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CHEMICAL COMPOSITION AND INSECTICIDAL ACTIVITY OF THE ESSENTIAL OILS OF *CRATEVA ADANSONII* DC. LEAF ON *SITOPHILUS ZEAMAIS AND CALLOSOBRUNCHUS MACULATUS*

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ABSTRACT

This study was conducted to determine the insecticidal activity of essential oil of *Crateva adansonii* against adult *S. zeamaise and C. maculatus.* The essential oil was obtained from the leaves of the plant using a Clevenger apparatus and analyzed by gas chromatography and mass spectrometry (GC/MS). The experimental data generated from the insecticidal assay results were analysed using statistical tool (ANOVA). GC/MS analysis revealed the presence of 52 compounds but with a low total yield of volatile fractions (0.1%). The major constituents of the oil were n-hexadecanoic acid (palmitic acid) (14.68%), Phytol (14.21%), 9,12-octadecanoic acid (Z,Z)- (linoleic acid)(5.81%), linalool (5.75%). Insecticidal bioassays showed that *Crateva adansonii* oil had a strong insecticidal activity on adult test insects they were exposed to at different concentrations and periods. An increase in the exposure time from 24 h to 72 h caused increase in mortality from 35.5% to 100% in *S. zeamais* and 31.1% to 100% in *C. maculatus*, respectively. According to these results, *Crateva adansonii* essential oil can be useful as an alternative for beans and maize protection against *C. maculatus and S. zeamais*.

Key words: *Insecticidal Bioassays, C. maculatus, S. zeamais, Crateva adansonii, Essential Oil,*

INTRODUCTION

Food security and increased productivity coupled with income are the major and primary aims of most poor farmers throughout Nigeria where the majority of them are subsistence farmers. Among the many challenges faced by farmers in Nigeria are insect pest infections which affect their food production, which if not controlled, may lead to total crop crisis now and in the future.

For many decades and up till now, farmers in Nigeria rely heavily on inorganic insecticides for the control of these insect pests. In resort to that, Nigerian farmers depend on importation of the total synthetic insecticide requirements resulting into expedition of large amounts of foreign exchange for the country and contributing in no small measure to the stagnantion and limitation of the economic growth. Apart from limitation and stagnation of Nigeria's economy, overuse of synthetic pesticides lead to problems such as environmental contamination, resistance development and health concerns that were not anticipated at the time of their introduction. Cancer, adverse effects on immune systems, neurodevelopment dysfunction, and metabolic diseases such as diabetes, endocrine system disruption and infertility are some of the health risks associated with continuous exposure to synthetic pesticides (Nicolopoulou-Stamati *et al*., 2016). The indiscriminate and haphazard usage of pesticides poses serious negative public health impacts and adverse environmental hazard too (Tripathi *et al*., 2009).

At the subsistence farmer level, convectional pesticides are costly and the distribution in rural areas is limited, and often most of the synthetics are adulterated by dilution, incorrect mixing and sold beyond their expiry date by the dealers (Stevenson *et al*., 2012). In some parts of the world, particularly Africa and Asia, there is no much effective regulation of pesticides, and many more toxic and hazardous products are still widely available and used. Illiteracy is one of the problems of synthetic pesticide use, due to the fact that many products are sold with English instructions and most of the rural farmers cannot read, therefore the probability of incorrect mixing and application can be high.

There has been a recent upsurge in the recognition given to plant essential oils as an important source of pesticides (Isman, 2013;Stevenson *et al.*, 2012), in the comestics industries and as components of disinfectants (Okoh *et al.*, 2010). *Crateva adansonii* DC (family: Capparidaceae) is a medicinal plant its common names are; sacred garlic pear tree, temple plant, Caper tree, Three-leaf caper, Obtuse Leaf. "Eegun orun" or "ajanaka" (Yoruba-western Nigeria), "Whotozinzen" (Egun/Ogun Western Nigeria) "Ungududu" (Hausa-northern Nigeria) and "Amakarode" (Igbo-eastern Nigeria). It is native to Japan, Australia, Southeast Asia and several South Pacific Islands (Akhtar and Isman, 2000). It is a small tree that grows up to 3 - 10 m tall with three-foliated leaves. The flowers are white and appear when the tree is completely without leaves, while the edible fruit which is 3.5 - 5 mm large is spherical in shape (Akhtar and Isman, 2000).

Various plant parts of *Crateva adansonii* are used in traditional medicine. The leaves are eaten as vegetable, soups or sauces and are applied externally to relieve joint pains; the fresh juice from the leaves is used for the relief of ear ache, eye infection and anodyne in toothache (Abdullai *et al.*, 2012). The bark is useful in disorders of urinary organs, urinary tract infections, pain and burning micturition, renal and vesical calculi (Arbonnier, 2004). Powder of bark is used in rheumatism, itch, epilepsy, and asthma (Sivarajan and Balachandran, 1994). The crude extracts showed moderate anti-trypanosomal activity. The bark yields ceryl alcohol, friedelin, lupeol, betulinic acid and diosgenin. Bark paste is used as a poultice on swellings (Burkill, 1990). Root is chewed at regular interval for a month to treat penis weakness (decreased libido) in males. The root is used as a febrifuge and in several treatments for syphilis. The dried, ground roots are used as an application to swollen parts of the body, while the seeds have unspecified medicinal uses (Burkill, 1990).

With all the aforementioned problems/drawbacks of synthetic pesticides, this research is focused on the development of insecticides from aromatic plant (*Crateva adansonii*) with diminished adverse effect on the environment and consumer.

EXPERIMENTAL

Plant Collection

Fresh leaves of *Crateva adansonii* were harvested from trees growing at the Agricultural Research Plantation of Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria between March and May 2017. The plant sample was identified as *Crateva adansonii* and authenticated at the Department of Forestry, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria (Keay *et al.*, 1964). The plant materials were washed with water, cut into smaller pieces and air dried (to prevent ultra-violet rays from inactivating the chemical constituents) for three weeks . The dried leaves were pulverized into coarse powder using pestle and mortar. The powdered materials were stored in air tight polyethene bags protected from direct sunlight until required for use.

Hydrodistillation of the Oil

Briefly, 180 g of pulverized sample was placed in a 2 L flask and distilled water was added to cover sample. Essential oil was obtained by hydrodistillation using glass modified Clevenger-type apparatus, according to the method of Guenther (1984). The process ran for 4 hours at normal atmospheric pressure and at 96-97 °C inside the extractor. The resulting oil was collected over hexane, and dried over anhydrous sodium sulphate and stored in sealed amber vials at 4 °C until analysis. The extraction process was carried out in triplicates.

2The Oil Yield

The total amount of the extracted oil from the leaves of *Crateva adansonii* was calculated using the following method. The dried weight basis was used for the calculation of the yield percentage by Guenther (1984):

$$\textit{Creteva adansonii oil yeild}\left(\frac{v}{w}\right)(\textit{dry weight}) = \frac{\text{volume of essential oils (mL)}}{\textit{weight of raw materials}} \times 100\% \quad (1)$$

Analysis of the essential oils

The *C. adansonii* essential oil was subjected to GC-MS analysis on an Agilent Technologies 7890 GC system coupled with MS of model 5975C. The GC column was an HP-5ms (30 m and internal diameter of 0.320 mm, film thickness 0.25 μm). The GC oven temperature program was initiated at 80 °C for 2 minutes then it was increased at 10 degrees per minute to 240 °Cand then held for 6 minutes. Helium was used as the carrier gas at 1.0 mL min^{-1} flow rate. Injector temperature was 250 °C. GC-MS analysis was conducted by a 7890 GC system coupled with a MS of model 5975C network mass selective detector with a capillary column as abovementioned, gas helium with a 1 mL min^{-1} flow rate as a carrier with a split ratio equal to 1/50, and programmed injector and oven temperature was identical to GC.

Identification of constituents of the oils: The oil compounds were identified by comparing their retention indices, mass spectra fragmentation with those of a mass special homemade library built up from pure substances and components of known oils and MS literature data and according to their Kovalt retention indexes. Secondary support for the identification compounds were done with the data given in the literature, National Institute of Standards and Technology (NIST), Wiley (McLafferty, 1989).

Determination of Insecticidal Activity

The maize and beans weevils were collected from agricultural field and stored in plastic boxes in an insect toxicology laboratory of Olabisi Onabanjo University, Ago-Iwoye at room temperature. The insecticidal activity of the essential oil against *Sitophilus zeamais* the maize weevil and *Callosobruchus maculatus* the beans weevil, was evaluated on filter paper disc by treating a Whatman number 1 filter paper with the oil diluted with DMSO. The filter paper was placed in a petri dish (diameter 7 cm, height 5.7 cm). Aliquot of the oil was diluted with 1 μL of DMSO and applied uniformly to the filter paper discs to obtained concentration of 40, 80, 120, and 150 μL/mL. The DMSO was allowed to evaporate after which sixteen unsexed adults (7-10 days old) each of *S. zeamais* and *C. maculatus* were introduced into each petri dish containing the food media (20 g) and sealed. The control seed were treated with 1 mL DMSO under the same conditions without the essential oil. Each concentration and control was replicated three times and experiments were conducted at room temperature. Insect mortality was determined by observing the recovery of immobilized insects in 12 h intervals up to 72 h after the application. Insects which did not respond to the gentle touch of a small probe were considered dead since no movements were observed (Su, 1991). Percentage insect mortality was calculated using Abbott's correction formula for natural mortality in untreated controls (Ibuodo *et al.*, 2010):

$$\%\ \text{Mortality (adjusted)} = \frac{\%\ \text{AC} - \%\text{AT}}{\%\ \text{AC}} = \frac{\%\ \text{DT} - \%\text{DC}}{100 - \%\text{DC}} \times 100 \quad (2)$$

Where: AC = Alive in control; DT = Dead weevil in test;
AT = Alive in treatment; DC = Dead weevil in control.

Statistical Analysis

The experimental data from the insecticidal assay were obtained in triplicates and were subjected to one way analysis of variance (ANOVA). *P* values $\leqslant$ 0.05 were regarded as significant. The percentage of mortality and lethal concentrations (LC_{50}) values for insecticidal activities were determined using Abbott's formula (Abbott, 1925).

RESULTS AND DISCUSSION

The essential oil obtained from the leaf of *C. adansonii* has a light yellow colour, fragrant aroma and insoluble in water but soluble in DMSO. The oil yield was calculated as 0.10% of dry plant materials. The chemical composition of the oil is shown in Table 1. GC/MS of the oil shows fifty-two components identified representing 99.98% of the total composition. The main classes of compounds present in the leaf oil were aliphatic acids. The chemical components of essential oil of the whole plant of specie of the plant *crateva religiosa Fost* have been previously reported (Ogunwande *et al.*, 2009) to have 43.5 and 41.1% oxygenated monoterpenes and aliphatic compounds, respectively. While major constituents are linalool (30.2%) and nonanal (17.2%) and it contains no sesquiterpene hydrocarbons, there were differences in the chemicals constituents of the essential oil of the leaves to that of the whole plant as reported. Insecticidal activity observed is likely due to the main volatile compounds in the oil acting alone or in synergy with other minor constituents, as reported by Lawal *et al.,* (2014).

The essential oil showed significant insecticidal activity against *S. zeamais and C. maculatus.* The activity of the oil was both dose dependent and exposure dependent; at a dose of 40 mg/mL, the essential oil produced 87.5% and 93.8% mortality after 72 h, respectively (Figure 1 and 2). At a dose of 80 μg/mL and after 24, 48, and 72 h exposure, the essential oil produced 62.5%, 75%, and 93.8% mortality for *S. zeamais* respectively and 68.8%, 75%, and 93.8% mortality for *C. maculatus* respectively. At a dose of 120 μg/mL and over the same duration of exposure, 56.3%, 81.3%, and 100% mortality were yielded for *S. zeamaize* and 62.5%, 81.3%, and 100% mortality were yielded for *C. maculatus* respectively (Figure 1and 2).

The highest concentration of 150 μg/mL produced a mortality of 81.3% and 100% after 48 and 72 h, respectively for both *S. zeamaize* and *C. maculatus* (Figures 1 and 2). Generally, the higher mortality was achieved when the insects were exposed to a higher dose of oil or a longer exposure period. The P-value for *S.zeamais* is 0.000535 and 0.006933 for *C. maculatus*, therefore there is a significant difference between concentration and exposure duration at 5% level of significance. Results indicated that the oil was relatively more toxic against *C. maculatus* than *S. zeamais*. The lowest concentration (40 mg/mL) of the oil yielded 93.8% mortality of *C. maculatus* after 72 h exposure but the mortality of *C.adansonii* at the same concentration was 87.5% after 72 h. This was in agreement with the earlier report (Chauhan, 2015).

Table 1: Chemical Composition of essential oil of *Crateva adansonii*

No	Compounds	Retention times (min)	Kovalt/Colum	% Composition
1.	Linalool	5.496	1101	**5.75**
2.	Levomenthol	6.463	1150	0.96
3.	L - α - Terpineol	7.772	1189	2.18
4	Safranal	6.846	1201	0.74
5	β - Cyclocitr al	7.144	1218	0.67
6	Nerol	7.287	1234	0.88
7	Geraniol	7.699	1228	1.61
8	1,1,5-Trimethyl-1,2 dihyronaphthalene	9.003		0.86
9	β- Damascenome	9.455	1391	0.72
10	Carryophyllene	9.930	1418	2.12
11	α- Ionone	10.045	1412	1.31
12	Hexane, 1-chloro-5-methyl	10.108		0.59
13	α- Famesene	10.428	1505	1.17
14	Geranyl acetone	10.428	1453	2.05
15	Germacrene D	10.737	1480	0.66
16	Trans-.beta.-Ionone	10.880	1485	**4.82**
17	γ - Muurolene	10.960	1477	0.60
18	2,4-Di-tert-butylphenol	11.195		0.65
19	Cadinene	11.275	1524	0.72
20	1,3-Benzenediol, 5-pentyl-	11.355		1.47
21	Nerolidol 2	11.796	1564	0.42
22	Caryophyllene oxide	12.059	1606	**3.48**
23	1,2 Epoxide-humulene	12.351	1606	0.38
24	10,10-Dimethyl-2,6-dimethylenebicy clo[7.2.0]undecan-5.beta.-ol	12.683		0.32
25	Cyclotetradecane	13.100		0.75
26	Tetrahydronaphthyl methyl carbamate	13.146		0.36
27	α- Costol	13.261	1785	0.39
28	1-Naphthalenol, 5,6,7,8-tetrahydro	13.375	1447	0.43
29	Tetradecanal	13.535	1611	0.59
30	Phenanthrene	14.256		1.51
31	Tetradecanoic acid	14.319	1768	0.74
32	Acridinone	14.771		0.49
33	6,10,14-Trimethyl ,2-Pentadecanone,	15.115	1849	**3.52**
34	2-Pyridinecarbonitrile	15.212	1079	0.75
35	1,2-Benzenedicarboxylic acid, bis(2 - methylpropyl)	15.366		0.65
36	Cis,cis,cis-7,10,13-Hexadecatriena	15.652		0.47
37	Fanesyl acetone	16.030	1922	**3.26**
38	Hexadecanoic acid, methyl ester	16.093	1928	1.60
39	Isophyto	16.350	1956	0.68
40	Dibutyl phthalate	16.568	1914	0.89
41	Palmitic acid	17.014	1950	**14.04**
42	Hexane, 1-chloro-5-methyl	18.216		1.18
43	10,13-Octadecadienoic acid, methyl ester	18.382	2092	0.79
44	Methyl linolenate	18.519	2098	2.87
45	γ- Palmitolactone	18.565		0.52
46	Phytol	18.902	1949	**14.21**
47	Linoleic acid	19.120	2173	**5.81**
48	Asclepic acid	19.440		2.99
49	Stearic acid	19.778	2172	**3.16**
50	Hexadecane	22.931	1600	0.44
51	Nonadecane	24.418	1900	0.91
52	Heptadecane	26.793	1700	0.81
	TOTAL			**99.98**
	Monoterpenoids			21.69
	Sesquiterpenoids			9.94
	Aromatic Compounds			6.45
	Diterpenoids			14.89
	Aliphatic esters			5.26
	Aliphatic ketones			6.78
	Aliphatic acids			26.78
	Aliphatic Hydrocarbons			4.68
	Others			3.51

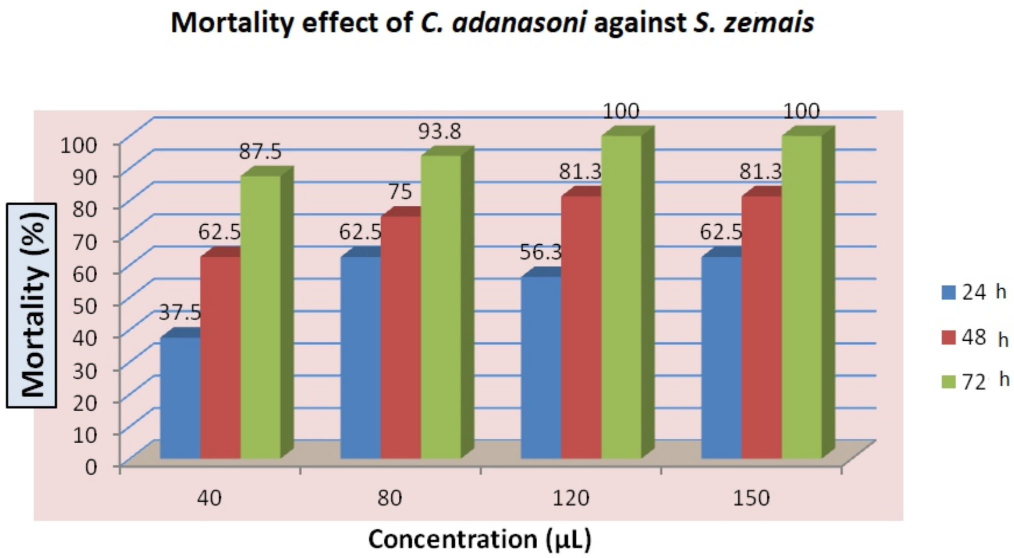

Figure 1: Percentage mortality effect of *C. adansonii* essential oil against the adult on *S. zeamaize*

Essential oil of leaves of *C.adansonii* contains monoterpenoids, such as linalool, which insecticidal properties have been demonstrated against *Tribolium confusum*, *T. castenaum*, *Sitophilus zeamais*, *C. maculatus and Rhyzopertha dominica* (Noudogbessi *et al.*, 2009; Ketoh *et al.*, 2006). Essential oils containing large amount of phytol are known to exhibit insecticidal activity against *S. zeamais* (NIST, 2009;Lawal *et al.*, 2014) and some other insects such as sweet potato weevil, *Cylas formicarius elegantulus* *Musca domestica* (Chahan *et al.*, 2015) and immature whitefly *Bemisia tabaci* (Cruz-Estrada *et al.*, 2013). Plant extracts and volatile oils containing a large amount of phytol, n-hexadecanoic acids and caryophyllene oxide were known to exhibit insecticidal activities against Coleopteran stored products (Cruz-Estrada *et al.*, 2013;Sha *et al.*, 2013). This validates the insecticidal activity of this plant against *C. maculatus* and *S, zeamais.*

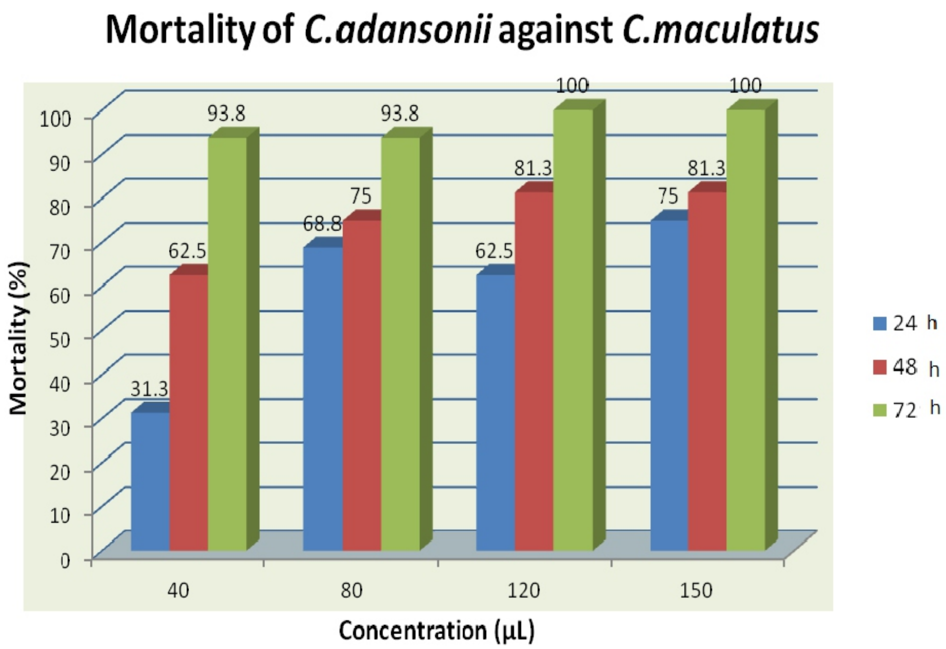

Fig. 2: Percentage mortality effect of *C. adansonii* essential oil against the adult on*C. maculatus*

Phytol and its isomer may be responsible anti-inflammatory activity of C. *procera* (Aliero *et al.,* 2013 Kumar *et al.,* 2001, Sangraula *et al.,* 2002). The presence of phytol in essential of *C. adansonii*, which is the major constituent of the oil (14.21%), is thus scientific evidence in support of the use of the plant in the treatment of rheumatoid arthritis and possibly other chronic inflammation. The plant can also be used to control malaria with the presence of, 6,10,14-trimethyl 2-pentadecanone in the essential oil. 6,10,14-Trimethyl 2-pentadecanone is a C-15 aliphatic methyl ketone have been reported to show repellence to arthropods including blood sucking insect (Ndungu *et al.,* 1995.; Mangwiro, 1996; Gikonyo *et al.,* 2002). The presence of fatty acids such as hexadecanoic acid, octadecanoic acid and 9–octadecenoic acid in the oil validated the plant to be use as anticancer, antioxidant, antimicrobial and hepatoproctective (Ogunlesi *et al.,* 2010; Omotosho *et al.,* 2014; Charles *et al.,* 2011). That since these compounds are known to have antimicrobial activity, antioxidant, hypercholesterolemic, anticancer and hepatoprotective activity, Caryophyllene is also reportedly used primarily in the treatment of chronic arthritic conditions and certain soft tissue disorders associated with pain and inflammation (Akhtar *et al.,* 2012).

CONCLUSION

The results from this study indicated that the essential oil from the leaves of *Crateva adansonii* exhibited effective toxicity to *S. zeamais* and *C. maculatus,* therefore, it can play an important role in stored grain protection and reduce the risks associated with the use of synthetic insecticides. The facts that the oil is made of mixture of compounds can make it potent against some other potentially destructive insects and also make it more effective than imported inorganic insecticides (Akhtar and Isman, 2000; Akhtar *et al.,* 2003;Akhtar *et al.,* 2012). To the best of our knowledge, no study has previously been reported on the insecticidal activity of volatile oil of *Crateva adansonii* against stored product pests. This study may be the first of its kind to provide literature information on the insecticidal effect of the essential oil of *Crateva adansonii*. In addition, further research to fully establish the potency as well as to fully explore the abundant insecticidal property of *Crateva adasoni* is necessary.

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