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MOLLUSCICIDAL ACTIVITY OF *ZYGOPHYLLUM SIMPLEX* (FAMILY: ZYGOPHYLLACEAE) AGAINST *BIOMPHALARIA ALEXANDRINA AND BULINUS TRUNCATUS*

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ABSTRACT

The molluscicidal activity of *Zygophyllum simplex* plant leaves powder on *Biomphalaria alexandrina* and *Bulinus truncatus* snails after 24 hours of exposure was evaluated under laboratory conditions. The results obtained indicated that the LC_{50} values for this plant were 42 ppm and 38 ppm and LC_{90} values were 65 ppm and 60 ppm for *B. alexandrina* and *B. truncatus* respectively. The sublethal concentrations (LC_0, LC_{10} and LC_{25}) were 4.2, 27 and 34 ppm for *B. alexandrina* and 3.8, 25 and 32 ppm, for *B. truncatus*. Continuous maintaining of snails in sublethal concentrations (LC_0, LC_{10} and LC_{25}) of *Z. simplex* led to an increase in mortality rate of the snails which was significantly higher than that of the control group.

The effect of the tested sublethal concentrations of *Z. simplex* on infection of *B. alexandrina* with *S. mansoni* miracidia was studied. The infection rate was significantly lower than that of control snails with reduction rates of 23.5%, 35.1% and 66.9% for snails exposed to LC_0, LC_{10} and LC_{25}, respectively. Prepatent period of exposed snails to LC_0, LC_{10} and LC_{25} of *Zygophyllum* was prolonged to be 30.2 ± 2.4, 32.4 ± 2.1 and 34.6 ± 3.1 days compared to 28.6 ± 3.6 days for the control group. Meanwhile, the duration of cercarial shedding was significantly shortened, being 22.8 ± 4.4, 18.3 ± 4.9 and 10.5 ± 3.8 days for LC_0, LC_{10} and LC_{25} respectively, compared with 44.2 ± 4.8 days for the control snails. Highly significant reductions of total cercarial production per snails and per stimulant were also detected in experimental snails in comparison with the control group.

INTRODUCTION

Screening of local plants for molluscicidal activity has received increasing attention by several authors (Sherif and El-Sawy,1962, El-

Gindy, 1969, and El-Emam, 1976, Mohamed *et al.*,1981, El-Sawy *et al.*,1983, 1984 and 1987 and Rawi *et al.*,1995 &1996). During the last two decades, several important reviews on plant molluscicides have been published (Kloos and McCullough, 1982; Marston and Hostettmann, 1985; Mott, 1987 and WHO, 1992). Triterpenoid saponins snd toxic flavonoids were reported in several *Zygophyllum* sp. (*Saber and Shoaib,* 1966). The aim of the present study is to assess the activity of the plant *Zygophyllum simplex* (Zygophyllaceae) against both *B. alexandrina* and *B. truncatus* snails.

MATERIAL AND METHODS

Zygophyllum simplex (Zygophyllaceae) was collected from the eastern desert, Egypt and identified by the Botany Department, Faculty of Science, Cairo University. The plant leaves were left to dry in air and then in an oven at 50°C and powdered by a mixer (Mahran *et al.,* 1977). Experimental solution was prepared freshly on the weight/volume basis in dechlorinated water to achieve the desired series of concentrations according to Rawi *et al.* (1995).

Biomphalaria alexandrina, Bulinus truncatus snails and *Schistosoma mansoni* ova used in this study were obtained from the Schistosome Biological Supply Program (SBSP) at Theodor Bilharz Research Institute, Imbaba.

The activity of the tested plant leaves against adult snails was determined according to the standard procedure recommended by WHO (1965). Ten adult healthy snails were immersed in each experimental concentration for 24 hr under laboratory conditions (24±1°C). Then the snails were washed carefully and kept for 24 hours in dechlorinated tap water as a recovery period. Thereafter, mortality counts were recorded and then computed to estimate the LC_{50} and LC_{90} according to the method of Litchfield and Wilcoxon (1949).

Prolonged exposure of snails to sublethal concentrations of the tested plant :

In this experiment, the snails were exposed continuously to sublethal concentrations of the aqueous solution of the dry powder of *Zygophyllum*. The sublethal concentrations used in this study are expressed as LC_0, LC_{10} and LC_{25}. Ten replicates, each of 30 lab-bred *B. alexandrina* snails (4-6 mm in diameter) were maintained in clean

glass container for each concentration. Concentrations were changed with newly prepared ones every 3 days. A control group (10 replicates) of 30 snails each was maintained in clean dechlorinated water under the same experimental conditions. The snails were daily provided with boiled lettuce leaves. Each container was provided with a polyethylene sheet for oviposition. Snails were continuously maintained in the sublethal concentrations till death of all snails. Observation was made daily on survivorship of the snails and number of laid eggs.

The effect of these sublethal concentrations on infection rate of *B. alexandrina* with *S. mansoni* miracidia and cercarial production were examined by exposing 3 groups each of 50 snails individually to *Schistosoma* miracidia with a dose of 10 miracidia/snail and maintained in each concentration of the tested plant (LC_0, LC_{10} and LC_{25}) for 24 hours under room temperature ($24 \pm 1^{\circ}C$) and ceiling illumination. After exposure to miracidia, snails were maintained in their corresponding sublethal concentrations. Another group of 50 snails was exposed to miracidia in the absence of the tested plant solutions and maintained under the same conditions (control group). Examination of snails for cercarial shedding was carried out twice weekly, 25 days post exposure, and the cercarial suspension was poured in a graduated Petri dish, then a few drops of Bouin's fluid were added and all cercariae were counted, using a dissecting microscope. Shedding snails were then isolated and kept in special aquaria in complete darkness.

Statistical analysis: for statistical analysis the student "t" test was used.

RESULTS

The molluscicidal activity of *Zygophyllum simplex* on *Biomphalaria alexandrina* and *Bulinus truncatus* snails after 24 hours of exposure under laboratory conditions is presented in Table (1). The data obtained indicate that the recorded LC_{50} values for this plant were 42 ppm and 38 ppm and LC_{90} values were 65 ppm and 60 ppm for *B. alexandrina* and *B. truncatus* respectively. The sublethal concentrations (LC_0, LC_{10} and LC_{25}) were found to be 4.2, 27 and 34

ppm for *B. alexandrina* and 3.8, 25 and 32 ppm, for *B. truncatus* respectively.

The results in Tables (2 and 3) showed a rapid increase in mortality rate of exposed snails to sublethal concentrations (LC_0, LC_{10} and LC_{25}) of *Z. simplex* which are significantly higher than that of control group. The data revealed that no *B. alexandrina* snails could survive more than 12, 10 and 7 weeks in groups maintained at LC_0, LC_{10} and LC_{25} respectively. Similarly, no *B. truncatus* snails could survive more than 10, 8 and 7 weeks in the experimental groups maintained at LC_0, LC_{10} and LC_{25} respectively, which are significantly lower ($p<0.01$) than that of control snails.

The effect of the tested sublethal concentrations of *Z. simplex* on infection of *B. alexandrina* with *S. mansoni* miracidia was presented in Table (4). The infection rate was significantly lower than that of control snails (82.6%), being 63.2%, 53.6% and 27.3% for snails exposed to LC_0, LC_{10} and LC_{25} respectively with a reduction rate 23.5%, 35.1% and 66.9% respectively.

Prepatent period (Table 5) of exposed snails to LC_0, LC_{10} and LC_{25} of *Zygophyllum* was prolonged to be 30.2 ± 2.4, $32.4 + 2.1$ and 34.6 ± 3.1 days compared to 28.6 ± 3.6 days for the control group. Meanwhile, the duration of cercarial shedding was significantly shortened among these snails, being 22.8 ± 4.4, 18.3 ± 4.9 and 10.5 ± 3.8 days for LC_0, LC_{10} and LC_{25} respectively, compared with 44.2 ± 4.8 days for control snails. Highly significant reductions of total cercarial production per snails and per stimulant were also detected in experimental snails in comparison with the control group.

DISCUSSION

The dry powder of the plant *Zygophyllum simplex* showed considerable molluscicidal effect against *Biomphalaria alexandrina and Bulinus truncatus.* The LC_{50} and LC_{90} were found to be 42 ppm & 65 ppm and 38 ppm & 60 ppm respectively.

The results showed that there was a significant increase in the mortality rates of snails exposed to sublethal concentrations of *Zygophyllum,* which were significantly higher than that of the control group. This finding agrees with those of El-Gindy (1969 a&b, 1975), Rawi *et al.*(1994 & 1996), Gawish (1997), Bakry and Sharaf El-Din (2000) and Tantawy *et al.*(2000). They showed a marked reduction in

the survival rate of snails treated with sublethal concentrations of different molluscicides compared to the control.

In this study, the infectivity of S. mansoni miracidia for B.alexandrina was greatly reduced by the tested sublethal concentrations of Zygophyllum. The reduction of infection rate was found to increase with the increase of sublethal concentrations. These results accord mostly with many authors working on various chemical and plant molluscicides (Warren and Weisberger,1966;Mohamed et al., 1981; Vianant et al., 1982; El-Emam et al., 1986; Mahmoud,1993;Rizk, 1995;Rawi et al.,1995; Gawish, 1997; El-Ansary et al., 2000 and Tantawy et al.,2000).

The present results showed that prepatent period of treated snails was prolonged compared to that of control group. Meanwhile, the duration of cercarial shedding was significantly shorter than control snails. This supports other authors on various molluscicides, e.g., El-Ansary *et al.*(2000) recorded longer prepatent period in *B. alexandrina* infected with *S. mansoni* in presence of *Ambrosia maritima*, and Gawish (1997) found that the period of cercarial shedding in snails treated with the experimental molluscicides during their exposure to miracidia are significantly shorter than that in control snails. This reduction in cercarial shedding period is probably due to rupture of snails' tissues through miracidial penetration in the presence of those molluscicides which increased the harmful effects of this plant. Also, Mahmoud (1993) found that the duration of *S. mansoni* cercarial shedding from infected *B. alexandrina* snails treated with Kelthane was shorter than that of corresponding control groups.

The results also indicated that treatment of snails continuously with sublethal concentrations of *Zygophyllum* resulted in highly significant reduction of total cercarial production per snails and the mean number of cercariae/snail/stimulant in comparison with control snails. These observations are in accordance with those of Yarinsky and Freele (1970) who reported that continuous exposure of *Australorbus glabratus* for 5 weeks to 1 ppm of lacanthone and 4 ppm of Hycanthone after infection resulted in high suppression of cercarial shedding. Also, El-Ansary *et al.(2000)* reported that *A. maritima* caused remarkable decrease in cercarial shedding in *Biomphalaria* snails treated with this plant powder.

In conclusion, the application of low doses of the dry powder of *Z. simplex* plant may play an important role in replacing the wide use of chemical molluscicides as they also show nearly the same effect on snail population without the severe degree of environmental damage.

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Table (1) : Molluscicidal activity of *Zygophyllum simples* on *Biomphalaria alexandrina* and *Bulinus truncatus* snails after 24 hours of exposure under laboratory conditions.

Snail species	LC_{50} ppm (Confidence limit)	LC_{90} ppm	Slope function	Sublethal concentrations		
				LC_0	LC_{10}	LC_{25}
B. alexandrina	42 (34-50.4)	65	1.2	4.2	27	34
B. truncatus	38 (31.2-48.4)	60	1.4	3.8	25	32

Table (2): Mortality (%) of *Biomphalaria alexandrina* snails maintained continuously in sublethal concentrationsof *Zygophyllum simplex*.

Duration of experiment (week)	Sublethal concentration of *Zygophyllum simplex*						Control	
	LC_0 (4.2 ppm)		LC_{10} (27 ppm)		LC_{25} (34 ppm)			
	Cumulative dead snails	Cumulative mortality (%)	Cumulative dead snails	Cumulative mortality (%)	Cumulative dead snails	Cumulative mortality (%)	Cumulative dead snails	Cumulative mortality (%)
0	0	0	0	0	0	0	0	0
1	6	12	8	16	12	24	3	6
2	12	24	16	32	20	40	5	10
3	19	38	22	44	28	56	8	16
4	25	50	29	58	40	80	14	28
5	31	62	36	72	44	88	17	34
6	33	66	39	78	48	96	20	40
7	37	74	43	86	50	100	25	50
8	40	80	46	92			28	56
9	42	84	48	96			32	64
10	45	90	50	100			36	72
11	49	98					36	72
12	50	100					36	72
13							36	72

Table (3): Mortality (%) of *Bulinus truncatus* snails exposed continuously to sublethal concentrations of *Zygophyllum simplex*

Duration of experiment (week)	Sublethal concentration of *Zygophyllum simplex*						Control	
	LC_0 (3.8 ppm)		LC_{10} (25 ppm)		LC_{25} (32 ppm)			
	Cumulative dead snails	Cumulative mortality (%)	Cumulative dead snails	Cumulative mortality (%)	Cumulative dead snails	Cumulative mortality (%)	Cumulative dead snails	Cumulative mortality (%)
0	0	0	0	0	0	0	0	0
1	8	16	12	6	18	36	2	4
2	18	36	22	44	25	50	4	8
3	24	48	27	54	32	64	7	14
4	28	56	33	66	44	83	13	26
5	36	72	42	84	48	96	16	32
6	40	80	45	90	50	100	21	42
7	42	84	48	96			23	46
8	45	90	50	100			26	52
9	49	98					30	60
10	50	100					32	64
11							37	74

Table (4) : Effect of sublethal concentrations of *Zygophyllum simplex* on infectivity of *Schistosoma mansoni* miracidia for *Biomphalaria alexandrina* snails.

Treatment	Number of exposed snails	Survived snails at first shedding		Infected snails		% Reduction
		Number	%	Number	%	
Control	50	46	92	38	82.6	
LC_0	50	38	76*	24	63.2	23.5*
LC_{10}	50	28	56***	15	53.6	35.1**
LC_{25}	50	22	36***	6	27.3	66.9 ***

Table (5) Effect of subiethal concentrations of *Zygophyllum simplex* on cercarial production of *Schistosoma mansoni* from infected snails.

Concentration (ppm)	Number of cercariae/ snail	Number of cercariae/snail/ stimulant	Prepatent period (days)	Duration of shedding (days)
LC_0	4547.35 ± 4321.1*	483.9 ± 383.1	30.2 ± 2.4	22.8 ± 4.4**
LC_{10}	1937.2 ± 1233.2**	253.5 ± 133.4*	32.4 ± 2.1	18.3 ± 4.9**
LC_{25}	866.4 ± 445.6***	186.4 ± 126.4***	34.6 ± 3.1*	10.5 ± 3.8***
Control	7194.9 ± 6430	584.6 ± 361.2	28.6 ± 3.6	44.2 ± 4.8

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