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EFFECT OF SOME INSECTICIDES ON BIOMPHALARIA ALEXANDRINA THE INTERMEDIATE HOST OF SCHISTOSOMA MANSONI IN EGYPT. I. TOXICITY AND BIOLOGICAL STUDIES

BY

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ABSTRACT

Six insecticides namely, triazophos, cyolane (organophophates), cypermethrin, baythroid (pyrethroids), methomyl and larvin (carbamates) which are widely used as agricultural isecticides, were tested for their molluscicidal activity against B. alexandrina snails. Three of which: triazophos, cyolane and cypermethrin were found to be most potent displaying LC_{50}'s of 5.00 ± 1.73, 5.58 ± 0.22 and 11.23 ± 2.26 ppm, respectively.

The laboratory investigation revealed that the latter three insecticides have pronounced effects on both the mortality and growth rates of the snails as well as on the egg laying capacity and hatchability rate of the eggs laid by pretreated snails. No significant ovicidal effect of the three insecticides on normal laid eggs was observed.

INTRODUCTION

Till 1957, the distribution of Biomphalaria alexandrina, the intermediate host of Schistosoma mansoni in Egypt, was limited to the Delta [6] and started to appear in Menia Governorate by 1968. Recently, the snails were found near the High Dam [2].

However, many investigators have tested the effect of several chemicals and molluscicides against the snail intermediate host of schistosomiasis, including sodium pentachlorophenate (NaPCP) against the eggs of B. boissyi [12] ; Bayluscide against eggs of B. alexandrina [21] ; Bayer-73 against B. glabrata [14]; copper sulphate, Niclosamide and Frescon against B. alexandrina [15]; magnesium carbonate against B. glabrata [13].

The present work aims to study the effect of some insecticides, widely used in plant protection, on various biological activities of B. alexandrina snails.

MATERIAL AND METHODS

1- Animals:

B. alexandrina snails were maintained under constant laboratory conditions (Temperature ; 25 ± 2°C ; Food, fresh lettuce). The method used for rearing the snails was followed as that described by Kamel [11].

2- Insecticides:

a) Triazophos ; 0,0-diethyl 0-1 phenyl 1-1,2,4 triazol-3-yl-phosphorothioate, an organophosphorous compound produced under the trade name Hostathion as emulsifiable concentrate of 42%.

b) Cyolane; 2-(diethoxyphosphinylimino)-1,3-dithiolane an organophosphorous compound produced as granules (50 g/kg).

c) Cypermethrin; RS-alpha-cyano-3-phenoxybenzyl (IRS)-cis, trans-3- (2,2 dichlorovinyl)-2,2-dimethyl-cyclophro-pane carboxylate, a synthetic pyrethroid provided as 40% emulsifiable concentrate under the trade name Ripcord.

d) Baythroid; cyno-(4-fluoro-3-phenoxyphenyl)-methyl-3-(2,2 dichloroethemyl)-2,2-dimethyl-cyclo- propanecarboxy-late; a synthetic pyrethroid provided under the name Cyflu-thrin as water soluble concentrate of 100 g/l.

e) Methomyl; S-methyl N-(methyl carbamoloxy) thio-acetimidate, a carbamate provided as 90% water soluble powder under the name Lannate.

f) Larvin; 3,7,9,13-tetramethyl-5, 11-dioxa-2,8,14-trithia-4.7,9,12-tetra-azapentadeca -3,12-dien -6, 10-dione; a carbamate provided as 75% wettable powder.

3. Assessment of the toxicity of the insecticides on the snails:

i) Test solution: All the insecticides used are water soluble and all test solutions were prepared in dechlorinated tap water (pH 7.5-7.7). For each insecticide a stock solution of 1000 ppm based on the content of the active ingredient was prepared of which several dilutions of 10, 20, 30, 40 and 50 ppm were prepared in order to determine the mortality rate.

ii) Screening tests: Screening tests were carried out to determine the percentage of mortality according to the standard method described elsewhere [22].

For each dose of the insecticides tested, 10 snails were immersed in 1000 ml of each of the tested solution in one-litre jars. The exposure period was 24 hours and replicates for each treatment were considered beside the control one. At the end of the exposure period both the control and treated snails were removed, washed throughly in dechlorinated water and transferred to containers containing fresh dechlorinated water. Observations were continued for further 24 hr. from the removal [11, 17, 22]. Death of snailcould be distinguished using sodium hydroxide solution [16].

For insecticides that displayed pronounced activity, five doses of each compound that expected to give mortalities between 10% and 90% were tested and the percentage of mortality was calculated and corrected on the basis of natural mortality in the untreated control snails using

Abott formula [1].

4. Biological investigation :

To study the effect of prolonged exposure of *B. alexandrina* snails to sub-lethal concentrations of the tested compounds on some biological activities, the following was carried out:

Four glass aquaria, each contained 15 litres dechlorinated water and 200 snails were prepared. Three of them were used for exposing the snails to the sub-lethal concentrations of each insecticide and the fourth one was used for the control group. The snails were exposed to the test solutions for different intervals according to the biological factor to be investigated.

i) Effect on shell diameter and total body weight:

The snails were treated for one week with the sub-lethal concentration of the tested insecticide solution. Twenty five snails were picked up from each test solution as well as from the control group. The snail diameter in mm. was measured at the begining of the experiment using calipers according to Chernin [4]. At the same time, the total body weight in mg. was determined using a torsion balance. The snails were kept individually in glass jars containing one litre dechlorinated water. The shell diameters and the total body weights were determined at following intervals of 7, 14, 21, 28, 35 and 42 days for both the control and the treated snails.

ii) Effect on the fecundity and hatchability of the snails:

Two groups each composed of 25 snails were picked up from each tested insecticide and the control. The first group was exposed for one week and the other was exposed for two weeks. The snails were kept individually in jars (one- litre capacity) containing dechlorinated water supplied with small plastic sheets for egg deposition [18].

Determination of egg cells/snail was carried out at intervals of 5, 10, 15, 20, 25, 30 and 35 days following different exposure periods. The hatchbility of the eggs laid by the snails was also determined. About 100 eggs from each group were collected and kept at least two weeks in small beaker at 24± 2 °C. Then the hatchability percentage was calculated for both the control and the exposed group.

iii) Determination of the ovicidal effect:

Freshly laid eggs were collected from laboratory maintained snails. The egg masses were immersed in beakers (100 ml capacity) containing the sub-lethal concentration of the corresponding tested insectcide. After exposure period of 7 days about 500 eggs were picked up from each group and immersed in beakers containing 250 ml dechlorinated water . The eggs were kept for at least two weeks at

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24 ±2°C. Then the hatching percentages was calculated for the control and exposed eggs.

Statistical analysis of the data obtained was carried out and the significance of differences was calculated using student's ''t'' test or X^2-test according to the design of the experiment.

RESULTS

1. Preliminary assessment of the toxicity of the insecticides:

The data presented in table (1) showed that the organophosphorous insecticides used at increasing concentration of 10, 20, 30, 40 and 50 ppm gave a mortality rates of 69, 79, 91, 100 and 100 respectively for triazophos and more or less the same mortality rates for cyolane. Concerning the pyrethroid insecticides used at the same concentrations they gave mortality percentages of 59, 69, 89, 100 and 100 respectively for cypermethrin, while no mortalities were recorded for baythroid at all concentrations used. Applying carbamate insecticides methomyl and larvin gave no mortalities at the low concentrations while at higher concentration i.e 40 and 50 ppm, the precentages of mortality were very low as shown in table (1)

2. Assessment of LC_{50}'s for the potent insecticides:

Only triazophos, cyolane and cypermethrin which gave high mortalities at low concentration were chosen for determining LC_{50} and LC_{90} whereas the other insecticides were excluded.

The results presentèd in table (2) showed that the LC_{50}'s of trizophos, cyolane and cypermethrin were 5 ± 73, 5.58 ± 0.22 and 11.23 ± 2.26 ppm, respectively, while the LC_{90}'s were 47.55 ± o.717, 27.42 ± o.643 and 30.20 ± 0.282 ppm, respectively.

3. Effect on growth rate:

Exposure of *B. alexandrina* snails to the sub-lethal concentrations of triazophos and cypermethrin for one week caused significant retardation in the growth rate of the shells of the snails at 35 and 42 days post exposure. But treatment with cyolane showed significant retardation in the growth rate of the shells at 28, 35 and 42 days post exposure compared with the control group as shown in table (3).

In respect to the effect of these isecticides on the total body weight, it was found that triazophos and cyolane showed significant retardation in the total body weight at 21, 28, 35, and 42 days, whereas cypermethrin has significant retardation only at 35 and 42 days post exposure as shown in table (4).

4. Effect on snail fecundity and hatchability:

i) Effect on egg production:

The results presented in table (5) showed that the snails pretreated with the sublethal concentrations (1/10 LC_{50}) of triazophos and cyolane for one week caused: significant decrease in the mean number of the egg-masses/ snail as well as the mean number of egg-cells from five days up to 35 days post exposure compared with the control snail. The treatment with cypermethrin decreased significantly the mean number of egg-mass/sainl at 20, 25, and 30 days post exposure. There also significant decrease in the egg-cells/snail but at 5, 20 and 25 days post exposure as presented in table (5).

ii) Effect on egg hatchability of B. alexandrina snails:

As presented in table (7) the statistical analysis of the data showed that there was no significant effect on the hatchability rate of the eggs collected from snails treated for one week with the sub-lethal concentration (1/10 LC_{50}) of any of the three insecticides. While significant reduction was found in the hatchability rate of the eggs collected from snails pretreated for two weeks with any insecticide compared with the control ones (Table 7).

5. The ovicidal effect on B. alexandrina eggs:

Exposure of freshly laid eggs of *B. alexandrina* snails to the sublethal concentration of triazophos,cyolane or cypermethrin for one week showed no significant ovicidal effect, as shown from the hatchability rate (97.23%, 95.81% and 98.96% respectively) compared with the hatchability rate of the unexposed control egg (98.02%).

DISCUSSION

The greastest problem in snail control after application of molluscicides is how to prevent reinfection of the habitat with snail. Reinfestation of the water bodies could be attributed either to a few survival snail egg-masses that were unaffected by the chemical or due to the invasion of a new stock of snails from other water sources. As a result of this, snails could repopulate a habitat to its former density in few months [23].

The drainage of chemicals such as pesticides in the snail water habitat may contribute a conspicuous role in the snail control. Therefore, the present investigation was promoted to evaluate the effect of six agricultural pesticides namely; triazophos, cyolane, cypermethrin, baythroid, methomyl and larvin, belonging to three different insecticide groups; organophosphorous, pyrethroid and carbamate.

The results obtained revealed that three out of the six compounds studied were found to have a pronounced effect against <u>B</u>. <u>alexandrina</u> snails, and those were triazophos, cyolane (organophosphorous) and cypermethrin (pyrethroid). The most effective one that showed the highest mortality rate was triazophos followed by cyolane then cypermethrin. These results agree with that of Oteifa and his Co-workers [19] who also found that the organophosphorous insecticides were the most potent compounds that have molluscicidal effect against *B. alexandrina* snails. Many workers [3,10,20]. have attributed the effectiveness of the organophosphorous compounds to their inhibitory effect on acetylcholinesterase activity allowing a consequential build-up of acetylcholine in the snail body.

On the other hand, results obtained for carbamates (methomyl and [illegible]vin) up to 50 ppm showed considerable low toxicity, a finding matches with that reported by El-Sebae and his Co-workers [7] who found that there was no significant toxicity of some other carbamates against *B. Alexandrina* snails.

However, the retardation in the growth rate of snails might be a result of the direct effect of the insecticides on the hormones regulating the growth rate of the snails and subsequently influenced their growth [8,9].

However, the reduction of the egg-masses, egg-cells and hatchability rate of the eggs laid by the snails pre-treated with the sub-lethal concentrations of the insecticides tested for two weeks is correlated to the direct effect of the tested compounds on the activity of the genital organs of the snails. This effect subsequently influenced the egg laying capacity and hatchability. These results could be explained in the view of El-Gindy [6] and Mohamed et al. [15] who mentioned that the molluscicides could affect spermatogenesis, oogenesis and vitelline glands in B. alexandrina and B. truncatus. However, there was no significant ovicidal effect on the tested insecticides on the freshly laid eggs. This could be attributed to the material surrounding the egg-masses which might prevent the insecticide from diffusing through it, and therefore stop any effect on the developing embryo. This finding is supported by the results previously reported by many authors [5,15,19] who found that the egg-masses exihibited a high resistance to most of the molluscicides except at concentrations above those which kill the snails.

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Table (1): Effect of insecticides on the mortality rate of *B. alexandrina* snails.

Conc. ppm	No. of Snails	Organophosphorous insecticides				Pyrethroid insecticides				Carbamate insecticides			
		Triatophos		Cyloane		Cypermethrin		Baythroid		Methomyl		Larvin	
		I	II	I	II	I	II	I	II	I	II	I	II
10	120	83	69.16%	84	70.00%	71	59.16%	0.0	0.0%	0.0	0.0%	0.0	0.0 %
20	120	95	79.16%	94	78.33%	83	69.16%	0.0	0.0%	0.0	0.0%	0.0	0.0 %
30	120	110	91.66%	109	90.83%	107	89.17%	0.0	0.0%	0.0	0.0%	12.0	10.0 %
40	120	120	100.00%	120	100.00%	120	100.00%	0.0	0.0%	12.0	10%	15.0	12.50%
50	120	120	100.00%	120	100.00%	120	100.00%	0.0	0.0%	26.0	21.67%	20.0	16.60%

I. No. of dead snails.

II. Mortality rate.

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Table (2): LC_{50}, LC_{90} for the various insecticides.

Compounds	LC_{50} ± Fidicial Limits ppm	LC_{90} ± Fidicial Limits ppm
Triazophos	5.00 ± 1.730	47.55 ± 0.717
Cyolane	5.58 ± 0.220	27.42 ± 0.643
Cypermethrin	11.23 ± 2.260	30.20 ± 0.282

Table (3): Effect of sub-lethal concentration (1/10 LC_{50}) of various insecticides on the growth of the shell diameter of *B. alexandrina* snails.

Days post-exposure	Rate of growth in mm/snail/day.			
	Control Mean ± S.D.	Triazophos Mean ± S.D.	Cyolane Mean ± S.D.	Cypermethrin Mean ± S.D.
7	0.039 ± 0.064	0.022 ± 0.050	0.039 ± 0.064	0.017 ± 0.046
14	0.039 ± 0.064	0.034 ± 0.061	0.034 ± 0.061	0.017 ± 0.046
21	0.022 ± 0.050	0.022 ± 0.052	0.011 ± 0.039	0.022 ± 0.050
28	0.060 ± 0.070	0.034 ± 0.061	0.017 ± 0.046*	0.032 ± 0.064
35	0.040 ± 0.060	0.011 ± 0.040*	0.011 ± 0.039*	0.011 ± 0.039*
42	0.062 ± 0.071	0.017 ± 0.064*	0.016 ± 0.046*	0.022 ± 0.052*

* Significantly retarded at 5% level than control (T-Test).

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Table (4): Effect of sub-lethal concentrations (1/10 LC_{50}) of various **insecticides** on the total body weight of B. alexandrina snails.

Days Post-exposure	Rate of growth in mg/snail/day			
	Control Mean ± S.D	Triazophos Mean ± S.D	Cyolane Mean ± S.D.	Cypermethrin Mean ± S.D
7	3.14 ± 2.19	2.05 ± 1.80	2.97 ± 2.49	3.13 ± 1.70
14	2.38 ± 3.14	1.21 ± 1.30	1.62 ± 0.75	1.55 ± 0.95
21	2.90 ± 1.98	1.70 ± 1.26*	1.67 ± 0.84*	1.81 ± 1.53
28	3.54 ± 2.54	1.55 ± 0.59*	1.73 ± 0.81*	2.27 ± 2.20
35	2.35 ± 1.86	1.45 ± 0.89*	1.54 ± 0.68*	1.45 ± 0.89*
42	2.49 ± 1.26	1.62 ± 0.86*	1.85 ± 0.69*	1.76 ± 0.89*

* Significantly retarded at 5% level than control (t - test).

Table (5): Effect of sub-lethal concentrations (1/10 LC_{50}) of various insecticides on egg production of B. *alexandrine* snails treated for one week.

Days Post-Expo.	Egg-production							
	Control		Triazophos		Cyolane		Cypermethrin	
	+ egg masses	++ egg cells	+ egg masses	++ egg cells	+ egg masses	++ egg cell	+ egg masses	++ egg cells
5	1.208 ± 0.415	20.833 ± 16.463	1.00 ± 0.294*	10.458 ± 10.172*	0.375 ± 0.388*	9.517 ± 8.270*	1.080 ± 0.503	17.292 ± 7.220
10	2.500 ± 2.157	54.333 ± 52.717	1.300 ± 0.637*	25.250 ± 24.788*	1.000 ± 0.720*	24.166 ± 18.220*	1.583 ± 1.059	34.417 ± 32.550
15	4.167 ± 3.085	105.666 ± 79.660	1.916 ± 1.213*	44.250 ± 40.074*	2.166 ± 1.52 *	57.042 ± 49.57 *	2.666 ± 2.200	53.917 ± 43.424*
20	3.375 ± 2.143	85.38 ± 55.407	1.958 ± 1.398*	50.000 ± 40.989*	2.166 ± 1.659*	52.000 ± 43.294*	2.208 ± 1.668*	50.500 ± 42.899*
25	3.958 ± 3.014	72.516 ± 62.532	1.916 ± 1.612*	49.333 ± 43.876	1.417 ± 0.88 *	32.083 ± 27.066*	1.625 ± 1.013*	43.250 ± 26.127*
30	2.583 ± 2.165	65.916 ± 55.465	1.583 ± 1.212*	40.458 ± 30.816	1.75 ± 1.159	35.417 ± 37.32 *	1.542 ± 0.830*	38.70 ± 16.01
35	1.958 ± 1.334	45.375 ± 36.493	1.330 ± 0.631*	28.833 ± 19.164	1.375 ± 0.641	23.623 ± 20.565*	1.083 ± 0.408	25.66 ± 13.528

+ Mean number of egg masses/ snail / 5 days.

++ Mean number of egg cells/snail/ 5 days.

* Significant decrease at 5% level than control (t - test).

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Table (6): Effect of sub-lethal concentrations (1/10 LC_{50}) of various insecticides on egg production of B. alexandrina snails treated for two week.

Egg-production							
Control		Triazophos		Cyolane		Cypermethrin	
+ egg masses	++ egg cells	+ egg masses	++ egg cells	+ egg masses	++ egg cell	+ egg masses	++ egg cells
1.200 ± 0.763	24.040 ± 20.741	1.040 ± 0.200	9.400 ± 8.961*	1.00 ± 0.000	6.462 ± 6.332*	1.200 ± 0.408	11.480 ± 4.560*
1.680 ± 0.802	31.360 ± 24.069	1.320 ± 0.748	20.800 ±18.037*	1.16 ± 0.374*	16.560 ± 14.365*	1.240 ± 0.435*	11.800 ± 3.476*
1.765 ± 0.760	35.760 ± 24.208	1.120 ± 0.332*	15.680 ±17.361*	1.04 ± 0.540*	20.440 ± 18.104*	1.000 ± 0.500*	16.040 ± 8.314*
1.840 ± 0.940	37.000 ± 19.676	1.166 ± 0.381*	19.560 ±13.482*	1.36 ± 0.489*	23.440 ± 17.975*	1.080 ± 0.571*	20.680 ± 13.700*
1.720 ± 1.100	38.440 ± [illegible]	1.200 ± 0.410*	15.720 ±14.665*	[illegible] ± [illegible]*	16.720 ± 14.472*	1.120 ± 0.600*	19.37[illegible] ± 1[illegible].392*
1.720 ± 1.339	40.040 ± 35.039	1.080 ± 0.277*	14.360 ±12.954*	1.090 ± 0.276*	13.720 ± 11.78*	1.120 ± 0.439*	13.400 ± 77.325*
2.500 ± 2.000	58.760 ± 52.970	1.200 ± 0.408*	16.360 ±11.019*	1.160 ± 0.374*	16.840 ± 15.472*	1.200 ± 0.408*	14.640 ± 5.945*

\+ Mean of egg masses / Snail / 5 days.

++ Mean of egg cells / snail / 5 days.

* Significant decrease at 5% level than control (T-Test).

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Table (7): Effect of sub-lethal concentrations (1/10 LC_{50}) of the various insecticides on the hatchability of freshly laid eggs.

	Control	Triazophos	Cyolane	Cypermethrin
Mean No. of freshly eggs/ egg-mass	27.77 ± 12.32	23.85 ± 7.47	21.74 ± 7.07	21.74 ± 6.31
Mean No. of fertilized eggs/egg-mass	27.22 ± 12.04	23.33 ± 7.34	20.91 ± 6.83	21.18 ± 6.15
Mean No. of hatched eggs/ egg-mass	27.22 ± 12.04	23.19 ± 7.24	20.83 ± 6.74	21.08 ± 6.05
Rate of hatching	98.02%	97.23%	95.81%	96.96%

تأثير بعض مبيدات الحشرات على قوقع البيومفلاريا الكسندرينا العائل الوسيط للبلهارسيا المانسونية فى مصر
١ـ دراسات سمية وبيولوجية

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تم اختيار كفاءة ستة مبيدات حشرية كمبيدات لقواقع البيومفلاريا الكسندرينا وهذه المبيدات هى الترايازوفس والسيولان (من المبيدات الفوسفاتية) والسيبرمترين والبايثرويد (من البيريثرويدات) والمثوميل واللارفين (من الكاربمات) وقد أوضحت الدراسة ان ثلاثة منها وهى الترايازوفس والسيولان والسيبرمترين ذوى فاعلية واضحة حيث كانت التركيزات المميتة لـ ٥٠ بالمائة من الحيوانات هى ٥,٠٠ ± ٠,٢٢ ، ١١,٢٣ ± ٢,٢٦ جزء فى المليون على التوالى :

وقد أوضحت الدراسة ايضا ان هذه المبيدات الثلاثة الاخيرة لها تأثير واضح على معدلى النمو والوفيات لهذه القواقع كذلك على كفاءة وضع البيض ونسبة فقسه للقواقع السابقة المعالجة بالمبيدات رغم انها لم تظهر اى كفاءة واضحة كمبيدات مقاومة للبيض٠