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MOLLUSCICIDAL AND BIOCHEICAL ACTIVITY OF SOME CRUDE PLANT EXTRACTS AGAINST THREE TYPES OF LAND SNAILS

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ABSTRACT

The effect of diluted ethanolic extracts of five crude extracts of wormseed (*Artemisia cinae*), lemongrass (*Cymbopogon citrates*), senna (*Cassia acutifolia*), geranium (*Pelargonium graveolens*) and sweet basil (*Ocimum basilicum*) in addition to methomyl as standard molluscicide were tested for their mortality effects against *Eobania vermiculata, Theba pisana* and *Monacha obstructa* snails at concentrations 25, 50, 75 and 100% and Biochemical studies were also conducted *in vivo* to determine the lower concentrations (5, 10, 25 and 50%) of the tested crude extracts and methomyl on the activity of ALT (Alanine Transaminase) and AST (Aspartate transaminase) as well as the total soluble protein in the three tested snails. The *Eobania vermiculata* was highly affected by lemongrass, wormseed and senna crude extracts but *Theba pisana* and *Monacha obstructa* were less sensitive. On the other hand, AST enzyme in *E. vermiculata* was higher stimulated by all the tested crude extracts than methomyl. The effect of methomyl on the activity of AST enzyme in *T. pisana* and *M. obstructa* was nearly similar with the effects of the tested extracts. ALT enzyme was weakly affected different crude extracts in addition to methomyl. Total soluble protein was very sensitive in *E. vermiculata* to senna extract and methomyl.

INTRODUCTION

Many scientists have considered the biological activities of different plant crude extracts to avoide the using of pesticides. Leaves and the bark of *Magnolia grandiflova* L. and an identified parthenolide compound were very toxic against fresh water snail, *Biomphalaria alexandrina* and terrestrial snails, *Theba pisana* and *Eobania vermiculata* (Abdelgaleil, 2005). Crude extracts of bark, root and leaves of neem (*Azadirachata indica*) at 500–700 mg/kg produced mortality against land snails, *Limcolaria auroa* and *Archachatino marginata* after exposure for 48 and 72 hour, respectively (Ebenso, 2004).

Singh and Singh (2001), reported that the seed powder of *Lawsonia inermis* was toxic against *Lymnaea acuminate* and *Indoplanorbis exustus* and it was more toxic when combined with cedar oil, neem, bulb garlic powder or ginger rhizome extract. *Calotropis* procera plant was found to have good molluscicidal activities against land snails, *Theba pisana* and *Eobania vermiculata* (El-Sebaii *et al.*, 1999). The water extract of *Anagallis arvensis* proved to be very effective against the helicid land snails, *Monacha obstructa, Helicelloa vestalis* and *Theba pisana* (Tolba, 1997). The present investigation was conducted to evaluate five crude extracts of wormseed (*Artemisia cinae*); Geranium (*Pelargonium graveolens*); Lemongrass (*Cymbopogon citrates*); sweet basil (*Ocimum basilicum*) and senna (*Cassia acutifolia*) against *Eobania vermiculata, Theba pisana* and *Monacha obstructa* snails. Biochemical studies were investigated to determine the *in vivo* efficiency of these plant extracts on total protein, Alanine transaminase (ALT) and Aspartate transaminase (AST) enzymes in the tested snails to know an idea about the specific target affected by these extracts. Methomyl was used as standard molluscicide for comparing with the tested plant extracts.

MATERIALS AND METHODS

I. Tested plants

Wormseed (*Artemisia cinae*), geranium (*Pelargonium graveolens*); lemongrass (*Cymbopogon citrates*); sweet basil (*Ocimum basilicum*) and senna (*Cassia acutifolia*). The tested plants were obtained form Horticulture Research Station at Sabahia area, Abbis, Alexandria.

II. Tested snails

The tested snails were *Eobania vermiculata, Theba pisana* and *Monacha obstrutca*. They were obtained from Agricultural Research Station Farm, at Abbis, Alexandria. The snails were kept in glass containers with suitable feeding for 14 days to be laboratory acclimatized.

III. Methods of Experiments:

A. Preparation of extracts

The tested plants were cleaned, completely sun dried and blended to be fine powder. Fifteen grams of each plant powder was soaked in 100 ml ethanol and extracted in 200 ml ethanol by soxhlet for 3 hrs. The extracted solution was evaporated by rotary evaporator up to 100 ml extract concentrate. The concentrate was diluted with water to 25, 50, 75% in addition the original concentrate (100%).

B. Mortality tests

Three replicates, each contained 10 snails were used for each treatment. The tested concentrations of each extract were 25, 50, 75 and 100%. The snails inside the mollusca shells were topically treated with 0.2 ml of the tested extracted/snail/for once using micropipette. The normal food was introduced after treatment. Untreated snails as control were concurrently conducted by the treatment with same ratio and volume of diluted ethanol free of the tested plant extract. The tested snails were examined for their response using stainless steel needle (WHO, 1965).

The number of dead snails were counted and mortality percentage after 72 hrs. were calculated. LC_{50} (Lethal concentration which causes 50% mortalities) was obtained using probit papers. Methomyl was used as standard molluscicide at the same rates for comparative studies.

C- Biochemical measurements

Alanine transaminase (ALT); Aspartate transaminase (AST) and total soluble protein were measured in the tested snails, *Eobania vermiculata, Theba pisana* and *Monacha obstructa*. Sublethal concentrations (5, 10, 25 and 50%) were selected according to the determined LC_{50}. Twenty snails were placed in glass container tightly covered with muslin secured with rubber band and used for each treatment. Each snail was topically treated inside the shell with 0.2 ml of the tested concentration and biochemical tests were carried out after 72 hr. after treatment.

The moullsca shells were removed and the soft tissues of snails were homogenized in 10 volumes (w/v) of 0.1 M phosphoate buffer, pH 7.4, using a polytron homogenizer for 30 seconds. The homogenates were centrifuged for 30 minutes at 4°C and 2200Xg (5000 r.p.m.) the supernatants were used for determination of ALT and AST enzymes activites (Reitman and Frankle, 1957), using Boehringer GmbH diagnostic kits, as IU/L and reduction or elevation in activities were calculated. Total soluble protein were detemined by folin method described by Lowery (1951). Standard curve of bovine serum albumin (BSH) and slope was determined to calculate total protein in g/dl. The data were statistically analyzed using complete random design and ANOVA to determine the significant differences between treatments (Steel and Torrie, 1980).

These biochemical studies were conducted at Institute of Graduate Studies and Research, Department of Environmental Studies.

RESULTS AND DISCUSSION

A- Mortality effects

The mortality percentages and LC_{50}'s obtained by the effect of the tested crude extracts against the tested snails are recorded in Tables 1, 2 and 3. the mortality percentages caused by the crude extracts of wormseed against *Eobania vermiculata, Theba pisana* and *Moncha obstructa* were found to be increased with increasing the tested concentrations, *A. cinae* crude extract showed LC_{50}'s values equal 55, 70, 80%, respectively. The crude extract of *Pelargonium graveolens* was less effective against the three tested snails which needed to use higher concentrations. However, this crude extracts gave LC_{50} value equal 90% against *Theba prsana*. Lemongrass crude extract was very active by increasing the tested concentrations against the *Eobania vermiculata, Theba pisana* and *Moncha obstructa* causing LC_{50}'s equals 52, 58 and 65%, respectively. The crude extract of sweet basil weakly affected the three tested snails with LC_{50}'s equals 90, 84 and 90%, respectively. Senna was very effective against the three tested snails with LC_{50}'s values equal 59, 45 and 82%, respectively. It could be said that, the crude extracts of lemongrass, wormseed and senna were very active in descending order against *Eobania vermiculata*, whereas *Thiba pisana* was highly affected by senna with LC_{50} equal 45% which nearly closed to LC_{50} of the standard molluscide, methomyl with LC_{50} equal 42%, followed by lemongrass crude extracts which showed LC_{50} equal to 58%. *Moncha obstructa* was slightly and weakly affected by the tested crude extracts at the tested concentration. Generally, the standard molluscicide methomyl was the most effective against *Eobania vermiculata, Theba pisana* and *Moncha obstructa* with LC_{50}'s values equal 30, 42 and 36%, respectively. These results in agreement with Abdelgaleil (2005) and Tolba (1997) that the tested three snails were affected by the crude extracts of different plants.

Table (1): Lethal effects of the tested crude extracts against *Eobania vermiculata* shown as mortality percentages and LC_{50}'s

Crude extracts	Mortality after 72 hr				L.C.₅₀ (%)
	Concentrations (%)				
	25	50	75	100	
Wormseed (*Artemisia cinae*)	20.00±0.0 bcd	40.0±16.3 bc	60.0±16.3 ab	80.0±28.3 ab	55
Geranium (*Pelargonium graveolens*)	6.67±9.4 cd	13.3±9.4 d	33.3±9.4 bc	46.6±24.9 b	>100
Lemongrass (*Cymbopogon citrates*)	33.30±9.4 ab	46.7±9.4 b	73.3±24.9 a	66.6±9.4 ab	52
Sweet basil (*Ocimum basilicum*)	6.70±9.4 cd	20.0±0.0 cd	20.0±0.0 c	53.3±18.9 ab	90
Senna (*Cassia acutifolia*)	26.7±18.9 abc	60.0±0.0 ab	60.0±16.3 ab	73.3±18.8 ab	59
Methomyl	46.6±9.4 a	73.3±9.4 a	86.7±18.6 a	93.3±9.4 a	30
Control	0.00±0.0 d	0.00±0.0 d	0.00±0.0 c	0.00±0.0 c	
$L.S.D._{0.05}$	21.61	22.9	32.4	36.97	

Table (2): Lethal effects of the tested crude extracts against *Thiba pisana* shown as mortality percentages and LC_{50}'s

Crude extracts	Mortality after 72 hr				L.C.₅₀ (%)
	Concentrations (%)				
	25	50	75	100	
Wormseed (*Artemisia cinae*)	20.00±0.0 ab	26.7±18.8 abc	53.3±9.4 ab	80.0±16.3 ab	70
Geranium (*Palargonium graveolens*)	13.3±9.43 ab	20.0±0.0 bc	33.3±9.4 b	53.3±24.9 b	90
Lemongrass (*Cymbopogon citrates*)	26.7±9.4 a	46.7±9.4 ab	66.7±18.4 a	66.7±9.4 ab	58
Sweet basil (*Ocimum basilicum*)	13.3±9.40 ab	20.0±0.0 bc	33.3±9.4 b	60.0±16.3 ab	84
Senna (*Cassia acutifolia*)	26.7±9.42 a	60.0±0.0 a	66.7±9.4 a	80.0±16.3 ab	45
Methomyl	20.0±16.3 ab	53.3±18.9 ab	86.7±16.9 a	100.0±0.0 a	42
Control	0.00±0.0 b	0.00±0.0 c	0.00±0.0 c	0.00±0.0 c	
$L.S.D._{0.05}$	20.22	32.4	26.47	31.51	

Table (3): Lethal effects of the tested crude extracts against *Moncha obstructa* shown as mortality percentages and LC_{50}'s

Crude extracts	Mortality after 72 hr				L.C.₅₀ (%)
	Concentrations (%)				
	25	50	75	100	
Wormseed (*Artemisia cinae*)	13.0±9.4 b	20.0±0.0 b	40.0±16.3 b	80.0±16.3 ab	80
Geranium (*Pelargonium graveolens*)	6.6±9.3 b	13.3±18.8 b	26.6±16.9 bc	46.7±18.6 c	>100
Lemongrass (*Cymbopogon citrates*)	13.3±11.5 b	26.7±18.9 b	53.3±24.9 b	86.6±9.4 ab	65
Sweet basil (*Ocimum basilicum*)	6.6±9.4 b	20.0±0.0 b	26.6±9.4 bc	60.0±16.3 bc	90
Senna (*Cassia acutifolia*)	6.6±4.7 b	20.0±0.0 b	33.3±9.4 b	66.6±9.4 bc	82
Methomyl	46.6±9.4 a	66.7±24.4 a	100.0±0.0 a	100.0±0.0 a	36
Control	0.00±0.0 b	0.00±0.0 b	0.00±0.0 c	0.00±0.0 d	
$L.S.D._{0.05}$	18.72	32.43	30.57	26.47	

B- Biochemical effects

1- Effect Alanine transaminase (ALT) enzyme

Effects of the tested crude extracts on ALT enzyme in the three tested snails are shown in Table (4). ALT enzyme weakly stimulated in *E. vermiculata* and *M. obstructa* by the crude extract of wormseed, which, weakly inhibited in *T. pisana* at the tested concentration. Lemongrass crude extract caused weak effects against ALT enzyme in both *E. vermiculata* and *M. obstructa* whereas it was slightly inhibited this enzyme in *T. pisana* snail. The crude extract of senna slightly stimulated the activity of ALT enzyme in *E. vermiculata*, but inhibited the activity at the lowest concentration (5%) and stimulated it and the highest one (50%) with –65.0 and +61.6%, respectively. This extract weakly stimulated and inhibited at the tested lower and higher concentrations. Methomyl as standard molluscicide had the same trend of stimulation or reduction of the tested crude extract against ALT enzyme in the three tested snails. These effects may be due to unsteady activities of ALT enzyme in the three tested snails.

2- Effects on Aspartate transaminase (AST) enzyme

The effect of the tested plant crude extracts on AST enzyme activity in the three tested terrestrial snails are shown in Table (5). The activity of AST enzyme was strongly stimulated in *E. vermiculata* by the effect of *A. cinae* crude extract, from +48.7 to +279.9% stimulation at the tested concentrations. The crude extract of lemongrass highly stimulated the activity of AST enzyme *E. vermiculata* to +162 and +112.7% at 5 and 10% concentrations, respectively and its stimulation decreased to +51.2 and +78.9% with increasing the tested concentrations at 25 and 50%., respectively. *C. citratus* crude extracts was very weak to stimulate or reduce the activity of AST enzyme in *T. pisana* whereas it highly stimulated AST enzyme with +147.4% at 5% then the stimulation decreased at 10% conc. And the activity weakly reduced with increasing the tested concentration in *M. obstructa*. The crude extract of senna was highly stimulant for AST enzyme in *E. vermiculata* specially at the lower tested concentrations. *T. pisana* was weakly affected by the tested concentration of senna against AST enzyme, which was slightly stimulated in *M. obstructa*. The standard molluscicide methomyl was moderately effective to stimulate AST enzyme at the lower two tested concentrations and the stimulation increased with increasing the tested concentrations in *E. vermiculata* whereas the activity of AST enzyme in *M. obstructa* was also stimulated at the lower concentrations but the stimulation decreased with increasing the tested concentration until it became weak reduction at the highest concentration. In *T. pisana* the activity of AST enzyme weakly reduced at the lowest and highest tested concentration and slightly stimulated at the other concentrations. It could be said that, AST enzyme in *E. vermiculata* was found to be more sensitive to stimulate by all the tested crude extracts more than methomyl. The effects of all crude extracts as well as methomyl against AST enzyme in both *T. pisana* and *M. obstructa* were nearly similar.

Table (4): Biochemical effects of the tested crude plant extracts on the activity of ALT enzyme (Alanine transaminase) in the tested three terristerial snails, shown as +ve (stimulation) or –ve (reduction) values

Crude extracts	Types of snails	Concentrations (%)			
		5	10	25	50
Wormseed (*Artemisia cinae*)	*E. vermiculata*	+3.2	+21.9	+13.3	+44.8
	T. pisana	–39.2	–46.6	–33.6	+16.8
	M. obstructa	0.0	+3.7	+0.8	+50.8
Lemongrass (*Cymbopogon citrates*)	*E. vermiculata*	+24.9	+9.0	+9.4	+40.6
	T. pisana	–64.0	–19.7	–28.3	–52.4
	M. obstructa	+31.9	+32.4	–2.9	–3.1
Senna (*Cassia acutifolia*)	*E. vermiculata*	+26.9	+72.6	+0.9	+33.6
	T. pisana	–65.0	–5.8	+17.9	+61.6
	M. obstructa	+17.0	+25.5	–54.5	–35.9
Methomyl 90%	*E. vermiculata*	–7.84	–3.2	+23.9	+28.9
	T. pisana	–4.7	–2.1	–21.5	–10.0
	M. obstructa	+53.7	+11.1	–54.5	–35.9

Table (5): Biochemical effects of the tested crude plant extracts on the activity of AST enzyme (Asparatate transaminase) in the tested three terristerial snails, shown as +ve (stimulation) or –ve (reduction) values

Crude extracts	Types of snails	Concentrations (%)			
		5	10	25	50
Wormseed (*Artemisia cinae*)	*E. vermiculata*	+48.7	+262.7	+110.2	+279.9
	T. pisana	+9.1	+4.0	20.4	+100.3
	M. obstructa	–3.5	+73.6	+121.8	+28.3
Lemongrass (*Cymbopogon citrates*)	*E. vermiculata*	+162.7	+112.7	+51.2	+78.9
	T. pisana	+33.3	+10.9	–9.6	–16.7
	M. obstructa	+147.4	+50.3	–33.9	–16.1
Senna (*Cassia acutifolia*)	*E. vermiculata*	+224.5	+201.6	+104.9	+78.9
	T. pisana	–14.6	+4.6	+6.5	–3.8
	M. obstructa	+53.5	+63.3	+28.3	+4.3
Methomyl 90%	*E. vermiculata*	+63.1	+54.1	+97.9	+117.2
	T. pisana	+25.9	+59.6	+32.6	–10.2
	M. obstructa	+163.3	+82.9	+72.1	–13.1

3- Effect on total soluble protein

The data in Table (6) showed that the alcoholic concentration 25% of methomyl, wormseed and lemongrass was highly stimulated the formation of total soluble protein in *E. vermiculata* snails with 117.0; 91.5 and 85.1% stimulation, respectively, whereas senna slightly stimulated the formation of protein with 42.5% in the same snail and concentration methomyl increased the formation of protein at 25% in *M. obstructa* with 143.6% stimulation. The crude extraction of lemongrass was moderately stimulated the formation of protein in *T. pisana* with 54.9 and 57.7% stimulation at the tested concentrations 25 and 50%, respectively. *E. vermiculata* was found to be very sensitive to stimulate the formation of total soluble protein by all the tested concentrations of both senna crude extract and methomyl.

It could concluded that *Eobania vermiculata* was very affected by the crude extracts of lemongrass, wormseed and senna whereas *T. pisana* and *M. obstructa* were less sensitive.

ALT (alanine transaminase) enzyme was weakly affected by both the tested crude extracts. Wormseed and senna as well as the standard molluscicide, methomyl. On the other hand, AST (aspartate transaminase) enzyme in *E. vermiculata* was highly stimulated by all the tested crude extracts more than methomyl, whereas this effect was nearly similar in both *T. pisana* and *M. obstructa*. Total soluble protein formation was found very sensitive in *E. vermiculata* to stimuli by *C. acutifolia* crude extract and methomyl.

Table (6): Biochemical effects of the tested crude plant extracts on the activity of protein in the tested three terristerial snails, shown as +ve (stimulation) or –ve (reduction) values

Crude extracts	Types of snails	Concentrations (%)			
		5	10	25	50
Wormseed (*Artemisia cinae*)	*E. vermiculata*	–25.5	–21.2	+91.5	–12.8
	T. pisana	–11.27	–23.9	–12.6	+2.8
	M. obstructa	+0.97	0.0	+35.9	+41.7
Lemongrass (*Cymbopogon citrates*)	*E. vermiculata*	+29.8	+23.4	+85.1	+27.6
	T. pisana	+35.2	+16.9	+54.9	+57.7
	M. obstructa	+16.5	–9.7	–8.8	+0.97
Senna (*Cassia acutifolia*)	*E. vermiculata*	+29.8	+76.5	+42.5	+74.5
	T. pisana	+23.9	+19.7	–4.22	–26.7
	M. obstructa	–18.5	+5.8	–40.7	–40.7
Methomyl 90%	*E. vermiculata*	+78.5	+72.3	+117.0	+30.8
	T. pisana	+11.3	+28.2	+1.4	–4.22
	M. obstructa	–4.8	–28.2	+143.6	+7.7

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النشاط الإبادي والبيوكيميائي لبعض المستخلصات النباتية ضد ثلاثة أنــواع مــن القواقع الأرضية

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أختير تأثير المستخلص الإيثانولي المخفف لخمسة مستخلصات نباتية هي الشيح البلدي، حشيشة الليمون، العتر البلدي، الريحان والذنسنامكي بالإضافة إلى مبيد الميثوميل كمبيد قواقع قياس من حيث السمية ونسبة الموت لهذه المستخلصات ضد ثلاثة أنواع من القواقع الأرضية وهي قوقع الحدائق البني *Eobanio vermiculata*، قوقع الحدائق الأبيض *Theba pisana* وقوقع البرسيم *Monacha obstructa* بتركيزات ٢٥، ٥٠، ٧٥، ١٠٠% وكذلك تم عمل دراسة بيوكميائية *in vivo* لتحديد فعالية التركيزات الأقل ٥، ١٠، ٢٥، ٥٠% في مستخلصات الشيح البلدي، حشيشة الليمون والسنامكي وأيضاً مبيد الميثوميل على نشاط إنزيم الـ ALT (الألنين ترانس أمينيز) وإنزيم AST (الأسبرتيت ترانس أمينيز) وأيضاً البروتين الكلي الذائب في الأنواع الثلاثة من القواقع الأرضية المختبرة. كان قوقع الحدائق البني *E. vermiculata* شديد التأثر بمستخلصات حشيشة الليمون والشيح البلدي والسنامكي بينما كان قوقع الحدائق الأبيض *T. pisana* وقوقع البرسيم *M. obstructa* أقل حساسية. ومن ناحية أخرى فقد زاد نشاط إنزيم الأسبرتيت في قوقع الحدائق البني بالمستخلصات المختبرة أعلى من مبيد الميثوميل وكان تأثير مبيد الميثوميل على نشاط إنزيم الاسبرتيت في كلا القوقعين الحدائق الأبيض والبرسيم مشابه تقريباً لتأثير المستخلصات المختبرة.

تأثير المستخلصات المختبرة ومبيد الميثوميل على نشاط وإنزيم الـ ALT (الألنين ترانس أمينيز) كان ضعيفاً بينما تأثر البروتين الكلي الذائب بشدة بمستخلص السنامكي ومبيد الميثوميل في قوقع الحدائق البني *Eobania vermiculata*.