neem seed oil (NSO) as a reference at the same dosages. Adult mortality of *C. maculatus* was assessed during a period of seven days, one day interval. Ovicidal and larvicidal toxicity was tested by treating freshly laid eggs and larvae at different immature stages of *C. maculatus*. Grain damage and weight loss were assessed after a storage period of four months. Repellency effects were detected in choice test using a linear olfactometer. 【Results】 Extracts had the same toxicity to *C. maculatus* adults and proved to be more toxic than the commercial NSO in treated cowpea. Hexane, acetone and methanol extracts caused 54.2% ± 3.9%, 62.9% ± 4.8% and 60.3% ± 4.5% adult mortality, respectively, within seven days of exposure to the dosage of 5 g/kg cowpea grain. Moreover, extracts evoked stronger repellency effects compared with the tested standard insecticide, irrespective of grain species. The products were the most toxic to eggs, and more toxic to 1st and 2nd instar larvae than to other developmental stages. Hexane extract was the most effective against the 2nd instar larvae (81.0% ± 5.3% mortality), while acetone extract (92.5% ± 7.5% mortality) and methanol extract (62.0% ± 15.7% mortality) were the most effective against the 1st instar larvae. Acetone extract had a similar toxicity with the tested standard insecticide against the 1st and 2nd instar larvae and was superior against the pupae. Cowpea grain damage and weight loss were reduced by more than three- and two-fold, respectively, after four months of storage. 【Conclusion】 The outcome of this study indicates the potential of extracts from *O. canum* as repellent and insecticide against *C. maculatus*, and because of its availability the plant is strongly recommended to be used in pest management strategies in Cameroon.

Key words: *Callosobruchus maculatus*; *Ocimum canum*; pulse; toxicity; repellency

1 INTRODUCTION

Pulses are one of the best and the least expensive solutions to quality food supply in developing countries, and many of them are staple foods in many developing countries (Ndjouenkeu *et al*., 2010; Guèye *et al*., 2011). Leguminous crops (Fabaceae) including cowpea (*Vigna unguiculata* (L.) Walp.) and soybean [*Glycine max* (L.) Merr.] are widely grown in the tropics and subtropics for human consumption as well as for animal feed. Malnutrition, particularly protein deficiency, is prevalent in many parts of Africa as animal protein is too expensive in most communities. Many leguminous crops such as cowpea and soybean are excellent sources of proteins, carbohydrates, dietary fiber, vitamins and minerals as well as edible oil from soybean (Adeyemi and Mohammed, 2014; IITA, 2016). Unfortunately, during storage, cowpea and soybean are respectively heavily and moderately damaged by insect pests, especially the pulse beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae). Amusa *et al*. (2014) recorded 40% to 100% cowpea grain damage and 29.68% to 83.20% cowpea grain weight loss caused by this insect pest after only 35

* Corresponding author, E-mail: kosinid@yahoo.fr
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d. After four months 14.63% soybean grain weight loss was also recorded (Bharathi *et al*., 2017). These losses are due to the different immature stages of the pulse beetle which develop inside the grains.

The control of *C. maculatus* is then crucial to the increased and sustainable production of pulses in tropical Africa. This control should target different immature stages of the pest since they are permanently present during storage. While there are several synthetic insecticides such as chemical grain protectants and fumigants for the control of *C. maculatus* in pulses, their use has not been sustainable owing to their harmful effects on nontarget organisms, including pesticide applicators, handlers, and anyone else exposed to the product. These demerits of synthetic chemicals have necessitated the search for alternative pest control methods. Research efforts are then being intensified on the use of botanicals as alternatives to commonly used insecticides because many plants demonstrate insecticidal activities against insect pests and plant products are more biodegradable, and thus pose fewer problems to the environment (Boeke *et al*., 2004; Isman, 2008; Jeon *et al*., 2011; Tofel *et al*., 2015).

Ocimum canum Sims (Lamiaceae), commonly known as wild basil, is a branched herm and its leaves are used locally as spice in an ethno dietary soup known as "Mbongo tchobi" (Madhiyazhagan *et al*., 2015). These authors reported that traditional practitioners in Cameroon apply this plant for abdominal pain, malaria, diarrhea, and stomach-related disorders. It has been demonstrated that its leaf essential oil shows antimicrobial (Bassole *et al*., 2005), mosquitocidal and antiplasmodial activities (Belong *et al*., 2013; Akono *et al*., 2014). Kéita *et al*. (2000) demonstrated the biological activity of *O. canum* leaf essential oil against *C. maculatus*. However, to the best of our knowledge, studies reporting on the biological activity of methanol, acetone and hexane extracts of *O. canum* against storage insects in general and *C. maculatus* in particular are not available, except that of Kosini *et al*. (2015) on *O. canum* leaf extract fractions to protect Bambara groundnut from infestation of *C. maculatus*.

The present research was carried out to determine the repellency efficacy, ovocidal, larvicidal and adulticidal toxicity of organic solvent extracts from leaves of *O. canum* against *C. maculatus* in stored cowpea and soybean. The ability of these extracts to reduce seed damage and seed weight loss was also assessed.

2 MATERIALS AND METHODS

2.1 Tested seeds

Cowpea seeds (variety Vya Moutouroua) were collected at harvest from farmers in Far North Cameroon, at Kossehone village in Mayo-Tsanaga division, while soybean seeds (variety Kodek) were purchased at IRAD (Agricultural Research Institute for Development), station of Garoua. The seeds were cleaned and disinfested as described in the previous study (Kosini and Nukenine, 2017). The seeds were then kept under experimental conditions for at least two weeks before use. The initial moisture contents of the seeds were 10.9% ± 0.1% and 8.8% ± 0.9%, respectively, for cowpea and soybean determined by the method of AFNOR (1982).

2.2 Collection and processing of plant materials

Leaves of *O. canum* were collected in November 2013 around Mogode sub-division, Mayo-Tsanaga Division, Far-north region of Cameroon (latitude 10°36′29.25″N and longitude 13°34.46″E, Sudano-Sahelian Savannah) and the identity of the plant was confirmed at the Cameroon National Herbarium in Yaounde, where voucher specimen (Serial number: 26811/SRFCam) was kept. The region is characterized by two seasons: a dry season from October to May and a wet season spanning June to September. Average annual temperature is 28 ± 7℃. Only green leaves were harvested and dried at room temperature for 72 h and then crushed in a mortar until the powder passed through a 0.4 mm mesh sieve. Powers were preserved in plastic bags in a refrigerator at 4℃ until used for extraction.

Hexane, acetone and methanol extracts were gotten by using the maceration method as described by Kosini *et al*. (2015). Extracts were stored in a refrigerator at 4℃ until needed for bioassay.

Azadirachta indica Juss. 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°33.160′N, longitude 14°815.040′E and altitude of 356 m.a.s.l.), Far-North region, Cameroon in August 2013. Seeds processing and oil extraction were done as described in previous study (Kosini *et al*., 2015).

2.3 Insect rearing

Insects used were originally collected in Mokolo market, Mayo-Tsanaga division in far north Cameroon and subsequently cultured in the laboratory on cowpea for at least two generations before they were used for the experiments.

2.4 Phytochemical screening of *O. canum* extracts

The plant extracts were phytochemically screened using standard techniques for the detection of sterols, saponins, cardiac glycosides, tannins, flavonoids, terpenoids and alkaloids (Adeniyi *et al.*, 2010).

2.5 Assessment of the toxicity of *O. canum* extracts to *C. maculatus* adults

Extracts were dissolved in the respective solvent used for extraction to get 250 mg/mL solutions. Two volumes, 0.2 and 1 mL corresponding to 0.05 and 0.25 g of each extract and NSO (cheek) at the same content level were separately mixed with 50 g cowpea or soybean grains in separate glass jars which correspond to 1 and 5 g/kg, respectively. The volume of 0.8 and 1 mL of solvents to each extract were added respectively to jars containing 0.2 mL of extract and to non-treated jars (negative controls) to bring up each volume added to 1 mL. The contents of each jar were shaken properly to ensure proper coating of the seeds with the product and the solvents were allowed to evaporate. A group of 20 adults of *C. maculatus*, aged 1 – 2 d, was added separately into the jars containing the treated grains. These were covered with a muslin cloth and perforated metal lid to facilitate proper aeration and prevent entry and exit of insects. Four replicates were made per treatment. Adult mortalities were recorded 1, 2, 3, 4, 5, 6 and 7 d after treatment. The experiment was carried out at Ngaoundere in laboratory conditions [25.4 ± 1.8℃ (22.0 – 30.5℃) and relative humidity (RH) of 72.8% ±4.1% (60.5% – 79.0%)].

2.6 Assessment of the effectiveness of *O. canum* extracts to control immature stages of *C. maculatus*

This trial was carried out under laboratory conditions (Table 1) and cowpea seeds were used as food material. Fifty couples of *C. maculatus* adults less than 2 d old were introduced on 500 g cowpea seeds in a glass jar to obtain the ovipositioned grains. Thirty grains with 1 – 2 eggs were selected 24 h after oviposition, weighted and introduced in petri dishes and treated at the egg stage and the 1st to 4th instar larval stage as well as at the pupal stage with the experimental design as that described for adult toxicity test. Two negative controls (grains treated with solvents or without any treatment) for each set of the treatment were designed. In parallel, 30 lots each of 30 untreated grains carrying 1 – 2 eggs were dissected using a stereo-microscope at a rate of one lot per day to observe the different developmental stages of insect which formed inside the grains, to determine the exact day when treatments would be applied for each life stage. Dissection began 5 d post infestation, since eggs hatch within 6 – 7 d of oviposition at temperature range of 18.1 to 27.1℃ and RH of 79.5% (Howe and Currie, 1964; Bhubaneshwari and Victoria, 2014).

Table 1 Laboratory conditions during the assessment of insecticidal efficacy of *Ocimum canum* extracts against different immature stages of *Callosobruchus maculatus*

Developmental stage	Temperature (℃)	Relative humidity (%)
Egg	23.1 ±0.9 (21.0 – 25.5)	79.2 ±1.3 (74.5 – 81.5)
1st instar larva	23.2 ±0.9 (21.0 – 21.5)	79.9 ±1.3 (74.5 – 82.5)
2nd instar larva	23.2 ±0.9 (21.0 – 25.5)	80.0 ±1.2 (84.5 – 82.5)
3rd instar larva	23.2 ±1.0 (21.0 – 25.5)	79.9 ±1.2 (74.5 – 82.5)
4th instar larva	24.0 ±1.5 (21.0 – 30.5)	77.8 ±3.0 (60.5 – 83.5)
Pupa	24.0 ±1.5 (21.0 – 30.5)	77.8 ±3.1 (60.5 – 83.5)

Data in parentheses are the ranges.

All the eggs were examined under the stereo-microscope and the viable eggs were recognized by their morphological aspect (Marcileyne *et al.*, 2004), since they become opaque as a function of their residue discharged by the larvae during penetration.

The percentage survival of the 1st, 2nd, 3rd and 4th instar larvae and pupae were respectively determined by counting the number of survived insects at the 2nd, 3rd and 4th larval stages, pupal stage and the number of emerged adults. Thus, direct examination of the grains was done with the aid of a stereo-microscope.

The description of the different developmental stages of *C. maculatus* was used as the main criteria for identification (Pajni, 1987; Bhubaneshwari and Victoria, 2014). The 1st instar larva is formed within the eggs prior to penetrating the seed coat with a convenient visual marker signaling that embryonic development is nearly complete. After the formation of the pigmented larval head capsule they borrowed from the egg through the seed coat and entered into the seed endosperm (Bhubaneshwari and Victoria, 2014). It is different from the 3 subsequent larval instars in possessing well formed thoracic legs, an H-shaped plate on the prothorax and a hatching spine on each lateral side of the first abdominal segment (Pajni, 1987). The 3rd instar larvae are the most active and fed on the entire endosperm voraciously. The 4th instar larvae are white, yellowish and somewhat C-shaped with a small head, similar to the 3rd instar larvae but with different size and shape.

In this instar the larvae seemed to become larger and fed on the entire endosperm voraciously. It burrowed into a position just underneath the seed coat prior to pupation (Bhubaneshwari and Victoria, 2014). During the time of pupation larval structures are broken down and adult structures developed; the rudiments of the wings appeared at the 1st day, at the 2nd day appendages such as legs, antenna and proboscis developed freely and at the 3rd day eyes, mouth part, forewing, hind wing and legs with cuticular hair developed.

2.7 Population increase and damage bioassay

The experiment was performed in a fluctuating laboratory conditions [23.98 ± 1.46℃ (21.0 – 30.5℃) and RH 77.91% ± 3.13% (60.5% – 87.5%)]. The same sampling units from the adult toxicity test were used in the bioassay to assess population increase and damage. After F_1 progeny production, all insects, dead and live as well as sample from each jar were kept in their respective jar on laboratory benches for a total period of four months from infestation to end. After the last day, the number of live and dead insects were recorded; the number of undamaged grains and those of damaged grains were separately weighted from each jar. The final moisture content of seeds was also determined. The weight of additional moisture to the initial moisture was assessed. The weight of final grains 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 follows:

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

The percent weight loss was determined as follows:

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

2.8 Repellency test

Repellency test was conducted according to Ngamo *et al*. (2007) with modification concerning the materials used. 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 seeds. One container contained seeds treated with plant materials, and the control container in the other end contained seeds treated with solvent. Two treatment concentrations (1 and 5 g/kg grains) were used. Twenty insects (not more than 48 h old) were separately introduced in the device at the hole level. The choice of insects was observed for a period of 2 h. 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 (1993) and Liu and Ho (1999) under ambient laboratory conditions [24.9 ± 1.7℃ (23.0 – 28.0℃) and RH 75.6% ± 1.7% (72.0% – 78.0%)].

Percent repellency (PR) = 2 × (C – 50); C = percentage of insects choosing the control end treated by hexane, acetone, or methanol as negative control either by neem seed oil as positive control. When PR > 0 the extract was repellent and when PR < 0 the extract was attractive.

2.9 Data analysis

Abbott's formula (Abbott, 1925) was used to correct for control mortality. Data on percent cumulative mortality of adults and immature stages, damage, weight loss and PR were arcsine-transformed [square root (x/100)]. The transformed data were subjected to the ANOVA procedure using the statistical analysis system (SAS Institute, 2003). Tukey multiple range test (P = 0.05) was applied for mean separation.

3 RESULTS

3.1 Chemical constituents of hexane, acetone and methanol extracts of *O. canum*

The result of the phytochemical screening (Table 2) revealed that the presence of chemical groups increased with the polarity of solvent used for extraction. Two, five and six chemical groups were present in hexane, acetone and methanol extracts, respectively. Saponins (moderately abundant) and steroids (abundant) were present in hexane extract; acetone extract was phytochemically characterized by

Table 2 Phytochemical components of three extracts from *Ocimum canum*

Chemical groups	Hexane extract	Acetone extract	Methanol extract
Total henolic compounds	–	+++	+++
Alkaloids	–	++	+
Saponins	++	++	+++
Tannins	–	–	++
Flavonoids	–	+	++
Steroids	+++	++	++
Triterpenoids	–	–	–
Cardiac glycosides	–	–	–

–: Absent; +: Present but not abundant; ++: Moderately abundant; +++: Abundant.

the presence of total polyphenolic compounds (abundant), alkaloids, saponins and steroids, each abundant and by not abundant presence of flavonoids, while total phenolic (abundant as in acetone extract), alkaloids (not abundant), saponins (abundant), tannins, flavonoids and steroids, each moderately abundant were detected in methanol extract of this plant species.

3.2 Toxicity of *O. canum* extracts to *C. maculatus* adults

All the insecticidal materials formulated and tested caused significant mortality of cowpea weevils in treated cowpea (Table 3) and soybean (Table 4), and this increased with ascending dosage (F = 4.22 to 352.46, P < 0.05 to 0.001 except hexane extract at 1 d post exposure, for treated cowpea; F = 14.07 to 745.23, P < 0.01 to 0.001 except methanol extract at 1 d exposure, for soybean) and time post exposure (F = 6.12 to 41.55, P < 0.001 to 0.01 for treated cowpea; F = 8.38 to 92.92, P < 0.001 for treated soybean). At the content of 1 g/kg, only the methanol extract caused significant mortality of *C. maculatus* adults in treated cowpea after 1 d exposure period and still more effective than acetone extract after 2 d exposure and than hexane extract after 4 d. At this content the highest adult mortality caused by *O. canum* did not exceed 32.4% ± 3.1% after 7 d post exposure. However, this plant species was more than 2-fold more effective than the commercial standard insecticide NSO at the lower content for cowpea seed treatment. At the content of 5 g/kg, the different extracts of *O. canum* had almost similar toxicity against *C. maculatus* in treated cowpea and the highest adult mortality recorded was 62.9% ± 4.8% within 7 d post exposure. At this content the standard insecticide NSO was so far less effective than *O. canum* to protect cowpea from infestation of *C. maculatus* and caused only 35.8% ± 2.2% adult mortality at the same highest point-time (7 d).

Table 3 Cumulative corrected mortality (means ± *SE* in percentage) of *Callosobruchus maculatus* adults in treated cowpea seeds (variety: Vya Moutourwa) with three extracts from *Ocimum canum*

Product	Dosage (g/kg)	Days after infestation						
		1	2	3	4	5	6	7
Neem seed oil	0	0.0 ± 0.0 b	0.0 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 c
	1	0.0 ± 0.0 Ab	2.6 ± 1.5 Aa	4.0 ± 2.5 Aab	5.4 ± 2.1Aab	8.3 ± 2.8 Aa	8.5 ± 2.8 Ab	9.0 ± 1.8 Ab
	5	5.1 ± 2.0 Ca	7.8 ± 3.4 Ca	10.8 ± 2.3 BCa	13.7 ± 3.5 BCa	19.3 ± 5.0 BCa	25.4 ± 3.7 ABa	35.8 ± 2.2 Aa
Hexane extract	0	0.0 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
	1	2.6 ± 1.5 Da	3.7 ± 2.4 Dab	5.1 ± 2.0 CDb	8.0 ± 1.5 CDb	15.6 ± 2.9 BCb	25.5 ± 3.3 ABb	32.4 ± 3.1 Ab
	5	8.8 ± 3.7 Ea	12.7 ± 4.4 DEa	23.2 ± 4.1 CDEa	29.4 ± 2.8 BCDa	33.8 ± 2.3 BCa	46.3 ± 4.3 ABa	54.2 ± 3.9 Aa
Acetone extract	0	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
	1	1.2 ± 1.2 Cb	3.9 ± 1.3 Cb	8.0 ± 2.7 BCb	9.5 ± 3.3 ABCb	9.7 ± 3.5 ABCb	19.5 ± 5.2 ABb	22.9 ± 3.1 Ab
	5	17.8 ± 3.5 Ca	19.5 ± 3.3 Ca	25.7 ± 2.8 Ca	30.2 ± 1.8 BCa	39.5 ± 2.3 Ba	49.3 ± 1.8 ABa	62.9 ± 4.8 Aa
Methanol extract	0	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
	1	6.2 ± 2.4 Ca	10.4 ± 0.3 Cb	10.5 ± 0.2 Cb	12.2 ± 1.5 BCb	13.6 ± 1.3 BCb	19.4 ± 2.6 ABb	25.4 ± 2.7 Ab
	5	10.1 ± 2.8 CDa	14.4 ± 1.5 Ca	15.8 ± 2.2 Ca	21.7 ± 0.5 Ca	27.1 ± 2.9 Ca	41.6 ± 2.7 Ba	60.3 ± 4.5 Aa

Means within the same column and line followed by different small and capital letters, respectively differ significantly at the 5% level by Tukey's test.

Table 4 Cumulative corrected mortality (means ± *SE* in percentage) of *Callosobruchus maculatus* adults in treated soybean (variety: Kodek) with three extracts from *Ocimum canum*

Product	Dosage (g/kg)	Days after infestation						
		1	2	3	4	5	6	7
Neem seed oil	0	0.0 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
	1	0.0 ± 0.0 Da	1.2 ± 1.2 Db	2.6 ± 1.5 CDb	4.0 ± 1.3 BCDb	7.2 ± 1.5 BCb	8.6 ± 1.7 Bb	17.4 ± 0.5 Ab
	5	2.6 ± 1.5 Ca	6.5 ± 1.3 Ca	15.8 ± 0.3 Ba	15.7 ± 2.0 Ba	15.7 ± 1.3 Ba	41.4 ± 1.1 Aa	48.0 ± 3.3 Aa
Hexane extract	0	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
	1	6.2 ± 2.4 Ba	11.5 ± 1.2 Bb	13.0 ± 1.6 Bb	25.1 ± 2.9 Aa	27.7 ± 1.9 Ab	28.1 ± 1.7 Ab	31.1 ± 1.6 Ab
	5	10.1 ± 0.1 Ea	18.0 ± 1.7 Da	19.6 ± 1.6 Da	31.6 ± 1.9 Ca	36.9 ± 0.8 BCa	40.0 ± 1.3 Ba	52.0 ± 2.0 Aa
Acetone extract	0	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 c
	1	2.6 ± 1.5 Db	5.1 ± 2.0 Db	10.5 ± 2.00 CDa	14.5 ± 1.4 BCa	18.3 ± 1.3 ABCa	18.6 ± 1.6 ABb	26.2 ± 2.1 Aa
	5	10.2 ± 2.0 Ca	14.2 ± 2.2 BCa	15.1 ± 3.3 BCa	22.3 ± 4.4 ABCa	26.6 ± 4.0 ABa	30.0 ± 1.2 Aa	35.3 ± 3.0 Aa
Methanol extract	0	0.0 ± 0.0 a	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 b
	1	3.7 ± 2.4 Da	7.6 ± 1.4 Db	9.0 ± 1.2 CDb	18.0 ± 3.0 BCa	20.5 ± 2.5 Ba	23.2 ± 2.2 Bb	42.5 ± 1.7 Aa
	5	3.8 ± 1.3 Da	12.7 ± 1.6 Ca	18.1 ± 1.1 BCa	22.0 ± 2.2 BCa	26.0 ± 2.5 Ba	38.3 ± 2.5 Aa	46.5 ± 3.8 Aa

Means within the same column and line followed by different small and capital letters, respectively differ significantly at the 5% level by Tukey's test.

O. canum was less effective against *C. maculatus* adults in treated soybean than in treated cowpea (Tables 3 and 4). Overall, soybean coated with hexane extract was the most toxic to *C. maculatus* adults than the seeds coated with acetone and methanol extracts. However, methanol extract had similar toxicity with hexane extract after 6 d post exposure. *O. canum* extracts were more effective than the standard insecticide NSO at the content of 1 g/kg and had similar toxicity at 5 g/kg soybean grains (Table 4).

3.3 Insecticidal efficacy of extracts of *O. canum* to immature stages of *C. maculatus*

Hexane, acetone and methanol extracts of *O. canum* were toxic to each immature stage of *C. maculatus*, however the sensitivity of different stages to different treatments did not follow the same trend (Fig. 1). The eggs, the 1st instar and 2nd instar larvae were the most sensitive while the 3rd instar larvae were the least sensitive. Overall, the standard insecticide NSO was the most effective compared to other products. However, it was less effective than hexane and acetone extracts against the 2nd instar larvae. Acetone extract was the most effective against the 4th instar larvae and pupae.

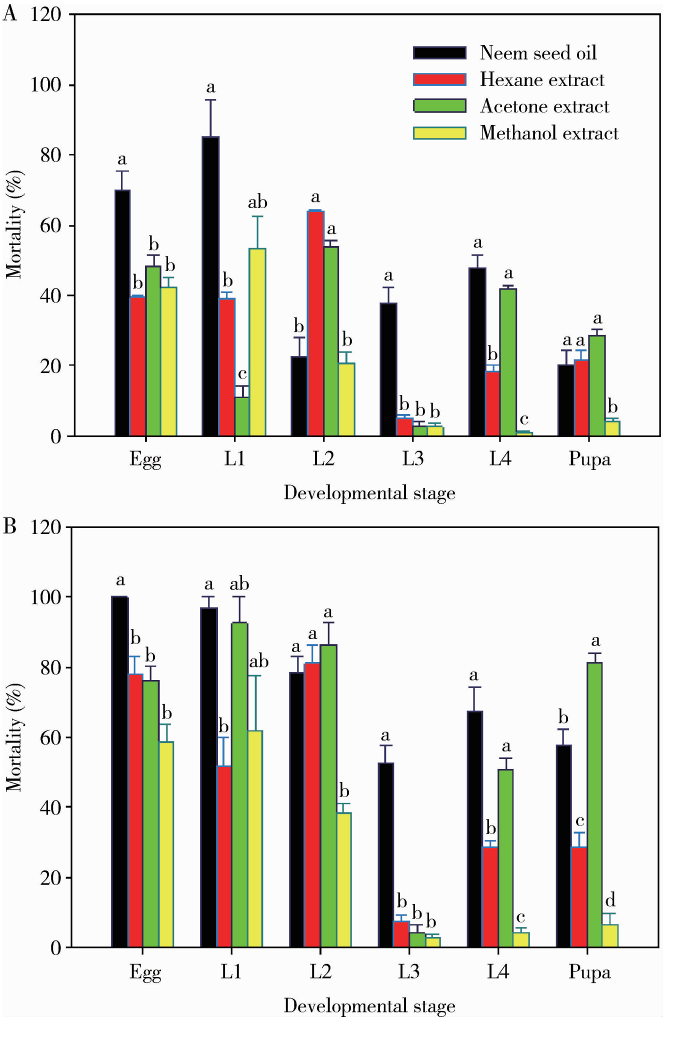

Fig. 1 Corrected mortality (Means ± *SE*) of immature stages of *Callosobruchus maculatus* feeding on treated cowpea with extracts from *Ocimum canum*

A: Mortality recorded at 1 g/kg; B: Mortality recorded at 5 g/kg. L1: 1st instar larva; L2: 2nd instar larva; L3: 3rd instar larva; L4: 4th instar larva. Bars with different letters at the top are significantly different at the 5 % level by Tukey's test.

3.4 Grain damage and weight loss reduction potential of *O. canum* extracts against infestation of *C. maculatus*

Untreated cowpea suffered from heavy damage with more than two holes per seed (severity of damage higher than two) caused by *C. maculatus* and consequently, the percentage of undamaged grains was very low and more than 32.8% of food was lost (Table 5). Untreated soybean was less damaged and more than 71% of seeds were not attacked by *C. maculatus*. Weight loss did not exceed 2.9%. The standard botanical insecticide NSO was more effective to protect cowpea from insect attack than extracts from *O. canum* and provided higher than 91.9% cowpea protection against *C. maculatus* attacks. However, all treatments reduced significantly grain weight loss compared to the untreated control both in cowpea and soybean. The severity of damage (number of holes per seed) was also significantly reduced, except in the case of treatment with hexane extract. Overall, the level of cowpea protection from insect attacks increased with ascending dosage and the associated weight loss decreased. At the content of 5 g/kg both methanol and acetone extracts reduced cowpea grain damage and weight loss by more than 3- and 2-fold, respectively. Treated soybean was free from damage and no weight loss was recorded.

Table 5 Undamaged grains, severity of damage and weight loss of cowpea and soybean treated with extracts from *Ocimum canum* against *Callosobruchus maculatus* attack

Product	Dosage (g/kg)	Cowpea			Soybean		
		PUDG	SD	PWL	PUDG	SD	PWL
NSO	0	13.6 ±4.2 b	2.6 ±0.2 a	32.8 ±1.3 a	71.9 ±2.4 b	1.1 ±0.0 a	2.7 ±0.2 a
	1	91.9 ±7.4 a	1.0 ±0.4 b	1.5 ±1.4 b	99.6 ±0.4 a	0.2 ±0.2 b	0.1 ±0.1 b
	5	99.8 ±0.1 a	0.5 ±0.3 b	0.0 ±0.0 b	100.0 ±0.0 a	0.0 ±0.0 b	0.0 ±0.0 b
OCH	0	9.9 ±2.7 b	2.3 ±0.2 a	32.9 ±1.0 a	74.1 ±4.6 b	1.2 ±0.1 a	2.9 ±0.8 a
	1	24.3 ±1.2 a	1.8 ±0.1 a	23.3 ±1.6 b	97.0 ±1.8 a	0.5 ±0.3 b	0.2 ±0.1 b
	5	32.8 ±2.9 a	1.1 ±0.1 a	21.7 ±1.2 b	100.0 ±0.0 a	0.0 ±0.0 b	0.0 ±0.0 b
OCA	0	13.6 ±4.2 b	2.6 ±0.2 a	32.8 ±1.3 a	71.9 ±2.4 b	1.1 ±0.0 a	2.7 ±0.2 a
	1	22.4 ±0.8 b	2.0 ±0.2 b	26.0 ±1.4 b	96.5 ±3.5 a	0.3 ±0.3 b	0.1 ±0.1 b
	5	46.4 ±3.6 a	1.8 ±0.1 b	16.0 ±1.0 c	100.0 ±0.0 a	0.0 ±0.0 b	0.0 ±0.0 b
OCM	0	9.4 ±4.2 b	2.7 ±0.2 a	33.2 ±1.8 a	70.4 ±2.3 c	1.1 ±0.1 a	2.6 ±0.5 a
	1	11.4 ±0.4 b	2.0 ±0.2 ab	27.2 ±1.7 b	98.5 ±0.3 b	1.0 ±0.0 a	0.2 ±0.1 b
	5	39.1 ±3.6 a	1.6 ±0.0 b	12.9 ±0.9 c	100.0 ±0.0 a	0.0 ±0.0 b	0.0 ±0.0 b

PUDG: Percentage of undamaged grains; SD: Severity of damage; PWL: Percentage weight loss; NSO: Neem seed oil; OCH, OCA and OCM are hexane, acetone and methanol extracts of *O. canum*, respectively. Means within the same column followed by different letters differ significantly at the 5% level by Tukey's test.

3.5 Repellent activities of *O. canum* extracts

Extracts of *O. canum* were repellent to the cowpea weevils and there was considerable variation in the repellent action of the extracts (Table 6). The degree of repellency increased with ascending dosages. Hexane extract was the most repellent than the other products including the commercial repellent NSO which was less effective. At 1 g/kg content, hexane extract was fairly repellent to *C. maculatus* in both treated cowpea and soybean; while acetone extract was averagely repellent and moderately repellent respectively in treated cowpea and soybean, methanol extract was moderately repellent in treated cowpea and averagely repellent in treated soybean. At 5 g/kg, hexane and acetone extracts were respectively very repellent and averagely repellent in treated cowpea and were fairly repellent in treated soybean, while methanol extract was averagely repellent in both two treated commodities.

Table 6 Mean percent repellency (PR) values for three extracts from *Ocimum canum* in relationship with grain type against *Callosobruchus maculatus*

Product	Dosage (g/kg)	Mean percent repellency			
		Cowpea	Class	Soybean	Class
Neem seed oil	1	−25.9 ±8.3	0	−28.3 ±5.6	0
	5	21.1 ±3.4	Ⅱ	34.9 ±8.2	Ⅱ
Hexane extract	1	63.8 ±8.0	Ⅳ	65.2 ±10.2	Ⅳ
	5	82.2 ±6.3	Ⅴ	71.9 ±3.0	Ⅳ
Acetone extract	1	47.5 ±10.3	Ⅲ	29.0 ±3.4	Ⅱ
	5	57.6 ±6.8	Ⅲ	68.9 ±10.1	Ⅳ
Methanol extract	1	37.1 ±6.0	Ⅱ	45.8 ±6.0	Ⅲ
	5	54.1 ±2.7	Ⅲ	50.2 ±7.2	Ⅲ

0 = Attractive, Ⅱ = Moderately repellent, Ⅲ = Averagely repellent, Ⅳ = Fairly repellent, Ⅴ = Very repellent.

4 DISCUSSION AND CONCLUSION

The worldwide trend to minimize the use of toxic substances on crops has led to several studies focusing on alternative postharvest technologies for crop protection during storage. Products from botanicals offer the cheapest sustainable alternative method (Mukanya *et al*., 2010) and their biological activities were widely reported with respect to their repellence, feeding and oviposition deterrence, growth inhibition, sterilant, toxicity, *etc*. (Adler *et al*., 2000; Isman and Akhtar, 2007). *O. canum* tested for some of these parameters in this study with the aim to fight against *C. maculatus* in stored pulses proved to be a good insecticide that may be exploited by farmers in Cameroon.

The insecticidal properties of the *O. canum* extracts against *C. maculatus* tested in this study might be connected with the phytochemical constituents such as total polyphenolic compounds, tannins, saponins, alkaloids, flavonoids and steroids which were reported to be toxic to insects (Singh and Pandy, 1982). To our knowledge, this is the second report for *C. maculatus* control by a bioactive extract from this plant; however it was tested successfully to kill *Cyperrus rotundus*, *Zabrotes subfasciatus*, *Acanthoscelides obtectus*, *Rhyzopertha dominica*, *Sitophilus oryzae* and *Tribolium castaneum* by previous researchers (Singh and Pandy, 1982; Weaver *et al*., 1991; Shimpi and Bendre, 2007) and the major active components were either linalool or camphor, two terpen secondary metabolites isolated in essential oil of the plant. The three extracts, hexane, acetone and methanol from *O. canum* might have the same toxicity and this could be attributed to their similarity in chemical composition, especially for polar acetone and methanol extracts. Hexane extract, the apolar extract which was poor in phytochemical groups, had the same toxicity with the other two extracts and this could be explained either by the higher proportion of steroids or by its physical property. Hexane extract had gluey aspect which acetone and methanol extracts lacked. Because of this property, insect might die by suffocation in treated grains with hexane extract.

Our investigations on the effect of extracts from *O. canum* on the hidden eggs and different immature stages of *C. maculatus* showed that all products greatly reduced egg hatchability and the percentage of larvae and pupae survivorship in a dose-dependent manner. This significant reduction confirms the possible presence of ovicidal and larvicidal constituents in the plant extracts. Rajasekaran and Kumaraswami (1985) reported that grains coated with plant extracts completely inhibited the development of insect by reducing the survival rates of larvae and pupae inside grain kernel, as well as adult emergence as reported by Koul *et al.* (2008). In fact, crude extract was reported to retard development and caused mortality of larvae, cuticle melanisation resulting in the disruption of the endocrine system controlling the growth and moulting of eggs and larvae, and high mortality in adults (Jamil *et al*., 1984).

The internal feeder insects like *C. maculatus* damages grains by developing inside kernels, feeding on the inner endosperm, and producing holes in the kernel through which adults exit. By consumption of grain endosperm, grain weight loss might result. Antinutritional factors exerting a deterrent activity on insects and disturbing their feeding (Boeke *et al*., 2004), may explain the significant reduction of grain damage recorded in this study with treated grains. Antifeedent property of tested products is confirmed by weight loss reduction of treated grains. NSO considerably reduced damage and weight loss of treated grains compared to extracts of *O. canum* and this may be attributed to its higher content in deterrent secondary metabolites than other products. This result finds support from other studies which showed feeding deterrence and insect growth inhibition property of the major compound limonoid of the Indian neem tree (Adeyemi, 2011; Adeyemi and Mohammed, 2014). The overall decrease in the number of hole per grain in treated grains might increase the market value and germination ability of seeds.

Further to their toxicity and deterrent properties, the organic solvent extracts from *O. canum* exhibited greater repellent action on *C. maculatus* irrespective of commodity than did NSO, indicating that *O. canum*, especially hexane extract, contains other components that increase its repellency. Shimpi and Bendre (2007) found ethanolic extract of plant fairly repellent (repellency class IV) against *T. castaneum*, *R. dominica* and *C. chinensis* and this support the results of our finding. The lower repellency (repellency class II) against *S. oryzea* of essential oil from the plant harvested in Cameroon and tested by Ngassoum *et al.* (2007) may be partially attributed to the physiological differences between insects. Repellents from plant origins are considered safe in pest control; minimize pesticide residues; ensure safety of the people, food, and environment (Taluckder *et al*., 2004; Taluckder, 2006; Maia and Moore, 2011). Therefore, products from *O. canum* may be strongly recommended to be used as grain protectants during storage.

The following points are summarized:

1. In sub-Saharan African countries like Cameroon, food storage is a matter of survival.

2. During storage cowpea and soybean are respectively heavily and moderately damaged by *C. maculatus* in northern Cameroon. The different immature stages of the insect are permanently present inside the grains during storage and control measures should target each developmental stage.

3. Extracts of *O. canum* are more potent than NSO against *C. maculatus* adults as insecticide and repellent on treated cowpea and soybean.

4. Extracts from *O. canum* as well as NSO are

effective to control different immature stages of *C. maculatus*, especially eggs, the 1st instar and 2nd instar larvae.

5. NSO is more effective than extracts from *O. canum* as grain protectant, however, these extracts reduced grain damage and weight loss by more than 3- and 2-fold over four months storage periods, respectively.

6. Due to their efficacy and availability, hexane, acetone and methanol extracts of *O. canum* are strongly recommended to be used in pest management strategies in Cameroon by rural farmers.

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樟脑罗勒生物活性提取物对储藏豇豆和大豆中四纹豆象的影响

Daniel KOSINI[1,*], Elias Nchiwan NUKENINE[2], Clément SAIDOU[3], Alembert Tiabou TCHINDA[4], Gabriel Agbor AGBOR[4]

(1. School of Health Sciences, Catholic University Institute of Saint Jérôme, Douala, Cameroon;
2. Department of Biological Sciences, Faculty of Science, University of Ngaoundere, Cameroon;
3. Department of Chemical Engineering and Environment, UIT of the University of Ngaoundere, Cameroon;
4. Medical Research Institute and Studies of Medicinal Plants (IMPM), Yaoundé, Cameroon)

摘要:【目的】本研究旨在评价樟脑罗勒 *Ocimum canum* Sims (唇形科)叶的正己烷、丙酮和甲醇提取物对四纹豆象 *Callosobruchus maculatus* (F.)的杀虫、保护和驱避潜力。【方法】用剂量为 1 和 5 g/kg豆粒的樟脑罗勒提取物包被处理豇豆和大豆豆粒,并以相同剂量的市售苦楝籽油进行包被处理作为参照。7 d 内每隔 1 d 检查处理豆粒中四纹豆象成虫的死亡率。在四纹豆象的各个未成熟期,通过处理新产卵和幼虫,进行杀卵毒性和杀幼虫毒性测试。贮存 4 个月后评价豆粒受损情况和重量损失。应用线型嗅觉仪,通过选择试验测定驱避效果。【结果】提取物对四纹豆象成虫的毒性与市售苦楝籽油相同,在处理的豇豆中,提取物比市售的苦楝籽油毒性更强。5 g/kg 豆粒提取物这一剂量处理 7 d 内,正己烷、丙酮和甲醇提取物分别引起 54.2% ±3.9%, 62.9% ±4.8%和 60.3% ±4.5%的成虫死亡。此外,与测试的标准杀虫剂相比,提取物激发这两种豆粒产生更强的驱避效果。这一产物对卵的毒性最强,对 1 和 2 龄幼虫的毒性比对其他发育阶段豆象的毒性强。正己烷提取物对 2 龄幼虫的毒性最强(致死率为 81.0% ±5.3%),而丙酮提取物和甲醇提取物对 1 龄幼虫的毒性最强,致死率分别为 92.5% ±7.5%和 62.0% ±15.7%。丙酮提取物对 1 和 2 龄幼虫的毒性与测试的标准杀虫剂相似,对蛹的毒性强于测试的标准杀虫剂。贮存 4 个月后,豇豆受损和重量损失分别减轻 3 倍和 2 倍多。【结论】本研究结果表明,樟脑罗勒提取物对四纹豆象具有潜在的驱避和杀虫活性,由于容易获得,因此强烈建议将该植物用于喀麦隆的害虫治理策略中。

关键词: 四纹豆象; 樟脑罗勒; 豆类; 毒性; 驱避性

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