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Research

Control of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) Using Fractionated Extracts from Cameroonian *Hemizygia welwitschii* (Lamiaceae) Leaf on Stored *Vigna unguiculata* (Fabales: Fabaceae)

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Abstract

Bioassays were conducted to assess the individually insecticidal activities of hexane, acetone, and methanol extracts from *Hemizygia welwitschii* Rolfe-Ashby leaves powder against *Callosobruchus maculatus* (F.). The extracts were applied at 2, 4, 6, and 10 g/kg of cowpea and the untreated seeds served as negative control. Treatments were arranged in a complete randomized design with four replications. Adult mortality, F_1 progeny emergence, as well as insect population increase, seeds damage, and seeds germination were carried out. The results obtained showed that the three extracts of *H. welwitschii* were very effective in protecting stored cowpea against *C. maculatus* at the highest dosage (10 g/kg) 7 d after treatment. There was no F_1 progeny emergence of *C. maculatus* in cowpea treated with hexane extract at the dosage of 10 g/kg, while, at the same dosage, acetone and methanol extracts almost completely inhibited the F_1 progeny emergence. Also, the different concentration levels significantly protected the seeds with regard to seed damage caused by *C. maculatus* compared with the untreated control after 3 mo storage. The viability of seeds was not affected by the extracts. Because of their effectiveness, the three extracts of *H. welwitschii* leaves powder could be a good candidate in pest management programs, especially against *C. maculatus* in stored cowpea grains, in Cameroon and other developing countries.

Key words: extract, *Hemizygia welwitschii*, effectiveness, *Callosobruchus maculatus*, storage

Résumé

Des bio-essais étaient menés afin d'évaluer individuellement les activités insecticides des extraits à l'hexane, à l'acétone et au méthanol de la poudre des feuilles de *Hemizygia welwitschii*, contre *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae). Les tests ont été réalisés dans des conditions ambiantes de laboratoire. Les extraits étaient appliqués aux doses de 2, 4, 6 et 10 g/kg et les grains non traités ont servi de témoin négatif. Les traitements étaient disposés au laboratoire en blocs complétement randomisés avec quatre répétitions. La mortalité, l'émergence de la progéniture F_1, aussi bien que la croissance de la population et les dégâts des graines, et la germination des graines étaient évalués. Les résultats obtenus ont montré que les trois extraits de la plante utilisés étaient très actifs dans la protection du niébé stocké contre *C. maculatus* à la plus grande dose (10 g/kg) 7 jours après traitement. Il n'avait aucune émergence de la progéniture F_1 de *C. maculatus* dans le niébé traité avec l'extrait à l'hexane à la plus grande dose de 10 g/kg. Tandis qu'à la même dose, les extraits à l'acétone et au méthanol ont presque complètement inhibé l'émergence de la progéniture F_1. De plus, les différentes doses ont significativement protégé les graines en ce qui concerne les dégâts des graines causés par *C. maculatus* par rapport au témoin non traité après trois mois de stockage. La viabilité des graines n'était pas affectée par les extraits. En raison de leur efficacité, les trois extraits de la poudre des feuilles de *H. welwitschii* pourraient être des bons agents de protection dans le programme de gestion des ravageurs spécialement contre *C. maculatus* dans les graines de niébé stocké au Cameroun et dans d'autres pays en voie de développement.

Mots clés: Extraits, *Hemizygia welwitschii*, efficacité, *Callosobruchus maculatus*, stockage.

About 870 million people in the world are undernourished because of inadequate intake of proteins, vitamins, and minerals in their diets (FAO 2012). Grain legumes are good source of proteins which offer a solution to malnutrition. In fact, they are the second most important group of crops worldwide after cereals. Among legumes, cowpea serves as a source of dietary protein for human feeding, mostly in developing countries where balanced diet is sometimes a problem (Ojo et al. 2013). The dry seed contains about 25% protein and 67% carbohydrate. Cowpea also contains calcium, iron, vitamins, and carotene (Adedire et al. 2011). Unfortunately, the grain is extensively infested by a number of insect pests including *Callosobruchus maculatus*, which is a primary field to store pest causing considerably great losses to farmers (Ojo et al. 2013) both in the field and storage (Gupta et al. 2011, Thakur 2012). Their larvae bore into the seed; now, the seed becomes unsuitable for human consumption and losses its viability during planting. To manage this cowpea pest, different methods have been used by the scientists or local farmers. Synthetic insecticides are effective to control stored insect pests in commercial storage facilities, but their availability and high cost limit their utilization by some local African farmers. Despite their positive effect on the pests, they continued to remain hazardous to man and the environment. With the perspective of providing quality food for population, the interest of researchers has been focused to find alternatives to synthetic chemicals which could be environmentally friendly and do not pose hazards to man (Alice and Srikanth 2013). As such, more attention is reverted to the usage of natural products, including plant extracts, powders, ashes, oils, and cow dung to control pests (Naumann and Isman 1995). Some plant materials have played an important part in those traditional methods in Africa where they have been mixed with stored grains and the use of these plant products has assumed significance as an important element of insect pest management because of their economic viability and eco-friendly nature. They hold promise as alternatives to chemical insecticides to reduce pesticide load in the environment. Phytochemicals possess a wide spectrum of biological properties against insects. Among them, *Hemizygia welwitschii*, an herbaceous and annual plant, is widely present in tropical environment such as West, East, Central, and parts of southern Africa (Otieno et al. 2006). Currently, a total number of 33 species are recognized in *Hemizygia* genus, including *H. welwitschii* (Rolfe) Ashby (Ashby 1935). The genus *Hemizygia* sp. has a wide range of use. In fact, in traditional medicine, aqueous extract of *H. bracteosa* (Benth) has shown promise alternative in the treatment of diabetes mellitus in Benin (Chabi et al. 2015). As insecticidal properties, the volatility and repellency effect of *H. welwitschii* essential oil and its formulations were evaluated against mosquitoes (Oyedele et al. 2000). In pest control, Fotso et al. (2018) recently shown the efficacy of *H. welwitschii* leaves powder against *C. maculatus* and *Sitophilus zeamais* in stored cowpea and maize, respectively. Their results showed that, at the content 40 g/kg, *H. welwitschii* caused significant mortality to *C. maculatus* (82.50%) and *S. zeamais* (81.25%) at 7 and 14 d after exposure, respectively. But the mode of use and type of botanical material vary from place to place, and appear to depend partly on the type and efficacy of suitable plants available in different locations (Chougourou et al. 2015). Plant extracts are commonly referred to as botanicals and are the secondary plant metabolites synthesized by the plants for protective purposes. Some of these compounds are toxic to insects. Since the presence of these different bioactive compound groups depend to the polarity of solvent used (Mahmoudi et al. 2013, the choice of solvents determines the nature of the compounds present in each extract fraction. In fact, under the same extraction time and temperature, solvent and composition of sample are known as the most important parameters (Do et al. 2014). Despite local availability of *H. welwitschii*, this plant is not yet used by farmers in pest control. In view of the alternatives to reduce conventional insecticides used in stored cowpea, this research was designed to study the efficacy of three fractionated extracts from *H. welwitschii* leaves powder for controlling *C. maculatus*.

Materials and Methods

Substrate: Cowpea

The cowpea seeds (Lori variety) used for this study were purchased from the Institute of Agricultural Research for Development (IRAD), Maroua, Cameroun. Broken seeds and particles were removed and then disinfested by keeping them in a freezer at −18°C for 30 d prior to bioassays. The cowpea was then kept under ambient experimental conditions for acclimation at least 2 wk before use. The moisture content (mc) of the seeds was determined according to the method used by AFNOR (1982). For that, 10 g (Mo) of cowpea were introduced into the oven at 120°C for 24 h; four replications were done. After this period, the grains were removed and reweighted (M_1), and the mc was calculated using the following formula and it was 9.88%.

$$\text{mc} \quad (\%) = \frac{\text{Mo} - \text{M1}}{\text{Mo}} \times 100$$

Insect Rearing

The original stock was purchased from infested grains in Ngaoundere market, Adamawa Region and maintained in the Laboratory of Applied Zoology of the University of Ngaoundere Cameroon. Cowpea weevils were mass reared on whole clean, undamaged, and disinfested cowpea in 5-liter transparent plastic jars. This was done under ambient laboratory conditions of [t ≈ 23 ± 2.02°C (18.50–31.50); relative humidity (r.h.) ≈ 63.69 ± 15.17% (21 − 88.5%)], registered with a thermo-hygrometer EL-USB-2 (RH/Temp Data Logger) (China). Four kilograms of cowpea seeds were introduced into a clean plastic jar and 200 unsexed adults of *C. maculatus* were then added into that jar containing cowpea and kept in the laboratory for 1 mo for the development of the insects. The subsequent progenies were used for all experimentations. All insects used for these experiments were not more than 2 d old.

Collection and Preparation of Plant Material

Green leaves of *H. welwitschii* were collected between August and October 2016 at Ngaoundere, Cameroon (latitude 7°22″ North and longitude 13°34″ East, altitude of 1,100 m a.s.l.), and shade-dried at the room temperature until they became crisp. The identification of the plant was done at the Cameroon National Herbarium in Yaounde. The sample was identified in comparison with voucher specimen of *H. welwitschii* (Rolfe) M. Ashby under the serial number 6910/SRFK. The dried leaves were hand crushed into powder using locally made pestle and mortar. Powder was stored in a deep-freezer at the temperature of −4°C until needed for extraction.

Extraction of the Plant Powder

The dried leaves powder of *H. welwitschii* (2.3 kg) was mixed with 10 liters of hexane (apolar solvent) in the perforated container and agitated for 30 min and allowed to stand for 24 h in the Phytochemistry Laboratory of Institute of Medical Research and Medicinal Plants Studies, Yaoundé, Cameroon, and then restirred again. The solvent was removed after 48 h by filtration. The residue obtained after filtration was put through the above process again and the filtrate was admixed with the one obtained initially. After the hexane extraction was done, the paste left was dried for 10 h at room temperature in the laboratory and then used for acetone (intermediate solvent) extraction and followed by methanol (polar solvent) extraction. The filtrates obtained with hexane, acetone, and methanol were then separately concentrated in a Rotavapor at 70, 60, and 65°C, respectively, at 120 rpm (Kosini et al. 2015). The extract yield of 3.42, 2.42, and 6.44% was obtained by using hexane, acetone, and methanol, respectively. Extracts were stored in a refrigerator at 4°C until needed for bioassays.

Phytochemical Screening of *H. welwitschii* Extracts

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

Toxicity Bioassay

Four dosages 0.1, 0.2, 0.3, and 0.5 g were introduced in 500 ml glass jar containing 50 g of cowpea corresponding to 2, 4, 6, and 10 g/kg. Untreated controls for each set of treatments consisted of grains treated with 2 ml of pure respective solvents. Each jar was then hand-shaken for 2 min to ensure uniform distribution of each extract or pure solvent to the entire seed mass. After this, the glass jars containing treated cowpeas or negative control were kept open for 2 h for complete solvent evaporation (Nukenine et al. 2010). After the solvent evaporation, 20 unsexed adults of *C. maculatus* were introduced in each glass jars. Each jar was then covered with cotton clothes to prevent insects from escaping and closed with a perforated metal lid for sufficient ventilation. All treatments were maintained under ambient laboratory conditions ($t \approx 24.62 \pm 1.04$°C; r.h. ≈ 76.36 ± 3.23%) in a complete randomized design with four replications. Mortality was recorded after 1, 3, 5, and 7 d of treatment. The percentage of control mortality was corrected according to Abbot (1925).

F_1 Progeny Bioassay

After 7 d mortality recorded, all insects were discarded, and jars containing treated and untreated cowpeas were maintained in the same laboratory conditions until progeny emergence. The counting of F_1 progeny was carried out once a week, for four consecutive weeks commencing fourth week after infestation. After each counting session, the insects were removed from the jars and recorded. The inhibition rate of F_1 progeny (%IR) was calculated as

$$\%\mathrm{IR} = \frac{\mathrm{Cn} - \mathrm{Tn}}{\mathrm{Cn}} \times 100$$

where Cn is the number of newly emerged insects in the untreated jar and Tn is the number of insects in the treated jar (Rajashekar et al. 2010).

Insect Population Increase and Seed Damage Assessment

Similar doses of each extract for toxicity bioassay were considered for 150 g cowpea grains. A group of 30 unsexed adult insects was introduced into each glass jar containing treated or untreated seeds. Control consisted of seeds with respective pure solvent. All treatments were maintained under ambient laboratory conditions in a complete randomized design with four replications. After 3 mo of storage, the numbers of live and dead insects were registered for each jar. Seed damage assessment was performed by counting the damaged and undamaged cowpea seeds. The seed damage percentage was calculated using the following formula:

$$\textbf{Seed} \quad \textbf{damage} = \frac{\textbf{Number} \quad \textbf{of} \quad \textbf{seeds} \quad \textbf{damged}}{\textbf{Total} \quad \textbf{number} \quad \textbf{of} \quad \textbf{seeds}} \times 100$$

Seed Viability Evaluation

To assess the viability of seeds, germination test was conducted using 20 undamaged seeds from each jar after the evaluation of seeds damage. The grains were placed in moistened sand in perforated plastic plates. Germination was counted and recorded after 10 d (Rao et al. 2006, Demissie et al. 2008) and percentage of seed germination was thereafter determined according to the following formula:

$$\% \quad \textbf{seed} \quad \textbf{germination} = \frac{\textbf{Number of germinated seeds}}{\textbf{Total number of seeds}} \times 100$$

Statistical Analysis

Data on % cumulative mortality, % reduction of F_1 progeny, % damaged grains, and % of germination were subjected to the analysis of variance (ANOVA) procedure using the Statistical Package for the Social Science (SPSS 16.0). Turkey's test (P = 0.05) was applied for mean separation. A logarithmic transformation (log10(x+1),{10, subscript, x italics} where x = content in %) was performed before regression analysis. Abbott's formula (Abbot 1925) was used to correct control mortality when mortality in the control is comprised between 3 and 10% before submission to the ANOVA. Probit analysis (Finney 1971; SPSS 16.0) was applied to determine lethal concentration causing 50% (LC_{50}) mortality of *C. maculatus* after 1, 3, 5, and 7 d of exposure.

Table 1. Phytochemical analyses of extracts from *Hemizygia welwitschii* leaf powder

Compounds	Extracts		
	Hexane	Acetone	Methanol
Phenolic compounds	—	+	+
Alkaloids	—	—	+
Saponins	+	+	+
Tannins	—	+	+
Flavonoids	—	+	+
Sterols	+	+	—
Terpenoids	+	—	—
Glycosides	—	—	—

— absent, + present.

Results

Phytochemical Constituents of *H. welwitschii* Extracts

Important phytochemicals, such as phenolic compounds, alkaloids, terpenoids, sterols, tannins, saponins, and flavonoids, were screened for their presence in *H. welwitschii* leaves powder using hexane (apolar), acetone (intermediate), and methanol (polar) as presented in Table 1. The qualitative phytochemical studies indicate that saponins, sterols, and terpenoids groups are found in hexane extract, whereas phenolic compounds, saponins, tannins, flavonoids, and sterols groups were present in the acetone extract. Concerning methanol extract, the chemical groups which were present are phenolic compounds, alkaloids, saponins, tannins, and flavonoids. It is noted that alkaloids were exclusively present in the methanol extract.

Adult Mortality

Adult mortality of *C. maculatus* caused on treated cowpeas by each leaf extract of *H. welwitschii* is presented in Fig. 1. The results show that extracts were toxic to *C. maculatus* adults, and this varies with ascending concentrations and exposure times. The highest concentration (10 g/kg) of each extract of *H. welwitschii* within 1 d caused less than 15% mortality of *C. maculatus*. At this same time point, mortality recorded in cowpea seeds treated with acetone extract was lower and had no significant effect on adult mortality compared with untreated control. Within 3 d of exposure, only the hexane extract recorded more than 50% of mortality at the highest dosage of 10 g/kg. At this same dosage and same time point, acetone and methanol extracts recorded 38.75 and 32.50% mortality, respectively. After 7 d of exposure, the mortality of *C. maculatus* increases significantly with concentrations (hexane extract: $F = 1766.55$; df = 4, 15; $P < 0.001$; acetone extract: $F = 397.37$; df = 4, 15; $P < 0.001$; methanol extract: $F = 1197.40$; df = 4, 15; $P < 0.001$). Treatments with hexane extract at the rate of 10 g/kg caused complete mortality within 7 d after treatment application. However, the mortality of *C. maculatus* recorded with methanol and acetone extracts, at this same exposure period and same dosage was 94.03 and 81.62%, respectively.

Table 2 shows the mortality parameters of *C. maculatus*. For 3, 5, and 7 d after exposure, hexane extract was more toxic to the bruchids. The lowest LC_{50} was obtained with the three extracts on cowpea within 7 d of exposure. The hexane extract (LC_{50} = 0.31 g/kg) was the most toxic to the bruchids than acetone (LC_{50} = 1.53 g/kg) and methanol (LC_{50} = 1.87 g/kg) extracts, respectively. Concerning the R^2 values, they ranged between 0.65 and 0.89 for the three extracts at all the exposure periods, except at 5 and 7 d exposure for hexane extract where the R^2 values were 0.57 and 0.48, respectively. Acetone extract within 1 d acted more rapidly (slope = 4.55 ± 0.74) than methanol extract (slope = 1.18 ± 0.21) and hexane extract (slope = 1.08 ± 0.42) and 5 d of exposure (slope = 1.25 ± 0.13). While within 3 d of exposure, the hexane extract acted more rapidly (slope = 1.19 ± 0.13) than acetone and methanol extract. In 7 d of exposure, methanol extract acted more rapidly (slope = 1.76 ± 0.14). All the χ^2 values were not significant for the three extracts at all the exposure periods, except for the hexane extract which was significant at 1 and 7 d of exposure.

F_1 Progeny Inhibition

The emergence of F_1 progeny was significantly affected by extract treatments compared with untreated control. The number of *C. maculatus* progeny emerged in the untreated control was significantly higher (number) than in the treated cowpea grains (236 adults for hexane, 292.75 adults for acetone, and 109.75 adults for methanol) (Table 3). Classification in terms of efficacy for each extract in progeny reduction on the treated cowpea seeds from the content of 4 g/kg was as follows: hexane extract > methanol extract >> acetone extract. At 4 g/kg, the number of F_1 progeny was 8.00, 11.00, and 28.25, respectively, for hexane, methanol, and acetone extracts. At the lower concentration (2 g/kg), the classification of extracts in terms of progeny inhibition was as follows: methanol extract > hexane extract > acetone extract with, respectively, 19.50, 19.75, and 36.75 *C. maculatus* emerged. Complete percentage in terms of inhibition rates of *C. maculatus* adult was obtained by hexane extract from the dosage of 6 g/kg, while with methanol extract the percentages of reduction were 95.68 and 98.40% at 6 and 10 g/kg, respectively. At the highest dosage (10 g/kg), acetone extract almost achieved complete inhibition of F_1 progeny (99.06%) (Table 3).

Population Increase and Grain Damage

The results of Table 4 indicate significant decrease of number of live insects among the three extracts tested compared with the untreated control. At the lowest dosage (2 g/kg), acetone extract recorded less live insects (13.25) compared with hexane and methanol extracts with 19.00 and 52.00 live insects, respectively. The hexane extract of *H. welwitschii* possesses the strongest effect against *C. maculatus* population growth with no live insects recorded at the highest dosage of 10 g/kg, followed by acetone extract (2 living insects) and methanol extract (14.25 living insects). The dead insects recorded in cowpea treated with hexane and acetone significantly decreased with the dosages compared with control. At the highest dosage (10 g/kg), hexane extract killed all the 30 insects introduced at the beginning of the experiment; acetone extract recorded 66.50 dead insects, while 167.27 dead insects were observed in cowpea treated with methanol extract.

Seeds Damage

Figure 2 shows that all treatments were effective to protect cowpea against the attack of *C. maculatus* and it is dose-dependent (damaged seeds decrease significantly with an increase of dosages (hexane extract: $F = 591.98$; df = 4, 15; $P < 0.001$; acetone extract: $F = 18.87$; df = 4, 15; $P < 0.001$; methanol extract: $F = 7.12$; df = 4, 15; $P < 0.001$). Higher seeds damage was recorded in untreated control (up to 39.38% seeds damage). Among the three extracts tested for their efficacy against *C. maculatus* in stored cowpea grains, hexane extract of *H. welwitschii* leaves powder was found to be the most protective by recording no seeds damaged at highest tested contents

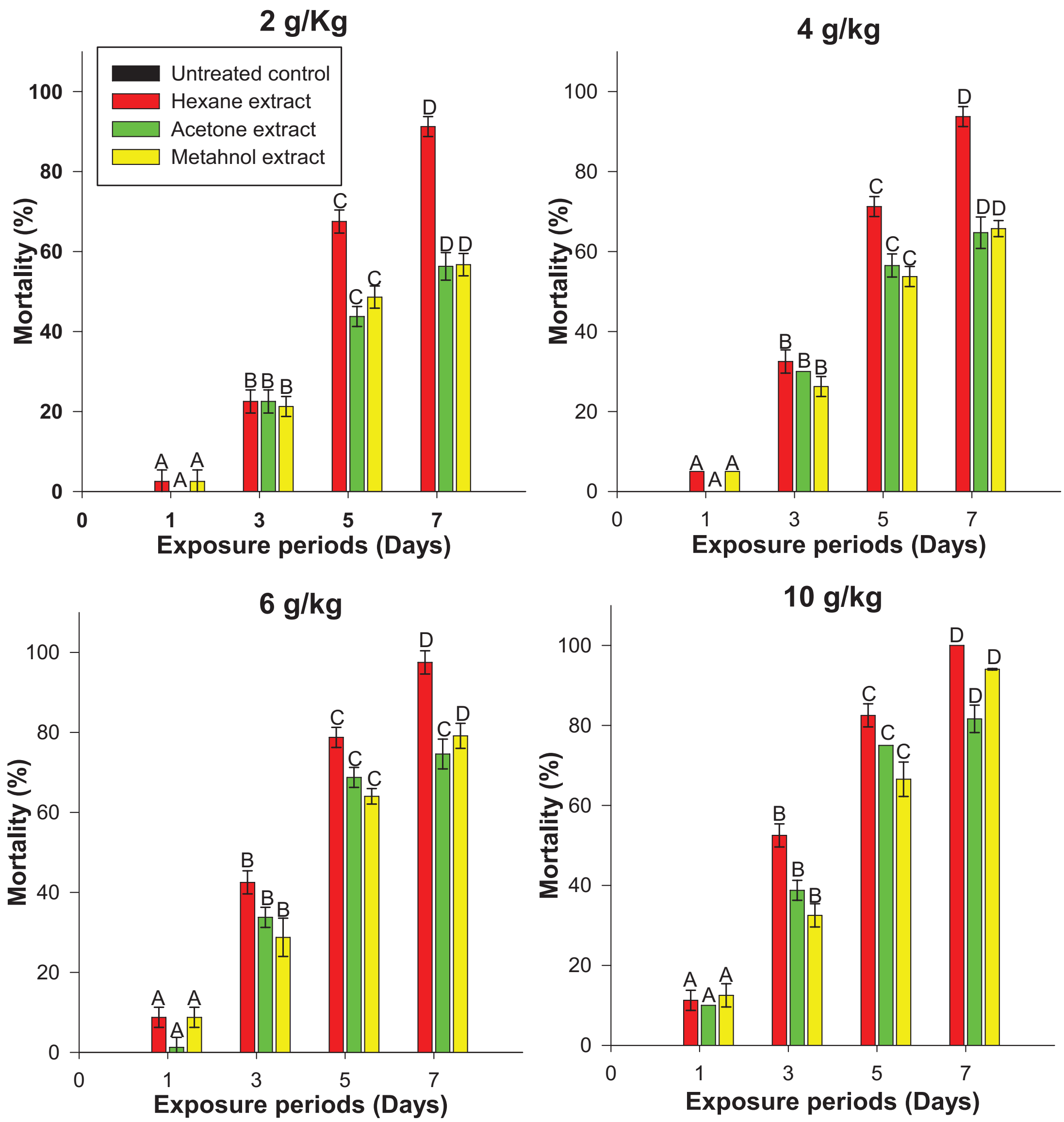

Fig. 1. Corrected cumulative mortality of *Callosobruchus maculatus* exposure to hexane, acetone and methanol leaf extracts of *Hemizygia welwitschii*.

of 10 g/kg. While at the same dosage, acetone and methanol extracts recorded 6.12 and 6.94% damaged seeds, respectively.

Germination Rate

The results obtained in this study for the viability test showed that the germination of cowpea seeds was influenced differently by concentrations and extract types of *H. welwitschii* (Fig. 3). There is significant difference of germination rate between the control and treated seeds (hexane extract: F = 4.86; df = 4, 15; P < 0.05; acetone extract: F = 142.30; df = 4, 15; P < 0.001; methanol extract: F = 18.69; df = 4, 15; P < 0.001). Germination rate of cowpea seeds in treatments ranged from 28.75% in the untreated control to 47.50% with hexane extract, 13.75% in the untreated control to 60% with acetone extract, and 15.00% in the untreated control to 41.25% with methanol extract. The highest percentage of germination was obtained in cowpea seeds treated with acetone extract (60%) at its highest tested content (10 g/kg), followed by cowpea seeds treated with hexane extract (47.50%) at the dosage 4 g/kg and the cowpea seeds treated with methanol extract (41.25%) at the lowest tested content of 2 g/kg.

Discussion

Phytochemical investigation of the extracts from the plant used in the present study showed that it is rich in many active compounds including phenolic compounds, alkaloids, saponins, tannins, triterpenes, flavonoids, and sterols groups. The presence of these different compound groups depend on the polarity of the solvent used. In fact, the solvent polarity used and the solubility determined the nature of the compounds present in each plant extract (Mahmoudi et al. 2013). Currently, many farmers in Africa and Asia are using botanicals to protect their legumes from attack by bruchids, with varying success degrees (Chellapa and Chelliah 1976), due to the type of formulations and the commodities used among others. Results from

Table 2. Toxicity parameters of adult *Callosobruchus maculatus* in grains treated with different extracts from *H. welwitschii* leaf powder

Products	N	Slope ± SE	R^2	LC_{50} (95% FL) (g/kg)	χ^2
			1 d		
Hexane extract	20	1.08 ± 0.42	0.81	123.22 (52.12–806.22)	15.07**
Acetone extract	20	4.55 ± 0.74	0.84	19.04 (15.66–27.53)	17.31^{ns}
Methanol extract	20	1.18 ± 0.21	0.84	91.07 (44.08–395.70)	15.51^{ns}
			3 d		
Hexane extract	20	1.19 ± 0.13	0.89	8.93 (7.64–11.05)	5.10^{ns}
Acetone extract	20	0.67 ± 0.13	0.76	25.86 (15.69–75.77)	3.18^{ns}
Methanol extract	20	0.49 ± 0.13	0.69	83.74 (30.12 ± 2234.77)	4.17^{ns}
			5 d		
Hexane extract	20	0.71 ± 0.13	0.57	0.51 (0.14–0.94)	6.73^{ns}
Acetone extract	20	1.25 ± 0.13	0.78	2.88 (2.43–3.28)	8.34^{ns}
Methanol extract	20	0.71 ± 0.12	0.65	2.36 (1.54–3.03)	7.28^{ns}
			7 d		
Hexane extract	20	1.56 ± 0.25	0.48	0.31 (0.03–0.69)	27.12*
Acetone extract	20	1.08 ± 0.13	0.69	1.53 (1.06–1.94)	9.76^{ns}
Methanol extract	20	1.76 ± 0.14	0.78	1.87 (1.43–2.24)	23.27^{ns}

$^{ns}P > 0.05$, $^{ns}P < 0.05$, $*P < 0.05$, $**P < 0.01$.

Table 3. Progeny production of *Callosobruchus maculatus* in grains treated with three extracts of *Hemizygyia welwitschii* leaf powder under ambient laboratory conditions

Products Contents (g/kg)	Mean number of F_1 adult progeny	% reduction in adult emergence relative to control
Hexane extract		
0	236.00 ± 10.73^c	0.00 ± 0.00^a
2	19.75 ± 2.22^b	91.62 ± 0.94^b
4	8.00 ± 2.5^a	96.60 ± 0.62^c
6	2.50 ± 3.00^a	98.92 ± 1.28^d
10	0.00 ± 0.00^a	100.00 ± 0.00^d
$F_{(4, 15)}$	1,599.18***	12,967.22***
Acetone extract		
0	292.75 ± 0.96^e	0.00 ± 0.00^a
2	36.75 ± 1.50^d	87.44 ± 0.54^b
4	28.25 ± 1.50^c	90.35 ± 0.49^c
6	11.00 ± 1.41^b	96.24 ± 0.49^d
10	2.75 ± 2.21^a	99.06 ± 0.75^e
$F_{(4, 15)}$	24,477.28***	26,068.39***
Methanol extract		
0	109.75 ± 0.96^d	0.00 ± 0.00^a
2	19.50 ± 3.00^c	82.22 ± 2.76^b
4	11.00 ± 1.41^b	89.98 ± 1.25^c
6	4.75 ± 0.96^a	95.68 ± 0.85^d
10	1.75 ± 0.50^a	98.40 ± 0.46^d
$F_{(4, 15)}$	3,158.53***	3,388.60***

Means within the column followed by the same small letter do not differ significantly at the 5% level according to Tukey test. *** $P < 0.001$.

this study showed that *H. welwitschii* leaves powder extracts also have significant insecticidal effects against *C. maculatus* which vary with the contents, exposure periods, and extracts. Among the tested extracts in the present investigation, it was found that the *H. welwitschii* hexane extract appeared to be more toxic to *C. maculatus* at its highest treatment (10 g/kg) than methanol and acetone extracts, respectively in 7 d of exposure. This result on adult mortality of *C. maculatus* is in accordance with the previous works done by Kosini and Nukenine (2017), which reported similar outcome on *C. maculatus* with *Gnidia kaussiana* hexane extract at 5 g/kg within 2 d of exposure. The results obtained in our study also agree with the findings of Mahama et al. (2018) and those of Danga et al. (2015). In the findings of these authors, hexane extract was also the most effective product against *C. maculatus* among the plant extracts they used. In addition to the presence of saponins and sterols, *H. welwitschii* hexane extract contains other secondary metabolites like triterpenoids, which exhibit antifeedant and/or lethal contact actions on insect. This can explain the high activity of hexane extract from *H. welwitschii* leaves powder. In fact, Kostyukovsky et al. (2002) reported that terpenoids cause symptoms that suggest a neurotoxic mode of action of these secondary metabolites which was observed to *C. maculatus* exposed to orange peel essential oil-treated seeds. However, before the current study, Fotso et al. (2018) recently shown the efficacy of *H. welwitschii* leaves powder against *C. maculatus* and *Sitophilus zeamais* in stored cowpea and maize, respectively. These authors recorded significant mortality (82.50%) of *C. maculatus* at the highest dosage (40 g/kg), in 7 d after exposure caused by *H. welwitschii* leaves powder. The present study demonstrated the potential of using *H. welwitschii* leaves powder extracts to control *C. maculatus* in stored cowpea but with the high effect pronounced when compared with *H. welwitschii* leaves powder. In fact, in this finding, the hexane and methanol extracts recorded more than 90% of *C. maculatus* which are more effective than *H. welwitschii* leaves powder plant extract. The effectiveness of the plant extracts indicates a possible contact action of the active constituents of *H. welwitschii* with the bruchids. In fact, volatile compounds of plant extracts contain many bioactive molecules, which may have contact and fumigant properties. The efficacy of these plant extracts could be attributed to the presence of phytochemical secondary metabolites present in each extract. Indeed, the plant secondary metabolites are responsible for diverse activities including their insecticidal properties (Rubabura et al. 2014). This shows that *H. welwitschii* leaves powder extracts are highly effective in controlling bruchids infestations, which could be due to the presence of some bioactive compounds, including phenolic compounds, alkaloids, saponins, tannins, flavonoids, triterpenoids, and sterols. Aniszewski (2007) found that alkaloids are the toxic secondary metabolites which can block ion

Table 4. Population increase of *Callosobruchus maculatus* in cowpea treated with of *Hemizygia welwitschii* leaf extracts and stored for 3 mo

	Insects		
Products	Number of insects (means ± standard error)		
Contents (g/kg)	Dead insects	Live insects	Total insects
Hexane extract	*Callosobruchus maculatus*		
0	244.00 ± 4.76^{c}	207.50 ± 5.74^{d}	451.50 ± 1.91^{d}
2	205.00 ± 49.89^{c}	19.00 ± 5.22^{c}	224.50 ± 44.78^{c}
4	114.00 ± 8.98^{b}	9.75 ± 0.96^{b}	123.75 ± 8.26^{b}
6	74.25 ± 5.38^{ab}	6.75 ± 1.26^{ab}	81.00 ± 4.24^{b}
10	30.00 ± 0.00^{a}	0.00 ± 0.00^{a}	30.00 ± 0.00^{a}
$F_{(4,15)}$	61.03***	2,526.37***	265.16***
Acetone extract			
0	290.25 ± 65.27^{c}	140.50 ± 12.50^{b}	430.75 ± 64.39^{c}
2	213.25 ± 76.42^{bc}	13.25 ± 3.30^{a}	223.50 ± 80.04^{b}
4	121.75 ± 57.95^{ab}	4.25 ± 3.77^{a}	126.00 ± 56.12^{ab}
6	109.25 ± 38.87^{ab}	3.75 ± 2.63^{a}	113.00 ± 41.00^{ab}
10	66.50 ± 26.45^{a}	2.00 ± 2.16^{a}	68.50 ± 27.96^{a}
$F_{(4,15)}$	10.40***	377. 79***	25.93***
Methanol extract			
0	197.50 ± 79.94^{a}	98.50 ± 6.76^{c}	296.00 ± 83.29^{a}
2	175.75 ± 78.64^{a}	52.00 ± 15.56^{b}	227.75 ± 78.53^{a}
4	131.00 ± 43.41^{a}	22.00 ± 4.08^{a}	153.00 ± 44.43^{a}
6	83.00 ± 17.26^{a}	17.75 ± 3.03^{a}	100.75 ± 19.72^{a}
10	167.25 ± 49.31^{a}	14.25 ± 2.87^{a}	181.50 ± 50.84^{a}
$F_{(4,15)}$	0.52^{ns}	77.97***	1.42^{ns}

Means within the column and line followed respectively by the same small letter do not differ significantly at the 5% level according to Tukey's test.
$^{ns}P < 0.05$; $***P < 0.001$.

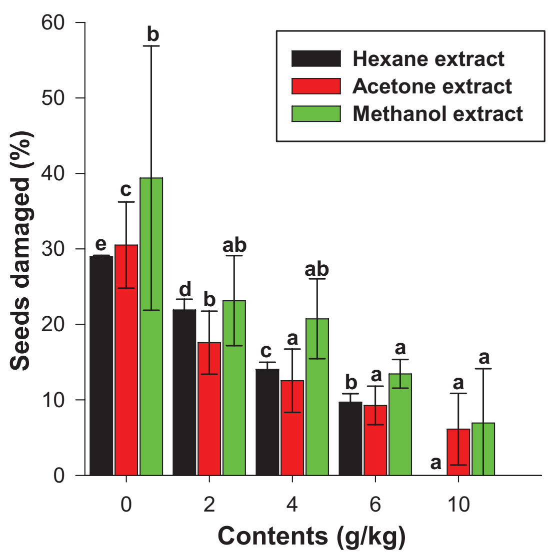

Fig. 2. Percentage of seeds damaged by *Callosobruchus maculatus* on cowpea treated with *Hemizygia welwitschii* leaf extracts and stored for 3 mo.

channels, inhibit enzymes, or interfere with neurotransmission, loss of coordination, and death. Alkaloid compounds were found only in methanol extract of *H. welwitschii*, which could be responsible for higher mortality caused by methanol extract than that caused by acetone extract in this study.

Faraway (2002) reported that in the Biological Sciences when the coefficient of determination $R^2 \geq 0.6$, then the favorable results are attributable to the products used. In the present study, most of the $R^2 \geq 0.6$. The few smaller values of coefficient of determination are linked to high doses of applied substances, which lead to complete or almost complete efficacy, with no variation in the insect responses (mortality). Therefore, the botanical extracts were greatly responsible for the responses of *C. maculatus* on the treated cowpea. The values of χ^2 were generally not significant for all the three extracts, implying that the obtained regression models approximate the theoretical model, concerning the toxicity of the used substances to *C. maculatus* (Finney 1971).

The *H. welwitschii* leaves powder extracts also reduced significantly the production and inhibited F_1 progeny emergence of *C. maculatus*. The same tendencies were recorded by other authors and plants extracted with the same solvents used in this investigation. Mahama et al. (2018) reported similar result with a significant reduction in the progeny F_1 production of *C. maculatus* on seeds treated with ***Eucalyptus camaldulensis* leaf extracts** on Bambara groundnut grains. Reduction in the F_1 progeny emergence of *C. maculatus* in the Bambara groundnut treated with *Ocimum canum* Sims leaf extract fractions was also obtained by Kosini et al. (2015). Chudasama et al. (2015) stated that the reduction in adult emergence could either be due to egg mortality, larval mortality, or even reduction in hatching of the eggs. This shows that *H. welwitschii* leaves extracts probably have oviposition deterrent, ovicidal, and lavicidal properties. The highest percentage of inhibition of *C. maculatus* F_1 progeny could be attributed to the highest mortality of the adult *C. maculatus* in the mortality test observed at the last day of exposure at these concentrations. According to Hertlein et al. 2011, effective control of protectants is attributed to the mortality of adult and/or immature stages, confirmed by lack of progeny generation.

Grain damage indicated the quantitative loss in stored grains due to insect feeding showing a direct relationship between insect population and grains damage. In our findings, all the extracts provide a significant reduction in seed damage activity compared with the untreated seeds. These effects resulted in reduced weight; especially,

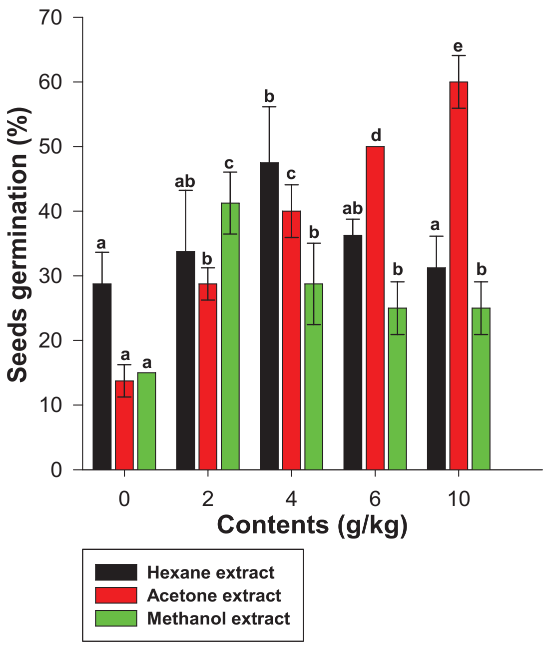

Fig. 3. Seed viability of cowpea treated with three extracts from *Hemizygia welwitschii* leaves powder after 3 mo storage.

in the control seeds and the feeding activities of *C. maculatus*; in particular, resulted in holes in the seeds.

The percentage seed germination recorded in the present study showed that there was a significant difference ($P > 0.05$) compared with the control. This may be that the three extracts have no effect on the seeds viability. This result is similar with the work of Umar (2008) who reported that the cowpea seeds remained viable which were treated with *Jatropha curcas* powder. The same tendency was also reported by Ojianwuna et al. (2014), which reported highest germination rate of cowpea viability when treated with crude extracts of *Ocimum suave* leaf oil. Our studies show that *H. welwitschii* leaf extracts cannot also have a negative effect on seed viability. But the lowest percentage of germination recorded in this study could be attributed to some factors such as environmental conditions and immature stages of bruchids. According to Couturon (1980), when environmental conditions are not well controlled, germination rate decreases quickly. The lowest percentage of germination recorded in this study could be related to the development of bruchids, which emerged at these level contents by feeding on seed germ, which prevents germination and reduces germination rate. Also, the seeds used for germination were nonperforated but they would contain the immature stages of bruchids that could destroy the parts of seed embryo.

The finding obtained in this study revealed that, all the three solvent extracts were toxic against *C. maculatus* adults in the protection of cowpea (*Vigna unguiculata*) and having significant inhibition of F_1 progeny emergence in treated grain. Among the extracts tested, hexane extract showed best result as insecticidal product on reduction in stored cowpea damage caused by *C. maculatus*. With the three extracts, the seeds did not lose their viability. Therefore, the use of *H. welwitschii* leaves powder extracts as insecticide of plant origin in postharvest protection should be encouraged in stored cowpea management because of its great toxicant potential against cowpea weevils in storage. However, further research is required to study the active ingredients of this plant, mode of action, and consumer safety, before promoting their use in stored product protection by the farmers who store cowpea for consumption and planting, and industrial level.

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