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Impact profenophos (pesticide) on infectivity of *Biomphalaria alexandrina* snails with *schistosoma mansoni* miracidia and on their physiological parameters

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ABSTRACT

Profenophos is an organophosphorus pesticide which are used effectively against cotton insects and mites.The present work was carried out to evaluate the molluscicidal activity of pesticides (Profenophos) against *Biomphalaria alexandrina* snails. Also, the effect of sublethal concentrations of pesticide on the infection of *B. alexandrina* with *Schistosoma mansoni* and some enzymes of energy metabolism were studied. The results showed that the infection of *B. alexandrina* with *schistosoma mansoni* was greatly reduced after exposure to LC_0, LC_{10}, LC_{25} of pesticide and also, reduction in number of cercariae per snail during the patent period and in the period of cercarial shedding. The present study indicated that the increase in levels of aminotransaminases, acid phosphate-se and alkaline phosphatases enzymes in haemolymph and soft tissue of snails and the activity level of lactate dehydrogenase, hexoki-inase and pyruvatekinase was also significantly reduced in response to treatment.

***Keywords*:** Biomphalaria Alexandrina; Profenophos; Schistosoma Mansoni; Physiology

1. INTRODUCTION

Excessive use of pesticides in agriculture has sparkled researchers' interest in investigating the harmful effects of these compounds. Consequently, there has also been an increase in the number of studies aiming at evaluating the action of the residues of such chemicals on non-target organisms. Pesticides are ubiquitous contaminants of the environment and have been found in air, soil, water, and human and animal tissues in samples from all over the world. These cover a wide range of compounds used in pest control, such as fungicides, herbicides, molluscicides, insecticides, rodenticides and others [1]. Pesticide may play an important role in the disappearance of snail vectors on reaching water bodies as residues from pesticide activities and consequently hindering schistosomiasis transmission in these sites [2].

Profenophos is an organophosphorus pesticide which are used effectively against cotton insects and mites. Improper use of pesticides combined with their persistence, volatilization and mobility lead to frequent detections of the pesticide residues in the atmosphere, soil, surface water and in vegetable fruits and cereal grains [3, 4].

The use of pesticides may engender biological effects beyond those for which they were originally manufactured [5,6]. As example of these is the agricultural insecticides which may interfere in the life, reproduction and infection of snail vectors of schistosomiasis when they reach water bodies [7]. Many authors studied the effect of agricultural pesticides that may reach water courses as residues on different biological parameters of snail vectors of schistosomiasis [8].

Organophosphate pesticide represent one of the world's most commonly used agrochemical. Consequently, many of its residues are frequently found in the environment. Of the organophosphates, Profenophos has been extensively used because of its low toxicity on non-targ organisms. The present work aimed to study the mode of action of Profenophos as molluscicides against *Biomphalaria alexandrina* as indicated by sublethal concentrations action on infection of *Biomphalaria alexandrina* to *Schistosoma mansoni* and some enzymes such as aminotransaminases, acid phosphatase and alkaline phosphatases enzymes in haemolymph and tissues of *B. alexandrina*. Moreover, lactate dehydrogenase (LDH), pyruvatekinase (PK) and, Hexokinase (HK) activity in soft tissue of treated snails.

2. MATERIAL AND METHODS

2.1. Snails

Laboratory bred *Biomphalaria alexandrina* snails (6 - 10 mm in shell diameter) were obtained from Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute (TBRI), Egypt.

2.2. Ova

Schistosoma mansoni ova were obtained from the Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute (TBRI), Egypt.

2.3. Pesticides (Profenophos)

Organophosphorus pesticide, having the chemical formula C11H15BrClO3PS [O-(4-Bromo-2-chlorophenyl)-O-ethyl-S-propy l phosphorothioate], Synonyms: Curacron; Polycron; Selecron; Nonacron and properties (M.Wt 373.6 and boiling point 110˚C). Profenophos was obtained from the Ministry of Agriculture, Egypt.

2.4. Biological study

2.4.1. Molluscicidal Screening

The efficacy of pesticide (Profenophos) against adult snails was determined according to the standard procedure recommended by WHO (9). Stock solution (1000 ppm) were prepared using dechlorinated water and a series of concentrations was prepared from each experimental pesticide on the basis of weight/volume that would permit the computation of LC_{50} and LC_{90} values were prepared [10]. Dead snails were counted and recorded. LC_{25} LC_{50} and LC_{90} values slop function and 95% confidence limits of compound were obtained from the curve in which serial concentrations in ppm were plotted against % mortality of snails [11]. While LC_0 was determined as 1/10 LC_{50} [9].

2.4.2. Effect of Sublethal Concentration of Pesticides on Infectivity of S. Mansoni Miracidia to B. Alexandrina Snails.

Exposing 3 groups of snails (each of 50 snails) individually to a dose of 10 miracidia/snail and maintained in each sublethal of concentrations of Pesticide (LC_0, LC_{10} and LC_{25} of Profenophos) for 24 h under room temperature (24 ± 1˚C). After exposure to miracidia, snails were maintained in their corresponding pesticide. Another group of 50 snails was exposed to miracidia in the absence of the tested pesticide and maintained under the same conditions (control group). The snails were daily fed lettuce (*Lactuca sativa* plant) leaves and dead ones were removed. Examination of snails for cercarial shedding was carried out twice weekly, 25 days post exposure, and the cercarial suspension was poured in a graduated Petri dish, then a few drops of Bouin's fluid (Dye for coloring cercariae) were added and all cercariae were counted, using a dissecting microscope. Shedding snails were then isolated and kept in special aquaria in complete darkness.

2.4.3. Effect of Sublethal Concentration of Pesticides on Physiological Parameters of B. Alexandrina Snails.

For studying some physiological parameters of *B. alexandrina* snails, four identical groups of snails (each of six replicates) of which three groups of snails were exposed for one month to sublethal of concentrations of Pesticide (LC_0, LC_{10} and LC_{25} of Profenophos). A four group was left unexposed under the same laboratory conditions as control. Snails surviving after exposure was used to study selected enzymatic activities were investigated both in treated and untreated snails. The measured enzymes included aminotransaminases [Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), (AST and ALT are the most frequently measured for hepatic diseases, the enzymes may be released from hepatocytes into the circulation by necrosis [12]), acid phosphatase and alkaline phosphatases enzymes (these are a lysosomal enzymes concerning with digestion of foreign substances and bacteria inside the cells and is involved in the defense mechanisms of both vertebrates and invertebrates [13]) in haemolymph and tissues of *B. alexandrina*. Moreover, lactate dehydrogenase (LDH), pyruvatekinase (PK) and Hexokinase (HK) activity [glycolytic enzymes] in soft tissue of treated snails.

2.4.3.1. Biochemical Analysis

For preparation of tissue extracts of treated and control snails, one gram of the soft tissues of the snail was homogenized in 5 ml distilled water, pH 7.5. A glass homogenizer was then used to grind the tissue, and the homogenate was centrifuged for 10 min at 300 rpm and the fresh supernatant was decanted. Haemolymph of infected and control snails was collected in accordance with techniques described by Michelson **[**14]. The haemolymph was obtained via small hole made in the shell into which capillary tube was inserted then it was drawn into tube by capillary suction. The haemolymph was pooled from 10 snails collected in a vial tube (1.5 ml) and kept in ice-bath.

2.4.3.2. Assay Methods

Aspartate aminotransferase (AST) activity in the haemolymph and soft tissues of exposed and unexposed

snails was determined according to the method of Reitman and Frankel [15], the oxaloacetate formed reacts with 2, 3-dinitrophenyl-hydrazine in alkaline solution(PH = 7.5 at 22˚C). The product of the reaction can then be determined photometrically at 500 nm to560 nm

Alanine aminotransferase (ALT) activities in the haemolymph and soft tissues of exposed and unexposed snails were determined according to the method of Reitman and Frankel [15], the pyruvate formed reacts with 2, 4 dinitrophenyl-hydrazine in alkaline solution(PH = 7.5 at 22˚C). The product of the reaction can then be determined spectrophometrically at 500 nm to 560 nm

Acid phosphatase (ADP) and alkaline phosphatase (ALKP) activities were determined spectrophotometer by measuring absorbance at 340 - 410 nm according to Bessey *et al*. [16], Acid phosphatases have pH optima below 7, whereas alkaline phosphatases are most active above pH 7 at 22˚C.

Hexokinase (HK) was determined spectrophotometer by measuring absorbance at 340 nm (PH = 7.5 at 22˚C) according to the method of Uyeda & Racker [17] in which glucose-6-phosphate formed by the hexokinase reaction is measured by adding glucose-6-phosphate dehydrogenase and NADP and following NADPH formation.

Pyruvatekinase (PK) activity was measured spectrophometrically as the rate of decrease in extinction at 340 nm (PH = 7.5 at 22˚C) due to the oxidation of NADH by coupling the system with excess of lactate dehydrogenase [18].

Lactate dehydrogenase activity was measured spectrophometrically by measuring absorbance at 340 nm ((PH = 7.5 at 22˚C) according to the method of Cabaud & Wroblewski [19]. In this method pyruvate is reduced by incubation with the enzyme in the presence of coenzyme nicotinamide adenine dinucleotide (NADH).

All physiological parameters determined in this study were determined spectrophotometrically, using reagent kits purchased from BioMerieux Company, France.

2.5. Statistical Analysis

Analysis of data was carried out by student's "t"-test for comparing the means of experimental and control groups Spiegel [20].

3. RESULTS

The molluscicidal activity of Profenophos on *Biomphalaria alexandrina* snails after 24 hours of exposure under laboratory conditions is presented in **Table 1**. The data obtained indicate that the recorded LC_{50} values for this pesticide was 4.6 ppm and LC 90 values was 10.7 ppm for *B. alexandrina*. The sublethal concentrations (LC_0, LC_{10} and LC_{25}) were found to be 0.46, 1.8 and 2.5 ppm for *B. alexandrina*.

The effect of the tested sublethal concentrations of Profenophos on infection of *B. alexandrina* with *S. mansoni* miracidia was presented in **Table 2**. The infection rate was significantly ($p < 0.001$) lower than that of control snails (55%) being 40%, 16.66% and 10% for snails exposed to LC_0, LC_{10} and LC_{25} of Profenophos respectively with a reduction rate –27.7%, –69.70% and 81.82% respectively.

There is no significant difference between prepatent period of the snails exposed to LC_0, LC_{10} and LC_{25} of pesticide and the control group. Prepatent period (**Table 3**) of exposed snails to LC_0, LC_{10} and LC_{25} of Profenophos was prolonged to be 36.2 + 4.8, 31.6 + 2.6 and 33.2 + 3.2 days compared to 35.6 + 4.1 days for the control group. Meanwhile, the duration of cercarial shedding was significantly ($p < 0.01$) shortened among these snails, being 8.2 + 1.2, 6.3 + 0.82 and 4.8 + 0.85 days for LC_0, LC_{10} and LC_{25}, respectively, compared with 16.2 + 4.3 days for control snails. Highly significant ($p < 0.001$) reductions of total cercarial production per snails was also detected in experimental snails in comparison with the control group.

The results showed that (**Table 4**.) the concentration of transaminase enzymes (AST and ALT) in haemo-

Table 1. Molluscicidal activity of profenophos (pesticide) against adult *Biomphalaria alexandrina* snails after exposure for 24 hours.

LC_{50} ppm*	Confidence limit of LC_{50} ppm	LC_{90} ppm*	Slope function	LC_0 ppm	LC_{10} ppm*	LC_{25} ppm*
4.6	5.98 - 3.54	10.7	1.64 - 3.54	0.46	1.8	2.5

***using SPSS computer program under windows.**

Table 2. Effect of sublethal concentrations of profenophos (pesticide) on infection of *Biomphalaria alexandrina* with *Schistosoma mansoni*.

Treatment	Number of exposed snails	Survived snails at first shedding		Infected snails		Reduction %
		Number	%	Number	%	
Control	50	40	80	22	55	
LC0	50	30	60	12	40	–27.7*
LC10	50	18	36	3	16.66	–69.70***
LC25	50	10	20	1	10	81.82***

$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 3. Effect of sublethal concentrations of profenophos (pesticide) on cercarial production of *Schistosoma mansoni* from infected snails.

Concentration (ppm)	Prepatent period (days)	Duration of shedding (days)	Number of cercariae/ snail
LC_0	36.2 ± 4.8	8.2 ± 1.2*	621.5 ± 55.5***
LC_{10}	31.6 ± 2.6	6.3 ± 0.82*	245.23 ± 44.2***
LC_{25}	33.2 ± 3.2	4.8 ± 0.85***	88.6 ± 12.4***
Control	35.6 ± 4.1	16.2 ± 4.3	2834.12 ± 213

lymph and soft tissue of experimental *B. alexandrina* snails showed significant ($p < 0.05$) increase than that in the control snails, The percentage of elevation 32.8%, 73.44% and 90.24% for AST and 37.11%, 41.41% and 61.71% for ALT in haemolymph and 29.33%, 83.47% and 88% for AST, and 27.77%, 43.77%, 72.22% for ALT in soft tissue of snails exposed to LC_0, LC_{10} and LC_{25} of pesticide, respectively than that in control snails.

The present results (**Table 5**) indicated that there were significant ($p < 0.05$) elevations in the level of acid phosphatase 21.57%, 27.45% and 39.22% in haemolymph and 27.27%, 31.82% and 40.91% in soft tissue of snail treated LC_0, LC_{10} and LC_{25} of pesticide, respectively. The increase of alkaline phosphatase was 40%, 56% and 92% in haemolymph and 12.5%, 27.1% and 41.67% in soft tissue of LC_0, LC_{10} and LC_{25} of pesticide, respectively than that in control snails.

The levels of hexokinase (HK), pyruvatekinase (PK), and lactate dehydrogenase (LDH) in the soft tissue in normal and treated snails are displayed in **Table 6**. The HK activity in snails exposed to sublethal concentrations of the pesticide for one month was 21.4 ± 1.4, 18.7 ± 1.1 and 13.5 ± 1.6 µmol/min/g, respectively. Such reduced values were statistically significant than those of the corresponding controls (30.2 ± 1.7). The activity levels of PK, LDH were also significantly reduced in response to treatment with plant extract, those of PK being 0.10 ± 0.018, 0.082 ± 0.011 and 0.066 ± 0.016 µmol/min/g wet tissue, as compared to the control being 0.12 ± 0.042 µmol/min/g.

4. DISCUSSION

Most of the chemical compounds residues found in the environment, such as pesticides, end up being discharged into water resources. In these environments, the substances become more and more diluted and are, therefore, usually found at low concentrations. In the current study, residual/environmental concentrations of commercial Profenophos were evaluated in order to investigate their possible effects on biological and physiological parameters of snails. The Profenophos (pesticide) showed considerable molluscicidal effect against *B. alexandeina*. The LC_{50} and LC_{90} were found to be 4.6 ppm & 10.7 ppm respectively. This result is supporting with Wafaa and Ragaa [8] indicated that Chlorpyrifos and Profenophos have a considerable killing effect against *B. truncatus* with LC_{50}, 1.32 ppm and 2.5 ppm of the two compounds, respectively. Similar results were obtained by El-Fiki and Mohamed [21], using the herbicides Gramaxone, Preforan and Trefla.

In this study, the infection of *B. alexandrina* with *S. mansoni* miracidia was greatly reduced by the tested sublethal concentrations of Profenophos. The reduction of infection rate was found to increase with the increase of sublethal concentrations of Profenophos. Thia agree with Wafaa and Ragaa, [8] found that increasing reduction of infection of snails maintained in LC_{25} of Chlorpyrifos and Profenophos pesticide. Similar inhibitory effects were seen in the results were obtained in literature by several authors working on various che- mical and plant molluscicides [22] using copper sulphate and Tributylin fluoride; [23] using Bayluscide; [24] using the plant *Calendula micrantha officinalis*; [25] using the

Table 4. Aspartate amino transferase (AST)), Alanine amino transferase (ALT) in *Biomphalaria alexandrina* exposed to sublethal concentrations of profenophos (pesticide).

Parameter Treatment	Aspartate amino transferase concentrations (µ/mg protein) % increase				Alanine amino transferase concentrations (µ/mg protein) % increase			
	Haemolymph mg/ml	% of change	Soft tissue mg/g	% of change	Haemolymph mg/ml	% of charge	Soft tissue mg/g	% of charge
Unexposed (control)	18.45 ± 2.3		37.5 ± 3.2		25.6 ± 4.1		50.4 ± 3.5	
lC_0	24.5 ± 1.1	32.8%	48.5 ± 4.3*	29.33%	35.1 ± 2.4*	37.11%	64.4 ± 8.2*	27.77%
LC_{10}	32 ± 5.1*	73.44%	68.8 ± 4.5**	83.47%	36.2 ± 3.2*	41.41%	72.43 ± 10.1*	43.77%
LC_{25}	35.1 ± 3.2**	90.24%	70.5 ± 4.6**	88%	41.4 ± 2.7**	61.71%	86.8 ± 6.3**	72.22%

Values are expressed as means ± SD of 4 independent experiments;Enzymatic activities expressed as µ mole NADH reacted/min/g wet. Tissue; *$p < 0.05$,**& $p < 0.01$

Table 5. Acid phosphatase (ADP), Alkaline phosphatase (ALKP) in *Biomphalaria alexandrina* exposed to sublethal concentrations of profenophos (pesticide).

Parameters Treatments	ADP concentration (μ mg protein)				ALKP concentrations (μ mg protein)			
	Haemolymph mg/ml	% of change	Soft tissue mg/g	% of charge	Haemolymph mg/m	% of change	Soft tissue mg/g	% of charge
Unexposed (control)	0.051 ± 0.05		0.44 ± 0.042		0.25± 0.34		0.048 ± 0.046	
LC_0	0.062 ± 0.07*	21.57%	0.56 ± 0.12*	27.27%	0.35 ± 0.17**	40%	0.054 ± 0.05*	12.5%
LC_{10}	0.065 ± 0.06*	27.45%	0.58 ± 0.33*	31.82%	0.39 ± 0.13**	56%	0.061 ± 0.086*	27.1%
LC_{25}	0.071 ± 0.048**	39.22%	0.62 ± 0.55**	40.91%	0.48 ± 0.64***	92%	0.068 ± 0033**	41.67%

Values are expressed as means ± SD of 4 independent experiments; Enzymatic activities expressed as μ mole NADH reacted/min/g wet. Tissue; *p < 0.05,**& p < 0.01

Table 6. Levels of lactate dehydrogenase (LDH), hexokinase (HK) and glucose phosphate isomerase (GPI) in the tissue of *Biomphalaria alexandrina* exposed to sublethal concentrations of profenophos (pesticide).

6	Enzyme activity (U/mg tissue)					
	LDH	% of change	HK	% of change	Pyruvate kinase (PK)	% of change
Unexposed (control)	44.2 ± 1.2		30.2 ± 1.7		0.12 ± 0.042	
LC_0	36.2 ± 2.1*	–18.1%	21.4 ± 1.4 *	–29.14%	0.10 ± 0.018*	–16.67%
LC_{10}	24.4 ± 1.8*	–44.80%	18.7 ± 1.1*	–38.1%	0.082 ± 0.011*	31.67%
LC_{25}	17.5 ± 2.1**	–60.41%	13.5 ± 1.6**	–55.30%	0.066 ± 0.016**	–45%

Values are expressed as means ± SD of 4 independent experiment; Enzymatic activities expressed as μ mole NADH reacted/min/g wet. Tissue; *p< 0.05,**& p< 0.01.

plants *Thymus capitatus* and *Piper nigrum*; [26], [11] using the plant *Synadenium grantii*. Tantawy [27] reported that the herbicides, Butachlor and Fluazifop-p-butyl reduced the survival and infection rates of *B. alexandrina* snails. Bakry [28] using plant extract.

However, there was no difference between the prepatent period of the snails exposed to sublethal concentration of Profenophos pesticide and the control. Despite that, a highly reduction in the duration of cercarial shedding and total cercarial production per infected snails were reported. This reduction in cercarial shedding period and total cercarial production per snail is probably due to rupture of snails' tissues through miracidial penetration in the presence of those pesticides which increased the harmful effects of these plants [8]. These observations are in accordance with many authors using different plant species as molluscicides. Thus, Ibrahim *et al*. [29] stated that low concentrations of the organophosphorus pesticides Chlropyrifos (Dursban) caused blockage of cercarial shedding of infected of *B alexandrina* snails. Sharaf El-Din *et al.* [30] obtained similar reduction in cercarial shedding and cercarial production from *B. alexandrina* treated with sublethal concentrations of aqueous suspension of *Zygophyllum simplex*. This supports other authors on various molluscicides, e.g.

El-Ansary *et al.* [31] recorded longer prepatent period in *B. alexandrina* infected with *S. mansoni* in presence of *Ambrosia maritima*, and Gawish [32] found that the period of cercarial shedding in snails treated with the experimental molluscicides during their exposure to miracidia are significantly short than that in control snails. The results also indicated that treatment of snails continuously with the methanol extract of *E.soongerica* plant resulted in highly significant reduction of total cercarial production per snails in comparison with control.

The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes in haemolymph and tissue of *B. alexandrina* snails were significantly increased than that in the control snails. This result is in accordance with Bakry *et al.* [33] who reported that the levels of AST and ALT in haemolymph of *Bulinus truncatus* are increased significantly when exposed to *oreopanax reticulum* and *Furcraea selloea* plants. El-mam and Ebeid [34] reported that the activity of AST and ALT of the heamolymph of *B. alexandrina* were significantly decreased by *S. mansoni* infection. Acid phosphatase enzyme plays an important role in the defense mechanism of host snails Sabry *et al.* [35].

The present results indicated that there are significant elevations in the level of acid phosphatase and alkaline phosphatase which can be explained by the destruction of internal snail cells.

This finding agrees with Bakry *et al.* [33] using *oreopanax reticulum* and *Furcraea selloea* plants. Also, Michelson and Dubois [36] found an increase in alkaline phosphatase levels in both the heamolymph and digestive gland from infected *B. glabrata* with *S. mansoni.* El-Emam and Ebeid [34] reported that the acid phosphatase activity in the heamolymph of *B. alexandrina* was increased by *S. mansoni* infection. The present study showed a significant decrease in LDH activity in the whole tissue extract of *Bulinus truncatus* in response to treatment with the methanol extract of *E.soongerica* plant. Several authors have reported a significant decline in LDH activity of tissues of vari]ous molluscs in response to some molluscicides [Aboul-Zahab & El-Ansary, [351 and Bakry *et al.* [38].

4. CONCLUSIONS

It is concluded that Profenophos pesticide are toxic to the intermediate snail host of *S. mansoni.* and therefore may have adverse effects on natural populations. Sublethal concentration of this pesticide play also a role in suppressing transmission of schistosomiasis by reducing the infection of snails with schistosomes. In addition, pesticides caused reduction in number of cercariae per snail during the patent period and in the period of cercarial shedding. The present study indicated that the increase in levels of aminotransaminases, acid phosphatase and alkaline phosphatases enzymes in haemolymph and soft tissue of snails and the activity level of lactate dehydrogenase, hexokinase and pyruvatekinase was also significantly reduced in response to treatment. According to the results obtained, we can observe that the Low concentrations of Profenophos tested (residues found in the environment) induced toxic to biological and physiological of snails.

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