Effect of Three Plant Extracts on Egg Hatchability and Larval Development of *Culex pipiens* L.

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ABSTRACT

The activity of methanolic plant extracts from *Rhazya stricta Cleome paradoxa* and *Heliotrobium bacciferum* has been investigated towards larval mortality and development of *Culex pipiens* L. Plant extracts exhibited variable biological activity. The greatest activity was observed for *R stricta* which showed LC 50s of 154.2 and 84.2 ppm, respectively after 2 and 10 days of exposure. Percentage of larval mortality was 95.33% in the treatment with extracts of *R stricta* and 84.8% in C. *paradoxa*. Egg hatchability was significantly reduced in *R stricta* concentrations. All concentrations of the plant extracts from *R stricta*, *C. paradoxa* (200 ppm and above) and *H. bacciferum* (400ppm and above) caused significantly high hindrance to the subsequent larval development and reduced both pupation and adult emergence. Drastic retardation of development was shown by *R stricta* extracts, where only 19.3% and 9 % of the larval managed to reach pupal and adult stages, respectively, when reared in very low concentration (100 ppm) of the extract. However, *C.paradoxa* and *H. bacciferum* were more effective at higher concentrations. Application of such plant extracts to mosquito breeding sites may have great practical importance in relation to non-synthetic chemical control of these serious disease vectors.

INTRODUCTION

Insect –transmitted diseases remain a major source of illness and death worldwide. Mosquitoes alone transmit diseases to more than 700 million people annually (Taubes 1997). These diseases including malaria, filariasis, yellow fever, dengue and Japanese encephalitis, contribute significantly to poverty and social debility in tropical countries (Jang, *et al.*, 2002 and Rajkumar and Jebanesan 2005). Control of such diseases is becoming increasingly difficult because the over production of detoxifying mechanisms of chemical insecticides has reported for *Culex* species (Severini, *et al.*, 1993).On the other hand, majority of mosquito species have developed high levels of resistance to microbial control agents (Rao, *et al.,* (1995). One alternative approach is the use of natural products from plant origin (Elhag, *et al.,* 1996). The botanical insecticides are generally pest specific and are relatively harmless to the non-target organisms including human beings. They are also biodegradable and harmless to environment. The phytochemicals derived plant resourses can act as larvicides, insect growth regulators, repellent and ovipositional attractant (Das et, al., 2003 and Venkatachalam and Jebanesan, 2001). One plant species may possess substances with a wide range of activities, like extracts from *Azadriachta indica* which showed antifeedent, antioviposition, repellent and growth- regulating activity (Schmutterer, 1995). Insecticidal activity of many plants against several insect pests has been demonstrated (Carlini and Grossi-de-Sa, 2002, Kundu, *et al.*, 2007, Boussada, *et al.*, 2008 and Bakhashwain and Zaitoun 2009). The three plants of *Heliotrobium bacciferum*, *Rhazya stricta and Cleome paradoxa* are available in Saudi Arabia and used in folk medicine (Migahid, 1978).

In this study the effects of Methanolic extracts of aerial parts from three plant species: *H. bacciferum*, *R. stricta* and *C. paradoxa* aerial parts, on egg hatchability and larval development of *Culex pipiens* mosquitoes, have been investigated.

MATERIALS AND METHODS

Insects: A *Culex pipiens* L. (Diptera: Culicidae) colony maintained in the laboratory for more than 10 years was used. Mosquitoes were held at 27±1 ◦C, 70±5% RH, and a photo regime of 14:10 (light:dark) hr. Adults were provided with a 10% sucrose solution as food source. A pigeon was introduced twice a week to the adults for blood feeding. Larvae were reared in dechlorinated water under the same temperature and light conditions and were fed daily with baby fish food. The experiment was carried out at the Faculty of Meteorology, Environment and Arid land Agriculture, King AbdulAziz University, Jeddah, Saudi Arabia..

Plant extracts: Plant materials of *Heliotrobium bacciferum*, *Rhazya stricta* and *Cleome paradoxa* were collected from different parts in Saudi Arabia. *H. bacciferum*, *C. paradoxa* and *R. stricta* leaves were air-dried for 48 hours, ground to fine parts and extracted with methanol at ambient temperature. A gentle warming to 35-40ºC was sometimes found necessary. The mixture was stirred for 30 min. by magnetic stirrer and left 24 hours. Then, it was condensed in a rotary vacuum evaporator of solvent in a water bath at 55ºC according to Chitra, *et al*. (1993).

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Test procedure: Stock solutions of the three plant methanolic extracts were prepared by dissolving the extracted in warm distilled water (at 0.5g/100ml water). Different concentrations of 100, 200, 300, 400 and 500 ppm were prepared from stock solution. Freshly laid egg mass (about twenty eggs) or ten second instars larvae were transferred from the culture into plastic cups (8cm diameter, 10 cm deep), each containing 30 ml of desired concentration. Treatments were triplicate and control had only distilled water. Larvae were fed *ad libitum* and kept under laboratory conditions. Egg hatchability was determined at 4 and 7 days after treatment. Larval mortalities were counted at 2, 4 and 10 days after treatment. Percentage of successful pupation and adult emergence were determined by monitoring on daily basis until all adults in the control have emerged.

Data were analyzed using maximum likelihood procedures and values of LC50 were calculated according to Finney (1971). Data were corrected for control mortality (Abbott, 1925). Data of egg hatchability were analyzed by analysis of variance. If significant differences ($p<0.05$) occurred, means were separated by Duncan's multiple range test.

RESULTS AND DISCUSSIONS

The mortality (%) of *C. pipiens* larvae treated with three plant extracts in methanol and their LC50 values and 95%confidence limits at 2, 4 and 10 days after treatment are shown in Tables 1 and 2. Data showed that 95.3 and 84.8% mortality of larvae reached after 10 days of exposure to 500 ppm *R. stricta* and *C. paradoxa* extracts, respectively. However, the lowest *R. stricta* concentration (100ppm) caused 45% mortality after 2 days of treatment. *H. bassifirum* extracts caused the lowest mortalities, wherase highest concentration (500ppm) caused 71.7% mortality after 10 days of treatment. LC50s and 95% confidence limits (CL) for each plant are given in Table 2. Data showed a significant differences. LC50 for second instar larvae were (154.2 and 84.2); 433.9 and 132.1) and (> 500 and 311.3) for *R. stricta*, *C. paradoxa* and *H. bassifirum*, respectively after 2 and 10 days from exposure. *R. stricta* was significantly more toxic at all exposure times than *C paradoxa* and *H. bassifirum*.

Egg hatchablity was significantly lower ($p<0.05$) in all extracts than control (Table 3). At 100 ppm concentration, *R. stricta* had the most severe effect on egg hatching rate which was reduced by about 31% compared with *C. paradoxa* and H. *bassifirum*. At their highest concentration (500 ppm), the three plant extracts reduced egg hatchability by about 83, 57 and 36%, for *R. stricta*, *C. paradoxa* and H. *bassifirum*, respectively. The effect of the three plant materials on growth and development of *C. pipiens*, larvae to adulthood are given in Table 4. There were considerable reduction in the percentage of larvae undergoing successful pupation in all treatments compared with control. No further larval development took place beyond the second instar in the *R. stricta* 500 ppm concentration.

Table 1. Mortality percentage of 2nd larvae of *Culex pipiens* larvae in media containing methanolic plant extracts at different exposure periods (2, 4 and 10 days)

Plant Extract	Conc. (ppm)	Mortality(%) 2d	4d	10d
Rhazya stricta	100	45.00	50.00	59.33
	200	53.31	60.00	75.00
	300	56.72	66.76	78.76
	400	64.31	76.00	90.00
	500	76.76	83.31	95.33
	Cont.	00.00	00.00	03.33
Cleome paradoxa	100	22.6	35.9	43.10
	200	27.8	42.6	61.00
	300	32.2	61.00	70.7
	400	48.2	68.6	76.7
	500	61.00	73.3	84.8
	Cont.	00.00	6.7	00.00
Heliotrobium bacciferum	100	8.20	17.3	27.8
	200	9.80	18.6	32.2
	300	12.5	26.3	42.60
	400	13.8	35.9	61.00
	500	18.6	46.6	71.7
	Cont	00.00	00.00	3.33

Table 2. LC_{50} values and 95% confidence limits of 2nd instar larvae of *Culex pipiens* larvae in media containing methanolic plant extracts

Plant Extract	Assay Time (days)	Slope	LC50 (95%CL)
Rhazya stricta	2	1.1	154.2(110.8- 214.1)
	4	1.3	113.2 (79.5- 160.7)
	10	1.8	84.3 (60.3- 117.7)
Cleome paradoxa	2	1.4	433.9 (95.4- 2004.4)
	4	1.6	232.8 (194.7-278.1)
	10	1.6	132.1 (103.3- 168.9)
Heliotrobium bacciferum	2		> 500 ppm
	4		> 500 ppm
	10	1.86	311.3 (78.4 – 1241.4)

Table 3. Egg hatchability percentage of *Culex* in media containing ethanolic plant extracts

Plant extract	Conc. (ppm)	Egg hatchability(%)*
Rhazya stricta	100	69.2cd
	200	58.0de
	300	49.3ef
	400	30.5g
	500	17.4h
	Control	98.0 a
Cleome paradoxa	100	81.7bc
	200	75.5c
	300	67.6cd
	400	50.0ef
	500	43.6ef
	Control	98.2a
Heliotrobium bacciferum	100	86.7b
	200	81.7bc
	300	75.5cd
	400	69.3cd
	500	63.0d
	Control	98.2a

*Means followed by the same letter are not significantly different at 5% level, Duncan multiple test.

On the other hand all plant extracts had an evident inhibitory effect even at their lowest concentrations (100 ppm), where the successful pupation were only 19.3, 39.3 and 68.1 for *R. stricta*, *C. paradoxa* and H. *bassifirum*, respectively. Complete suppression of adult emergence was evident in the 500 ppm concentration of *R. stricta* and *C. paradoxa*. The adult emerging percentages from the 100 ppm treatments were 9.0, 14.9 and 39.0% for *R. stricta*, *C. paradoxa* and H. *bassifirum*, respectively.

Considerable biological activity related to toxicity and hindrance of growth and development of the larvae of C. pipiens has been observed in this study. Of the three plant extracts, *R. stricta* was found to cause higher rate of mortality compared to other plant extracts. Zaitoon ,2001 found that *R. stricta* was very effective against Varroa destructor In the present study, the activity of *R. stricta* extracts has been attributed in part to the saponins and alkaloids in the seeds (El-Shanwani, 1996). *C. paradoxa* and H. *bassifirum* exhibited a relatively mild acute effect on mosquito larvae especially in its lower concentrations. However, after 10 days of exposure the toxicity was almost high above 200 ppm. The larvicidal activity of some plant extracts, essential oils and phytochemicals against *C. pipiens* have been demonstrated (Traboulsi *et al.*, 2005; Abdelgaleil 2006; Michaelakis *et al.*, 2007; Radwan *et al.*, 2008).

The results obtained in this investigation demonstrate the importance of toxic, growth and development-retarding influence of the extracted plant materials specially *R. stricta* 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.

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

Plant Extract	Conc.(ppm)	% successful pupation and adult emergence	
		Pupation	Adult emergence
Rhazya stricta	**100**	19.3	9.0
	200	16.6	7.3
	300	9.4	5.1
	400	6.1	2.2
	500	0.0	0.0
	Control	100.0	97.6
Cleome paradoxa	**100**	39.3	14.9
	200	23.9	8.6
	300	15.3	5.6
	400	8.6	2.2
	500	1.0	0.0
	Control	100	100
Heliotrobium bacciferum	**100**	68.1	39.0
	200	58.3	20.2
	300	50.0	19.6
	400	31.4	10.0
	500	10.0	3.9
	Control	96.6	93.2

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الملخص العربي

تأثير ثلاث من المستخلصات النباتية على فقس البيض وتطور اليرقات في بعوضه الكيولكس

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تم دراسة النشاط الأبادى للمستخلص الميثانولى لكل من *Rhazya stricta, Cleome paradoxa* و *Heliotrobium bacciferum* وذلك ضد يرقات وتطور بعوضة الكيوليكس. أظهرت المستخلصات النباتية المختلفة تأثيرات بيولوجية مختلفة.وكان الأكثر تأثيرا هو مستخلص نبات *Rhazya stricta* والذي أظهر فعل حاد بعد يومين وعشره أيام من المعاملة حيث كان التركيز القاتل ل50% من العشيرة 154.2 و84.2 جزء في المليون على التوالي. كانت نسبة الموت في اليرقات 95.33% عند المعاملة بمستخلص *R. stricta* و84.8% عند المعاملة بمستخلص *C.paradoxa* . انخفض فقس البيض بصوره كبيره في المعاملات الخاصة ب *R. Stricta* . كل التركيزات المستخدمة من *R. stricta* و *C. Paradoxa* (200جزء في المليون وأعلى) و400 جزء في المليون وأعلى من *H. bacciferum* سببت مشاكل في الانسلاخ بالنسبة لليرقات وكذلك قللت من تعذير اليرقات وتحولها إلى حشره كاملة. وقد أظهر مستخلص *R. stricta* أكبر خفض في التحول حيث كانت نسب التحول إلى عذراء 19.3% فقط بينما تحول 9% إلى حشره كاملة وذلك عندما كان التركيز المستخدم 100جزء في المليون. وقد وجد أيضا أن مستخلصات كل من *C. paradoxa* و *H. bacciferum* كانت فعالة عند التركيزات الأعلى. ومن هنا نجد أن المعاملة بهذه المستخلصات في بيئة نمو اليرقات تعطى نتائج أفضل من استخدام المواد الكيمائية التي تسبب ضررا للبيئة والإنسان والحيوان وكذلك قد تبدى الحشرات مقاومة لها.