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Effects of *Hemizygia welwitschii* leaf extract fractions on postharvest infestation of maize by *Sitophilus zeamais* Motsculsky (Coleoptera: Curculionidae)

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Abstract

As part of on-going efforts to use eco-friendly alternatives to chemical pesticides, leaf powder of *Hemizygia welwitschii* was sequentially extracted in hexane, acetone and methanol. Bioassays were carried out to establish the most active fraction(s) against *Sitophilus zeamais* in maize. Maize grains (50 g) were treated with concentrations within the range 2, 4, 6, and 10 g/kg of extract and *Azadirachta indica* seed oil (positive control) in the laboratory. The total number of progeny emerging from grains infested separately with *S. zeamais* eggs, larvae and pupae were recorded. Adult mortality counts were carried out 1, 3, 7 and 14 d post-exposure. Acetone extract was more toxic to the eggs, larvae and pupae than the other extracts, inhibiting progeny production by 90.90%, 88.10% and 100%, respectively, at the concentration 10 g/kg. For the same concentration, *A. indica* seed oil reduced progeny production by 100% for eggs, 96.08% for larvae and 70.93% for pupae. Hexane extract was more potent to the adult weevil than the other extracts, recording 100% mortality for the concentration 10 g/kg within 14 d. LC_{50} values were 0.78 (Hexane), 5.52 (acetone) and 1.69 g/kg (methanol). Extracts of *H. welwitschii* leaves had sufficient efficacy to be a component of storage pest management package for *S. zeamais*.

Key words: Leaf powder, Mortality, Grain damage, Pest management

1. Introduction

Maize (Zea mays L.) is a staple food for a large proportion of the world with significant economic importance. It is currently the third most-cultivated and traded cereal after wheat and rice (FAO, 2006). The highest amounts of maize consumed as food are found in Southern Africa at 85 kg/capita/year as compared to 27% in East Africa and 25% in West and Central Africa (Smale *et al.,* 2011). The crop is characterized by the diversity of its consumption forms: fresh, boiled, roasted, and "foufou" (Ndjouenkeu *et al.,* 2010). A world challenge is to increase the global maize production to feed nine billion people by 2050 (Godfray *et al.,* 2010).

The production and storage of maize have faced many constraints throughout developing countries such as scarcity of rain, diseases and lack of inputs (Brisibe *et al.,* 2011), and most important constraint being the field-to- store infestations of maize weevil *Sitophilus zeamais* (Coleoptera: Curculionidae) (Akob and Ewete, 2007). This insect inflicts severe damages leading to weight loss and reduction of the economic value, grain viability and nutritive value of maize (Akunne *et al.,* 2013). According to Obeng-Ofori and Amiteye, (2005) and Yuya *et al.,* (2009), about 20 to 40% of maize grain is lost due to the attack of *S. zeamais*. The control of insect pests of stored products in general, relies mainly on the use of residual synthetic chemical insecticides. This practice, although effective in the fight against insects, is toxic to consumers, pollutes the environment and induces resistance in pests (Arnaud *et al.,* 2001). The use of increasing amounts of pesticides represents a real danger since it leads to the stage where the insecticide is completely ineffective against the pest. Also, obsolete synthetic chemicals are found in our local markets, which cause serious health hazards (Bambara and Tiemtoré, 2008).

Alternative solutions to the application of synthetic chemicals is the use of phytochemicals (reduced-risk insecticides of plant origin) which is presently being encouraged in stored grain protection because there are more biodegradable, and thus may pose less environmental hazards. *Hemizygia welwitschii* Rolfe Ashby (syn. *Orthosiphon welwitschii* Rolfe) (Lamiaceae) is a bushy aromatic perennial herb, widely available in the Adamawa region of Cameroon, and used in folk medicine for the treatment of skin diseases (Ngassoum *et al.,* 1999). The essential oil is known to possess antibacterial properties and repellence activity against mosquitoes (Oyedele *et al.,* 1992, 2000). The chemical composition of the essential oil from the powdered leaf of *H. welwitschii* harvested in Ngaoundere, Cameroon, were mainly 1-octen-3-01 (14.1%), 3-octanol (4.5%) and linalool (2.6%) (Ngassoum *et al.,* 1999). To date, no scientific publication has reported the efficiency of *H. welwitschii* leaf extracts on stored product insect pests. The study was therefore aimed at determining the most active fraction of *H. welwitschii* against the eggs, larvae, pupae and adults of *S. zeamais*.

2. Materials and Methods

2.1. Collection and extraction of *Hemizygia welwitschii*

Fresh leaves of *H. welwitschii* were collected from the surroundings of University of Ngaoundere in the Vina Division, Adamawa region, Cameroon between August and November 2016 and shade-dried naturally at the room temperature for five days where they became crisp dry. The identity of the plant was confirmed at the Cameroon National Herbarium in Yaounde, where a voucher specimen (Serial number: 6910/SRFK) was deposited. The dried leaves were crushed in a mortar until the powder passed through a 0.4 mm mesh sieve. The powder was stored in a deep-freezer at the temperature of -18°C until needed for bioassay.

Two thousand three hundred grams of *H. welwitschii* powder were mixed with 7.5 L of hexane and stirred for 30 min and allowed to stand for 24 hours in the laboratory of the Institute of Medical and Medicinal Plant Research (IMPM), Yaounde, Cameroon, and then re-stirred again. After 48 hours the mixture was then filtered with a filter paper (Whatman no. 1). The residue obtained after filtration was put through the process above 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 extraction and followed by methanol extraction. The filtrates obtained with hexane, acetone and methanol were then separately concentrated in a Rotavapor at 70°C, 60°C and 65°C, respectively at 120 rpm. Extracts were stored in a refrigerator at 4 °C until needed for bioassay. *Azadirachta indica* seed oil from the study of Tofel *et al.,* (2016), which was stored in a deep freezer at -18°C, was used as a positive control.

2.2. Insects

The parent adults of *S. zeamais* for the adult toxicity test were obtained from colonies maintained at the Applied Zoology laboratory of the University of Ngaoundere while those for the immature stages toxicity were taken from colonies maintained at JKI, Institute for Ecological Chemistry, Plant Analysis and Stored Products Protection, Berlin since 2007 and 1968, respectively. In Ngaoundere, *S. zeamais* were reared on Shaba maize variety while in Berlin they were reared on Ricardino variety.

2.3. Bioassays

For the adult mortality bioassay, four different masses of 0.1, 0.2, 0.3 and 0.5 g each of the hexane, acetone and methanol extracts were separately mixed with 50 g grains in glass jars, which corresponded to the concentrations of 2, 4, 6 and 10 g/kg, respectively. The negative controls consisted of grains with solvent alone. The content of each jar was hand-shaken properly to ensure complete coating of the grains with the extracts. The grains were then air-dried for 2 hours to evaporate the solvent. Groups of 20 *S. zeamais* were added to glass jars containing treated or untreated maize. Glass jars were securely covered with muslin cloth and were tightly held in place with rubber bands to ensure adequate ventilation. All treatments were arranged in a completely randomized design on shelves under fluctuating laboratory condition in Ngaoundere, Cameroon and each treatment had four replications. Mortality was recorded 1, 3, 7 and 14 days after treatment. Insects were considered dead when no movement was observed after touching them with forceps twice within two or three minutes.

Concerning the toxicity test on immature stages, individual lots of 50 g maize grains in 250 ml glass jars, containing eggs, different larval stages or pupae of *S. zeamais* were coated with *A. indica* seed oil (positive control) or the hexane, acetone and methanol extracts at the rates 1, 4 and 10 g/kg, with the aid of a rotatory shaker. The jars were closed with perforated metal lids. The negative controls consisted of grains with solvent alone. The grains were then dried for 10 min in a ventilated fume chamber to evaporate the solvent. The treated and untreated grains, which were replicated four times were keep in a controlled environment at 25 ± 1°C and 65 – 70% r.h. in a complete randomized design in Berlin, Germany for F_1 progeny emergence. All the F_1 progeny were counted.

2.4. Data analysis

Data on % cumulative corrected mortality and % reduction in F_1 progeny, were arcsine [(square root(x/100)] transformed to homogenise the variance. The transformed data were subjected to the ANOVA procedure using the Statistical Analysis System (version 9.2). Tukey (HSD) test (P = 0.05) was applied for mean separation**.** Probit analysis (Finney, 1971) was applied to determine lethal concentrations causing 50% (LC_{50}) mortality of *S. zeamais* at 1 and 7 days, after treatment application. Abbott's formula (Abbott, 1925) were used to correct for control mortality before probit analysis and ANOVA.

3. Results

3.1. Toxicity to adult

All the *H. welwitschii* extract fractions generally caused significant mortality to adult *S. zeamais* compared to the control. Mortality increased with ascending content levels and time exposure, irrespective of extract fractions. Overall, significant difference was observed among the fractions. Hexane extract was more potent to the adult weevil than the other extracts, recording the maximum 100% mortality for the concentration 10 g/kg within 14 days post infestation. Within the same time and at the same dosage, acetone and methanol extracts recorded respectively 64.87 and 75.98% adult mortality. Within 1day after infestation the methanol extract caused higher mortality of 16.25% at the dose of 10 g/kg. The lowest tested dose (2 g/kg) achieved 63.32, 51.91 and 83.14% *S. zeamais* adult mortality respectively for the methanol, acetone and hexane fractions within14 days post-exposure.

The results of the evaluation of toxicity of the different extract fractions of *H. welwitschii* s are shown in Table 1. All the extracts proved to be toxic to adult *S. zeamais* although the acetone fraction was less effective with the LC_{50} of 410.18 g/kg one day after treatment. Adult S. *zeamais* was more susceptible to the hexane fraction with LC_{50} of 0.78 g/kg (7 day) followed by the methanol fraction with LC_{50} of 1.69 g/kg (7 day). At day one, the slope of the hexane extract (4.55 ± 0.74) was steeper than that of methanol (1.89± 0.24) and acetone (0.83 ± 0.21) extracts while they seemed similar at seven days. In general, the coefficients of determination (R^2) of the extracts were between 0.60 and 0.94. The values of chi-square (Chi^2) were not significant for all the extracts except the methanolic fraction after day one exposure.

Tab. 1 Toxicity of leaf extracts from *Hemizygia welwitschii* to adult *Sitophilus zeamais* in maize grains at different exposure periods

Extract	Slope ± S.E.	R^2	LC_{50} (g/kg)	Chi^2
Day 1				
Hexane	4.55 ± 0.74	0.70	19.04	17.31ns
Acetone	0.83 ± 0.21	0.72	410.18	15.27ns
Methanol	1.89± 0.24	0.84	34.70	30.79***
Day 7				
Hexane	0.96 ± 0.14	0.60	0.78	6.47ns
Acetone	1.15 ± 0.13	0.83	5.52	4.90ns
Methanol	1.02 ± 0.13	0.69	1.69	12.26ns

Ns $P > 0.05$, ***$P < 0.001$

3.2. Inhibition of offspring production from eggs and immature stages

Table 2 shows the result of ability of *H. welwitschii* extract to inhibit the emergence of progeny in grains containing eggs, larvae and pupae of *S. zeamais*. All the extracts significantly influenced the production of the weevil (P < 0.05). Overall, the bioefficacy of these extracts on the eggs and immature stages was dose-dependent. *A. indica* seed oil was more efficient in inhibiting the development of eggs at all doses (100% inhibition). Acetone extract was more toxic to the eggs, larvae and pupae than the other extracts, inhibiting progeny production by 90.90%, 88.10% and 100%, respectively, at the concentration 10 g/kg. Contrariwise, hexane fraction had less affects the eggs, larvae and pupae development, reducing them by only 25%, 33.75% and 52%, respectively, at the concentration 10 g/kg. Methanol extract was most effective on pupal stage (73.50% inhibition) at its lowest dose (1 g/kg) than at its highest content of 10 g/kg with 58.50 % emergence reduction.

Tab. 2 Inhibition of adult emergence in grains containing eggs and immature stages of *Sitophilus zeamais* and treated with extracts from the leaves of *Hemizygia welwitschii*

Product	Dose (g/kg)	Inhibition of offspring production from three life stages (%)		
		Egg	Larva	Pupa
Neem seed oil	1	100 ± 0.00 [a]	43.50 ± 6.95	70.25 ± 8.33 [b]
Hexane	1	8.50 ± 5.06 [c]	37.25 ± 11.95	49.75 ± 6.12 [b]
Acetone	1	61.00 ± 16,47 [b]	32.00 ± 4.56	95.50 ± 2.63 [a]
Methanol	1	83.50 ± 6.06 [ab]	30.75 ± 4.61	73.50 ± 4.87 [b]
$F_{3, 12}$		15.50***	0.45ns	17.09***
Neem seed oil	4	100 ± 0.00 [a]	82.25 ± 3.92 [a]	89.00 ± 3.00 [b]
Hexane	4	8.25 ± 8.25 [b]	15.00 ± 9.00 [b]	38.75 ± 8.34 [c]
Acetone	4	88.75 ± 6.57 [a]	37.50 ± 6.59 [b]	100 ± 0.00 [a]
Methanol	4	39.00 ± 18.73 [b]	16.75 ± 11.31 [b]	63.50 ± 8.51 [c]
$F_{3, 12}$		15.85***	14.86***	34.90***
Neem seed oil	10	100 ± 0.00[a]	96.25 ± 1.65 [a]	72.25 ± 5.50 [b]
Hexane	10	25.00 ± 10.16[b]	33.75 ± 14.30 [b]	52.00 ± 1.15 [c]
Acetone	10	93.75 ± 6.25 [a]	91.00 ± 4.12 [a]	100 ± 0.00 [a]
Methanol	10	79.50 ± 7.35 [a]	25.50 ± 6.33 [b]	58.50 ± 1.70 [c]
$F_{3, 12}$		23.74***	20.19***	109.79***

ns < P > 0.05, ***$P < 0.001$

Means ± S.E. followed by the same letter in a column do not differ significantly at $P < 0.05$ (Tukey's test). Each datum represents the mean of four replicates.

4. Discussion

Plant products and their secondary metabolites are receiving increasing attention in stored product management (Zettler and Arther, 2000). Several researchers have evaluated the insecticidal, repellent or antifeedant and development inhibiting effects of various plant parts and plant extracts on *S. zeamais* with varying degrees of success (Arannilewa et *al.,* 2006). Boulogne *et al.,* (2012) mentioned that, 656 plant species worldwide, distributed into 110 families, were identified to have a significant insecticidal activity. The most cited family is the Lamiaceae in which *H. welwitschii* belongs, with 181 species distributed into 48 genera, counting for 28 % of the plant families with an insecticidal activity. In the present study, the different extracts of *H. welwitschii* which caused significant mortality to *S. zeamais s*uggests that they contain insecticidal compounds. Compounds like linalool known for insecticidal activity and present in the leaves as reported by Ngassoum *et al.* (1999) may have played a significant role. The hexane extract was the most effective against adult *S. zeamais.* This could be speculated that the fraction may possess compound with high insecticidal potency. Kosini *et al.* (2015) reported similar results with *Ocimum canun,* plant of the same family. They mentioned that the high mortality caused by hexane extract may be partially related to the gummy aspect of the hexane extract, which acetone and methanol extracts lacked. The extract may be glued to the insect's wing cover and legs and hindered mobility. The insect thus lost vigor by trying to get loose and when combined with the toxic effects of compounds in the leaf extracts, may lead to the death of *S. zeamais*. The essential oil of the studied plant was repellent against mosquitoes preventing them from feeding (Oyedele *et al.,* 2000). This repellency property may have averted the maize weevils in the present study from food intake and death occurred by starvation since mortality was time dependent. More studies are needed to elucidate the active ingredients in each fraction and to understand the mode of action of these against insect pests of stored products. Also, microscopic observations will help to clarify how and where the glue of the extract fixes on the insect.

One of the basic characteristics of an effective phytochemical is its ability to reduce progeny emergence in treated grains (Khoshnoud et al., 2008). Results of inhibition of progeny production showed that extract fractions from *H. welwitschii* inhibited adult emergence of *S. zeamais,* showing their ability to control the development of the insect. The fractions might have acted physically by asphyxiation or chemically on eggs or immature stages, depending on the compounds present in each extract. The coating of the grains with extracts might have prevented the eggs from adhering unto the grains. Afful *et al.,* (2012) indicated that the methanol extract of root of *Securidaca longipendonculata* inhibited the development of eggs and larvae of the maize weevil. This reduction in emergence is an indication of the presence of ovicidal and larvacidal compounds which need to be determined.

The results of the present investigation based on the laboratory experiments, revealed that the extracts of *H. welwitschii* leaves had sufficient efficacy to be a component of storage pest management package for *S. zeamais,* especially for low income farmers since the plant species are cheap and widely available.

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Chemical properties and efficacy of Sweet orange essential oil nanoemulsion applied as cold aerosol against two stored product beetles

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463
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463

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