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EVALUATION OF THE MOLLUSCICIDAL ACTIVITIES OF *ATRIPLEX STYLOSA* AND *AGAVE FEROX*

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تم تقييم فعالية المعلق المائى لنباتى اتريبلكس استيلوزا (عائلة كينوباديسى) وأجاف فيروكس (عائلة أجافيس) كمبيدات لقواقع بيموفلاريا الكسندرينا وبولينس ترنكاتس وليمنيا كايودى العوائل الوسيطة للبلهارسيا والدودة الكبدية وكانت قيم LC_{90} لنبات الاتربلكس هى ١٨٠ ، ١٦٧ ، ١٦٢ جزء فى المليون خلال زمن تعرض قدره ٢٤ ساعة بينما كانت للاجاف هى ١٩٢ ، ١٨٥ ، ١٧٩ للثلاث قواقع على التوالى.

بدراسة فعالية بعض مستخلصات النباتين وجد أن المستخلص الميثانولى للنباتين له فعالية عالية ضد قواقع بيموفلاريا الكسندرينا حيث كانت LC_{90} لنبات أتربلكس هى ٨٠ جزء فى المليون بينما كانت لنبات أجاف فيروكس هى ٩٢ جزء فى المليون خلال زمن تعرض قدره ٢٤ ساعة.

وجد أن للنباتين تأثير سام ضد السركاريا الناقلة للبلهارسيا المعوية.

تمت دراسة تأثير بعض العوامل البيئية مثل (التغير فى درجات الحرارة ، أشعة الشمس ، الطمى ، التغير فى قيم pH ، التخزين) على فعالية النباتين وقد وجد أنهما لا يتأثران بتلك العوامل مما يرشح النباتين لمزيد من الدراسة لفصل المكونات الفعالة لهما مع دراسة فعاليتهم على المستوى الحقلى.

The molluscicidal activities of aqueous suspension of Atriplex stylosa (Family Chenopodiaceae) and Agave ferox (Family Agavaceae) were evaluated against Biomphalaria alexandrina, Bulinus truncatus and Lymnaea cailliaudi snails, the intermediate hosts of Schistosomiasis and Fascioliasis (LC_{90} for A. stylosa were 180, 167 and 162 ppm while for A. ferox were 192, 185 and 179 ppm) within 24 hours against the three snails respectively. Also, screening of some different extracts of the two plants against B. alexandrina snails revealed that the methanol extracts of both plants were most active (LC_{90} was 80 ppm for A. stylosa and 92 ppm for A. ferox) within 24 hours. On the other hand, it was found that both plants are toxic against Schistosoma mansoni cercariae.

Studying the effect of some simulated field conditions of the aqueous suspension of the two plants such as (different water temperatures, sun radiation, mud particles, different exposure times and pH values) proved that the activities of both plants are nearly stable under these conditions, therefore, it is recommended to evaluate these plants in the field as molluscicides and to isolate their active constituents.

INTRODUCTION

Schistosomiasis is one of the major health problems in many tropical countries, especially in Africa. The infection is transmitted by fresh water snails acting as intermediate hosts. Despite the success of some control programmes, the prevalence of this disease remains constant, largely because population growth and development of manmade water resources is continuing.[1] Treatment of water bodies with molluscicidal compounds is considered as important element in an integrated strategy for morbidity control, but as the use of synthetic molluscicides is impeded by the high costs, there is a demand for inexpensive alternatives.[2] Therefore, the attention was drawn to the use of botanical molluscicides with the hope to be cheaper, safe from environmental pollution and eeasy for application.[3-5]

In continuation to our investigations in the field of botanical molluscicides,[6-11] the present

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work reports on the molluscicidal and cercaricidal properties of two plants; *Atriplex stylosa* and *Agave ferox* as well as study the effect of some simulated field conditions on the potency of the two plants.

MATERIALS AND METHODS

Plant materials

Atriplex stylosa (Family Chenopodiaceae) was collected in June 1996 from Borg El-Arab, Alexandria, Egypt while *Agave ferox* (Family Agavaceae) was collected in March 1996 from El-Orman Botanical Garden Giza, Egypt. The two plants were identified by Prof. Dr. N. El-Hadidi, Professor of Plant Taxonomy, Cairo University as well as by specialists at El-Orman Botanical Garden. The plants were shade dried and finally powdered by electric mill.

Snails

The three snails under investigation; *Biomphalaria alexandrina* (shell diameter 8-11 mm) the intermediate host of *Schistosoma mansoni* and *Bulinus truncatus* (shell height 4 mm), the intermediate host of *Schistosoma haematobium* and *Lymnaea cailliaudi* (shell length 7-8 mm) the intermediate host of *Fasciola gigantica* in Egypt were collected from irrigation canals in Abou-Rawash, ten kilometers from Giza Governorate, Egypt which were not treated with any molluscicides. *B. alexandrina* and *B. truncatus* were maintained in aquaria filled with dechlorinated tap water and left under laboratory conditions (Temp. 25°C, pH 7.0-7.7) for three weeks before used in experimental tests while *Lymnaea* snails were used immediately. Dried lettuce leaves are added daily.

Preparation of *Atriplex stylosa* and *Agave ferox* extracts

50 g dry powder of each plant was separately extracted with petroleum ether, chloroform, benzene, ethyl acetate and methanol. Each extract was dried under reduced pressure. Molluscicidal activity of each extract was determined against *Biomphalaria alexandrina* snails.

Testing for molluscicidal activity

Series of dilutions of both aqueous suspensions and different extracts that would permit the computation of LC_{50} and LC_{90} were prepared. For each dilution, ten snails were added. The exposure time was 24 hours, followed by 24 hours as recovery period. Three replicates were run in each case. Procedures and statistical analysis of data were carried out according to the WHO 1953 and 1965 and Litchfield and Wilcoxon method.[12-14] Studying the effect of some simulated field conditions such as different water temperature, sun radiation, mud particles, different exposure times, storage and pH values on the molluscicidal activity of the aqueous suspension of the two plants under investigation were carried out according to the previously reported methods by Lemma (1970).[15]

Preliminary phytochemical screening of *A. stylosa* and *A. ferox*

The dry plant powder of the plants were screened for the presence of different constituents using the reported methods.[16-19]

Cercariae materials

Schistosoma mansoni cercariae were obtained from experimentally infected *B. alexandrina* snails. Infected snails were allowed to shed cercariae by exposing them in a small amount of dechlorinated water to artificial light at 28°C. The obtained cercariae were directly used in experiments.

Cercaricidal activity

The dry powder was tested for its cercaricidal activity using the techniques by Pellergrino and De Maria.[20] The cercariae were transferred to a small petri dish and aqueous plant suspension was added. Microscopical observation was carried out and a cercariae was presumed dead when all motion ceased. Two replicates were run in each case. The number of dead cercariae was determined after 30, 60, 90 and 120 min of exposure. Thereafter, few drops of Bouin's fluid were added to the solutions containing cercariae to kill the remaining living

ones. Thereafter, all exposed cercariae were counted and mortality rates after various periods of exposure were computed. Cercarial solutions containing no plant material were taken as control.

RESULTS AND DISCUSSIONS

Results of comparison of the molluscicidal potencies of aqueous suspensions of *Atriplex stylosa* and *Agave ferox* in Table 1 exhibited that:

- The activity of *A. stylosa* against the three snails; *B. alexandrina, B. truncatus* and *L. cailliaudi* was found to be higher than *A. ferox* (LC_{90} values for *A. stylosa* within 24 hours were 180, 167 and 162 ppm while for *A. ferox* were 192, 185 and 179 ppm).
- *Lymnaea cailliaudi* snails were more sensitive to the action of the two tested plants than *B. alexandrina* snails.
- *Bulinus truncatus* showed moderate sensitivity to the action of both plants (LC_{90} was 167 for *A. stylosa* and 185 for *A. ferox*).
- The activity of both plants increased by increasing the exposure period from 24 hours to 48 hours and this result is in full agreement with Abdel-Gawad *et al.* (1995) and El-Amin *et al.* (1992).[10,11]

In order to identify the nature of the active constituents of the two plants under investigations, their different extracts were tested against *B. alexandrina*. Only methanolic extracts of both plants were most toxic (LC_{90} was 80 and 92 ppm for *A. stylosa* and *A. ferox* respectively) whereas the other extracts; petroleum ether, benzene, chloroform and ethyl acetate showed no activity up to 300 ppm. This result suggests that the activity of both plants is associated with the presence of more polar substances and this conclusion was supported by results of preliminary phytochemical investigations which were carried out on the two plants and showed that the major constituents of *A. stylosa* are triterpenoidal saponins while *Agave ferox* has steroidal sponins. These results are in full agreement with the previous studies on other *Atriplex* and *Agave* species.[21-24]

Table 1: Molluscicidal activities of the aqueous suspensions of *Atriplex stylosa* and *Agave ferox* dry powders against *Biomphalaria alexandrina, Bulinus truncatus* and *Lymnaea cailliaudi* snails.

Snail species	Exposure time (hrs)	*Atriplex stylosa*			*Agave ferox*		
		LC_{50}	LC_{90}	S	LC_{50}	LC_{90}	S
B. alexandrina	24	148 (137.41-158.31)	180	1.41	154 (136.32-169.2)	192	1.24
	48	135 (126.21-142.5)	164	1.16	144 (132.81-153.63)	186	1.25
B. truncatus	24	138 (129.87-152.92)	167	1.13	142 (128.12-159.4)	185	1.22
	48	131 (120.81-143-92)	158	1.14	138 (125.0-152.21)	179	1.23
L. cailliaudi	24	133 (122.26-146.50)	162	1.15	136 (123.25-148.52)	179	1.21
	48	128 (118.25-137.52)	152	1.16	132 (119.36-143.81)	172	1.22

Before we recommend any plant for field trials, it is necessary to study the effect of some simulated filed conditions such as different water temperatures, sun radiation, mud particles, different pH values and storage on the potency of this tested plant. Therefore, the two plants under investigations were submitted to the effect of these factors and the results were discussed as follows:

1- Effect of different water temperatures

Different dilutions of aqueous suspensions of both plants were prepared and kept at 10°C, 25°C and 35°C. *Biomphalaria alexandrina* snails were exposed to these dilutions for 24 hours followed by 24 hours recovery period.

Results in Table 2 exhibited that at low temperature (10°C) a remarkable decrease in the molluscicidal activity of both plants was recorded while at high temperature (35°C), the mortality of snails recorded high percent. This means that the plant activity increases by increasing the water temperature and this result is in full agreement with the fact that the solubility of the active constituents of both plants (triterpenoidal or spirostanol saponins) increased by increasing the water temperature.[22-25]

2- Effect of sun radiation and mud particles

After exposing the different dilutions of aqueous suspensions of the two plants to sun radiation for 6 hours. *Biomphalaria alexandrina* snails were added to these concentrations for 24 hours followed by 24 hours as recovery period. Results in Table 2 showed that the activities of the two plants were not nearly affected by sun radiation.

Different dilutions of aqueous suspensions of both plants using water containing 10,000 ppm of mud particles were prepared then *Biomphalaria alexandrina* snails were exposed to these concentrations. From the results in Table 2, it is evident that plant activity recorded a small depression in the presence of mud particles compared with the control without mud. This depression may be due to the absorption or desorption of the active constituents on mud particles.[9,25]

3- Effect of pH values and storage

Biomphalaria alexandrina snails were exposed to different dilutions that were previously adjusted to pH 4, 7 and 9. Tests involving 24 hours followed by a similar recovery period were carried out. Results in Table 3 exhibited that the activities of the two plants recorded a small depression in acidic medium (pH 4) whereas it showed nearly the same percent of snail mortality in alkaline (pH 9) and neutral (pH 7) media. This depression in acidic medium may be attributed to partial hydrolysis of the active constituents (triterpenoidal or spirostanol) of both plants.[7-10]

Different dilutions of aqueous suspension of both plants were stored for one week at laboratory conditions then *B. alexandrina* snails were exposed to these dilutions for 24 hours followed by 24 hours as recovery period. The activity of both plants disappeared up to 300 ppm. This result may be due to complete biodegradation of the active constituents of both plants.[7,9,26]

Cercaricidal activity of *Atriplex stylosa* and *Agave ferox*

The effect of the two tested plant against *Schistosoma mansoni* cercariae (the living stage of *S. mansoni*) was evaluated and the results were listed in Tables 4 and 5. These results exhibited that:

- The two plants showed a considerable cercaricidal activity, but *Atriplex stylosa* has stronger activity than *Agave ferox* as total death of cercariae was obtained after being exposed to the plant molluscicidal concentration (180 ppm) for 1½ hour while in case of *Agave ferox* only 62.3% cercarial mortality were obtained after exposure to 200 ppm for the same time.
- Increasing the plant concentration reduced the time necessary to get higher cercarial mortality with the two plants. The cercaricidal activities of the two plnats are in complete accordance with that of *Phytolacca dodecandra, Tetrapleura tetraptura* and *Zingiber officinal*.[27,28]

Table 2: Effect of different water temperatures, sun radiation and mud particles on the molluscicidal activities of aqueous suspensions of *Atriplex stylosa* and *Agave ferox* dry powders against *B. alexandrina* after 24 hours exposure time.

Concentration (ppm)	Percent mortality of snails									
	Atriplex stylosa					*Agave ferox*				
	Temperature levels			Sun radiation	Mud particles	Temeprature levels			Sun radiation	Mud particles
	10°C	25°C	35°C			10°C	25°C	35°C		
300	100	100	100	100	100	100	100	100	100	100
250	90	100	100	100	100	60	100	100	100	80
225	60	100	100	100	100	40	100	100	100	70
200	40	100	100	100	80	20	100	100	100	50
180	20	100	100	100	60	10	80	100	90	20
160	10	80	100	90	30	0	50	80	60	0
150	0	50	80	60	10	0	20	50	30	0
140	0	30	60	30	0	0	0	20	10	0
130	0	10	30	10	0	0	0	0	0	0
120	0	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	0

Table 3: Effect of pH values and storage on the molluscicidal activities of aqueous suspensions of *Atriplex stylosa* and *Agave ferox* dry powders against *B. alexandrina* snails after 24 hours exposure time.

Concentration (ppm)	Percent mortallity of snails							
	Atriplex stylosa				*Agave ferox*			
	pH values			Storage	pH values			Storage
	4	7	9		4	7	9	
300	100	100	100	0	100	100	100	0
250	100	100	100	0	100	100	100	0
225	100	100	00	0	100	100	100	0
200	90	100	100	0	70	100	100	0
180	60	90	100	0	50	90	100	0
160	40	70	90	0	20	70	80	0
150	20	50	70	0	10	30	40	0
140	0	20	40	0	0	20	30	0
130	0	10	20	0	0	10	10	0
120	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0

Table 4: Cercaricidal activity of *Atriplex stylosa* dry powder against *Schistosoma mansoni* cercariae.

Exposure time (hrs)	Percent mortality of cercariae after exposure to the following concentrations						
	120	140	160	180	200	240 ppm	cont*
½	12.3	32.8	38.6	48.5	87.4	100	4.5
1	16.4	56.7	69.3	70.4	100	100	6.7
1½	19.4	82.2	89.5	100	100	100	9.5
2	25.5	90.3	100	100	100	100	11.5

* Control = Mortality of cercariae in tap water.

Table 5: Cercaricidal activity of *Agave ferox* dry powder against *Schistosoma mansoni* cercariae.

Exposure time (hrs)	Percent mortality of cercariae after exposure to the following concentrations						
	120	140	160	180	200	240 ppm	cont*
½	6.2	10.2	14.2	17.8	20.2	32.9	2.2
1	8.3	19.3	28.6	39.2	47.3	62.3	5.3
1½	13.2	25.3	34.2	48.6	62.3	86.5	10.3
2	18.5	37.2	48.5	59.2	78.5	100	14.2
2½	30	49.3	70.6	93.6	100	100	15.8

* Control = Mortality of cercariae in tap water.

Owing to the high molluscicidal and cercaricidal activities of the two plants under investigations as well as because of their stability under the effect of different semifield conditions, it is worthy to subject these plants for further comprhensive laboratory investigations.

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