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Semi-field and field trials to control *Biomphalaria alexandrina* and *Bulinus truncatus*, intermediate host snails of schistosomiasis in Egypt by the plant molluscicide Luowei/TDS 4%

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ABSTRACT

Controlling the snail intermediate hosts of schistosomiasis is an efficient and rapid method for reducing or eliminating the transmission of this disease. The present study aimed to assess the molluscicidal activity of Luowei/TDS 4% against *Biomphalaria alexandrina* and *Bulinus truncatus* under semi-field and field conditions in Egypt. Moreover, its effect on *Schistosoma mansoni* miracidial viability and infectivity to *B. alexandrina* snails and on cercarial production from infected snails were evaluated. In the present study, miracidia of *Schistosoma mansoni* and *S. haematobium* were exposed to sublethal concentrations of Luowi/TDS 4% and miracidial mortality rates in the test and control groups were recorded. The effect of exposure to LC_{10} and LC_{25} of Luowei/TDS 4% after 1, 2 and 3 weeks of *B. alexandrina* infection with miracidia on their cercarial production was determined. The molluscicidal activity of $2LC_{90}$ Luowei/TDS 4% against *B. alexandrina* and *B. truncatus* was tested under semi-field and field conditions. Luowei/TDS 4% exhibited a promising molluscicidal potency against *B. alexandrina* and *B. truncatus* as their LC_{90} values were considerably low, 2.851 and 1.936 mg/L, respectively, after 24 h of exposure. Moreover, infection rates of *B. alexandrina* with *S. mansoni* and cercarial production from snails exposed to LC_{10} and LC_{25} of Luowei/TDS 4% post miracidial exposure were reduced. Moreover, Luowei/TDS 4% at semi-field and field trials proved to be a potent molluscicidal agent against schistosomiasis intermediate host snails as mortality rates of free and caged sentinel snails from these trials were considerably high ranging from 87% to 100% after 24 h of treatment with 5.702 mg/L ($2LC_{90}$ for *B. alexandrina* snails). Luowei/TDS 4% should be considered as a candidate molluscicide in schistosomiasis control programs. Implementation of this plant molluscicide in operational schistosomiasis control strategies will minimize the ecological side-effects associated with using synesthetic chemicals.

INTRODUCTION

Schistosomiasis is one of the major communicable diseases with socio-economic and health importance in the developing world **(King *et al.*, 2015)**. Improvement of agricultural schemes has created new breeding sites of snails and consequently potential foci for schistosomiasis transmission **(WHO, 2017)**.

The absence of an effective and safe vaccine for schistosomiasis and the appearance of some *S. mansoni* batches resistant to the antischistosomal drug praziquantel support snail control programs **(Hamed, 2010)**. In this concept, molluscicides either synthetic or of plant origin are effective and rapid method for snail control. Molluscicides of plant origin appear to be environment friendly with several advantages over the synthetic ones **(El-Ansary, 2001; Mantawy and Mahmoud, 2002; Mahmoud *et al.,* 2011)**.

In Egypt, several plant species were screened for molluscicidial activity against *B. alexandrina* and *B. truncatus*, the intermediate hosts of *S. mansoni* and *S. haematobium*, respectively. Only, two of them; *Anagallis arvensis* and *Ambrosia maritima* were evaluated under semi-field conditions showing a considerable activity against different snail species **(Belot *et al.*, 1991; El-Emam *et al.*, 1996)**. Their molluscicidal properties are due to sesquiterpenes in *Artimisia maritima* **(Geerts *et al.*,1991)** and saponins in *Anagallis arvensis* **(Abd El-Gawad *et al.*, 2000)**.

Recently, in China field trials proved that the compound Luowei/TDS 4% is promising in controlling the amphibious snail *Oncomelania hupensis*, the intermediate host of *S. japonicum* **(Jia *et al.*, 2013; Zhang *et al.*, 2013)**. The authors reported that in immersion trials 100% death of snails was recorded after 2 h of exposure to 3 g/m^3, while 90.3% death was observed after 15 days of spraying with 5 g/m^2 of this compound. In natural water bodies it is easily degraded to non-toxic carbohydrates and saponins **(Jia *et al.*, 2013)**.

Preliminary bioassay tests of TDS 4% plant-derived molluscicide against *B. alexandrina* and *B. truncatus* snails revealed that it has a promising molluscicidal activity with LC_{50} values of 1.975 and 1.396 mg/L, respectively after 24 h of exposure **(Jia *et al.*, 2019)**. Therefore, the present study was planned to evaluate the toxicity of the plant molluscicide Luowei/TDS 4% to *B. alexandrina* and *B. truncatus* under semi-field and field conditions in Egypt. In addition, its effect on infection of *B. alexandrina* with *S. mansoni* was determined in the laboratory.

MATERIALS AND METHODS

SNAILS

Biomphalaria alexandrina and *Bulinus truncatus* were collected from irrigation canals in Giza governorate, Egypt, transferred to laboratory, washed and examined for natural trematode infections. Healthy non-infected snails were maintained at Medical Malacology Department, Theodor Bilharz Research Institute, 10-20 snail/L dechlorinated water (24±1 ºC), for at least 3 weeks before using in the tests. They were fed oven dried lettuce leaves and dead snails were removed daily and water was changed weekly.

SCHISTOSOMA MIRACIDIA

Fresh hatched *Schistosoma mansoni* and *S. haematobium* miracidia used were from Schistosome Biological Supply Center, Theodor Bilharz Research Institute.

ETHICAL STATEMENT

The study followed all the ethical guidelines of Theodor Bilharz Research Institute, Egypt.

LUOWEI/TDS 4%

TDS 4% was extracted from the tea seeds pomace of the plant *Camelia oleifera,* that left after commercial pressing of seeds for tea oil, by Hubei Jinhaichao Science and Technology Company, Ltd, Hubei Provence, P. R. China (Liu *et al.*, 2007; Yuan *et al.,* 2008). A full description of the compound and its structural formula are available in Jia *et al.* (2019).

TOXICITY TO *SCHISTOSOMA* MIRACIDIA

The selected concentrations for *S. mansoni* miracidia were 1.369, 1.628 and 1.975 mg/L corresponding to LC_{10}, LC_{25} and LC_{50} of *B. alexandrina* snails. Those for *S. haematobium* were 1.006, 1.175 and 1.394 mg/L (LC_{10}, LC_{25} and LC_{50} of *B. truncatus*). Five ml of water containing 30 fresh hatched miracidia were mixed in a divided Petri dish with 5 ml of double concentration from each of the selected concentrations. Another 10 ml dechlorinated tap water containing 30 fresh hatched miracidia was kept as control (Mohamed *et al.,* 2012). After intervals of ¼, ½, ¾, 1 and 2 h, alterations in miracidial mobility were observed under a dissecting microscope. Stationary miracidia were considered dead and mortality rates in the test and control groups were recorded (Mahmoud *et al.*, 2011).

EFFECT ON INFECTION OF *B. ALEXANDRINA* WITH *S. MANSONI*

B. alexandrina snails (5-7 mm) were exposed to *S. mansoni* miracidia in mass for 24 h under illumination. Then, they were transferred and maintained in clean dechlorinated water (24±1 ºC). After 1, 2 and 3 weeks of snails exposure to miracidia, survived snails were exposed for 24 h to LC_{10} (1.369 mg/L) and LC_{25} (1.628 mg/L) of Luowei (TDS 4%), then they were re-maintained in clean water up to shedding cercariae. They were daily fed oven dried lettuce leaves and dead snails were removed. Water was changed weekly and surviving snails were individually examined once weekly for cercarial shedding 24 days post miracidial exposure. Then cercarial production/infected snail was recorded (Bakry *et al.,* 2017).

TOXICITY TO SNAILS UNDER SEMI-FIELD AND FIELD CONDITIONS

Semi-field trials

Two semi-field trials were carried out at the Snail Research Station of Medical Malacology Department in Egypt, to evaluate the potency of Luowei/TDS 4% against the two snail species under natural controlled aquatic ecosystem (Mostafa *et al.*, 2005). The Snail Research Station is composed of 18 parallel ditches, each of 30 m length and 1.85 cm width at water surface.

The first trial in muddy ditches (LC_{90} and $2LC_{90}$)

Five ditches in the Snail Research Station were provided with underground water. One section (10 m in length) from each ditch was used for this experiment. The 1st and 2nd sections (3500 and 3810 L, respectively) were for testing the effect of *B. alexandrina* snails LC_{90} (2.851 mg/L) and $2LC_{90}$ (5.702 mg/L), respectively. The 3rd and 4th (3700 and 4600 L, respectively) were for *B. truncatus* snails (LC_{90}= 1.936 mg/L & $2LC_{90}$= 3.872 mg/L, respectively). The 5th (3300 L) was for control snails of both species. In each section, 3 replicates were prepared, each of a large plastic net (2 m width and 4 m length) which was quiescence fixed onto the bottom and sides of the ditch/section. Thereafter, 400 snails were introduced in each replicate. In addition, another 3 replicates of cylindrical plastic cages (15 cm length) were introduced into each ditch/section with 20 sentinel snails each. Exposure and recovery periods were 24 h each. Water temperature ranged 20 to 28 ºC, pH 7.1 to 8.6 and conductivity was from 300 to 750 µS.

The second trial in muddy and cement lined ditches ($2LC_{90}$)

This experiment was done to evaluate the lethal effect of $2LC_{90}$ concentration on *B. alexandrina* and *B. truncatus* in muddy and cement lined ditches. Four ditches were used, two muddy and two lined with cement (sides and bottom). These ditches were provided with underground water. One of the muddy ditches (12200 L) was for testing the compound Luowei/TDs 4%, and the other (11900 L) served as a control. Also, one of the cement lined ditches (12000 L) was for the test and the other (1390 L) was used as a control. In each ditch a large plastic net with 400 snails (as in the 1st trial) was introduced. In addition, 1500 *B. alexandrina* were added as free snails all over the length of the ditch (30 m). After 24 h of exposure the snails of the plastic nets were collected and samples of free snails were collected by dip net all over the length of the ditch (a fixed number of dip nets; 20), then they were transferred to clean underground water for 24 h of recovery before calculating the death rates. Regarding *B. truncatus* snails, the same technique was followed in muddy and cement-lined ditches. For each ditch 1000 snails were introduced as free snails and 400 snails were added in to each plastic net/ditch. Snails in the control ditches (free and net groups) were prepared in parallel to treated snails.

Field trials ($2LC_{90}$)

Two field trials were done in Egypt in natural irrigation canals during November 2016 for the assessment of Luowei/TDS 4% (5.702 mg/L) as a molluscicidal agent under field conditions. The 1st trial was done in 343.75 m^3 of Saadan secondary canal, Tanta, Al-Gharbia Governorate. The 2nd trial was carried out in 370.5 m^3 of Wahet Boush secondary canal, Nasser, Beni Swef Governorate. The selected areas (50 m/canal) were surveyed pre-treatment for snail species. Caged sentinel *B. alexandrina* were introduced into each of the selected areas, 10 cages each of 20 snails. After treatment (exposure 24 h) the areas were surveyed for snails by dip-net, the collected samples of snail species were washed, sorted, counted and mortality rates were recorded.

STATISTICAL ANALYSIS

The data were statistically analyzed for the significance of differences between treated and control group by using student "*t*" test and values were expressed as means ± S.D. or means ± S.E. A chi-square test was used to examine the differences in mortality between groups.

RESULTS

1. Miracidicidal activity

Exposure of *S. mansoni* and *S. haematobium* miracidia to three sublethal concentrations of Luowei/TDS 4% that represent the values of LC_{10}, LC_{25} and LC_{50} to *B. alexandrina* and *B. truncatus*, respectively, caused a gradual increase in the mortality rates of *S. mansoni* and *S. haematobium* miracidia in a time and concentration dependent manner (Table 1). Thus, *S. mansoni* miracidia exposed to 1.628 mg/L (LC_{25} for *B. alexandrina*) suffered from 50% and 80% death after 30 min and an hour, respectively. Similarly, 50% death of *S. haematobium* miracidia was seen after 30 min of exposure to 1.006 mg/L (LC_{10} for *B. truncatus*), while no miracidia were alive by elongating the exposure period to an hour (100% death). Also, the mortality rates of miracidia were increased within the same exposure period by raising the concentration. So, after 30 min mortality rate of *S. mansoni* miracidia was increased from 40% to 66% by raising the concentration from 1.628 mg/L to 1.975 mg/L (LC_{50}), compared to 0% mortality in control group. It is worth mentioning that survived miracidia of both *Schistosoma* species showed slow movement and less viability after 45 min of exposure to the tested concentrations.

Table 1. *Schistosoma* miracidial mortality % post exposure to Luowei/TDS 4%.

Exposure time	***Schistosoma* species/ Concentrations (mg/L)**							
	S. *mansoni*				***S. haematobium***			
	Control	**LC_{10} (1.369)**	**LC_{25} (1.628)**	**LC_{50} (1.975)**	**Control**	**LC_{10} (1.006)**	**LC_{25} (1.175)**	**LC_{50} (1.396)**
15 min	0	0	26.7	40	0	13.3	10	26.7
30 min	0	40	50	66	0	50	43.3	63.3
45 min**	0	60	73.3	73.3	0	63.3	66.7	86.7
1 h	6.7	80	80	86.7	10	100	100	100
2 h	6.7	100	100	100	-	-	-	-

** Living miracidia move very slow

2. Effect on infection of *B. alexandrina* with *S. mansoni* and cercarial production

During molluscicidal application in natural water courses, some snails may be exposed to sub-lethal concentrations of the molluscicide due to their presence in specific and separate spots (sites). In addition, other treated snail populations may be in the prepatent period of the parasite (i.e. snails exposed to miracidia and the parasite is developing within snails' tissues) or in the patent period (i.e. snails shedding cercariae). Therefore, the following tests were carried out to assess the effect of sublethal concentrations from Luowei/TDS 4% on cercarial production from *B. alexandrina* snails exposed to *S. mansoni* miracidia.

From Table (2), the survival rates of snails exposed to *S. mansoni* miracidia and thereafter re-exposed to Luowei/TDS 4% at 1-week, 2-weeks, and 3-weeks post miracidial exposure (PME) were significantly less than that of control group. The

survival rate of snails exposed to 1.369 mg/L after two weeks of miracidial exposure was 48% compared to 88% for control group ($p<0.001$). It was also noticed that survival rate exhibited more reduction by increasing the tested concentration from 1.369 mg/L to 1.628 mg/L. So, at 1-week PME it was reduced from 60% to 32% for snail groups exposed to these concentrations, respectively.

Regarding the infection rate of *B. alexandrina,* although most of the survived snails in the treated groups shed cercariae, yet their infection rates were generally less than that of control groups. The infection rate was 63% for each of the snail groups exposed to 1.628 mg/L at 1- and 3-weeks PME compared to 86% for control snails ($p<0.001$).

The prepatent period was 33.3 days for control snails which is approximately similar to those of snail groups treated with the molluscicide at one and two-weeks post miracidial exposure (Table 2). However, it was elongated to 38.7 days for snails exposed to 1.369 mg/L at 3-weeks PME compared to 33.3 days control group ($P<0.05$).

Table 2. Survival rate, infection rate and cercarial production from *Biomphalaria alexandrina* exposed to sublethal concentrations (LC_{10} and LC_{25}) of the molluscicide Luowei/TDS 4% post exposure to *Schistosoma mansoni* Miracidia.

Experimental group	Conc. (mg/L)	Survival rate (%)	Infection rate (%)	Prepatent period (days) Mean±SD	Patent period (days) Mean±SD	Cercariae/snail Mean±SE
Control	0.0	88	86	33.3±4.88	19.16±6.53	1231.11± 168.46
One-week	LC_{10} (1.369)	60***	73*	31.27±2.83	17.82±3.66	521.82±86.80***
post miracidial exposure	LC_{25} (1.628)	32***	63***	31.40±3.13	14.0±0.0**	388.40±50.19***
Control	0.0	85	80	30.3±3.58	21.10±4.43	1451.21± 108.70
Two-weeks	LC_{10} (1.369)	48***	83	33.50±3.69	17.50±8.89	1286.29±406.76
post miracidial exposure	LC_{25} (1.628)	28***	71*	34.20±3.83	15.40±9.13*	1012.20±481.76**
Control	0.0	86	85	26.90±5.02	19.09±7.03	1732.43± 98.94
Three-weeks	LC_{10} (1.369)	44***	73*	38.75±6.20*	12.25±4.95***	358.25±117.62***
post miracidial exposure	LC_{25} (1.628)	32***	63***	37.0±0.0**	22.40±5.86	1664.80±561.16

* & *** = Significantly different compared to control at $P<0.05$ & $P<0.001$, respectively

Cercarial production/snail for snail groups treated at 1-week PME was significantly less than that of control group (Table 2 and Fig. 1). It recorded 388.4 cercariae/snail for snail group exposed to 1.628 mg/L compared to 1231.11 cercariae/control snail ($P<0.001$). Similarly, this parameter was reduced to 358.25 cercariae/snail for snails at 1.369 mg/L three-weeks post miracidial exposure ($P<0.001$). However, snails exposed to 1.628 mg/L at the same period post miracidial exposure

exhibited higher cercarial production (1664.8 cercariae/snail) than that of control group but without significant differences (P>0.05).

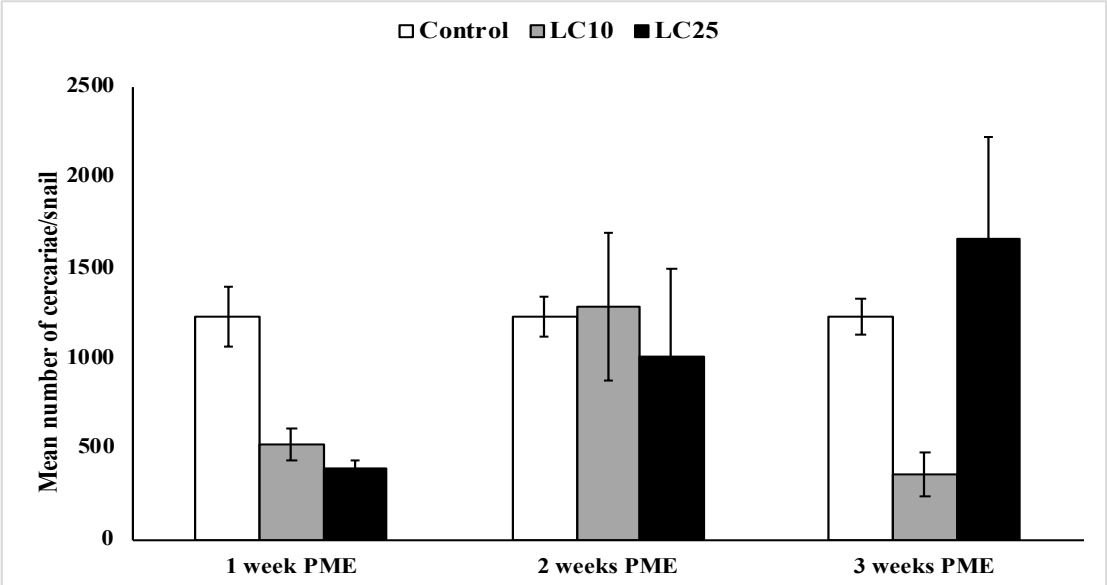

Fig. 1. Mean number of *Schistosoma mansoni* cercariae/*B. alexandrina* snail exposed to the molluscicide Luowei/TDS 4% following 1, 2- and 3-weeks post miracidial exposure (PME).

3. Semi-field trials

According to the promising results of laboratory tests for evaluating the toxic effect of Luowei/TDS 4% on *B. alexandrina* and *B. truncatus*, the semi-field trials were carried out in Snail Research Station as follows:

3.1. The first trial in muddy ditches (LC_{90} and $2LC_{90}$)

B. alexandrina exposed to LC_{90} (2.851 mg/L) in the net and cylindrical cages survived well with mortality rates of 7.3% and 16.7%, respectively. Meanwhile, those exposed to $2LC_{90}$ (5.702 mg/L) showed 97% mortality rate for snails in the net and 100% mortality for caged snails compared to 0.3% and 0% mortality rates in control groups, respectively. For *B. truncatus* snails, approximately similar observations were recorded (Table 3 and Fig. 2).

Table 3. Effect of the molluscicide Luowei/TDS 4% on *Biomphalaria alexandrina* and *Bulinus truncatus* at Snail Research Station (natural controlled aquatic ecosystem).

Snail species	Concentration (mg/L)	Mortality (%) M±SD	
		Net snails	Caged snails
Biomphalaria alexandrina	Control	0.3±0.2	0.0±0.0
	LC_{90} (2.851)	7.3±3.5*	16.7±5.8***
	$2LC_{90}$ (5.702)	97.0±1.0***	100.0±0.0***
Bulinus truncatus	Control	0.0±0.0	0.0±0.0
	LC_{90} (1.936)	8.9±2.2**	0.0±0.0
	$2LC_{90}$ (3.872)	99.3±1.3***	100.0±0.0***

*, ** & *** = Significantly different from control at P<0.05, P<0.01 & P<0.001, respectively

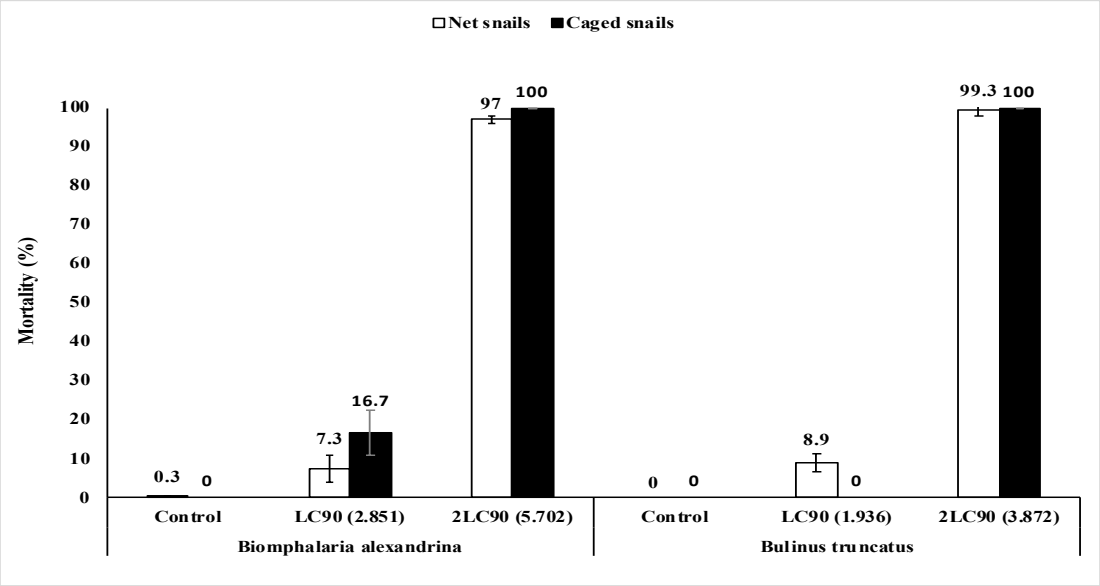

Fig. 2. Preliminary testing of Luowei/TDS 4% toxicity (LC_{90} & $2LC_{90}$ vs Control) to *B. alexandrina* and *B. truncatus* at Snail Research Station (natural controlled aquatic ecosystem).

3.2. The second trial in muddy and cement lined ditches ($2LC_{90}$)

Data in table (4) show that the morality rates of *B. alexandrina* exposed to $2LC_{90}$ (5.702 mg/L) in cement lined ditch were 96.9% and 99% for collected free snails and net snail groups, respectively, compared to 9.1% and 0% for control groups. Similarly, snails exposed in muddy ditch recorded high mortality rates of 98.4% and 94.2% for free collected and net snail groups, respectively. *B. truncatus* exposed to $2LC_{90}$ (3.872 mg/L) in muddy and cement lined ditches, no snails were alive (100% death) among collected free snails and net snail groups compared to 7.2% and 4.2% mortality for control groups, respectively (Table 4 and Fig. 3).

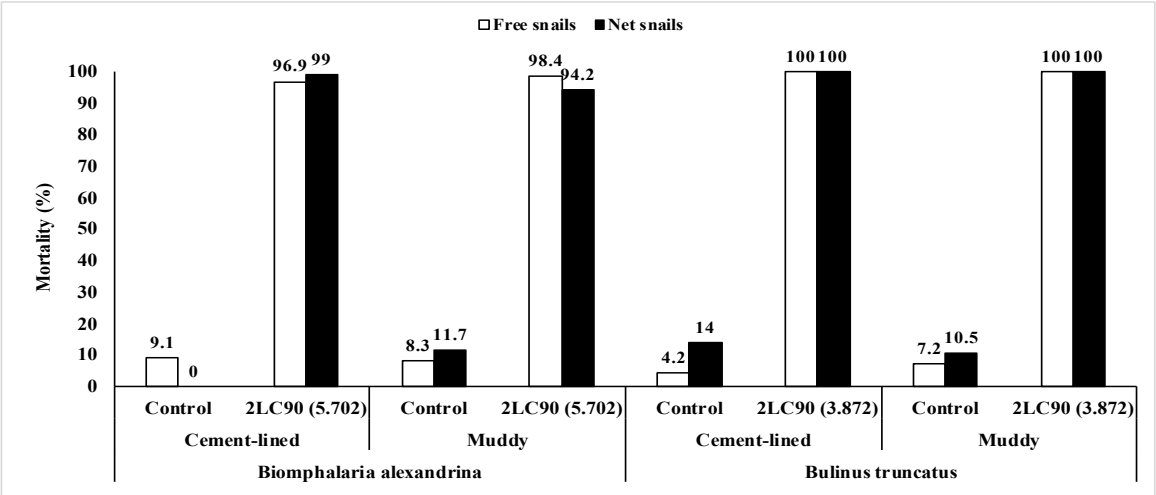

Fig. 3. Effect of $2LC_{90}$ from the molluscicide Luowei/TDS 4% on *Biomphalaria alexandrina* and *Bulinus truncatus* in cement-lined and muddy ditches at Snail Research Station (natural controlled aquatic ecosystem).

Table 4. Mortality of *Biomphalaria alexandrina* and *Bulinus truncatus* post exposure to the molluscicide Luowei/TDS 4% at Snail Research Station in cement lined and muddy ditches (natural controlled aquatic ecosystem)

Snail species	Concentration	Type of ditch	Mortality (%)	
			Net snails	Caged snails
Biomphalaria alexandrina	Control	Cement-lined	9.1	0.0
		Muddy	8.3	11.7
	$2LC_{90}$ (5.702)	Cement-lined	96.9	99.0***
		Muddy	98.4	94.2***
Bulinus truncatus	Control	Cement-lined	4.2	14.0
		Muddy	7.2	10.5
	$2LC_{90}$ (3.872)	Cement-lined	100.0***	100.0***
		Muddy	100.0***	100.0***

*** = highly significant (P<0.001)

4. Field trials ($2LC_{90}$)

snails survey conducted in the selected canals before TDS 4% application revealed the presence of five snail species in Saadan secondary canal, Tanta, Al-Gharbia Governorate namely; *B. alexandrina, Planorbis planorbis, Physa acuta, Lymnaea natalensis* and *Lanistes carinatus*, in association with the water weeds, *Eichhornia crassipes, Lemna gibba* and *Echinochloa stagnina.* While, in Wahet Boush secondary canal, Nasser, Beni Swef Governorate two snail species were found, *B. alexandrina* and *L. natalensis* associated with the water weeds *E. crassipes* and *E. stagnina* (Table 5).

Table 5. Molluscicidal potency of Luowei/TDS 4% under field conditions in irrigation canals in Al-Gharbia and Beni Swef governorates, Egypt during November 2016

Location:	**Al-Gharbia Governorate**	**Beni Swef Governorate**
Village, district	Kafr-Dawood, Tanta	Boush, Naser
Water course description		
Type:	**Saadan secondary canal**	**Wahet Boush secondary canal**
Length (m)	50	50
Mean width at water surface (m)	5.50	5.70
Mean depth (m)	1.25	1.30
Water temperature (°C)	23.6	23.5
Water pH	7.7	7.5
Water conductivity (µS)	0.44	0.48
Total dissolved solids (ppt)	0.22	0.25

Water volume (m^3)	343.75	370.50
Weight of 4%TDS used (kg)	1.836	1.979
Aquatic weeds:		
Eichhornia crassipes	++	+++
Lemna gibba	++	+
Echinochloa stagnina	+++	+++
Number of snail species collected pre-treatment (100 dips by dip net):		
Biomphalaria alexandrina	285	549
Planorbis planorbis	32	-
Lymnaea natalensis	27	12
Physa acuta	49	-
Lanistes carinatus	12	-
Number of caged *B. alexandrina* introduced (10 cages, 20 snails each)	200	200
Number (n/N) and mortality (%) of snail species collected post-treatment:		
Biomphalaria alexandrina	193/193 (100%)	530/612 (86.6%)
Planorbis planorbis	21/21 (100%)	-
Lymnaea natalensis	13/13 (100%)	9/9 (100%)
Physa acuta	28/28 (100%)	-
Lanistes carinatus	7/7 (100%)	-
Caged *B. alexandrina*	200/200 (100%)	198/200 (99%)

Following 24 h of TDS 4% treatment, the treated area from each selected canal was re-surveyed for snails. It was found that in the first trial in Saadan secondary canal, Al-Gharbia Governorate, the collected snail species from the treated area were dead as well as caged sentinel snails (100% death). In the second trial, Wahet Boush secondary canal, Beni Swef Governorate, it was observed that the water level in the selected area was higher post-treatment than that recorded pre-treatment due to introduction of water from the main canal to the trial canal according to the governmental systematic irrigation cycle in this village. Accordingly, the treated area was infested with snails from untreated upstream area. However, the mortality rates of samples collected from free snail in the treated area were 86.6% for *B. alexandrina* and 100% for *L. natalensis.* Meanwhile, 99% mortality of caged *B. alexandrina* snails was recorded which means that cages were not infested with snails from untreated upstream area.

DISCUSSION

Although chemotherapy is one of the most valuable methods for the control of schistosomiasis, there is still a need for more selective and efficient molluscicides for control the snail intermediate hosts of this parasite **(WHO, 2010)**. In this concept, the

compound Luowei/TDS 4% (pentacyclic triterpenoid saponin), which was extracted from the plant *C. oleifera* exhibited a promising molluscicidal potency against *B. alexandrina and B. truncatus*, the snail intermediate hosts of schistosomiasis in Egypt. This is coincided with the biological and pharmacological properties of the crude saponins extracted from the seeds of the plant *C. oleifera* **(Chen *et al.*, 2010)**. It was previously stated that the high molluscicidal activity of Endod was due to the presence of monodesmosidic saponin with an oleanolic acid glucoside base in the pericarp of immature fruit of the plant *Phytolacca dodecandra* **(Lemma, 1983; Ali, 2010)**. Similarly, the high concentration of saponins and flavonoids in the plants *S. sesban* **(Mahmoud *et al.*, 2011)**, *E. splendens* **(Bakry, 2009)** and *Cestrum purpureum* and *Yucca filamentosa marginata* **(Hamed *et al.*, 2015a,b)** were beyond their remarkable toxicity to *B. alexandrina*. The high toxicity of saponins to treated organisms could be due to their properties as protease inhibitors and interacting with cholesterol forming insoluble substances that alter cells' activities, causing cytotoxicity and death of treated organism **(Chaieb, 2010)**.

The present mortality rates of *S. mansoni* and *S. haematobium* miracidia were time and concentration dependent. This agrees with **Ibrahim *et al.* (2004)** who found that water suspension from the dry powder of the plants *D. kerchoveana, S. nigrum* and *P. repens* killed *S. mansoni* miracidia within few hours. **Abdel Kader *et al.* (2005)** recorded that low concentrations of molluscicides (synthetic or of plant origin) caused 100% death of *S. mansoni* miracidia after 6 h of exposure. However, **Mahmoud *et al.* (2011)** concluded that *S. mansoni* miracidia were dead post 90 min of exposure to snails LC_{90} (62.44 ppm) of the plant *S. sesban*.

The current data revealed that survival rates of *B. alexandrina* snails at 1st shedding post 24 h of exposure to Luowie/TDS 4% were less than their corresponding control groups. Similar conclusion was observed by **Massoud *et al.* (1973)** on *B. truncatus* snails exposed to Bayluscide and infected with *S. haematobium* and **Mahmoud *et al.* (2011)** on *B. alexandrina* infected with *S. mansoni* and exposed to the dry powder of the plants *D. stramonium, and S. sesban.* However, **Hira and Webbe (1972)** recorded *B. glabrata* snails treated with triphenyl lead acetate post 20 days of exposure to *S. mansoni* miracidia, had a similar mortality at 1st shedding as control group. Also, a significant decrement was recorded for *S. mansoni* infection rates and cercarial output from *B. alexandrina* snails treated with Luowei/TDS 4% post miracidial exposure. This may be due to snails' exposure to the molluscicide at the 1st week post miracidial exposure raised the harmful stress and injury to snails' cells occurred during miracidial penetration of their skin leading to high mortality rates during the prepatent period, reducing infection rates and cercarial production of infected treated snails. Moreover, suppression of cercarial output from snails treated with Luowei/TDS 4% could be due to its accumulation in the snails' head-foot region especially for those treated at the 1st week post miracidial exposure where mother sporocysts are still present **(Malek, 1980)**, hence their subsequent developmental stages probably deteriorated leading to a decrement in cercarial production and shortening the patent period (shedding period) of infected snails. This was recorded by **Mahmoud *et al.* (2011)** on *S. mansoni* cercarial production from *B. alexandrina* snails treated with the dry powder of the plants *D. stramonium and S. sesban* post miracidial exposure. Similarly, **Gawish (2008), Bakry (2009),** and **Rizk *et al.* (2012)** stated that exposure of *B. alexandrina* snails to the plants *Syzygium jambos,*

Euphorbia splendens, Atriplex stylosa, and H. tuberculatum has an obvious negative effect on duration of *S. mansoni* cercarial shedding and cercarial production/infected snail.

The importance of systematic semi-field and field trials of promising molluscicides post laboratory and toxicity tests was recommended by several authors. In this concept, the acute toxicity of Luowei/TDS 4% to mammals and birds is very low and it has no mutagenic activity according to the methods of **Chinese Ministry of Agriculture (1995)** and **National Environmental Protection Agency (1989)**. However, it is lethal to fish as the other currently molluscicides either synthetic e.g. Niclosamide **(Zinada, 2000; Bellete, 2015)** or of plant origin e.g. Endod **(Lemma, 1983)** and *Guaiacum officinale* **(Mendes *et al.*, 1993)**. To overcome the possible antagonistic effects of biotic and abiotic factors in snails' natural habitats against the activity of molluscicides during semi-field and field trials higher concentrations than those recorded in laboratory tests are required. This was reported by **Mendes *et al.* (1993)** that although LC_{90} of the plant *G. officinale* against *B. glabrata* was 15 ppm in laboratory tests, it was necessary to use 40 ppm to get 100% death of snails in field trials. Accordingly, the present work evaluated the high concentration $2LC_{90}$/24 h of Luowei/TDS 4% against *B. alexandrina* and *B. truncatus* snails in semi-field and field trials in Egypt and the results were promising, 87%-100% mortalities of free and caged sentinel snails under semi-field conditions, in addition to 100% mortality of free and caged sentinel snails in the 1st field trial, Al-Gharbia Governorate. Meanwhile, for the 2nd field trial, although 99% and 100% mortality rates were recorded for caged sentinel *B. alexandrina* and free *L. natalensis* snails, respectively, only 86.6% mortality rate for free *B. alexandrina* snails was observed. This could be, mainly, due to introduction of untreated water from the main canal to the trial canal after treatment, according to the governmental irrigation cycle in this village. Consequently, the treated area was infested with snails from untreated upstream areas and this was supported by high death rate of caged sentinel snails which means no infestation occurred in these caged snails. Similar observations were recorded in semi-field trials using the plants *Fagonia arabica* **(El-Nahas *et al.*, 2003)** and *Calendula micrantha* and *Anagallis arvensis* **(Mostafa *et al.*, 2005)**. Also, pilot field trials were done using the plants *A. arvensis* against *B. alexandrina* in Egypt **(El-Emam *et al.*, 1996)**, *E. splendens* against *B. glabrata* in Brazil **(Schall *et al.*, 2001)** and *P. dodecandra* (Endod) against *B. pfeifferi* in Ethiopia **(Abebe *et al.*, 2005)**.

CONCLUSION

It is concluded from the foregoing data that Luowei/TDS 4% could be considered as a candidate molluscicide of plant origin. Its molluscicidal properties under laboratory, semi-field and field conditions are in harmony with WHO recommendations **(WHO, 1983)** on plant molluscicides. Besides its molluscicidal activity, reduction of *S. mansoni* cercarial production from infected snails was observed and toxicological tests proved it is safe to mammals and birds. Therefore, it should be considered in schistosomiasis control programs in order to sustain the impact of chemotherapy in an integrated control strategy of this parasitic disease.

REFERENCES

Abdel-Gawad, M.M.; El-Amin, S.M.; Ohigashi, H.; Watanabe, Y.; Takeda, N.; Sugiyama, H. and Kawanaka, M. (2000). Molluscicidal saponins from *Anagallis arvensis* against schistosome intermediate hosts. Japan J. Infect. Dis., 53(1): 17-19.

Abdel-Kader, A.; Hamdi, S.A. and Rawi, S.M. (2005). Biological and biochemical studies on *Biomphalaria alexandrina* snails, treated with low concentrations of certain molluscicides (Synthetic and of plant origin). J. Egypt. Soc. Parasitol., 35(3): 841-858.

Abebe, F.; Erko, B.; Gemetchu, T. and Gundersen, S.G. (2005). Control of *Biomphalaria pfeifferi* population and schistosomiasis transmission in Ethiopia using the soap berry endod (*Phytolacca dodecandra*), with special emphasis on application methods. Trans. R. Soc. Trop. Med. Hyg., 99(10): 787-794.

Ali, S.A. (2010). Natural products as therapeutic agents for schistosomiasis. Res. J. Med. Plant, 5: 1-9.

Bakry, F.A. (2009). Use of some plant extracts to control *Biomphalaria alexandrina* snails with emphasis on some biological effects. Pestic. Biochem. Physiol., 95: 159-165.

Bakry, F.A.; El-Garhy, M.; Abd El-Atti, M.S. and Atwa, M. (2017). Effects of extracts of *Euphorbia pulcherima* and *Atriplex nummularia* on the infection rate and the infectivity of *S. haematobium* to *B. truncatus* snails. Adv. Biomechanics, 1(2): 34-41.

Belot, J.; Bornarel, P.; Diouf, M. and Polderman, A. (1991). *Ambrosia maritima* L: molluscicidal effects on the local snails *Lymnaea natalensis*, *Bulinus forskalii*, *Bulinus globosus* and *Biomphalaria pfeifferi* from Senegal. Plant Sci., 74(2): 167-170.

Chaieb, I. (2010). Saponins as insecticides: a review. Tunisian J. Plant. Protection, 5: 39-50.

Chala, B. and Torben, W. (2018). An Epidemiological Trend of Urogenital Schistosomiasis in Ethiopia. Front. Public Health, 6: 60.

Chen, Y.F.; Yang, C.H.; Chang, M.S.; Ciou, Y.P. and Huang, Y.C. (2010). Foam properties and detergent abilities of the saponins from *Camellia oleifera*. Int. J. Mol. Sci., 11(11): 4417-4425.

El-Ansary, A.; Mohamed, S.M. and Mohamed, A.M. (2001). Induced changes in energy metabolism of *Biomphalaria alexandrina* snails using two potent plant molluscicides. Bull. NRC Egypt, 26: 425-439.

El-Emam, M.A.; El-Amin, S.M.; El-Sayed, M.M. and El-Nahas H.A. (1996). Field trials to control the snail vectors of schistosomiasis and fascioliasis by the plant *Anagallis arvensis* (Primulaceae). Egypt J. Bilh., 18: 89-100.

El-Nahas, H.A.; El-Sayed, M.M.; El-Deeb, F.A. and El-Wakil, E.A. (2003). Laboratory and semi-field assessment of three wild Egyptian plants as molluscicidal agents. Egypt. J .Appl. Sci., 18(9):102-120.

Gawish, F.A. (2008). Activity of the plant *Syzygium jambos* against *Biomphalaria alexandrina* snails` reproduction and infection with *Schistosoma mansoni.* New Egypt. J. Med., 39: 103-110.

Geerts, S.; Belot. J.; Sabbe, F.; Triest, L. and Sidhom, M. (1991). *Ambrosia maritima:* effects on molluscs and non-target organisms. J. Ethnopharmacol., 33(1-2): 1-12.

Hamed, M.; El-Amin, S. and Abdel Fattah, A.S. (2015a). Molluscicidal activity of some constituents isolated from *Cestrum purpureum*. Pharmacol. Online, 2: 59-72.

Hamed, M.; El-Amin, S. and Abdel Fattah, A.S. (2015b). Isolation and identification of some compounds from molluscicidaly active plant *Yucca filamintosa marginata*. Pharmacol. Online, 2: 142-147.

Hamed, M.A. (2010). Strategic control of schistosome intermediate host. Asian J. Epidemiol., 3(3): 123-140.

Hira, D.R. and Webbe, G. (1972). The effect of sublethal concentrations of the mollucicide triphenyl lead acetate on *Biomphalaria glabrata* (Say) and on the development of *Schistosoma mansoni* in the snail. J. Helminthol., 46: 11-26.

Ibrahim, A.M.; El-Emam, M.A.; El-Dafrawy, S.M. and Mossalem, H.S. (2004). Impact of certain plant species on *Schistosoma mansoni-Biomphalaria alexandrina* system. Proc. 3rd Int. Conf. Sci., 3: 390-413.

Jia, T.W.; Sun, L.P.; Hong, Q.P.; Tao, B.; Cai, J.X.; Li, Y.S.; Lin, D.D.; Yu, X.L. and Zhou, X.N. (2013). Field evaluation of a novel plant molluscicide "Luo-Wei" against *Oncomelania hupensis* I- molluscicidal effect by the spraying method in lake and marshland regions. Chin. J. Schisto. Control, 25(2): 125-129.

Jia, T.W.; Wang, W.; Sun, L.P.; Lv, S.; Yang, K.; Zhang, N.M.; Huang, X.B.; Liu, J.B.; Liu, H.C.; Liu, R.H.; Gawish, F.A.; Habib, M.R.; El-Emam, M.A.; King, C.H. and Zhou, X.N. (2019). Molluscicidal effectiveness of Luo-Wei, a novel plant-derived molluscicide, against *Oncomelania hupensis*, *Biomphalaria alexandrina* and *Bulinus truncatus*. Infect. Dis. Poverty, 8: 27.

King, C.H. and Bertsch, D. (2015). Historical perspective: snail control to prevent schistosomiasis. PLoS Negl. Trop. Dis., 9: e0003657.

Lemma, A. (1970). Laboratory and field evaluation of the molluscicidal properties of *Phytolacca dodecandra*. Bull. World Health Organ, 42(4): 597-612.

Liu, R.H.; Wei, T.E.; Yuan, H. and Zhang, N.M. (2007). Study on the purification of tea saponin. Appl. Chem. Industry, 36: 649-650.

Mahmoud, M.B.; Ibrahim, W.L.; Abou-El-Nour, B.M.; El-Emam, M.A. and Youssef, A.A. (2011). Biological and biochemical parameters of *Biomphalaria alexandrina* snails exposed to the plants *Datura stramonium* and *Sesbania sesban* as water suspensions of their dry powder. Pestic. Biochem. Physiol., 99: 96-104.

Malek, E.A. (1980). Snail-Transmitted Parasitic Diseases. CRC Press Inc Boca Raton, FL. pp.179-307.

Mantawy, M.M. and Mahmoud, A.H. (2002). Effect of *Allium sativum* feeding on glucose, glycogen, protein bands profile and phenol oxidase activity in *Biomphalaria alexandrina.* J. Egypt Soc. Parasitol., 32: 271-283.

Massoud, J.; Arafaa, F. and Chu, K.Y. (1973). Effect of Bayluscide (Bayer 73) on the development of *Schistosoma haematobium* in *Bulinus truncatus.* Bull. Soc. Pathol. Exot., 66(4): 544-547.

McCullough, F.S.; Gayral, P.; Duncan, J. and Christie, J.D. (1980). Molluscicides in schistosomiasis control. Bull. World Health Organ., 58(5): 681-689.

Mendes, N.M.; Gómez, J.D.; Araújo, N.; Zani, C.L. and Katz, N. (1993). Preliminary trials of *Guaiacum officinale* L. as a molluscicide. Rev. Inst. Med. Trop. Sao Paulo, 35(6): 509-513.

Chinese Ministry of Agriculture. (1995). Toxicological Test Methods of Pesticides for Registration. GB 15670-1995, MoA.

Mohamed, A.M.; El-Emam, M.A.; Osman, G.Y.; Abdel Hamid, H. and Ali, R.E.M. (2012). Biological and biochemical responses of infected *Biomphalaria alexandrina* snails with *Schistosoma mansoni* postexposure to the pesticides Basudin and selecron and the phytoalkaloid colchicine. J. Evolutionary Biol. Res., 4(2): 24-32.

Mostafa, B.B.; El-Khayat, H.M.; Ragab, F.M. and Tantawy, A.A. (2005). Semi field trials to control *Biomphalaria alexandrina* by different modes of exposure to certain plant and chemical molluscicides. J. Egypt. Soc. Parasitol., 35(3): 925-940.

National Environmental Protection Agency. (1989). The Examinational Rule of Security Evaluation of Chemical Pesticide. *Beijing:* China Environmental Science Press 1989.

Rizk, M.Z.; Metwally, N.S.; Hamed, M.A. and Mohamed, A.M. (2012). Correlation between steroid sex hormones, egg laying capacity and cercarial shedding in *Biomphalaria alexandrina* snails after treatment with *Haplophyllum tuberculatum*. Exp. Parasitol., 132(2):171-178.

Schall, V.T.; Vasconcellos, M.C.; Rocha, R.S.; Souza, C.P. and Mendes, N.M. (2001). The control of the schistosome-transmitting snail *Biomphalaria glabrata* by the plant Molluscicide *Euphorbia splendens var. hislopii* (syn milli Des. Moul): a longitudinal field study in an endemic area in Brazil. Acta Trop., 79(2): 165-170.

WHO. (1983). Report of scientific working group on plant molluscicide and guidelines for evaluation of plant molluscicide, Geneva: WHO; (TDR/SCH – SWE (4)/ 83.3)

WHO. (2010). First WHO report on neglected tropical diseases 2010: working to overcome the global impact of neglected tropical diseases. ISBN 9789241564090.

WHO. (2017). Field use of molluscicides in schistosomiasis control programmes: an operational manual for programme managers. ISBN 978-92-4-151199-5.

Yuan, H.; Liu, R.H. and Zhang, N.M. (2008). A simple method for purification of crude tea saponin. Chin. J. Bioprocess. Eng., 6: 18-20.

Zhang, Z.H.; Fang, R.; Yu, B.; Liu, Z.S.; Zhao, Z.J.; Xu, X.W.; Huang, X.L. and Jia, T.W. (2013). Field evaluation of a novel plant molluscicide "Luo-wei" against *Oncomelania hupensis* snails. II- Molluscicidal effect in the field areas in Hanchuan City, Hubei Province. Chin. J. Schisto. Control, 25(2): 481-484.

Zinada, O.A. (2000). Effect of niclosamide on the marketable fish *Liza ramada* (Risso, 1826) concerning accumulation in muscles and activities of three metabolic liver enzymes. J. Egypt. Soc. Parasitol., 30: 791-802.