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Volatile oils from *Cedrela odorata* L. As Protectants against *Sitophilus zeamais* (Coleoptera: Curculionidae)

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Abstract
Synthetic chemicals have undesirable effects on man and his environment, when used in controlling insect pests. Volatile oils from plants possess numerous biological activities against insect pests, but information on the use of volatile oils from *Cedrela odorata* against stored product insect pests, is scanty. Volatile oils were identified from ethanol extract of *Cedrela odorata* stem bark using the gas chromatography - mass spectrometry (GC/MS). The effects of six of the standard compounds, each at 1.0% (v/v) namely: Caryophyllene oxide, Ethyl oleate, Ethyl hexadecanoate, Guaicol, Hexanal and Methyl linoleate on mortality, oviposition and repellence of *Sitophilus zeamais* as well as on germination of maize seeds were determined relative to a synthetic chemical, pirimiphos-methyl and ethanol, as positive and negative control, respectively. All data collected were analyzed using ANOVA and means were separated with the DNMRT at 5% significant level. Twenty-nine constituents were identified in the volatile oil. The most abundant compounds were ethyl hexadecanoate (19.76%), ethyl oleate (17.88%) and methyl hexadecanoate (10.38%) while minor compounds included α-curcumene (0.57%), α-muurolene (0.53%) and spinasterone (0.43%). The compounds tested were better than the control in causing mortality and reducing oviposition by female *Sitophilus zeamais*. Mortality and oviposition of *S. zeamais* on grains treated with ethyl hexadecanoate, ethyl oleate and methyl linoleate were not significantly different from those of pirimiphos-methyl. The compounds were repellent to *S. zeamais* with methyl linoleate, exhibiting the highest. This study revealed that the volatile oils from *Cedrela odorata* stem bark have potential as biopesticide for *Sitophilus zeamais*.

Keywords: *Volatile oil; weevil mortality; gas chromatography; ethyl hexadecanoate; biopesticides*

1. Introduction
The maize weevil, *Sitophilus zeamais* Motschulsky is a serious cosmopolitan pest of stored cereal grains, causing up to 30% yield loss to stored maize thereby threatening food security. It is capable of penetrating and infesting intact kernels of grain, in which immature stages develop [1] leaving the maize emptied of its nutritional and seed value and culminating in outright rejection of the product at the local and international markets [2]. The damage produced on grains also favors the occurrence of secondary pests and fungi [3, 4]. The control of storage insects like *S. zeamais* has relied on the widespread use of various synthetic chemical insecticides and fumigants, leading to a number of serious problems such as environmental pollution, pesticide residue in food grains, pesticide resistance and toxicity to non-target organisms [5].These problems necessitated the search for eco-friendly and cheaper insect pest control alternatives which included the use of powdered plant parts and their extracts [6,7]. Fortunately, most of these plants can be sourced locally in the tropics. Research on the evaluation of local materials for stored product protection is important as maize production is basically left in the hands of resource-poor farmers in the rural areas of Nigeria. *Cedrela odorata* Linnaeus (Meliaceae) is native to South America and the West Indies. The tree is known for its red, rot-resistant wood that is used to make furniture and guitars [8]. In Africa, the decoction of the bark is used to cure malaria and/or fever [9, 10]. Some studies have demonstrated that the plant possesses insect antifeedant activity and toxicity. Ewete *et al* reported ethanol extract of *C. odorata* stem bark in artificial diet significantly reduced growth in the European corn borer, *Ostrinia nubilalis* Hubner at a concentration of 1.0% [6]. Asogwa and Osisanya reported that the leaf and wood extract of the plant caused between 12-20% and 6-16% mortality of adult *Sitophilus zeamais* for both topical application and residual action test [12] while Akinbuluma and Adeyemi reported that ethanol extract of *Cedrela odorata* caused about 95% mortality, reduced the number of oviposition and adult emergence in *Callosobruchus maculatus* [12].

Although, volatile oils from *Cedrela odorata* in its crude form has been reported to show antifeedant, repellent and toxic effects on a wide range of insects, information is scanty about the identification and evaluation of individual component as protectant against stored produce insect, such as *Sitophilus zeamais*. This study was carried out to identify the volatile oil components from stem bark of *Cedrela odorata* L. and evaluate their potential as protectant against *Sitophilus zeamais* in stored maize.

2. Materials and Methods

2.1 Insects and plant materials

Culture of adult *S. zeamais* was established on TZPB SR-W maize variety in the Entomology Research Laboratory of the Department of Crop Protection and Environmental Biology (27 ± 2 °C and r.h: 65 ± 5%) from an initial culture from the laboratory. Teneral adults were removed from the culture and sexed using the rostrum as the character for sex differentiation [13, 14] and the culture was maintained as source of weevils for all bioassay. Stem bark of *Cedrela odorata* was obtained from the Forest Research Institute of Nigeria (FRIN), Ibadan.

2.2 Extraction and chromatographic techniques

Prior to extraction, the plant materials were washed, cut into small pieces and air dried. About 500 g of the sample was ground and extracted using 95% ethanol in a Soxhlet apparatus and concentrated *in vacuo* on a rotary evaporator at 40 ºC and kept at 4 ºC for further analysis. A glass column (length = 85 cm, internal diameter = 6.0 cm) was used in the purification of the samples. The column was half filled with hexane and glass wool inserted down the bottom of the column to ensure no air bubble was trapped. About 500 g of 230-400 µm mesh size silica gel was mixed with hexane to form the slurry. The mixture was poured into the column and constantly tapped to prevent trapping of air bubbles. One hundred grams (100 g) of the crude extract was dissolved in acetone and slurry pre-adsorbed using silica gel. The solvent was dried by keeping in the hood and stirring at intervals of 5 mins until fine powder of sample was formed. Sample was later loaded into the column and the mobile phase of increasing polarity was initiated and consisted of successive elution of hexane (100%), hexane: ethyl acetate (9:1-1:9), to ethyl acetate (100%) at a constant flow rate. The eluates (40 ml) were successively collected in 100 mL beakers, concentrated under pressure and monitored by a Thin Layer Chromatography (TLC) carried out on pre-coated silica gel plates. The samples were pooled together based on their TLC characteristics, labeled and kept for further analysis.

2.3 Determination of Components of volatile samples from *Cedrela odorata* stem bark extract

Volatile sample (0.01 mg) was weighed using a Metler weighing scale (0.0001 mg) and dissolved in one milliliter (1 mL) of dichloromethane and the mixture was vortexed for 30 seconds. Sample was sonicated for five minutes using Branson 2510E-DTE sonicator and later centrifuged for five minutes at 13000 rpm at room temperature. The sample was then transferred to 1.5 mL auto-sampler vials and analyzed on a 7890 A gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA) linked to a 5975 C mass selective detector (Agilent Technologies, Inc., Santa Clara, CA, USA). The Instrumental conditions used were as reported in [15] with some modifications. An inlet temperature, 270 °C; transfer line temperature of 280 °C and column oven temperature programmed from 35 to 285 °C with the initial temperature maintained for 3 minutes then 10 °C $min^{-1}$to 280 °C min^{-1}. The GC was fitted with an HP-5 MS low bleed capillary column (30 m × 0.25 mm i.d., 0.25 µm) (J&W, Folsom, CA, USA). The carrier gas was helium at a flow rate of 1.25 mL min^{-1}. The mass selective detector was maintained at ion source temperature of 230 °C and a temperature of 180 °C. Electron impact (EI) mass spectra were obtained at the acceleration energy of 70 eV. Fragment ions were analyzed over 40-550 *m/z* mass range in the full scan mode. The filament delay time was set at 3.3 min. Preliminary identification of constituents was based on computer matching components of mass spectral data against the standard Wiley and NIST library spectra, constituted from spectra of pure substances and components of the known essential oils, and literature MS data [16, 17]. They were confirmed by their GC retention time in comparison with those of reference compounds, peak enhancement as well as Co-injection/Co-elution with authentic standards. The total running time of GC-MS was 50 min. The standards used were obtained from Aldrich Chemicals UK.

2.4 Biological activities of volatile oils from *C. odorata* stem bark

2.4.1 Mortality bioassay

Six volatile oil standards namely; caryophyllene oxide, ethyl hexadecanoate, ethyl oleate, guiacol, hexanal and methyl linoleate were obtained from the Chemistry Laboratory of the Behavioural and Chemical Ecology Department, International Centre of Insect Physiology and Ecology, Nairobi. One percent (1.0%) (v/v) of each of the standards was formulated in absolute ethanol and was taken as separate treatment. The synthetic insecticide, pirimiphos-methyl, at a concentration of 0.015 g/30 g grains was taken as a positive control while the negative control contained ethanol only. Consequently, there were eight treatments replicated three times. Fifty grams (50 g) of maize grain was weighed into twenty-four Kilner jars. Each concentration (0.5 mL) was applied to the grains with a micro syringe and shaken for 5 minutes to allow for effective coverage of the surface. Ten-day-old (1♀: 1♂) adult *S. zeamais* were transferred into the infested grains containing the test and control solutions and mortality was monitored up to 72 hours.

2.4.2 Oviposition bioassay and viability test

Twenty grains from each of the jars in the experiment above were removed after 10 days and the number of eggs laid was determined using the egg-plug staining/detection technique described by [18]. The number of eggs on the grains was recorded. Germination test was also conducted on 20 randomly selected undamaged seeds from each jar. Seeds from each treatment were placed on a moistened filter paper in plastic Petri-dishes in three replications. The number of germinated seeds was counted and recorded in each of the treatments at five days after the set-up and percentage viability of the seeds were determined as follows:

$$\text{Percentage (\%)viability} = \frac{\text{Number of germinated seed}}{\text{Number of seed sown}} x\ 100$$

2.4.3 Repellency test

Repellent activity of the volatile components was evaluated on *S. zeamais* using the area preference method described by [19]. Briefly, the test area consisted of 11.0 cm Whatman No. 1 filter papers cut in halves and 200 µL of each of the components was applied uniformly to half-filter paper disc

with a microsyringe. The other filter paper halves were treated with ethanol (control). The half filter papers were air-dried for 10 minutes to allow the solvent to evaporate completely. Full filter papers were re-made by attaching together the treated and untreated halves of the same dimensions with cellotape and each placed in a Petri dish and ten adult weevils released separately at the center of each filter paper in the Petri dish. The number of insect present on the control (untreated) and treated half discs were recorded after 30 minutes exposure. Percent Repellency (PR) for each replicate was estimated as:

$$PR = \frac{(Nc - Nt)}{(Nc + Nt)} x\ 100 \quad [14]$$

Where (Nt) = number of insects present on the treated half disc and; (Nc) = number of insects present on the untreated (control) half disc, A negative PR value was taken as zero and data on percent repellency were analyzed.

2.5 Statistical analysis
All data were analyzed using analysis of variance (ANOVA) and means were separated with the Duncan's New Multiple Range Test, using the DSAASTAT version 1.101 at 5% level of significance.

3. Results
3.1 Volatile fractions and components identified from *Cedrela odorata* stem extract
The eluting solvent system for each fraction from column chromatography, number of components present and weights of the different fractions obtained are presented in Table 1. Six samples were obtained from a total of 34 fractions of 40 mL based on their TLC characteristics. The six samples obtained were labeled as *Cedrela odorata* fractions (COF) 1 to (COF) 6. COF-5 which was eluted with 70% ethyl acetate in hexane had the highest weight (9.88 g) while the least weight (0.74 g) was observed with COF 1. GC-MS analysis of the volatile oil components from the samples afforded the identification of twenty-nine constituents comprising the fatty acid derivatives and steroids (Table 2). The most abundant compounds were ethyl hexadecanoate (19.76%), ethyl oleate (17.88%) and methyl hexadecanoate (10.38%) while minor compounds included α-curcumene (0.57%) and α-muurolene (0.53%) and spinasterone (0.43%).

Table 1: Eluting solvent systems, number of components and weight of fractions obtained from column chromatography

Fractions	Code	Solvent system	No. of spot(s)	Weight (g)
1 – 6	COF-1	100% Hexane	2	0.74
7 – 11	COF-2	20% EtOAc in Hexane	3	1.24
12 – 13	COF-3	50% EtOAc in Hexane	3	2.21
14 – 19	COF-4	50% EtOAc in Hexane	4	6.33
20 – 27	COF-5	70% EtOAc in Hexane	3	9.88
28 – 34	COF-6	100%EtOAc	2	4.19

COF = *Cedrela odorata* fraction

Table 2: Chemical constituents of volatile components of *Cedrela odorata* stem extract

Compounds	Retention Time (mins)	Composition (%)
Hexanal	7.11	0.63
Hexanoic acid	11.29	0.94
Guaiacol (para-vinyl)	16.53	0.35
α-copaene	17.40	1.09
β-elemene	17.59	0.85
β-gurjunene	18.18	5.60
Curcumene (ar-)	18.73	0.57
α-muurolene	18.99	0.53
Phenol,2,4-bis(1,1-dimethylethyl)-	19.05	0.65
γ –cadinene	19.27	1.67
(-)-Mellein	19.62	1.16
Caryophyllene oxide	20.07	0.72
Globulol	20.08	0.88
α –muurolol	20.75	1.07
α-cadinol	20.86	3.32
Oplopanone	21.81	0.76
Methyl hexadecanoate	23.56	10.38
Ethyl hexadecanoate	24.22	19.76
Methyl linoleate	25.19	1.34
Methyl octadecenoate	25.46	3.27
Oleic acid	25.63	5.05
Octadecanoicacid	25.81	1.31
Ethyl oleate	25.85	17.88
Ethyl octadecenoate	26.07	8.29
Campesterol	37.51	0.98
Stigmasterol	38.20	1.66
Gamma.Sitosterol	39.55	6.84
Spinasterone	41.27	0.43
Stigmast-4-en-3-one	42.76	1.81

3.2 Biological assays
Mortality of *Sitophilus zeamais* in grains treated with the volatile compounds from *Cedrela odorata* was significantly ($p < 0.05$) higher than in the solvent-treated control (Table 3). Adult mortality increased with the length of exposure, in all the treatments. There was no significant difference ($P > 0.05$) in *S. zeamais* mortality on grains treated with ethyl hexadecanoate, ethyl oleate, methyl linoleate and the synthetic chemical pirimiphos-methyl, at 72 h after infestation. The number of eggs laid by *S. zeamais* was reduced on grains treated with the volatile oils (Table 4). Although the least oviposition was recorded on pirimiphos-methyl–treated

grains, oviposition was not significantly different ($P < 0.05$) from those on grains treated with ethyl hexadecanoate (0.67±0.29), ethyl oleate (1.33±0.29) and methyl linoleate (2.00±0.50). Table 4 also showed the repellent effect of volatile compounds from on *S. zeamais* as well as on germination of maize seeds. Methyl linoleate elicited the highest repellence (50%) while the least repellent compound was ethyl oleate (6.7%). There was no significant difference ($P > 0.05$) in the germination of maize seeds in all the treatments and the control.

Table 3: Effects of volatile components from *Cedrela odorata* stem extract on mortality of *Sitophilus zeamais*

Volatile oils from *C. odorata*	Percentage mean mortality (± S.D) over 3 days post treatment		
1.0% (v/v)	**24 h**	**48 h**	**72 h**
Caryophyllene oxide	0.00 ± 0.00^{a}	10.00 ± 5.00^{a}	16.67 ± 10.41^{b}
Ethyl hexadecanoate	73.33 ± 10.41^{c}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}
Ethyl oleate	90.00 ± 5.00^{c}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}
Guaiacol	16.67 ± 5.77^{ab}	26.67 ± 5.77^{b}	40.00 ± 5.00^{c}
Hexanal	0.00 ± 0.00^{a}	3.33 ± 2.89^{a}	16.67 ± 2.89^{b}
Methyl linoleate	36.67 ± 11.55^{b}	66.67 ± 7.64^{c}	96.67 ± 2.89^{d}
Ethanol (control)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Pirimiphos-methyl	93.33 ± 2.89^{c}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}

Means followed by the same letters in the column are not significantly different

Table 4: Effects of compounds from *Cedrela odorata* stem extract on oviposition and repellence to adult *Sitophilus zeamais* and germination of maize seeds

Volatile oils from *C. odorata*	No. of	%	%
1.0% (v/v)	**Eggs laid**	**Repellence**	**Germination**
Caryophyllene oxide	3.33 ± 0.58^{cde}	46.67 ± 15.28^{b}	73.33 ± 5.09
Ethyl hexadecanoate	0.67 ± 0.29^{a}	46.67 ± 5.77^{b}	70.00 ± 5.00
Ethyl oleate	1.33 ± 0.29^{ab}	6.66 ± 5.77^{a}	76.67 ± 7.64
Guaiacol	3.00 ± 0.87^{bcd}	33.33 ± 5.77^{ab}	76.67 ± 7.64
Hexanal	4.33 ± 0.29^{de}	20.00 ± 17.32^{ab}	76.67 ± 7.64
Methyl linoleate	2.00 ± 0.50^{abc}	50.00 ± 5.00^{b}	66.67 ± 5.77
Ethanol (control)	5.00 ± 0.50^{e}	0.00 ± 0.00^{a}	76.67 ± 5.77
Pirimiphos-methyl	0.33 ± 2.89^{c}	-	†

Means followed by the same letters in the column are not significantly different at 5% level using the Duncan's New Multiple Range Test
†= means are not significantly different
Values after ± represent standard deviations from mean

4. Discussions

The results of *Cedrela odorata* volatile composition characterized by gas chromatography, in this study, indicated that the compound contained fatty acid derivatives and steroids. Ayvaz *et al* reported that the insecticidal constituents of many plant extracts and essential oils are monoterpenoids [20] while Asekun and Ekundayo identified twenty-six volatile constituents from the leaf of *Cedrela odorata* L. and reported that sesquiterpenoids such as *α*-santalene and *β*-elemene were the dominant compounds [21]. In this study, the esters, ethyl hexadecanoate and ethyl oleate were the major components, while the steroid, spinasterone was observed as the minor constituent. Oliveira *et al* observed that the percentage composition of volatile oils varies with plant part used and the condition in which the extraction is performed [22]. The volatile oils composition of *Cedrela odorata* obtained were extracted from the stem bark of *C. odorata* using the Soxhlet extraction method and followed by Column Chromatography. The volatile oil components in this study caused high mortality, reduced oviposition and were repellent to the weevils. Earlier reports showed that plant extracts and volatile oils might be of importance for controlling stored-product insects due to their high volatility [23, 24]. In a study, Savaris *et al* observed efficiency of 100% of essential oil of *Cunila angustifolia* in the control of *S. zeamais*, and the major component was pulegone with participation of 56.1% in the composition of its oil [25]. As well, the essential oil of *Ocimum grattissimum* leaves was reported to be moderately repellent to *S. zeamais* [26, 27]. The toxicity might be by penetrating the insect body through the respiratory system [28]. Volatile oil components tested in this study did not affect the germination of sampled maize kernels. This agrees with the reports of [29] that the essential oils from the leaves of *Laureliopsis philippiana* at concentrations below 2.0% did not show phytotoxic effects maize kernels. This study shows that volatile oils from *Cedrela odorata* caused high mortality, reduced oviposition and were repellent to *Sitophilus zeamais.* The use of volatile components from *C. odorata* thus represents a promising alternative to be used under storage conditions for the integrated management of *Sitophilus zeamais* and as a cue to other insect pests of stored produce.

5. Conclusions

The result obtained from this study revealed that stem bark of *Cedrela odorata*is rich in volatile oil components that have potential as biopesticide for *Sitophilus zeamais.* These components can be used as an alternative to synthetic chemical in the management of *Sitophilus zeamais* on stored produce. This will reduce the attendant problems by synthetic chemicals on stored food.

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