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TOXIC AND FEEDING DETERRENT EFFECTS OF *HYPTIS SUAVEOLENS* AND *HYPTIS SPICIGERA* EXTRACTS ON COWPEA WEAVILS (*CALLOSOBRUCUS MACULATES*)

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ABSTRACT

In the present work, two plants *Hyptis suaveolens* and *Hyptis spicigera* were studied to identify their toxic and anti-feeding effects on cowpea weevils *(Callosobruchus maculatus* F.). Active ingredients of both plant samples were separately extracted and partitioned using ethanol, chloroform and distilled water solvents. Extracts and fractions obtained were tested on cowpea weevils for toxic and anti-feeding effects using cowpea seeds by two-choices bioassay technique. Results indicated that extracts and fractions from the plant samples were effective in restricting cowpea weevils from feeding on cowpea seeds. Also, extracts and fractions from the plant samples demonstrated cowpea weevil mortalities. These were indications that the plant samples contained toxicants and anti-feedants which prevented the weevils from feeding on the cowpea seeds. Thus, ingredients from *Hyptis suaveolens* and *Hyptis spicigera* should be used to protect cowpea seeds and other grains from infestations by *Callosobrucus maculatus* and other grain infesting insects. Chloroform soluble fraction of the two plants recorded the least percentage consumption index of cowpea seeds and the highest percentage mortality of cowpea weevils which confirmed that it contained the most active ingredients. Hence, chloroform solvent should be used to isolate anti-feedants and pesticide compounds from *Hyptis suaveolens* and *Hyptis spicigera* by column chromatographic method.

Keywords: Extracts, fractions, toxicants, anti-feedants, % mortality and consumption index.

INTRODUCTION

In 1939, Paul Muller discovered that diphenyl dichloro trichloroethane (DDT) and other synthetic compounds were very effective pesticides. His discovery prompted many manufacturers in the 1940’s to produce synthetic pesticides in large amounts because they were widely used (Daly *et al*., 1998).

The use of DDT and other synthetic chemicals as pesticides began to pose serious threats to human health and the environment. In the 1960’s, it was discovered that DDT was preventing many birds from reproducing, which was a serious threat to biodiversity (Lobe, 2006).

The agricultural use of DDT, lindane, and karate, is now banned under the Stockholm Convention on Persistent Organic Pollutants, but these pesticides are still used in some developing countries to store grains and prevent tropical diseases by spraying on interior walls and fields to kill or repel insects (Lobe, 2006).

Surveys conducted in vegetable growing areas in Ghana identified lindane, karate, unden and dithane as the most used pesticides by farmers. However, the agricultural use of these compounds has been banned and the pharmaceutical use of lindane is prohibited in some countries because it causes damage to the central nervous system and weakens the immune system (Glover-Amengor and Tetteh, 2007).

There are concerns that some pesticides used on food crops to control pests are harmful to people who consume foods from such crops because the pesticides contain small amounts of toxins which remain in the foods even when they are washed with water or peeled. Sometimes, these toxins are left in the soil as residues for long periods after the application and could be carried by rain into water bodies or absorbed by plants. These cause serious health effect and environmental problems.

The cummulative effects resulting from the use of synthetic chemical compounds had diverted the attention of most chemists and environmentalists to the search of compounds from natural sources which are directly or indirectly non-toxic to humans, wildlife and the ecosystem. In the 17th century, nicotine sulphate was extracted from tobacco leaves for use as an insecticide. The 19th century saw the introduction of two more natural pesticides, pyrethrum which is derived from

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chrysanthemums and rotenone which is derived from the roots of tropical vegetables (Miller, 2002).

The objective of this study is to identify solvents that can extract active ingredients from *Hyptis suaveolens* and *Hyptis spicigera*, which can be used to isolate pesticides by column chromatographic technique. It is the aim of the work to prove that *Hyptis suaveolens* and *Hyptis spicigera* have feeding deterrent and pesticide potentials. This can be proven by applying extracts of these two plants on cowpea seeds and subjecting these seeds to cowpea weevils (*Callosobruchus maculates F.*).

MATERIALS AND METHODS

Materials

The materials used in this work included digital chemical balance, separatory funnels, beakers (600 and 300ml), retort stand, funnels, Whatman no. 1 filter paper, vials, micro liter syringes, cowpea seeds, cowpea weevils *(Callosobruchus maculatus F.)*, screen cages demarcated into compartments, Winchester bottles, Aluminium foil, 95% ethanol, methanol, chloroform, petroleum ether and distilled water. Reagents used were analytical grades bought from Timster Laboratory Suppliers Limited, Accra, Ghana.

Plant collection and treatment

Hyptis suaveolens Lam and *Hyptis spicigera Lam* were used for the experiment due to their local use in some African countries and Northern Ghana in particular to protect grains from insect infestations. Both plants were randomly harvested behind the Microbiology Laboratory of University for Development Studies, Navrongo Campus, Ghana in November, 2008. The entire plants were separately air dried for twenty one days and later pulverized with the aid of a clean mortar and pestle.

Inhalation of the powdered samples during pulverizing caused dizziness and severe sneezing. The pulverized samples were stored in cleaned air-tight containers and kept in a cool dry place.

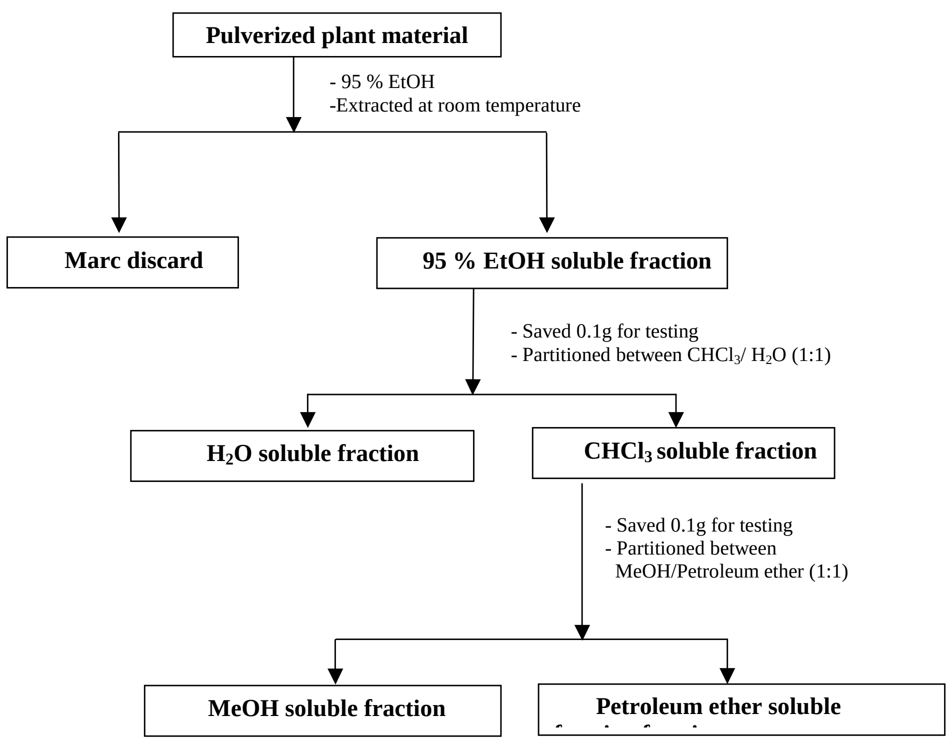
Extraction

The *Hyptis suaveolens* and *Hyptis spicigera* pulverized samples (400 and 500g) were separately soaked in 2200 millilitres of 95% ethanol with intermittent shaking for two weeks. Percolates were evaporated to dryness at room temperature to give crude extracts of the two plants which were each subjected to partition process.

Partition process

Crude extracts obtained as described above were partitioned between chloroform and distilled water (1:1, 100ml) using separating funnels. The chloroform and distilled water soluble fractions of each plant sample were separately evaporated to dryness at room temperature.

The chloroform soluble fraction of each plant was later partitioned between methanol and petroleum ether (1:1, 100ml). The methanol and petroleum ether soluble fractions were then separated and concentrated. The



fractions derived in the whole process were transferred into vials and used in a two- choices cowpea weevil bioassay (Abdullahi *et al*., 1995). The flow chart for the extraction and partition processes is as shown:

The two-choices cowpea weevil bioassay

The extracts and fractions employed in this research were in each instance weighed (10mg) and dissolved in 1ml of methanol solvent. 250µl of the solution formed was poured into a vial and kept overnight to evaporate to dryness. To the residue obtained, 5 ml of methanol solvent was added to re-dissolve it to form a concentration of 500µg/ml, and 40 cowpea seeds were then immersed in the solution. The vial and its contents were shaken for a few seconds and allowed to dry at room temperature. Also, concentrations of 250µg/ml and 125µg/ml were prepared in the same manner as the 500µg/ml concentration by transferring 125µl and 62.5µl of the solution in to two distinctive vials and topping each of them with 5ml of methanol solvent. Control cowpea seeds were introduced into 5 ml of methanol in a similar manner as the treated seeds and the treated seeds were prepared in duplicates. Both the treated and control cowpea seeds were separately placed in compartments demarcated by cardboards at the bottom of a screen cage and the seeds were infested with weevils. At the end of two weeks interval, the perforated and unperforated cowpea seeds, and dead weevils were counted.

The percentage consumption index (% C.I) and mortality were calculated using the following formulae:

% C.I = % treated cowpea seeds perforated ÷ (% treated cowpea seeds perforated+% control cowpea seeds perforated) ×100

% mortality = (% test mortality – % control mortality)÷ (100 – % control mortality) × 100

RESULTS AND DISCUSSION

From table 1, there were zero % consumption indices for both the 250 and 500µg/ml concentrations of the chloroform soluble fraction of *Hyptis suaveolens*. This implied that the solvent soluble fraction protected the cowpeas from being infested by the weevils. The 125µg/ml concentration of the chloroform soluble fraction recorded the least consumption index of 21.42% when

Table 1. Feeding deterrent activity of *Hyptis suaveolens* extracts on cowpea weevils.

SOLUBLE SOLVENT EXTRACT/ FRACTION	CONCENTRATION (µg/ml)	NUMBER OF PERFORATED SEEDS		% TREATED SEEDS PERFORATED	% CONTROL SEEDS PERFORATED	% CONSUMPTION INDEX (% C.I)	MEAN % CONSUMPTION INDEX
		TREATED	CONTROL				
ETHANOL	500	2	9	10	45	18.18	26.29
	250	3	9	15	45	25.00	
	125	5	9	25	45	35.71	
CHLOROFORM	500	0	11	0	55	0.00	7.14
	250	0	11	0	55	0.00	
	125	3	11	15	55	21.42	
DISTILLED WATER	500	1	10	5	50	9.09	21.83
	250	3	10	15	50	23.08	
	125	5	10	25	50	33.33	
PETROLEUM ETHER	500	1	9	5	45	10.00	21.92
	250	3	9	15	45	25.00	
	125	4	9	20	45	30.76	
METHANOL	500	1	8	5	40	11.11	21.48
	250	2	8	10	40	20.00	
	125	4	8	20	40	33.33	

Table 2. Feeding deterrent activity of *Hyptis spicigera* extracts on cowpea weevils.

SOLUBLE SOLVENT EXTRACT/ FRACTION	CONCENTRATION (µg/ml)	NUMBER OF PERFORATED SEEDS		% TREATED SEEDS PERFORATED	% CONTROL SEEDS PERFORATED	% CONSUMPTION INDEX (% C.I)	MEAN % CONSUMPTION INDEX
		TREATED	CONTROL				
ETHANOL	500	0	8	0	40	0.00	20.20
	250	3	8	15	40	27.27	
	125	4	8	20	40	33.33	
CHLOROFORM	500	0	9	0	45	0.00	11.67
	250	1	9	5	45	10.00	
	125	3	9	15	45	25.00	
DISTILLED WATER	500	1	11	5	55	8.33	19.65
	250	2	11	10	55	15.34	
	125	6	11	30	55	35.29	
PETROLEUM ETHER	500	1	8	5	40	11.11	23.90
	250	3	8	15	40	27.27	
	125	4	8	20	40	33.33	
METHANOL	500	1	10	5	50	9.09	20.24
	250	3	10	15	50	23.07	
	125	4	10	20	50	28.57	

Table 3. Effects of *Hyptis suaveolens* extracts on the mortality rates of cowpea weevils.

SOLUBLE SOLVENT EXTRACT/ FRACTION	CONCENTRATION (µg/ml)	TEST MORTALITY	CONTROL MORTALITY	% TEST MORTALITY	% CONTROL MORTALITY	% MORTALITY	MEAN %MORTALITY
ETHANOL	500	6	2	60	20	50.00	29.16
	250	4	2	40	20	25.00	
	125	3	2	30	20	12.50	
CHLOROFORM	500	6	1	60	10	55.56	40.74
	250	6	1	60	10	55.56	
	125	2	1	20	10	11.11	
DISTILLED WATER	500	6	1	60	10	55.56	29.63
	250	3	1	30	10	22.22	
	125	2	1	20	10	11.11	
PETROLEUM ETHER	500	6	1	60	10	55.56	29.63
	250	3	1	30	10	22.22	
	125	2	1	20	10	11.11	
METHANOL	500	5	2	50	20	37.50	25.00
	250	4	2	40	20	25.00	
	125	3	2	30	20	12.50	

Table 4. Effects of *Hyptis spicigera* extracts on the mortality rates of cowpea weevils.

SOLUBLE SOLVENT EXTRACT/ FRACTION	CONCENTRATION (µg/ml)	TEST MORTALITY	CONTROL MORTALITY	% TEST MORTALITY	% CONTROL MORTALITY	% MORTALITY	MEAN % MORTALITY
ETHANOL	500 250 125	5 4 2	1 1 1	50 40 20	10 10 10	44.44 33.33 11.11	29.627
CHLOROFORM	500 250 125	6 4 2	1 1 1	60 40 20	10 10 10	55.56 33.33 11.11	33.333
DISTILLED WATER	500 250 125	5 4 3	1 1 1	50 40 30	10 10 10	44.44 33.33 22.22	33.330
PETROLEUM ETHER	500 250 125	6 5 4	3 3 3	60 50 40	30 30 30	42.85 28.57 14.28	28.567
METHANOL	500 250 125	5 4 3	2 2 2	50 40 30	20 20 20	37.50 25.00 12.50	25.000

compared to all the other tested extracts of the same concentration. This clearly gave a clue that the chloroform soluble fraction of *Hyptis suaveolens* contained the most active components which prevented the weevils from feeding on the cowpea seeds.

From the same table, the ethanol soluble extract at 125 and 500µg/ml concentrations recorded the highest consumption indices of 35.71 and 18.18% respectively when compared to all the other tested soluble fractions at these concentrations. This indicated that the ethanol soluble extract was the least active in terms of deterring the weevils from feeding on the cowpea seeds. A contributing factor to the least activity of the ethanol soluble extract was the presence of components or impurities in its content which masked its active ingredients, thus preventing it from exhibiting its complete feeding deterrent property.

Table 2 results showed that the chloroform soluble fraction of *Hyptis spicigera* had the least consumption indices at the tested concentrations when compared to all the other soluble extracts or fractions. Hence, it was a clear indication that the chloroform soluble fraction contained the most active component(s) of the plant, and was capable of deterring the weevils from feeding on the cowpea seeds. On the contrary, the petroleum ether soluble fraction of *Hyptis spicigera* gave the highest average consumption index of 23.90% when compared to those of all the other tested extracts or fractions. Therefore, this fraction contained the least active component(s) of the plant which deterred the weevils from feeding on the cowpea seeds.

Table 3 results showed the chloroform soluble fraction of *Hyptis suaveolens* with the highest average mortality of 40.74% and the ethanol soluble extract with the least average mortality of 29.16%. These values revealed that the chloroform soluble fraction contained the most active component(s), while the ethanol soluble extract contained the least active component(s) of the plant which caused dead of the weevils.

From table 4 results, the chloroform soluble fraction of *Hyptis spicigera* recorded the highest average mortality of 33.333% and was closely followed by the distilled water soluble fraction with an average mortality of 33.330%. The petroleum ether soluble fraction of the plant presented the least average mortality of 28.56%. These stated values explained that the chloroform soluble fraction contained the most active component(s) of the plant, while the petroleum ether soluble fraction contained the least.

Thus, the chloroform soluble fraction was the most active ingredient for both plants, while the ethanol soluble extract and the petroleum ether soluble fraction were the least active for *Hyptis suaveolens* and *Hyptis spicigera* respectively.

CONCLUSION

Results for the anti-feeding and mortality tests revealed that extracts/fractions of the two plants were toxic to cowpea weevils by killing and preventing them from feeding on the cowpea seeds. In all, the chloroform soluble fraction was identified to contain the most active ingredient responsible for protecting the cowpea seeds from being infested, and for causing dead of the cowpea weevils because of the least percentage consumption indices and the highest percentage mortalities it recorded. There is a correlation between the feeding deterrent activity and toxicity of this fraction to cowpea weevils. Hence, chloroform solvent should be used to isolate anti-feedants and pesticides from *Hyptis suaveolens* and *Hyptis spicigera* by column chromatographic method.

RECOMMENDATION

It is recommended that extracts/fractions of these plants should be used to protect cowpea seeds and other grains from damage by cowpea weevils and other grain infesting insects. Also, further research should be conducted on both *Hyptis suaveolens* and *Hyptis spicigera* by the use of chloroform solvent to help isolate active compounds which could serve as pesticides for keeping the desirable qualities of cowpea seeds. This will go a long way to avoid the use of synthetic pesticides that leave residues on plants and in the soil that may be harmful to both humans and the environment at large.

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