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Potentiality of Fractionated Extracts from *Hemizygia welwitschii* Rolfe-Ashby (Lamiaceae) Leaf to Protect Maize against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in Storage

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Authors' contributions

This work was carried out in collaboration among all authors. Authors TGF, WFGDK and ENN designed the study and wrote the protocol. Authors TGF, MCZ and NT made the laboratory bioassays. Authors TGF and TG performed the statistical analysis. Authors TGF and MNL managed the analyses of the study. Authors TGF and DJL managed the literature searches. Author TGF wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Laboratory tests were conducted in order to assess the potential insecticidal effect of hexane, acetone and methanol fractionated extracts from *Hemizygia welwitschii* leaves against *Sitophilus zeamais* Motschulsky, important stored maize pest. The three extracts used individually were applied at four different concentrations (2, 4, 6 and 10 g/kg of maize) against the weevil. The efficacy of the treatment was evaluated by recording adult mortality after 1, 3, 7 and 14 days of infestation, and the F_1 progeny emergence. Population increase, grain damage, and seeds germination were also assessed after three months of storage. The results showed that, among

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the three fractionated extracts, hexane extract was the most effective with 100% mortality recorded within 14 days after infestation at the concentration of 10 g/kg of maize, followed by acetone extract with 83.75% and methanol extract with 79.21% of mortality. The LC_{50} values decrease with the increase of exposure periods. The 14-day LC_{50} values were 0.78 g/kg, 1.58 g/kg and 3.10 g/kg respectively for hexane, acetone and methanol extract. The three extracts achieved significant inhibition of F_1 progeny at all the concentrations. Among them, the hexane and methanol extracts induced complete inhibition of F_1 progeny emergence at 10 g/kg of maize, acetone extract recorded 82.33% of inhibition. Significant reductions of insect population growth and percentage of seed damage were recorded after three months of storage on the maize treated with each extract at all the concentrations compared to negative control. Furthermore, no alive insects was recorded in maize seeds treated with the three extracts at the concentration of 10 g/kg. It is noticed that, percentage of gain damage were similar (0.15%) in maize treated with hexane and methanol extracts at 10 g/kg, while acetone extract recorded 0.76% of grain damage. In general, these extracts had no negative effect on the germination capacity of maize grains at the end of storage. Overall, the results obtained indicate that the use of these fractionated extracts could serve as an alternative to synthetic insecticides.

Keywords: Sitophilus zeamais; Hemizygia welwitschii; fractionated extracts; conservation; storage.

1. INTRODUCTION

Maize (*Zea mays* L.) is a cereal with world grain production ranking second next to wheat [1,2]. This crop is staple foods in many developing countries [3]. In sub-Saharan African countries, maize production is mainly done only within the rainy season, but the products are consumed and marketed all year round [4]. Proper food storage becomes therefore a matter of survival. One of the major problems encountered in agriculture in developing countries is postharvest losses which usually occur during storage [5]. Postharvest losses then vary greatly among commodities, production areas and seasons. Maize weevil, *Sitophilus zeamais* is a major pest that attacks stored maize grains in the tropics and temperate regions of the world [6,7]. In stored maize, heavy infestation of this pest may cause weight losses of as much as 30-40% [1,8]. The weevil has been reported to cause up to 80% grain damage in Africa during storage [9]. However, declining food production, worsened by huge losses resulting from *S. zeamais* attack during maize storage expose farmers to different magnitudes of food shocks [10]. Chemical insecticides are the method of choice for controlling stored grain pests. A rapid and efficient control of the pests is generally provided with chemical insecticides. Nevertheless, the chemical insecticides cause many environmental hazards and are detrimental to human health [11]. There is therefore a need to develop safer products that are environment friendly and less dangerous to consumers. Crop protection agents of natural origin also have the advantage of possessing novel modes of action against insects and thus have the potential to reduce the risk of cross-resistance while offering new leads for the design of target-specific molecules [12]. Botanicals are widely reported for their efficacy as insecticides, with the dominance of essential oils, pure compounds or solvent extracts [13]. Different plant extracts are valuable source of components that can be used as pest control against various insects [14,15]. The insecticidal activity of plant extracts against different stored-product pests has been evaluated [16–19]. Among these various plants, there is *Hemizygia welwitschii* Rolfe-Ashby (Lamiaceae), which is a good source of biological insecticides. It contains many secondary metabolites like alkaloids, steroids, phenolic compounds, flavonoids, tannins, terpenoids, saponins which are present in all insecticidal plants used against insect pests [18]. Recently, the leaf powder of *H. welwitschii* was tested against *Callosobruchus maculatus* and *S. zeamais* [20]. Fractionated extracts from *H. welwitschii* leaf have been also tested against *C. maculatus* in storage [18]. Since efficacy of plants has been documented for a number of insect species on various stored grains, this often varies with the plant formulations, and commodities. Therefore, results on plants efficacy obtained on a particular commodity may not be transferable to other commodities. In this study, we examined the potential of *H. welwitschii* leaves extracts to protect maize against *S. zeamais* in storage.

2. MATERIALS AND METHODS

2.1 Maize

Maize grains were collected from the Institute of Agricultural Research for Development at

Ngaoundere (Cameroon). The maize variety used was ‘’Shaba’’ the composite mostly used by Adamawa farmers and mostly susceptible to the infestation of *S. zeamais*. The grains were thoroughly cleaned to remove kernels with visible damage symptoms then disinfested by keeping in a freezer at -18°C for 30 days [18]. The grains were then kept in ambient conditions of laboratory for two weeks to allow its acclimatization prior to bioassays. The moisture content (mc) of the seeds was determined by the method described by AFNOR [21]. For that, 10 g (Mo) of maize were introduced in the oven at 120°C for 24h. After this period, the grains were removed and reweighted (M_1), and the mc were calculated followed the formula below and it was 11.65%. Four replications were done.

$$\mathrm{mc}\,(\%) = \frac{\mathrm{Mo} - \mathrm{M_1}}{\mathrm{Mo}}\ \mathrm{x}\ 100$$

2.2 Insects Rearing

S. zeamais parent was obtained from infested maize grains in Ngaoundere market, Adamawa Region. Maize weevils were mass reared on whole clean, undamaged and disinfested maize in 5 liter transparent plastic jars. This was done by weighing 4kg of maize grains into clean plastic jars. 200 mixed sex adults of *S. zeamais* were then introduced into the plastic jar containing maize and kept in the laboratory for one month for the insects to lay eggs and multiply. This culture was maintained under ambient laboratory conditions [t ≈ 23±2.02°C (18.50 – 31.50); r.h. ≈ 63.69±15.17% (21 – 88.5%)], recorded with an EL-USB-2 thermo-hygrometer (RH/TEMP DATA LOGGER, China), and used as source of *S. zeamais* for all bioassays. All insects needed for the bioassays were not more than 14 days old.

2.3 Collection of *Hemizygia welwitschii* and Extracts Preparation

Green leaves of *H. welwitschii* were collected from surroundings of the University of Ngaoundere in the Vina Division, Adamawa region, Cameroon around the point of latitude 7°22" N and longitude 13°34" E, altitude of 1,100 m.a.s.l. between August and November 2016 and shed-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 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. The extraction with the hexane, acetone and methanol was made separately according the method of extraction used by Fotso et al. [18].

2.4 Phytochemical Screening of *Hemizygia welwitschii* Extracts

The standard technique described by Adeniyi et al. [22] was used for the detection of phenolic compounds, sterols, saponins, glycosides, tannins, flavonoids, terpenoids, and alkaloids.

2.5 Toxicity Test

For testing adults mortality, four quantities concentrations 0.1, 0.2, 0.3 and 0.5 g of each fractionated extract were introduced individually in 500 mL glass jar containing 50 g of maize (Fotso *et al*., 2019). Negative controls consisted of maize grains treated with 2 mL of pure solvent (hexane, acetone or methanol). Each jar was hand-shaken for 5 min to ensure uniform adherence of each extract to the entire grain mass. After this, the glass jar containing treated grains or negative control were kept open during 2 h for complete solvent evaporation [23]. Then, 20 mixed-sex adults of *S. zeamais* (not more than 14 day-old) were introduced in each glass jar. Each jar was then covered with cotton clothes to avoid insects from escaping and closed with a perforated metal lid for sufficient ventilation. The experiments were arranged in a Completely Randomized Design in the laboratory with four replications. All treatments were kept under ambient laboratory conditions (t ≈ 24.62±1.04°C; r.h. ≈ 76.36±3.23%). Mortality was evaluated after 1, 3, 7 and 14 days of treatment and the percentage of insect mortality was calculated after counting the live and dead insects. The percent mortality was corrected for control mortality according to Abbott’s [24].

2.6 F_1 Progeny Emergence

After 14th day (Section 2.5), all insects were removed in each jar, and the number of live and dead weevil was recorded. All treatments were exposed to the same temperature and relative humidity conditions. The counting of F_1 progeny was carried out once a week for 5 weeks, commencing five weeks after infestation. After each counting session, the insects were removed from the jars and recorded. Percentage reduction in adult emergence of F_1 progeny or inhibition rate (%IR) was calculated as:

$$\%\mathrm{IR} = \frac{(\mathrm{Cn} - \mathrm{Tn})}{\mathrm{Cn}}\ x\ 100$$

Where, Cn is the number of newly emerged insects in the control jar and Tn is the number of insects in the treated jar.

2.7 Population Increase and Grain Damaged

Similar concentrations of each fractionated extract as for the toxicity test (section 2.5) were considered for 150 g grain of maize. A group of 30 adult insects were introduced into each glass jar containing treated or negative control grain. Negative control were consisted of grains with each pure solvent (hexane, acetone or methanol corresponding to each extract). Each treatment was repeated four times. After three months of storage, the number of live insects and dead ones were recorded for each jar. Grain damage assessment was performed by counting the damaged and undamaged maize grains. The percentage of grain damage was calculated using the formula:

$$\text{Grain damage } (\%) = \frac{\text{Number of grain damage}}{\text{Total number of grains}} \text{ x } 100$$

2.8 Germination Test

Germination test was assessed to evaluate the viability of maize grains treated with each fractionated extract of *H. welwitschii* after three months of storage. This was done by using twenty undamaged grains taken randomly from each treatment after separation of damaged grains from the undamaged one (Section 2.7). Then, the grains were placed in moistened fine sand in perforated plastic plates. Each treatment was replicated four times. Germination was counted and recorded after 10 days [18,25,26]. Percentage of seed germination was thereafter determined using the following formula.

$$\text{Grain germination } (\%) = \frac{\text{Number of germinated grains}}{\text{Total number of grains}} \text{ x } 100$$

2.9 Data Analysis

Abbott‘s formula [24] was used to correct control mortality when mortality in the control is comprised between 3% and 10% before submission to the ANOVA. Data on cumulative mortality, reduction of F_1 progeny, grain damage and grain germination were subjected to the ANOVA using the Statistical Package for the Social Science (SPSS 16.0). Turkey's test ($p \leq 0.05$) was applied for mean separation. A logarithmic transformation [$\log_{10}(x+1)$, where x = content in %] was performed before regression analysis. Probit analysis [27] (SPSS 16.0) was applied to determine lethal concentration causing 50% (LC_{50}) mortality of *S. zeamais* after 1, 3, 7 and 14 days of exposure.

3. RESULTS

3.1 Chemical Constituents of Extracts of *Hemizygia welwitschii*

The qualitative phytochemical studies indicate that saponins, sterols, and terpenoids groups are found in hexane extract (apolar), whereas phenolic compounds, saponins, tannins, flavonoids, and sterols groups were present in the acetone extract (intermediate). Concerning methanol extract (polar), 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 (Table 1).

3.2 Effect of the Three Extracts from *Hemizygia welwitschii* on the Mortality of *Sitophilus zeamais*

Adult mortality rate due to each extract was concentration dependent and increased with concentrations and exposure time (Fig. 1). Treatments with hexane extract of *H. welwitschii* resulted in higher mortality (97.50% from the concentration of 2 g/kg in 14 d of exposure) of *S. zeamais* adults compared to methanol and acetone extracts. After 1-day exposure, the methanol extract was more toxic to the weevil than hexane and acetone extracts. Four g/kg methanol extract treatment caused significant higher mortality in 1-day of exposure ($F_{(4;\ 15)} = 107.13$; $p < 0.001$). At the highest concentration (10 g/kg), hexane extract also caused significant mortality (10.0%) to adult of *S. zeamais* within 1-day of exposure. While, no significant difference in term of mortality was recorded in the maize treated with acetone extract after 1-day of exposure. Within 14-days of exposure all the *H. welwitschii* leaf extracts resulted in significant higher mortalities at all the concentrations (hexane extract: $F_{(4;\ 15)}$ = 4743.00; P < 0.001; acetone extract: $F_{(4;\ 15)}$ = 982.05; P < 0.001; methanol extract: $F_{(4;\ 15)}$ = 836.60; P < 0.001). At all the concentrations, mortality caused by hexane extract on *S. zeamais* was complete after 14-days of exposure. While, the highest cumulative mortalities at the same time point caused by methanol and acetone extracts were 79.21 and 83.75% respectively, at the highest concentration of 10 g/kg (Fig. 1).

Table 1. Chemical composition of the hexane, acetone and methanol extracts of *Hemizygia welwitschii* leaf powder

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

- absent, + present

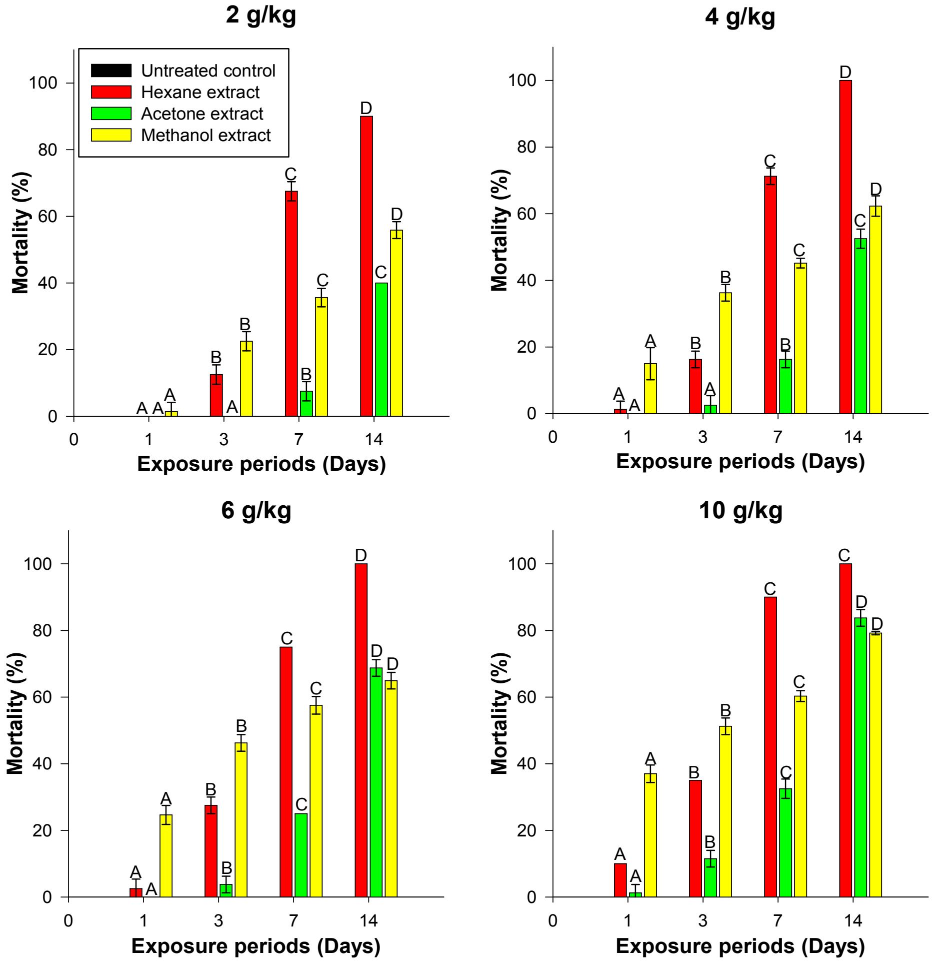

Fig. 1. Corrected cumulative mortality of adults *Sitophilus zeamais* exposed to *Hemizygia welwitschii* extracts

The LC_{50} values decrease with the increase of exposure periods. The 14-day LC_{50} values were 0.78 g/kg, 1.58 g/kg and 3.10 g/kg respectively for hexane, acetone and methanol extracts (Table 2). The R^2 values ranged between 0.65 and 0.94 at all the exposure periods, except for acetone extract (R^2 = 0.14) in 1-day after exposure and hexane extract (R^2 = 0.42) in 14-days of exposure. The slopes were higher for acetone extract (1.42 – 3.58) within the first three periods of exposure compared to hexane (1.01 – 2.72) and methanol (0.96 – 2.29) extracts. But, within 14-days of exposure, the slope was higher for hexane (4.68±2.19), than those of acetone (1.76±0.13) and methanol (0.87±0.13). All the Chi–square (χ^2) values were not significant for the three extracts at all the exposure periods, excluding those of hexane and methanol extracts which were significant within 1-day of exposure, and acetone extract within 3-day after exposure respectively (Table 2).

3.3 Effect of Each Extract of *Hemizygia welwitschii* on the Emergence of F_1 Progeny Production of *Sitophilus zeamais*

Progeny production of *S. zeamais* was significantly affected by each extract treatment ($p \leq 0.001$). 64.25±5.44 to 64.75±8.34 adult *S. zeamais* emerged in the maize grains treated with pure solvent, whereas 0.00±0.00 to 44.50±10.77 emerged in treated ones (Table 3). Adult emergence decreased with increase in concentration for the three extracts. The F_1 progeny reduction was highest for methanol and hexane extracts (Table 3). As low as 4.00, 4.75 and 44.50 F_1 progeny was recorded on maize grains treated with hexane, methanol and acetone extracts respectively at the lowest concentration (2 g/kg). After 49-days post treatment, complete suppression of F_1 *S. zeamais* progeny was obtained with hexane and methanol extracts at 10 g/kg (Table 3).

3.4 Population Increase and Grain Damage in Maize Treated with the Three Extracts of *Hemizygia welwitschii*

3.4.1 Population increase

There was significant reduction in the insect population growth in the treated maize grains ($p \leq 0.05$). Adult emergence decreased with increase in concentration (Table 4). Hexane and methanol extracts from *H. welwitschii* recorded complete suppression of *S. zeamais* adults at all extract concentrations (Table 4). As low as 1.00 and 0.25 live insects was recorded on maize grains treated with 6 and 10 g/kg concentration of *H. welwitschii* acetone extract.

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

Products	N	Slope±SE	R^2	LC_{50} (95% FL) (g/kg)	*Khi*^{2}
1 day					
Hexane extract	20	2.72±0.42	0.74	29.78 (18.56 – 113.24)	31.27**
Acetone extract	20	3.58±2.04	0.14	/	19.69^{ns}
Methanol extract	20	2.29±0.18	0.93	12.89 (10.80 – 16.77)	26.02*
3 days					
Hexane extract	20	1.72±0.14	0.93	21.61 (15.96 – 34.87)	9.00^{ns}
Acetone extract	20	2.28±0.33	0.77	33.92 (21.06 – 102.64)	24.25*
Methanol extract	20	1.15±0.12	0.84	8.32 (7.19 – 14.06)	6.29^{ns}
7 days					
Hexane extract	20	1.01±0.14	0.65	0.89 (0.48 – 1.27)	18.85^{ns}
Acetone extract	20	1.42±0.15	0.94	19.77 (15.37 – 28.74)	7.05^{ns}
Methanol extract	20	0.96±0.12	0.76	4.79 (4.11 – 5.59)	5.78^{ns}
14 days					
Hexane extract	20	4.68±2.19	0.42	0.78 (0.00 – 1.21)	9.67^{ns}
Acetone extract	20	1.76±0.13	0.88	3.10 (2.78 – 3.40)	10.72^{ns}
Methanol extract	20	0.87±0.13	0.68	1.58 (0.98 – 2.08)	10.35^{ns}

*^{ns}: p>0.05, * p< 0.05, ** p < 0.01; / : Estimated LC values are too large or estimation impossible due to inadequate mortality; N: Number of insects per replication*

Table 3. Progeny production of *Sitophilus zeamais* in grains treated with each extract of *Hemizygia welwitschii* leaf powder under ambient laboratory conditions

Products Concentrations (g/kg)	Mean Number of F_1 adult progeny	% reduction in adult emergence relative to control
Hexane extract		
0	64.75±8.34^{b}	0.00±0.00^{a}
2	4.00±2.16^{a}	93.65±3.84^{b}
4	2.25±1.89^{a}	96.41±3.29^{bc}
6	1.00±1.41^{a}	98.56±1.90^{bc}
10	0.00±0.00^{a}	100.00±0.00^{c}
$F_{(4, 15)}$ value	199.03***	1297.66***
Acetone extract		
0	64.25±5.44^{d}	0.00±0.00^{a}
2	44.50±10.77^{c}	30.86±15.57^{bc}
4	40.00±10.95^{bc}	37.20±18.55^{bc}
6	24.25±4.65^{ab}	62.14±7.08^{c}
10	11.50±3.87^{a}	82.33±4.60^{d}
$F_{(4, 15)}$ value	26.79***	29.95***
Methanol extract		
0	64.50±7.33^{b}	0.00±0.00^{a}
2	3.00±4.76^{a}	95.80±6.60^{b}
4	1.00±1.15^{a}	98.58±1.64^{b}
6	0.50±1.00^{a}	99.30±1.39^{b}
10	0.00±0.00^{a}	100.00±0.00^{b}
$F_{(4, 15)}$ value	204.55***	804.19***

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

Table 4. Number of insects emerged in maize treated with *Hemizygia welwitschii* leaves extracts and stored for three months

	Insects		
Products	**Number of insects (Means±Standard Error)**		
Concentrations (g/kg)	**Dead insects**	**Live insects**	**Total insects**
Hexane extract			
0	50.00±0.00^{c}	122.00±0.00^{b}	172.00±0.00^{c}
2	38.25±2.87^{b}	0.25±0.50^{a}	38.50±2.89^{b}
4	33.75±1.71^{a}	0.00±0.00^{a}	33.75±1.71^{a}
6	32.00±2.45^{a}	0.00±0.00^{a}	32.00±2.45^{a}
10	30.00±1.00^{a}	0.00±0.00^{a}	30.00±1.00^{a}
$F_{(4, 15)}$ values	68.35***	237901.00***	4202.86***
Acetone extract			
0	53.25±1.71^{b}	202.25±2.21^{c}	255.50±2.89^{c}
2	54.25±4.03^{b}	27.50±22.31^{b}	82.00±25.73^{b}
4	47.25±5.12^{b}	13.00±14.90^{ab}	60.25±19.19^{ab}
6	37.00±2.45^{a}	1.00±2.00^{a}	38.00±3.56^{a}
10	32.25±1.71^{a}	0.25±0.50^{a}	32.50±1.73^{a}
$F_{(4, 15)}$ values	35.43***	205.24***	162.59***
Methanol extract			
0	42.25±2.63^{b}	177.50±9.26^{b}	219.75±6.70^{b}
2	32.75±1.50^{a}	0.00±0.00^{a}	32.75±1.50^{a}
4	31.25±1.89^{a}	0.00±0.00^{a}	31.25±1.89^{a}
6	30.75±0.96^{a}	0.00±0.00^{a}	30.75±0.96^{a}
10	31.00±1.41^{a}	0.00±0.00^{a}	31.00±1.41^{a}
$F_{(4, 15)}$ values	30.62***	1471.11***	2643.33***

*Means within the column followed by the same letter do not differ significantly for the same extract at the 5% level according to Tukey's test. *** $p < 0.001$*

3.4.2 Grain damage

Fig. 2 shows that the grain damage caused by the feeding activity of both larvae and adults of *S. zeamais* was significantly ($p<0.001$) higher in the solvent treated control compared to grains treated with each extract of *H. welwitschii* leaf powder (Fig. 2). Grains treated with each concentration of the extracts exhibited better protection against infestation by *S. zeamais* compared to the untreated grains. The least grain damage (0.15%) was recorded on grains with highest concentration (10 g/kg) of hexane and methanol extracts. The better protection with acetone extract (0.76%) was recorded also at the concentration of 10 g/kg (Fig. 2).

3.5 Germination Assessment

There was no significant difference ($p>0.05$) in the germination capacity of maize grains treated with hexane extract and solvent treated control. While significant differences ($p<0.001$) were observed between the maize grains treated with acetone and methanol extracts and their respective untreated control. Generally, the germination of maize grains ranged from 76.25 – 80.00% for hexane, 48.75 – 72.50% for acetone and 53.75 – 83.75% for methanol extracts. However, the lowest germination rate was recorded in the grains treated with acetone extract at 4 g/kg (48.75%), and the highest germination was observed in the maize seeds treated with methanol extract (83.75%) at 2 g/kg (Fig. 3).

4. DISCUSSION

Plants are considered as a rich source of bioactive chemicals and may be an alternative source of insect control agents so as to ensure food security in developing countries such as Cameroon. The most prominent active constituents from plants which are responsible for the botanical insecticides properties are alkaloids, non-proteic amino acids, steroids, phenols, flavonoids, glycosids, glucosinolates, quinones, tanins, terpenoids, salanine, meliantrol, azadiractin, piretrolone, cinerolone and jasmolone [28]. Some of these important bioactive compounds are present in each fractionated extract of *H. welwitschii* leaf tested in the present study according the solvent polarity used for extraction.

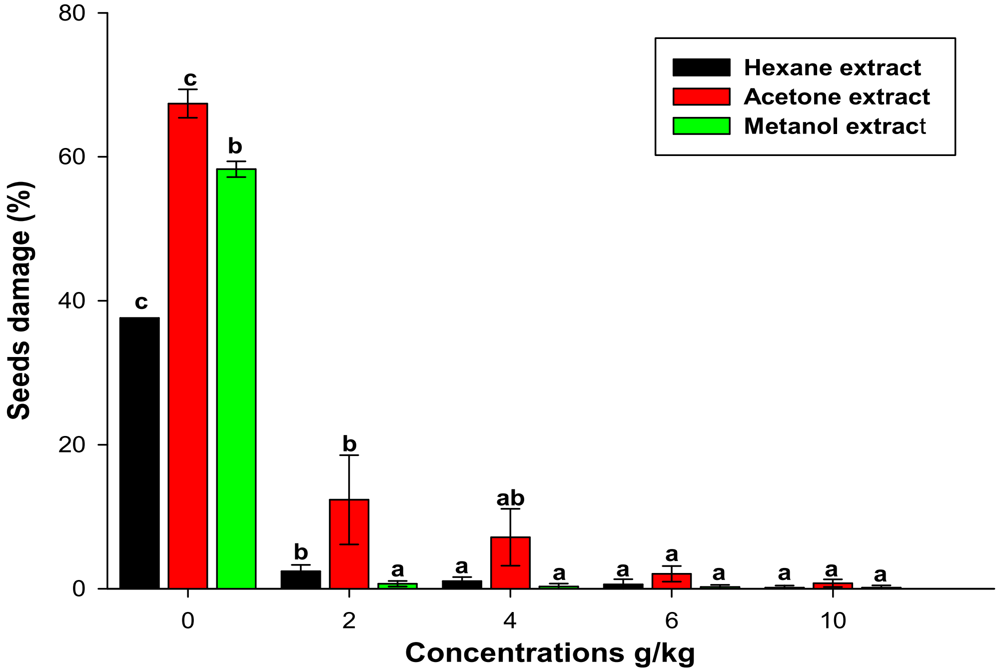

Fig. 2. Grain damage caused by *Sitophilus zeamais* on maize treated with four concentrations of each extract from *Hemizygia welwitschii*, and stored for three months

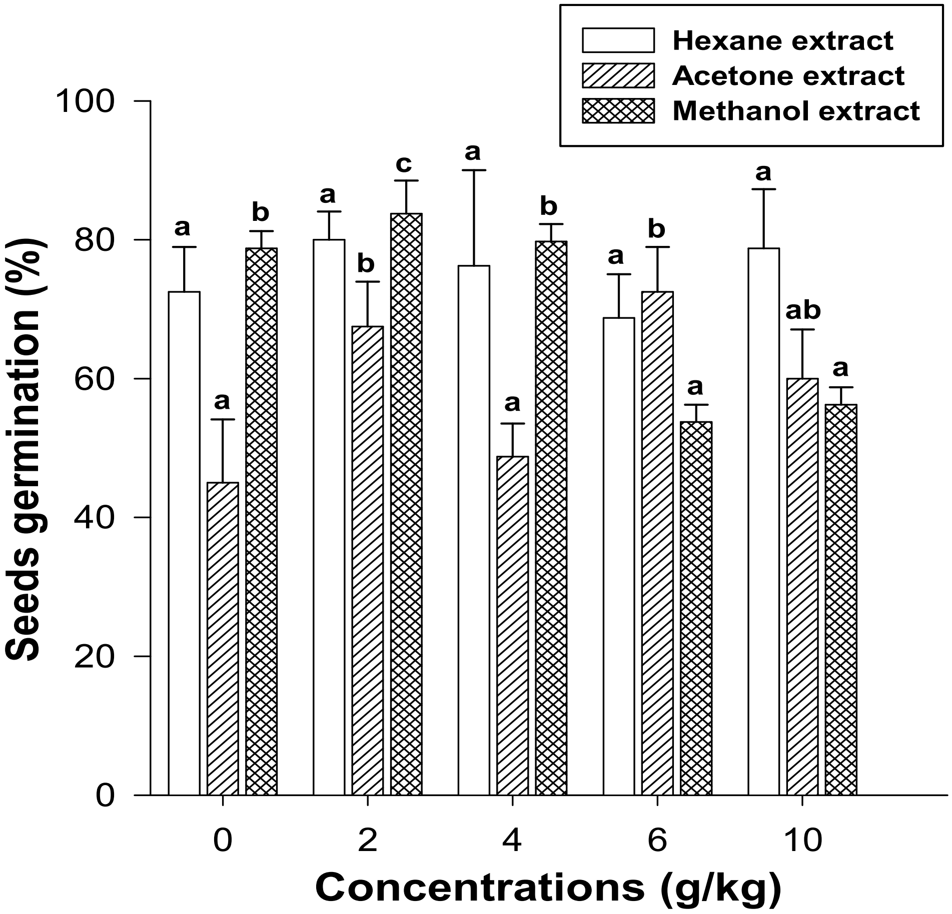

Fig. 3. Percentage of maize grains germination admixed with four concentrations of *Hemizygia welwitschii* hexane, acetone and methanol extracts

In this study, a very high potency of *Hemizygia welwitschii* leaf fractionated extracts was observed against *S. zeamais*. In fact, *H. welwitschii* leaf extracts caused mortality of adult *S. zeamais*, with the hexane extract inducing highest adult mortality of 100%. Moreira *et al*. [29] observed that, among the two solvents used for the extraction of *Ageratum conyzoides*, only the hexane crude extract showed insecticidal activity, with 76 and 88.67% adult *Rhyzopertha dominica* mortality within 4 and 24 h after the exposure, respectively. In addition to the presence of saponins and sterols, *H. welwitschii* hexane extract contains other secondary metabolites like terpenoids that exhibit considerable toxicity to insects [30] like antifeedant and/or lethal contact actions. This could explain the high activity of hexane extract from *H. welwitschii* against the *S. zeamais*. In fact, Kostyukovsky et al. [31] reported that terpenoids cause symptoms that suggest a neurotoxic mode of action of these secondary metabolites. The higher susceptibility of adults *S. zeamais* to the hexane extract of *H. welwitschii* leaf on treated maize within concentration of 4 g/kg agrees with the previous findings indicating that, 100% mortality of *C. maculatus* F. (Coleoptera: Chrysomelidae) adults were recorded with this same extract at 10 g/kg [18]. The efficacy of the *H. welwitschii* fractionated extracts used in this study could be attributed to the presence of secondary metabolites in each extract. Indeed, the plant secondary metabolites are responsible of diverse activities including their insecticidal properties [32]. The combination with the actions of saponins and sterols may explain the higher toxic effect of *H. welwitschii* leaf hexane extract against the maize weevil shown in the present work. Aniszewski [33] found that, alkaloids are the toxic secondary metabolites which can block ion channels, inhibit enzymes or interfere with neurotransmission, loss of coordination and death of insects. Alkaloid compounds were found only in methanol *H. welwitschii* leaves extract [18] and could be responsible for higher mortality caused by this extract in this study. In additional to causing mortality, Faraway [34] reported that in Biological Sciences when the coefficient of determination, $R^2 \geq 0.6$, then the favorable results are attributable to the insecticidal products used. In the present study most of the $R^2 \geq 0.6$. The few smaller values for the 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 mortality. Therefore, the three extracts were greatly responsible for the responses of *S. zeamais* on the treated maize. The χ^2 were generally no significant for all the extracts, suggesting that the obtained regression models approximate the theoretical model, concerning the toxicity of the used substances to *S. zeamais* [27].

One of the basic characteristics of an effective grain protectant is its ability to reduce progeny production in treated grains [35]. Adult emergence of *S. zeamais* was significantly affected by the three fractionated *H. welwitschii* leaf extracts. Inhibition percentage of F_1 progeny ranging between 96.65 and 100% (hexane extract), 30.86 and 82.33% (acetone extract) and 95.80 and 100% (methanol extract) were recorded in this study. These results are in agreement with previous studies that showed various plant extracts as valuable source of potential grain protectant against development of all life stages of a number of postharvest grain pests [17,36]. Fotso et al. [18] observed zero emergence of *C. maculatus* F_1 progeny in cowpea treated with *H. welwitschii* hexane extract at the concentration of 10 g/kg, while acetone and methanol extracts almost completely inhibited the F_1 *C. maculatus* progeny emergence. Acetone extract of *H. welwitschii* leaf achieved at least 82.33% of inhibition of F_1 *S. zeamais* at the concentration of 10 g/kg. This could be explained by the presence of phenolic compounds in this extract. The reduction in adult emergence could be also a result of high adult mortality of *S. zeamais* recorded in treated maize grains with each tested fractionated extract of *H. welwitschii*. In addition, active phytochemicals such as alkaloids have been found to disrupt growth and reduce larval survival by hindering loss of exoskeleton during larval development [37]. Other active principles such as isoflavonoids, flavonoids and terpenoids have also been reported to inhibit reproduction and fertility among coleopterans [38,39]. The associate effects of these secondary metabolites in hexane and methanol extracts could explained the higher percentage of inhibition of *S. zeamais* F_1 progeny recorded in the maize grains treated individually with these two fractionated extracts. Since the three fractionated extracts of *H. welwitschii* leaf possess these different phytochemicals, it could be concluded that, they also have inhibitory effects against the F_1 progeny of *S. zeamais*.

Results of grain protection showed that the three *H. welwitschii* extracts caused significant reduction in insect population growth of *S. zeamais* at all the concentrations after three months of storage. This may be due to the presence of saponins in each extract. In fact, saponins directly affect growth and reproduction of insect pests due to their repellent or deterrent activity. They increase mortality levels by lowering food intake and affecting movement of food in the insect gut due to toxicity and less digestibility [40]. The Mode of action for plant derivatives used for insect pests management is as contact poisons, ingestion or stomach poisons, feeding deterrences, repellents and confusants, which paralyze nerve activity, respiratory arrest, and act on the central and peripheral nervous system leading to convulsions and finally death of the insect victims [41,42].

The least grain damage in maize grains treated with the *H. welwitschii* leaf extracts after three months of storage confirmed that secondary compounds in the extracts of this plant produced repellent or antifeeding activity in adults *S. zeamais*. Since saponins decrease digestive capabilities by affecting microflora living in the insect gut, they form complexes with the digestive enzymes like proteases and thereby, apparently affect digestion process in the insect gut [43]. Saponins have many commercial applications due to their wetting, emulsifying and foaming properties [44]. These compounds also have insecticidal activities. They have been used in stored grains to minimize food grain damage and loss due to insect pests [43,45,46]. This could explained the lowest percentage of grain damage recorded in the maize treated with each tested extract in this study, since these secondary metabolites are present in the three *H. welwitschii* extracts.

The germination percentage of grain treated with fractionated extracts of *H. welwitschii* at different concentrations was highest and recommended for maize seed treatment. Pingsutiwong and Wattanakij [47] suggested that, percentage of seed germination in field corn (*Zea mays* Linn.) must not be lower than 75%. The results of this experiment clearly showed that *H. welwitschii* fractionated extracts generally conserved maize seed germination ability. Further, our study proved that there were no significant differences between control grain germination and those treated with hexane acetone and methanol extracts. The lack of adverse effect of hexane, acetone and methanol *H. welwitschii* fractionated extracts on seed germination is highly desirable for grain protection.

5. CONCLUSION

In conclusion, results of this study revealed that all the tested *H. welwitschii* leaf extracts exhibited insecticidal action against maize weevil, which was most susceptible to the hexane extract and did not have negative effect on the potential of seeds viability. In the light of the findings of present study, it could be stated that botanicals especially *Hemizygia welwitschii* fractionated extracts possess insecticidal properties against insects and have shown promising effects for plants and seeds protection, so these might be used in pests population management in crops and stored grains.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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