Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 23(5): 267 - 283 (2019)
www.ejabf.journals.ekb.eg

Effect of Water Bodies Lining on the Efficacy of Molluscicides Against *Biomphalaria alexandrina* Snails with Emphasis to their Integrated Control Measures

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ARTICLE INFO

Article History:
Received: Oct.17, 2019
Accepted: Nov.29, 2019
Online: Dec. 2019

Keywords:
Lining water courses
B.alexandrina
Molluscicides
Control measures
Copper sulphate
Helisoma duryi
Niclosamide

ABSTRACT

The effect of lining snails'aquaria with different materials on efficacy of the molluscicides niclosamide and copper sulphate on *Biomphalaria alexandrina* snails under laboratory and semi-field conditions was studied. Under laboratory conditions, the survival rate of *B. alexandrina* kept in aquaria lined with cement or plastic sheets and exposed to the sub-lethal concentration(LC_{50}) of niclosamide showed a highly significant reduction (p<0.001) represented by 5% and 0.0, respectively comparing with those in mud and $control_1$ (50% and 40%, respectively). Similarly, snails kept in aquaria with substratum of cement or plastic and exposed to copper sulphate (LC_{50}) showed 100% mortality compared to those with muddy substratum and $control_1$ (65% and 50%, respectively). The Semi-field experiment showed 100% and 96% snails mortality in canals lined with cement or plastic, respectively after exposing to copper sulphate (LC_{25}) compared to 38% for snails in muddy canal. The mortality of control snails (snails without molluscicide) was recorded as 2%, 52% and 48% in canals with mud, cement and plastic, respectively. By using the sublethal concentration (LC_{25}) of the niclosamide, there was a highly significant increase in snails mortality in both of canals lined with cement and plastic (100%) compared to 40% in snails maintained in muddy canals (p<0.001). A highly significant reduction (p<0.001) was recorded in the survival rate of *B. alexandrina* kept with the competitor snails *Helisoma duryi* in both of the aquaria with mud and $control_1$ groups either in the case of free snails (40% and 53.3%, respectively) or those in cages (53.3% and 46.7%, respectively) compared to those of *H. duryi* (80% and73.3% for free snails and those in cages, respectively). The same pattern was observed in the semi-field experiment. Laboratory study on the role of lining on the integrated control of snails maintained in mud substratum with the competitor snails and molluscicide indicated that there was a highly significant reduction (p<0.001) in snails mortality represented by 55% in case of both of the two molluscicides compared to those snails exposed in the presence of lining materials cement and plastic (92.5% and 100%., respectively). In addition, 100% mortality of *B. alexandrina* snails was observed after exposing to the molluscicide copper sulphate and *H. duryi* snails in both of the canals lined with cement and plastic compared to snails maintained in muddy canals (68%). From the view of snail integrated control measures, it could be concluded that lining of canals with cement or plastic sheets improve and increase the efficacy of the molluscicides and the bio-control agents (snail competitors) against snail borne parasitic diseases.

INTRODUCTION

One of the environmental modifications for controlling snail vectors is the lining of water canals (Martin, 1997). Ohmae *et al*. (2003) stated that as measures of snail control, cement-lining of ditches was most effective in Japan. The lined and unlined water bodies in some governorates in Egypt were studied by Ismail (2009) for the distribution and abundance of fresh water snails. The density of all recorded pulmonate snails (except *Bulinus truncatus*) in the lined sites were lower than those of the corresponding un-lined ones. *Biomphalaria* spp. were disappeared from the examined lined sites but it presented in only %11.8 of all unlined ones. Many authors stated that soil types of the water bodies substratum may play an important role in success of molluscicіding operations and may explain the failure in complete eradication of snails by molluscicides in some areas. This depression of the molluscicidal activity may be due to absorption and/or adsorption of some toxic constitute of the molluscicides on mud particles (Abdel Hameed, 2003; Ahmed,2003; El Said,2004; El Said *et al*., 2009). Furthermore, snails have a remarkable capability to survive long periods (5-8 months) in moist sand or mud (aestivation), their main source of food is organic matter originating from decaying submerged or emerged vegetation, different species of algae, bacteria and fungi (Martin,1997). So lining water bodies by suitable materials is important to improve the molluscicіding methods for snail control (El Said *et al*, 2009). Successful control of schistosomiasis should be based on an integrated approach which includes the control of intermediate snail hosts (Adewunmi *et al*., 1993; WHO, 1993). The use of combined control methods was carried out by many investigators in Tanzania (Lwambo and Moyo, 1991**)**, Brasil (Giovanelli *et al*., 2001) and Egypt (Allam, 2000; Mostafa, *et al*., 2005; El-deeb and Ismail, 2007).

El-Deeb and Ismail (2007) investigated that under laboratory and semi field conditions, the total numbers of harmed snails due to application of consecutive methods of control (plant or chemical molluscicides followed by adding fish) was significantly higher than the number of harmed or consumed snails exposed to any of these methods of control alone. Mostafa *et al*. (2005) suggested that the plant molluscicide *Calendula micrantha* and chemical molluscicide (niclosamide and copper sulphate) can be used together with the non-target snails *Melanoides tuberculata* and *Helisoma duryi* which serves as bio-control agents against *Biomphalaria alexandrina* in a combined effect for controlling this snails. Similarly, Giovanelli *et al.* (2001) found that the LC_{90} of the latex of *Euphorbia seplendenshislopii* plant against *M. tuberculata* snails was 13.8 times greater than that for *B. glabrata*. They added that the use of such plant in the presence of *M. tuberculata* snails has a synergic effect on reduction of *B. glabrata*. Lwambo and Moyo (1991) suggested that the application of ground seed pods of *Swartizia madagascariensis* (at a concentration inimical to kill snail's hosts of schistosomiasis) inhabitants with *Marisa carnuarietis* snails (a competitor and /or predator of snails bearing *Schistosome*) may not adversely affect the competitor snails. Thus, they recommended the use of such application as a combined method of control against snail hosts. Thus, the control of snail hosts by chemical, environmental and/or biological means can still play a significant supporting role in many endemic situations.

The present work aims to study three approaches under laboratory and semifield conditions. Firstly, the impact of lining materials on the efficacy of the chemical molluscicides (copper sulphate and Niclusamide) against *B. alexandrina* snails was investigated. Secondly, the effect of lining materials on the biological control agent *Helisoma duryi* (as a competitor snails) against *B. alexandrina* snails was also studied. Finally, the effect of integrated control measures as: biological (by competitor snails), chemical (by the molluscicides) and environmental (by lining canals) on *B. alexandrina* snails was carried out.

MATERIALS AND METHODS

Laboratory study:

Snail species:

Biomphlaria alexandrina (Ehrenberg, 1831) and *Helisoma duri* (Wetherby, 1879) snails were used in the present study. The two snail species were collected from irrigation canals in Giza Governorate. Snails were kept in plastic containers, then transported to the laboratory. At the laboratory, alive snails were washed in de-chlorinated tap water. Individuals of each species were separated and maintained in stock plastic aquaria provided with 20 liters de-chlorinated tap water at a water temperature of 25-28 ºC. They were provided with dried powdered lettuce (*lactuca stativa* L) twice a week. The water was changed every week to avoid bacterial growth and pollution. Each snail species had been acclimatized and maintained under accommodated laboratory conditions for at least three weeks before being used. (El-Emam and Ebeid, 1989; El-Sayed, 2006; Mosaad *et.al*., 2012).

Chemical molluscicides:

Copper sulphate ($CuSo_4$-$5H_2O$, 98% Technical powder, El-Nasr company for Chemical Drugs, Egypt). Niclosamide (Baylsucide) 2,5 dichloro 4´nitro salcylanilide, 70% Wettable powder, Bayer Pflanzenschutz Leverkusen, Germany).

Experiments:

Bioassay tests*:*

Screening tests of the chemical molluscicides (copper sulphate and Niclosamide) were carried out according to the methods recommended by Crossland *et al*. (1965). For each molluscicide, a series of concentrations was prepared from the dry powder of the chemical molluscicides (dissolved in de-chlorinated tap water on the basis of weight/volume). Thirty snails of *B. alexandrina* were immersed in one liter of the experimental concentrations for 24 hrs exposure in three replicates each of 10 snails for each concentration. Three replicates of control snails were maintained under the same experimental conditions without exposure to the tested molluscicide. After 24 hrs, snails were removed, washed thoroughly with fresh dechlorinated tap water and transferred to containers for recovery period. Percentage of mortality was calculated against the concentration used. Mortality regression lines were established by SPSS computer Program for the treated and control snails. The LC_{50} and LC_{90} values of the chemical molluscicides were determined from the toxicity lines.

Preparation of the experimental aquaria:

For each chemical molluscicide, an experimental set was conducted. Each set consisted of ten glass aquaria (25x20x14 cm) and designed as follows: two replicates were prepared for each substratum type and control aquaria as: the 1st and the 2nd ones provided with mud substratum, the 3rd and 4th ones lined in the bottom and inside walls with plastic sheets, the 5th and 6th ones lined with cement. The 7th, 8th, 9th and 10th aquaria kept without any lining or mud substratum and represented as: the 7th and 8th aquaria for the first control (snails with molluscicide) and the 9th and 10th aquaria for the second control (snails without any molluscicide). Then, each aquarium was provided with 2 liter of de-chlorinated tap water and all aquaria were maintained under the ambient room temperature of 25±2ºC throughout the experimental periods and the following experiments were conducted:

Effect of lining on the efficacy of the chemical molluscicide Copper sulphate against *Bimophlaria alexandrina* snails:

Four sub-lethal concentrations (0.02, 0.05, 0.27and 0.51ppm for LC_5, LC_{10}, LC_{25}, LC_{50}, respectively) of copper sulphate were used. Thus, four experimental sets were conducted. For each concentration, a number of *B. alexandrina* snails (size: 7-14mm in shell diameter) was introduced to each prepared aquarium in two replicates (20 snails/replicate). Then, the prepared concentration was added to each replicate except the 9th and 10th aquaria for the second control (snails without any molluscicide). The percentage of snails mortality was recorded and determined after one week of exposure.

Effect of lining on the efficacy of the chemical molluscicides Niclosamide against *Bimophlaria alexandrina* snails:

The same set of experiment described above was carried out with the exception of using the sub-lethal concentrations (0.08, 0.11, 0.17and 0.23 ppm for LC_5, LC_{10}, LC_{25}, LC_{50} respectively) of the molluscicide Niclosamide instead of copper sulphate.

Effect of lining materials on the biological control agent *Helisoma duryi* (as a competitor snails) against *B. alexandrina* snails:

Five glass aquaria (70x32x40 cm) were set up to test the potentiality of *H. duri* as a biological control agent against *B. alexandrina* snails. These aquaria were designed as: the 1st one was provided with mud substratum, the 2nd was covered in the bottom and inside walls with plastic sheets; the 3rd one was lined with a cement substratum and the 4th and 5th aquaria kept without lining as control ones. An equal number of 30 snails of both *B. alexandrina* and *H. duryi* were provided together to each aquarium (except the 5th aquarium which was provided with only *B. alexandrina*) in cages (30 snails each) and another 30 snails were introduced as free ones in the aquarium (outside cages). The dead snails were removed daily and their numbers were recorded and the mortality rate was calculated weekly for each snail species in all aquaria. The experimental period was three weeks. Results were presented in tabulated and graphic forms and the comparison between the different types of lining was observed.

Effect of integrated control on *Biomphalaria alexandrina snails*:

Four aquaria (38.5x32x20 cm) were prepared, they designed as, the first one lined with cement, the second one lined with plastic, the third one provided with mud as substratum. The fourth aquarium was considered as control (snails without lining substratum and molluscicide). Two replicates of each designed aquaria were prepared. Each one was provided with de-clorinated tap water, 20 snails of both *B. alexandrina* and *H. duryi*, and the sublethal concentrations of copper sulphate (LC_{25} against *B. alexandrina* except the control ones).The number and percentage of mortality of *B. alexandrina* and *H. duryi* were determined after one week of exposure and the results were presented in tabulated and graphic forms to investigate the effect of the three means of control measure (lining materials, biological control agent and the chemical control by molluscicide) on *B. alexandrina* snails.

The same set of experiment described above was carried out with the exception of using the sub-lethal concentrations LC_{25} of the molluscicide Niclosamide instead of copper sulphate.

Semi-field studies

Descriptions of the outdoor station:

The semi-field study was carried out at the Snails Research Station, (TBRI), in El-Qanater El-Khayria, Qaluobyia Governorate (Fig. 1). This station lies at 25Km to the north of Cairo near El-Qanater city. It has a field laboratory and 18 parallel ditches with muddy bottom and sloping banks, each is 30 meters long, 150 cm wide at water level, and 50 cm wide at the bottom. The water depth in ditches was about 50-60cm. Two channels at right angle to the ditches served as common feeding and draining channels. At the inner side of each channel, there is a base in which a series of leveled tubes is installed opposite to the ditches. The tubes of the common feeding channel act as water inlets and are located a few centimeters higher than those of the common draining channel which act as outlets, to allow the flow of water in one direction. All outlet tubes were guarded by plastic sieves to prevent loss of snails.

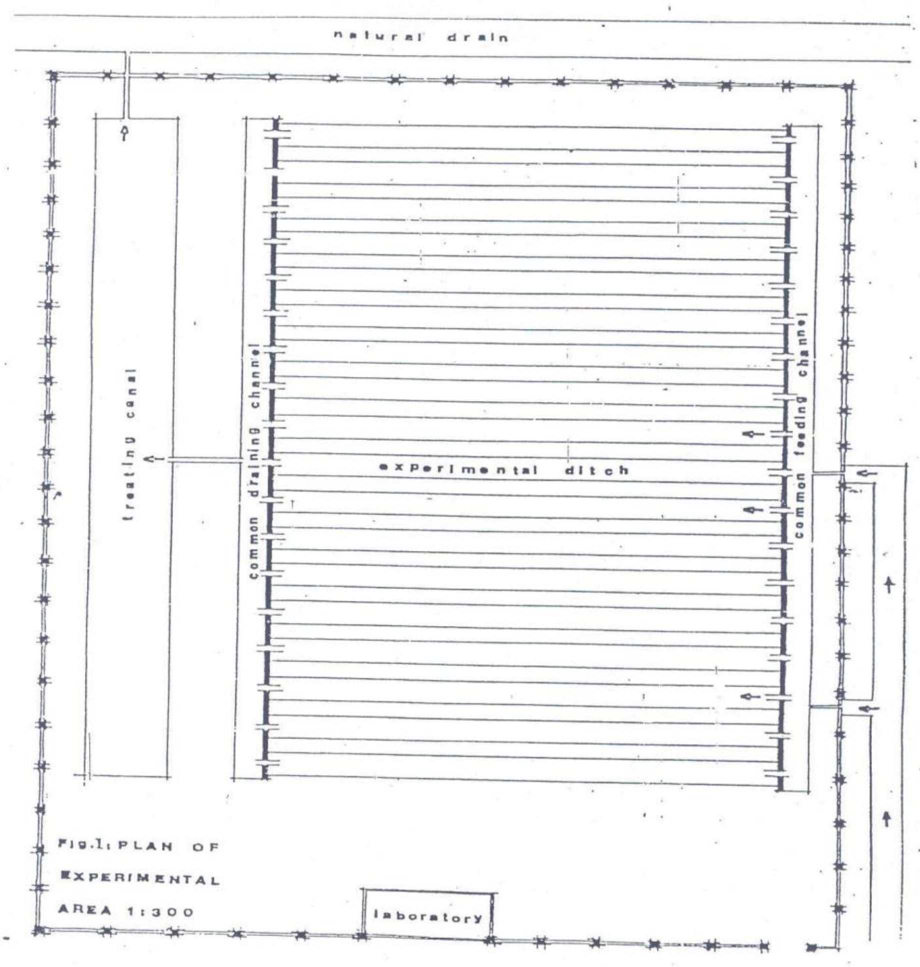

Fig. 1: A diagram for the Snail Research Station, Theodor Bilharz Research Institute (TBRI), in El-Qanater EL-Khayria, Qaluobia Governorate.

This system of experimental ditches was supplied with underground water (40 m depth). The ditches were shaded equally for about 60% of water surface. Vegetation was allowed to grow in all ditches but partial clearing was done to maintain almost equal vegetation density. The ditches so constructed provided parallel, more or less controlled environmental conditions not only for the volume, level and quality of water but also for temperature, light, wind action and content of aquatic flora.

Design of the experiments:-

Before the start of the experiments, four canals (30meter long x 2meter width) were selected. Then the water of these canals were drained, allowed to dry for 3 weeks (Gergis *et al*., 2008). Each one was divided into 6 small parts (about 5 meter in length each). These parts were designed as: the 1st and 2nd parts lined with plastic, the 3rd and 4th parts kept as muddy, the 5th and 6th parts was lined with cement. The canal filled again with water. Water level at the canals was maintained at the same depth by adding water as necessary.

Experiments:

Effect of lining on efficacy of copper sulphate on *B. alexandrina* snails:

The first divided canal (30 meter) was used in this experiment. Then, a number of 50 *B. alexandrina* snails (5-16mm in diameter) was added into each part. The 1st, 3rd and the 5th parts were provided with the sub-lethal concentration of the copper sulphate as chemical molluscicides (LC_{25} against *B. alexandrina*). The 2nd, 4th and the 6th parts was left without molluscicide as control ones. The number of survived snails were recorded and counted after one week of exposure to the molluscicide in the six parts. The results were represented in tabulated and graphic forms.

Effect of lining on efficacy of niclosamide on *B. alexandrina* snails:

The same procedure carried out above in case of copper sulphate was followed using the molluscicide niclosamide in the 2nd divided canal.

Effect of lining on *Biomphalaria alexandrina* snails and the biological control agent *Helisoma duryi* (competitor snails):

One divided canal (30 meter: 6 parts) was used in this experiment. A number of 50 *B. alexandrina* snails (5-15 mm in diameter) were added into each part. The 1st, 3rd and the 5th parts were provided with 50 of snails *Helisoma duryi*. The number of survived snails was counted weekly for 4 weeks in the six parts of canal. The results were presented in tabulated and graphic forms.

Effect of the integrated control measures against *B. alexandrina* snails under semi-field conditions:-

One divided canal (30 meter: 6 parts) was used in this experiment. A number of 50 *B. alexandrina* snails (size: 5-15 mm in diameter), 50 *Helisoma duryi* snails (size 5-17mm in diameter) and a sub-lethal concentration of copper sulphate (LC_{25} against *B. alexandrina*) were added to each part to determine the effect of three means of control as: biological (by competitor snails), chemical (by the molluscicide) and environmental (by lining canals) on *B. alexandrina* snails. The experimental period was one week. The average number and the percentage of snails mortality in the two replicates in the plastic, mud and cement parts was recorded regularly during the experiments. Results was presented in tabulated and graphic forms (Ismail and El-Deeb, 2007 & El-Deeb and Ismail, 2007).

Statistical analysis:

Quantitative data was analyzed by using F-test (computerized calculations) and student t-test to measure difference means and stander deviation of 2 groups while qualitative data was analyzed by using Z-test to test proportion of 2 mutually exchanged groups. Mortality regression lines were established by SPSS computer Program (version 17) for the treated and control snails.

RESULTS

Laboratory study:-

Bioassay tests of the tested molluscicides against Biomphalaria alexandrina snails:

Probit analysis of the toxic effects of the synthetic molluscicides Niclosamide and copper sulphate against *B. alexandrina* snails showed that the LC_{50} and LC_{90} of the two molluscicides were 0.23 and 0.33ppm for niclosamide; 0.51and 0.97ppm for copper sulphate, respectively after 24 h of exposure (Table 1).

Table 1: Probit analysis of the toxic effect of Niclosamide and copper sulphate against *Biomphalaria alexandrina* snails (24 h exposre).

Compounds tested	LC_{50} (ppm)	95% Confidence limit		LC_{90} (ppm)
		Lower	Upper	
Niclosamide	0.23	0.17	0.26	0.33
Copper sulphate	0.51	0.42	0.59	0.97

In general, there was a gradual decrease in the survival rate of *B. alexandrina* snails maintained in different substratum of mud, cement, plastic and control (snails exposed to the molluscicides without lining) with increasing the concentrations (LC_5, LC_{10}, LC_{25} and LC_{50}) of the two tested molluscicides (Tables 2, 3 & Figs 2, 3).

Effect of lining on the efficacy of Niclosamide against *Bimophlaria alexandrina* snails:

There was a highly significant reduction ($p<0.001$) in the survival rate of *B. alexandrina* kept in both substratum of cement (25%) and plastic (20%) when exposed to the concentration of 0.17ppm (LC_{25}**)** of niclosamide compared to those snails maintained in mud (75%) and control ones (65%). These reduction was increased with increasing the concentration to 0.23ppm (LC_{50}) which represented by value of 5% and 0.0 of snails′ survival in both aquaria lined with cement and plastic, respectively comparing with those in mud and $control_1$, represented by 50% and 40%, respectively (Table 2 & Fig. 2).

Table 2: Effect of lining materials on the efficiency of the mollusicide Niclosamide on *Biomphalaria alexandrina* snails under laboratory conditions.

Concentrations (ppm)	% of survived snails				
	Mud	Cement	Plastic	$Control_1$	$Control_2$
LC_5 (0.08)	85	45***	35***	80	100
LC_{10} (0.11)	85	40***	30***	75	100
LC_{25} (0.17)	75	25***	20***	65	100
LC_{50} (0.23)	50	5***	0.0	40	100

$Control_1$: *B. alexandrina* snails kept in aquaria with molluscicide and no lining
$Control_2$: *B. alexandrina* snails kept in aquaria without molluscicide and lining
*** highly significant ($p<0.001$) relative to snails in mud and control.

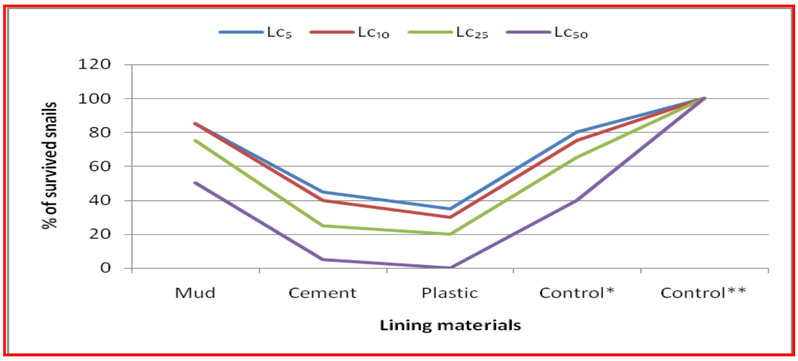

Fig. 2: Effect of lining on efficiency of the molluscicide Niclosamide on *Biomphalaria alexandrina* under laboratory conditions. (control*: *B. alexandrina* snails kept in aquaria with molluscicide and no lining, control**: *B. alexandrina* snails kept in aquaria without molluscicide and lining).

Effect of lining on the efficacy of copper sulphate against *Bimophlaria alexandrina* snails:

The same pattern was observed by exposing *B. alexandrina* snails to the sub-lethal concentrations of copper sulphate (LC_5, LC_{10}, LC_{25} and LC_{50}) in different substratum of mud, cement, plastic and control ones (Table 3 & Fig. 3). At the concentrations of 0.27 and 0.51 ppm (LC_{25} and LC_{50}), a highly significant reduction ($p<0.001$) was recorded in the survival rate of *B. alexandrina* kept in both aquaria lined with cement (35% and 0.0, respectively) and plastic (30% and 0.0, respectively) compared to those snails kept in aquaria with mud substratum (85% and 65%, respectively) and $control_1$ ones (75% and 50%, respectively). Moreover, $control_2$ snails (*B. alexandrina* kept in aquaria without lining and molluscicides) showed 100% of survival rate in all experiments.

Table 3: Effect of lining on efficiency of the molluscicide copper sulphate on *Biomphalaria alexandrina* under laboratory conditions.

Concentrations (ppm)	% of survived snails				
	Mud	Cement	Plastic	$Control_1$	$Control_2$
LC_5 (0.02)	100	95	95	97.5	100
LC_{10} (0.048)	100	90**	90**	95	100
LC_{25} (0.27)	85	35***	30***	75	100
LC_{50} (0.51)	65	0.0	0.0	50	100

$Control_1$: *B. alexandrina* snails kept in aquaria with molluscicide and no lining
Control : *B. alexandrina* snails kept in aquaria without molluscicide and lining
*** highly significant ($p<0.001$) relative to snails in mud and control.

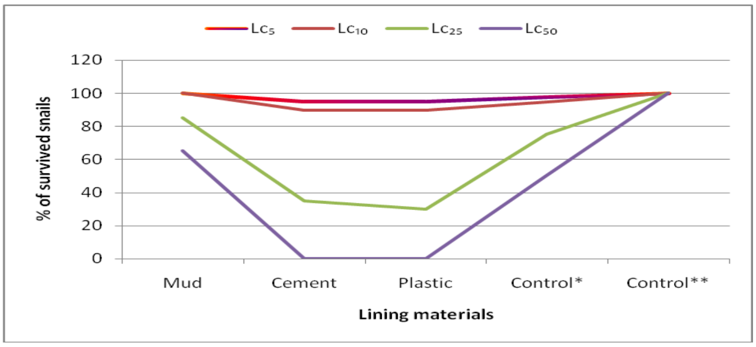

Fig. 3: Effect of lining on efficiency of the molluscicide copper sulphate on *Biomphalaria alexandrina* under laboratory conditions (control*: *B. alexandrina* snails kept in aquaria with molluscicide and no lining , control**: *B. alexandrina* snails kept in aquaria without molluscicide and lining).

Effect of lining materials on the biological control agent *Helisoma duryi* (as a competitor snails) against *Biomphalaria alexandrina* snails:

The effect of lining materials on both of *B. alexandrina* and the competitor snails *H. duryi* was illustrated in Table (4). Free *B. alexandrina* snails which maintained together in contact with their competitor *H. duryi* in aquaria with mud substratum and those lined with cement and plastic showed a highly significant decrease in survival rate (60%, 53.3% and 66.7%., respectively) compared to those in $control_1$ (both snail species maintained in glass aquaria without lining) represented by 86.7% at the 1^{st} week of the experiment. Moreover, the survival rate of free *H. duryi* showed a highly significant reduction in both aquaria lined by cement and plastic (33.3% and 20%, respectively) after three weeks of experiment. In contrast, another pattern was observed for snails maintained in mud and $control_1$ which approximately sharing the same survival rate ranging between 80% and 93.3% . On the other hand, a highly significant reduction ($p<0.001$) was recorded in the survival rate of *B. alexandrina* compared to that of *H. duryi* in both aquaria with mud and control groups. In contrast, the survival of *H. duryi* was lower than those of *B. alexandrina* in aquaria lined with cement and plastic during the experimental period.

Table 4: Effect of the bio-control agent (*Helisoma duryi*) on *Bimophlaria alexandrina* snails maintained on different lining substratum under laboratory conditions.

weeks	snails	**Percentage of survived snails**									
		Mud		Cement		Plastic		$control_1$ *		$Control_2$ **	
		Free snails	Snails in cages	Free snails	Snails in cages	Free snails	Snails in cages	Free snails	Snails in cages	Free snails	Snails in cages
0	B.a	100	100	100	100	100	100	100	100	100	100
	H.d	100	100	100	100	100	100	100	100	0	0
1	B.a	60	80	53.3	73.3	66.7	86.7	86.7	86.7	100	100
	H.d	93.3	100	40	60	60	80	93.3	100	0	0
2	B.a	46.7	80	46.7	53.3	60	66.7	73.3	73.3	100	100
	H.d	80	86.7	33.3	40	60	60	93.3	80	0	0
3	B.a	40	53.3	40	53.3	40	53.3	53.3	46.7	100	100
	H.d	80	73.3	33.3	40	20	13.3	80	73.3	0	0

control* : snails of *B. alexandrina* and *H. duryi* maintained in aquaria without lining.
Control**: snails of *B. alexandrina* only maintained in aquaria without lining.

Also, results in Table (4) show the survival rate of *B. alexandrina* and *H. duryi* when put together in cages (indirect contact with the lining substratum).The results indicated that the lowest survival rate of both *B. alexandrina* and *H. duryi* at the 1st week (73.3% and 60%, respectively) was observed in aquaria lined with cement. At the 3rd week, *B. alexandrina* showed a highly significant reduction in the survival rate (53.3% and 46.7% in aquaria with mud and $control_1$, respectively) compared to *H. duryi* (73.3%). In contrast, the survival of *B. alexandrina* (53.3%) was significantly higher than those of *H. duryi* in cement and plastic aquaria represented by 40% and 13.3%, respectively.The survival of *B. alexandrina* snails in $control_2$ (only *B. alexandrina*) was 100% throughout the whole period of the experiment (3 weeks).

Effect of integrated control on *Biomphalaria alexandrina snails*:

Results in Figures (4), (5) illustrated the effect of integrated control measure against *B. alexandrina* snails by using three methods of control: chemical (by using LC_{25} concentrations of the tested molluscicides copper sulphat and niclosamide), biological (*H. duryi* as a competitor agent) and physical (by lining the aquaria). It was found that there was a highly significant reduction ($p<0.001$) in the percentage mortality of *B. alexandrina* maintained in mud substratum represented by 55% for both two molluscicides compared to those snails exposed in the presence of lining materials cement (97.5% and 100%, for copper sulphate and niclosamide, respectively) and plastic (92.5% and 95%, respectively).

On the other hand control snails (only *B. alexandrina* kept in aquaria without lining , molluscicides and biological agent) showed 100% survival.

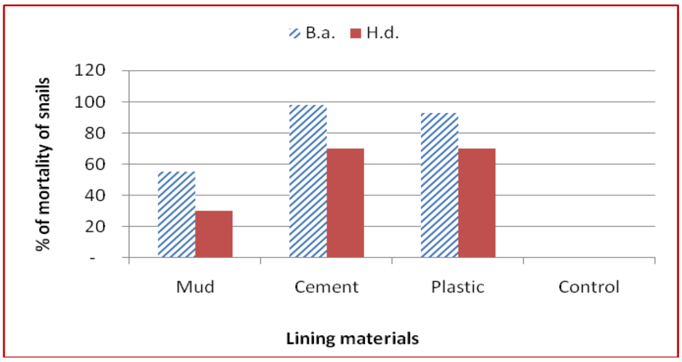

Fig. 4: Mortality (%) of *Biomphalaria alexandrina* snails under different lining materials exposing to the molluscicide copper sulphate and the biological agent *Helisoma duryi* snails (integrated control) under laboratory conditions.

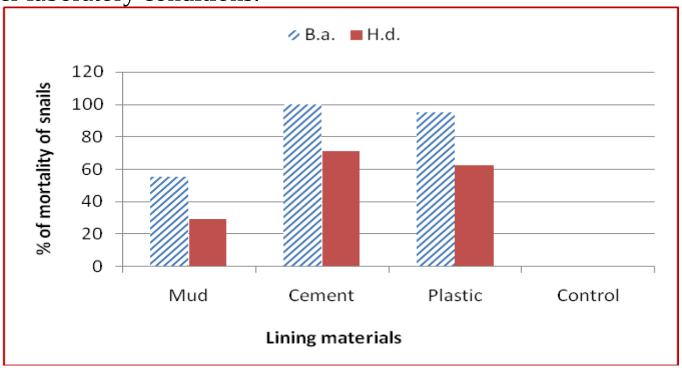

Fig. 5: Mortality (%) of *Biomphalaria alexandrina* snails under different lining materials exposing to the molluscicide niclosamide and the biological agent *Helisoma duryi* snails (integrated control) under laboratory conditions.

Semifield study:

Effect of lining on efficacy of the molluscicides Copper sulphate and Niclosamide on *Biomphalaria alexandrina* snails:

Results in Fig. (6) show that the percentage of mortality of *B. alexandrina* snails treated with the sublethal concentration of copper sulphate (LC_{25}) under cement and plastic lining (100% and 96%, respectively) at the semi-field habitat was highly significant higher than those snails in mud substratum (38%). While the percentage of mortality of control snails in mud, cement and plastic (snails without molluscicide) was 2%, 52% and 48%, respectively. On the other hand, there was a highly significant difference ($P<0.001$) in the percentage of mortality of *B. alexandrina* snails treated with the sublethal concentration (LC_{25}) of the molluscicide niclosamide under different lining materials at semi-field habitat represented by 40% for snails in canals with mud substratum compared to 100% for both snails in canals lined with cement and plastic. Meanwhile, the percentage mortality of control snails in mud, cement and plastic (snails without molluscicide) was 2%, 54% and 50%, respectively (Fig. 7).

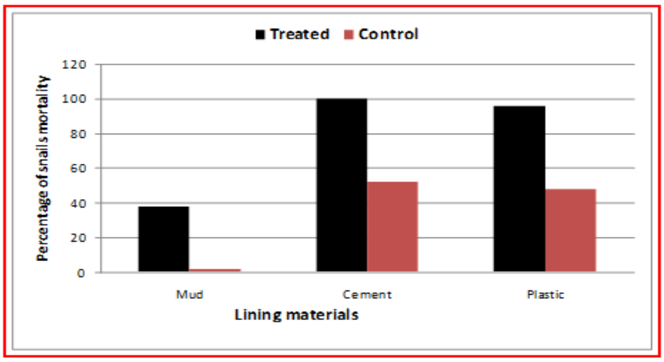

Fig. 6: Mortality (%) of *Biomphalaria alexandrina* snails treated with the sublethal concentration (LC_{25}) of the molluscicide copper sulphate under different lining materials at semi-field habitat.

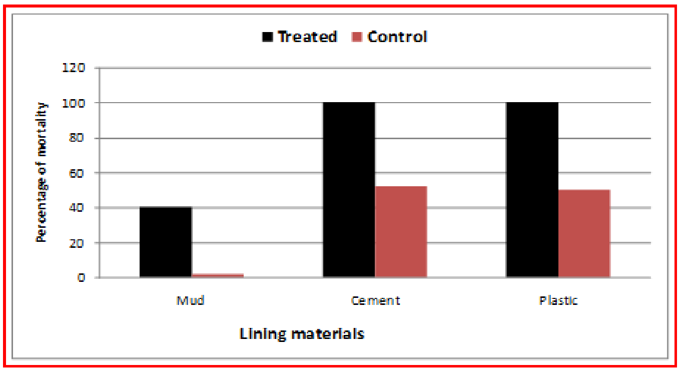

Fig. 7: Mortality (%) of *Biomphalaria alexandrina* snails treated with the sublethal concentration (LC_{25}) of the molluscicide Niclosamide under different lining materials at semi-field habitat.

Effect of lining on *Biomphalaria alexandrina* snails and the biological control agent *Helisoma duryi* (competitor snails):

Results in Table (5) showed that the effect of lining materials (cement and plastic) on the efficiency of the competitor snails *H. duryi* against *B. alexandrina* was highly significant reduced than those snails maintained in mud. The percentage of survived *B. alexandrina* exposed to competition by *H. duryi* in mud after four weeks was 36% compared to 92% of control snails (*B. alexandrina* alone). In contrast, the lining materials affected both snail species in which the percentage of survival was decreased for the two snail species in case of *B. alexandrina* exposed to competition by *H. duryi* and *B. alexandrina* alone.

Table 5 : Percentage survival of both *Biomphalaria alexandrina* snails and the biological control agent *Helisoma duryi* maintained in different lining materials at semi-field habitat.

Weeks	Snail Spps	% of snails survival					
		Mud		Cement		Plastic	
		B.a &H.d.	*B.a*	*B.a &H.d.*	*B.a*	*B.a &H.d.*	*B.a*
W0	*B.a.*	100	100	100	100	100	100
	H.d.	100		100		100	
W1	*B.a.*	74	100	56**	58	64$^{n.s}$	66
	H.d.	96		52		58	
W2	*B.a.*	60	96	36**	46	44*	48
	H.d.	86		30		36	
W3	*B.a.*	50	94	18***	20	16***	16
	H.d.	84		10		10	
W4	*B.a.*	36	92	0.0***	0.0	0.0***	0.0
	H.d.	78		0.0***		0.0***	

*** extremely highly significant relative to mud group (*B.a &H.d.*) ($P<0.001$).
** highly significant relative to mud group (*B.a &H.d.*) ($P<0.01$).
* significant relative to mud group (*B.a &H.d.*) ($P<0.05$).
n.s : non significant. *B.a.*: *Biomphalaria alexandrina* , *H.d.*: *Helisoma duryi*.

Effect of the integrated control measures (chemical, biological and environmental) against *B. alexandrina* snails:

The percentage mortality of *B. alexandrina* snails after exposing to the molluscicide (copper sulphate as a chemical control agent) and the biological control agent (*Helisoma duryi* snails as a competitor agent) under both lining materials (cement and plastic) was higher than those snails maintained in canals with mud substratum without lining. This represented by 68% mortality for snails in canals with mud compared to 100% for those snails maintained in canals lined with cement and plastic (Fig. 8). Thus, the lining of canals is efficient for completing the control measure and act as an environmental modification for snail control.

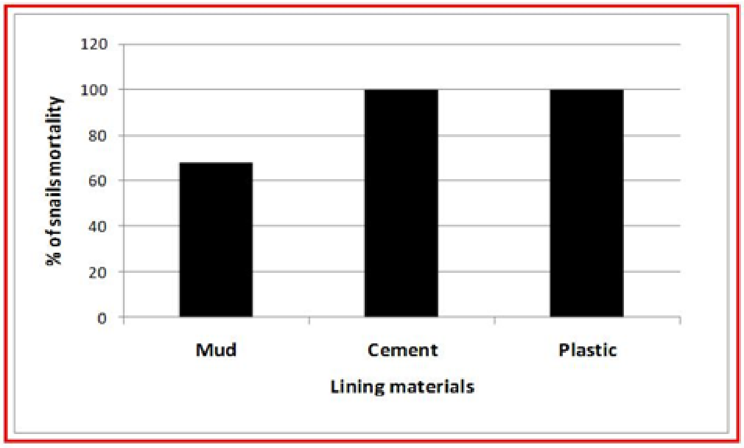

Fig. 8: Mortality (%) of *Biomphalaria alexandrina* snails under different lining materials exposing to the molluscicide copper sulphate and the biological agent *Helisoma duryi* snails (integrated control) at semi-field habitat.

DISCUSSION

Under laboratory conditions, the effect of lining the snails' aquaria on efficacy of the molluscicides niclosamide and copper sulphate against *Biomphalaria alexandrina* snails was studied in the present study. Generally, there was a gradual decrease in the survival rate of *B. alexandrina* snails maintained in different substratum of mud, cement, plastic and the exposed control$_1$ (snails exposed to the molluscicides without lining) with increasing the concentrations of the two tested molluscicides. There was a highly significant reduction in the survival rate of *B. alexandrina* kept in both cement and plastic substratum when exposed to the sub-lethal concentrations ($LC_{25\&}$ LC_{50}) of each of the two molluscicides compared to the snails maintained in mud and the exposed control groups. These present results at the laboratory was confirmed with those obtained under the semifield experiments which showed that the percentage of mortality of *B. alexandrina* snails treated with the sublethal concentrations(LC_{25}) of both copper sulphate and niclosamide in canals lined with cement and plastic was highly significant higher than those snails in canals with mud substratum. These results agree with those of Ritchie (1973) and El-Said (2004) who investigated that niclosamide effectiveness was slightly reduced when tested in alluvial soil by adsorption on mud colloidal particles. El Said *et al.* (2009) stated that soil types of the water bodies substratum may play an important role in success of mollusciciding operations and may explain the failure in complete eradication of snails by molluscicides in some areas. This depression of the molluscicidal activity may be due to absorption and/or adsorption of some toxic constitute of the molluscicides on mud particles. They suggest that lining water bodies by suitable materials is important to improve the mollusciciding methods for snail control. Also, the present results are in line with those obtained by several authors (El-Deeb, 1986; El Said, 1987; El-Wakil, 2001; Abdel Hameed, 2003; Ahmed, 2003) who stated that the molluscicidal activity of some plant molluscicides decreased in the presence of mud particles.

Helisoma duryi (Planorbid snails) has been suggested as biological control agent against the snail intermediate hosts of schistosomiasis by several authors (Madsen, 1983; Jordan, 1985; Yousif *et al*., 1993; 1999; El-Sayed and Sharaf *et al*., 2001).

The effect of lining materials on both *B. alexandrina* and competitor snails *H. duryi* was studied in the present work by putting snails in cages and free ones in the aquaria. A highly significant reduction ($p<0.001$) was recorded in the survival rate of *B. alexandrina* compared to that of *H. duryi* in both of the aquaria with mud and $control_1$ groups either in the case of free snails or those in the cages. This may be attributed to the effect of the competitor snails *H. duryi* against *B. alexandrina*. In contrast, the survival rate of *H. duryi* showed a highly significant reduction compared to that of *B. alexandrina* in aquaria lined by plastic followed by those maintained in aquaria lined with cement after three weeks of experiment for both of free snails and those in cages. The results at the laboratory confirmed the present ones obtained at the semifield experiments which showed that with increasing the time of exposure of snails to lining materials (cement and plastic), the survival rate of both *B. alexandrina* and *H. duryi* decreased. Thus, the lining materials affected the two snail species in case when putting together or on *B. alexandrina* alone. In addition, when the two snail species maintained in canal with mud substratum, the survival rate of *B. alexandrina* snails (36%) after 4 weeks was significantly lower than those of *H. duryi* (78%) compared to the control *B. alexandrina* snails (92%). These observations may due to the effect of only biological control agent (*H. duryi*) against *B. alexandrina* in canal with mud. These results are in agreement with those obtained by Yousif *et al*. (1993) who reported that the range of distribution of *H. duryi*, a potential competitor to schistosomiasis snail vector in Egypt, was determined in the south of Nile Delta. They recorded that *Helisoma* was found in canals and drains coexisting in various degrees with other snail species including schistosomiasis vectors (*Bulinus truncatus* and *B. alexandrina*). The population denisity of *H. duryi* and associated *B. truncatus*, *B. alexandrina* and *Physa acuta* were studied for one year in three channels. Statistical analysis of results showed that *Helisoma* has a significantly negative correlation with schistosomiasis vectors in these channels. Many authors suggested the use of integrated control measures for controlling snail vectors (Pieterse, 1977; Lwambo and Moyo,1991; Slootweg *et al*., 1994; Allam,2000; Giovanelli *et al*., 2001; Mostafa *et al*., 2005; El-Deeb and Ismail, 2007). Snails habitat alteration and appropriate water management can reduce their breeding and refuge sites, where natural or introduced competitors and predators put further pressure on snail populations (Slootweg *et al*., 1994). The effect of the integrated control measures against *B. alexandrina* snails by using three methods of control: chemical (using LC_{25} concentrations of copper sulphate and niclosamide), biological (using *H. duryi* snails as a competitor agent) and environmental (by lining the aquaria and canals) was studied in the present work under laboratory and semifield conditions. Laboratory study indicated that the mortality percentage of *B. alexandrina* maintained in mud substratum with the competitor snails and molluscicides was significantly ($p<0.001$) lower (represented by

55% in case of both two molluscicides) than those exposed in the presence of lining materials cement and plastic which ranged between 92.5% and 100%, respectively.

This was supported by the results of the semifield experiments. It was observed that the mortality percentage of *B. alexandrina* snails after exposing to the molluscicide (copper sulphate as a chemical control agent) and *H. duryi* snails (as biological control agent) under the lining materials, cement and plastic (as mean of environmental control) was higher than those snails maintained in canals with mud substratum without lining. This represented by 68% mortality for snails in canals with mud compared to 100% for those snails maintained in canals lined with cement and plastic. Thus, the lining of canals is efficient for integrating the control measure and act as an environmental modification for snails control. It may be seen as part of an integrated approach where the using of the sublethal concentrations of molluscicides can decreased the survival rate of snails and where the biological control agents put an additional effect on their survival. These present results are in agreement with those obtained by El-Deeb and Ismail (2007) who studied the effect of the biological and chemical control methods of the snails *B. alexandrina*, *Planorbis planorbis, Physa acuta* and *Lymnaea natalensis* under laboratory and semi field conditions. They stated that the total number of harmed snails due to application of plant or chemical molluscicides followed by adding fish was significantly higher than the number of harmed or consumed snails exposed to any of these methods of control alone.

Also, Mostafa *et al.* (2005) stated that the plant molluscicide *Calendula micrantha* and the chemical molluscicides niclosamide and copper sulphate at a concentration lethal to *B. alexandrina* snails was ineffective for the non-target snails *Melanoides tuberculata* and *H. duryi* which serve as bio-control agents against *B. alexandrina*. They suggested that these molluscicides can be used together with the non-target snails in a combined effect for controlling *B. alexandrina*. Similarly, Giovanelli *et al*. (2001) found that the LC_{90} of the latex of *Euphorbia seplendens hislopii* plant against *M. tuberculata* snails was 13.8 times greater than that for *B. glabrata* (the intermediate host snails of *S. mansoni* in Brasil). They added that the use of such plant in the presence of *M. tuberculata* snails has a synergic effect on reduction of *B. glabrata*. Also, the present data confirmed those of Allam (2000) who evaluated different means of *B. alexandrina* snails control e.g. chemical (Bayluscide), biological (plant molluscicides: *Ambrosia maritima* or Damsissa and *Azolla pinnata*), and physical (clearing of vegetations). The best results for control of *B. alexandrina* snails was obtained by clearing vegetation together with application of Damsissa.

Moreover, Lwambo and Moyo (1991) suggested that the application of ground seed pods of *Swartizia madagascariensis* (at a concentration inimical to kill snail´s hosts of schistosomiasis) in a water course inhabitants with *Marisa carnuarietis* snails (a competitor and /or predator of snails bearing *Schistosome*) may not adversely affect the competitor snails. Thus, they recommended the use of such application as a combined method of control against snail hosts. Pieterse (1977) stated that the use of biological agents in an integrated control program that includes the combination of biological methods with mechanical and chemical means of control is well known in many fields of research. It could be concluded that cement and plastic lining improve and increase the efficacy of the molluscicides against snails. Thus, the use of integrated control of snails including lining of canals is more effective for control of *B. alexandrina* snails.

ACKNOWLEDGMENT

The authors heartily dedicated this work to the late Dr. Mohamed Nagy Mohamed Mosad Professor of Physiology, Zoology Department, Faculty of Science, Benha University. The authors thanks all staff of the Environmental Researches Laboatory, Theodor Bilharz Research Institute, Egypt. Funding: This work was supported by the Environmental Researches Department, Theodor Bilharz Research Institute, Egypt in the frame of project 84M.

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ARABIC SUMMARY

تأثير تبطين القنوات المائية على فاعلية مبيدات القواقع ضد قواقع *Biomphalaria alexandrina* مع الاشارة الى طرق المكافحة المتكاملة للقواقع

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تمت دراسة تأثير مواد التبطين على فاعلية المبيدات نيكلوساميد وكبريتات النحاس على القواقع *Biomphalaria alexandrina* تحت ظروف المعملية وشبه الحقلية. أظهر معدل البقاء لقواقع *Biomphalaria alexandrina* في كل من الاحواض المائية المبطنة بالإسمنت والبلاستيك والمعرضة للتركيز تحت المميت (يقتل ٥٠% من القواقع : LC_{50}) للنيكلوساميد انخفاضًا كبيرًا (p<0.001) ممثلاً بنسبة ٥٪ و ٠.٠ ، على التوالي مقارنةً بتلك التى في تربة طينية والمجموعة الضابطة (٥٠ ٪ و ٤٠ ٪ ، على التوالي). كما أظهرت القواقع الموجودة في احواض مبطنة بكل من طبقة الأسمنت والبلاستيك والمعرضة لكبريتات النحاس (LC_{50}) وفيات بنسبة ١٠٠ ٪ مقارنة مع تلك الموضوعة فى احواض طينية والمجموعة الضابطة (٦٥ ٪ و ٥٠ ٪ ، على التوالي). وقد أظهرت التجربة الشبه الحقلية نفوقًا للقواقع بنسبة ١٠٠٪ و ٩٦٪ في القنوات المبطنة بالإسمنت والبلاستيك ، على التوالي بعد تعريضها لكبريتات النحاس (LC_{25}) مقارنة بـ ٣٨٪ للقواقع في القناة الطينية. وسجلت وفيات القواقع فى المجموعة الضابطة (القواقع دون المبيدات) بنسبة ٢ ٪ ، ٥٢ ٪ و ٤٨ ٪ في القنوات الطينية والمبطنة اسمنت وبلاستيك، على التوالي. باستخدام التركيز تحت المميت (يقتل ٢٥% من القواقع : LC_{25}) للنيكلوساميد ، كانت هناك زيادة معنوية عالية في وفيات القواقع في كل من القنوات المبطنة بالإسمنت والبلاستيك (١٠٠ ٪) مقارنة مع ٤٠ ٪ في القواقع الموجودة في القنوات الطينة (p<0.001).تم تسجيل انخفاض معنوىكبير (p<0.001) في معدل البقاء لقواقع *Biomphalaria alexandrina* الموضوعة مع القواقع المنافسة هيلوسوما ديورای في كل من الاحواض المائية الطينية والمجموعة الضابطة إما في حالة القواقع الحرة (٤٠ ٪ و ٥٣.٣ ٪ ، على التوالي) أو تلك الموجودة في أقفاص (٥٣.٣ ٪ و ٤٦.٧ ٪ على التوالي) مقارنة مع قواقع *Helisoma duryi* (٨٠ ٪ و ٧٣.٣ ٪ للقواقع الحرة وتلك الموجودة في أقفاص ، على التوالي). وقد لوحظ نفس النمط في التجربة الشبه حقلية. وقد أوضحت الدراسة المعملية دور التبطين في المكافحة المتكاملة لقواقع *Biomphalaria alexandrina* ، حيث سجلت تلك الموجودة في الاحواض الطينية مع القواقع المنافسة والمبيدات المستخدمة انخفاضا معنويا كبيرًا (p<0.001) في وفيات القواقع بنسبة ٥٥٪ في حالة اى من المبيدين مقارنة لتلك القواقع المعرضة في الاحواض المبطنة بالأسمنت والبلاستيك التى سجلت ٩٢.٥ ٪ و ١٠٠ ٪ ، على التوالي. بالإضافة إلى ذلك فقد لوحظ فى التجربة الشبه حقلية وفاة ١٠٠٪ من قواقع *Biomphalaria alexandrina* بعد تعريضها لكل من كبريتات النحاس وقواقع هيلوسوما ديوراى في كل من القنوات المبطنة بالإسمنت والبلاستيك مقارنة مع تلك الموجودة في القنوات الطينية (٦٨٪). يمكن أن نخلص إلى أن تبطين القنوات المائية بالأسمنت والبلاستيك ممكن ان تحسن وتزيد من فعالية المبيدات ضد القواقع. وبالتالي ، فإن استخدام المكافحة المتكاملة للقواقع بما في ذلك تبطين القنوات المائية يكون أكثر فعالية للحد من قواقع *Biomphalaria alexandrina*.