

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

Ahmed T. Sharaf El-Din, Fayez A. Bakry and Ahmed. A. Tantawy
Theodor Bilharz Research Institute, P.O. Box 30 Imbaba. Egypt

Key words : Molluscicides, *Zygophyllum*, *Biomphalaria alexandrina*,
Bulinus truncatus.

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 LC₅₀ values for this plant were 42 ppm and 38 ppm and LC₉₀ values were 65 ppm and 60 ppm for *B. alexandrina* and *B. truncatus* respectively. The sublethal concentrations (LC₀, LC₁₀ and LC₂₅) 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 (LC₀, LC₁₀ and LC₂₅) 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 LC₀, LC₁₀ and LC₂₅, respectively. Prepatent period of exposed snails to LC₀, LC₁₀ and LC₂₅ 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 LC₀, LC₁₀ and LC₂₅ 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 and 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 LC₅₀ and LC₉₀ 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 LC₀, LC₁₀ and LC₂₅. 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 (LC₀, LC₁₀ and LC₂₅) for 24 hours under room temperature (24 ± 1°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 LC₅₀ values for this plant were 42 ppm and 38 ppm and LC₉₀ values were 65 ppm and 60 ppm for *B. alexandrina* and *B. truncatus* respectively. The sublethal concentrations (LC₀, LC₁₀ and LC₂₅) 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 (LC₀, LC₁₀ and LC₂₅) 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 LC₀, LC₁₀ and LC₂₅ respectively. Similarly, no *B. truncatus* snails could survive more than 10, 8 and 7 weeks in the experimental groups maintained at LC₀, LC₁₀ and LC₂₅ 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 LC₀, LC₁₀ and LC₂₅ respectively with a reduction rate 23.5%, 35.1% and 66.9% respectively.

Prepatent period (Table 5) of exposed snails to LC₀, LC₁₀ and LC₂₅ 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 LC₀, LC₁₀ and LC₂₅ 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 LC₅₀ and LC₉₀ 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 *Australorbis 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.

REFERENCES

- Bakry, F.A. and Sharaf El-Din, A.T. (2000). Effect of sublethal concentrations of Bayluscide on *Biomphalaria alexandrina*. J. Egypt. Germ. Soc. Zool., 31 : 15-25.
- El-Ansary, A; El-Bardicy, S.; Solima, S.M. and Zayed, N. (2000). *Ambrosia maritima* (Damsissa) affecting compatibility of *Biomphalaria alexandrina* snails to infection with *Schistosoma mansoni* through disturbing some glycolytic enzymes. J. 1st Inter. Cong. Biolg. Sci., (ICBS) Tanta University
- El-Gindy, H.I. (1969a). Effect of molluscicide application against *Bulinus truncatus* in Iraq on the structure of eggs produced by treated snails. J. Egypt. Med. Assoc., 52: 141-150.
- El-Gindy, H.I. (1969b). Copper sulphate as a molluscicide against *B. truncatus* eggs in Iraq. J. Egg. Med. Assoc., 52 (4): 245-257.
- El-Gindy, H. (1975). Effect of Bayluscide or Mollutox application against *Biomphalaria alexandrina* from Egypt on the viability and size of egg masses produced by treated snails. J. Egypt. Soc. Parasitol., 4 & 5 : 127-137.
- El-Sawy, M.F.; Duncan, J.; De C. Marchall, T.F.; Bassiouny, H.K. and Shehata, M.A. (1983). The molluscicidal properties of *A. maritima* L. (Compositae) 1. Design for a molluscicide field trial. Tropenmed Parasit., 34: 11-14. Georg Thieme Verlag Stuttg art. New York.
- El-Sawy, M.F.; Duncan, J.; De C. Marchall, T.F.; Shehata, M.A.R. and Brown, N. (1984). The molluscicidal properties of *A.*

maritima L. (Compositae) 2. Results from a field trial using Dry plant material Tropemed. Parasit., 35: 100-104.

El-Sawy, M.F.; Duncan, J.; Amer, S.; El-Roweini, H. Brown, N. and Hills, M. (1987). The molluscicidal properties of *Ambrosia maritima* L.(Compositae). 3. A comparative field trial using dry and freshly-harvested plant material. J. Trop. Med. Parasit.,38: 101-105.

Gawish, F. M.(1997). Evaluation of combination of certain molluscicides against *Biomphalaria alexandrina* and the free living stages of *Schistosoma mansoni*. Ph.D. Thesis. Zoology Dept., Girls Coll., & For Arts & Sci., Ain Shams University.

Kloos, H. and McCullough, F.S.(1982). Plant molluscicides. J. Med. Plant. Res. (Planta medica),46 :195-209.

Litchfield, J.T. and Wilcoxon, F. (1949). A simplified method of evaluating dose-effect experiment. J. Pharmc. and Exper. Therap., 96: 99-113.

Mahmoud, M.B. (1993). Effect of certain pesticides on *Biomphalaria alexandrina* and the intramolluscan larval stages of *Schistosoma mansoni*. M.Sc. Thesis, Fac. Sci., Cairo Univ.

Mahran, G.H.; El-Hossary G.A.; Saleh, M.; Mohamed, A.M. and Motawe.H.M.(1977).Contribution to the molluscicidal activity of *Canna* species growing in Egypt. J.African Med. Plants, 1: 147-156.

Marston, A. and Hostettmann, K.(1985). Plant molluscicides. Phytochem., 24 : 639-652.

Mohamed, A.M.; El-Fiki, S.A.;El-Sawy, M.F. and El-Wakil, H. (1981). Effect of prolonged exposure of *B. alexandrina* to low concentrations of some molluscicides. J.Egypt. Soc. Parasit., 11 (2):295-311.

- Mott, K.E.(1987). Plant molluscicides. Edited by K.E. Mott. Published on behalf of the UNDP/World Bank/WHO.
- Rawi; S.M.; El-Gindy, H.I. and Abdel Kader, A. (1994). The effect of some fresh water pollutants on the survival and egg production of the snail *B. alexandrina*. J. Egypt. Ger. Zool.,13 (D): 273-288.
- Rawi; S.M.; El-Gindy, H; Haggag, A.M. Abou El-Hassan. A. and Abdel Kader, A.(1995). New possible molluscicides from *Calendula micrantha officinalis* and *Ammi majus* plants. I Physiological effect on *B.alexandrina* and *B.truncatus*. J. Egypt. Ger. Soc. Zool .,16 (D): 69-75.
- Rawi; S.M.; El-Gindy, H.I. and Abdel Kader, A. (1996). New possible molluscicides from *Calendula micrantha* and *Ammi majus*. II. Molluscicidal, physiological and Egg laying effects against *Biomphalaria alexandrina* and *Bulinus truncatus*. J. Eco toxicol. Environmental Safety, 35 (3): 261-267.
- Rizk, E.T. (1995). Studies on the effect of certain molluscicidal agents on the snail intermediate host of *Schistosoma mansoni*. Ph.D. Thesis, Fac. Sci., Tanta Univ. Egypt, 120 pp .
- Sherif, A.F. and El-Sawy, M.F. (1962). Molluscicidal action of an Egyptian herb. I. Laboratory experiment. Alex. Med. J., 8(2): 139-147.
- Shoeb, H.A. and El-Emam, M.A. (1976). The molluscicidal properties of natural products from *A. maritima*. Egypt. J. Bilh.,3 (2): 157-167.
- Tantawy, A.; Sharaf El-Din, A.T. and Bakry, F.A. (2000). Laboratory evaluation of the mollusciciding activity of *Solanum dubium* (Solanaceae) against *Biomphalaria alexandrina* snails. J. 1st Inter. Cong. Biolg.Sci.(ICBS) Tanta Univ.
- Viyanant, V.; Thirachantra,S. and Sornmani, S.(1982).The effect of controlled release copper sulphate and tributyltin fluoride

on the mortality and infectivity of *Schistosoma mansoni* cercariae. J. Parasitol., 56 :85-92.

Warren, K.S. and Weisberger, A.S. (1966). The treatment of molluscan *Schistosomiasis mansoni* with chloramphenicol. Amer. J. Trop. Med. Hyg., 15 :342-350.

Yarinsky, A. and Freele, H. (1970). A comparison of molluscicidal and mollusc inhibitory activity Hycathone and lacanthone and the effect of the drugs on the snail intermediate host, *Autoralorbis glabratus*. J. Trop. Med. Hyg., 73 : 23-37.

WHO (1965). Snail control in the prevention of Bilharziasis. Monograph Ser., 50 : 11-161.

WHO (1992). Mollusciciding in schistosomiasis control. WHO/Schisto/ 92. 107.

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

Duration of experiment (week)	Sublethal concentration of <i>Zygophyllum simplex</i>						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 sublethal 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.