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Evaluation of the insecticidal properties of fractionated extracts of *Ocimum canum* and *Laggera pterodonta* on stored maize against the infestation of *Sitophilus zeamais* (Coleoptera: Curculionidae)

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Abstract

The weevil, *Sitophilus zeamais* (Motschulsky), is a devastating pest of stored maize, especially in the warm humid tropical regions. The objective of this study was to test the insecticidal properties of fractionated *Ocimum canum* and *Laggera pterodonta* extracts used as natural alternatives to synthetic insecticides in the management of maize *S. zeamais* weevils. Fractionated extracts were obtained by maceration of the leaves of *O. canum* and *Laggera pterodonta*. Cyclohexane, acetone and methanol were used as extraction solvents and the tests were carried out at 1, 5 and 10 g/kg on adult *S. zeamais*, for mortality, progeny production inhibition, feeding deterrence and repellence with a control that received no extract treatment. Adult mortality was recorded within 1, 7 and 14 days post-exposure. All the fractions showed insecticidal activity that increased with dosage and period of post-treatment. At 10 g/kg the *Laggera* methanol fraction caused a mortality of 20 % within 14 days of exposure, and a progeny production inhibition rate of over 84 %. *Laggera* Cyclohexane fraction (5 g/kg) that showed repellence index of 75.44 % greater than neem oil (*Azadirachta indica*) that served as the positive control referenced plant.

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Introduction

Maize (*Zea mays* L.) is an important cereal and widely cultivated crop in the tropics; both for human consumption and as livestock feed. After wheat and rice, maize is the third most grown cereal (Lyon, 2000). The United States, China, Brazil and Mexico account for 70 % of global production. Africa accounts for about 7 % (49 million tons) of global (736 million tons) maize production (FARA, 2009). It is estimated that by 2050, the demand for maize in developing countries will double, and by 2025 maize will be the crop with the greatest production globally as well as in developing countries (CIMMYT and IITA, 2010).

Storage is an important aspect of food security and sustainable food crop production in poor countries. In the Sahelian Regions for instance, where they have long dry season, post-harvest storage is very important (Mikolo *et al.,* 2007) since it assures the future availability of consumable and marketable food as well as seedlings (Ngamo and Hance, 2007). Increasing the supply of the maize crop is marred by losses during storage caused principally by insects and fungi, with losses due to insects being the highest (Ngamo *et al.,* 2007) especially *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) (Dobie *et al.,* 1984). Losses due to *S. zeamais* infestation on untreated maize in storage could get up to 80 % in five months (Nukenine *et al.,* 2002; Ngamo *et al.,* 2007).

Together with other insects weevils cause an estimated 24.5 % loss of maize with untold damage (Asawalam *et al.,* 2008; Napoleao *et al.,* 2013). Methods involved in the control of these pests include: biological control, the hygienic state of storage structures, physical, chemical (synthetic organic pesticides), varietal resistance, integrated and the use of botanicals (CIMMYT and IITA, 2010).

The life cycle shows the female of *S. zeamais* copulates immediately after emergence and starts laying eggs. It takes about 6 weeks to be completed (Throne and Eubanks, 2002; Hill, 2008). A female may lay a total of 150 to 400 eggs during her life span (Bosque-Perez, 1992).

Synthetic organic pesticides promote faster development of resistance, destroy natural enemies, harm other non-target species and contaminate food. The development of strategy to ensure food safety still remains a global challenge most especially in sub-Saharan Africa where a third of the population is under fed (FAO, 2006).

There is a renewed interest in the search for natural alternative anti-pest agents. Plant-derived pesticides (botanicals) can be used for crop protection, especially for small-scale farmers (Maribet *et al.,* 2008). Plants are the sources of natural pesticides that provide excellent leads for new pesticide development (Vetrivel *et al.,* 2009; Napoleao *et al.,* 2013) . *Ocimum canum* Sims. (Lamiaceae) is a semi-perennial plant found in Africa and Asia (Vieira *et al.,* 2003). It has a wide range of culinary (Ngassoum *et al.,* 2004), medicinal (Nyarko *et al.,* 2002; Ngassoum *et al.,* 2004; Naghibi *et al.,* 2005) and cosmetic properties (Ngassoum *et al.,* 2004) while *Laggera pterodonta* L. (Asteraceae) spread throughout the sub-Saharan Africa and the tropical countries of Asia, especially Southeast Asia (Burkill, 1985; Wu *et al.,* 2011), and has medical and insecticidal properties properties (Njan Nlôga *et al.,* 2007; Ngamo *et al.,* 2007).

Njan Nlôga *et al.* (2007) results showed that the aptitudes of essential oils of both *L. pterodonta* and *O. canum* can be used as insecticides against *Anopheles gambiae*. The insecticidal properties of essential oils, aqueous and methanolic extracts and powders of *O. gratissimum* and *O. basilicum* have been proven in the laboratory to control *S. zeamais*, *Tribolium castaneum* and *Callosobrochus maculatus* (Martins *et al.,* 1999; Osei-Akrasi, 1999; Owusu *et al.*, 2008).

In this work, fractionated extracts of *O. canum* (Lamiaceae*)* and *L. pterodonta* (Asteraceae) in Cyclohexane, acetone and methanol (apolar, intermediate and polar solvents respectively) were evaluated for their insecticidal properties on the protection of maize against the infestation of *Sitophilus zeamais*. Specifically we evaluated: insect mortality, progeny production control, repellence and feeding deterrence.

Materials and methods

Elimination of infestation and moisture balance of seeds

Maize seeds (variety: Shaba) were obtained from the Dang local market in the Ngaoundere III district, Cameroon. The seeds were washed in distilled water and sterilized in 70 % ethanol and kept in a freezer at -20°C for 20 days and later kept in the laboratory for two weeks for acclimatization (Prates *et al.,* 1998; Akob and Ewete, 2010) The moisture content after acclimation of the seeds was 12.08 %, determined using the method of AFNOR (1982). All tests were carried out under the following laboratory conditions: t≈25.21 ± 1.83°C, r.h.≈ 72.72 ± 3.98 %.

Insect rearing

Adults of *S. zeamais* were obtained from infested untreated maize from farmers' stocks in the Dang residence of Ngaoundere III District, Cameroon. The weevils were reared on disinfested maize in 900-mL glass jars. 30 adult weevils were introduced into the jars, and kept under laboratory conditions (temp.: 17.3–28.8°C and relative humidity: 56.3–97.8 %) recorded with a Voltcraft temperature and humidity data logger (model DL-121TH, Voltcraft, Hirsham, Germany). The adult weevils were removed after a 2-week ovipositional period by sieving the grains through a 2 mm mesh, locally made sieve (Ngamo *et al.,* 2001)[23]. The glass jars containing the infested maize (eggs and immature stages) were kept in the same laboratory conditions until the adults emerged.

Collection and preparation of Ocimum canum and Laggera pterodonta leaf powders

Young green leaves of *O. canum* were collected in December 2014 at Mogodé in the Mayo-Tsanga Division of the Extreme-North Region of Cameroon, while *Laggera pterodonta* was collected in January 2015 at the Dang district of the University of Ngaoundere Campus. The plant was identified at the National herbarium in Yaounde, Cameroon were the identification voucher number of 11612SFR/CAM and 11615/SFR/CAM respectively. These were dried at room temperature for 7 days and crushed in a traditional wooden mortar. The powder obtained was then sieved through a 0.4 mm mesh sieve and then stored by deep freezing at -20°C, until need for solvent extraction.

Obtaining extracts of O. canum and L. pterodonta

Cyclohexane (apolar), acetone (intermediate) and methanol (polar) extracts were prepared by maceration of 2 kg of *O. canum* powder in 7.5 L of cyclocyclohexane for 48 hours and filtered with the use of Whatman No 8 filter paper. The residue was re-filtered and the filtrate dried for 10 hours at room temperature of 25 ± 1 °C in the laboratory. The residue left was used for acetone extraction followed by methanol. This was in an effort to extract all the active soluble chemicals present with the help of the three solvents. The filtrates obtained with cyclohexane, acetone and methanol were separately concentrated in a rota vapour at 70 °C, 60 °C and 65 °C respectively, at 120 r.p.m (Kaushik & Vir, 2000). The same process was carried out for *Laggera pterodonta*. After complete evaporation of the solvents, the different fractions of the extracts were weighed, stored in air-tight containers and kept in a refrigerator at 5°C till needed for bioassays.

Toxicity tests

Mortality

Three different concentrations of the extracts (1, 5 and 10 g/kg of grain: w/w + 1 ml respective solvents) were added separately to 25 g of maize in 500 ml glass jars and manually stirred for 2 minutes. The jars were then left exposed to air in the laboratory for 45 minutes to permit complete evaporation of the solvents. Maize grains treated with only 1 ml of the solvents served as negative controls. After complete evaporation, a group of 20 unsexed insects (Nukenine *et al.,*2010), aged between 7-14 days were added to each glass jar and covered with a porous cloth to allow air circulation, with a metal lid. Each treatment was repeated 4 times. Mortality indices were evaluated after 1, 7 and 14 days. The calculation of mortality rate was corrected for control mortality according to Abbott's formula:

$$Mc = (Mo - Mc/100 - Me) \times 100$$

Where, Mo = Observed mortality rate of treated adults (%), Me = mortality rate of control (%), and Mc = corrected mortality rate (%).

F_1 progeny test

On the 14th day, all the live as well as dead insects were removed and the glass jars closed and kept in the same ambient conditions for F*1* progeny assessment. The recording of F1 progeny was done once a week for 5 weeks commencing 6weeks post-infestation (Nukenine *et al.,* 2007).

Percentage reduction in adult emergence or inhibition rate (% IR) was calculated as

$$\%IR = (C_n - T_n)100/C_n,$$

where C_n is the number of newly emerged insects in the untreated (control) jar and T_n is the number of insects in the treated jar.

Repellency test

The repellent activities of the extracts were investigated using a method modified from that by Maribet *et al.*, 2008 in arenas consisting of two plastic containers (350mL linked using 150mm transparent pipes). At one end of the linear olfactometer was attached a 350 ml plastic bottle containing maize treated separately with the respective extracts (1, 5 and 10 g/kg) or with Neem oil as the check, and at the other end was maize treated only with 1 ml of the respective solvents (acetone, cyclocyclohexane or methanol). A hole in the middle tube was used to introduce 20 unsexed insects delicately with the use of forceps. Thereafter, the hole was covered with transparent adhesive tape. The set up was kept in the dark for two hours for the insects to make a choice of which direction to move to. Only insects in the bottles were considered to have made a choice. The percentage repellence was calculated using the formula by Talukder and Howse (1995) given by PR=2 × (C-50) where: C is the percentage of insects in the negative control bottle. The results were interpreted following the scale by McDonald *et al.* (1970).

Feeding deterrence

Maize flour cylindrical disks were prepared using the method of Xie *et al.* (1996) and used as test food. Only a unique concentration of 10 g/kg of the cyclocyclohexane and methanol extracts were used with neem oil as check. Negative control disks were prepared by mixing the flour with 1 ml of the respective solvents using a micro syringe and then air-dried for 24 h; first in the cylindrical tubes used and later on petri dishes. Each treatment had four repetitions. To monitor the hygroscopic nature of maize flour, similar set ups were made with untreated corn flour and weighed before and after the test to check the quantity of water absorbed. Maize flour disks were then weighed placed into petri dishes and 10 unsexed weevils aged up to 24 hours were introduced into each petri dish; the disks were reweighed after 72 hours. Food consumption of weevils was recorded under two conditions: 1) composed untreated disks (C); 2) on the treated (T) disk. According to the amount of food consumed in the three different treatments (C, T, and CT) the feeding deterrent activity coefficients was calculated using the formula by Isman *et al.,* 1990 as follows:

Feeding Deterrence Index (%) = (C - T/C + T)*100

The results were then interpreted as by Xie *et al.* (1991).

Data analysis

Data on % cumulative mortality, and % reduction of adult emergence were arcsine-transformed ($\sin^{-1}\sqrt{(x/100)}$) while the number of *F1* progeny produced was transformed ($\sqrt{(x+0.5)}$). Where x = number of individuals %. The transformed data were subjected to two way ANOVA procedure using the Statistical Analysis System (Zar, 1999; SAS Institute, 2003). Mean separation was done using Tukey Studentized Range HSD test (P = 0.05) and the student *t* test. Bar charts were drawn using Microsoft Excel 2010 software. All values were represented as mean ± SE.

Results and discussion

Toxicity tests

Generally, mortality increased with concentration and duration of exposure (Tables 1and 2). The differences in bioactivity between whole plant extracts as well as fractions were usually as a result of the various compounds present in the extracts. Rahman *et al.* (2006) demonstrated that compounds present in plant extracts may independently or jointly contribute to cause toxic and repellent (as well as inhibitory and anti-feedant) action against *S. oryzae*.

Never the less, the relatively high mortality of *S. zeamais* observed with *O. canum* could be attributed to elevated concentrations of oxygenated monoterpenes as well as sesquiterpenes (Ngassoum *et al.,* 2001 ; Ngamo *et al.,* 2007a ; Nukenine *et al.,* 2010a).

Vaddi *et al.,* 2002 proved the solubility of cervacrol, linalool and α-terpineol in ethanol. This could explain the general high mortality experienced in the methanol fractions (Tables 1 & 2).

Table 1. Mortality (Mean ± S.E) of *Sitophilus zeamais* due to treatment of maize seeds with fractionated extracts of *Ocimum canum*.

Exposure period (days)	Concentration (g/kg)	Mortality			F
		Ocim Hex	Ocim Acet	Ocim MeOH	
	0	0.00± 0.00	0.00 ± 0.00	0.00± 0.00	/
1	1	0.00 ± 0.00	0.00 ± 0.00	1.25 ± 1.25	1.00^{NS}
	5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	/
	10	0.00 ± 0.00	0.00 ± 0.00	1.25 ± 1.25	1.00^{NS}
	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	/
7	1	2.50 ± 1.44	0.00 ± 0.00	6.25 ± 2.39	3.80^{NS}
	5	2.50 ± 1.44	5.00 ± 2.04	2.50 ± 1.44	0.49^{NS}
	10	3.75 ± 2.39	5.00 ± 3.54	15.00 ± 0.00	3.98^{NS}
	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	/
14	1	7.50 ± 2.50	10.00 ± 3.54	12.50 ± 1.44	1.14^{NS}
	5	11.25± 5.15	15.00 ± 2.04	10.00 ± 2.04	0.63^{NS}
	10	11.25 ±2.39	15.00 ± 4.56	17.50 ± 1.44	1.04^{NS}

NS: P>0.05. Each datum represents the mean of four replicates of 20 insects each.

Table 2. Mortality (Mean ± S.E) of *Sitophilus zeamais* due to treatment of maize seeds with fractionated extracts of *Laggera pterodonta*.

Exposure Period (days)	Concentration (g/kg)	Mortality			F
		Lag Hex	Lag Acet	Lag MeOH	
	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	/
1	1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	/
	5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	/
	10	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}	10.00 ± 3.54^{A}	8.68^{**}
	0	0.00 ± 0.00	0.00 ± 0.00	0.00± 0.00	/
7	1	0.00 ± 0.00^{B}	10.00 ± 2.89^{A}	1.25 ± 1.25^{B}	14.64^{**}
	5	1.25 ± 1.25^{B}	11.25 ± 4.73^{A}	1.25 ± 1.25^{B}	6.35^{*}
	10	3.75 ± 2.39^{B}	$12.50\pm .23^{AB}$	15.00 ± 2.04^{A}	5.70^{*}
	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	/
14	1	3.75 ± 1.25^{B}	11.25 ± 2.39^{AB}	15.00 ± 3.54^{A}	5.63^{*}
	5	7.50 ± 1.44^{B}	15.00 ± 3.54^{A}	5.00 ± 3.54^{B}	3.36^{*}
	10	8.75 ± 2.39^{B}	17.50 ± 1.44^{AB}	20.00 ± 3.54^{A}	6.06^{*}

NS P>0.05;*P<0.05; **P<0.001. Each datum represents the mean of four replicates of 20 insects each. NB: Means with the same letter in a column are not significantly different after comparison with Tukey's Test.

F_1 progeny production

All the extracts and their fractions showed good inhibitors of progeny emergence (Table 3). For the inhibition of progeny production monoterpenes found in essential oils of these plants have been proven to inhibit reproduction in insects (Regnault-Roger *et al.,* 1993; Adjou *et al.,* 2012; Egharevba *et al.,* 2012), while alkaloids inhibit larval growth and development (Ngamo *et al.,* 2001).We could guess that these active substancesmight be more in *Ocimum* cyclohexane than in the other extracts.

Table 3. Pooled F_1 progeny production of adult *Sitophilus zeamais* on maize seeds treated with fractionated extracts of *Ocimum canum* and *Laggera*.

	Concentration (g/kg)	*Ocimum canum*	*Laggera pterodonta*	t
Mean number of F1 adults progeny	0	32.58 ± 2.40^{a}	32.58 ± 2.40^{c}	/
	1	21.83 ± 2.40^{b}	19.75 ± 1.84^{b}	0.69^{ns}
	5	9.17 ± 1.45^{c}	11.50 ± 1.53^{a}	1.10^{ns}
	10	6.25± 1.19^{c}	8.42 ± 0.99^{a}	1.39^{ns}
F		41.27***	35.29***	
Mean		12.42 ± 1.51	13.22 ± 1.16	0.42^{ns}
% Inhibition of adult emergence	0	0.00 ± 0.00^{c}	0.00 ± 0.00^{a}	/
	1	30.00 ± 7.87^{b}	36.94 ± 7.02^{b}	0.66^{ns}
	5	70.75 ± 4.79^{a}	63.37 ± 5.52^{c}	1.01^{ns}
	10	79.14 ± 4.03^{a}	73.09 ± 3.67^{c}	1.11^{ns}
F		60.53***	64.62***	
Mean		59.96 ± 4.88	57.80 ± 4.05	0.34^{ns}

Means ± S.E. in the same column for the same category of insecticide, followed by the same letter do not differ significantly at P = 0.05 (Tukey's test). Each datum represents the mean of four replicates of 20 insects each. ns: not significant (P>0,05) ; *: significant (P<0,01) ; ***: highly significant (P<0,001).

Table 4. Percentage Feeding Deterrence Indices (Mean ± S.E) of *Sitophilus zeamais* due to treatment of maize flour discs with fractionated extracts of *Ocimum canum*, *Laggera pterodonta* and *Azadirachta indica* Oil as the check.

Extract	Concentration (g/kg)	PFDI	Designation	Interpretation
Ocimum Cyclohexane	0	0.00 ± 0.00	+	Inactive
	10	19.18± 5.28	+	Inactive
Ocimum Methanol	0	0.00 ± 0.00	+	Inactive
	10	12.34± 3.28	+	Inactive
Laggera Cyclohexane	0	0.00 ± 0.00	+	Inactive
	10	5.68 ± 2.43	+	Inactive
Laggera Methanol	0	0.00 ± 0.00	+	Inactive
	10	16.60±2.36	+	Inactive
Neem Oil	0	0.00 ± 0.00	+	Inactive
	10	27.60± 3.02	+	Active

An index T of 50-100 is designated ++, and of 0-50 +; PFDI: percentage feeding deterrence index. PFDI<30: substance inactive.

Feeding deterrence

Except for the check, all fractions were inactive (Table 4). *Ocimum* cyclohexane was the most active fraction. Mazen *et al.,* 2012 proved antifeedant capabilities of flavonoids (Nyarko *et al.,* 2002) in *Erisoma lanigerum* on *Aphilenus mali* (an aphid). Owusu *et al.* (2008) found that methanol extracts of *Allium sativum, O. canum, O. gratissimum, Sporobolus pyramidalis* and *Zanthoxylum xanthoxyloides* roots have antifeedant effects against termites.

Repellency

All extracts fairly repellent with *Laggera* cyclohexane (5 g/kg) was the most repellent (Table 5). Asawalam *et al.,* 2008 proved that 1,8 cineole found in the essential oils of *Ocimum grattissimum* and *Vernonia amygdalina* leaves was moderately repellent to the *S. zeamais*. With the repellent behaviour of *Laggera* it has been proven by Luciana *et al. (*2013) that insect olfactory organs have a level of saturation beyond which odours are not sensed. Christos *et al.* (2006) and Quintin *et al.* (2012) found out that following "saturation" the refuge-seeking behavior occurred independently of the chemical stimuli.

Table 5. Percentage Repellences (Mean ± SE) of *Sitophilus zeamais* due to treatment of maize seeds with different concentrations of fractionated extracts of *Ocimum canum*, *Laggera pterodonta* and *Azadirachta indica* oil as the check.

Extract	Concentration	PR	Class	Interpretation
Ocimum Cyclohexane	1	46.11 ± 4.84	III	Moderately repellent
	5	66.22 ± 11.36	V	Very Repellent
	10	46.97 ± 8.67	III	Moderately repellent
Ocimum Acetone	1	35.04 ± 12.33	III	Moderately repellent
	5	42.45 ± 10.86	III	Moderately repellent
	10	48.93 ± 17.10	IV	Repellent
Ocimum Methanol	1	45.00 ± 7.34	III	Moderately repellent
	5	42.54 ± 11.95	III	Moderately repellent
	10	48.29 ± 15.72	IV	Repellent
Laggera Cyclohexane	1	33.33 ± 9.07	II	Weakly repellent
	5	75.44 ± 16.40	V	Very repellent
	10	44.52 ± 11.45	IV	Repellent
Laggera Acetone	1	20.39 ± 12.17	II	Weakly repellent
	5	51.46 ± 13.36	IV	Repellent
	10	50.83 ± 17.08	III	Repellent
Laggera Methanol	1	45.39 ± 5.84	III	Moderately repellent
	5	40.62 ± 3.14	III	Moderately repellent
	10	36.07 ± 4.89	II	Weakly repellent
Neem Oil	1	52.57 ± 5.87	III	Moderately repellent
	5	52.70 ± 4.86	III	Moderately repellent
	10	62.76 ± 4.73	V	Very repellent

Conclusion

The study showed that the methanol fraction of *Laggera pterodonta* was the most promising phyto-insecticide with the *Laggera* cyclohexane that exhibited a very good *Sitophilus zeamais* repellent. *Ocimum canum* extracts generally proved to be good inhibitors of F_1 progeny emergence; however *Laggera* methanol extract showed maximum inhibitory properties. Finally, *Ocimum* cyclohexane fractions were comparatively the most effective anti feedants. The others were still active since these observations were just a part of a plethora of positive ones made from manipulations using these two plants' fractionated extracts. These plants have shown good indicators for exploitation as alternatives to synthetic insecticides and have the potential for application by most subsistent farmers in low resource countries in sub Saharan Africa. These plants are readily available to the local farmers and have been shown by earlier studies to be environmentally friendly, affordable and less toxic to the humans.

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