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Sherifat A Aboaba
Department of Chemistry, University of Ibadan, Ibadan, Nigeria

Idowu O Okunlola
Department of Chemistry, University of Ibadan, Ibadan, Nigeria

Mobolade D Akinbuluma
Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria

Correspondence
Sherifat A Aboaba
Department of Chemistry, University of Ibadan, Ibadan, Nigeria

Chemical constituents and insecticidal activity of the essential oils from *Cardiospermum grandiflorum* (Sweet) *Sapindaceae* on *Sitophillus zeamais*

Sherifat A Aboaba, Idowu O Okunlola and Mobolade D Akinbuluma

Abstract
This present work aims to study for the first time the chemical composition and evaluate insecticidal effects of essential oils of *Cardiospermum grandiflorum*, against *Sitophillus zeamais*, an insect pest of stored maize seeds as alternatives to synthetic pesticides. The essential oils were obtained from the leaf, stem and root of *Cardiospermum grandiflorum* using hydro distillation, and the oils were analysed by Gas Chromatography-Mass Spectrometry (GC-MS). Insecticidal analysis was carried at ambient temperature of 27 ± 2 ^{0}C and relative humidity of 78 ± 5% using adult *Sitophillus zeamais*. Parameters including mortality and viability of seeds were assessed and all data were analysed using Analysis of Variance (ANOVA), the differences were compared at 0.05 significant level using Least Significant Difference (L.S.D). The percentage yield of essential oils from the leaf, stem and root were 0.37%, 0.42% and 0.33% respectively. A total of 27, 14 and 8 constituents were characterised in the leaf, stem and root with isogermacrene (14.05%), caryophyllene oxide (29.49%) and α-octadecene (49.36%) being predominant compounds in the essential oils respectively. Insecticidal activities of the essential oils of *Cardiospermum grandiflorum* leaf, stem and root on *Sitophillus zeamais* showed that 25.00% concentration was most toxic at 12 - 24 hour post treatment. All the treated maize seeds were viable when treated with the essential oils from the leaf, stem and the root. The essential oils from the leaf, stem and root of *Cardiospermum grandiflorum* had significant mortality effect on *Sitophillus zeamais,* this suggests that the essential oils could render insecticidal activity in the control of *Sitophillus zeamais* and related stored seed products.

Keywords: Essential oils, *Cardiospermum grandiflorum*, maize pest, mortality, viability

1. Introduction

Essential oils are volatile concentrated aromatic compounds obtained from different parts of plants containing mainly 10 carbon atom monoterpenes, as well as 15 carbon atom sesquiterpines and higher terpenes as minor constituents [1]. They are used extensively in perfumery, aromatherapy, medicine flavouring food and drinks as well as in household cleaning products [2]. Balloon vine, *Cardiospermum grandiflorum* is a woody vine climber that grows throughout the year having numerous lateral branches that climbs by means of tendrils and grows up to 5-8 m in length. Different parts of the plant have been reported to be useful in traditional medicine. For example, the roots are used to give laxative effect, induce vomiting, and also to increase the rate of urine excretion and the leaves can be made into medicines to alleviate swelling, oedema and pulmonary complications [3]. The plant, *Cardiospermum grandiflorum* have also been popularly grown as an ornamental plant across the globe [4].

The weevil *Sitophillus zeamais* is the most common pest for stored maize seeds that causes a major damage to seeds when they are not treated making them unfit for human consumption or planting. Major side effects have been observed in the use of conventional insecticides and fumigants compounds, such as selected specimens resistant to the chemicals and toxicity for humans and the environment. Therefore, there is the need to develop new insecticides that are safer, thereby replacing the use of conventional insecticides and fumigants for stored products. However, pesticides from plant essential oils have been reported to have potential for controlling stored product pest like *S. zeamais.* Pesticides from plant-based extracts have been suggested as an alternative to synthetic insecticides [5] and are thus being encouraged among resource-poor farmers in the developing nations.

This study was carried out to evaluate the Efficacy of the essential oils of *C. grandiflorum* on *S. zeamais* in stored maize seeds. Parameters including mortality, repellence and viability of seeds were assessed using adult *S. zeamais*.

2. Materials and Methods

2.1 Plant material and distillation of the essential oils

Fresh *C. grandiflorum* leaf, stem and root were harvested at Amina way, University of Ibadan, South west, Nigeria, between December 2017 and April, 2018. The plant samples were identified at the herbarium, Botany Department, University of Ibadan and voucher specimens (UIH-22680) was deposited at the herbarium for further reference. The plant samples were air-dried for the period of one week in a shade away from direct sunlight after which they were pulverised and weighed. The essential oils of *C. grandiflorum* were obtained by hydro-distillation using a modified Clevenger's type apparatus [6]. Pulverised samples (200 g each) were used for the extraction. The oils were collected and stored in an amber colored sample vial while the yield of the oils obtained were calculated and expressed as percentage weight/volume (%w/v) then stored in the refrigerator at 4 ^{o}C prior to analysis.

2.2 Gas Chromatography - Mass Spectrometry Analysis (GC - MS)

Gas Chromatography-Mass Spectroscopy analyses of the essential oils were performed with a Varian CP 3800 Gas chromatograph equipped with a HP-5MS capillary column (30 m x 0.25 mm x 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were met by making the injector and transfer line temperatures at 250 ^{0}C and 240 0C, respectively; oven temperature was programmed from 50 0C- 240 ^{0}C and 3 ^{o}C/min; carrier gas used was helium at a flow rate of 1 mL/min. The volume of the sample injected was 1 µL and the split ratio was 50:1 with split flow at 70.615ml/min. The total chromatogram was auto-integrated and the constituents were identified by comparison of the GC-MS data with published mass spectral database (NIST 11.L) and literature data [7, 8].

2.3 Source of maize seeds and treatment

Untreated maize were harvested from a farm in Ogbomosho, Oyo state, Nigeria and identified at the Institute of Agricultural Research and Training (IAR&T), Moor Plantation Ibadan, Oyo State, Nigeria. The seeds were disinfested in a deep freezer at a temperature of about 5 ^{0}C for one week prior to experiments and later air-dried in the laboratory, after which they were cleaned and kept for one week under experimental conditions until acclimation.

2.4 Sitophillus zeamais culture

The research was carried out in Department of Crop protection and Environmental Biology (Entomology research Laboratory), University of Ibadan, Oyo State, Nigeria. The insects were cultured under laboratory conditions of 27 ± 2 ^{0}C ambient temperature and 78 ± 5% relative humidity. Initial culture of adult *S. zeamais* was raised from infested maize seeds bought from Bodija market, Ibadan. Fifty pairs of adult S, zeamais, were introduced into 1 L Kilner jars containing 250 g of the disinfested maize and left in the laboratory for 30 days till the insects multiply. New generations of the maize seed beetles were later reared on cleaned disinfested maize in the laboratory and culture was maintained throughout the experiment. The jar was covered with wire mesh lid to allow for aeration and replicated three times for ready availability of insects throughout the experiment. Jars were placed on a table whose stands were dipped in the essential oils and prevented from contamination by ants.

2.5 Serial dilution of samples

Crude essential oil (0.4 mL) was diluted with (1.6 mL) of 95% ethanol to obtain a stock solution. From the stock solution, serial dilution was done to obtain 20.0%, 40.0%, 60.0% and 80.0% with a control containing 0.00% (v/v) ethanol only.

2.6 Mortality Assay

Twenty grammes (20 g) of maize seeds were weighed into forty five plastic jars. Exactly 1.0 mL of concentrations 20.0%, 40.0%, 60.0% and 80.0% (v/v) of the extract were applied each to the grains using a 1 mL syringe and shaken to allow homogeneity of the oil within the grains. There were four treatments with three replicates and the seeds in the jar used as control were treated with ethanol only. The seeds were infested with twenty adult *S. zeamais* per jar and covered with mesh to allow aeration and prevent escape of maize beetles. The death rate (mortality) was recorded at 6, 12, 18 and 24 hours after infestation. The insect were considered dead once they do not move when probed with a brush. The dead beetles were removed at the different time intervals, counted and recorded. Percentage mortality were calculated using Abbott's (1925) formula:

$$PT = \frac{Po\ -Pc}{100\ -Pc} \times 100$$

Where PT = corrected mortality (%).
PC = control mortality (%)
PO = observed mortality (%)

2.7 Viability Assay

From the mortality assay above, all the grains were removed from the jars after four days and twenty grains were randomly selected from each jars after which the same concentration were re-applied on the selected grains to determine viability of the seeds with each concentrations. The seeds were placed in a Petri dish which was moistened from time to time in order to make germination condition available. Viability was recorded after four days and the numbers of germinated seeds were recorded. The data recorded were processed in percentages using the formula;

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

2.8 Data analysis

The results obtained were analysed using Analysis of Variance (ANOVA) and significant mean values for each concentrations 0.00%, 20.0%, 40.0%, 60.0% and 80.0% were compared using the Least Significant Difference (LSD) at 0.05 significant level.

3. Results

The yields of the essential oils obtained by hydro distillation were 0.37%, 0.42%, and 0.33% for the leaves, stem and root Respectively. The oils of the stem and root were colour less while that of the leaf was light yellow at the time of extraction. Gas Chromatography- Mass Spectrometry (GC – MS) analysis of the essential oil of *C. grandiflorum* leaf, stem, and root resulted in [27, 14, 8] constituents representing 84.34%, 80.04% and 70.78% of the total oil respectively (table 1). The leaf essential was dominated by β-caryophyllene (5.95%), valerena-4, 7 (11)-diene (11.44%), neophytane (12.45%), isogernacrene (14.05%) and (E)-5-eicosene (12.37%) while 6, 10, 14, trimethyl-2-pentadeanone (7.96%), (E)-5-eicosene (12.37%), and caryophyllene oxide (14.05% were the major compounds found in the stem oil. The root oil had largely hydrocarbons with α-octadecene (49.36%) as the most prominent.

Table 2 shows the mortality effect of the essential oils on *S. zeamais* with the leaf oil having the highest mortality at 80.00% concentration while the viability study (table 3) revealed that the leaf and root oils does not affect germination of the maize seed to any significant extent.

Table 1: Chemical constituents of essential oil of *Cardiospermum grandiflorum* (sweet) leaf, stems and leaf.

RT	Constituents	% Composition		
		CGLE	CGST	CGRT
7.84	Nonanal	-	1.20	-
9.44	Methyl salicylate	-	6.33	-
11.68	α-Longipinene	1.56	1.18	-
11.95	α-Ylangene	2.42	1.71	-
12.01	α-Copaene	0.69	-	-
12.16	β-Bourbonene	0.61	-	-
12.68	β-Caryophyllene	5.95	-	-
12.79	γ -Curcumene	0.67	-	-
13.03	6,10-dimethyl-,(E)-5,9-undecadiene	1.27	-	-
13.14	β-Humulene	1.16	-	-
13.24	Alloaromadendrene	1.46	-	-
13.30	9-epi-β-Caryophyllene	-	1.65	-
13.38	Pentadecane	-	-	1.75
13.41	γ-Muurolene	-	1.64	-
13.56	Valerena-4,7(11)-diene	11.44	-	-
13.71	α-Maaliene	0.90	-	-
13.77	α-Cuprene	1.27	-	-
14.04	(E)-Calamenene	1.16	-	-
14.34	α-Calacorene	0.90	1.97	-
14.51	Aromadendrene	1.71	-	-
14.69	Thujpsadiene	-	3.39	-
14.98	Isogermacrene	14.05	-	-
15.32	Caryophyllene oxide	2.34	29.49	-
15.44	α-Bisabolene epoxide	0.74	-	-
15.73	(-)-Eudesma-1,4(15),11-triene	-	4.52	-
16.04	Heptadecane	-	-	2.18
16.17	8,9-Dehydroneoisolongifolene	1.16	-	-
16.20	n-Tetradecane	-	-	2.41
16.40	n-Hexadecanal	-	-	1.98
16.43	1H-Cyclopropa[a]naphthalene	-	1.43	-
17.48	4-Tetradecyl ester methoxyacetic acid	-	-	1.76
17.99	Octadecamethyl cyclononasiloxane	0.99	-	-
18.27	Neophytadiene	0.84	-	-
18.43	Perhydrofarnesyl acetone	1.67	-	-
18.45	6,10,14-Trimethyl-2-pentadecanone	-	7.96	-
19.68	Hexadecanoic acid methyl esther	1.43	-	-
22.15	2-Hexyldecanol	-	-	2.41
22.36	α-Octadecene	-	-	49.36
22.37	(E)-5-octadecene	13.20	-	-
22.38	(E)-5-Eicosene	-	12.37	-
22.88	Neophytane	12.45	-	-
23.01	Methyl stearate	2.61	-	-
26.83	(Z)-9-octadecenamide	-	5.61	-
31.07	Squalene	-	-	8.93
	Total	83.81	80.04	70.78

RT = Retention time in minutes, **CGLE** *Cardiospermum grandiflorum* leaves, **CGST =** *Cardiospermum grandiflorum* stems, **CGRT =** C*ardiospermum grandiflorum* roots

Table 2: Mortality effect of the essential oil of *Cardiospermum grandiflorum* on *Sitophillus zeamais*

Plant Part	Time (HR)	Concentrations					
		0.00%	20.00%	40.00%	60.00%	80.00%	L.S.D
LEAF	6	0	18.33	70.00	31.67	60.00	45.96
	12	0	25.00	71.67	31.67	60.00	49.27
	18	0	25.00	71.67	31.67	61.67	49.99
	24	0	25.00	71.67	31.67	61.67	49.99
STEM	6	0	48.33	33.33	20.00	23.33	49.82
	12	0	48.33	33.33	20.00	25.00	49.43
	18	0	48.33	33.33	20.00	25.00	49.43
	24	0	48.33	33.33	20.00	25.00	49.43
ROOT	6	0	55.00	35.00	71.67	30.00	56.56
	12	0	56.67	41.67	71.67	33.33	53.71
	18	0	56.67	41.67	71.67	36.67	56.42
	24	0	56.67	41.67	71.67	36.67	56.42

Means within the same row are compared at (P<0.05) using Least Significant Difference (LSD)

Table 3: Viability activity of essential oil of *Cardiospermum grandiflorum* leaf, stem and root on maize seeds.

Concentration of essential oils (%)	Viability (%)		
	Leaf	Stem	Root
0.00	48.33	51.67	33.33
20.00	56.67	56.67	55.00
40.00	61.67	65.00	51.67
60.00	43.33	50.00	50.00
80.00	56.67	33.33	53.33
LSD $_{(0.05)}$	34.92	17.73	26.90

Means within the same column are compared at (P<0.05) using Least Significant Difference (LSD).

4. Discussion

The chemical profile of the essential oils of *C. grandiflorum* generally reveal the presence of majorly sesquiterpenoid and aliphatic hydrocarbons. A total of 83.81% of the leaf oil was characterised and out of this were 53.33% sesquiterpene /sesquiterpenoid compounds; such as α-longipinene (1.56%), ylangene (2.42%), β-caryophyllene (5.95%), isogermacrene (14.05%), β-humulene (1.16%), and valerna-4,7(11)-diene (11.44%). The insecticidal constituents of many plant extracts and essential oils are mainly monoterpenoids and sesquiterpenoids compounds [9] and hydrocarbons [10]. Adjalian *et al*. [9] reported the presence of β-caryophyllene, globulol, germacrene, and β-humulene, octen-3-ol as constituents present in the essential oils of two Premna species that exhibited significant insecticidal and repellent effects. Likewise, Ibrahim, 2011 [10] reported the repellent and insecticidal activity of some plant essential oils that contained appreciable quantity of aliphatic hydrocarbons (N-octadecane (11.02%), eicosane (12.21%), and N-nonadecane (8.62%) amongst other constituents against some stored grain insects. The mortality rate exhibited by the leaf essential oil of *C. grandiflorum* manifested by contact adult insect S. zeamais may be linked to the main volatile compounds characterised. In a related work, analysis of the essential oil of Molsa soochowensis showed that the major constituent is β-caryophyllene (12.82%) and it also displayed contact toxicity against *S. zeamais* and *T. castaneum* adult (LC_{50} values, 25, 45, and 10.23 g/adult, respectively) [11]. Similarly, the stem oil was characterised by 53.51% sesquiterpenoid with caryophyllene oxide (29.49%) as the most significant in quantity. Caryophyllene oxide, is the oxidized form of caryophyllene (or β-caryophyllene). It has been reported to be the only terpene in Cannabis that has the ability to successfully bind with the human body's endocrine CB2 receptors. In addition to giving the cannabis plant its scent and flavours, caryophyllene oxide also serves as a natural insecticide and anti-fungi to further protect the pH. It is found in large amount in the essential oils of many common spices and food plants such as Oregano (*Origanum vulgare* L), Cinnamon (*Cinnamonum* spp), Clove (*Sygium aromaticum*), rosemary (*Rosmarinus offinalis* L), thyme (*Thymus serpyllum*), black pepper (*Piper nigrum* L), [12, 13, 14, 15, 16, 17].

In addition, isogermacrene detected in the leaf oil is a compound typically produced in a number of plants species for their antimicrobial and insecticidal properties, though they also play a role as insect pheromones. Generally, constituents of plant essential oil work synergistically to give the observed activities because the mixture of the chemical components of essential oils are more effective than the individual pure compounds [18]. On the other hand, the 8 compounds representing 70.78% of the total content in the root essential oil, 65.32% were aliphatic hydrocarbon with α-octadecene (49.36%) as the most dominant. This compound have also been detected in many essentials that displayed good insecticidal properties [10, 19, 20].

Mortality (table 2) was significantly higher (P<0.05) in some of the concentrations of the essential oils from the leaf and root of *C. grandiflorum* against Sitophillus zeamais compared to the control. The highest mortality (71.67%) was obtained on seeds treated with 40% (v/v) concentration of the leaf oil and 60% (v/v) concentration of the root oil at 12–18 hour and at 6-24 hour post treatment respectively. Similar trends were reported on the insecticidal activity of extracts of Trichilia gilgiana against Sitophilus zeamais with significant mortality rate at different doses considered [20]. Although the viability study (table 3) reveals that the highest percentage germination (65%) was recorded in grains treated with 40% (v/v) concentration of the essential oils from the stem of *C. grandiflorum*, this value was not significantly different (p>0.05) from the percentage germination observed in grains treated with other concentrations of the oil and the control.

5. Conclusion

The essential oils of the leaf, stem and root essential oils of *C. grandiflorum* consists majorly of sesquterpene/sesquiterpenoids and aliphatic hydrocarbons that had significant insecticidal effects. The observed insecticidal activities is an indication of the possibility of exploring the plant essential oils as natural insecticides which have less adverse effects on environment compared to synthetic pesticides.

All the treated maize seeds were viable when treated particularly with the essential oils from the leaf. The essential oils from the leaf, stem and root of *Cardiospermum grandiflorum* had significant mortality effect on Sitophillus zeamais, this suggests that the essential oils could render insecticidal activity in the control of *Sitophillus zeamais* and related stored seed products. However, the toxicity and mechanism of the action of these essential oils need to be ascertained for effective use as insecticide.

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