

Evaluation of Some Wild Herb Extracts for Control of Mosquitoes, (Culicidae, Diptera)

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Abstract. The mortality and effects on developmental stages of *Culex pipiens*, in lyophilized aqueous extracts of local wild herbs (*Fagonia cretica*, *Chrozophora verbascifolia*, *Heliotropium bacciferum*, *Euryops arabicus*, *Rhazya stricta*, *Capparis spinosa*, *Calligonum comosum*, *Tribulus terrestris* and *Artemisia judaica*) were investigated in the laboratory. It was found that egg hatchability was significantly impaired, while emerging larvae suffered severe mortality in some extracts. Late instar larvae and pupae were more tolerant. However, at higher concentrations, significant larval mortality and decreased pupation were observed. Some of the herbs such as *R. stricta* and *C. comosum* may be promising in providing an environmentally safe mean of mosquito control.

Introduction

In spite of the fact that synthetic organic pesticides appear to provide a solution to the problems of pest control, it has become apparent that excessive reliance on their use is challenged by health, environmental and economic concerns, and emergence of pesticide resistant strains in many insect species in agriculture and in public health. In this respect, it was reported an increase in the DDT tolerance and dieldrin resistance in mosquitoes [1, p.341]. Similar studies demonstrated that *Culex pipiens* had resistance to DDT and dieldrin [2, p.731-744]. Numerous reports provided evidence that strains in several mosquito species developed considerable resistance to commonly used insecticides [3-10]. The use of non-conventional insecticides (aromatic sulphides, sulphoxides and sulphones) to avoid the development of resistance by mosquitoes revealed that sulphone derivatives were more toxic than their corresponding sulphide [11]. Numerous agents are currently being researched and extensively tested in private and public laboratories as potential candidates for replace-

ment of chemical control. There is great awareness for a need for new pesticides with a new or different mode of action and/or improved effectiveness, or safety over those currently in use, whose usefulness became limited. One approach to the control of pests is the use of biodegradable natural plant compounds. Many of these plant products have been shown to possess properties that would make them efficient pest control tools [12].

Our goal is to evaluate the biological activity of several species of Saudi wild herb-extracts as possible sources of botanical insecticides. These herbs are known to have some biological activity as being poisonous to higher animals, e.g. rats [13], or of medicinal importance where many of these herbs have been used by folk medicinal practitioners. For example, *Heliotropium bacciferum* is used topically or internally as a drink for scorpion bites; *Rhazya stricta* is used for the treatment of diabetes; *Caligonum comosum* is used as a decoction to treat toothache; *Artemisia judaica* decoction is used to treat fevers as well as skin diseases [14]. *Capparis spinosa* is used as a decoction to treat arthritis [15], and *Euryops arabicus* has a diuretic activity [16].

Materials and Methods

Materials

Nine Saudi wild herbs namely; *Fagonia cretica*, *Chrozophora verbascifolia*, *Heliotropium bacciferum*, *Euryops arabicus*, *Rhazya stricta*, *Capparis spinosa*, *Caligonum comosum*, *Tribulus terrestris* and *Artemisia judaica*, were collected from different areas of the Qassim province (Table 1).

Preparation of the compounds

Powdered air dried parts of each plant (200 g) were extracted with hot distilled water (1l) four times in a conical flask. The mixture was shaken then followed by filtration. The combined aqueous extracts were then freeze-dried using a Labconco Freeze dryer - 18 model 75018 for 48-72 hr. Yields (%) of lyophilized aqueous extracts is listed in Table 1. Stock solution was prepared from the lyophilized residue.

Insects

Eggs and larvae were obtained from a laboratory bred strain of *Culex pipiens* maintained on pigeon blood, at the College of Agriculture and Vet. Med. Experimental Station KSU, at Meleida.

Table 1. Botanical source (Families), Major constituents, parts used and local names of plants used

Botanical source (family)	Local name	Major constituents	Parts used	Yield (%) of lyophilized extracts
1 - <i>Fagonia cretica</i> (Zygophyllaceae)	Shoaikah	Saponins, Alkaloids,	Aerial parts	12.5
2 - <i>Chrozophora verbascifolia</i> (Euphorbiaceae)	Tannoum	Flavonoids	Aerial parts	20.35
3 - <i>Heliotropium bacciferum</i> (Boraginaceae)	Ramram	Alkaloids	Aerial parts	18.5
4 - <i>Euryops arabicus</i> (Compositae)	Kabour	Sesquiterpene, lactones, flavonoids	Leaves	20.5
5 - <i>Rhazya stricta</i> (Apocynaceae)	Harmal	Alkaloids, Flavonoids	Leaves	19
6 - <i>Capparis spinosa</i> (Capparaceae)	Shafallah	Glucosinolates, Flavonoids, Alkaloids	Leaves	18.5
7 - <i>Calligonum Comosum</i> (Polygonaceae)	Arta	Anthraquinones, Tannins	Leaves	19
8 - <i>Tribulus terrestris</i> (Zygophyllaceae)	Sharshar	Saponins, Flavonoids	Aerial parts	18.85
9 - <i>Artemisia judaica</i> (Compositae)	Ghazalah	Sesquiterpene, lactones, Flavonoids	Aerial parts	19.5

Procedure

Nine stock solutions of lyophilized aqueous extracts of the herbs were prepared in water (0.5 g/100 ml). Different concentrations of 100, 200, 300, 400, and 500 ppm were prepared from the stock solutions of *R. stricta*, *E. arabicus*, *H. bacciferum*, *C. verbasifolia* and *F. cretica*. Another series of concentrations (200, 400, 600, 800, and 1000 ppm) were made from the stock solutions of *C. spinosa*, *C. comosum*, *T. terrestris* and *A. judaica*. Freshly laid eggs were transferred from the stock culture into petri dishes (10 cm diam.) each containing 20 ml of the required concentration. Larvae were also transferred at the designated age from their stock cultures into the petri dishes containing the concentrations tested, each treatment was replicated at least three times. Larvae (including those emerged from the treated eggs) were fed *ad libitum* and kept under prevailing laboratory conditions.

Egg hatchability was determined at 24, 48, and 96 hr. after treatment. Mortality percentages were determined after 48 and 96 hrs and 10 days after treatment. Successful pupation and adult emergence percentages were recorded.

All tests were carried out under laboratory conditions (temperature $25^{\circ}\text{C} \pm 3$, R.H. 60 ± 5). Data were subjected to probit analysis and the effectiveness was expressed as LC_{50} [17, p. 333].

Results and Discussion

Effect on egg hatchability

The effect of the lyophilized aqueous extracts on egg hatchability is given in Table 2. The results showed that the hatchability of eggs was reduced with the concentration gradient. The extract of *R. stricta* was the most effective product on the rate of hatchability followed by *E. arabicus* and *F. cretica*. The corresponding hatchability percentages were 19.2, 54.8, and 61%, respectively at the concentration of 500 ppm of the last mentioned plant extracts. However, the lowest effective extract was *T. terrestris* (90% at 1000 ppm). These findings indicate that *R. stricta* gives the most promising effect as a botanical product against mosquito eggs. In comparison with the control, its extract reduced the egg hatchability percentage to 21.8%.

Effect on the larvae

The mortality percentages of mosquito larvae following plant extracts treatments at 2, 4, and 10 days were listed in Table 3. Data indicated that *R. stricta*, *C. comosum*, *F. cretica*, and *E. arabicus* extracts, were more effective against the mosquito

Table 2. Effect of some wild herb extracts on egg hatchability of *Culex pipiens*

Herbs	Concentrations (ppm)							
	100	200	300	400	500	600	800	1000
	Hatchability %							
<i>R. stricta</i>	70.1	66.7	52.8	37.2	19.2	N.T.	N.T.	N.T.
<i>E. arabicus</i>	76.3	76.2	72.7	59.5	54.8	N.T.	N.T.	N.T.
<i>H. bacciferum</i>	91.3	92.2	94.2	85.4	84.8	N.T.	N.T.	N.T.
<i>Ch. verbascifolia</i>	96.9	94.3	92.2	92.3	92.0	N.T.	N.T.	N.T.
<i>F. cretica</i>	96.4	86.6	81.1	70.3	61.0	N.T.	N.T.	N.T.
<i>C. spinosa</i>	N.T.	81.5	N.T.	81.0	N.T.	74.6	70.8	71.7
<i>C. comosum</i>	N.T.	85.7	N.T.	83.6	N.T.	85.9	77.9	68.3
<i>T. terrestris</i>	N.T.	97.5	N.T.	90.3	N.T.	93.8	94.8	90.0
<i>A. judaica</i>	N.T.	94.7	N.T.	92.1	N.T.	100	94.4	70.8
Control	97.4							

N.T. = Not tested

Table 3. Effect of some wild herb extracts on the mortality percentage of *Culex pipiens*

Conc. ppm	<i>R. stricta</i>			<i>E. arabicus</i>			<i>H. bacciferum</i>			<i>C. verbascifolia</i>			<i>F. cretica</i>			<i>C. spinosa</i>			<i>C. comosum</i>		
	(Days after treatment)																				
	2	4	10	2	4	10	2	4	10	2	4	10	2	4	10	2	4	10	2	4	10
Mortality %																					
100	16.7	35	38.3	4.6	9.1	10.6	0	0	3.3	0	0	0	0	1.6	3.3		N.T.			N.T.	
200	19.1	36.5	55.6	5	8.3	16.7	0	0	3.4	0	0	0	5.4	10	15	0	0	3.5	7.8	16.7	22.3
300	60.1	75	85.0	22.6	30.7	33.9	3.3	6.7	8.3	0	0	3.3	11.7	18.3	23.3		N.T.			N.T.	
400	80.3	91.8	100	21.7	33.3	41.9	6.7	6.7	11.7	0	4.8	4.8	15.9	20.6	28.6	0	0	8.6	8.1	54.4	61.2
500	84.1	96.9	100	23.3	46.7	58.3	11.7	15	18.3	1.7	8.3	11.7	25.0	43.3	51.7		N:T.			N.T.	
600		N.T.			N.T.			N.T.			N.T.			N.T.		5.4	16	18.3	11.4	60	71.5
800		N.T.			N.T.			N.T.			N.T.			N.T.		13.5	23.8	28.4	17.5	67.2	75.1
1000		N.T.			N.T.			N.T.			N.T.			N.T.		16.2	37.2	44.5	28.1	88.4	100

N.T = Not tested.

larvae. Table 4 summarizes the LC_{50} values of the herb extracts against the mosquito larvae 2, 4, and 10 days following application.

Table 4. LC_{50} values of lyophilized aqueous extracts of some herbs against larvae of *Culex pipiens*

Species	LC_{50} values (ppm)		
	2 days	4 days	10 days
<i>R. stricta</i>	280	230	190
<i>E. arabicus</i>	> 1000	600	520
<i>H. bacciferum</i>	> 1000	> 1000	> 1000
<i>F. cretica</i>	1000	580	490
<i>C. spinosa</i>	> 1000	> 1000	820
<i>C. comosum</i>	780	440	210

The results showed that 10 days LC_{50} values for the extracts of *R. stricta* and *C. comosum* were obtained at 190 and 210 ppm, respectively, indicating that they are relatively more toxic to the larvae compared to *H. bacciferum* and *C. spinosa* extracts whose respective LC_{50} values were > 1000 and 820 ppm, respectively. It seems logical to conclude that *R. stricta* and *C. comosum* are good candidates as botanical larvicides against mosquitoes, where they can serve as biodegradable natural plant products.

Effect on pupation

The percentage of larvae going into successful pupation after the herb extracts exposure is shown in Table 5. These findings demonstrated that no pupation took place following the treatment with the extract of *R. stricta* at 400 ppm level, while the rest of the herb extracts showed varying levels of pupation at the same concentration level (400 ppm). The pupation percentages of other extracts were varying, and ranged from 2.2 for *C. comosum* to 27.6 for *A. judaica*. In comparison, the pupation percentage in the control was 50.4%.

These results indicate that decline or failure in pupation is attributable to the larval exposure in the herb extracts.

Effect on adult emergence

The percentage of adults emerging following larval exposure in plant extracts are given in Table 5. The results indicated that adults did not emerge in treatments

Table 5. Percentage of successful pupation and adult emergence after treatment of *Culex pipiens* larvae with herb extracts

Concs. ppm	<i>R. stricta</i>		<i>E. arabicus</i>		<i>H. bacciferum</i>		<i>C. verbascifolia</i>		<i>F. cretica</i>		<i>C. spinosa</i>		<i>C. comosum</i>		<i>T. terrestris</i>		<i>A. judaica</i>	
	P	Ad	P	Ad	P	Ad	P	Ad	P	Ad	P	Ad	P	Ad	P	Ad	P	Ad
100	11.7	0	37.7	3.2	26.2	4.8	29.2	15.4	26.5	8.4	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
200	6.9	0	15.7	0.9	12.5	3.1	24.3	11.4	17.5	6.1	22.7	15.7	1.4	0	20.3	11.4	22.2	0
300	4.3	0	8.1	2.0	10.1	2.5	10.6	7.8	15.0	5.7	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
400	0	0	3.6	0	12.2	0	9.4	6.0	9.4	4.5	10.6	9.5	2.2	0	15.5	9.5	27.6	15.5
500	0	0	0	0	10.0	0.34	8.0	10.7	4.2	0	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
600	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	18.0	2.0	1.8	0	18.7	0	29.2	8.3
800	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	15.9	3.2	0	0	12.3	0	22.4	10.4
1000	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	11.6	23.3	0	0	14.3	15.9	63.3	4.1

P = Percentage pupation.

Ad = Percentage adults emerging.

N.T. = Not tested.

with extracts of either *R. stricta* or *C. comosum* at all concentrations tested. This is due to the fact that most of the larvae died before or during pupation.

Also, most of the larvae that were treated with 400, 500 and 600 ppm of *E. arabicus*, *E. cretica* or *T. terrestris* extracts completed their development to adulthood. Different percentages of adult emergence were observed at different treatment levels.

Finally, we can conclude that the Saudi wild herbs, *R. stricta* and *C. comosum* are promising as natural botanical products against all stages of *Culex mosquitoes* and could replace chemical insecticides if they have new modes of action and proved to be safe compounds. More research in the nature of these extracts, their site of action in insects, their effect on non-target organisms and environmental aspects, is needed before they can be recommended for large scale trials.

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اختبار كفاءة مستخلصات بعض النباتات البرية في مكافحة البعوض

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(قُدّم للنشر في ٢٢/٤/١٤١٥هـ؛ وقبل للنشر في ٦/٨/١٤١٥هـ)

ملخص البحث . أجريت دراسة معملية لتقويم أثر وكفاءة مستخلصات بعض النباتات البرية المحلية وهي شويكة، تنوم، رمرام، قابور، حرمل، شفلح، أرطي، شرشر، وغزالة، على بيض ويرقات وعذارى بعوضة الكيوليكس . أظهرت الدراسة تأثيراً سلبياً لبعض المستخلصات على فقس البيض، كما أن نسبة الموت بين اليرقات حديثة الفقس كانت عالية. رغم أن اليرقات المتقدمة في العمر والعذارى أظهرت تحملاً واضحاً لتأثير المستخلصات إلا أنه قد لوحظ وجود نسبة عالية من الموت بين هذه اليرقات وانخفاض في التعذر عند التركيزات العالية. يمكن اعتبار بعض هذه المستخلصات مثل مستخلص نبات حرمل، أرطي واعدة في تقديم بديل نظيف وسليم بيئياً لمكافحة البعوض.

