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LABORATORY ASSESSMENT OF THE MOLLUSCICIDAL ACTIVITIES OF TWO LOCAL EGYPTIAN PLANTS

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ABSTRACT

In the search for new plant molluscicides, it was found that the dry powder of two diterpene -containing plants; *Euphorbia peplus* and seeds of *Azadirachta indica* exhibited high molluscicidal activity when tested against *Biomphalaria alexandrina* and *Lymnaea cailliaudi* snails; the Egyptian intermediate hosts of *Schistosoma mansoni* and *Fasciola* species. The recorded LC_{90} values of *E. peplus* were 50 ppm and 47 ppm against the two snails respectively , while that of *A. indica* were 100 ppm and 91 ppm. Different ages of *B. alexandrina* snails showed different susceptibility towards the action of both plants, with the newly hatched snails being the most sensitive age. Increase in temperature within the optimal range and increasing time of exposure improved the potency of the plants, while presence of mud and alkaline media reduced it . Storing the molluscicidal solutions of the plants for one week greatly reduced the plants toxicities against the snails . However, storing they dry powder of the plants for a whole year did not affect their potencies . Also, the effect of repeated application of LC_0 of the plants on the susceptibility of *B. alexandrina* was investigated . The absence of ovicidal activity of the plants against *B. alexandrina* eggs was discussed.

INTRODUCTION

As the chemotherapy of bilharziasis and fascioliasis has been always met with toxicity problems, combating the disease through snail control still considered as a main factor [1,2] . Plant molluscicides appear to have several advantages over synthetic chemicals , being simple, inexpensive and readily available in endemic areas [3-5]. Obviously, diterpenes are a group of natural products widely distributed in higher plants [6]. Diterpenes- containing plants ; *Croton macrostachys* and *Annona senegalensis* were known to possess strong molluscicidal potency since 1976 [6,7]. Later on, the dry powder of *wedelia scaberrima, Jatropha gossypiifolia* and *Baccharis trimera,* known in the traditional medicine, were found to have high molluscicidal activity [9-11].

Numerous *Euphorbia* species with diterpenoidal constituents such as *E. cotinifolia* [12,13], *E. pulcherrima* [14] , *E. lactea* [15], *E. royleana* [16,17], *E. aegyptiaca* [18] , *E. tirucalli* [19] , *E. antisyphilitica* [20] , *E. pseudocactus* [21] and *E. splendens* [22] were reported to possess strong molluscicidal properties .

Euphorbia peplus , the most common *Euphorbia* sp. in Egypt is small annual glabrous weed. It is often used in many medicinal purposes in folk medicine [23, 24]. It has been also used in treatment of asthma and catarrh and its extracts are believed to be mitotic poisons and have proteolytic activities [25]. Recently **Osman** [26] reported on the molluscicidal activity of methanol and chloroform extracts of this plant against *B. alexandrina*, however, the molluscicidal properties of its dry powder needs to be studied.

On the other hand, *Azadirachta indica,* popularly known as Neem is an important traditional world wide medicinal plant. It exhibits antimalarial [27], fungicidal [28], antibacterial [29] and pesticidal activities [30]. Also the mosquito repellent action of its oil has been studied [31].

The present study deals with laboratory screening of the molluscicidal properties of the dry powder of two diterpenes - containing plants ; *E. peplus* and *A. indica* against *Biomphalaria alexandrina* and *Lymnaea cailliaudi* sanils . The effect of some environmental factors such as age of snails and repeated treatments on the plants activity were studied. Also, the ovicidal potency of the plants was examined. So this study, to our knowledge is a new contribution to previous work on Egyptian molluscicidal plants which addes more informations in this field .

MATERIALS AND METHODS

Plant materials :

The plants used in this study, *Euphorbia peplus* (Fam. Euphorbiaceae) and *Azadirachta indica* (Fam. Meliaceae) were collected from clover fields in Giza Governorate and from Cairo University Garden respectively. They were identified and different parts of each plant beside the whole plants were dried individually in shade and powdered .

Snail Materials :

Biomphalaria alexandrina (shell diameter 7-11mm) and *Lymnaea cailliaudi* snails (shell height 8-12 mm); the Egyptian intermediate hosts of *Schistosoma mansoni* and *Fasciola sp.* respectively were used in this study. Snails were collected from irrigartion schemes, not previously treated with molluscicides, in Giza Governorate. They were examined successively for natural trematode infections. Infected snails were discarded and adult healthy snails

were maintained to be accomodated to laboratory conditions for three weeks before being used in screening tests. They were fed on green lettuce leaves and exposed to normal day light.

Molluscicidal screening of the plants:

The procedures used for molluscicidal screening were that recommended by **WHO** [32] . Ten snails were added to each dilution and three replicates were used for each concentration. Snails were exposed to the molluscicidal solutions at 25 ± 2°C for 24 hours, at the end of it, snails were washed thoroughly with water and transfered to jars containing fresh dechlorinated tap water for another 24 hrs as recovery period. Dead snails are counted and recorded LC_{50} and LC_{90} of each part of the plant were determined, and the slope function in each case was calculated according to the method of **Litchfield and Wilcoxon** [33].

The effect of snail age on their susceptibility :

The snails used in laboratory toxicity tests are usually of moderate size , but in the field different ages of snails are encountered, so different ages of *B. alexandrina* were used . They were classified according to the following categories : newly hatched (1-2 mm) , juvenile (3-4 mm) , adult (7-11mm) and old snails (15-17mm). Snails were subjected to a series of different concentrations of the plants dry powder for 24 hrs followed by 24 hrs recovery period. Mortality of snails were thereafter recorded .

Testing the ovicidal activity :

Snails aquaria were supplied by floating plastic sheets, so snails could deposit their eggs on them. Then sheets containing *B. alexandrina* egg masses at different stages of development were transfered to the molluscicidal solutions of the plants for 24 hrs. Thereafter, treated and control egg masses were microscopically examined to define embryos mortality inside each egg mass.

Effect of some environmental factors:

Before field trials are started , more laboratory testing is also necessary and this was carried out according to **Lemma** [34] as follows :

i) Effect of time factor : Snails were exposed to the molluscicidal solutions of the plants for varying exposure periods 3,6,24 and 48 hrs.

ii) Effect of temperaterture: Different concentrations of the tested plants were prepared, supplied with snails and incubated at different temperatures 15, 25, 35°C for 24 hrs.

iii) Effect of pH : Dechlorinated tap water previously adjusted to different pH values (4, 7, 10) were used in preparing the molluscicidal solutions and then snails were added to them for 24 hrs: No follow up measuring of pH values was made .

iv) Effect of mud : 10,000 ppm of mud concentration was added to each of the molluscicidal concentration, thereafter snails were exposed to these concentrations for 24hrs.

v) Effects of storage: Solutions of freshly collected plants were prepared and stored at room temperature for 3 and 7 days. Also plants collected and stored as dry powder for a year were used to prepare other molluscicidal suspensions. Then snails were exposed to these concentrations for 24 hrs.

At the end of each of the above experiment , the recovery period was 24 hrs and snail mortality was recorded .

Effect of repeated application of LC_0 of the plants :

This experiment was designed to test the effect of repeated applications of sublethal concentrations (LC_0) of the two plants on the susceptibility of *B. alexandrina* snails . Large group of adult snails was subjected to the LC_0 of the two plants (10 ppm of *E. peplus* and 30 ppm of *A. indica* seeds) for 24 hrs. After the exposure period, snails were washed with water thoroughly and transfered to fresh water for three weeks . At the end of the three weeks, some of the snails were treated with the molluscicidal concentrations of the plants for 24 hrs (second treatment) to test their susceptibility . The other snails were subjected to LC_0 of the plants for another 24 hrs thereafter these snails were allowed to recover in fresh water for 3 weeks. After this recovery period, snails were exposed to the plants molluscicidal solutions for 24 hrs (the third treatment).

RESULTS

The molluscicidal activity of the plants :

Comparing the different LC_{90} values of different parts and the whole plant of *E. peplus* (table 1) showed that the leaves are the most active organ with LC_{90} =35 and 31 ppm against *B. alexandrina* and *L. cailliaudi* respectively , while seeds and roots were the least active organs as its LC_{90} were >200 ppm against the two snail species respectively . The whole plant and stem dry powder proved to posses marked molluscicidal activity having LC_{90} = 50 and 120 ppm respectively against *B. alexandrina* and 47 and 114 ppm respectively against *L. cailliaudi*. Also data presented in the same table proved that the outer shell of *A. indica* seeds was the most molluscicidally active organs as its LC_{90}, after 24 hrs of exposure of *B. alexandrina* and *L. cailliaudi* were 80 ppm and 72 ppm while LC_{90} of the inner part of the seeds was above 200 ppm against both snails . The dry powder of the whole seeds also proved to possess high molluscicidal potency since their LC_{90} were 100 ppm and 90 ppm for the two snails . However, the recorded lethal concentrations of the leaves and stems were over 200 ppm.

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Table (1) : Molluscicidal screening of *Euphorbia peplus* and *Azadirachta indica* dry powder against *Biomphalaria alexandrina* and *Lymnaea cailliaudi* snails.

Plant (part tested)	Biomphalaria alexandrina			Lymnaea cailliaudi		
	LC_{50} (ppm)	LC_{90} (ppm)	Slope function	LC_{50} (ppm)	LC_{90} (ppm)	Slope function
Euphorbia peplus						
Roots	>200			>200		
Stems	60 (33.57-67.2)	120	1.69	56 (51.38-62.5)	114	1.37
Leaves	23 (18.55-28.52)	35	1.38	19 (16.7-22.56)	31	1.29
Seeds	>200			>200		
Whole plant	26 (19.4-34.84)	50	1.34	23 (18.2-26.52)	47	1.52
Azadirachta indica						
Stem	>200			>200		
Leaves	>200			>200		
Seeds (outer shell)	50 (40.96-63.9)	80	1.5	43 (37.5-49.3)	72	1.41
Seeds (inner part)	>200			>200		
Whole seeds	60 (48.0-75.3)	100	1.54	52 (43.5-59.3)	91	1.37
Whole plant	100 (89.28 - 112.5)	140	1.24	93 (78.4-99.5)	134	1.32

Molluscicidal effect of the plants on different ages of snails :

Results listed in table (2) proved that the newly hatched snails were the most sensitive to the action of the two plants, showing 100% mortality at concentration of 30 ppm of *E. peplus* and 60 ppm of *A. indica* . It was followed by old snails as they were completely killed at 40 ppm and 80 ppm of the two plants respectively. While snails of medium age are less susceptible to the effect of the plants . Using *E. peplus* , juvenile are more sensitive than adult snails , while using *A. indica*, the contrary was noticed and juvenile were more tolerant.

The ovicidal activity :

Results concerning the effect of exposing *B. alexandrina* eggs to the plants dry powder proved that both plants have no ovicidal activity since all egg masses treated with different molluscicidal solutions showed no death among them and hatched to viable young snails after the same incubation period.

Effect of environmental factors :

Data concerning the effect of some environmental factors on the toxic action of the plants (tables 3 and 4) proved that concentrations of the plants required to get 100 % snail kill are time dependant. At 3 and 6 hrs of exposure, even at high concentrations up to 140 ppm, low snail mortality were obtained. However, increasing the exposure period from 24 to 48 hrs increased slightly the toxic action of the plants as snails exposed to 40 ppm *E. peplus* showed 70% and 80% mortality after 24 and 48 hrs respectively. Also exposing snails to 50 ppm of *A. indica* for 24 and 48 hrs gave 40% and 50% mortality respectively. Results also

Table (2) : Effect of the dry powder of *Euphorbia peplus* and Azadirachta indica *on different age groups of* B. alexandrina

Plant Concentration (ppm)	% Mortality of snails			
	Newly hatched (1-2mm)	Medium age		Old snails (15-17mm)
		Juvenile (3-4mm)	Adult (7-11mm)	
E. peplus				
60	-	100	100	-
50	-	100	100	-
40	100	100	90	100
30	100	80	70	100
20	100	50	40	70
10	80	30	20	50
	50	10	0	20
A. indica				
100	-	100	00	-
90	100	80	90	100
80	100	60	80	100
60	100	40	60	80
40	80	0	20	50
30	70	0	0	50
20	50	-	-	30

showed that the increase in temperature markedly and positively affects the lethal action of the plants since it reaches its maximum action at 35°C. While lowering the temperature of the molluscicidal solution to 15°C greatly depressed the plants toxicities. Comparing the potency of the plants in different pH media many authors (4, 7 & 10) showed that at alkaline pH both plants activities are reduced and it reached zero at 30 ppm (10). At acidic media (pH 4) ; the activity of *A. indica* exceeds that at neutral medium, while in case of using *E. peplus* the activity is depressed.

The presence of 10.00 ppm of mud concentrations in the molluscicidal solutions of the two plants has a limited effect on the toxicity of the plants as there was a negligible decrease in snail mortality in presence of mud.

Storing the plants as aqueous suspensions for 3 and 7 days and testing their potency after these periods proved that both plants suffered from partial loss of activity after 3 days and great loss after 7 days. However, storing the plants for a year as a dry powder and screening their activity after this long period showed that there is a limited depression in their potency yet they are still active.

Table (3) : Effect of time and different water temperatures on the molluscicidal activity of Euphorbia peplus and Azadirachta indica dry powder against Biomphalaria alexandria.

Plant Concentrations (ppm)	% mortality of snails after exposure to the following plante													
	Euphorbia peplus							Azadirachta indica						
	Time				Temperature			Time				Temperature		
	3	6	24	48 hrs	15	25	35°C	3	6	24	48 hrs	15	25	35°C
140	50	70	-	-	100	-	-	10	50	-	-	60	100	-
120	30	40	-	-	100	-	-	0	20	100	100	50	100	-
100	10	20	-	-	90	-	-	0	10	100	100	30	90	100
80	0	10	-	-	80	-	-	0	0	80	100	20	70	100
70	0	0	100	-	70	100	-	-	-	60	80	10	60	80
60	-	0	100	100	50	100	100	-	-	60	70	0	50	70
50	-	-	90	10	40	90	100	-	-	40	50	0	30	50
40	-	-	70	80	20	70	100	-	-	20	40	-	10	20
30	-	-	40	60	0	30	50	-	-	0	10	-	0	10
20	-	-	20	30	0	10	30	-	-	0	0	-	0	0
10	-	-	0	10	0	0	10	-	-	-	0	-	-	0
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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Table (4) : Effect of pH values, mud particles and storage on the molluscicidal activity of Euphorbia peplus and Azadirachta indica dry powder against Biomphalaria alexandrina.

Plant Concentrations (ppm)	% mortality of snails after exposure to the following plante												
	Euphorbia peplus							Azadirachta indica					
	pH values			Mud	Storage for			pH values			Mud	Storage for	
	4	7	10		3	7 days	1 year	4	7	10		3	7 days 1 year
120	100	100	100	100	70	30	100	100	100	1000	100	50	0 100
100	100	100	100	100	70	20	100	100	100	80	80	30	0 100
80	100	100	80	90	50	0	100	100	90	60	70	20	0 70
70	90	100	70	80	40	0	100	90	70	30	60	0	0 70
60	70	100	50	60	30	0	90	80	60	20	40	0	- 60
50	50	90	30	30	0	-	70	80	50	20	40	-	- 40
40	30	70	10	10	0	-	50	50	30	10	20	-	- 20
30	20	40	0	0	-	-	30	20	0	0	0	-	- -
20	0	20	0	0	-	-	10	10	0	0	-	-	- -
10	0	0	0	0	-	-	0	0	-	-	-	-	- -
Control	0	0	0	0	0	0	0	0	0	0	0	0	0 0

Table (5) : Effect of repeated application of 10 ppm of *Euphorbia peplus* and 30 ppm of *Azadirachta iindica* on the susceptibility of *Biomphalaria alexandrina* snails.

Plant Concentration (ppm)	% mortality of snails exposed to the plants at:		
	1st treatment	2nd treatment	3rd treatment
E. peplus			
60	100	100	100
50	90	100	100
40	60	80	80
30	30	40	60
20	20	30	40
10	0	20	20
A. indica			
100	100	100	100
90	80	100	100
80	70	80	90
60	50	60	60
40	30	50	60
30	0	20	40
20	0	10	30

Effect of repeated applications of the plants :

Comparing the mortality percentage of adult *B. alexandrina* snails exposed to LC_0 for the first, second and third treatment of *E. peplus* (table 5) showed that snails in the second treatment are more susceptible than smails exposed to the plant for the first time . Also snails at the third treatment are less tolerant than snails at the first two treatments as they recorded high mortality ratio (60%) at the same molluscicidal concentration (30ppm).

Similar results were also obtained by exposing snails for three successive treatments to *A. indica* dry powder. Snails became very sensitive to the toxic action of the plant at the third treatment as they suffered very high death rate.

DISCUSSION

Attention is drawn to the restrictions of applying conventional methods of controling trematode infections such as schistosomiasis and fascioliasis. Plant molluscicides could have an important role in the furture control of these infections (35).

In the present study, screening the molluscicidal activities of the dry powder of *E. peplus* and *A. indica* against *B. alexandrina* and *L. cailliaudi* snails proved

that the two plants showed an excellent power to kill both snails at a considerably low concentrations. The present results support the previous reported data on *E. peplus* methanol and chloroform extracts [26]; however, using the whole powder of the plant without extraction would be more feasible and productive in application . Also it agrees with the results of the toxicity of *A. indica* to other snail species ; *B. pfeifferi* ; *L. natalensis* ; *L. acuminata* and *Indoplanorbis exustus* [36,37]. Comparing the potency of *E. peplus* with other Euphorbia plants proved that its molluscicidal activity is higher than many other *Euphorbia species* [12-22] . It can also be concluded that the leaves of *E. peplus* is the most active organ having LC_{90} = 35 and 31 ppm against both snails while in case of *A. indica* , the outer shell of the seeds was the most active part of the plant with LC_{90} =80 and 72 ppm against the two snails respectively. However, as *E. peplus* whole plant and *A. indica* whole seeds posses also a marked high potency and because of simplicity of application, these parts of the plants were chosen for carrying out laboratory comprehensive screeing. It can also be noticed from data in table (1) , that *L. cailliaudi* snails proved to be less tolerant to the action of the tested plants than *B. alexadrina* anails. This is in a full agreement with the previous results on the effect of *A. indica* on other *Biomphalaria* and *lymnaea sp.* as *Biomphalaria pfeifferi* proved to be more resistant than *Lymnaea natalensis* [36]. So detailed studies were carried out on *B. alexandrina* as the lethal concentrations necessary to kill it would be also lethal to the other less sensitive species *(L. cailliaudi)* .

Studying the phytochemical constituents of the plants proved that both plants posses more than one chemical constituents; diterpenes, carbohydrates ... etc., although they seem to be rich in diterpenes specially. These results are in good agreement with **Osman** [26] who separated three diterpenoid compounds from *E. peplus* and with **Siddiqui et al.,** [29] who reported on seven terpenoids in *A. indica* . This richness of terpenes in the two plants seem to be responsible for the high moulluscicidal influences of the two plants.

Different ages of snails showed different susceptibility to the toxic action of the plants . This is in complete accordance with the previous results [38]. It is reasonable that newly hatched snails ; being delicae and old senile snails are more sensitive to the action of the plants . So they must be excluded from any laboratory molluscicidal test as they will give fales results. The absence of ovicidal activity of the plants against *B. alexandrina* eggs resemble most of the other plant molluscicides [4] as their active constituents is characterized by molecules of high molecular weight which may hinder the molecular penetration of the thick gelatinous cover of the egg mass. However the non- ovicidal activity of the plant molluscicide can be overcome by repeated application of the plants because embryos which would not die from the toxic effect of the plant would die as soon as they hatch in the plant medium.

Investigations on molluscicides had shown that their efficacy depends not only on the concentration used and time of application, but also on some environmental factors which might affect the activity of any molluscicide [1&3] . In this respect , studying the effect of some of these factors , indicated that water temperature positively affects the molluscicidal potency of the tested plants . This agrees with the results obtained on other plant molluscicides [39]. This planomena could be explained by the increase in both the solubility of the active constituents of the plants and the vital activity of the snails associated with elevation of temperatue , so snails would be subjected to more active constituents at high temperature . Mud particles almost have no effect on the plants. Different pH values have shown different effects on the plants according to the nature of their constituents. Storing the plants as aqueous suspensions for one week depressed their activity greatly and this may be attributed to the great biodegradation of the plants active constituents in water. While storing the plants as a dry powder for over one year did not affect their potency which adds to the numerous advantages of the plants since *E. peplus* is an annual weed and can be collected , stored and applied at any time of the whole year without being affected or deteriorated .

Also snails that were previously subjected to several treatments of the two plants were more sensitive to the tested plants, which may give primary indications of no possibility of developing snail resistance so there would be no limitations on repreating application of these plants in the field . A major disadvantage of many synthetic molluscicides is that snails may acquire resistance to these molluscicides after being exposed to them for more than one time [40].

Many reporters discussed the safety of neem and proved that it may be recommended for consumption by humans [41] . It was also found that neem seeds represent a big ratio (45%) of the total weight of the plant [41]. , so we can obtain big amount of the neem seeds powder from its tree . At last , both plants are not only molluscicidal but also possess other numerous bioactivities [23-31], and that they are almost stable when subjected to semifield factors is another privilege. It can thus be concluded from all previous advantages of the two plants; *E. peplus* and *A. indica* that these plants could be excellent molluscicides for use in areas where focal transmission of schistosomiasis and fascioliasis are taking place .

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التقيم المعملى لنباتين محليين كمبيدات للقواقع

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معمل الكيمياء العلاجية . معهد تيودوربلهارس . أمبابه . جيزة

استمراراً للجهود المبذولة للتوصل إلى مبيد جديد من أصل نباتى وجد أن البودرة الجافة لاثنين من النباتات المحلية هما ايفوربيا بيبولس وبذور نبات ازادیراکتا اندیکا لهما قدرة ابادية عالية ضد قوقعى بيومفلاريا الكسند وليمنيا كايودى العائلين الوسيطين لطفيلى البلهارسيا المعوية والدودة الكبدية فى مصر – حيث كانت الجرعة المميته لنبات ايفوربيا بيبولس هى ٠,٥ ، ٤٧ جزء فى المليون ضد القوقعين على التوالى بينما الجرعة القاتلة لنبات ازادیراکتا اندیکا هى ١٠٠ ، ٩١ جزء فى المليون. ومن جهه أخرى بأختبار القدرة الابادية للنباتين ضد الاعمار المختلفة للقواقع تبين أن القواقع حديثة الفقس هى الاكثر تأثيراً بالنباتين . أيضاً بدراسة تأثير بعض العوامل البيئية على فاعلية النباتين تبين أنه بزيادة درجة الحرارة (حتى ٣٥°م) وكذلك زيادة زمن تعرض القواقع للمبيد زادت نسبة وفيات القواقع بينما قلت تلك النسبة فى وجود الطمى وفى الوسط القلوى . ويتخزين محاليل النباتين لمدة أسبوع أدى ذلك إلى أختفاء فاعليتها . أما بتخزين البودرة الجافة للنباتين لمدة عام كامل فأن فاعليتها لم تتأثر تقريباً . كما تمت دراسة تأثير التعرض المتكرر للجرعة الغير مميتة (LC_0) من النباتين على حساسية قواقع بيومفلاريا الكسندرينا ، وضح أن القواقع أصبحت أكثر حساسية لمبيدات . ومن جهة أخرى وضح أن بيض قواقع بيومفلاريا الكسند رينا لم تتاثر بالجرعة المميته من النباتين