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TOXICOLOGICAL STUDIES OF SOME PLANT EXTRACTS AGAINST GLASSY CLOVER SNAIL; *Monacha obstructa* (PFIFFER)

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ABSTRACTS

Plants of Santonia (Foliage), Red Pepper (fruits) and Pomegranate (fruit's Skin peel) were extracted in boiled water, filtered and the obtained extracts were used as crude extracts. Also, the acetone extracts of these plants in addition to Fennel (seeds), Black pepper (Fruits) and Neem (seeds) had been screened in laboratory for their molluscicidal activities against glassy clover snail; *Monacha obstructa* (Pfiffer), three concentrations of each plant crude extract were tested as bait, residual film, leaf-dipping and repellency technique. Results indicated that, water extracts had no effect on snails, and no mortality of snail individuals was recorded. Acetone extract of Fennel exhibited more toxic effect than other plant extracts on bait and dipping technique. The efficiency of Fennel and Pomegranate was the highest followed by Black pepper, Santonia, Red pepper and Neem on residual film technique, while in the repellency tests, Santonisa and Neem exhibited high repellent action than other plant extracts.

In order to identify the plant extract components, the extracts were subjected to thin layer chromatography technique.The extracts of Black pepper, Pomegranate, Fennel, Santonia, Red pepper and Neem contained 5, 2, 3, 2, 6 and 3 spots, respectively. The acetone extract of Black pepper yielded 5 fractions having (RF) values of 0.4, 0.5, 0.6, 0.8 and 0.9 cm. Other plant extracts showed different developed fractions.

Keywords: Land snail, plant extracts, *Monacha obstructa*, Fennel, Black pepper, Santonia, Red Pepper, Pomegranate and Neem.

INTRODUCTION

The terrestrial snails become an economic serious pest in Egypt during the last few years (Khidr et al., 2005). they attack field crops, vegetables, orchards, as well as ornamental plants (Kassab and Daoud 1964, Ghamry *et al.*, 1993 and Ismail, 1997). Such animals were recorded with a relatively high population density on the majority of economic crops cultivated in the northern newly reclaimed desert land due to the availability of favorable weather conditions (mainly, temperature and relative humidity), sandy soils and manuring. (El-Okda, 1980;Godan, 1983 and Nakhla *et al.*, 1993).These animals cause serious yield reduction of infested crops and fruits, as well as destroying plant seedlings.

These pests were controlled chemically by using molluscicides or insecticides (Crowell, 1967 and El-Okda, 1981).These chemical compounds cause health and environmental pollution in addition to the toxic effects to non-target organisms. Some natural compounds e.g. Neemix (crude plant

extract of Neem) and Vertemic biocide (abamectin) proved promising efficiency for control of land snail species (Gabr et al., 2006).This is a suitable method of control snails without harmful effects on the human and animals. The aim of this study was to estimate the efficiency of some plant extracts against glassy clover snail *Monacha obstructa* (Pfiffer).

MATERIALS AND METHODS

Tested snail :-

Adults of glassy clover snail *Monacha obstructa* (Pfiffer) were collected directly, from infested fields in Fayoum Governorate. The collected snails were transferred to the laboratory, kept under room conditions at 20 ± 3° C and 80 ±5 R.H%. Healthy and similar individuals were chosen and kept in glass terrariums , (40 cm long × 25cm wide and 40 cm deep) which was filled with moist soil adjusted at 75% of water field capacity and provided daily with fresh green lettuce leaves for two weeks before treatment for acclimatization (Godan, 1983).

Plant extracts

Three plants were chosen to be extracted by boiled water. These plants are; foliage of Santonia (*Artemisia herba . alba- Asso*), fruits of Red Pepper (*Capsicum annum L.*), fruit's Skin Peel of Pomegranate (*Punica granatum L.*). These plants, in addition to seeds of Fennel (*Foeniculum vulgare I.*), Fruits of Black pepper (*Piper nigrum I.*) and Seeds of Neem (*Azadirachta-indica*) were also extracted by solvent (Acetone) .

Extraction procedure :

The parts of each plant (250 gm) were ground in a food grinder and extracted using water and acetone. The used plant parts of Santonia, Red Pepper, Pomegranate were put in 350 cm water, boiled for 24 minuets, then filtered and the obtained extracts were used as crude extracts. The solvent (Acetone) was used to extract the six plants at rate of 2 ml /g plant material, compared with the method described by (Su and Horvat 1981). After 24 hours, the extracts were filtered and evaporated to dryness under vacuum using a rotary evaporator in a water bath at 50 ° C. The crude extracts were then weighted and adjusted to 10 ml. volumes with acetone, and kept in a refrigerator until testing.

Bioassay Tests :-

Laboratory evaluation of the different plant extracts (crude extracts) were tested against land snail *M. obstructa* using four different techniques; three concentrations of each acetone plant extract were used for each testing technique.

a- Bait technique:

Bait technique was prepared according to the method described by Ebenso,(2004).

Experiments were conducted to evaluate the efficacy of six plant extracts .The poison baits were prepared by mixing (tested concentrations of each plant extracts + 5% molasses + 80 % bran + water) . Sample of five grams of the poison bait was put on plastic sheet, placed on the surface of the soil in each box. Fifteen animals of *M. obestructa* adults were divided into three replicates and exposed to the candidate concentration of the tested plant extracts. A control test was parallel conducted using the same technique without any treatment. Mortality percentages were calculated after one, four and seven days and corrected by Abbott's formula, (Abbott,1925). LC_{50}, LC_{90} and slope values were calculated for each experiment.

b- Residual film technique:

Thin layer film technique was used as a method of application according to Ascher and Mirian (1981), whereas the tested concentrations were applied in Petri-dishes of 5 cm diameter. One ml of each concentration of the tested plant extracts were spread on inner surface of a Petri-dish, by moving the dish gently in circles. Acetone was evaporated under room condition in a few minutes leaving a thin layer film of tested material on the inner surface of Petri-dish. Five Adults of *M. obestracta* were exposed to the candidate concentration film for 24 hours in Petri-dishes. A parallel control test was conducted using acetone only. Mortality percentages were calculated after 24 hours.

c- Leaf Dipping technique:

Leaf discs of lettuce (3cm diameter) were immersed in the tested extracts for 3 seconds. After that, the discs were air dried then each disc was placed with 5 snails which were over night starred in a box fillet with soil. Snails were allowed to feed on the treated lettuce for one week. Mortality percentages were calculated after one, four and seven days, as described by (Salama and Radwan, 1995).

d- Repellency tests :

Repellency tests were conducted according to McDonald *et al.* (1970) with some modifications. Substrates were prepared from 9 cm diameter filter papers (Whatman No. 1) which were cut in two halves. One ml of the desired plant extracts concentration was applied to a half filter paper as uniformly as possible with a pipette. The treated half discs were then air-dried to evaporate the solvent completely. Full discs were then remade by attaching treated halves to untreated (treated with acetone only) halves of same dimensions with cellotape. Precautions were taken so that an attachment did not prevent the free movement of snails from one half to another. Ten snails were put at the center of each filter – paper disc and the Petri dish was covered. Three replicates were conducted for each concentration. Counts of the snails present on each strip were done after 1 and 6 hours. The results were converted to express Repellency percentage (RP) by the formula of Talukder and Howse (1994).

$$RP\ \% = \frac{(N - C)}{C} \times 100$$

Where:

N = the number of snails present in the control half.

C = half the number of total snails present.

Positive values (+) expressed repellency and negative values (-) expressed attractancy

Repellency percentages values were assigned repellency classes by using the following scale : classes 1, 11, 111, 1V and V designated repellency percentages values of < 0 - 20, 20.1 - 40, 40.1 - 60, 60.1 - 80 and 80.1 – 100, respectively

Identification of Molluscicidal components in plant extracts using thin layer chromatography:-

A layer of silica gel, about 0.75 mm. thickness, was spread on 20x20cm glass plates. Aslurry of 7.5 grams of silica in about 15 ml. water was prepared and spread over the plate with the applicator. The plate was allowed to stand for 2 hours at room temperature, and then activated in an oven for 2 hours at 110°C. Marks were made near the edge of each plate at a distance of 1.5 cm. to define the spotting line. Spots of the plant extracts were put along the plate. The developing solvent system; consisted of Toluene: Acetic acid (9:1), was used for all tests. The developing solvent added to the chamber to a depth of 10 mm, and spotted plate was placed in the chamber so that the bottom edge was in contact with the solvent, and the lid was then replaced when the solvent was developed to a high of about 15 cm, the plate was removed and the solvent was allowed to evaporate.

The dry plates were exposed to iodine vapors as a general detection. The visualization with iodine was carried out by placing the developed, dried plates in a jar containing crystalline iodine. After closing the jar, the iodine vapors was absorbed into the dreas of the layer containing organic compounds, yielding brown spots on a white background. The spots become darker when left in the iodine, but generally fade rapidly when the layer is removed from the chamber.

RESULTS AND DISCUSSION

Boiled water extracts of the three plants; Santonia, Red Pepper and Pomegranate were tested against adults of glassy clover snail *Monacha obstructa.* Four bioassay techniques were conducted; bait technique, residual film technique, leaf dipping technique and repellency tests. Results revealed that all crude boiled water extracts had no effect on snails and no mortality individuals was recorded during the tests period. These results were in agreement with Ghamry (1994).

Crude Acetone extracts of the above mentioned plants in addition to Fennel, Black pepper and Neem plants were tested against *M. obstructa*,using the above mentioned techniques. A control test (check) was paralleled conducted using the same techniques without any treatments. Results revealed that, No mortality in snail individuals was recorded in control tests during the test period for bait, residual film and dipping techniques.

Data tabulated in table (1) show the effect of six plant extracts against *M. obstructa* using four bioassay techniques at three levels of concentrations after 1, 3 and 7 days of treatment. Results revealed that mortality percentage increased with the increasing of plant extract concentrations and the period of exposure. Fennel extract exhibited more toxic effect than the other plant extracts when using bait technique, while Neem exhibited the least toxic one. Values of LC_{50}'s for Fennel, Black pepper, Santonia, Red Pepper, Pomegranate were 0.08, 0.42, 0.2, 1.55 and 0.7 respectively. When using residual film technique, after 24h of treatment for the different concentrations, the efficiency of Fennel and Pomegranate showed higher effect followed by Black pepper, Santonia, Red pepper and Neem. Mortality show the percentages of *M.obstracta* adults exposed to lettuce leaves which were dipped in Fennel, pomegranate, Black pepper, Santonia, Red pepper and Neem extracts were (26.7, 60.0 and 86.7%), (20.0, 40.0 and 80.0%), (20.0, 53.3 and 80.0%), (13.3, 33.3 and 60.0%) and (20.0, 46.7 and 73.3%) and (6.7, 20.0 and 33.3%) after 7 days of treatments, respectively. values of LC_{50}'s for these plant extracts were 0.04, 0.79, 0.51, 3.1, 0.75 and 22.91 respectively.

The average repellency and attractancy action of plant extracts against adults of *M. obstructa* after 1-6 hours of treatment was tabulated in table (1). Santonia and Neem plant extracts were revealed strong repellency than other plant extracts. After 6 hours, the Repelency percentage (RP %) of Santonia (0.5 gm / ml) reached to 90% (class V), while Neem at concentration of (15%) exhibit 27% RP (class II).

General identification of the toxic components in plant extracts using thin layer chromatography:

In order to identify the plant extract components, the extracts were subjected to thin layer chromatography (TLC) technique. TLC indicates the different fractions obtained from acetone plant extract. The extracts of Black pepper, Pomegranate, Fennel, Santonia, Red pepper and Neem contained 5, 2, 3, 2, 6 and 3 spots, respectively. The acetone extract of Black pepper yielded 5 fractions having (RF) values of 0.4, 0.5, 0.6, 0.8 and 0.9 cm. Other plant extracts showing the different developed fractions as presented in fig (1). Hussen *et al*, (1994) isolate the active ingredient in *Calotropis procera* responsible for the molluscicidal activity from its latex by solvent extraction.

Several authors used plant extracts to control land snail pests. Hamdy and El-Wakil, (1993); Ghamry, (1994 and 1997). Zidan *et al* (2001) used several plant extracts against *M. obstructa*, *Eobania. vermiculata* and *Theba pisana*,Ebenso. (2004) used Neem extract and reported that, there is no effects on the snails exposed to Neem seeds oil extract. Gaber *et al*. (2006) used Neem extract against land snails; *M. obstructa* and *E. vermiculata*.

From the foregoing results, it could be concluded that, plant extracts can be successfully used in controlling the injurious land snails, using the bait technique, especially extracts of Fennel and Pomegranate as they exhibit more than 90% mortality after 7 days of treatment.

Table (1): Toxic action and average repellency and attractions of some plant extracts against *M. obstructa* by using four different techniques.

Plant extracts	Bait technique				Residual film technique		Dipping technique				Repellency test Exposure time hours		
	Conc g/ml	%Mortality after 1day	4days	7days	Conc. g/ml	%mortality after 24 h	Conc. g/ml	% Mortality after 1day	4days	7days	Conc. g/ml	After6h	class
Fennel	0.05	6.7	26.7	33.3	0.01	26.7	0.025	0.0	20.0	26.7	0.02	-73	IV
	0.1	13.3	53.3	66.7	0.05	53.3	0.05	6.7	33.3	60.0	0.05	13	I
	0.3	26.7	73.3	93.3	0.1	100.0	0.1	20.0	53.3	86.7	0.1	80.0	IV
Pomegranate	0.33	13.3	26.7	33.3	0.15	33.3	0.5	6.7	13.3	20.0	0.075	-60.0	III
	0.65	20.0	40.0	73.3	0.3	60.0	0.7	13.3	26.7	40.0	0.33	33.3	II
	1.0	33.3	60.0	93.3	0.5	93.3	1.2	20.0	46.7	80.0	0.65	80.0	IV
Black pepper	0.1	0.0	13.3	26.7	0.3	20.0	0.3	6.7	13.3	20.0	0.03	-66.7	IV
	0.3	6.7	40.0	60.0	0.6	46.7	0.6	13.3	26.7	53.3	0.3	6.7	I
	0.6	20.0	53.3	86.7	0.75	80.0	0.75	26.7	46.7	80.0	0.55	66.7	IV
Santonia	0.5	0.0	6.7	13.3	1	0.0	1	0.0	13.3	13.3	0.13	15	I
	1.0	0.0	13.3	26.7	2	6.7	2	13.3	20.0	33.3	0.25	40.0	II
	1.5	13.3	33.3	53.3	4	13.3	4	20.0	40.0	60.0	0.5	90.0	V
Red pepper	0.3	0.0	13.3	20.0	0.5	13.3	0.5	0.0	6.7	20.0	0.05	-80.0	IV
	0.6	13.3	33.3	46.7	0.7	40.0	0.7	6.7	26.7	46.7	0.1	0.0	I
	1.3	20.0	46.7	73.3	1.5	86.7	1.0	13.3	33.3	73.3	0.3	60.0	III
Neem*	5	0.0	0.0	0.0	5	13.3	5	0.0	0.0	6.7	5	18	I
	10	0.0	0.0	13.3	10	33.3	10	0.0	0.0	20.0	10	22	II
	15	0.0	0.0	53.3	15	66.7	15	0.0	0.0	33.3	15	27	II

* = Concentrations (%).

Table (2): LC_{50}, LC_{90} and Slope values of some plant extracts used against *Monacha obstructa* by four different techniques

Plant extracts	Bait technique			Residual film technique			Dipping technique		
	LC_{50}	LC_{90}	Slope	LC_{50}	LC_{90}	Slope	LC_{50}	LC_{90}	Slope
Fennel	0.08	0.3	2.26	0.02	0.07	2.71	0.04	0.11	2.85
Pomegranate	0.42	0.92	3.72	0.2	0.5	3.43	0.79	1.62	4.13
Black pepper	0.2	0.8	2.19	0.5	1.1	4.13	0.51	1.0	4.25
Santonia	1.55	5.24	2.42				3.1	11.2	2.3
Red pepper	0.7	2.5	2.3	0.9	1.6	4.58	0.75	1.4	4.6
Neem				12.3	32.1	3.08	22.91	84.9	2.25

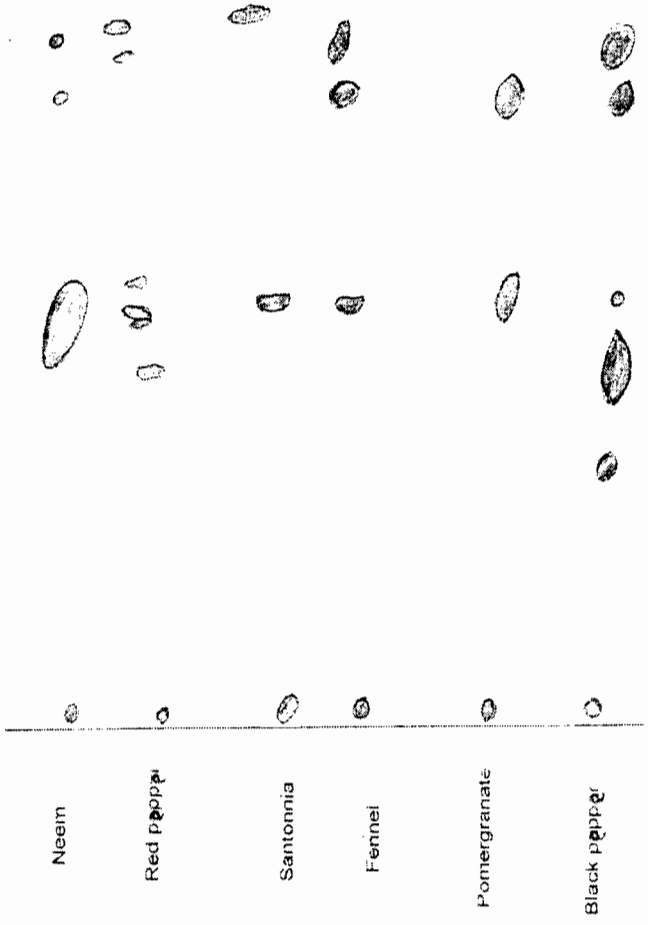

Fig (1):Thin layer chromatograms of plant extracts.

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دراسات تكسوكولوجية على بعض المستخلصات النباتية ضد قوقع البرسيم الزجاجي

عبد الله محمد عفيفي[١] ، نبيل محفوظ ابراهيم [٢] ، أحمد غازى السيسى [٣] و سهير محمد عبد الحليم[١]

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تناول البحث تقييم فاعلية ثلاث مستخلصات نباتية باستعمال الماء المغلى وهى نباتات الشيح البلدى والفلفل الأحمر وقشر الرمان . كما تم تقييم هذه النباتات بالإضافة إلى نباتات الشمر والفلفل الأسود والنيم باستخدام مذيب الأسيتون ضد قوقع البرسيم الزجاجى فى المعمل. تم معاملة القواقع بأربع طرق مختلفة لمعرفة التأثير السام والطارد و الجاذب و هذه الطرق هى طريقة الطعوم السامة وطريقة الغمر لورق الخس و طريقة الفيلم الرقيق و طريقة التأثير الطارد والجاذب. و قد أوضحت النتائج أن مستخلصات الماء المغلى لهذه النباتات كان غير سام للقواقع ولم يحقق أى نسبة موت لها وكان غير فعالا فى التأثير الطارد والجاذب . أما مستخلص الأسيتون للشمر كان أكثر كفاءة فى طريقتى الطعوم السامة والغمرلورق الخس. بينما فى طريقة الفيلم الرقيق فقد حقق مستخلص الشمر وقشر الرمان أعلى كفاءة فى التأثير على القواقع.و فى طريقة التأثير الطارد والجاذب أظهر مستخلص الشيح البلدى والنيم كفاءة أعلى عن باقى المستخلصات فى التأثير الطارد على القواقع

وفى محاولة لتعريف وتحديد المواد الفعالة فى هذه المستخلصات تم استخدام طريقة التفريد الكروموتوجرافى بالسيلكاجيل وأظهرت مستخلصات الفلفل الأسود و قشر الرمان و الشمر و الشيح البلدى و الفلفل الأحمر و النيم ٥ ، ٢ ، ٣ ، ٢ ، ٦ ، ٣ بقع على الترتيب.