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Evaluation of Some Medicinal Plants for Control of *Culex pipiens* Mosquitoes.

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ABSTRACT

The study evaluated the efficacy of methanolic extracts of *Ruta chalepensis* L. (Rutaceae)*, Withania somnifera* (L.) Dunal (Solanaceae)*,Cleome paradoxa* (B. Br.) (Capparaceae) and *Heliotropium longiflorum* (Hochst & Steud. ex A. DC.) Jaub.&Spach (Boraginaceae) aerial parts against C*ulex pipiens* larvae*.* Different concentrations (100-500ppm) of the methanolic extracts of the plants were tested towards larval mortality and development of C*. pipiens* separately. Larval mortalities were counted at 2, 4 and 10 days after treatment. Egg hatchability was determined at 4 and 7 days after treatment. Percentage of successful pupation and adult emergence were determined by monitoring on daily basis until all adults in the control had emerged. All plants extracts exhibited variable activities. The greatest effect was with *R. chalepensis* which showed acute (2 days) and chronic (10 days) LC_{50} of 132.6 & 96.56 ppm, respectively. Larval mortality up to 84.47% &85.53%, were observed with C. *paradoxa* and *R. Chalepensis* respectively. Egg hatch was significantly reduced about equal with *R. chalepensis* and *W. somnifera* extracts. Concentration levels of *C. paradoxa* (≥200 ppm) and *H. longiflorum* (≥400ppm) showed significant hindrance to the larval development and reduction to resulting pupae and adults. Drastic development retardation was shown with extract of *C. paradoxa* leaves (300ppm), but only 15.3% & 5.6% of larvae reach pupal and adult stages respectively. The larvicidal activity of methanolic extracts of *R. chalepensis, W. somnifera, C. paradoxa*, *H.longiflorum* proved to be effective against C*. pipiens* larvae.

Keywords: Biological larvicides, *Culex pipiens, Ruta chalepensis, Withania somnifera, Cleome paradoxa*, *Heliotropium longiflorum*.

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INTRODUCTION

Undoubtedly, mosquitoes play most serious role in transmission of many zoonotic diseases worldwide, mainly in Tropic and Sub-tropic countries [1]. These diseases are parasitic as malaria and filariasis [2] or viral as Yellow fever, dengue [3],West Nile Valley and Rift Valley fever[4]. These infectious diseases are more or less encountered in the Middle East Countries [5]. Worldwide all water sources are common habitats for the immature stages of vector mosquito species and reducing mosquito-diseases morbidity in both urban and rural areas where a sufficient proportion of larval habitats can be targeted [6]. So, control of mosquito larvae is a public health importance in preventing emerging adults and their emerging pathogens [7]. Many plant derivatives proved to be effective against a wide array of insect species including mosquitoes [8] . Plants are a rich source of bioactive compounds as phenolics, terpenoids, coumarins and alkaloids [9]. These compounds including active specific target insects are biodegradable to non-toxic products and potentially suitable for the development of new classes of friendly insecticidal agents [10].

This study evaluated the efficacy of four plants; *Ruta chalepensis, Withania somnifera, Cleome paradoxa* and *Heliotropium longiflorum* as insecticidal agents for *Culex pipiens* larvae.

MATERIALS AND METHODS

Aerial parts of *R. chalepensis* L*., W. somnifera* (L.) Dunal**.**, *C. paradoxa R.Br. and H. longiflorum* (Hochst &Steud.ex A.DC.) Jaub.&Spach were collected from many parts in Al Taif District and kindly identified by Dr.Saleh Bazaid. Samples of these plants were deposited in the herbarium of Natural Products and Alternative Medicine, Faculty of Pharmacy, King AbdulAziz University (No.: RC1133, HL1021, CP1039 & WS1154, respectively).

Dried powdered plant materials (aerial parts, 5000g each) were separately extracted with methanol (3x700ml) at room temperature. The solvent was distilled off under reduced pressure, extracts were then freeze dried using a Labconco Freeze Dryer-18, model 75018 for 48-72hr and kept at 4^{o}C till needed.

Insects

Adults and 2nd instar larvae were from culture of *C. pipiens*, at the laboratory, reared on pigeon blood and 10% sucrose solution. Larvae were put in tap water. The experiment was done at Faculty of Meteorology, Environment and Arid land Agriculture.

Plants Extraction

Methanolic extracts of the four plants were prepared by dissolving the extract in warm distilled water (0.5g/100ml) using a sonication. Concentrations of 100, 200, 300, 400 & 500 ppm were prepared from each stock solution. Twenty freshly laid eggs and ten 2nd instar larvae were transferred from the culture into plastic cups (8x10cm), each with 30ml desired concentration. Treatments were in triplicate and control used only distilled water. Larvae were fed *ad-libitum* and kept under laboratory condition. Egg hatching was determined at 4th&7th days post-treatment. Mortalities were recorded at 2nd, 4th& 10th days; post-treatment. Pupation and emerging adult percent was determined by monitoring on daily basis.

Statistical analysis

Data were analyzed using maximum likelihood method& LC_{50} were calculated [11] and corrected for mortality [12]. Egg hatch was analyzed by variance. Significant differences ($p<0.05$) means were separated by Duncan's multiple range test [13].

RESULTS

Larvicidal activity

The mortality percentage of *C. pipiens* larvae treated with the four plant extracts and their LC_{50} values and 95% confidence limits (CL) at 2nd, 4th and 10th days of treatment were shown in Tables 1 and 2. Data

showed that larvae suffered up to 86 and 85% mortality after 10 days of exposure to 500 ppm for *R. chalepensis* and *C. paradoxa* extracts, respectively. However, the lowest concentration (200ppm) of *R. chalepensis* caused 42% mortality after 2 days of treatment. *H. longiflorum* extracts caused the lowest mortalities, while the highest concentration 500 ppm caused 72.2% mortality after 10 days of treatment. LC_{50} and 95% CL for each plant are given in Table 2. Data showed significant differences. Acute toxicity with the plant extracts ranged between132.60 and462.70 while chronic toxicity ranged between 96.56 and 249.70.

The results showed that 10 days LC_{50} values for *R. chalepensis* and *W. sominfera* were obtained at 96.56 and 132.81 ppm, respectively, indicating that they were relatively more toxic to the larvae compared to *C. paradoxa* and *H. longiflorum* whose respective LC_{50} values were 170.1 and 249.7 ppm. Therefore, we can conclude that *R. chalepensis* and *W. sominfera* are good candidates as botanical larvicides against mosquitoes, where they can serve as biodegradable natural plant products.

Ovicidal activity

Egg hatchability was significantly lowered ($p<0.05$) in all extracts than control (Table 3). At 100 ppm concentration, *W. sominfera* had the most severe effect on egg hatching was reduced by 29%. At the500 ppm, the methanolic extracts of the four plants reduced egg hatch by 79.4, 78.4, 56.43 & 36.67 % for *R. chalepensis*, *W. sominfera*, *C. paradoxa*and *H. longiflorum*, respectively. The results showed that egg hatchability was reduced in a concentration gradient. *R. chalepensis* and *W. sominfera* were the most effective plants on the inhibition of hatchability followed by *C. paradoxa* and *H. longiflorum.* These finding indicate the *R. chalepensis* and *W. sominfera* give the most promising effects as botanical products against mosquito eggs.

Pupicidal activity

The effect of the four plant materials on growth and development of *C. pipiens* larvae to adulthood were given in Table 4. There was considerable reduction in the percentage of larvae undergoing successful pupation in all treatments compared with control. No further larval development took place beyond the 2nd instar in the *R. chalepensis* 500ppm concentration. On the other hand all plant extracts had an evident inhibitory effect even at lowest concentrations 100ppm, where the successful pupations were only 20.07, 21.20, 39.27 & 68.13 for *R. chalepensis*, *W. somnifera*, *C. paradoxa* and *H. longiflorum*, respectively. Complete suppression of adult emergence was evident at 500 ppm concentration of *R. chalepensis* and *C. paradoxa*. The adult emerging percentages from 100ppm treatments were 10.0, 10.63, 14.879 & 39.0% for *R. chalepensis*, *W. somnifera*, *C. paradoxa* and *H. longiflorum*, respectively, compared with control.

Considerable biological activity related to the toxicity and hindrance of growth and development of the larvae of *C. pipiens* has been observed in this study. Of the four plant extracts, *R. chalepensis* was found to cause higher rate of mortality compared to other plant extracts. Previously, *R. chalepensis* was reported to be very effective for control of parasitic bee mite *Varroajacobsoni* [13](Zaitoon, 2001). Activity of *R. chalepensis* extracts could be attributed in part to alkaloidal content [14](El-Shanwani, 1996). *C. paradoxa* and *H.longifolium* exhibited a relatively mild acute effect on mosquito larvae especially in its lower concentrations. However, its chronic toxicity was more than 200 ppm. The results obtained in this investigation demonstrated the importance of toxic, growth and development-retarding influence of the plant extracts, *R. chalepensis* and *C. paradoxa* on *C. pipiens* mosquitoes. Moreover, application of these materials is not likely to leave harmful residues in the environment since they are naturally occurring among the local flora. A striking observation on the four plant materials investigated in the present work was that the length of exposure time of all extracts resulted in increased mortality, indicating that larvae cannot tolerate long exposures to such materials.

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Table 1: Mortality percentage of *Culex pipiens* larvae reared in media containing methanolic plant extracts.

Plant extract	Conc.(ppm)	Mortality		
		2d	4d	10d
R. chalepensis	100	$41.90\pm4.186^{a\ A}$	$45.00\pm5.00^{ab\ A}$	$52.23\pm5.084^{b\ A}$
	200	$42.20\pm8.404^{a\ A}$	$51.10\pm3.81^{a\ A}$	$55.57\pm13.891^{a\ AB}$
	300	$61.10\pm3.81^{a\ B}$	$65.53\pm5.084^{a\ B}$	$69.97\pm5.773^{a\ BC}$
	400	$67.77\pm11.693^{a\ B}$	$72.2\pm11.688^{a\ B}$	$78.90\pm10.179^{a\ C}$
	500	$73.3\pm8.825^{a\ B}$	$75.53\pm7.736^{a\ B}$	$85.53\pm3.868^{a\ C}$
W. somnifera	100	$40.00\pm8.825^{a\ A}$	$43.33\pm6.65^{a\ A}$	$45.57\pm5.095^{a\ A}$
	200	$41.133\pm7.678^{a\ A}$	$45.53\pm6.925^{a\ A}$	$47.77\pm3.868^{a\ AB}$
	300	$46.67\pm5.773^{a\ A}$	$48.90\pm6.965^{a\ A}$	$53.33\pm3.35^{a\ BC}$
	400	$52.20\pm1.905^{a\ A}$	$61.13\pm5.095^{b\ B}$	$68.87\pm5.095^{b\ C}$
	500	$70.00\pm6.7^{a\ B}$	$74.43\pm5.095^{ab\ C}$	$82.20\pm1.905^{b\ D}$
C. paradoxa	100	$22.23\pm3.868^{a\ A}$	$35.57\pm5.095^{b\ A}$	$43.33\pm3.35^{b\ A}$
	200	$27.77\pm5.085^{a\ AB}$	$43.33\pm3.35^{b\ A}$	$61.13\pm5.095^{c\ B}$
	300	$32.23\pm6.926^{a\ B}$	$61.13\pm5.095^{b\ B}$	$71.13\pm5.095^{b\ C}$
	400	$47.77\pm5.085^{a\ C}$	$68.87\pm5.095^{b\ BC}$	$76.67\pm3.35^{b\ C}$
	500	$61.10\pm1.905^{a\ D}$	$73.33\pm3.35^{b\ C}$	$84.47\pm3.868^{c\ D}$
H. longiflorum	100	$8.90\pm1.905^{a\ A}$	$17.8\pm1.905^{b\ A}$	$28.9\pm1.905^{c\ A}$
	200	$10.00\pm3.3^{a\ A}$	$18.90\pm1.905^{b\ A}$	$33.33\pm3.35^{c\ A}$
	300	$13.33\pm3.35^{a\ A}$	$28.90\pm1.905^{b\ B}$	$43.33\pm3.35^{c\ A}$
	400	$14.43\pm3.84^{a\ A}$	$35.57\pm1.963^{b\ C}$	$61.13\pm5.095^{c\ A}$
	500	$21.10\pm1.905^{a\ B}$	$46.67\pm3.35^{a\ D}$	$52.23\pm39.47^{a\ A}$

Conc. (ppm) concentration (parts per million)
Results were expressed as mean ± SE
The presence of different small letters in the same row indicating a significant difference in mortality
The presence of different capital letters in the same column indicating a significant difference between concentration by using Two Way ANOVA followed by Duncan's multiple comparison test at p 0.05>

Table 2: LC_{50} value and 95% confidence limits for *Culex pipiens larvae* in media containing methanolic plants extract

Plant extract	Assay time (days)	Slope	LC_{50} (95%CL)
R. chalepensis	2	1.40	132.60 (178.87-98.28)
	4	1.47	115.95 (159.23-84.28)
	10	1.80	96.56 (130.98-71.66)
W.somnifera	2	1.03	191.44 (257.91-111.91)
	4	1.17	149.45 (205.75-108.35)
	10	152	132.81 (174.71-100.82)
C. paradoxa	2	0.76	300.50 (195.6- 467.9)
	4	1.35	233.60 (131.3-387.9)
	10	1.44	170.10 (101.2- 321.5)
H.longiflorum	2	1.03	462.70 (305.9- 601.1)
	4	1.76	301.50 (190.3- 463.6)
	10	1.86	249.70 (141.3- 397.2)

LC_{50}= lethal concentration (ppm) at which 50%of the larvae showed mortality

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Table 3: Egg hatchability percentage of *Culex pipiens* in media containing methanolic plant extracts.

Plant extract	Conc.(ppm)	Mean ± S.D
R. chalepensis	100	72.43±2.503 e
	200	62.03±1.595 d
	300	52.47±1.290 c
	400	32.63±2.450 b
	500	20.60±3.195 a
	Control	98.10±0.100 f
W. somnifera	100	71.067±1.626 e
	200	63.133±4.202 d
	300	54.4±3.724 c
	400	32.8±2.961 b
	500	21.60±4.158 a
	Control	98.10±0.100 f
C. paradoxa	100	81.63±0.404 e
	200	75.267±0.252 d
	300	67.60±0.200 c
	400	50.10±0.361 b
	500	43.57±0.252 a
	Control	98.10±0.100 f
H. longiflorum	100	86.67±0.351 e
	200	81.73±0.153 d
	300	75.50±0.300 c
	400	69.33±0.351 b
	500	63.33±0.416 a
	Control	98.23±0.252 f

All values are represented as mean ± Standard Deviation. *= There is a significant effect of time by using One Way ANOVA at p< 0.05
The same letter means that there is no significant difference by using Duncan multiple comparison test at p<0.05
The different letters means that there is a significant difference by using Duncan multiple comparison test at p<0.05

Table 4: Successful pupation and adult emergence of *Culex pipiens* larvae reared in media containing methanolic plant extracts

Plant extract	Conc. (ppm)	Mean ± S.D (pupation)	Mean ± S.D (emergence)
R. chalepensis	100	20.07±1.704 c	10.00±5.00 c
	200	17.83±2.122 c	9.33±2.309 c
	300	6.90±2.307 b	6.90±2.307 bc
	400	3.63±1.193 a	3.63±1.193 ab
	500	1.10±1.100 a	0.00±0.00 a
	Control	100.00±0.100 f	100.00±0.100 f
W. somnifera	100	21.2±1.50 e	10.63±2.99 d
	200	17.83±2.403 d	7.93±1.686 cd
	300	11.77±1.474 c	6.767±1.33 bc
	400	7.63±1.665 b	3.767±0.702 b
	500	1.30±1.353 a	0.33±0.577 a
	Control	100.00±0.100 f	100.00±0.100 f
C. paradoxa	100	39.27±0.252 e	14.87±0.153 e
	200	29.2±9.354 d	8.600±0.200 d
	300	15.33±0.252 c	5.400±0.400 c
	400	8.57±0.351 b	2.233±0.252 b
	500	1.00±0.100 a	0.00±0.00 a
	Control	100.00±0.100 f	100.00±0.100 f
H. longiflorum	100	68.13±0.153 e	39.00±0.300 e
	200	67.3±15.762 d	20.20±0.200 d
	300	50.00±0.300 c	19.60±0.200 c
	400	31.43±0.252 b	10.00±0.300 b
	500	10.00±0.20 a	3.90±0.100 a
	Control	96.60±0.300 f	93.00±0.200 f

All values are represented as mean ± Standard Deviation. *= There is a significant effect of time by using One Way ANOVA at p< 0.05
The same letter means that there is no significant difference by using Duncan multiple comparison test at p<0.05
The different letters means that there is a significant difference by using Duncan multiple comparison test at p<0.05

DISCUSSION

Ruta chalepensis (Rutaceae) is a perennial herb widely used in folk medicine as an antirheumatic, antispasmodic, treatment for snake bites, headaches and wounds [14], and many biological activities such as insecticidal[15], larvicidal [16,17],and repellent activity [18]. Phytochemical studies revealed the presence of alkaloids, coumarins and flavonoids [19-22]. Toxic effect of *R. chalepensis* was also previously reported on whitefly and *Spodoptera littoralis*(Boised) [23,24]. Although the toxic mode of action of *R. chalepensis* on insects is not yet known, it might be attributed to its high content of alkaloids [25].

Withania somnifera (Solanaceae) is used as aphrodisiac, tonic, anthelmintic and narcotic by traditional medicine practitioners [26-28]. *W. somnifera*is rich mainly in alkaloids and anolides [29-32].The larvicidal potential against mosquitoes were proved [33-35], as well to its insecticidal effect on two termite species[36].

Few studies were reported the chemistry or biological activity of *C.paradoxa* (Cleomaceae). Some was investigated its anti-diabetic activity and isolated two flavonoids from the active ethyl acetate fractions[37], andisolated a new alkaloid and a new cembranoid diterpene from chloroformic fraction[38]. Different species of *Cleome* possess anthelmintic, insecticidal activity on *Spodopteralitura* [39], and larvicidal on cotton leaf-worm, *S. littoralis* [40]. Larvicidal potential of wild mustard (*Cleome viscosa*) against mosquito vectors was also investigated [41]**.**

Pyrrolizidine alkaloids as heliotrine, cynoglossine were reported in genus *Heliotropium* [42,43]. Wide variety of biological activities were reported for *Heliotropium* species as antitumor, antibacterial, antifungal, antispasmodic, mydriatic, mutagenic, teratogenic, hepatotoxic activity, insecticide and antifeedant activity [44-46]. *Heliotropium indicum* exhibited high potential against resistant and sensitive III & IV instar larvae of *C.quinquefasciatus* and *Anopheles gambiae* [46]. From the active methanolic fraction of *H. indicum* as anti-feedant, a new isoquinoline was isolated with comparable with those of standard insecticides [45].

Considerable biological activity related to the toxicity and hindrance of growth and developed larvae of *C. pipiens* was noticed. *R. chalepensis* caused high mortality rate compared to others. Activity of *R. chalepensis* extracts was attributed in part to alkaloidal content [47,48]. *C. paradoxa* and *H.longifolium* exhibited a relatively mild acute effect on mosquito larvae especially in lower concentrations. But, its chronic toxicity was more than 200ppm. The results showed the importance of toxic, growth and development-retarding influence of *R. chalepensis* and *C. paradoxa* on *C. pipiens*. Besides, application of these materials was not likely to leave harmful residues to environment since they are naturally local flora. A striking observation on the four plant materials investigated in the present work was that the length of exposure time of all extracts resulted in increased mortality, indicating that larvae cannot tolerate long exposures to such materials.

Many promising, economical and eco-friendly botanical larvicides were reported from the families' *viz.*Apiaceae, Ruta- ceae and Solanaceae [49,50]. Several phytochemicals as alkaloids, phenolics& terpenoids exit in plants [51] which may jointly or independently contribute to the generation of mosquito larvicidal activities [52]. There is continued interest in plants and plant extracts which are effective as control against mosquitoes developmental stages with various active compounds asazadiractins, plumbagin, β-sitosterol and others which are toxic against mosquitoes [10,14,16,18][53-55]. Quinoline and pyrrolizidine alkaloids are chemical composition of these plants' extracts with larvicidal activity. For successful application of these phytochemicals ingredients, one must understand the mechanisms of action in the target insects as well as the spectrum of insects affected by them.

CONCLUSION

This is a primary study on larvicidal activity of methanolic extracts of *R. chalepensis,W. somnifera,C. paradoxa*, *H.longiflorum.* The promising larvicidal, ovicidal and pupicidal activities were recorded for *R. chalepensis* and *W. somnifera.* Application of such extracts to mosquito breeding sites is practical importance as non-synthetic chemical control agents. More studies are ongoing to isolate and identify the active components of the promising extracts to be developed into effective formulations to be utilized in integrated vector control and to explore the multiple medicinal properties of these plants.

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