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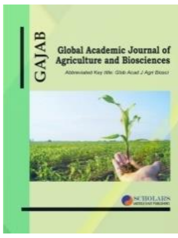

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Original Research Article

Comparative Composition of *Chromolena odorata* and *Aspilla africana* Oils against Cowpea Beetles (*Callosobrunchus maculatus* F.)

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Abstract: The composition of *Chromaleana odorata and Aspilla africana* leaf oil against cowpea seed storage bruchid, *Callosobruchus maculatus* F. was evaluated in the laboratory under the ambient temperature of 28±2^{0}C and 70±5% relative humidity. The bioactivity of the vital oil removed by hydro distillation from *Chromaleana odorata and Aspilla africana* leaves was evaluated as well as 12: 12h light: dark regimes for its biological activity. Specific components of the important oil were recognized through GC, GC-MS as well as GC-Co injection with accurate standards. A total of 17 and 30 basic compounds identity of the vital oil of the two plants were established as well as their relative proportion determined. 6 – phenylhexanoic acid had highest percentage composition in *Chromaleana odorata* essential oil (24.53%), while 3 – Hydroxy-alpha-ionene is highest in *Aspilia Africana* oil (21.68). The main components were found to be basically responsible for the toxic action of its vital oil against bruchids. The uppermost dosage of the vital oil of the plant materials tested evoked the highest mortality after 7 days post treatment. The two botanicals were expressively effective at the bioactive concentration of 0.25ml invoked weevil bruchid mortality in treated cowpea seeds. The adult bruchid mortality improved slowly with botanical concentration as well as with the exposure time. There is need to expand the use of botanicals particularly in the agricultural management pest sector.

Keywords: *Callosobruchus maculatus* F, *Chromaleana odorata and Aspilla africana* oil, Soxhlet extractor, Bioactivity, Concentration.

INTRODUCTION

Callosobruchus maculatus is a species of beetle commonly known as cowpea beetle or cowpea seed beetle due to its affinities for stored legumes. It is a cosmopolitan polyphagous insect pest found in most parts of the tropics and sub tropics. The beetle most likely originated in West Africa and moved around the globe with the trade of legumes and other crops. As only a small number of individuals were likely present in legumes carried by people to distant places, the populations that have invaded various parts of the globe have likely gone through multiple bottlenecks. Despite these bottlenecks and the subsequent rounds of inbreeding, these populations persist. This ability to withstand a high degree of inbreeding has likely contributed to this species' prevalence as a pest (Tran, *et al.,* 1995). Cope *et al.*, (2003) stated that temperature and humidity in legume storage areas are relatively constant and the food density is high. The females lay eggs on legume in the field or in the storage.

The beetle is known for attacking the cowpea but it readily attacks other beans and peas such as mung bean and adzuki bean (Beck and Blumer, 2013). The adult beetles do not feed on stored produce, are very short lived, usually not more than 12 days under optimum conditions. During this time the females lay many eggs up to 115. *Callosobrunchus maculatus* begins laying eggs on ripening cowpea pods in the field and these beans beetles frequently enter cowpea pods through holes created by other pests and lay eggs directly on the seeds.

The initial infestation of cowpea seeds occurs in the fields and the population build-up rapidly under storage, store infestations are frequently derived from the harvested pods or seeds (Booker, 1967) but may also come from a hidden infestation in the store bruchid frequently destroys cowpea grains that have not been properly protected during the first 10-12 months of storage (Umeozor, 2005)

Objective: To evaluate composition of the pesticidal plants

MATERIALS AND METHODS

Material and Reagents for Extraction and G.C/MS

G.C/ MS is the Agilent 6890N Gas Chromatograph with Agilent 5975 Mass Selective Detector Auto sampler vials, 150mL vial inserts, and crimp seals, vial crimper and decrimper 2.5 mL airtight syringe or 3ml disposable hypodermic syringe and 10mL autosampler syringe, 30-mm x 0.25-mm or 0.32-mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5 or equivalent), 1-um film thickness Soxhlet extractor.

Reagents - Air-zero grade, Helium gas -- UHP grade, n-hexane, Anhydrous sodium sulfate and Standard plant chemicals (internal standard)

Extract Preparation

The plant extract was prepared using a Soxhlet extractor. The collected leaves were thoroughly rinsed in distilled water to remove dirt and were allowed to air dry on a wire mesh for a period of 2 weeks. The dried leaves were then milled into powder using a grind mill. 80g each of the dried and milled leaves of *Chromolena odorata* and *Aspilla africana* were weighed separately into Vials and taken for extraction. Extraction was done with the use of n-hexane in the Soxhlet extractor to produce a stock solution of each of the botanicals that was used for the study. A 20g aliquot of sample is homogenized, and a 10g aliquot is spiked with the labeled compound. The sample is mixed with anhydrous sodium sulfate, allowed to dry for a minimum of 30 minutes and extracted for 18-24 hours using methylene chloride in a Soxhlet extractor. The extract is evaporated to dryness and the liquid content is determined. For a 12ml volume of extract that was used for the experiment, the stock solutions were serially diluted (extract: water) into different concentrations 1:2v/v (50%), 1:4v/v (25%), 1:8v/v (12.5%) and 0% concentration which was water as control.

Procedure for Gas Chromatography

Extracts for phytochemicals analysis were subjected to a sequential methylene chloride - n-hexane (1:1) cleanup specifically for these analytes. 1uL of the sample was injected into a gas chromatograph equipped with either a narrow or wide-bore fused-silica capillary column and either an electron capture detector (GC/ECD) or an electrolytic conductivity detector (GC/ELCD).

Phytochemical Analysis

GC-MS analysis was carried out using an Agilent 6890 gas chromatograph with a 5975 MS detector equipped 30-m x 0.25-mm or 0.32-mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5 or equivalent), 1-um film thickness (Agilent). The following temperature ramp was used: injector at 250 C, oven initially at 200C, held for 1 minute and heated to 230C (1.5C min-1, then held for 10min). The characterization and identification of phytochemicals, from the sample was completed in the SCAN mode with the m/z range varied from 35 to 450. The flow rate of the helium as carrier gas was 1mLmin-1; manual injection; the injection volume was 1mL. Interpretation of mass spectrum of GC-MS was done using data base of National Institution Standard and Technology (NIST). The mass spectrum of unknown component was compared with spectrum of the known component stored in the NIST library. Major components were identified by the authentic standards and by the recorded from computerized libraries film thickness.

RESULTS

Table 1 showed various doses of the essential oils (2.0 ml, 1.5 ml and 1.0 ml and 0.5 ml) used to identify the minimum doses, which gave varied percent mortality of the *C. maculatus*. The laboratory tests results displayed that bruchid mortality was immediate as the concentration of each botanical increased as well as the exposure period. Therefore, the bruchids proportion knocked down or dead at numerous concentrations of different botanicals as the exposure and dosages increased. The uppermost dosage of the vital oil of the plant materials tested convinced the highest mortality in the weevils after 7 days treatment. Therefore at (1.5ml) (2.0ml) concentration of the *C. odorata* oil (25.42%) and *A. africana* oil (20.59%) were effective in achieving adult weevil mortality after 7 days post treatment. By the third days exposure the botanicals has become poisonous to the bruchids and gave immediate knock down the insect. At 1.0 ml the *C. odorata* and *A. africana* oil except control become more potent and knocked down some of the weevils within 7 days of exposure compared with other concentrations. *C. odorata* and *A. africana* was expressively effective at the bioactive concentration of 1.0 ml invoked bruchid mortality in cowpea seeds. The adult bruchid mortality was brisk with botanical by the increased concentrations as well as with the exposure time; whereas the least adult bruchid mortality was recorded by control (11.25%).

Table 1: Mortality of *Callosobruchus maculatus* to various concentrations of *C. odorata* and *A. africana* botanical insecticide

Concentration	*Chromolena odorata*	*Aspilia africana*
2.00 ml	22.57a	20.59a
1.50 ml	25.42a	18.33a
1.00 ml	24.35a	20.24a
0.5 ml	17.38a	14.70a
Control	11.25b	25.89a
Mean	20.19	19.95

Means with same alphabet are not significantly different at 0.05 level

Botanicals Composition

The analysis of the botanicals revealed a complex mixture of constituents. A total of 17 and 30 compounds were identified in *Chromolena odorata* and *Aspilla africana* respectively (Table 2 & 3) by GC -MS using data based on NIST. Compounds such as 6 – phenylhexanoic acid had highest percentage composition in *Chromaleana odorata* essential oil (24.53%), while 3 – Hydroxy-alpha-ionene is highest in *Aspilia Africana* oil (21.68) which is an alkaloid that present in the plant oil. These are the main components found to be basically responsible for the toxic action of its vital oil against bruchids.

Table 2: Major identified constituents of *Chromalena odorata* leaf and their relative proportion in the extract

GC peak number	Component	Peak area (%)	Retention time
1	2-Ethyl-2-hexen-1-al	5.48	8.394
2	p-Cresidine	2.69	8.814
3	Hexadecanoic acid	9.17	9.563
4	2-Ethylhex-3-enal	2.33	9.806
5	1H-Indole-4-carboxaldehyde	6.54	10.193
6	9,12,15-Octadecatrien-1-ol	0.82	10.718
7	Dimethylester	5.93	10.963
8	4-methoxybenzoic acid	2.42	12.273
9	3-Ethyl-2-pentanol	4.76	12.811
10	6-Phenylhexanoic acid	24.53	13.369
11	Octacosane	7.47	13.708
12	Diethylphthalate	2.66	14.246
13	Methyl-8-oxooctanoate	1.21	14.653
14	4-Hydroxy-2-methoxybenzaldehyde	15.36	16.336
15	Cyclooctane,1,4-diol, cis	2.59	16.817
16	Nopylacetate	1.36	17.294
17	2-Phenylcyclohexane-1,4-diol	4.68	17.605

Table 3: Major identified constituents of *Aspilia africana* leaf extract and their GC peak number and retention time in the extract

GC peak number	Component	Peak area (%)	Retention time
1	Panaxydol	3.39	8.475
2	Germacrene D	9.84	8.937
3	3-Methylbenzylalcohol	2.57	9.194
4	1,5-Heptadiene,2,5-dimethyl-3-methylene	1.31	9.628
5	5-Ethyl1-2,2,3-trimethylheptane	3.98	10.350
6	Cyclododecanone, 2-methylene	10.42	10.807
7	7-Hydroxyfarnesen	5.70	12.341
8	3-Hydroxy-alpha-ionene	21.68	12.769

GC peak number	Component	Peak area (%)	Retention time
9	Falcarinol	4.42	12.907
10	n-Hexadecanoic acid	2.19	14.326
11	Tributylacetylcitrate	6.37	14.844
12	Caryophyllene	3.42	15.525
13	2-Carene	1.48	15.913
14	Trans-1,3-diisopropenylcyclobutane	13.16	16.462
15	2,4-Dimethyl-3-nitrobicyclo [3,2,1] octan-8-one	6.78	16.893
16	Bicyclo[2.2.1]heptanes,7,7dimethyl-2-methylene	0.53	18.247
17	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b(3-methylbut-2-enyl) cyclohexane trans	2.76	18.713
18	Phytic acid	2.63	23.77
19	Myrcene	7.21	23.91
20	Lobeline	9.07	24.18
21	Beta-Caryophyllene	1.45	24.66
22	Sparteine	3.27	26.45
23	Pelletierine	4.31	26.76

DISCUSSION

Post-harvest losses caused by *Callosobruchus maculatus* are regarded as a major constraint in cowpea production; thus inaccurate application or use of synthetic insecticides by most farmers especially subsistent farmers has resulted to environmental and health hazards as well as development of insecticide resistance in insects (Hossain *et al.,* 2014). Post harvest losses caused by *C. maculatus* is a major constraint on cowpea production and the improper use of synthetic insecticides by so many farmers have resulted to health and environmental hazards and the development of insect resistance (Ajayi *et al*., 2020) The result obtained from this study showed that the *Chromolena odorata* and *Aspilla africana* oil applied to cowpea seeds significantly achieved adult bruchid mortality. Therefore, the highest mortality was recorded with high dosage of the plant oil. This is corroborated by the findings of Enobakhare and Azeez, (2016) who reported that higher concentrations of Tobacco leaf powder and aqueous extracts were observed to be most effective as cowpea seed protectant. The result obtained from this study showed that the *Chromolena odorata* and *Aspilla africana* oil applied to cowpea seeds significantly achieved adult bruchid mortality. Therefore, the highest mortality was recorded with high concentration on treated cowpea seeds. This is corroborated by the findings of Enobakhare and Azeez, (2016) who reported that effective control of *Azadirachta indica* and *Nicotiana tabacum* was higher dosage dependent as cowpea seed protectant. Other workers confirmed that plant products exerted toxic effects by distrupting physiological process like feeding, post –embryonic development and considerable reduction in egg laying, and increased mortality. Furthermore, the immediate knock down effect of bruchds few days of insect introduction was attributed to toxic effect exerted by distrupting normally respiration activity of the bruchids thus results in asphyxiation and subsequent death (Grainge *et al*., 1985)

The mean grain damage percent (number basis) with plant material was significantly different ($p<0.05$) among the treatments after 7 days post treatment. However, only the higher concentration of the plant oil employed completely evoked weevil mortality suggesting that botanical oil has strong insecticidal effect on adult bruchids. This agrees with the findings of ovicidal and larvicidal effects which prevent eggs or larva development or both (Ofuya, 1990). The serial mortality rate in relation with rise in plant extracts focus as stated by many researchers previous agreed with this study and this may be because of the combination of active compounds were in the best proportional mixture and extraction solvent for insecticidal activities. Phytochemical analysis of the botanical displayed that the botanical established valuable bio-active compounds which are alkaloids, amides, monoterpenes, sesquiterpenes, phenyl results etc with therapeutic agents which can be credited to the fatal effect of these plants. Since these botanicals proved lethal to the test insect and contained phytochemicals that are effective in the control of pests. The component of plant, terpenoids is concerned as accountable for the toxic effect used by the botanical oil. The plant compounds toxic effect has been stated by numerous authors (Bouda *et al*., 2001; Belmain *et al*., 2001) who ascribed their effect to diverse terpene as well as alkaloid components of the botanicals. This toxic effect has been attributed by various authors to the presence of some chemical compounds of triterpenoids and indole alkaloid that have been identified in the material (Philipson *et al*., 1987).

The tested plant products significantly reduced the development of bruchid that could lead to the damage of cowpea seeds. This agrees with the similar findings of Abdulai and Shepard (2000) that cowpea seed damage was significantly lower in cowpea seeds or plant treated with garlic oil and turmeric powder. From this research, the use of *Chromolena odorata* and *Aspilla africana* oil have been found to inhibit the growth of bruchids and caused their mortality rate. Agreeing to Raupp *et al*., (2014), who stated, the lasting insecticide effect of the botanical on insect pests as well as usual enemies?; Zacharia (2011), and natural great mammalian toxicity as well as ecological safety of the insecticides use. This underscored the valuable bioactive compounds with therapeutic agents identified (Adesina *et al*., 2003) which could be used for the control of pest.

CONCLUSION

Chromolena odorata and *Aspilia africana* oil proved to be more effective as 1. 0 ml and 1.5 ml are better bioactive concentration against cowpea damage irrespective of protectant used for the control of *C. maculatus*. This study highlights the potential of botanicals to effectively suppress infestation of cowpea by *C. maculatus* during storage. It has shown that *Chromolena odorata* and *Aspilia africana oil* proved *to* be more effective in reducing adult bruchid mortality.

RECOMMENDATIONS

Availability and safety given that *Chromolena odorata* and *Aspilla africana* botanicals are biodegradable and is therefore recommended for its adoption for the management of *C. maculatus* on cowpea seeds. Farmers are therefore advised to try this inexpensive strategy to reduce and prevent post harvest loss in cowpea production. These botanicals can be integrated into other management practices and methods.

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