Bioactivity of Novel Botanical Insecticide From Gnidia kaussiana (Thymeleaceae) Against Callosobruchus maculatus (Coleoptera: Chrysomelidae) in Stored Vigna subterranea (Fabaceae) Grains

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Abstract

Hexane, acetone, and methanol extracts from Gnidia kaussiana Meisn (Thymeleaceae), each at two dosages (0.2 and 1 ml/50 g grains corresponding, respectively to 1 and 5 g/kg), and neem seed oil (NSO), used as standard insecticide were evaluated for repellence, toxicity to Callosobruchus maculatus (F.) (Coleoptera: Chrysomelidae) adults, F1 progeny inhibition, persistence and as grain protectant during storage. Experiments were laid out at complete randomized design with five replications for repellence test and four for others. All the extracts were effective in protecting stored Bambara groundnut (Vigna subterranea L. Verdcourt) from insect attack; however, their bioactivities were inversely correlated with solvent polarity. No adult survival was recorded in treated grains with hexane extract at 5 g/kg dosage within 2 d exposure. Also at 5 g/kg, all extracts hindered adults emergence, grain damage and weight loss after 4 months storage. Moreover, hexane extract was more repellent and exhibited averagely repellency. The insecticidal effectiveness of hexane extract did not decreased provided that the exposure time of insects to the product was high (7 d). The potency of acetone and methanol extracts decreased with storage time, although not linearly and remained significantly toxic to C. maculatus up to 60 d of storage. Therefore, hexane and acetone extracts are good candidates for incorporation in integrated pest management programs for control of cowpea weevils in stored grains by poor-resourced farmers and store keepers in Cameroon and other developing countries.

Key words: Vigna subterranea, Callosobruchus maculatus, Gnidia kaussiana, toxicity, repellence

World’s population stands at about 8 billion (FAO 2015) and it is projected to increase at 2.2% per year to around 11.5 billion by 2100, with 87% living in the developing countries of Africa, Asia and Latin America (Penning de Vries 2001). The high population growth rate, particularly in the developing countries, and the changing diets will lead to a much higher food demand by 2020 (Penning de Vries 2001). Enhancing food availability in sub-Saharan Africa could be realized not only by increasing agricultural productivity through the use of sustainable good agricultural practices, but also by reducing pre- and post-harvest crop losses (Tschamntke et al. 2012). In the dry African Sahelian countries agricultural production is seasonal while demands for agricultural commodities are more evenly spread throughout the year (Mikolo et al. 2007). In this circumstance, grains need to be stored from one harvest to the next in order to maintain its constant supply all year round and to preserve its quality until required for use (Nukenine 2010). The reduction of postharvest grain losses, especially those caused by insects, microorganisms, rodents, and birds, can increase available food supplies, particularly in less developed countries where the losses are largest and the need is greatest. Amongst these living organisms, insects are responsible for the greatest storage losses in cereals and pulses.

Traditionally, the grain weevils, Sitophilus spp. (Coleoptera: Curculionidae) and the Angoumois grain moth, Sitotroga cerealella (Olivier) (Lepidoptera: Gelechiidae) on cereals and three genera of bruchids, Acanthoscelides, Zabrottes, and Callosobruchus spp. on pulses are the most important pests of stored grains in Africa (Abate et al. 2000). Identifying the specific pest found within a sample is the first step in controlling insect pests, because insects have different damage potentials, biology, behaviors, growing temperatures, moisture requirements, and reproductive potentials (Mason and McDonough 2012). As in other countries of sub-Saharan Africa, Callosobruchus maculatus was reported to be a leading pest of Bambara groundnut in Cameroon (Kouninki et al. 2014; Kosini et al. 2015). Infestation of Vigna subterranea by C. maculatus leads to a significant reduction in the viability of planting seeds, which could be >50%, after the emergence of only the F1 progeny.
The weight loss of more than 21% and grain damaged of 100% were recorded due to C. maculatus attack within only 4 months storage (Kosini et al. 2015).

Toxic synthetic insecticides being applied in solid and liquid forms against these insect pests are generally accepted as effective but carcinogenic, hazardous to nontarget organisms and the environment. There is therefore, the growing interest in adapting naturally existing plant in crop protection to stem the trend of food shortage as a result of insect infestation. In recent years, researchers have been focusing on the secondary compound of plants to be used as alternatives for chemical insecticides (Moreira et al. 2012, Adeyemi 2010, Derkyi et al. 2010, Kosini et al. 2015). In fact, insecticides from botanical origin are more biodegradable and have, with a few exceptions for some pure compounds (Golob et al. 1999; Bakkali et al. 2008; Suthisut et al. 2011a,b), low mammalian toxicity (Regnault-Roger et al. 2012, El-Wakeil 2013). Gnidia kaussiana Meisn (Thymeleaceae) is an herbaceous, perennial plant with ~30–40 cm in height. The alternated leaves are acuminate on the base and lanceolate in shape with entire margins. Plant is clumped and stems are generally pink in color at the base. Capitulum inflorescence in top position is intense yellow; they appear 1 month after the first rains. Plant has a heavy root like cassava root with or without ramifications. G. kaussiana is mainly used in local storage structures by communities of the far north region of Cameroon to protect their stored grains from insect infestation. However, there is a dearth of information concerning the plant. No study considering G. kaussiana and insect control is found in the literature. Therefore, research is needed to show the effectiveness of the insecticide from this local botanical to kill insects, yet be safe for workers and consumers.

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The present study was undertaken to investigate the insecticidal effects and phytochemical screening of hexane, acetone and methanol extracts from the root powder of that plant against C. maculatus in Bambara groundnut.

Materials and Methods

Seeds and Insect Rearing

The Bambara groundnut seeds (variety Kodek) were collected from farmers at Mokolo during harvest time. The seeds were disinfested by keeping them in a freezer at 4°C for 3 wk prior to the bioassays. The seeds were then kept under experimental conditions for at least 2 wk before use. The initial moisture content of the seeds was 10.66 ± 1.15%, determined by the method of AFNOR (1982).

Insect used were originally collected in Mokolo market, Mayo-Tsanaga division in far north Cameroon and subsequently insects were cultured in the laboratory on cowpea, the most suitable host plant for the cowpea weevil development.

Collection and Processing of Plant Materials

The roots of G. kaussiana were collected in XI-2013 around Mogode in the Mayo-Tsanaga Division, Far-North region of Cameroon (latitude 10°36.25’ N and longitude 13°34.46’ E, altitude 1,005 m above sea level [m.a.s.l]). The identity of the plant was confirmed at the Cameroon National Herbarium in Yaounde, where voucher specimen (Serial number: 38259/HNC) was kept. The Far-North region is in the Sudano-Sahelian agro-ecological zone of Cameroon (IRAD 2007). This agro-ecology is characterized by two seasons: wet (June to September) and dry (October to May). Annual rainfall ranges between 800 and 1,000 mm. Annual mean temperature is 29°C, with a maximum of 39°C in March and minimum of 17°C in January. Average annual RH stands at 67%. The roots were dried in a room at Mokolo under ambient conditions for 7 d and then crushed in a mortar until the powder passed through a 0.4 mm mesh sieve. Powders were stored in a deep-freezer at the temperature of 4°C until needed for extraction, to avoid degradation.

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°C until needed for bioassay.

Asadirachta 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°33.16’ N, longitude 14°815.04’ E and altitude of 356 m.a.s.l.), Far-North region, Cameroon in XIII-2013. Seeds processing and oil extraction were done as described in previous study (Kosini et al. 2015).

Phytochemical Screening of G. kaussiana Extracts

The plant extracts were phytochemically screened using standard techniques for the detection of Sterols, saponins, Cardiac glycosides, tannins, flavonoids, terpenoids and alkaloids as described in Adeniyi et al. (2010).

Adult Toxicity, Progeny Production, and Persistence Bioassay

Extracts were dissolved in the respective solvent used for plant 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 (check) at the same weights were separately mixed with 50 g grains in separate glass jars which correspond to 1 and 5 g/kg, respectively. The volumes of 0.8 and 1 ml of solvents, respective 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 solution added to 1 ml. The content of each jars were shaken properly to ensure proper coating of the seeds with the extract or NSO and the solvents allowed to evaporate (Kosini et al. 2015). 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 mortality was recorded 1–7 d post-exposure. After the 7-d mortality recordings, all the live and dead insects were separated from the grains and discarded. The grains were left inside the bottles on laboratory shelves and all the F1 progeny were counted. The experiment was carried out in ambient laboratory conditions (temp. = 24.11 ± 1.54°C [21.00–30.50°C], RH ≈ 77.30 ± 3.18% [60.50–83.50%]).

Products persistence was laid out by treating 25 g seeds at 5 g/kg content and infested with 20 insects after 0, 7, 14, 30, and 60 d storages. At each persistence time assessment, the experiment was similar to that of adult toxicity.

Population Increase and Damage Bioassay

The experimental units from the adult toxicity test and progeny production above were used in the bioassay to assess population increase and damage. After the F1 progeny recordings, all the insects, dead and live, as well as the grains from each jar were left in their respective jars on laboratory shelves for a total period of 4 months starting from infestation. The undamaged grains and damaged grains were counted and weighed for each jar. The final moisture content of the grains was also determined. The final grain weight (FGW) 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.
Table 1. Repellency scale from the less to the most repellent = 0 to V

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

Table 2. Phytochemical analyses of extracts from *G. kaussiana*

| Phytochemicals | HE | AE | ME |
|----------------|----|----|----|
| Total phenolic compounds | – | ++ | + |
| Alkaloids | – | ++ | + |
| Saponins | – | – | + |
| Tannins | – | + | ++ |
| Flavonoids | – | ++ | + |
| Steroids | – | – | – |
| Triterpenoids | +++ | + | + |
| Cardiac glycosides | – | – | +++ |

HE, hexane extract; AE, acetone extract; ME, methanol extract.

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

The weight of additional moisture content was expressed as follow:

\[
\text{Sample initial weight} \times \frac{\text{final moisture content (%)} - \text{initial moisture content (%)}}{100 - \text{final moisture content (%)}}
\]

- Percent weight loss was determined as follows:

\[
\left(\frac{\text{IGW} - \text{FGW}}{\text{IGW}}\right) \times 100, \text{where IGW was the initial grain weight}
\]

Repellency Test

Repellency test was conducted using the olfactometer method (Ngamo et al. 2007). A linear olfactometer made of 30-cm plastic tube, having 2 cm diameter with a hole at its middle was used. At each end, a small container was placed with 10 g of Bambara groundnut grains. One container contained grains treated with plant materials and the other the control containing seeds treated with the respective solvent used to dilute each of the extract: hexane for hexane extract, acetone for acetone extract and methanol for methanol extract. Two treatment dosages (1 and 5 g/kg grains) were used. Twenty insects (<48-h old) of mixed sex were separately introduced in the hole at the middle of the olfactometer. The choice of insects was observed for a period of 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), Liu and Ho (1999) under ambient laboratory conditions [24.87 ± 1.72% RH (72.00–78.00% RH)].

Percentage repellency (PR) = \(2 \times (C - 50)/C\); C = percentage of insects choosing the control end treated by hexane, acetone, or methanol as negative control either by NSO as positive control. When PR > 0 the extract was repellent and when PR < 0 the extract was attractive.

The average values were then categorized according to the scale in Table 1 (Juliana and Su 1983).

Data Analysis

Abbott’s formula (1925) was used to correct for control mortality. Data on % cumulative corrected mortality, % reduction in progeny production, % grain damage, % weight loss and PR were arcsine-transformed \([\text{square root}(x/100)]\) 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 (SAS Institute 2003). Tukey’s (Honest Significant Difference) multiple range test \((P = 0.05)\) was applied for mean separation.

Results

Phytochemical Constituents of *G. kaussiana* Fractions

The result of the phytochemical screening (Table 2) reveals that the presence of chemical groups increased with the polarity of solvent used for extraction. Methanol extract tested positive for total phenolic compounds, alkaloids, saponins, tannins, flavonoids, terpenoids, and cardiac glycosides. Except saponins, all these phytochemicals were also detected in acetone fraction. Only one phytochemical group, terpenoid compounds was detected in hexane fraction. Terpenoids were abundant in hexane fraction and not abundant in acetone and methanol fractions. Acetone extract was more concentrated in alkaloids and flavonoids than methanol extract which contained abundant and moderately abundant cardiac glycosides and tannins, respectively.

Adult Toxicity and Persistence

Extracts from *G. kaussiana*, using hexane (apolar), acetone (intermediate) and methanol (polar) solvents were toxic to *C. maculatus* adults at different doses and exposure periods (Table 3). The insecticidal efficacy of *G. kaussiana* was not the same for hexane, acetone and methanol extracts either at the content of 1 g/kg \((F = 19.2–56.3; \text{df} = 3, 12; P < 0.001)\) or 5 g/kg \((F = 48.4–293.9; P < 0.001)\). Hexane extract was the more toxic to *C. maculatus* and methanol the least toxic at both tested contents. At the content of 1 g/kg, the standard insecticide NSO was so far less effective than extracts from *G. kaussiana*. Hexane extract caused 84.7 ± 7.3% *C. maculatus* within 4 d exposure while NSO caused only 12.0 ± 2.6% at the same point-time exposure. Also, the lethal time (LT) to mortality highlighted that hexane extract \((LT_{50} = 0.6 \text{ d and } LT_{95} = 5.8 \text{ d})\) was very fast acting than NSO \((LT_{50} = 18.2 \text{ d and } LT_{95} = 171.5 \text{ d})\) (Table 4). However, hexane extract and NSO had similar efficacy \((LT_{50} = 0.6 \text{ d, } LT_{95} = 1.1 \text{ d both})\) and achieved 100.0% *C. maculatus* mortality within 2 d postexposure at the content of 5 g/kg (Tables 3 and 4). At this content, acetone and methanol extracts caused respectively 90.5 ± 3.4 and 63.9 ± 3.6% insect mortality within 4 d exposure.

During 2-month storage period, the toxicity of the tested products did not remain the same (Table 4). However, hexane extract remained the more toxic and caused 90% mortality of *C. maculatus* within 5 d exposure after 60 d storage of treated Bambara groundnut. Higher toxicity was also recorded for acetone extract than for methanol extract and NSO which caused respectively 41 and 48% insect mortality at the same storage and exposure periods.

F1 Progeny Production

The two doses of each extract and NSO reduced *C. maculatus* offspring significantly (Table 4). At the content of 1 g/kg NSO, hexane and acetone extracts had similar activity and 94.5 ± 1.4 – 97.8 ± 1.4% progeny inhibition was recorded and only 66.5 ± 3.1% for methanol. At 5 g/kg dose, hexane and acetone extracts suppressed completely progeny production by cowpea weevils and this was not
The untreated control had no mortality. Means within the same line followed by the same letter (s) did not differ significantly (P < 0.05; Tukey’s test).

*P < 0.001; NSO, neem seed oil.

Table 4. Persistence, LT to mortality (LT50 and LT95, days) values and inhibition of C. maculatus offspring by extracts from G. kaussiana on treated Bambara groundnut

| Product | Dose (g/kg) | LT50 (95%) CI5, days | LT95 (95%) CI5, days | Offspring production of control | Inhibition of offspring (%) | Mortality at 5 d (%) after 60 d in storage |
|---------|-------------|----------------------|----------------------|-------------------------------|-----------------------------|------------------------------------------|
| NSO     | 1           | 18.2 (12.3–39.1)    | 171.5 (66.9–1,134.3) | 31.5 ± 0.7                    | 96.9 ± 2.1                  | –                                        |
| Hexane  | 1           | 1.8 (1.4–2.1)       | 5.8 (4.8–8.0)        | 32.3 ± 1.3                    | 97.8 ± 1.4                  | –                                        |
| Acetone | 1           | 4.1 (3.7–4.6)       | 23.1 (17.3–35.1)     | 31.5 ± 0.7                    | 94.5 ± 1.4                  | –                                        |
| Methanol| 1           | 8.7 (7.5–10.7)      | 50.6 (33.2–94.8)     | 30.5 ± 0.3                    | 66.5 ± 3.1                  | –                                        |
| F1,12   |             | 0.8^a               |                      |                               |                             |                                          |
| NSO     | 5           | 0.6 (0.3–0.7)       | 1.1 (1.0–1.3)        | 31.5 ± 0.7                    | 97.8 ± 1.4                  | 48^c                                      |
| Hexane  | 5           | 0.6 (0.2–0.7)       | 1.1 (1.0–1.3)        | 32.3 ± 1.3                    | 100.0 ± 0.0                 | 90^a                                      |
| Acetone | 5           | 1.5 (1.3–1.7)       | 4.5 (4.0–5.4)        | 31.5 ± 0.7                    | 100.0 ± 0.0                 | 69^b                                      |
| Methanol| 5           | 2.6 (2.2–3.0)       | 21.4 (14.9–37.4)     | 30.5 ± 0.3                    | 95.0 ± 3.2                  | 41^c                                      |
| F1,12   |             | 0.8^a               |                      |                               |                             | 45.2^a                                    |

The untreated control had no mortality. ns, not significant; 
*P < 0.001. Means within the column followed by the same letter did not differ significantly (P < 0.05; Tukey test).

significantly different compared with the rate of inhibition recorded for methanol extract and NSO for the same dosage.

Damage and Weight Loss

The population increase of C. maculatus was significantly reduced by NSO, hexane and acetone extracts either at low or at high content relative to the negative control. At 5 g/kg these products protected Bambara groundnut completely from insect infestation within 4 months storage (Table 5). Methanol extract was the least effective product to protect grains from insect infestation; however, significant reduction, either at the higher content (2.8 ± 1.7 insects) or at the lower one (210.0 ± 32.42 insects), was recorded compared with the number of insects (508.8 ± 63.84) recorded in the control. Apart from methanol extract at the content of 1 g/kg, no live insects were recovered after the period of storage.

Four months after storage, 100.0 ± 0.0% untreated Bambara groundnut grains were severely damaged (6.4 ± 0.8 holes per grain) by cowpea weevil and 18.8 ± 0.9–21.4 ± 0.9% grain weight loss were recorded. Significant reduction of grain damage (F = 132.26–24,168.90; df = 2,9; P < 0.001) as well as grain weight loss (F = 62.66–442.12; df = 2,9; P < 0.001) was recorded in treated grains. Hexane extract at both 1 and 5 g/kg contents including acetone extract and NSO at 5 g/kg were more potent to C. maculatus and averted weevil damage. No weight was lost from these treatments. Treated grains with acetone and NSO at the content of 1 g/kg were also significantly protected from insect attack, and these products had the same efficacy. Methanol extract was also more potent at the higher content and protected almost completely Bambara groundnut from weight loss caused by bruchid’s attack.

Repellency

An exponential increased for NSO and acetone extract repellency activity with ascending dosage was recorded (Table 6). These products were respectively moderately (class II) and slightly repellent (class I) at 1 g/kg content and were fairly (class IV) and averagely (class III) at 5 g/kg. Hexane extract was the more repellent (class III) tested product to C. maculatus at the lower content. At higher content (5 g/kg), the positive control (NSO) was more repellent to cowpea weevil. Methanol extract was the least repellent with moderately and slightly repellency activity, respectively and the lower and at the higher contents suggesting that its repellence efficacy decreases with increasing dosage.
Acetone
Hexane
NSO
Concn (g/kg) Repellence (%) Class Interpretation treated Bambara groundnut against C. maculatus

Table 5. Population increase of C. maculatus in treated Bambara groundnut with NSO and extracts from G. kaussiana, grains damaged and grain weight loss, at 4 months after storage

| Prod. (g/kg) | Population increase (mean ± SE) | Grains damaged (%) | Grain weight loss (%) |
|--------------|---------------------------------|--------------------|-----------------------|
|              | Dead insects | Live insects      |                       |                       |
| NSO          |               |                    |                       |                       |
| 0            | 472.3 ± 25.3a | 45.0 ± 2.9a       | 100.0 ± 0.0a          | 21.4 ± 0.9a           |
| 1            | 2.2 ± 1.9b    | 0.0 ± 0.0b        | 1.3 ± 1.2b            | 0.7 ± 0.7b            |
| 5            | 1.2 ± 0.9b    | 0.0 ± 0.0b        | 0.3 ± 0.1b            | 0.0 ± 0.0b            |
| F 2,9        | 343.7*        | 300.0*            | 6,805.5*              | 327.1*                |
| Hexane       |               |                    |                       |                       |
| 0            | 445.2 ± 13.7a | 35.5 ± 3.5a       | 100.0 ± 0.0a          | 18.8 ± 0.9a           |
| 1            | 1.3 ± 0.9b    | 0.0 ± 0.0b        | 0.0 ± 0.0b            | 0.1 ± 0.0b            |
| 5            | 0.0 ± 0.0b    | 0.0 ± 0.0b        | 0.0 ± 0.0b            | 0.0 ± 0.0b            |
| F 2,9        | 1,044.4*      | 101.5*            | 24,168.9*             | 442.1*                |
| Acetone      |               |                    |                       |                       |
| 0            | 472.3 ± 25.3a | 45.0 ± 2.9a       | 100.0 ± 0.0a          | 21.4 ± 0.9a           |
| 1            | 5.0 ± 3.7b    | 0.0 ± 0.0b        | 2.9 ± 1.2b            | 1.0 ± 0.9b            |
| 5            | 0.0 ± 0.0b    | 0.0 ± 0.0b        | 0.0 ± 0.0b            | 0.0 ± 0.0b            |
| F 2,9        | 336.6*        | 300.9*            | 6,540.4*              | 155.9*                |
| Methanol     |               |                    |                       |                       |
| 0            | 457.0 ± 70.9a | 51.7 ± 7.8a       | 100.0 ± 0.0a          | 20.0 ± 1.9a           |
| 1            | 192.0 ± 32.2b | 18.0 ± 2.3b       | 92.1 ± 7.9a           | 12.0 ± 0.9b           |
| 5            | 2.8 ± 1.7c    | 0.0 ± 0.0b        | 2.1 ± 1.6c            | 0.3 ± 0.2c            |
| F 2,9        | 25.8*         | 31.5*             | 132.3*                | 62.7*                 |

Prod., product.
* P < 0.001, means within the column followed by the same letter did not differ significantly (P < 0.05; Tukey’s test).

Table 6. Mean PR values of three fractions of G. kaussiana in treated Bambara groundnut against C. maculatus

| Conc (g/kg) | Repellence (%) | Class Interpretation |
|------------|----------------|---------------------|
| NSO        |                |                     |
| 1          | 27.75 ± 6.63   | II Moderately repellent |
| 5          | 73.56 ± 6.43   | IV Fairly repellent |
| Hexane     |                |                     |
| 1          | 40.16 ± 5.45   | III Averagely repellent |
| 5          | 50.39 ± 4.40   | III Averagely repellent |
| Acetone    |                |                     |
| 1          | 11.74 ± 3.45   | I slightly repellent |
| 5          | 50.66 ± 7.82   | III Averagely repellent |
| Methanol   |                |                     |
| 1          | 34.87 ± 7.76   | II Moderately repellent |
| 5          | 10.21 ± 2.98   | I slightly repellent |

Discussion

Chemical insecticide compounds extracted from botanicals are responsible for the biological activities such as toxicity and persistence, growth inhibition and grain protectant ability against insect pests (Adler et al. 2000, Isman 2008). G. kaussiana extracts tested for these parameters with the aim to control C. maculatus in stored pulses proved to be a good insecticide candidate that may be exploited by farmers worldwide in general and in Cameroon in particular.

In this investigation, the activity of hexane extract was higher than that of other fractions. This may suggest that the effect of G. kaussiana extracts on C. maculatus mortality may be mostly attributed to its terpenoid compounds. Important terpenoids include camphor, methanol, and pyrethrin (Mungenge et al. 2014). Pyrethrin is fast acting, providing almost immediate “knockdown” of insects following an application. Since hexane fraction of G. kaussiana contained terpenoid compounds as secondary metabolites and was fast acting, its insecticidal property may be due to monoterpenoid pyrethrin or another chemical compound similar in biological activity. Alkaloids are also toxic secondary metabolites which can block ion channels, inhibit enzymes or interfere with neurotransmission, loss of coordination and death (Aniszewski 2007). This activity may be responsible for higher mortality caused by acetone extract than that caused by methanol extract in this study. At low concentration, hexane and acetone extracts of G. kaussiana showed higher insecticidal activity than NSO. This suggests that active compounds are either more concentrated in G. kaussiana extracts or C. maculatus adults are more sensitive to active compounds (especially terpenoids and alkaloids) from that plant than NSO.

One of the most important steps in the use of novel insecticidal products from plant origin is to ascertain the persistence of their insecticidal activity. Activity of products decreased with the time because of the presence of volatile active compounds or because of the oxidation of nonvolatile compounds which lead to the reduction of the insecticidal efficiency. However, NSO had great persistent because of the low volatility of its main insecticidal compound, Azadirachtin as reported by Tamgno and Ngamo (2014). Hexane extract of G. kaussiana was also more persistent with more rapid action against C. maculatus adults than NSO. Hexane extract of G. kaussiana and NSO could then protect Bambara ground nut grains against the infestation of C. maculatus for at least 2 months. Acetone extract could also adequately protect grains during this period of storage. Methanol extract did not remain effective to protect grains against insect infestation 1 wk after storage. However, its efficacy has been increased 1 wk after storage. In fact, total phenolic compounds were more abundant in this extract and transformation processes of phenolic compounds led to the increase of toxicity of individual compounds by the formation of electrophilic metabolites that may bind and damage DNA or enzymes (Michalowicz and Duda 2007).
In addition to adult toxicity, extracts of *G. kaussiana* exhibited significant inhibition of the F1 progeny emergence and grain protective effects with the hexane and acetone extracts producing the best results (similar to that produced by NSO), irrespective of tested content. Thus, the extracts of *G. kaussiana* as well as the positive control (NSO) either inhibited oviposition and/or killed the larvae at developmental stages after eggs laid on the grains. In fact, crude extracts have been 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 larvae (Jamal et al. 1984), induced by some secondary metabolites like terpenoids, alkaloids, and flavonoids (Slama 1979, Salunke et al. 2005, Acheuk and Doumandji-Mitiche 2013) present in the tested extracts. In the similar way at the physiological level, azadirachtin, the active principal component of neem oil blocks the synthesis and release of molting hormones (ecdysteroids) from the prothoracic gland, leading to incomplete ecdisis in immature insects (El-Wakeil 2013). Consequently, hexane and acetone extracts of *G. kaussiana* reduced the rate of population increase of cowpea weevils as did the NSO during 4 months of storage, with total suppression achieved at the content of 5 g/kg. The protection of the grains against *C. maculatus* damage by the extracts of *G. kaussiana* indicates that these products, especially hexane and acetone extracts could be of value in storage protection against the bruchids. At higher content (5 g/kg), no grain damage or weight loss was recorded.

Extracts of *G. kaussiana* have also been found to have effective repellent action on *C. maculatus*. Comparatively in earlier studies (Xie et al. 1995, Boeke et al. 2004, Nukenine et al. 2009, Kosini et al. 2015), plant extracts, powders, and essential oil from different bioactive plants were reported as repellent against stored grain pests. The repellency is an important feature of extracts for the management of insect pests, since they play a role directly in the reduction of egg-laying and hence the emergence of adults, making difficult the establishment of pest populations in grain storage facilities.

**Conclusion**

This study shows that the extracts of *G. kaussiana* could be a potential toxicant and grain protective, against *C. maculatus* and having total suppression of progeny emergence in treated grain. Moreover, the persistence of the toxicity indicates that these extracts are stable enough and could protect Bambara groundnut grains against insect pests attack for at least 2 months. This novel botanical insecticide, especially its hexane fraction has a similar or higher biological activity than the most popular botanical insecticide from *A. indica* against *C. maculatus* in treated Bambara groundnut. As the bitter taste of NSO restricts its use and unsuitied on stored-products meant for human consumption, insecticidal products from *G. kaussiana* could be exploited for the development of novel molecules with precise targets for sustainable insect pest management in stored grain as alternative to neem oil. However, further studies are needed, especially to isolate and identify the active principle component and to evaluate the cost/benefit ratio regarding its use for insect control in grain stores. Finally, the effects on end use quality and toxicity of the products to mammals would need to be determined before commercialization.

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