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Research

Fractionated Extracts From *Gnidia kraussiana* (Malvales: Thymeleaceae) as Bioactive Phytochemicals for Effective Management of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) in Stored *Vigna unguiculata* (Fabales: Fabaceae) Seeds

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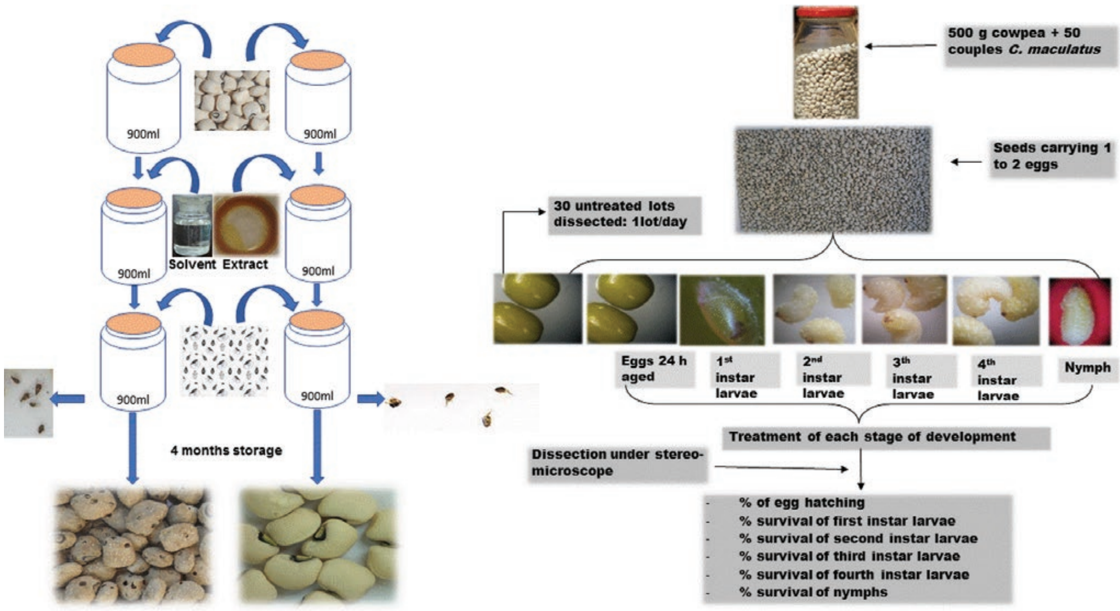
Abstract

One of the most important global problems is protecting food from insect pests. The negative effects of synthetic insecticides on human health led to a resurgence of interest in botanical insecticides due to their minimal ecological side effects. Therefore, the insecticidal potential of hexane, acetone, and methanol extracts of *Gnidia kraussiana* Meisn roots at 1 and 5g/kg, and neem seed oil (NSO), used as standard insecticide, were evaluated. Ovicidal and larvicidal toxicity was tested by treating freshly laid eggs and larvae at different immature stages of *Callosobruchus maculatus* (F.). Cowpea (*Vigna unguiculata*) (L.) Walp seed damage and weight loss were assessed after a storage period of 4 mo. Repellency effects were detected in choice test using a linear olfactometer. All the fractions were toxic to *C. maculatus*; however, their bioactivities were inversely correlated with products polarity. Extracts proved to be more toxic than the commercial NSO. The acetone extract was more effective against immature stages of *C. maculatus* than the methanol extract; eggs, first-, and second-instar larvae being the more susceptible. No cowpea seed damage and weight loss were recorded from the seeds treated with hexane and acetone extracts at the dosage of 5 g/kg, after 4 mo of storage. Extracts evoked stronger repellency effects compared with the tested standard insecticide. According to the above, hexane and acetone extracts are good candidates for incorporation in integrated pest management programs for the control of *C. maculatus* in stored cowpea seeds.

Graphical Abstract



Key words: cowpea, cowpea weevils, *Gnidia kraussiana*, toxicity, repellency

One person in every four, in sub-Saharan Africa, lack adequate food for a healthy and active life (Bremner 2012). One of the prerequisites for achieving food security and reducing undernourishment is the consistent availability of sufficient quantities of appropriate foods. Consistent food availability is determined by food production, food trade, and food preservation.

Food preservation is an increasing challenge in Africa due to postharvest losses causing a significant threat to food security and household incomes. In the developing countries, 15–25% postharvest loss occurs during storage (Abass et al. 2014); storage loss due to insect pest infestations being a problem of major concern (Hengsdijk and Boer 2017). Cowpea (*Vigna unguiculata*) (L.) Walp seeds known in developing countries as the ‘meat of poor people’ (Kosini et al. 2020) are heavily damaged by insect pests during storage (Deshpande et al. 2011). Seed loss is generally due to the different immature stages of *Callosobruchus maculatus* (F.), which develop inside the seeds (Kosini et al. 2017). After insect emergence, seeds are left hollow with an unpleasant odor, rendering them objectionable for consumption.

Hence, the management of *C. maculatus* targeting its different developmental stages is a prerequisite for constant availability of cowpea seeds. The first four decades of the 20th century saw significant progress in the synthesis of insecticides, which were used unsuccessfully due to acute and chronic poisoning of applicators, and even consumers; and the evolution of resistance to insecticides in pest populations (Perry et al. 1998). As the negative side effects of synthetic insecticides continue to increase in prevalence and severity, there is a renewed interest in natural insecticides as an eco-chemical approach in insect pest control (Isman 2004).

Botanical insecticides are found to be an effective alternative to conventional insecticides because they are known by farmers and more selective to insect pests and less aggressive with the natural enemies; some are plants with medicinal applications and not phytotoxic; there is rapid degradation of their active products; resistance to these compounds is not developed as quickly as with synthetic insecticides (El-Wakeil 2013). *Gnidia kraussiana* Meisn is locally used in far north region of Cameroon, in storage structures, by farmers to protect their stored seeds from insect infestation. The insecticidal properties of that plant against *C. maculatus* infesting Bambara groundnut were reported for the first time in the previous study (Kosini and Nukenine 2017). However, there are several factors that can affect the efficacy of insecticidal product. One of the crucial factors is seed species (Athanassiou et al. 2008). Thus, there is a need to test this novel botanical insecticide against insect pests infesting various seed species, for recommendations to be followed by farmers and store keepers.

The present study was undertaken to assess the insecticidal potential of hexane, acetone and methanol extracts from the root powder of *G. kraussiana* against the different developmental stages of *C. maculatus* in cowpea seeds.

Materials and Methods

Experimental Conditions

Experiments were carried out under ambient conditions in the Laboratory of Applied Zoology of the Department of Biological Sciences, University of Ngaoundéré. 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.

Table 1. Temperature and relative humidity of laboratory during the assessment of bioactivities of *Gnidia kraussiana* extracts against *Callosobruchus maculatus*

Bioassay	TempERATURE (°C)	Relative humidity (%)
Toxicity		
Egg	23.1 ± 0.9 (21.0–25.5)	79.2 ± 1.3 (74.5–81.5)
first instar larva	23.2 ± 0.9 (21.0–21.5)	79.9 ± 1.3 (74.5–82.5)
second instar larva	23.2 ± 0.9 (21.0–25.5)	80.0 ± 1.2 (74.5–82.5)
third instar larva	23.2 ± 1.0 (21.0–25.5)	79.9 ± 1.2 (74.5–82.5)
fourth 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)
Adult	25.4 ± 1.8 (22.0–30.5)	72.8 ± 4.1 (60.5–79.0)
Damage and weight loss	24.0 ± 1.5 (21.0–30.5)	77.9 ± 3.1 (60.5–87.5)
Repellency test	24.9 ± 1.7 (23.0–28.0)	75.6 ± 1.7 (72.0–78.0)

Data in parentheses are the ranges.

Tested Seeds

Cowpea seeds (variety Vya Moutourwa) were collected at harvest from farmers at Kossehone in Mayo-Tsanaga division, far north region of Cameroon. The seeds were checked individually and damaged seeds were excluded. Cleaned seeds were placed in a plastic bag and kept in a freezer at −4°C during 3 wk to eliminate eggs, larvae, and pupae of *C. maculatus* and parasitoids. 'The seeds were then kept under experimental conditions for at least two weeks before use' as described in the previous study (Kosini et al. 2017).

Insect Rearing

Callosobruchus maculatus were collected in Mokolo market, Mayo-Tsanaga division in far north Cameroon. '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', as described in the previous study (Kosini et al. 2020). They were reared for two generations and freshly emerging adults were selected from the culture and used for the experiment.

Collection and Processing of Plant Material

Roots from *Gnidia kraussiana* were collected in the wild in XI–2013 around Mogode (latitude 10°36.25′ N and longitude 13°34.46′ E, 1,005 m a.s.l). The plant was identified by the Cameroon National Herbarium, where a voucher specimen (Serial number: 38259/HNC) is deposited. Collected roots were dried in a room under ambient conditions for 7 d and then crushed in a mortar until the powder passed through a 0.4-mm mesh sieve as described in the previous study (Kosini and Nukenine 2017). The powdered material was kept at 4°C in a deep-freezer until needed for extraction. With the aim to fractionate the different compounds of botanicals according to their polarity, hexane, acetone, and methanol extracts were gotten by using the maceration method as described in the previous study (Kosini et al. 2015). Extracts were stored in a refrigerator at 4°C until needed for bioassay.

Azadirachta indica Juss. seed oil processed and extracted as described in the previous study (Kosini et al. 2015) was used as standard insecticide.

Phytochemical Screening of Extracts

Extracts were tested for presence of bioactive compounds by utilizing standard techniques (Adeniyi et al. 2010) for the detection of sterols, saponins, cardiac glycosides, tannins, flavonoids, terpenoids, and alkaloids.

Assessment of the Toxicity of Extracts on *C. maculatus*

The bioassay for insecticidal activity of hexane, acetone, and methanol extracts of *G. kraussiana* against the adult stage of *C. maculatus* was tested in the laboratory by applying extracts at rates of 1 and 5 g of extract/kg of sterilized seeds introduced in a glass jar. Before their application, 'extracts were dissolved in the respective solvent used for plant extraction to get 250mg/ml solutions', as described in the previous study (Kosini and Nukenine 2017). Neem seed oil (NSO) was used at the same rates as standard (check) insecticide while 1 ml of each solvent (hexane, acetone, and methanol) was used as negative control. The content of each jar was shaken to ensure proper mixture and the solvent was allowed to evaporate during 2 h. Twenty unsexed adults of *C. maculatus* (1- to 2-d old) were introduced into the treated and control seeds, and covered with a muslin cloth and perforated metal lid to facilitate proper aeration and prevent entry and exit of insects. 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 four times. The number of dead insects in each container was counted 1- to 7-d postexposure to estimate mortality.

The toxicity of extracts on the development of eggs, larvae, and pupae of *C. maculatus* in seeds was also investigated. Five hundred grams of sterilized cowpea placed in 1-liter glass jars were infested with 50 adults of *C. maculatus* (sex ratio: 1:1) to allow for egg laying. The parent adults were removed after 24 h. As described in the previous study (Kosini et al. 2017), 30 lots each of 30 seeds with one to two eggs selected 24-h postoviposition were weighted and treated in Petri dishes by applying acetone and methanol extracts as well as NSO at the egg stage, first- to fourth-instar larval stage, and at the pupal stage. Two doses (1 and 5 g/kg of seeds) of each product were applied. Another three lots of seeds treated with acetone extract, methanol extract, or without treatment were considered as negative controls. The experimental design for toxicity against immature stages was a 1 insecticidal plant × 2 solvent extracts × 2 concentrations × 6 developmental stages factorial, arranged in a Complete Randomized Design, with each treatment replicated four times. The different developmental stages of larvae (L1, L2, L3, and L4) and pupa, and their respective percentage survival were determined as described in the previous study (Kosini et al. 2017).

Damage and Weight Loss Assessment

The experimental units from the adult toxicity test above were used to assess seed damage and weight loss. After adult mortality recordings, all the insects, dead and alive, were discarded, whereas seeds from each jar were left in their respective jars on laboratory shelves for a total period of 4 mo. At the end of the 4-mo 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 (Kosini and Nukenine 2017).

Repellency Test

The device for area preference test described in the previous study (Kosini and Nukenine 2017) was used to evaluate the repellent action of extracts against *C. maculatus*. The device consisted of a 'linear olfactometer made of 30-cm plastic tube, having 2 cm diameter with a hole at its middle. At each end, a small container was placed'. One container contained seeds treated with plant materials at rates of 1 and 5 g/kg seeds, whereas the other one (control) contained seeds treated with solvent alone (hexane or acetone or methanol). Treatments were air-dried to evaporate the solvent completely and then, 20 insects (≤2 d old) of mixed sex were released at the center of the olfactometer through the hole at its middle. For each trial, five replications were made. The setup was kept in the dark for 2 h, and then, the number of insects present in the control and treated containers were recorded.

Percent repellency (PR) values were determined as follows: PR = 2 x (C50); where C is the percentage of insects choosing the control end treated by hexane, acetone, or methanol as negative control.

When PR > 0, the extract was repellent, and when PR < 0, the extract was attractive. The average values were categorized according to the scale described by Juliana and Su (Juliana and Su 1983; Table 2).

Statistical Analysis

Abbott's formula (Abbott 1925) was used to correct collected data with respect to the control mortality. Data on % cumulative corrected mortality of different developmental stages, % grain damage, % weight loss, and PR were tested for normality and heterogeneity of variance by using Levene's test and then were arcsine-transformed [square root (×/100)]. To identify significant effects of the treatments on the variable measured, the transformed data were subjected to the analysis of variance procedure of the statistical analysis system (SAS Institute 2003). For comparison of means, Tukey (Honest Significant Difference) multiple range test and the parametric Student *t*-test were applied with a significance threshold of 0.05.

Results

Phytochemicals From Three Fractions of *G. kraussiana* Root Extract

From the qualitative findings presented in Table 3, it is observed that only terpenes were present and abundantly in hexane fraction of root extract of *G. kraussiana*. That chemical group was also found but not abundantly in acetone and methanol fractions. Saponins were only tested positive for methanol fraction. Total phenolic compounds, alkaloids, tannins, flavonoids, and cardiac glycosides were detected in acetone and methanol fractions, but not in the same quantity. Alkaloids and flavonoids were more abundant in acetone fraction, whereas tannins and cardiac glycosides were more abundant in methanol fraction.

Toxicity of *G. kraussiana* Extracts on *C. maculatus* Adults

The root of *G. kraussiana* was active to protect cowpea seeds from *C. maculatus* infestation. Overall, among the three tested fractions, hexane extract was the most toxic to weevils, whereas methanol extract was the less effective (F = 8.85–202.89, df = 3,12, P = 0.0001–0.0023; Table 4). Six days after treatment with hexane fraction at 5 g/kg, cowpea seeds were free from insect infestation. There was not another treatment including the standard insecticide able to kill all the insects infesting treated seeds, even at 7-d posttreatment. The efficacy of hexane (Student t-test = 4.98–10.65; P = 0.000–0.004) and methanol (Student t-test = 5.31–11.64; P = 0.001–0.059) fractions was dose-dependent, whereas that of acetone fraction (Student t-test = 0.46–2.52; P = 0.045–0.660) did not vary significantly with dosage rates. The standard insecticide NSO was so far less effective than the different fractions of *G. kraussiana*.

Toxicity of *G. kraussiana* Extracts on *C. maculatus* Immature Stages

The results of the toxicity tests against immature stages of *C. maculatus* showed that acetone and methanol fractionated extracts of *G. kraussiana* caused significant mortality to the different developmental stages (Table 5). The effectiveness of the tested products including the standard insecticide NSO differed significantly (F = 20.76–115.89; df = 2,6; P = 0.0001–0.0020), except at the content of 5 g/kg against the first-instar larvae (F = 0.53; P = 0.5118). Acetone fraction was more effective than its counterpart, methanol fraction, and had the same efficacy with the standard insecticide NSO on first- and third-instar larvae as well as pupae. Moreover, it was more effective than NSO against the second-instar larvae, especially at the content of 1 g/kg.

The response of the different immature stages to the treatments was not the same (F = 18.42–106.4; df = 2,12; P = 0.0001). Eggs and first-instar larvae were more sensitive to the different treatments including the standard insecticide NSO. The second-instar larvae were also highly sensitive to acetone fraction.

Seeds Damage and Weight Loss

The extracts caused a significant reduction in the damage and weight loss caused by *C. maculatus* to treated cowpea seeds compared with the control (Table 6) and the action was dose-dependent (F = 27.80–315.24; df = 2,9; P = 0.0001). Four months after storage, 86–91% of untreated seeds were damaged, accounting for 32.8–32.9% of seed weight loss. Thus, the untreated seeds were severely damaged (2.3 ± 0.2–2.7 ± 0.2 holes per seed). Hexane and acetone extracts of *G. kraussiana* averted completely seed damage and weight loss as did the standard check NSO, at their content of 5 g/kg. Significant reduction was also recorded at their content of 1 g/kg. Methanol extract was the least effective product. However, a significant reduction

Table 2. Repellency scale from the less to the most repellent

Class	Repellence rate (%)	Interpretation
0	>0.01 to <0.1	Non repellent
I	0.1 to 20	Very low 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

Table 3. Qualitative phytochemical analysis

Chemical	Hexane fraction	Acetone fraction	Methanol fraction
Total phenolic compounds	–	+++	+++
Alkaloids	–	++	+
Saponins	–	-	+
Tannins	–	+	++
Flavonoids	–	++	+
Steroids	–	–	–
Terpenoids	+++	+	+

–, absent; +, present but not abundant; ++, moderately abundant; +++, abundant.

Table 4. Corrected cumulative mortality (mean ± SE) of *Callosobruchus maculatus* adults in cowpea seeds treated with fractionated root extracts of *Gnidia kraussiana*

DAI	Mortality				$F_{3,12}$
	NSO	Hexane fraction	Acetone fraction	Methanol fraction	
1 g/kg					
1	0.0 ± 0.0	11.4 ± 4.3	6.3 ± 4.7	3.8 ± 1.3	2.30^{ns}
2	2.6 ± 1.5^{B}	17.8 ± 5.1^{A}	20.4 ± 2.7^{A}	7.6 ± 1.4^{AB}	8.85^{**}
3	4.0 ± 2.5^{B}	37.5 ± 5.3^{A}	29.8 ± 1.6^{A}	14.3 ± 3.6^{B}	18.19^{***}
4	5.4 ± 2.2^{C}	54.4 ± 7.4^{A}	37.1 ± 2.9^{AB}	20.1 ± 2.1^{B}	24.60^{***}
5	8.4 ± 2.8^{D}	66.0 ± 5.6^{A}	43.8 ± 4.0^{B}	26.7 ± 1.1^{C}	43.06^{***}
6	8.5 ± 2.8^{D}	73.0 ± 5.3^{A}	55.07 ± 4.7^{B}	29.0 ± 0.4^{C}	55.58^{***}
7	9.0 ± 1.8^{C}	75.8 ± 3.3^{A}	67.2 ± 6.1^{A}	35.3 ± 2.1^{B}	67.06^{***}
5 g/kg					
1	5.1 ± 2.0^{B}	51.0 ± 4.5^{A}	10.1 ± 6.8^{B}	10.1 ± 0.1^{B}	36.71^{***}
2	7.8 ± 3.4^{C}	82.1 ± 3.2^{A}	26.8 ± 3.7^{B}	13.9 ± 2.3^{BC}	112.00^{***}
3	10.8 ± 2.3^{C}	93.4 ± 4.0^{A}	36.4 ± 2.1^{B}	34.2 ± 1.1^{B}	187.20^{***}
4	13.7 ± 3.5^{C}	94.6 ± 3.1^{A}	43.9 ± 2.3^{B}	40.9 ± 1.3^{B}	154.69^{***}
5	19.3 ± 5.0^{C}	98.5 ± 1.5^{A}	50.7 ± 2.4^{B}	46.5 ± 1.3^{B}	124.43^{***}
6	25.4 ± 3.7^{C}	100.0 ± 0.0^{A}	69.1 ± 3.3^{B}	53.6 ± 2.5^{C}	202.89^{***}
7	35.8 ± 2.2^{D}	100.0 ± 0.0^{A}	77.7 ± 3.6^{B}	60.1 ± 2.3^{C}	198.96^{***}

DAI, day after infestation.

The untreated control had the no mortality. Means within the same line followed, respectively, by the same letter(s) did not differ significantly ($P < 0.05$; Tukey's test).

Table 5. Corrected mortality (means ± SE) of immature stages of *Callosobruchus maculatus* feeding in treated cowpea with acetone and methanol extracts of *Gnidia kraussiana*

Stage	Mortality			$F_{2,6}$
	NSO	Acetone fraction	Methanol fraction	
1 g/kg				
Eggs	70.1 ± 5.5^{Aab}	49.8 ± 2.1^{Bb}	30.4 ± 3.2^{Ca}	26.13^{***}
L1	85.3 ± 10.5^{Aa}	81.3 ± 3.6^{Aa}	19.9 ± 2.8^{Ba}	30.91^{***}
L2	22.5 ± 5.4^{Bc}	78.1 ± 0.5^{Aa}	4.3 ± 3.0^{Cb}	115.89^{***}
L3	37.8 ± 4.7^{Ac}	33.9 ± 5.8^{Ac}	3.6 ± 0.8^{Bb}	30.57^{***}
L4	47.9 ± 3.8^{Abc}	4.2 ± 2.0^{Bd}	1.9 ± 1.0^{Bb}	103.05^{***}
Pupa	20.3 ± 4.0^{Ac}	16.2 ± 1.4^{Ad}	1.2 ± 1.2^{Bb}	20.76^{**}
$F_{5,12}$	18.42^{***}	106.64^{***}	29.47^{***}	
5 g/kg				
Eggs	100.0 ± 0.0^{Aa}	96.6 ± 0.2^{Ba}	57.8 ± 4.8^{Cb}	163.15^{***}
L1	97.0 ± 3.0^{Aab}	95.3 ± 4.7^{Aa}	90.9 ± 5.1^{Aa}	0.53^{ns}
L2	78.6 ± 4.8^{Abc}	82.4 ± 2.4^{Aa}	8.7 ± 3.8^{Bc}	118.28^{***}
L3	52.8 ± 4.8^{Ad}	40.6 ± 1.9^{Abc}	16.8 ± 2.1^{Bc}	34.73^{***}
L4	67.4 ± 6.7^{Acd}	26.3 ± 8.2^{Bc}	2.0 ± 0.4^{Cc}	56.86^{***}
Pupa	57.8 ± 4.4^{Acd}	45.0 ± 3.0^{Ab}	7.1 ± 1.9^{Bc}	66.99^{***}
$F_{5,12}$	25.80^{***}	83.92^{***}	106.44^{***}	

L1: first-instar larva; L2: second-instar larva; L3: third-instar larva; L4: fourth-instar larva.

Means within the column and the line followed respectively by the same small and capital letter(s) did not differ significantly ($P < 0.05$; Tukey test).

ns: $P > 0.05$; $^{**}P < 0.001$; $^{***}P < 0.001$.

of seed damage and seed weight loss was recorded at its content of 5 g/kg.

Repellency

Gnidia kraussiana was repellent to *C. maculatus* and fractions showed different level of repellency (Table 7). Hexane fraction was most repellent, whereas methanol fraction was least repellent. The level of repellency of hexane and acetone fractions was dose-dependent, whereas that of methanol fraction was the same at the both tested doses (class II). At the content of 1 g/kg, the three fractionated extracts were more active to repel cowpea weevils than the standard check NSO which was attractive. At the content of 5 g/kg, the less effective extract, i.e., methanol fraction had the same efficacy with NSO.

Discussion

Discovery and development of novel ecologically safe insecticides from botanicals continues to expand. *Gnidia kraussiana*, recently reported by Kosini and Nukenine (2017) to have insecticidal

Table 6. Seeds damaged and seed weight loss at four months after storage of treated cowpea with fractionated extracts of *Gnidia kraussiana*

Product (g/kg)	Seeds damaged (%)	Seed weight loss (%)
NSO		
0	86.4 ± 4.2[a]	32.8 ± 1.3[a]
1	8.1 ± 7.4[b]	1.5 ± 1.4[b]
5	0.2 ± 0.1[b]	0.0 ± 0.0[b]
$F_{2,9}$	93.04***	292.45***
Hexane fraction		
0	91.0 ± 2.7[a]	32.9 ± 1.0[a]
1	39.9 ± 6.8[b]	9.0 ± 1.6[b]
5	0.0 ± 0[c]	0.0 ± 0.0[c]
$F_{2,9}$	115.22***	238.04***
Acetone fraction		
0	86.4 ± 4.2[a]	32.8 ± 1.3[a]
1	49.7 ± 5.0[b]	9.1 ± 1.0[b]
5	0.0 ± 0.0[c]	0.0 ± 0.0[c]
$F_{2,9}$	132.36***	315.24***
Methanol fraction		
0	90.6 ± 4.2[a]	33.2 ± 1.8[a]
1	78.2 ± 1.8[a]	26.9 ± 1.5[a]
5	19.9 ± 11.6[b]	4.3 ± 2.8[b]
$F_{2,9}$	27.80***	51.04***

Means within the same column followed by the same superscript letter(s) did not differ significantly ($P < 0.05$; Tukey test).

***$P < 0.001$.

Table 7. Mean percent repellency values of fractionated extracts of *Gnidia kraussiana* in treated cowpea against *Callosobruchus maculates*

Dose (g/kg)	Repellence (%)	Class	Interpretation
Neem seed oil			
1	-25.93 ± 8.32	–	Attractive
5	21.14 ± 3.45	II	Moderately repellent
Hexane fraction			
1	54.89 ± 11.62	III	Averagely repellent
5	77.15 ± 9.44	IV	Fairly repellent
Acetone fraction			
1	39.78 ± 4.88	II	Moderately repellent
5	49.75 ± 7.06	III	Averagely repellent
Methanol fraction			
1	31.15 ± 5.50	II	Moderately repellent
5	34.64 ± 5.42	II	Moderately repellent

properties, was tested, in the present study, effective as toxicant and repellent to protect stored cowpea against *C. maculatus* infestation. In fact, unlike conventional pesticides that are based on a single active ingredient, botanical insecticides comprise an array of chemical compounds, with a wide range of activities, which act concertedly to kill and repel insect pests, and to prevent seed damage and weight loss (Salunke et al 2005; Kosini et al. 2015, 2017; Saira et al 2017).

In the present study, chemical groups extracted from *G. kraussiana* by using non polar (hexane), intermediate (acetone), and polar (methanol) organic solvents showed, respectively, different levels of toxicity in treated cowpea against *C. maculatus*. Insect mortality declined with increasing polarity of the solvent as reported by Overgaard et al. (2014). The active compounds of hexane fraction were terpenes, which proved to be more toxic than the mixture of other extracted chemicals as reported in the previous study (Kosini and Nukenine 2017). The insecticidal property of two diterpenes, excoecariatoxin and wikstrotoxin D, extracted from the methanol extract of *G. kraussiana* was reported (Bala et al. 1999). Contact toxicity of terpenes against stored grain pests had been proven also by other searchers (Herrera et al. 2015, Kanda et al. 2017). Some of the reasons for the poor market penetration of botanical insecticides in developing countries are their relatively slow action, lack of persistence, and inconsistent availability (Isman 2008). However, *G. kraussiana* is widely available in Cameroon, its persistence was reported to be at least 2 mo (Kosini and Nukenine 2017) and was very toxic to insect pests compared with various botanical extracts tested by other researchers including our previous studies (Bisseleua et al. 2008, Kosini et al. 2017, Langsi et al. 2017, Fotso et al. 2019). Thus, hexane fraction of *G. kraussiana* is an excellent candidate for the development of eco-friendly insecticides to protect cowpea against *C. maculatus* infestation. Acetone fraction may have also an important role to protect cowpea against *C. maculatus* compared with the well-known botanical insecticide NSO. Undoubtedly, the major toxic constituents of acetone fraction were alkaloids and flavonoids. This is consistent with the findings of other searchers who reported insecticidal activity of flavonoids against *Callosobruchus chinensis* (Upasani et al. 2003) and that of alkaloids against *Spodoptera litura* (Ge et al. 2015). The low toxicity of methanol fraction could be that the higher extractive yield of methanol gives more inactive material, thus diluting the active components. This is confirmed by the results recorded from our preliminary investigation, which revealed that the powder of *G. kraussiana* roots might present very low biological activities against *C. maculatus*. However, in contrast to our finding, methanol extract of roots of *G. kraussiana* showed potent insecticidal activity against *Aphis gossypii* and *Drosophila melanogaster* (Bala et al. 1999). This difference in susceptibility was not surprising because the insects are from different families.

There is an emphasis in the botanical insecticide literature on adulticidal effects against *C. maculatus*, and dearth reports on larvicidal toxicity. Control measures targeting the different immature stages of the pest should also receive much attention since larvae are permanently present during storage and are responsible for seed damage and weight loss. To the best of our knowledge, this is the first study investigating the ovicidal and larvicidal effects of *G. kraussiana*. The reduction of egg hatchability and the percentage of larvae and pupae survivorship in treated cowpea showed that *G. kraussiana* contained ovicidal and larvicidal components. The higher toxicity of acetone fraction highlights its higher concentration in active components than methanol fraction. This finding confirms the previous report (Kosini et al. 2017), where acetone fraction of *Ocimum canum* was more active than methanol fraction against immature stages of *C. maculatus*. The effectiveness of that fraction against the first to third instars larvae and pupae was similar to that of the standard larvicide NSO. The major active components were probably flavonoids and especially alkaloids mainly present in acetone fraction. Thus, acetone solvent may be more appropriated than methanol for chemical extraction for larvicide development. The larvicidal activities of these chemical groups extracted from different plant families were reported (de Souza Wiuillda et al. 2019). Different flavonoids are found to alter molting in insect, causing death; they either act as anti-estrogens or inhibit cytochrome P450 isozyme expression and activity (Salunke et al. 2005). However, our further research will focus on the determination of the mode of action of these metabolites extracted from *G. kraussiana*.

Significant reduction of seed damage and weight loss was recorded in this study as consequence of reduction of egg hatchability and larvae survivorship in treated seeds. In fact, losses are mainly due to the consumption of cowpea seed cotyledons by larvae, resulting in increased seed perforation and reduced seed weight (Desande et al. 2011). The antifeedant activity of tested products might be also partially responsible for the reduction of seed damage and weight loss, because the antifeedant activity of phenolics, alkaloids, and terpenoids, present in extracts of *G. kraussiana,* was reported (Koul 2008). Hexane and acetone fractions of *G. kraussiana* may be used to protect cowpea against *C. maculatus* infestation within 4 mo at least, without any damage and weight loss. Damage reduction and weight loss might increase the nutritional and market values, and the germination ability of seeds.

Terpenoids secondary metabolites of *G. kraussiana* exhibited high repellency against *C. maculatus* in treated cowpea. The repellent activities of acetone and methanol fractions might be due to the presence of active components like alkaloids in addition to terpenoids. The repellent activities of these metabolites were reported by other searchers (Ulubelen et al. 2001). Compared with our previous finding (Kosini and Nukenine 2017), the repellent potential of these chemicals extracted from *G. kraussiana* might vary according to the treated seed species. In fact, smell from allelochemicals released by treated seeds might interact synergistically or antagonistically with repellent botanical insecticide to repel insect pests. The repellency is an important feature of botanical insecticides, because repellents are substances that act locally or at a distance, deterring a pest from egg laying, and prevent it to locate and/or recognize its host (Deletre et al. 2016).

An ideal pesticide should be highly toxic to target insect, but safe to humans and domestic animals. Botanicals with medicinal uses are generally less toxic to mammals. *Gnidia kraussiana* showed a wide range of potentially useful biological activities (Bhandurge et al. 2013). The roots are used for chest complaints (Hutchings et al. 1996, McGaw 2008), stomach ache, measles, dropsy, anorexia, and ulcer (Gelfand et al. 1985; Amusan et al. 2002, 2007), as a drastic purgative, and to treat gastrointestinal pain (Smet 1998, Bala et al. 1999), as a remedy for anorexia and antipsychotic (Wild and Gelfand 1959), treatment of burns, snake bites, stomach complaints, constipation, to ensure easy birth (Watt and Breyer-Brandwijk 1933, Hutchings et al. 1996, Varga and Veale 1997), and have been reported to have antineoplastic activities (Borris and Cordell 1984). Nevertheless, the whole plant has been reported to be toxic to mammals (Wink and Van Wyk 2008). Therefore, further research is required to elucidate the health safety of residues that remain on treated seeds.

In view of the above, the application of hexane and acetone fractionated extracts of *G. kraussiana* may be promising in protecting of stored seeds against cowpea weevils. In fact, this botanical insecticide has higher biological activities than the most popular botanical insecticide from *Azadirachta indica* against *C. maculatus* in treated cowpea. In light of the rising problem of insecticide resistance, there is an urgent need for the development of biologically safe insecticide from *G. kraussiana*, especially in low-income country like Cameroon, where the plants are widely available. Since the use of any botanical with insecticidal activity is likely to involve some unwanted exposure of human and domestic animals to toxic substances, the potential toxicity to nontarget organisms of hexane and acetone extracts of *G. kraussiana* will need to be undertaken before the adoption of the results of this study. Being highly toxic to insect pests, hexane or acetone extract of *G. kraussiana* may be recommended at the content not exceeding 5-g/kg grains to minimize unwanted effects on human.

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Author Contributions

This work was carried out in collaboration among all authors. Authors D.K. and E.N.N. designed the study, wrote the protocol and performed the statistical analysis. Authors D.K., G.A.A., A.T.T., J.P.A., J.A.G.Y. and T.K.K. managed the analyses of the study. Author D.K. wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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