Sub-Lethal Effect of Four Plant Extracts on Growth And Reproductive Parameters of *Culex pipines molestus* Forskal (Diptera: Culicidae) *

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التأثير تحت القاتل لمستخلصات أربع نباتات في نمو ومؤشرات تكاثر البعوض *Culex pipiens moletus* Forskal

الخلاصة

أجريت هذه الدراسة من اجل تقييم التأثير غير القاتل للمستخلص الايثانولي لأربعة انواع من النباتات في العمر اليرقي الرابع والعذارى وبعض مؤشرات التكاثر في بالغات البعوض Culex pipiens molestus Forskal التي تطورت عنها . اذ نتج عند التركيز 20 ج ف م لمستخلصات كارسيا *Carissa grandiflora* وشجرة الجنة *Alianthus altissima* و السذاب *Ruta graveolens* زيادة معنوية لمدة العمر اليرقي الرابع من 7,3 ايام للضابطة الى 9.7 و10.3 و12.8 يوما على التوالي فيما سبب الحنظل *Citrullus colocynthis* عند نفس التركيز نقصانا واكتمل بمدة 3.4 ايام. وقد اكتمل طور العذراء وبمدة اطول من المجموعة الضابطة وبشكل معنوي للمستخلصات الاربعة.

لقد أحدث التأثير تحت القاتل للمستخلصات الأربعة نقصا معنويا في عدد البيض للإناث المعاملة في عمرها اليرقي الرابع بالمستخلصات الأربعة وعند جميع المستخلصات بتراكيز (5، 10، 15، 20 ج ف م) المستخدمة. وقد اتبع النقص في عدد البيض نقصا في الخصوبة والانتاجية للإناث، كما وازدادت نسبة العقم مع زيادة تراكيز تلك المستخلصات لتبلغ عند التركيز 20 ج ف م 65.2 66.7 و73.19 و76.5% لكل من مستخلصات شجرة الجنة والكارسيا والحنظل والسذاب على التوالي.

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* البحث مستل من رسالة الباحث الثاني This paper was quoted from M.Sc. thesis of second researcher

ABSTRACT

The ethanol extracts of leaves *Alianthus altissima*, *Carrisa grandiflora*, fruits of *Citrullus colocynthis* and aerial parts of *Ruta graveolens* was tested against fourth instar larvae of the mosquito, *Culex pipines molestua* Forskal through sublethal doses at the concentrations 5, 10, 15 and 20 ppm. The extracts of *C. grandiflora, A. altissima* and *R. graveolens* were prolonged IV-P time with 9.7, 10.3 and 12.8 days at 20 ppm. Whereas, the period shorted to 4.3 days for *C. colocynthis* in comparison with control 7.3 days. All the plant extracts extended pupal period with significant time, but not more 3.1 days, and 2.1 days for untreated pupae. The reproductive parameters as egg laying hatchability, fecunidity, fertility and sterility percent were showed significant values of emerged adults treated with the plant extracts at IV larval instar.

Keywords: *Culex pipiens molestus*, growth disruption, fecunidity, fertility, sterility .

INTRODUCTION

Many mosquitoes species are agent vectors of the most threating diseases in tropics and subtropic areas like malaria, yellow fever, dengue fever, encephalitis and west Nile virus [1]. Also, mosquitoes transmit diseases among animals like fouwl pox of chicken, meyxomalosis of rabbits, rift valley of sheep and encephalitis of horses. Moreover, their hovering sound cause great discomfort[2].

In Iraq, there were 16 *Anopheles* spp. and 18 *Culex* spp. Distributed in all Iraq regions [3]. *Culex pipiens* L. represents mosquito species complex, the principle members of the complex are : *pipiens* and *molestus* [4,5]. *Culex pipiens* biotype *molestus*, the anthropophilic biotype of the *Culex pipiens* complex [6]. Secondary metabolic compounds of plants act as repellent, oviposition or food deterrent, growth inhibitors and significant prolongation of larval and pupal duration [7]. Plant bioinsecticides are alternatives to costly organic pesticides and usually safe to non-target organisms [8,9]. They are autodegradable and considered one of the safely methods of pests and vectors control [10,11]. Application of phytochemicals in mosquitoes control were in use since 1920 [12].

The objective of the present study was to examine effect of some native and implanted plants on larval and pupal duration of *C. pipiens molestus* and evaluate the effectiveness of some plant extracts against some biological aspects of this adult mosquito.

MATERIALS AND METHODS

Plant extracts

Leaves of *Carissa grandiflora* (Eckl.) A. DC (Apocynaceac), fruits of *Citrullus colocynthis* L. (Cucurbitaceae), leaves of *Alianthus altissima* L. (Simaroubaceae) and aeriel parts of *Ruta graveolens* L. (Rutaceas) were collected from the Mosul University park except *C. colocynthis* fruits were getting from the local market. The plant parts were washing and dried in shadow place then powdered with electrical grinder.

Method of the plant powers extractions was modified after Mekhlif [13], as fellows, 150 ml of 96% ethanolic alcohol was added to 50 gms. of each plant sample, the mixture was left for 48 hr for maceration in refrigerator at 4°C. the mixture was stirred for 24 hr. and filtrated through Whatman No.1 filter paper, the solvent was evaporated by lifting exposed to atmosphere over night. The dried extracts were kept in dark and constant 4°C until experimental treatment.

The mosquito,*Culex pipiens molestus* colony was established in the insectarium at Biology Department, Education College, Mosul University, Mosul City. The insectarium was adjusted at 27+2°C temperature, 70%+5 relative humidity under 16:8 light and dark photoperiod cycle. The larvae were fed with artificial food (powdered mixture of mouse diet and dried yeast powder in the ratio of 3:1) [14]. The molted pupae were transferred to new trays containing tap water and placed in screened cages (1x1xl m), where adults emerged. Adult mosquitoes were fed on a 10% sucrose solution, the three-day starved females fed from fresh blood of pigeon. The egg rafts produced due to adults mating were kept to continue next generation.

Bioassay tests

The fourth instar larvae of *C. pipiens molestus* were tested with different concentrations (5, 10, 15 and 20 ppm.) of selected plant extracts according to the modified WHO [15]. A total of 25 larvae were introduced in 250 ml plastic cups each containing one of the various concentrations of the applied plant extracts, the treatment were replicated three times.

Fecundity, fertility and sterility percentages of the developed adults were calculated as follow :

$$\text{Fecundity} = \frac{\text{Ova No. of treated female}}{\text{Ova No. of control female}} \times 100 \quad [16]$$

$$\text{Fertility} = \frac{\text{Larvae counted of the treated female}}{\text{Ova no. of the treated female}} \times 100 \quad [17]$$

Sterility = [1-($F_t \times F_{et} / F_c \times F_{ct}$)] x 100 [18]

F_t = fecundity females treated.
F_{et} = fertility females treated.
F_c = fecundity females control.
F_{ct} = fertility females control.

Pupal malformation percent was estimated by any change in color, size, shape or failure to develop to adult stage. Pupal malformation % = C/A x 100, where C = No. of malformed pupae and A = number of tested pupae.

RESULTS AND DISCUSSION

Effect of the extracts on the life cycle

Table 1 show significant disruption of fourth larval instar and pupal periods, the disruption in the life cycle ranged between elongation and shortening for those periods. The disruption range depends upon the plant species and its concentration. In comparison with the control period (7.3 days) extracts of *C. granliflora, A. altessima* and *R. graveolens* were extended IV-larval instar period especially *R. graveolens* (12.8 days at 20 ppm.). while *C. colocynthis* shorted this period up to 4.3 days, at 20 ppm. In spite of short pupal period (2.1 days) of normal pupae, but this stage may extended between 2.5-3.1 days as result of water of the pupae treated with 10, 15 and 20 ppm. of the plant extracts (Table 1). The active metabolic compound of the extracts were could be behaved as growth regulators as Agonist and antagonist hormones, so that, accelerated the molting hormones or inhibited them through interference with molting hormone receptors. [19,20]. This hormonal disturbance would led to either shortening nor extending of one or both larva and pupa duration with significance time, also Table 1 indicated to very notable malformation percent of obtained pupae from treated larvae. These malformations were significantly correlated with increasing of the extracts concentrations at 15 and 20 ppm.

As shown from the results of Table 1, the emergence average and emergency percent were affected by the plant extract and its concentration, where the adult emergence reduced to 68.0, 54.0, 54.0 and 48.0% at 20 ppm of *C. grendiflora, A. altissima, C. colocynthis* and *R. graveolens* respectivaly, compared to 100% of untreated group. The emergence failure of *C. pipiens molestus* adults was mainly related with the proteinaceous eclusion hormone inhibition, due to its denaturation through the extracts applications.

Table 1: Effect of different plant extracts on some aspects immature mosquito. *Culex pipiens molestus* reared in media containing different extract concentrations.

Plant species parts used	Conc (ppm)	IV-larva Duration	Pupal** duration	Malformed pupae %	Emergence average	Emergence %
C. grandifloral **(Leaf)**	**5**	***7.7 ± 0.6 fg**	**2.2 ≠ 061**	**5.2**	**23.7± 0.6 b**	**94.8**
	10	**8.3 ± 0.6 efg**	**2.2 ± 1.5 h**	**12.0**	**22 ± 1.0 c**	**88.0**
	15	**8.7 ± 0.6 def**	**2.4 ± 0.6 f**	**21.1**	**19.7 ±0.6 e**	**78.8**
	20	**9.7 ± 0.6 cd**	**2.5 ± 1.5 e**	**32.0**	**17.0 ± 1.0 hi**	**68.0**
C. colocynthis **(fruits)**	**5**	**6.3 ± 0.6 ih**	**2.3** ± 1.2 g	**14.8**	**21.3 ± 0.6 ed**	**85.2**
	10	**5.7 ± 0.6 i**	**2.5 ± 1.5 g**	**20.0**	**20.0 ± 1.5 e**	**80.0**
	15	**5.3 0.6 ij**	**2.7 ± 0.6 d**	**30.8**	**17.3 ± 1.2 gh**	**69.2**
	20	**4.3 ± 0.6 j**	**2.9 ± 1.5 b**	**45.2**	**13.7 ± 0.6 j**	**54.8**
A. attissiam **(leaf)**	**5**	**7.7 ± 0.6 fg**	**2.2 ± 0.6 h**	**18.8**	**20.3 ± 0.6de**	**81.2**
	10	**8.7 ± 0.6 def**	**2.4 ± 0.6 fg**	**26.8**	**18.3 ± 0.6 fg**	**69.9**
	15	**9.2 ± 1.0 cde**	**2.6 ± 1.2 e**	**36.0**	**16.0 ± 1.0 i**	**64.0**
	20	**10.3 ± 0.6 c**	**2.8 ± 0.6 c**	**41.2**	**14.7 ± 0.6 j**	**58.8**
R. graveolens **(Aried parts)**	**5**	**8.8 ± 1.0 def**	**2.4 ±1.0 fg**	**22.8**	**19.3 ± 0.6 ef**	**77.2**
	10	**9.8 ± 0.8 cd**	**2.5 ± 1.0 e**	**32.0**	**17.0 ± 1.0 hi**	**68.0**
	15	**11.7 ± 0.6 b**	**2.8 ± 1.15 c**	**41.8**	**14.3 ± 0.6 j**	**57.7**
	20	**12.8 ± 0.8 a**	**3.1 ± 1.53 a**	**52.0**	**12.0 ± 1.0 k**	**48.0**
Control	**0**	**7.3 ± 0.6 gh**	**2.12 ± 1.0 i**	**0.0**	**25.0 ± 0.0 a**	**100**

* - Different superscripts in the column indicate significance difference at P 0.05 level by Duncan's test.

** - Values are mean ± SD of 25 immature stages observed

Mosquito reproduction after the treatment :

Exposure of the 4th instar larvae of the mosquito, *C. pipiens molestus* to sublethal doses (5, 10, 15 and 20 ppm.) of the plant extracts were significantly reduced the mean number of eggs oviposited by obtained females. Table 2 indicated that mean of control was oviposited 227.5 and that number significantly reduced at all the applied concentrations. At 5 ppm treatment, the egg laying of the females decreased with 66, 79, 94 and 101 eggs for *A. altissima, C. grandiflora, C. colocynthis* and *R. graveolens* respectively. Also the fertility were slightly reduced at 5 and 10 ppm concentration, but those reductions decreased at 20 ppm from 94.9% for control to 77.4, 76.7, 67.1 and 61.5% for *A. altissima, C. grandiflora, C. colocynthis* and *R. graveolens* respectively. Also for the applied concentrations(5, 10, 15 and 20 ppm) in table 2, the sterility percent for the developed adults formerly treated with the extract at the larval stage ranged between 33.4-65.2% and 37.5-66.7% for *A. altissima* and C. *grandiflora* respectively. On the other hand, extracts of *R. graveolens* and *C. colococynthis* more effective and their sterility percent ranged between 46.5-73.1% and 50.4-76.5%.

Table 2 : Efficacy comparison among plant extracts through reproductive parameters of *Culex prpiens molestus*.

Plant extract	Conc. (ppm)	Eggs No./ Female **	Larvae No. Female /	Fecundity %	Fertility %	Sterility %
Carissa grandifora	5	*148.0 ± 5.6 b	135.0 ± 5.7 b	65.1	91.2	37.5
	10	129.5 ± 4.0 cd	113.0 ± 4.0 c	56.9	87.3	48.2
	15	111.0 ± 5.6 efg	93.0 ±5.9 def	48.8	83.8	56.9
	20	94.3 ± 4.1 hi	72.3 ± 4.1 gh	41.7	76.7	66.7
Alianthus altissima	5	159.0 ± 10.6 b	143.8 ± 10.5 b	69.96	90.4	33.4
	10	126.8 ± 7.0 cde	108.8 ± 7.27 cd	55.7	85.8	49.6
	15	113.5 ± 5.2 defg	93.03 ± 5.1 def	49.9	82.2	56.8
	20	97.3 ± 6.6 ghi	75.3 ± 6.7 g	42.7	77.4	65.2
Citrullus colocynthis	5	133.0 ± 5.7 c	115.5 ± 5.4 c	58.5	86.8	46.5
	10	116.8 ± 3.7 cdef	95.0 ± 3.7 de	51.3	81.4	56.0
	15	102.0 ± 4.2 fgh	77.5 ± 44.8 fg	44.8	75.9	64.1
	20	86.5 ± 3.9 hi	58.0 ± 3.4 hi	38.5	81.2	73.1
Ruta graveolens	5	126.0 ± 5.5 cde	107.0 ± 5.5 cd	56.4	84.9	50.4
	10	111.2 ± 2.2 efg	89.3± 2.2 efg	48.9	80.2	58.7
	15	101.3 ± 3.4 fgh	75.3 ± 3.4 g	44.5	74.3	65.1
	20	82.2 ± 2.1 i	50.8 ± 1.7 i	36.3	61.5	76.5
Control	0	227.5 ± 21.0	216.0 ± 22.2 a	100	94.9	0.0

** Values are mean + SD of 6 females

* Means followed by the same letter (vertically) are not significantly different at P 0.05 level by Duncan's test.

CONCLUSION

The extensive use of chemical pesticides inducing resistance in mosquitoes beside residues contamination of human food and environmental pollution. So the present study was designed to evaluate the sublethal effect of the plant extracts. An insecticide does not need to cause high mortality on target organisms in order to be acceptable. Besides toxic effect of the plant extract their active ingredients has interfering ability with the hormonal system of the insect and causing hormonal disruption, so cause extending of the life cycle, this meaning reducing the generations numbers of *C. pipiens moloustes* as multivolatine insect or reducing the females fecunidity through acceleration of growth and produce sterile or less fertile ones[7,19]. Due to gonads of the adult insects were histolysed after larval diet application with plant extracts [21]

The sub-lethal effect of the plant extracts contributes the toxic effect of the plant extracts and enhances their eco-friendly roles through sterility and reducing population density.

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