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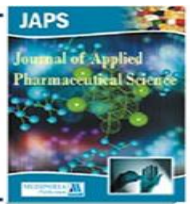

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Biological studies on *Biomphalaria alexandrina* snails treated with *Furcraea selloa marginata* plant (family: Agavaceae) and *Bacillus thuringiensis kurstaki* (Dipel-2x)

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ABSTRACT

Effect of the dry leaves powder water suspension of the plant *Furcraea selloa marginata*, belonging to family *Agavaceae* and *Bacillus thuringiensis kurstaki* (Dipel-2x) was evaluated against non-infected and *Schistosoma mansoni*-infected *Biomphalaria alexandrina* snails as well as their efficacy against the free larval stages of *S. mansoni*. The obtained results indicated that the LC_{50} and LC_{90} values after 24 hrs exposure were 53.66 & 84.35 ppm for *F. selloa marginata* and 392.3 & 483.64 ppm for *B. thuringiensis kurstaki* against adult *B. alexandrina* snails, respectively. The plant *F. selloa marginata* and *B. thuringiensis kurstaki* have a larvicidal activity against *S. mansoni* larvae (miracidia and cercariae), the plant *F. selloa marginata* was more toxic against larvae than *B. thuringiensis kurstaki*, the miracidia were more sensitive towards the toxic action of the tested agents than cercariae and the mortality percent of miracidia and cercariae is directly proportional to the time and the tested concentrations. The results revealed that the tested sub-lethal concentrations (LC_o, LC_{10} and LC_{25}) reduced the survival, growth rates and egg laying capacity of both non-infected and *S. mansoni*-infected snails during 12 weeks of exposure in comparison with their control group. The hatchability percent of *B. alexandrina* eggs of one, three and six days old exposed to LC_o, LC_{10}, LC_{25}, LC_{50} & LC_{90} concentrations of *F. selloa marginata* and *B. thuringinesis kurstaki*, significantly decreased by increasing their age and the tested concentrations. Exposing *B. alexandrina* snails to sub-lethal concentrations of the tested agents for 24 hours either pre-, during or post exposure of snails to *S. mansoni* miracidia caused a marked reduction in the infection rate and decreased the mean total number of shedding cercariae/snail. Also, elongated their prepatent period (cercarial incubation period) and shortened the duration of cercarial shedding in comparison with their control group. Under semi-field conditions the more time of exposure to the concentration (LC_{90}= 84.35 ppm) of the plant *F. selloa marginata* the more mortality among snails. The mortality rates of the snails were 0%, 2%, 18% and 30% at 3, 6, 12 and 24 hrs post exposure, respectively.

Keywords: Biomphalaria snails- Biocontrol agents- Furcraea selloa marginata- Bacillus thuringiensis kurstaki (Dipel-2x).

INTRODUCTION

Schistosomiasis remains one of the most prevalent parasitic infections in the world. It has been estimated that more than 200 million people in 76 countries are infected and approximately 500 - 600 million people at risk of infection (Borch *et al.*, 2009). Snail's control is an essential

part in the combat against schistosomiasis and the biological control of snail populations offers an expensive and environmentally acceptable alternative to chemical molluscicides (Jobin *et al*., 1977).

In fact, molluscicides of plant origin seem to be less expensive, readily available, rapidly biodegradable, have low toxicity to non - target organisms and probably easily applicable with simple techniques appropriate to developing countries (Adewunmi *et al*., 1990a & b and Ibrahim *et al*., 2004).

During the last two decades several important investigations on plant molluscicides were carried out (Mott, 1987 and Mohamed and Abdel Gawad, 2005). Among the promising plants for snail control are *Millettia thonningii* (Evans *et al*., 1986), *Anagallis arvensis* (Shoeb *et al*., 1989), *Cestrum parqui* and *Hedra canariensis* (El- Emam *et al.*, 1990), *Dialium guineese* (Odukoya *et al*., 1996), *Calendula micrantha officinalis* (Mostafa and Tantawy, 2000), *Solanum dobium* (Tantawy *et al*., 2000), *Solanum nigrum* and *Panicum repens* (Ibrahim *et al*., 2004). Also the plants belonging to *Agavaceae* were found to have good molluscicidal properties by several investigators as shown by Shoeb *et al*., 1983 (*Agave angustifolia* & *Agave celsii*), 1992a (*Agave lophantha*), 1992b (*Agave attenuata*) and El-Sayed *et al*., 1995 (*Agave filifera*).

Biological agents have been exploited for snail's control (Osman and Mohamed, 1991). For example, bacteria were suggested as biocontrol agent against schistosomiasis vector snails. The bacterial pathogen, *Bacillus thuringiensis*, is one of the most common biological control agents in use today. It was known for along time as insecticide (Ignoff *et al.*, 1981and Hassanain *et al.*, 1997). It has been also used as anthelmintic (Hassanain *et al.*, 1998; Abdel-Rahman *et al.*, 1998). El-Emam *et al.* (1996 b) reported that *Bacillus thuringiensis israelensis* has a highly suppressive effect on the population growth of *B. alexandrina* snails, thus affecting the availability of these snails in *S. mansoni* transmission. Gamal *et al.* (2000) reported that prolonged exposure of snails to *B. thuringiensis* (R153/78) (Bt1) and *B. thuringiensis kurstaki* 32000 IU/mg (Bt2) at concentrations of 0.8-1 gL^{-1} resulted to complete loss of the hatchability of *B. alexandrina* snails.

This study aimed to evaluate the effect of the plant *Furcraea selloa marginata*, belonging to family *Agavaceae* and *Bacillus thuringiensis kurstaki* (Dipel-2x) against non-infected and *S. mansoni*-infected *Biomphalaria alexandrina* snails as well as it's efficacy against the free larval stages of *S. mansoni*.

MATERIALS AND METHODS

Experimental animals used in the present study were *Biomphalaria alexandrina* snails; *Schistosoma mansoni* miracidia and cercariae and male white albino mice (CD1) brought from Schistosome Biological Supply Center (SBSC) Theodor Bilharz Research Institute, Giza, Egypt. The experimental snails were maintained under experimental laboratory conditions (25± 2ºC) according to the method described by (WHO, 1965).

The plant used in this study *Furcraea selloa marginata* (Family: *Agavaceae*). It was collected from EL-Orman Garden, Giza during full growing season. The collected plant leaves were transferred to the laboratory, shade dried, then in an oven at 50°C and finely powdered using an electrical grinder. The dry powder of each experimental plant was stored in a clean dark glass bottle till use. Therefore, they were evaluated against *B. alexandrina* snails as aqueous suspensions on basis of weight / volume using dechlorinated tap water (WHO, 1965).

Commercial *Bacillus thuringiensis kurstaki* (Dipel-2x), 32,000 I.U. / mg was kindly provided from Central Agricultural Pesticides Laboratory, Ministry of Agriculture, Dokki, Giza, Egypt. Dilutions of the experimental powder were prepared on the basis of weight / volume using dechlorinated tap water according to Osman and Mohamed (1991).A series of concentrations that would allow the computation of LC_{50} and LC_{90} values were prepared according to WHO (1965), while the sub-lethal concentration (LC_o) was calculated as it equals 1/10 LC_{50} (WHO, 1965).

Experimental Infection

Mice infection

CD1 mice were individually exposed to 80-100 freshly emerged *S. mansoni* cercariae by paddling method, in dechlorinated tap water for 1-2 hrs at 22-25 ºC.

Snail infection

Infected mice 6-8 weeks post infections were dissected, the infected livers and intestines were homogenized and eggs were extracted washed in saline. *S. mansoni* miracidia hatched under illumination from the isolated eggs (Chernin, 1970). *B. alexandrina* snails were individually infected each with 4-5 miracidia in glass test tubes filled with 1 ml dechlorinated tap water for 2 hours (Anderson *et al.*, 1982).

Cercaricidal and miracidicidal effect

Twenty five ml of dechlorinated tap water containing 100 freshly hatched miracidia or cercariae were mixed with 25 ml double concentrations of LC_o, LC_{10}, LC_{25}, LC_{50} & LC_{90} values of the tested materials. Fifty ml of dechlorinated water containing 100 freshly hatched miracidia were used as a control (Ritchie *et al*., 1974). During treatment period, microscopical observations on the movement and mortality of the miracidia and cercariae were recorded at time intervals of 1/4, 1/2, 3/4, 1, 2, 3, 4, 5 & 6 hrs.

Prolonged exposure of snails to sub-lethal concentrations (LC_o, LC_{10} and LC_{25}) of the tested materials

Sets of 180 mature snails with (8-10 mm) shell diameter were divided into six groups, each of 30 snails. The 1st group was kept as non-treated and non-infected group (control). The 2nd group was treated with *F. selloa marginata* whereas the 3rd ones was exposed to *S. mansoni* miracidia (control infected). The 4th group was exposed to both *F. selloa marginata* and *S. mansoni* miracidia (treated-infected I) while the 5th group was treated with *Bacillus thuringiensis kurstaki* (Dipel-2x). The last group 6th was exposed to both (Dipel-2x) and *S. mansoni* miracidia (treated-infected II). Snails were maintained in 1000 ml of the experimental

concentration in two-liter capacity plastic containers. For 12 weeks the concentrations were changed with freshly prepared ones every week. Fresh lettuce leaves were provided as the daily food. Observations were recorded weekly for mortality, number of egg masses laid and the shell diameter (growth rate).

Effect on hatchability

For studying the effect of the tested materials on the hatchability of *B. alexandrina* eggs, three replicates of egg masses, each of about 60 eggs of one, three and six days old were used. Egg masses were obtained from healthy *B. alexandrina* snails, which were laid on foam pieces, maintained in the laboratory. Egg masses were continuously exposed to 100 ml of LC_o, LC_{10}, LC_{25}, LC_{50} & LC_{90} concentrations of the tested materials in Petri dishes until hatching. Another group of about 60 eggs was maintained in dechlorinated tap water as a control (Frik and Dejmenez, 1963 and Oteifa *et al*., 1975). Egg masses were examined daily during the experimental period under a steromicroscope and the number of normal viable eggs and hatched embryos were recorded (Oliver *et al*., 1962). At the end of the experiment, the percentage of hatchability was calculated.

Effect on infectivity

B. alexandrina snails were exposed to sub-lethal concentrations (LC_o, LC_{10} & LC_{25}) of the tested materials for 24 hours either pre-, during or post exposure of snails to *S. mansoni* miracidia (Badawy, 2007).Snail exposure to miracidia was carried out in mass, i.e. for each experimental concentration three replicates, each of 10 snails/ L in glass container, were exposed to miracidia freshly hatched from ova at a dose of 10 miracidia/snail, either with or without the experimental concentrations. Another group untreated with the tested concentrations, but exposed to miracidia was maintained as a control. After 25 days of miracidial exposure, surviving snails were individualy examined for cercarial shedding in multi-dishes under artificial light for 1 hr and 2 ml dechlorinated water /snail to stimulate cercarial shedding (Meuleman, 1972), the mean number of cercariae, the incubation period (prepatent period), duration of cercarial shedding (patent period) and the infection were calculated for each snail.

Effect of F. selloa marginata on B. alexandrina Snails under Semi-Field Conditions

This experiment was carried out in the Snails Research Station of TBRI , El-Qanater El-Khayria, Qalubia Governorate, Egypt according to the protocol of (Mostafa *et al*., 2005). Statistical analysis: Student t-test was carried out to determine the significance between control and experimental groups.

RESULTS

Molluscicidal activity of the plant *Furcraea selloa marginata* and *Bacillus thuringiensis kurstaki* (Dipel-2x) against adult *B. alexandrina* snails is presented in Table (1). The data revealed that the LC_{50} and LC_{90} values were 53.66 & 84.35 ppm for *F. selloa marginata* and 392.3 & 483.64 ppm for *B. thuringiensis kurstaki*, respectively after 24hrs. The results also showed that tested materials displayed a larvicidal activity against *S. mansoni* miracidia and cercariae as shown in Tables (2 and 3). The miracidia are more sensitive towards the toxic action of the tested agents than cercariae during 6 hours of exposure and the mortality percent of miracidia and cercariae is directly proportional to the time and the tested concentrations.

Table 1: Molluscicidal activity of *Furcraea selloa marginata* plant (family: *Agavaceae*) and *Bacillus thuringiensis kurstaki* (Dipel-2x) on adult *Biomphalaria alexandrina* after 24 hours of exposure.

Tested Materials	LC_{50} (ppm)	LC_{90} (ppm)	Slope function	Sublethal concentrations (ppm)		
				LC_o	LC_{10}	LC_{25}
Furcraea selloa marginata	53.66	84.35	1.66	5.37	22.96	37.50
Bacillus thuringinesis kurstaki	392.31	483.64	1.21	39.23	300.98	344.24

Table 2: Miracidicidal effect of the tested materials on *S. mansoni* miracidia.

Concentrations (ppm)	%Mortality of miracidia after 4 hours	
	F. selloa marginata	***B. thuringiensis kurstaki***
LC_o	95	89
LC_{10}	100	94
LC_{25}	100	97
LC_{50}	100	100
LC_{90}	100	100
Control	6	6

Table 3: Cercaricidal effect of the tested materials on *S. mansoni* cercariae.

Concentrations (ppm)	%Mortality of cercariae after 6 hours	
	F. selloa marginata	***B. thuringiensis kurstaki***
LC_o	93	78
LC_{10}	97	84
LC_{25}	100	88
LC_{50}	100	92
LC_{90}	100	95
Control	5	5

Figures (1, 2, 3 and 4) illustrate the effect of prolonged exposure of snails to sub-lethal concentrations (LC_o, LC_{10} and LC_{25}) of *Furcraea selloa marginata* and *Bacillus thuringiensis kurstaki*. The obtained results showed a significant decrease on survival rate, growth rate and egg laying capacity in both non-infected and *S. mansoni*-infected snails during 12 weeks of exposure compared to untreated control group.

Regarding to the hatchability of *B. alexandrina* eggs, the obtained results indicated that the eggs of different ages can hatch in all tested concentrations (LC_o, LC_{10}, LC_{25}, LC_{50} and LC_{90}) of *F. selloa marginata* and *Bacillus thuringiensis* but with different rates ,except eggs at three and six days ages exposed to LC_{90} of *B. thuringiensis kurstaki* didn't hatch in the examined solution (Tables 4 A & B). The results illustrated in (Table 5 A & B) revealed that the treatment of infected *B. alexandrina* snails with sub-lethal concentrations (LC_o, LC_{10} & LC_{25}) of *F. selloa marginata* and *Bacillus thuringiensis* for 24 hours either pre-, during or post exposure of snails to *S. mansoni* miracidia caused a marked reduction in the infection rate and the mean total number of shedding cercariae/snail. Also, elongated their prepatent period (cercarial incubation period) and shortened the duration of cercarial

Table (4): Effect of *F. selloa marginata* (A) and *B. thuringiensis kurstaki* (B) on hatchability of *B. alexandrina* eggs of different ages.

(A) *F. selloa marginata*

Conc. (ppm)	% of hatchability of *B.alexandrina* eggs		
	1 day old	3 days old	6 days old
LC_0 (5.37)	92.98	88.89	85.29
LC_{10} (22.96)	89.66	84.38	81.25
LC_{25} (37.50)	84	80	73.08
LC_{50} (53.66)	77.14	75	70.83
LC_{90} (84.35)	72	66.67	62.07
Control	95.45	96.36	96.89

(B) *B. thuringiensis kurstaki*

Conc. (ppm)	% of hatchability of *B.alexandrina* eggs		
	1 day old	3 days old	6 days old
LC_0 (39.23)	88.57	86.36	73.33
LC_{10} (300.98)	61.76	48.21	43.14
LC_{25} (344.24)	18.52	10.64	7.34
LC_{50} (392.31)	14.50	8.82	4.69
LC_{90} (483.64)	1.70	0	0
Control	95.45	96.36	96.89

shedding in comparison with their control group. From the results in Table (6) it is clear that the more time of exposure the more mortality among snails treated with the plant *F. selloa marginata* (LC_{90}= 84.35 ppm) under semi-field conditions.

DISCUSSION

The present study indicated that the plant *F. selloa marginata* and *Bacillus thuringiensis kurstaki* (Dipel-2x) have molluscicidal and larvicidal activities against adult snails and *S. mansoni* larvae, respectively.

The considerable toxic effect of the plant *F. selloa marginata* might be due to steroidal saponins, the main constituents of *Agavaceae* (Mahato *et al*., 1982), that are known to possesss molluscicidal activities, due to their ability to form complexes with cholesterol and decrease its level in the plasma and increase cholinesterase activity or may be decrease the frequency of cardiac contractions (El-Gengaihi *et al.*, 1988). The results obtained by Diaz and Ferrer (1996) proved that the heart rate of *Biomphalaria havanensis* snails was highly reduced post their exposure to an aqueous extract of *Agave fourcroydes.*

B. thuringiensis kurstaki (Dipel-2x) also showed molluscicidal activity against adult *B. alexandrina* snails, and this might be due to the fact that it has a hazardous effect on the digestive tract or probably due to one or more of the toxins produced by bacteria (Abdel-Rahman and Hassanian, 1999). The author showed that Dipel-2x caused great alterations in the stomach and digestive tubules of *Lymnaea natalensis* snails. The present data concerning the molluscicidal activity of Dipel-2x are in agreement with those obtained by Ducklow *et al*. (1980) who reported that the bacterium *Vibrio parahaemolyticus* was found to be pathogenic for the schistosome intermediate host *B. glabrata*. Also, Abdel-Megeed and Abdel-Aziz (1999) revealed that the Dipel-2x concentration which induced 50% mortality (LC_{50}) of *Physa acuta* snails after 24hr of exposure was 270 mg/L.

Results of the current study also revealed that the miracidial mortalities are greater than that of cercariae after the same time intervals. This observation is in agreement with the study of Mahmoud (1993) on Kelthane that killed miracidia faster than cercariae. Also, Ibrahim *et al*. (2007) showed that miracidial mortality were greater than that of cercariae during application of Hinsan and the plant *T. terrestris* after the same time intervals.

The tested materials showed a significant reduction on the survival rate, growth rate and egg laying capacity of both adult non-infected and *S. mansoni*-infected *B. alexandrina* snails. Such reduction of snail's survival and fecundity may arise as a result of the action of the tested agents upon the steroid hormones, the harmful effect on the male and female genital tract, or may arise from metabolic disorders as has been described by Mohamed *et al.* (1981) who tested the efficacy of low concentrations of some organometallic compounds that may alter the reproduction of *B. alexandrina* snails as a result of reduction of the growth of the male and female organs of the genetal tract and endocrine disruption, which reduce or stop their oviposition. These findings are in a harmony with results obtained by Abdel-Hafez *et al*. (1997); Bakry *et al*. (2001); Tantawy (2002 and 2008); Tantawy *et al*. (2004); El-Sayed (2006) and Bakry *et al*. (2007) on testing the plants; *Azolla pinnata*, *A. franzosinii*, *Atriplex halimus*, *Commiphora molmol*, *Synadenium grantii*, *Cupressus macrocarpa* and *Azadirachta indica* for mollusciciding activities. Abdel–Rahman and Hassanain (1999) revealed that *B. thuringiensis kurstaki* (Dipel-2x) has a potent effect on the survivor and egg laying capacity of *L. natalensis* snails. Also, investigations by El-Emam *et al*. (1996b) reported that *B. thuringiensis israelensis* bacteria have a highly suppressive effect on the population growth of *B. alexandrina* snails.

Ibrahim (2006) stated that, at 4th week post infection, additional demonds consume the energy of the *B. alexandrina* snails, i.e. cercarial emergence, thus leaving low amounts for survival, growth, detoxification and reproduction.

The current study also revealed that the tested materials have aconsiderable effect on the hatchability of *B. alexandrina* eggs of different ages. Several authors recorded a similar harmful and remarkable reduction in hatchability of *B. alexandrina* eggs treated with different molluscicides (El-Bolkiny *et al*., 2000; Rizk *et al*., 2001 and Al-Mathal and Fouad, 2006).

The present results indicated that 24 hours of snails exposure to the tested materials either pre-, during and post miracidial exposure reduced their infection rates, the mean total number of shedding cercariae/snail. Also, elongated their prepatent period and shortened the duration of cercarial shedding in comparison with their control group. The same results were observed by Ahmed and Ramzy (1997); Mostafa and Tantawy

Table 5: Effect of sublethal concentrations of F.selloa marginata (A) and *B. thuringiensis* on infectivity of *S.mansoni miracidia* to *B. alexandrina* snails.

(A) ***F. selloa marginata***

Treatment related to miracidial exposure	Concentration (ppm)	Total exposed snails	No. of alive snails	No. of shedding snails	Infection rate (%)	Prepatent period (day)	Duration of shedding (day)	Mean no. of cercariae/snail
One day pre-exposure	LC_0 (5.37)	30	26	24	92.31	33.92*** ± 1.22	35.00 ± 5.31	733.62 ± 193.70
	LC_{10} (22.96)	30	20	17	85*	34.33*** ± 0.91	24.90*** ± 3.30	512.86** ± 171.24
	LC_{25} (37.50)	30	17	12	70.59***	35.24*** ± 0.64	20.00*** ± 3.50	419.57** ± 216.97
	Control	30	24	23	95.83	32.0 ± 0.00	37.00 ± 0.91	795.70 ± 379.91
During exposure	LC_0 (5.37)	30	18	18	100	28.0 ± 0.00	23.33*** ± 10.69	401.60* ± 198.56
	LC_{10} (22.96)	30	13	13	100	28.0 ± 0.00	17.50*** ± 4.95	290.40*** ± 124.71
	LC_{25} (37.50)	30	15	15	100	28.33 ± 1.53	14.00*** ± 9.90	162.11*** ± 39.59
	Control	30	16	16	100	28.0 ± 0.00	42.40 ± 0.41	635.00 ± 345.92
One day post-exposure	LC_0 (5.37)	30	14	10	71.43***	38.21** ± 1.00	28.00*** ± 4.50	567.62 ± 234.76
	LC_{10} (22.96)	30	16	11	68.75***	39.24*** ± 1.10	31.93*** ± 7.00	558.80 ± 93.91
	LC_{25} (37.50)	30	13	7	53.85***	39.33 ± 3.21	21.00*** ± 2.71	354.99*** ± 91.34
	Control	30	12	12	100	36.82 ± 0.95	46.22 ± 4.73	601.00 ± 163.19

(B) ***B. thuringiensis kurstaki***

Treatment related to miracidial exposure	Concentration (ppm)	Total exposed snails	No. of alive snails	No. of shedding snails	Infection rate (%)	Prepatent period (day)	Duration of shedding (day)	Mean no. of cercariae/snail
One day pre-exposure	LC_0(39.23)	30	20	19	95.00	33.10*** ± 0.52	31.00*** ± 5.53	695.00 ± 163.60
	LC_{10}(300.98)	30	23	21	91.30	32.60*** ± 0.40	34.66* ± 4.10	515.12** ± 273.55
	LC_{25}(344.24)	30	25	23	92.00	33.30*** ± 0.60	26.00*** ± 4.54	624.67 ± 200.41
	Control	30	24	23	95.83	32.0 ± 0.00	37.00 ± 0.91	795.70 ± 379.91
During exposure	LC_0(39.23)	30	15	11	73.33***	28.00 ± 0.00	14.38 *** ± 11.06	535.00 ± 412.82
	LC_{10}(300.98)	30	12	8	66.67***	28.00 ± 0.00	11.80*** ± 4.13	530.00 ± 390.72
	LC_{25}(344.24)	30	18	10	55.56***	30.36 ± 5.26	8.45*** ± 3.24	418.33* ± 183.66
	Control	30	16	16	100	28.0 ± 0.00	42.40 ± 0.41	635.00 ± 345.92
One day post-exposure	LC_0(39.23)	30	17	17	100	36.80 ± 0.91	45.43 ± 2.61	480.00 ± 309.10
	LC_{10}(300.98)	30	20	20	100	37.90* ± 1.44	41.60** ± 2.11	596.20 ± 197.05
	LC_{25}(344.24)	30	18	13	72.22***	38.20* ± 1.00	44.70 ± 1.30	323.00** ± 230.11
	Control	30	12	12	100	36.82 ± 0..95	46.22 ± 4.73	601.00 ± 163.19

Significant difference compared to control group at $p < 0.05$, :Non Significant ($p > 0.05$), *:Significant ($p < 0.05$), **:Highly Significant ($p < 0.01$), ***: More highly Significant ($p < 0.001$).

Table 6: Effect of *F. selloa marginata* against *B. alexandrina* snails under semi-field conditions.

	%Mortality of *B. alexandrina* after indicated periods (hours)			
	3	6	12	24
Furcraea selloa marginata	0	2 %	18 %	30 %
Control	0	0	0	0

(2000); Bakry *et al*. (2001 and 2007); El-Ansary *et al*. (2001); Massoud *et al*. (2004) and El-Sayed *et al*. (2006) on different molluscicides at different periods of *B. alexandrina* snail's exposure to *S. mansoni* miracidia. Under semi-field conditions the more time of exposure the more mortality among snails treated with the plant *F. selloa marginata*. The importance of semi-field and field trials of successful laboratory studies was recommended by several scientists. Lemma *et al*. (1978) reported that a systemic field trial needed to demonstrate that a candidate molluscicidal operation can kill snails under local field conditions when applied by simple means. Some plant species were applied in field trials and used in snail control programs, as *T. tetraptera* in Nigeria (Adewunmi, 1991), *E. splendens* in Brazil (Baptisa *et al.*, 1994 and Mendes *et al.*, 1997 and Schall *et al*., 2001) and *P. dodecandra* (endod) in Zimbabwe (Erko *et al*., 2002). In Egypt, *A. maritima* (damsissa) (El-Sawy *et al.*, 1981 and 1989) and *Anagallis arvensis* Emam *et al.* (1996a).

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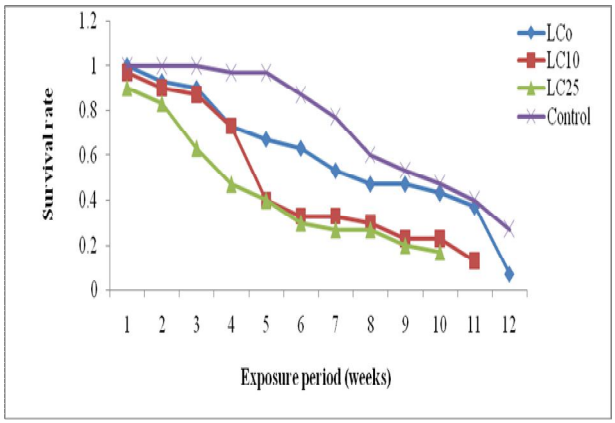

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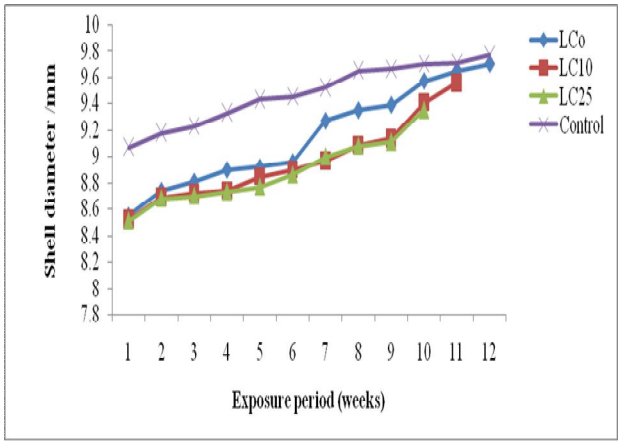

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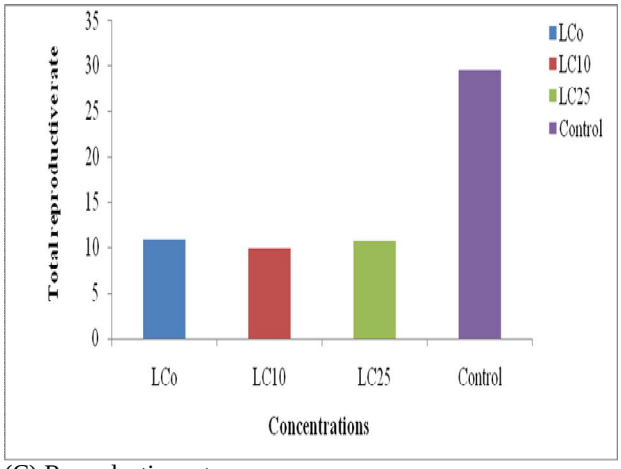
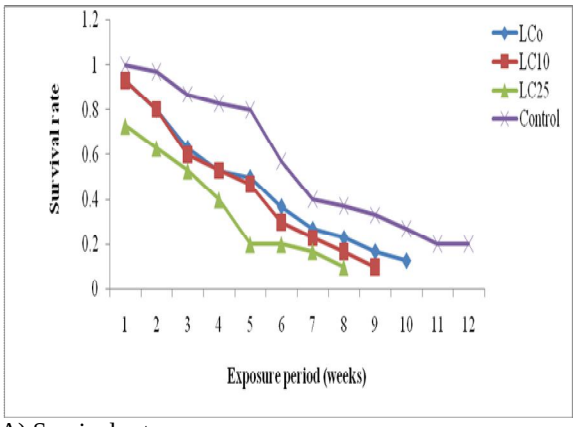


(C) Reproductive rate

Fig 1: Effect of sub-lethal concentrations of *F. selloa marginata* on survival rate (A), growth rate (B) and reproductive rate (C) of adult non-infected *B. alexandrina* snails.



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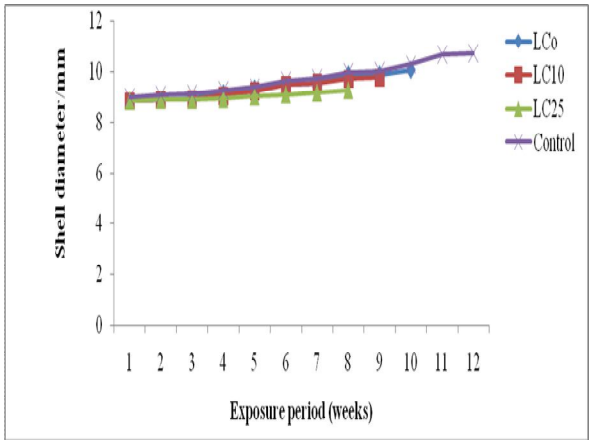

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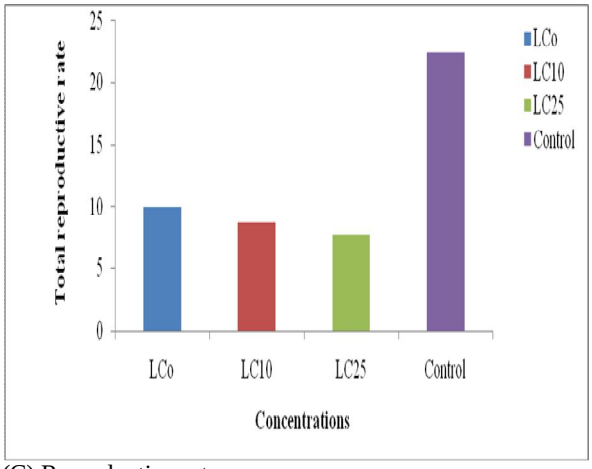
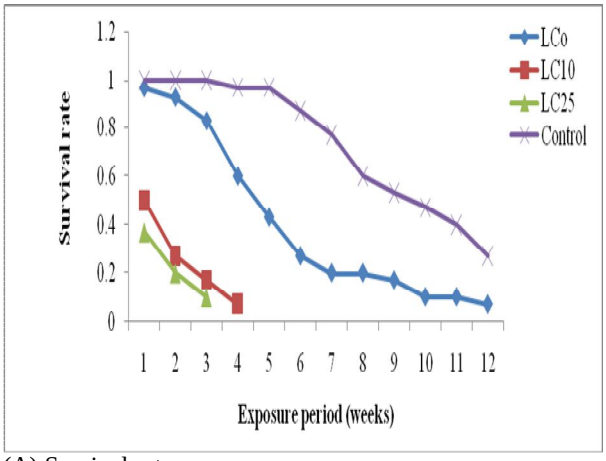


(C) Reproductive rate

Fig 2: Effect of sub-lethal concentrations of *F. selloa marginata* on survival rate (A), growth rate (B) and reproductive rate (C) of adult *S. mansoni* infected *B. alexandrina* snails.



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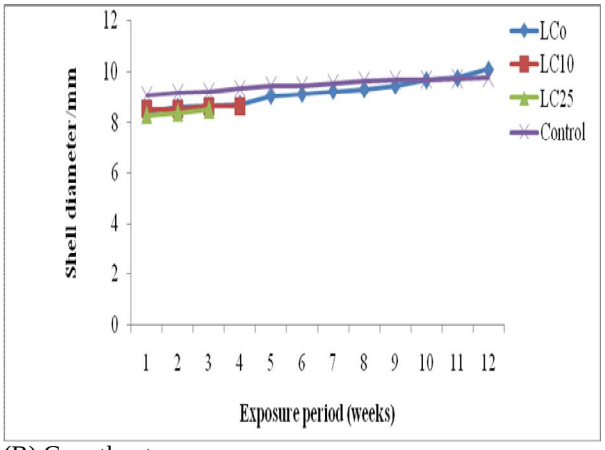

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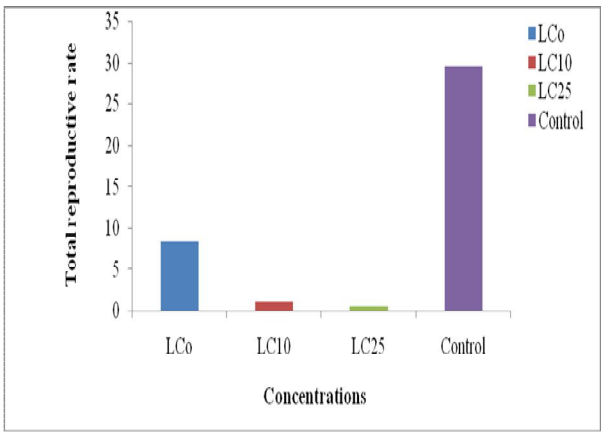
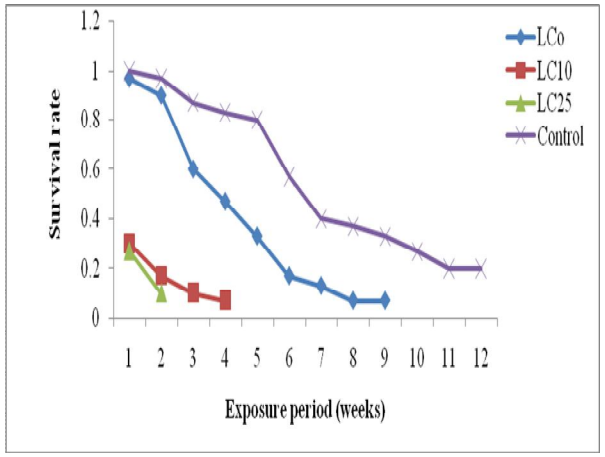


(C) Reproductive rate.

Fig 3: Effect of sub-lethal concentrations of *B. thuringiensis kurstaki* on survival rate (A), growth rate (B) and reproductive rate (C) of adult non-infected *B. alexandrina* snails.



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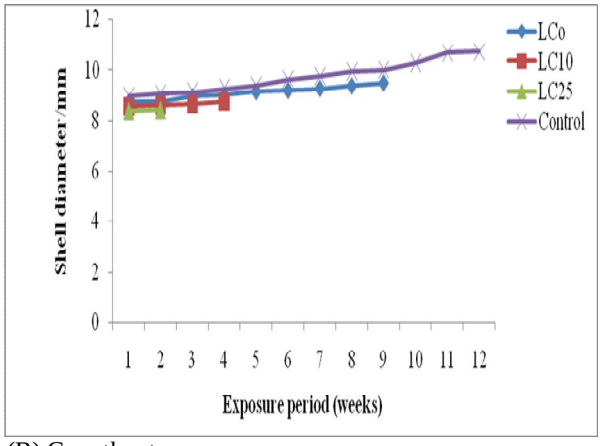

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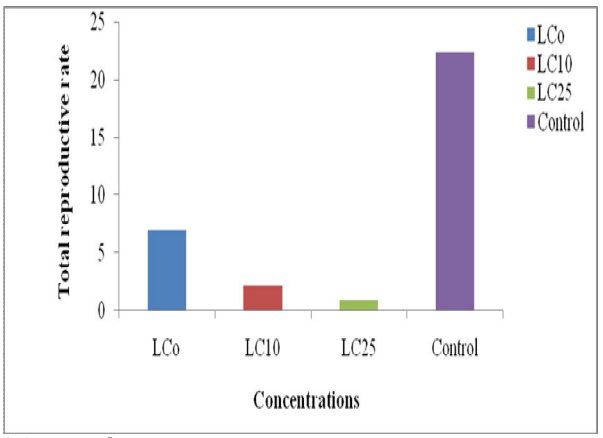

(C) Reproductive rate

Fig 4: Effect of sub-lethal concentrations of *B. thuringiensis kurstaki* on survival rate (A), growth rate (B) and reproductive rate (C) of adult *S. mansoni* infected *B. alexandrina* snails.