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EVALUATION OF SOME MEDICINAL PLANTS IN CONTROLLING *CULEX PIPIENS*

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Abstract

The study evaluated the efficacy of methanolic extracts of *Ruta chalepensis* (Rutaceae), *Withania somnifera* (Solanaceae), *Cleome paradoxa* (Capparaceae) and *Heliotropium longiflorum* (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 & 10 days after treatment. Egg hatchability was determined at 4 & 7 days after treatment. Successful pupation and adult emergence percentage were recorded daily till all control adults 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 without any human or animal risk.

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

Introduction

Undoubtedly, mosquitoes play most serious role in transmission of many zoonotic diseases worldwide, mainly in Tropic and Sub-tropic countries (El Bahnasawy *et al*, 2013). These diseases are parasitic as malaria and filariasis (El Bahnasawy *et al*, 2010) or viral as Yellow fever, dengue (Aziz *et al*, 2014), West Nile Valley and Rift Valley fever (Himeidan *et al*, 2014). These infectious diseases are more or less encountered in the Middle East Countries (Mackey *et al*, 2014). Generally, all water sources are common habitats for the immature stages of mosquito species and reducing mosquito-diseases morbidity in both urban and rural areas where a sufficient proportion of larval habitats can be targeted (Kobayashi *et al*, 2008). So, control of mosquito larvae is important in preventing adults emerging and their pathogens (Osório *et al*, 2014).

Many plant derivatives proved effective against a wide array of insect vectors as mosquitoes (El-Hag *et al*, 1996). Plants are a rich source of bioactive compounds as phenolics, terpenoids, coumarins and alkaloids (Abdel-Hady *et al*, 2014). These compounds include active specific target insects are biodegradable to non-toxic products and potentially suitable for the development of new classes of friendly insecticidal agents (Mansour *et al*, 2004).

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

Materials and Methods

Aerial parts of *R. chalepensis*, ***H.** longiflorum*, *C. paradoxa* and *W. somnifera* were collected from many insecticide-free areas in Al Taif District and kindly identified by Dr. Saleh Bazaid. These plants and others 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 Dryer-18, model 75018 for 48-72hr and kept at 4°C till needed. Adults & 2nd instar larvae were from culture of *C. pipiens*, at the laboratory, reared on pigeon blood & 10% sucrose solution. Larvae were put in tap water. Experiments were done at Faculty of Meteorology, Environment and Aridland Agriculture.

Methanol extracts of all plants were prepared by dissolving the extract in warm distilled water (0.5g/100ml) using a sonication. Concentrations of 100, 200, 300, 400 & 500ppm were prepared from each stock solution. Twenty freshly laid eggs and ten 2nd instar larvae from culture were put 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. The 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 (Finney, 1971), and corrected for mortality (Abbott, 1925). Egg hatch was analyzed by variance. Significant differences ($p<0.05$) means were separated by Duncan's multiple range test.

Results

Table1: Mortality percentage of *Culex pipiens* larvae reared in media with methanolic plant extracts.

Plant extract	Conc.(ppm)	Mortality		
		2 days	4 days	10 days
R. chalepensis	100	41.90±4.186^{aA}	45.00±5.00$^{ab\ A}$	52.23±5.084^{bA}
	200	42.20±8.404^{aA}	51.10±3.81^{aA}	55.57±13.891^{aAB}
	300	61.10±3.81^{aB}	65.53±5.084^{aB}	69.97±5.773^{aBC}
	400	67.77±11.693^{aB}	72.2±11.688^{aB}	78.90±10.179^{aC}
	500	73.3±8.825 ^{aB}	75.53±7.736^{aB}	85.53±3.868^{aC}
W.somnifera	100	40.00±8.825^{aA}	43.33±6.65$^{ab\ A}$	45.57±5.095^{aA}
	200	41.133±7.678^{aA}	45.53±6.925^{aA}	47.77±3.868^{aAB}
	300	46.67±5.773^{aA}	48.90±6.965^{aA}	53.33±3.35^{aBC}
	400	52.20±1.905^{aA}	61.13±5.095^{bB}	68.87±5.095^{bC}
	500	70.00±6.7 ^{aB}	74.43±5.095^{abC}	82.20±1.905^{bD}
C. paradoxa	100	22.23±3.868^{aA}	35.57±5.095^{bA}	43.33±3.35^{bA}
	200	27.77±5.085^{aAB}	43.33±3.35^{bA}	61.13±5.095^{cB}
	300	32.23±6.926^{aB}	61.13±5.095^{bB}	71.13±5.095^{aC}
	400	47.77±5.085^{aC}	68.87±5.095^{BC}	76.67±3.35^{bC}
	500	61.10±1.905^{aD}	73.33±3.35^{bC}	84.47±3.868^{cD}
H.longiflorum	100	8.90±1.905^{aA}	17.8±1.905$^{b\ A}$	28.9±1.905^{cA}
	200	10.00±3.3^{aA}	18.90±1.905^{bA}	33.33±3.35^{cA}
	300	13.33±3.35^{aA}	28.90±1.905^{bB}	43.33±3.35^{cA}
	400	14.43±3.84^{aA}	35.57±1.963^{bC}	61.13±5.095^{cA}
	500	21.10±1.905^{aB}	46.67±3.35^{aD}	52.23±39.47^{aA}

Conc. (ppm) concentration (parts per million), presence of different small letters in same row =a significant difference in mortality, presence of different capital letters in column = 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 & 95% confidence limits for *C. pipiens larvae* in media with methanolic extract

Plant extract	Assay time (days)	Slope	LC_{50}(95%CL)= lethal*
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)
R. chalepensis	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 larvae showed mortality

Larvae suffered up to 86 & 85% mortality after 10 days exposure to 500ppm for *R. chalepensis* and *C. paradoxa* extracts, respectively. But, 200ppm of *R. chalepensis* caused 42% mortality after 2 days. *H. longiflorum* extracts gave the lowest mortalities, while 500ppm gave 72.2% mortality after 10 days post-treatment, with significant differences. Acute toxicity with the plant extracts ranged between132.60 & 462.70 while chronic one ranged between 96.56 & 249.70. The 10 days LC_{50} values for *R. chalepensis* and *W. sominfera* were at 96.56 & 132.81 ppm, respectively, showed more toxic to larvae compared to *C. paradoxa* and *H. longiflorum* with LC_{50} values were 170.1 & 249.7 ppm, respectively. So, *R. chalepensis* and *W. sominfera* are good candidates as botanical larvicides against mosquitoes, where they can serve as biodegradable natural plant products.

Table 3: Egg hatchability percentage of *C.pipiens* in media with methanolic extracts.

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

All values represented as mean ± Standard Deviation, *significant effect of time by using One Way ANOVA at p< 0.05, same letter = no significant difference by using Duncan multiple comparison test at p<0.05, different letters = a significant difference by using Duncan multiple comparison test at p<0.05

The egg hatchability was significantly low ($p<0.05$) in all. At 100ppm, *W. sominfera* gave most severe effect on egg hatching as reduced by 29%. At 500 ppm, the four plants' extracts reduced egg hatching by 79.4, 78.4, 56.43 & 36.67% for *R. chalepensis*, *W. sominfera*, *C. paradoxa* and *H. longiflorum*, respectively. Egg hatching was reduced in a concentration gradient. *R. chalepensis* & *W. sominfera* were the effective ones in hatchability inhibition followed by *C. paradoxa* & *H. longiflorum.* These showed that *R. chalepensis* and *W. sominfera* gave the promising effects against mosquito eggs.

Table 4: Successful pupation and adult emergence of *C.pipiens* larvae reared in media with methanol extracts

Plant extract	Conc. (ppm)	M±S.D (pupation)	M±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 represented as mean ± Standard Deviation, *significant effect of time by using One Way ANOVA at $p< 0.05$
Same letter = no significant difference by using Duncan multiple comparison test at $p<0.05$, different letters = a significant difference by using Duncan multiple comparison test at $p<0.05$

Considerable reduction was in percentage of larvae successful pupation in all treatments. No larva developed beyond the 2nd instar in *R. chalepensis* 500ppm. All plant extracts had an evident inhibitory effect even at 100ppm, as 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 at 500ppm with *R. chalepensis* and *C. paradoxa*. Adult emerging percentages with 100ppm were 10.0, 10.63, 14.879 & 39.0% for *R. chalepensis*, *W. somnifera*, *C. paradoxa* and *H. longiflorum*, respectively.

Discussion

Ruta chalepensis (Rutaceae) is a perennial herb widely used in folk medicine as an antirheumatic, antispasmodic, and a treatment for snake bites, headaches and wounds (Ghazanfar, 1994), and many biological activities such as insecticidal (Jeon *et al,* 2013), larvicidal (Mookey *et al,* 2002, Al-Myah *et al,* 2012), and repellent activity (Hadis *et al,* 2003). Phytochemical studies revealed the presence of alkaloids, coumarins and flavonoids (Ulubelen *et al,* 1994; El-Sayed *et al,* 2000; Farag *et al,* 2005; Emam and Mahmoud, 2005). Toxic effect of *R. chalepensis* was reported on whitefly and *Spodoptera littoralis* (Boised) (Al-mazraawi and Ateyyat, 2009; Emam *et al,* 2009). 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 (Shah *et al,* 1991).

Withania somnifera (Solanaceae) is locally known as *Sum El Far* or *Sum el firakh* and used as aphrodisiac, tonic, anthelmintic and narcotic by the traditional medicine practitioners (Patwardhan *et al,* 1988; Sharma and Dandiya, 1992; Karmegam *et al,* 1997), also described as an adaptive that enhances survival during stress (Singh *et al,* 1982). *W. somnifera*is riches with alkaloids and anolides (Schröter *et al,* 1966; Vitali *et al,* 1996; Ali *et al,* 1997). Its larvicidal potentiality against mosquitoes were proved (Banasal *et al,* 2011; Arora *et al,* 2011; Ghosh *et al,* 2012), as well as insecticidal effect on two termite species (El Sayed, 2011).

Few studies reported the chemistry or biological activity of *C. paradoxa* (Cleomaceae). Abdel-Sattar *et al.* (2009) studied its anti-diabetic activity and isolated two flavonoids from the active ethyl acetate fractions. Abdel Monem (2011) isolated a new alkaloid and a new cembranoidditerpene from chloroformic fraction. Different species of *Cleome* possess anthelmintic, insecticidal activity on *Spodoptera litura* (Phowichit *et al,* 2008), and larvicidal action on cotton leaf-worm, *S. littoralis* (Ladhari *et al,* 2013). Larvicidal potential of wild mustard (*Cleome viscosa*) against mosquito vectors was also reported (Bansal *et al,* 2014).

Heliotropium longiflorum (Boraginaceae) is a herbal plant. Genus *Heliotropium*is is well known with toxic unsaturated pyrrolizidine alkaloids as heliotrine, cynoglossine (Stegelmeier, 1999; Huxtable, 2001). Wide variety of biological activities were reported for *Heliotropium* species as antitumor, antibacterial, antifungal, antispasmodic, mydriatic, mutagenic, teratogenic, hepatotoxic activity, insecticide and antifeedant activity (Rizk, 1991; Dolui *et al,* 2011; Azokou *et al,* 2013). *Heliotropium indicum* exhibited high efficacy against resistant and sensitive 3rd & 4th instar larvae of *C. quinquefasciatus* and *Anopheles gambiae* (Azokou *et al,* 2013). From the active methanol fraction of *H. indicum* as anti-feedant, a new isoquinoline was isolated with comparable with those of standard insecticides (Dolui *et al,* 2011).

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. *R. chalepensis* has action against parasitic bee mite *Varro ajacobsoni* (Zaitoon, 2001). Activity of *R. chalepensis* extracts was attributed in part to alkaloidal content (El-Shanwani, 1996). *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, the application of these materials did not have any 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, Rutaceae and Solanaceae (Jacobson, 1989; Sivagnaname and Kalyanasundaram, 2004). Several phytochemicals as alkaloids, phenolics & terpenoids exit in plants (Wink, 1993) which may jointly or independently contribute to the generation of mosquito larvicidal activities (Hostettmann and Potterat, 1997).

There is continued interest in plants and plant extracts which are effective as control against mosquitoest developmental stages with various active compounds as azadiractins, plumbagin, β-sitosterol and others which are toxic against mosquitoes (Ghazanfar, 1994; Park *et al* 2000; Hmamouchi, 2000; Jang *et al*, 2002; Mookey *et al*, 2002; Hadis *et al*, 2003; Mansour *et* *al*, 2004). 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. challepensis, W. somnifera, C. paradoxa*, *H. longiflorum.* The promising larvicidal, ovicidal and pupicidal activities were *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 principals of these promising extracts to be developed into effective formulations utilized in integrated vector control and to explore the multiple medicinal properties of these plants.

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