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Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2010, 2(4):387-397

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Assessing the effectiveness of the non-polar extract of *C*rotalaria *retusa* in the control and management of the flea beetle, [*Podagrica uniformis* (L)] on okro, [*Abelmoschus esculentus* (L) moench]

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ABSTRACT

Five formulations made from the non-polar extracts of C. retusa have been used as test agents on the flea beetles (P. uniformis). The effectiveness of each formulation (prepared by using Omo detergent solution and petroleum ether) was assessed by the estimation of the number of okro leaf discs (dipped in the respective formulations) eaten by P. uniformis and their mortality within the experimental period. The results of this study have showed that, non-polar constituents of the extract of C. retusa have antifeedant properties on flea beetles since they significantly reduced the feeding of P. uniformis on Okro leaf discs. The most effective formulation from the non-polar extract was found to be 0.3g of the extract in 40ml petroleum ether, while the least effective formulation was 0.6g of the extract in 40ml petroleum ether. There was a weak positive correlation (P>0.05) between the number okro leaf discs consumed and the mortality of the P. uniformis. This research suggests that the non-polar extract of C. retusa may contain some active ingredients for controlling the flea beetle, P. uniformis.

Key words: *Crotalaria retusa*, *Podagrica uniformis*, Okro plant, *Abelmoschus esculentus*, non-polar extracts, ANOVA.

INTRODUCTION

The use of *Crotalaria retusa* extracts as insect pest control agents could be practicable, considered against the background that it is hardly touched by foraging insects (pers.ob), and that several alkaloids are reported to be extracted from the species. In South Africa, the alkaloids usramine nilgirine and intergerrimine were the constituents of the seeds of C. *lanceolata* Dill [1]. Whilst in Nigeria, Mattocks and Nwude [2] also reported finding pyrrolizidine alkaloid from two other species of *Crotalaria*; *C. lachnosema* Staff and C. *naragutensis* Hutch. In C. *naragutensis* seeds and seed pods specifically, the researchers found nilgirine together with intergerrimine, usaramine and acetyl intergerrimine [2].

Five species of *Crotalaria*, *C. spectabilis* Roth, *C. juncea* Linn, *C verrucosa* Linn, *C. striata* DC (*C. pallida*) and *C. derecha* Dill were assayed for their pyrrolizidine alkaloid content by researchers in Cuba. *C derecha* and *C. verrucosa* were identified to contain Monocrotaline which according to the researchers is metabolized to the hepatotoxic pyrrolic derivatives [3].

In spite of the evidence of the presence of toxic alkaloids in most *Crotalaria spp*. not much attempt has been made to assess the effect of the extracts from *Crotalaria* spp. as agents for insect pest control. However few species have been assessed against some pests. For example, under greenhouse conditions, *C.retusa* and *C. juncea* have been found to be resistant to attack by the nematode, *Pratyylenchus zeae* and also that *C. retusa* has also shown a high degree of resistance to attack by the nematode, *Rotylenchus reniformis* Linford and Oliveira [4].

Again in Puerto Rico a [5] a number of *Crotalaria spp., specifically C. stipularia* Dill and C. lanceolata were found to be not susceptible to attack by the lima bean pod borer, [*Etiella zinckenella* (Trectschke)].

In a recent survey in Ghana, West Africa researchers from the University of Cape Coast found that the aqueous extract of the devil bean, *C. retusa* exhibited great potential at controlling the flea beetle, *Podagrica uniformis* on the Okro plant, *Abelmoschus esculentus* (L) Moench [6]. It is therefore a matter of expediency to find out which part of the Crotalaria extract may contain active ingredients. Hence the non – polar extract of *Crotalaria retusa* is being assessed to find out whether it may contain factors effective at controlling or managing the flea beetle.

EXPERIMENTAL SECTION

2.1. Extraction of the non-polar constituent

Three kilograms of freshly harvested *C.retusa* shoot was cut up into pieces and oven dried at 60 ^{0}C. The *C. retusa* shoot was dried and weighed daily until the weight became constant (2kg). Thus all the moisture had evaporated. The dried material was milled.

Two round bottom quick fit flasks, each with a capacity of 100 ml were filled with 100 g of the milled dried *C. retusa* shoot. About 100ml of petroleum ether (60- 80 ^{0}C grade) was then poured unto the plant material in each flask and the flasks were fitted to a shaker for 24hours to hasten the extraction processes. The extract was then decanted and filtered to remove all solid particles with a suction filter. The filtrate was then concentrated to an oily semi-solid brownish green substance using a Rotary Evaporator.

2.2. Formulations for application

Two formulations were made from the non-polar constituent: a detergent formulation and a petroleum ether formulation. The detergent formulation was made by dissolving (i) 0.6 g and (ii) 0.3 g of the non-polar constituent in a solution of Omo (a detergent) with a concentration of 0.5 g Omo in 40 ml of water. Thus two different concentrations of the detergent formulations were obtained.

The petroleum ether formulations were made as follows (i) 0.6 g of the non-polar substance was dissolved in 40 ml of petroleum ether, (ii) 0.3g of non-polar extract was dissolved in 40 ml of petroleum ether and (iii) 0.2g of the non-polar extract was dissolved in 40 ml of petroleum ether. Thus three different concentrations of petroleum ether-formulations were obtained.

2.3. The effects of the non-polar extracts on the feeding of p. uniformis.

The various formulations made from the Non-Polar extracts of *C. retusa* were used as test agents. In each case, 10 discs of Okro leaves were placed in a set of four Petri-dishes. Each disc had a diameter of 10 mm. The discs were first dipped in the respective formulations made from the non-polar constituents of the shoot extract. The discs were left to air-dry for a period of 30 minutes to one hour, and then placed in each Petri-dish which had a base of white paper. 10 beetles were placed in each of the four Petri-dishes containing the treated Okro leaf discs. The four Petri-dishes each containing10 treated discs of Okro leaves and 10 beetles constituted one set for one experiment. Each experiment was replicated three times. Petroleum ether (60^o-80^o) and Omo-solution were also used as test agents in order to ascertain that they were not in any way adding to the effect of the extract.

Control experiments were also carried out with discs dipped in water. Each disc was considered as a unit so that if a disc had only one nibbled hole, it was counted as having been eaten. The number of discs eaten and the number of discs not eaten, together with the mortality of *P. uniformis* in each experiment was recorded after 24 hours.

2.4. Data analyses

The experimental data were subjected to statistical analyses using SPSS software (Version 16). The normality of the data was checked using Shapiro-Wilk procedure. One way ANOVA was applied to compare the means of mortality and consumption or damage to leaves for the different formulations. The critical differences among the different formulations and control were examined using Least Significant difference (for equal variances assumed variables) and Games-Howell Post-Hoc (for equal variances not assumed variables) multiple comparisons tests. The linear relationship between mortality and the number of leaves consumed were measured using regression analysis.

RESULTS

3.1 Disc consumed

The results from the study of the effect of the non-polar constituents of the shoot extract of C. *retusa* on the feeding and mortality of the flea beetles are presented on table 1 and 2. The variation of the mean percent mortality of flea beetles caused by consuming various formulations of non-polar extract and control; and mean percent okro disc consumed by flea beetles is depicted on Fig. 1 and 2 respectively.

Table 1. Omo and non-polar extract formulations summary statistics [minimum, maximum and Mean (Standard deviation)] for the consumption pattern and mortality of *Podacrica uniforms*

Variable	0.6g. NPE O solution		0.3g. NPE in O solution		O solution		Water (Control)	
	A	B	A	B	A	B	A	B
Min	5	0	35	0	30	0	100	5
Max	37.5	17.5	40	8.8	62	7.5	100	10
Mean(±SD)	25.83±14.77	9.17±7.16	36.67±2.36	4.90±3.66	48.17±13.41	3.33±3.12	100.0±0.0	7.50±2.04

NPE=Non-polar Extracts **O=OMO** **A=Percent Disc consumed** **B=Mortality (%)**

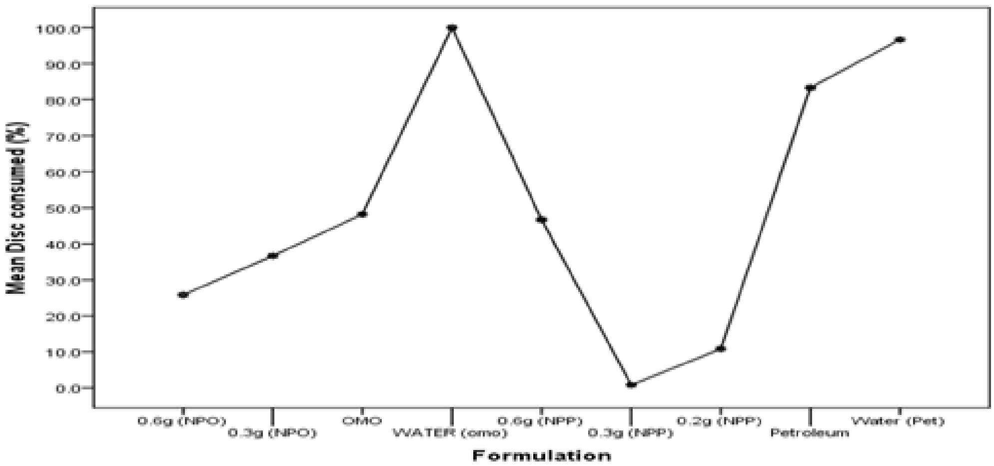

Fig. 2. Comparisons of mean percent okro disc consumed by flea beetles.

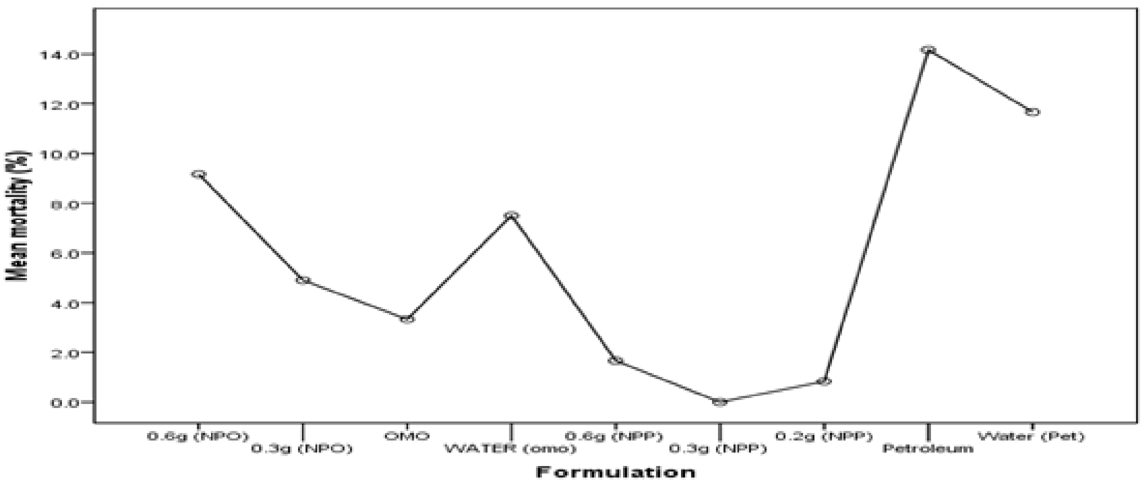

Fig. 1. The variations of mean percent mortality of flea beetles caused by consuming various formulations of non-polar extract and control

Table 2. Petroleum ether and non-polar extract formulations summary statistics [minimum, maximum and Mean (Standard deviation)] for the consumption pattern and mortality of *Podacrica uniforms*

Variable	0.6g. NPE in P		0.3g. NPE in P		0.2g. NPE P		P solution		Water (Control)	
	A	B	A	B	A	B	A	B	A	B
Min	42.5	2.5	0	0	7.5	0	75	12.5	95	2.5
Max	52.5	0	2.5	0	15	2.5	100	15	100	17.5
Mean(±SD)	46.67±4.25	1.67±1.18	0.83±1.18	0	10.83±3.12	0.83±1.18	83.33±11.79	14.17±1.18	96.67±2.36	11.67±6.56

NPE=Non-polar Extracts P=Petroleum ether A=Percent Disc consumed B=Mortality(%)

The results revealed that flea beetles on the average ate 25.8±14.8% and 36.7±2.4% of Okro leaf discs treated with Omo-base formulations which were prepared with 0.6 g non-polar extract/40 ml Omo solution and 0.3 g non-polar extract/40 ml Omo solution respectively. Also, flea beetles on the average ate 48.3±7.85% and 100% of the discs treated with Omo solution (with no non-polar extract) and water respectively. The observed differences between the means of the damage caused to the leaf discs were significant as revealed by the results of one way ANOVA test ($p<0.05$) [Table 3]. However, the noted differences with respect to the means of the percent number of discs consumed by the flea beetles treated with the two different concentrations of the non-polar extract in the Omo-base formulations, were not statistically significant (as shown on Table 5; $p=0.832$). This may suggests that the two different concentrations of the Omo-base formulations of non-polar extract have similar effect in reducing feeding by the flea beetles.

Table 3. Results of One Way ANOVA analysis

Variable		Sum of Squares	df	Mean Square	F	Sig.
Percentage mortality	Between Groups	803.38	8	100.42	7.18	0.000
	Within Groups	377.72	27	13.99		
	Total	1181.10	35			
Disc consumed	Between Groups	42066.49	8	5258.31	81.98	0.000
	Within Groups	1731.83	27	64.14		
	Total	43798.33	35			

There was also a significant difference ($p=0.031$) between the means of the damage caused to the Okro leaf discs treated with water and those treated with Omo solution (Table 5). The results suggest that the non-polar extract and even the Omo solution alone reduced feeding on Okro by the flea beetles.

Table 5. Results of Games-Howell test on percent okro disc (with Non-polar extract in OMO formulations and control) consumed by flea beetles

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.6g (NPO)	0.3g (NPO)	-10.835	7.47682	0.832	-57.5977	35.9277
	OMO	-22.335	9.97636	0.479	-67.0621	22.3921
	WATER (omo)	-74.16750*	7.38335	0.015	-122.077	-26.258
	0.6g (NPP)	-20.835	7.68295	0.368	-65.6354	23.9654
	0.3g (NPP)	25	7.40683	0.250	-22.6041	72.6041
	0.2g (NPP)	15	7.54615	0.617	-31.0226	61.0226
	Petroleum	-57.50000*	9.44649	0.013	-100.4789	-14.5211
	Water (Pet)	-70.83500*	7.47682	0.015	-117.5977	-24.0723
0.3g (NPO)	0.6g (NPO)	10.835	7.47682	0.832	-35.9277	57.5977
	OMO	-11.5	6.81196	0.739	-53.7951	30.7951
	WATER (omo)	-63.33250*	1.17851	0.000	-70.9797	-55.6853
	0.6g (NPP)	-10	2.42956	0.097	-22.0361	2.0361
	0.3g (NPP)	35.83500*	1.31762	0.000	29.1119	42.5581
	0.2g (NPP)	25.83500*	1.95434	0.000	16.8607	34.8093
	Petroleum	-46.66500*	6.00925	0.025	-83.53	-9.8
	Water (Pet)	-60.00000*	1.66667	0.000	-67.4473	-52.5527
OMO	0.6g (NPO)	22.335	9.97636	0.479	-22.3921	67.0621
	0.3g (NPO)	11.5	6.81196	0.739	-30.7951	53.7951
	WATER (omo)	-51.83250*	6.70924	0.031	-95.3678	-8.2972
	0.6g (NPP)	1.5	7.0376	1.000	-38.7911	41.7911
	0.3g (NPP)	47.33500*	6.73507	0.039	4.1343	90.5357
	0.2g (NPP)	37.335	6.88799	0.068	-4.185	78.855
	Petroleum	-35.165	8.92951	0.087	-75.3097	4.9797
	Water (Pet)	-48.50000*	6.81196	0.034	-90.7951	-6.2049
WATER (omo)	0.6g (NPO)	74.16750*	7.38335	0.015	26.258	122.077
	0.3g (NPO)	63.33250*	1.17851	0.000	55.6853	70.9797
	OMO	51.83250*	6.70924	0.031	8.2972	95.3678
	0.6g (NPP)	53.33250*	2.12459	0.001	39.5463	67.1187
	0.3g (NPP)	99.16750*	0.58926	0.000	95.3439	102.9911
	0.2g (NPP)	89.16750*	1.55902	0.000	79.0512	99.2838
	Petroleum	16.6675	5.89256	0.360	-21.5684	54.9034
	Water (Pet)	3.3325	1.17851	0.360	-4.3147	10.9797

NPO= Non-polar extract in OMO *. The mean difference is significant at the 0.05 level. NPP=Non-polar extract in Petroleum ether

3.2. Mortality

As shown on table 1, nearly (7.5±2.04%) as many flea beetles died when they were fed with the Okro leaf discs treated with only water, as when they were fed with the 0.6g non-polar extract in the Omo solution (9.17 ± 7.16%). The results of the least significant difference (LSD) post hoc test (Table 4) indicate that the differences in the percent mortality of the flea beetles which fed on okro leaf discs treated with 0.6g, 0.3gNon-polar extracts (omo formulation) and water (control), were not statistically significant ($p>0.05$). The non-polar extract in the Omo solution, therefore did not seem to cause mortality of the flea beetles. However, the difference in percentage mortality of the flea beetles between 0.6g non-polar extract (Omo formulation) and Omo (only) was statistically significant ($p=0.036$).

Table 4. Results of Least Significant Difference (LSD) test of percent mortality of flea beetles due to Non-polar extract in OMO formulations and control

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.6g (NPO)	0.3g (NPO)	4.2675	2.64477	0.118	-1.1591	9.6941
	OMO	5.83500*	2.64477	0.036	0.4084	11.2616
	WATER (omo)	1.6675	2.64477	0.534	-3.7591	7.0941
	0.6g (NPP)	7.50000*	2.64477	0.009	2.0734	12.9266
	0.3g (NPP)	9.16750*	2.64477	0.002	3.7409	14.5941
	0.2g (NPP)	8.33500*	2.64477	0.004	2.9084	13.7616
	Petroleum ether	-5	2.64477	0.069	-10.4266	0.4266
	Water (Pet)	-2.5	2.64477	0.353	-7.9266	2.9266
0.3g (NPO)	0.6g (NPO)	-4.2675	2.64477	0.118	-9.6941	1.1591
	OMO	1.5675	2.64477	0.558	-3.8591	6.9941
	WATER (omo)	-2.6	2.64477	0.334	-8.0266	2.8266
	0.6g (NPP)	3.2325	2.64477	0.232	-2.1941	8.6591
	0.3g (NPP)	4.9	2.64477	0.075	-0.5266	10.3266
	0.2g (NPP)	4.0675	2.64477	0.136	-1.3591	9.4941
	Petroleum	-9.26750*	2.64477	0.002	-14.6941	-3.8409
	Water (Pet)	-6.76750*	2.64477	0.016	-12.1941	-1.3409
OMO	0.6g (NPO)	-5.83500*	2.64477	0.036	-11.2616	-0.4084
	0.3g (NPO)	-1.5675	2.64477	0.558	-6.9941	3.8591
	WATER (omo)	-4.1675	2.64477	0.127	-9.5941	1.2591
	0.6g (NPP)	1.665	2.64477	0.534	-3.7616	7.0916
	0.3g (NPP)	3.3325	2.64477	0.218	-2.0941	8.7591
	0.2g (NPP)	2.5	2.64477	0.353	-2.9266	7.9266
	Petroleum ether	-10.83500*	2.64477	0	-16.2616	-5.4084
	Water (Pet)	-8.33500*	2.64477	0.004	-13.7616	-2.9084
WATER (omo)	0.6g (NPO)	-1.6675	2.64477	0.534	-7.0941	3.7591
	0.3g (NPO)	2.6	2.64477	0.334	-2.8266	8.0266
	OMO	4.1675	2.64477	0.127	-1.2591	9.5941
	0.6g (NPP)	5.83250*	2.64477	0.036	0.4059	11.2591
	0.3g (NPP)	7.50000*	2.64477	0.009	2.0734	12.9266
	0.2g (NPP)	6.66750*	2.64477	0.018	1.2409	12.0941
	Petroleum ether	-6.66750*	2.64477	0.018	-12.0941	-1.2409
	Water (Pet)	-4.1675	2.64477	0.127	-9.5941	1.2591

NPO=Non-polar extract in OMO   *. The mean difference is significant at the 0.05 level   NPP=Non-polar extract in Petroleum ether

DISCUSSION

4.1. Disc consumed

Table 2 presents the response of the beetles to Okro leaf discs treated with petroleum ether formulations of the non-polar constituent of the *C. retusa* extract. The petroleum ether formulations with 0.6g, 0.3g and 0.2g non-polar extracts yielded percentage okro leaf discs consumptions of 46.67±0.58; % 0.83±1.18%; 10.83±3.12% respectively. This shows that the effectiveness of the petroleum ether formulations to reduce feeding by the flea beetles improved at the concentration of 0.3g of the non-polar extract in the petroleum ether. The differences in the percentage consumption of the Okro leaf discs treated with the different formulations of the non-polar extract petroleum ether were also significant ($p<0.05$).

On the other hand, Petroleum ether and water (control) only formulations recorded percentage Okro leaf discs consumption of 83.33±11.79% and 96.67±2.36% respectively. This result shows that petroleum ether formulations with non-polar extracts of the applied concentrations significantly reduced the feeding of the flea beetles on the okro leaf discs in comparison with consumption of the Okro leaf discs treated with water and petroleum ether without the extracts. This was also supported by the outcome of the Games-Howell post hoc test (P<0.05) shown by Table 7.

Table 7. Results of Games-Howell test on percent okro disc (with Non-polar extract in petroleum ether formulations and control) consumed by flea beetles

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.6g (NPP)	0.6g (NPO)	20.835	7.68295	0.368	-23.9654	65.6354
	0.3g (NPO)	10	2.42956	0.097	-2.0361	22.0361
	OMO	-1.5	7.0376	1.000	-41.7911	38.7911
	WATER (omo)	-53.33250*	2.12459	0.001	-67.1187	-39.5463
	0.3g (NPP)	45.83500*	2.20479	0.001	32.8951	58.7749
	0.2g (NPP)	35.83500*	2.63523	0.000	23.6643	48.0057
	Petroleum ether	-36.66500*	6.26387	0.042	-71.5208	-1.8092
	Water (Pet)	-50.00000*	2.42956	0.000	-62.0361	-37.9639
0.3g (NPP)	0.6g (NPO)	-25	7.40683	0.250	-72.6041	22.6041
	0.3g (NPO)	-35.83500*	1.31762	0.000	-42.5581	-29.1119
	OMO	-47.33500*	6.73507	0.039	-90.5357	-4.1343
	WATER (omo)	-99.16750*	0.58926	0.000	-102.9911	-95.3439
	0.6g (NPP)	-45.83500*	2.20479	0.001	-58.7749	-32.8951
	0.2g (NPP)	-10.00000*	1.66667	0.037	-19.1699	-0.8301
	Petroleum ether	-82.50000*	5.92195	0.005	-120.3579	-44.6421
	Water (Pet)	-95.83500*	1.31762	0.000	-102.5581	-89.1119
0.2g (NPP)	0.6g (NPO)	-15	7.54615	0.617	-61.0226	31.0226
	0.3g (NPO)	-25.83500*	1.95434	0.000	-34.8093	-16.8607
	OMO	-37.335	6.88799	0.068	-78.855	4.185
	WATER (omo)	-89.16750*	1.55902	0.000	-99.2838	-79.0512
	0.6g (NPP)	-35.83500*	2.63523	0.000	-48.0057	-23.6643
	0.3g (NPP)	10.00000*	1.66667	0.037	0.8301	19.1699
	Petroleum ether	-72.50000*	6.09531	0.005	-108.5536	-36.4464
	Water (Pet)	-85.83500*	1.95434	0.000	-94.8093	-76.8607
Petroleum ether	0.6g (NPO)	57.50000*	9.44649	0.013	14.5211	100.4789
	0.3g (NPO)	46.66500*	6.00925	0.025	9.8	83.53
	OMO	35.165	8.92951	0.087	-4.9797	75.3097
	WATER (omo)	-16.6675	5.89256	0.360	-54.9034	21.5684
	0.6g (NPP)	36.66500*	6.26387	0.042	1.8092	71.5208
	0.3g (NPP)	82.50000*	5.92195	0.005	44.6421	120.3579
	0.2g (NPP)	72.50000*	6.09531	0.005	36.4464	108.5536
	Water (Pet)	-13.335	6.00925	0.531	-50.2	23.53
Water (Pet)	0.6g (NPO)	70.83500*	7.47682	0.015	24.0723	117.5977
	0.3g (NPO)	60.00000*	1.66667	0.000	52.5527	67.4473
	OMO	48.50000*	6.81196	0.034	6.2049	90.7951
	WATER (omo)	-3.3325	1.17851	0.360	-10.9797	4.3147
	0.6g (NPP)	50.00000*	2.42956	0.000	37.9639	62.0361
	0.3g (NPP)	95.83500*	1.31762	0.000	89.1119	102.5581
	0.2g (NPP)	85.83500*	1.95434	0.000	76.8607	94.8093
	Petroleum ether	13.335	6.00925	0.531	-23.53	50.2

NPO=Non-polar extract in OMO *. The mean difference is significant at the 0.05 level. NPP=Non-polar extract in Petroleum ether

The difference between okro leaf discs consumption with respect to the water (control)[96.67±2.36%] and that for the petroleum ether (83.33±11.79%,) formulations above was not significant (p=0.531). Thus the petroleum ether alone was not effective in reducing feeding of the flea beetles.

Table 6. Results of Least Significant Difference (LSD) test on percent mortality of flea beetles due to Non-polar extract in Petroleum ether formulations and control

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.6g (NPP)	0.6g (NPO)	-7.50000*	2.64477	0.009	-12.9266	-2.0734
	0.3g (NPO)	-3.2325	2.64477	0.232	-8.6591	2.1941
	OMO	-1.665	2.64477	0.534	-7.0916	3.7616
	WATER (omo)	-5.83250*	2.64477	0.036	-11.2591	-0.4059
	0.3g (NPP)	1.6675	2.64477	0.534	-3.7591	7.0941
	0.2g (NPP)	0.835	2.64477	0.755	-4.5916	6.2616
	Petroleum ether	-12.50000*	2.64477	0.000	-17.9266	-7.0734
	Water (Pet)	-10.00000*	2.64477	0.001	-15.4266	-4.5734
0.3g (NPP)	0.6g (NPO)	-9.16750*	2.64477	0.002	-14.5941	-3.7409
	0.3g (NPO)	-4.9	2.64477	0.075	-10.3266	0.5266
	OMO	-3.3325	2.64477	0.218	-8.7591	2.0941
	WATER (omo)	-7.50000*	2.64477	0.009	-12.9266	-2.0734
	0.6g (NPP)	-1.6675	2.64477	0.534	-7.0941	3.7591
	0.2g (NPP)	-0.8325	2.64477	0.755	-6.2591	4.5941
	Petroleum ether	-14.16750*	2.64477	0.000	-19.5941	-8.7409
	Water (Pet)	-11.66750*	2.64477	0.000	-17.0941	-6.2409
0.2g (NPP)	0.6g (NPO)	-8.33500*	2.64477	0.004	-13.7616	-2.9084
	0.3g (NPO)	-4.0675	2.64477	0.136	-9.4941	1.3591
	OMO	-2.5	2.64477	0.353	-7.9266	2.9266
	WATER (omo)	-6.66750*	2.64477	0.018	-12.0941	-1.2409
	0.6g (NPP)	-0.835	2.64477	0.755	-6.2616	4.5916
	0.3g (NPP)	0.8325	2.64477	0.755	-4.5941	6.2591
	Petroleum ether	-13.33500*	2.64477	0.000	-18.7616	-7.9084
	Water (Pet)	-10.83500*	2.64477	0.000	-16.2616	-5.4084
Petroleum ether	0.6g (NPO)	5	2.64477	0.069	-0.4266	10.4266
	0.3g (NPO)	9.26750*	2.64477	0.002	3.8409	14.6941
	OMO	10.83500*	2.64477	0.000	5.4084	16.2616
	WATER (omo)	6.66750*	2.64477	0.018	1.2409	12.0941
	0.6g (NPP)	12.50000*	2.64477	0.000	7.0734	17.9266
	0.3g (NPP)	14.16750*	2.64477	0.000	8.7409	19.5941
	0.2g (NPP)	13.33500*	2.64477	0.000	7.9084	18.7616
	Water (Pet)	2.5	2.64477	0.353	-2.9266	7.9266
Water (Pet)	0.6g (NPO)	2.5	2.64477	0.353	-2.9266	7.9266
	0.3g (NPO)	6.76750*	2.64477	0.016	1.3409	12.1941
	OMO	8.33500*	2.64477	0.004	2.9084	13.7616
	WATER (omo)	4.1675	2.64477	0.127	-1.2591	9.5941
	0.6g (NPP)	10.00000*	2.64477	0.001	4.5734	15.4266
	0.3g (NPP)	11.66750*	2.64477	0.000	6.2409	17.0941
	0.2g (NPP)	10.83500*	2.64477	0.000	5.4084	16.2616
	Petroleum ether	-2.5	2.64477	0.353	-7.9266	2.9266

NPO=Non-polar extract in OMO *. The mean difference is significant at the 0.05 level. NPP=Non-polar extract in Petroleum ether

4.2. Mortality

The mortalities of the flea beetles fed with the Okro leaf discs treated with the petroleum ether formulations of the non-polar extract, petroleum ether and water (control) ranged between 0-

14.17%. High mortalities were recorded for the petroleum ether (14.17±1.18%) and the control (11.67±6.56%). No deaths were recorded for the 0.3g/40ml formulation. As shown by Table 6, the difference in mortalities for the various petroleum ether formulations was not significant ($P > 0.05$). Also, the difference in mortalities of the flea beetles caused by petroleum ether formulation (without extract) and water (control) was not significant ($P > 0.05$). However, the differences in the mortalities caused by the various petroleum ether/extract formulations, the petroleum ether alone, and the water (control), was statistically significant($p < 0.05$) [Table 6]. This implies that the non- polar extract of C. retusa in the petroleum ether was not insecticidal. The percentage mortality and the percentage number of okro leaf discs consumed by the flea beetles were inversely proportional to each other. That is, the formulation that prevents damage to okro leaf discs produces the least number of deaths to the flea beetles.

4.3 Differences between omo and petroleum ether formulations

Figure 3 depicts the ranking of the various formulations of the non-polar extracts in Omo and petroleum ether. The experiment reveals that non-polar extract in 0.3g/40ml petroleum ether is the most effective formulation which was able to prevent the flea beetles from eating the Okro leaf discs. The relative least effective formulation which prevented the flea beetles from eating okro leaf discs was found to be 0.6g non-polar extract in 40ml petroleum ether.

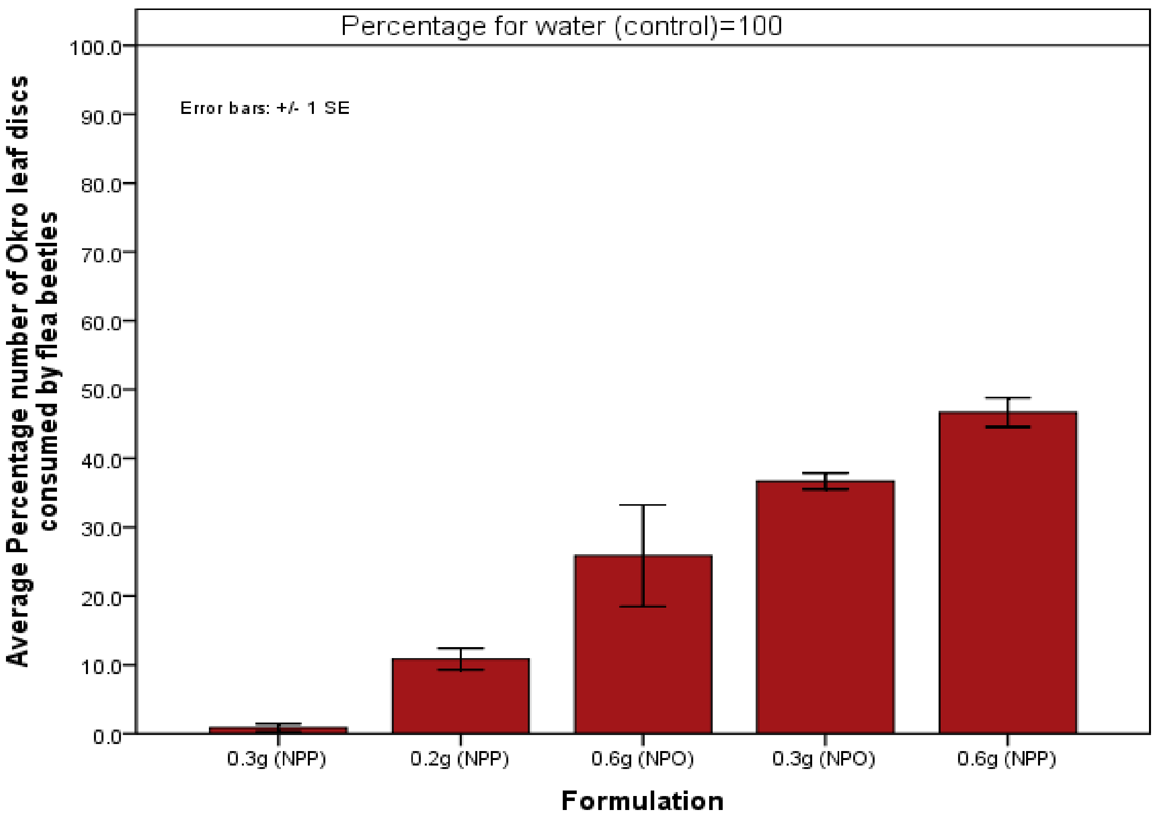

Fig. 3. Ranking of effective formulation based on the number of discs damaged by flea beetles

CONCLUSION

The effectiveness of the non-polar constituents of the extract of *Crotalaria retusa* have been assessed by feeding the flea beetles with Okro leaf discs treated with the non-polar extracts. The five formulations made from the non-polar extracts of *C. retusa* were used as test agents on the flea beetles. Petroleum ether, Omo solution and water without the non-polar extracts, were also used in the control experiment, in order to ascertain that they were not in any way adding to the effect of the extract. The effectiveness of each formulation (prepared by using Omo detergent solution and petroleum ether) was determined by the evaluation of the number of okro leaf discs (treated with the respective formulations) eaten by flea beetles and their mortality within the experimental period.

The results from this study have revealed that, non-polar constituents of the extract of *C. retusa* have antifeedant properties on flea beetles since they greatly reduces the feeding of *P. uniformis* on Okro plants. The most effective formulation from the non-polar extract was found to be 0.3g of the extract in 40ml petroleum ether, whereas the least effective formulation was 0.6g of the extract in 40ml petroleum ether. There was a weak positive correlation between the number okro leaf discs consumed and the mortality of the flea beetles.

This research suggests that the non-polar extract of *C. retusa* may contain some active ingredients for controlling or managing the flea beetle, *P. uniformis* (L). It is envisaged that the results of this study will enrich the discussion and understanding of the how non-polar extract of *C. retusa* could be used in pest management. It is recommended that lethal dose investigations are integrated into future studies *C. retusa* non-polar extracts.

Acknowledgements
The authors are grateful to all who contributed to this research. Special thanks to L. K. Boamponsem of Department of Laboratory Technology, University of Cape Coast for his assistance in the data analysis.

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