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Effect of some plant extracts on *Culex pipiens* L. (Diptera: Culicidae)

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ABSTRACT: Four plants extracts were evaluated against *Culex pipiens* mortality and sub-lethal effects. All extracts killed the larvae increasingly with concentration. *Lantana camara* was the most active with LC_{50} equaled 29.3 ppm, followed by *Acalypha fruticosa*. and *Rhazya stricta* with 435.6 and 611.9 LC_{50} values, respectively. *Ruta chalepensis* caused <50%mortality. Pupation of larvae reached 95-36%, 86-14%, 94-29% and 97-51% by *A. fruticosa*, *L. camara*, *R. stricta* and *R. chalepensis* extracts with EC_{50} equaled 711.1, 355.7, 602.8 and >1000 ppm with adult emergence inhibition with EC_{50} values of 435.2, 363.3, 291.2 and 614.4 ppm, respectively. Several malformations in all stages were recorded.

Keywords: *Culex pipiens, Acalypha fruticosa* Forssk, *Ruta chalepensis* L, *Rhazya stricta* Decne, *Lantana camara* Robex, insecticidal *activity*

I. Introduction

Undoubtedly, mosquitoes are medically important playing serious role in transmission of many zoonotic diseases worldwide as malaria and filariasis [1], yellow fever and dengue [2], West Nile Valley and Rift Valley fever in the Middle East [3]. *Culex* genus is the vector of encephalitis and filariasis [4]. It transfers Cache-Valley (CV) and West Nile Virus, which causes infertility and malformations in ruminants [5]. Synthetic insecticides are effective in controlling mosquitoes but they destruct the environment and kill the non-target biota disrupting natural biological control [6] in addition to the insect resistance [7] development

Several studies have been using herbal substances for controlling mosquitoes [8] as a rich source of bioactive compounds as phenolics, terpenoids, coumarins and alkaloids [9] that are biodegradable to non-toxic products developing new classes of friendly insecticides [10]. Oleo-gum-resin from *Commiphora molmol* (Myrrh) harmfully affected the larval fat, muscles, gut and nervous tissues [11]. *C. pipiens* L. oviposition, hatchability and larval viability were reduced by *Solenostemma argel* (Del.) Hayne aerial parts extract when incorporated into rearing media[12]. Larvicidal activity of *Teucrium divaricatum* Sieber, *Mentha longifolia* (L.) Huds., *M. pulegium* L., *Melissa officinalis* L. and *Salvia sclarea* L. oils were more than that of temephos insecticide [13]. Oils of thyme, catnip, amyris, eucalyptus and cinnamon revealed larvicidal and repellent effects on this pest [14]. Its developmental periods, pupation rates and adult emergences were altered by several plant extracts [15]. *Acokanthera spectabilis* (Apocynaceae) fruits constituents showed

insecticidal potency against *Culex pipiens* 4th larval instar mortality, pupation and adult emergency [16]. *Acalypha indica* leaf extracts showed larvicidal and ovicidal activities on *Anopheles stephensi* [17].

This study was carried out to study the insecticidal effect of four plant species against *Culex pipiens* 4th larval instar mortality, pupation and its adopted adult emergency as well as their malformation effects against the different stages were recorded as an alternative control method of synthetic insecticides to avoid their resistance and environmental hazardous.

Table 1: Larvicidal effects of the tested plant extracts against *Culex pipiens* 4th larval instar; shown as mortality%± SD and LC_{50} values

Plant species	Mortality% at different concentrations (ppm)						LC_{50} ppm	95 % CL		γ^2	Slope± SE	RT
	0	100	300	500	700	1000		Lower	Upper			
A. fruticosa	3	5	17	36	51	64	435.6	374.4	506.3	5.0	1.8 ± 0.19	**1.48**
L. camara	0	14± 2.9	36± 4.8	62± 6.4	77± 10.8	86± 2.7	293.4	248.7	338.6	6.1	1.99 ± 0.2	**1.0**
R. stricta	4	6	15	47	56	71	611.9	539.3	705.5	1.3	2.2 ± 0.23	**2.09**
R. chalepensis	4	3	5	21	37	49	>1000					

Data are average of five replicates**;** **RT**, Relative toxicity among plant species; Degree of freedom = **3**

Table (2): Statistical comparison among the tested extracts larval mortality

Lethality of	The tested plant species									
	A. fruticos			***L. camara***			***R . stricta***			***R. chalepensis***
	Value (ppm)	95% CL Lower	95% CL Upper	Value (ppm)	95% CL Lower	95% CL Upper	Value (ppm)	95% CL Lower	95% CL Upper	Value (ppm)
25	186.8	141.7	228.3	134.3	100.3	166.5	305.9	253.2	353.8	-
50	435.6	374.4	506.3	293.4	248.7	338.6	611.9	539.3	705.5	>1000
75	1015.7	833.8	1332.0	640.7	549.9	772.6	1223.9	1013.7	1593.8	>1000
90	2176.2	1605.6	3396.4	1294.1	1031.8	1767.2	2284.2	1728.7	3434.9	>1000
95	3433.4	2359.2	5990.4	1970.9	1485.1	2935.3	3318.2	2368.9	5462.0	>1000
99	8074.9	4829.5	17457	4338.5	2916.4	7666.6	6684.3	4262.6	13082	>1000

95% CL, 95% confidence limit (ppm)

2. Materials and methods

I- Collecting and extraction of plant samples:

Four plant species (shrubs) were collected from the campus of Faculty of Science, King Abdel-Aziz University and classified by the plant herbarium in Department of biology, Faculty of Science, King Abdel-Aziz University the university. The tested plant species are *Acalypha fruticosa* Forssk., Family (Euphorbiaceae), *Lantana camara* Robex., Family (Verbenaceae), *Rhazya stricta* Decne., Family (Apocynaceae) and *Ruta chalepensis* L., Family (Rutaceae). The leaves of the studied plant species were collected, washed from dust and left for complete drying under the room temperature in shadow. The complete dried leaves were electrically pulverized to fine powder and 30 grams were individually extracted continuously using Soxhlet extractor with 100 ml of absolute ethanol at 40^{O}C. The resulted ethanolic extract was filtered to remove the un-extracted plant debris and the organic solvent was completely evaporated with rotary evaporator under vacuum at less than 40^{O}C to dryness. 1.0 gram of the produced dried extract was dissolved in 100 ml of water containing 0.5 ml of Triton X-100 as original toxicant solution of each plant species that were kept under cooling in opaque bottles till using**.**

II- Treated Animal

The insect *Culex pipiens* L. (Diptera, Culicidae) was used for evaluating the insecticidal activity of the tested plant species. The eggs was kindly provided by the unit of Dengue favor and vectors control, Biology Department, Faculty of Science, King AbdulAziz University, KSA. The eggs were cultured under the laboratory conditions at 27 ± 2.0 ^{O}C and 60-70% R.H. to enough censuses for the experimental use.

III- Insecticidal activity measurement:

The tested plant extracts were prepared by adding 1.0 ml to 99 ml of deionized water contains 0.5 ml of Triton X-100 was used for complete miscibility of the extract with water. The reared larvae were treated under laboratory conditions of 27 ± 2.0 ^{O}C and 60-70% R.H. The WHO standard test method for mosquito larvae [18] was used to test the insecticidal activities of the obtained plant extracts against the 4th instar larvae of mosquito (*Culex pipiens* L.) at the concentrations of 100, 300, 500, 700 and 1000 ppm. Twenty five larvae were used in each replicate and five replicates were considered as one treatment. Control was concurrently conducted under the same conditions. The larval lethality was determined daily and the dead larvae were removed. Mortality percents were determined [19]. LC_{50} (Lethal concentration which caused 50% mortality) was also calculated [20]. Inhibition of pupation and adult emergence were also studied. Pupation and adult emergence percentages were calculated from the alive larval numbers by the end of exposure time. Dead stages were investigated for their morphological malformations under an anatomical microscope attached with a digital camera connected to a Computer set.

IV- Statistical analysis:

Mortality and larval weight reduction percents were analyzed using the analysis of variance (ANOVA) and Student-Newman-Kules Test. LC_{50} and EC_{50} values were determined using probit analysis method [24] at 95% confidence limit and 0.05% significance.

3. Result and dissection:

I-Insecticidal effects:

A. Larval mortality effects

In Table (**1**), the tested plant extracts caused relative lethal effects against the treated mosquito population in a function of the tested plant extract and the tested concentration. The lethality of the tested extracts increased with increasing their tested concentrations. *Lantana camara* Robex., Family (Verbenaceae) appeared significantly the most active extract as it caused lethal concentration of 50% (LC_{50}) of the treated population value equaled 293.4 ppm, followed by *Acalypha fruticosa* Forssk., Family (Fabaceae) and *Rhazya stricta* Decne., Family (Apocynaceae) with 435.6 and 611.9 LC_{50} values, respectively. *A. fruticosa* Forssk significantly exceeded the tested extract of *R. stricta* Decne. The extract of *Lantana camara* Robex. proved to be more lethal than *A. fruticosa* Forssk and *R. stricta* Decne extracts against the tested larvae based on the relative toxicity. The used ethanolic extract of *Ruta chalepensis* L., Family (Rutaceae) caused mortality percent less than 50% on the treated larval population at the highest used concentration, so it needs to increase its used concentration as it achieved LC_{50} value of > 1000 ppm.

Following the larval lethality caused by the tested plant extracts by determining the lethal concentration (LC) of 25, 50, 75, 90, 95 and 99% of the treated population emphasized the obtained results and proved that *L. camara* Robex. overcame *A. fruticosa* Forssk., which exceeded *R. stricta* Decne.in its effect. However *R. chalepensis* L., was less mortal than other tested extracts (Table 2).

B. Effect on pupation and adult emergence

The tested plant extracts affected the larval pupation of the treated insect population at the tested concentration. All the tested plant extracts weakly reduced the pupation of the still alive larvae at the lowest used concentration. This reduction effect increased with increasing the used concentration. *A. fruticosa*, *L. camara*, *R. stricta* and *R. chalepensis* tested extracts, respectively reduced larval pupation with 5- 64, 14-86, 6-71 and 3-49% of the originally treated population, achieving effective concentration on 50% (EC_{50}) of the treated larval population equaled 711.1, 355.7, 602.8 and >1000 ppm in the same order. The adult emergence from the pupated larvae was differently inhibited based on the used tested extract and even its applied concentration. This inhibition changed from 0 -78%, 0-98%, 0-91% and 0-69% of the treated population comparing with control. In case of *A. fruticosa*, *L. camara*, *R. stricta* and *R. chalepensis* leaves ethanolic extracts with EC_{50} values of 435.2, 363.3, 291.2 and 614.4 ppm in the same arrangement (Table **3**). It was noticed that although *L. camara* used extract was the most lethal against the treated larval population, the tested *R. stricta* extract exceeded it in its inhibitory effects against pupation and adult emergence, which may be referred to their different bioactive components. These results go with [21], who proved that 3.3% of the *Culex pipiens* larvae pupated and no adults emerged at 200 ppm of *R. stricta* methanol extract and *Lantana camara* and *Ruta chaliphenses* reduction of both pupation and adult emergence of *A. aegypti*.

C. Malformation of the stages

On the other hand magnified photos proved some malformations in all stages of the treated insect. Comparing with the untreated larva that appeared normally in normal skeleton shape, the treated larvae appeared with some abnormalities including disturbance in pigments secretion, elongation in cephalothorax region and in some cases, the body nods are shortened and contracted (stunning effect). The produced pupae from the treated larvae showed Larval-pupal intermediate showing larval siphon and pupal trumpets (incomplete molting), disturbance in molting (pupation) as well as Un-melaninized pupa (albino pupa). Some pupae showed larval siphon attached to pupal paddles region. The produced adult stage was also malformed in more than one appearance including incomplete molting from pupa with several adhesions features of the body trinctiles with the pupal molting skeleton (Figure **1**).

The obtained results agreed with [22], who proved the insecticidal effects of *A. fruticosa* leaf extracts on *C. quinquefasciatus* larvae. This effect may be due to its content of alkaloids, tannins, saponins, flavonoids, resins and glycosides [23]. The lethal effect of the *L. camara* leaves extract may be referred to its essential oil sesquiterpenes mainly β-caryophyllene as α-Copaene, Germacrene D&B, α–Cubebene, β–Elemene, α–Guaiene, α– humulene, Aromadendrene, β–Selinene, α–Selinene, Caryophyllene oxide, Nerolidol, Spathulenol and included large amounts of δ–Cadinene [24]. It is proved that different contents of these compounds indicate different *L. camara* chemotypes or different harvest time [25]. *R. stricta* showed marked acute and chronic toxic effects, having an LC_{50} of 251and 140; 467 and 211 ppm, for the methanol and ether extracts, respectively [21]. The obtained effects of *R. stricta* Decne may be due to its contents of alkaloids, glycosides, flavonoids, tannins and triterpenes [26]. *R. chalepensis* showed acute and chronic *C. pipiens* larval mortality with LC_{50} of 132.6 and 96.56 ppm, respectively reducing egg hatch [27] and decreasing its growth index. Sublethal concentrations affected sterility in its progeny.

Our obtained LC_{50} and EC_{50} values differed from literature because of using different insect or solvent system in extraction as [28] proved that against *Culex pipiens* larvae, the toxicity of *R. chalepensis* differed according the used solvent in the extraction.

Regarding the environmental impact of the tested plant species, *R. stricta* is used in folk medicine for treatment of fever, sore throat, rheumatism, diabetes, inflammatory conditions and syphilis and similar types of pains [29] with anticancerous properties [30]. The family Rutaceae is used for treating snake bites, stomatitis, rheumatism, bronchitis and other diseases [31]. *Lantana camara* is one of the important medicinal plants [32].

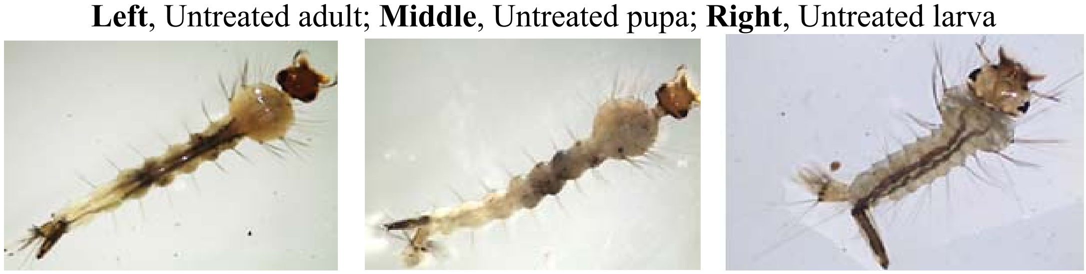
Table 3: Effects of the tested plant extracts against *Culex pipiens* pupation and adult emergence

Extract	Effect on	Effect at different concentrations (ppm)						EC_{50} ppm	95 % CL		γ^2	Slope± SE
		0	100	300	500	700	1000		Lower	Upper		
A. fruticosa	Pupae %	100	95	83	64	49	36	711.1	613.4	824.7	2.1	-2.1± 0.06
	Adults%	100	84.2	68.4	49.5	33.7	21.1	435.2	375.4	504.5	4.8	-1.8± 0.04
	% Adults inhibition	0	15.8	31.6	50.5	66.3	78.9					-1.8± 0.04
L. camara	Pupae %	100	86± 2.9	64± 4.8	38± 6.4	23± 5.9	14± 2.7	355.7	312.5	404.9	3.9	-2.2± 0.04
	Adults%	100	82± 1.6	48± 3.5	31± 4.7	14± 2.5	2.0± 0.7	363.3	230.9	300.3	8.2	-2.5± 0.04
	% Adults inhibition	0	18± 1.5	52± 1.9	69± 4.7	86± 2.5	98± 2.1					2.5± 0.04
R. stricta	Pupae %	100	94	85	53	44	29	602.8	531.5	683.4	7.3	-2.3± 0.06
	Adults%	100	78	57	33	24	9	291.2	250.3	338.7	6.3	-2.0± 0.04
	% Adults inhibition	0	22	43	67	76	91					2.0± 0.04
R. chalepensis	Pupae %	100	97	95	79	63	51	>1000				
	Adults%	100	95	79	59	43	31	614.4	539.2	700.1	1.2	-2.3± 0.06
	% Adults inhibition	0	5	21	41	57	69					2.3± 0.06

Figure 1: Several malformation of the treated C. pipiens stages

A. Un-treated stages

Left, Untreated adult; **Middle**, Untreated pupa; **Right**, Untreated larva



B. Malformations of the treated larvae

Left, disturbance in pigments secretion; **Middle**, elongation in cephalothorax region; **Right**, the body nods are shortened and contracted (stunning effect)

C. Malformations of the treated pupae

Left, larval pupal stage (incomplete molting); **Middle**, disturbance in molting (pupation); **Right**, Un-melaninized pupa (albino pupa)

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تأثير بعض المستخلصات النباتية على بعوضة الكيولكس

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الخلاصة:

تم تقييم اربعة مستخلصات نباتية على ابادة (موت)الطور الرابع ليرقات بعوضة الكيولكس *Culex pipiens* و مدى امكانية تعذير هذه اليرقات المعاملة و نشوء الأطوار الكاملة منها بالإضافة إلى التشوهات التى قد تعترى مختلف أطوار هذه الآفة. كل المستخلصات المختبرة سببت موت العشيرة المعاملة بصورة متزايدة مع زيادة التركيز المستخدم. ظهر مستخلص الـ Lantana camara الأكثر كفاءة يليه الـ Acalypha fruticosa يليه الـ *Rhazya stricta* محققين تركيز لازم لقتل 50% من العشيرة المعاملة قدره 29.3 ، 435.6 ، 611.9 جزء فى المليون. جاء مستخلص الـ*Ruta chalepensis* أقلهم فاعلية حيث حقق موت < 50% على أعلى تركيز مستخدم. أختزل تعذير العشيرة المعاملة من اليرقات بالمستخلصات المسختبرة فكان 95–36%, 86–14%, 94–29% and 97–51% فى حالة مستخلصات الـ *A. fruticosa*, *L. camara*, *R. stricta* and *R. chalepensis* على الترتيب بتركيز لازم للتأثير على 50% من الأفراد المعاملة قدره 711.1، 355.7، 602.8 و > 1000 على الترتيب. ثبطت هذه المستخلصات المختبرة عملية انسلاخ العذارى إلى طور الحشرة الكاملة بنسب تثبيط بلغت 78% و 98% و 91% و 69% على الترتيب بتركيز لازم لتثبيط 50% من العشيرة المعاملة قدره 435.2 و 363.3 و 291.2 و 614.4 جزء فى المليون على الترتيب. أظهرت كل المستخلصات المختبرة عدة تشوهات على كل أطوار الحشرة المعاملة.