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The Effect of Various Plant Products on Mosquitoes Larvae *Anopheles arabiensis* and *Culex quinquefasciatus* (Diptera: Culicidae)

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Abstract:

This research aimed to study the larvicidal potentialities of three different local poisonous plants: ward elhameer *N*, ushar *U* and castor oil *R* (using different parts) leaves *L*, flowers *F* and seed *S*) against two important species of mosquitoes (*Anopheles arabiensis* and *Culex quinquefasciatus*), by using two different methods of application (powder P and aqueous extract aq-ex). The standard methods of WHO for rearing and assessment of mosquitos' susceptibility were carefully followed. The results revealed that, for *Anopheles* larvae, and according to the LD50 values, the products can be ranked from the more potent toward the least potent ones as follows: *RS*, *NL*, *UL*, *NF* and lastly *UF*. The potentiality arrangement for *Culex* larvae are as follows: *RS*, *UL*, *NL*, *NF* and lastly *UF*.

Introduction:

The order Diptera is of prime importance in medical entomology, because it contains the blood sucking forms, disease vectors and various casuals of nuisances to man and his livestock. When foraging, a blood -thirsty female mosquito flies upwind searching for the scent trail of an attractive host (White, 1991).The genus *Anopheles* includes over 400 species. *A. gambiae* Giles complex is among the major vectors of malaria and filariasis (White, 1974), while *A. arabiensis* Patton is the principal malaria vector in the Gezira area (El Safi, 1994). *A. pharoensis* is an important insect species in the Gezira, where it causes a great deal of discomfort by biting humans (El Safi and Haridi, 1986).

Culex quinquefasciatus Say is generally regarded as a subspecies. It is a tropical vector of urban filariasis (White, 1989). *C. quinquefasciatus*, a worldwide pest, the most nuisance mosquito, and the tropical vector of arboviruses, is widely distributed in the Gezira (Brown, 1986).The wide spread use of different types of insecticides for malria control has created serious problems through contamination of the environment. For all insecticides, fishes were found to be the least tolerant, followed by arthropods and then snails (Abdel Karim *et al*., 1985).

Natural products are substances or combinations of substances and elements found in nature, and used for the purpose of maintaining or improving health, treating or preventing diseases and control of vectors ((Balbaa, 1978).

Nerium oleander L. (Oleander: Apocynaceae) is green shrub bears a thick, leathery, whorled, arrow like leaves and various colored flowers. The latex contains some glycosides: Neriin, Oleanderin and Folinerin. This plant is very toxic because of its cardioactive glycoside Oleanderin (AOAD, 1988). Common names, geographical distribution, botanical description, parts used, chemical constituents, medicinal effects, cancer treatment and toxicology of *Nerium oleander* were well reviewed by: Rashan and Al-Allaf (1997) ; Moon and Park (1994) and Al and Al. (2000).

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Usher: *Calotropis procera* Ait. (Asclepiadaceae) is a desert milky plant that bears large, simple and opposite arrangement leaves and a violet green flower. The plant contains a caoutchouc substance. The latex contains very toxic cardiac glycosides (AOAD, 1988). *C. procera* is rich in different chemical compounds. The active principles of juice were calctin, calotropin, calotoxin and uscaridin, which are poisonous in nature (Chouhan *et al*., 1992). It has been used against the toxins of snakes and scorpions (Joshi, 1993). Castor *Ricinus communis* L. (Euphorbiaceae) is a green shrub with large palmate leaves and apical racemose flowers. Castor oil is composed of glycerides of several fatty acids, whereas ricinoleic acid is of most important. The oil contains also the ricinine alkaloid. The seed contains a very toxic resin (AOAD, 1988). *R. communis* is known to either stop the cell division proliferating tissues, resulting in sterility in insects, or to disrupt their physiological functions (Qureshi, *et al.*, 1997). Seed oil contains a very toxic lectin, ricin and ricinine (Holfelder *et al*., 1998).

Objective:

This work aimed to study the larvicidal potentialities of three different local poisonous plants: ward elhameer *Nerium oleander*, ushar *Calotropis procera* and castor oil *Ricinus communis* (using different parts: leaves *L*, flowers *F* and seed *S*) against two larvae of mosquitoes (*Anopheles arabiensis* and *Culex quinquefasciatus*), by using two different methods of application (powder and aqueous extract).

Materials and Methods:

1- Collection of Plant Materials:

All plant materials were collected from Wad Medani district (the central part of the Gezira area). Plant materials were included Oleander *Nerium oleander* (leaves (*NL*) and flowers (*NF*)), Giant milkweed *Calotropis procera* (leaves (*UL*) and flowers (*UF*)) and castor (*Ricinus communis*) seeds (*RS*). These plant parts were collected at early morning, manually cleaned, shade dried and crushed to fine powder using manual mortar and pestle. These plant materials were used later to test their larvicidal potentialities against mosquitoes larvae as raw powder and as aqueous extract.

2- Collection and Maintenance of Mosquitoes:

Larvae of *A. arabiensis* and *C. quinquefasciatus* were collected by means of dipping using a dipper net and half filled enamel container. The collected larvae were transferred and reared in the laboratories according to the standard method of rearing of mosquitoes (WHO, 1980).

3- Preparation of Aqueous Extracts:

The aqueous extracts for these plant parts were carefully prepared as was described by Balbaa,1978. 10 g of the powder of each plant part was soaked within 100 ml tap water. After 24 hour, the solution was filtered using ordinary filter paper and then used directly against the mosquitoes larvae in a series of concentrations.

4- Sustained Toxicity Tests:

One liter of tap water was kept in one-liter beakers, and to each beaker 20 larvae (3rd or early 4th instar) *A. arabiensis* or *C. quinquefasciatus* were placed. This represented a replicate. Certain weight of each crushed dried product (3 g/L) was placed on the water surface of each beaker. Beakers were marked and left under room conditions in the laboratory. Data was taken as dead larvae after 24 hour. Dead and alive larva were removed from the beaker after the 24 hour. New sets of larvae were introduced to the same jars. Tests were performed to at least for

7 days on the same jars, with the same natural product, or continued until obtaining 0% larval mortality. Each test was repeated 2-3 times. Control patches, in similar jars, were consistently used so as to correct the larval mortality against each product using Abbott, formula.

5- Toxicity Tests of Aqueous Extracts:

Twenty of mosquitoes' larvae (either *Anopheles* or *Culex*) were put in a beaker containing 150–300 ml water. 0.5 - 2.0 ml from the original concentration of each aqueous extract were dropped gently on these beakers so as to obtained series of tested concentrations of each aqueous extract. No new sets of larvae were substituted in this test. Tests were replicated 2-3 times. After 24 hours, the dead larvae were counted as percentage mortalities. A control batch was usually designed and was used to correct the tested mortalities.

6- Calculations and Statistics:

6.1. Sustained toxicity data:

Tables were drawn to illustrate the sustained toxicity effect of each product against both species of mosquito larvae in terms of Day No. (for the successive days of the experiment), and % Corrected mortality (for the means of the corrected mortalities of each day). Corrections always depend on the % mortality of the control batches according to Abbott's formula.

Excluding the zeros, data of the sustained toxicity of each powder against each species were subjected to a normal descriptive statistics (size, variance and mean). Descriptive statistics were used to conclude the significant differences in the susceptibilities between the two mosquito species or even between two products *via* simple t-test.

6.3. Aqueous extract toxicity tests

To each product, data were subjected to probit analysis followed Kehail, 2004. Probit analysis were placed in special cells at the end of the sustained toxicity tables.

Results:

1. Toxicity and sustained toxicity of *N. oleander*:

1.1- Flowers:

Table (1) showed that, 100% mortality was happened in the 6th day of the test against *Anopheles*, while it was 60% on *Culex* at that day. Mortality of *Anopheles* ranged between 0% - 100%. Mortality of *Culex* species ranged between 0% - 95%. No significant difference between susceptibility of *Anopheles* and *Culex* larvae (t_{cal} = 0.23, $t_{0.01}$ = 2.552).

When 3 g of flowers powder was thrown to one liter tap water, the maximum concentration expected was 670.5 mg/L (polar substances = 22.35 %). The LD50 of *Anopheles* larvae (281.54 ppm) was little more than that of *Culex* (267.79 ppm) and the LD95 of *Anopheles* larvae (610.85 ppm) was less than that of *Culex* (749.31 ppm). With respect to the R^2 values, *Anopheles* larvae (R^2 = 0.979) exhibited low homogeneity than *Culex* larvae (R^2 = 0.987).

Considerable mortalities were observed for 9 days in *Anopheles* larvae, while it sustained for 7 days against *Culex* larvae.

1.2- Leaves:

Table (1) showed that, mortality of *Anopheles* species ranged between 0% - 100%. Mortality of *Culex* species ranged between 0% - 92.5%. Considerable mortalities were observed for 10

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days in *Anopheles* larvae, while it sustained for 7 days against *Culex* larvae.

There is a significant difference between susceptibility of *Anopheles* and *Culex* larvae (t_{cal} = 1.99, $t_{0.05}$ = 1.725).

When 3 g of leaves powder was thrown to one liter tap water, the maximum concentration expected was 420.6 mg/L (polar substances = 14.02%). The LD50 for *Anopheles* larvae (127.83 ppm) was less than that of *Culex* (144.49 ppm), and so also the LD95 (512.34 and 679.15 ppm, respectively) and accordingly leaves product is more potent towards *Anopheles* larvae than towards *Culex* larvae.With respect to the R^2 values, *Anopheles* larvae (R^2 = 0.842) exhibited more homogeneity than *Culex* larvae (R^2 = 0.778).

2. Toxicity Of Usher (*Calotropis procera*) Powder:

2.1. **Flowers:**

Mortalities of *Anopheles* ranged between 0 and 100%, while those of *Culex* ranged between 0 and 60% when 3g of the powdered material was added to 1L of water (Table, 2). Total mortality (100%) was reported in first seven days of the *UF* P) for *Anopheles* larval populations.

When 3 g of *UF* P was added to one L tap water, the maximum expected water-soluble fraction concentration was 692.4 mg/L (polar substances = 23.08%). The LD50 for *Anopheles* larvae (328.8 ppm) was smaller than that of *Culex* (415.57 ppm) larval populations. The LD95 of *Anopheles* larval populations (955.5 ppm) was also lower than that of *Culex* (2314.23 ppm). Therefore, *UF* P proved to be more potent against *Anopheles* larvae than towards *Culex* larvae. *Anopheles* larvae (R^2 = 0.842) exhibited more homogeneity than *Culex* larvae (R^2 = 0.790).

Table (1): Toxicity of 3 g of *N. oleander* leaf and flower powders per 1-L tap water on *A. arabiensis* and *C. quinquefasciatus* larvae.

Day no.	% Corrected Mortality			
	Leaves		Flowers	
	Anopheles	Culex	Anopheles	Culex
1	67.5	92.5	86.67	95.00
2	85.0	92.5	80.00	85.00
3	50.0	70.0	80.00	55.00
4	60.0	70.0	40.00	58.33
5	100.0	45.0	40.00	60.00
6	100.0	17.5	100.00	60.00
7	100.0	17.5	75.00	63.33
8	100.0	10.0	70.00	0
9	100.0	70.0	40.00	0
10	72.5	50.0	5.00	0
11	0	0	0	0
Summary descriptive statistics				
No	10	10	10	7
Mean	83.50	53.50	61.67	68.09

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Variance	379.44	936.39	884.17	238.38
Aq-ex probit analysis				
Equation LD50 LD95 R^2 Slope SE-Y SE-X	Y=- 0.73+2.72X 127.83 mg/L 512.34 mg/L 0.842 0.78 1.359 0.589	Y=- 0.27+0.44X 144.49 mg/L 679.15 mg/L 0.778 0.75 1.809 0.751	Y=- 3.01+3.27X 281.54 mg/L 610.85 mg/L 0.979 2.48 0.698 0.276	Y=- 3.91+3.67X 267.79 mg/L 749.31 mg/L 0.987 1.88 0.624 0.246

2.2. Leaves:

When 3 g of *ULP* was mixed with one L of tap water, the maximum expected water-soluble concentration was 544.8 mg/L (18.16%). The LD50 of *Anopheles* larvae (166.7 ppm) was greater than that of *Culex* (122.3 ppm), while the LD95 of *Anopheles* larvae (410.6 ppm) was smaller than that of *Culex* (764.7 ppm), and accordingly *ULP* is considered to be more potent towards *Anopheles* larvae than towards those of *Culex*.

The R^2 values for *Anopheles* larvae (0.925) exhibited little homogeneity than *Culex* larvae (0.990). For the larval population of both species, the LD50s and the LD95s values demonstrated that *ULP* was more effective than *UFlP*.

3- Toxicity of *Ricinus communis* Seeds:

The effect of the 3g/L (137.4 ppm; 6.87%) persisted for eight days for both species larval populations (Table 3). Means of 62.8 and 47.5% were obtained for *Anopheles* and *Culex*, respectively. Mortality of *Anopheles* and *Culex* ranged between 7.5- 100% for the former species, and 5- 100% for the latter. The LD50 were 74.1 and 84.8 ppm, respectively, whereas the LD95s, following the same order were 121.7 and 122.2 ppm (*i.e*. equitoxic). The respective slopes were 3.27 and 3.48. There are no significant differences in susceptibilities between both larval species (t_{cal} = 0.76, $t_{0.01}$= 2.583).With respect to the R^2 values, *Anopheles* larval populations (0.958) exhibited lower homogeneity towards the treatment than their Culex counterparts (0.987).

Table (2): Toxicity of 3 g of *Calotropis procera* leaves and flowers powder per 1-L tap water to the larvae of *A. arabiensis and C. quinquefasciatus*.

Day no.	**% Corrected Mortality**			
	Leaves		**Flowers**	
	Anopheles	Culex	Anopheles	Culex
1	100	100.0	100.00	60.00
2	100	100.0	95.00	3.33
3	100	100.0	88.33	1.67
4	100	100.0	58.33	0.00

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5	100	100.0	88.33	15.00
6	100	100.0	100.00	0
7	100	70.0	70.00	0
8	90	90.0	0	0
9	20	40.0	0	0
10	5	22.5	0	0
11	0	0	0	0
Summary descriptive statistics				
N mean variance	10 81.51 1344.72	10 82.25 828.40	7 85.71 250.82	5 16.00 746.29
Aq-ex probit analysis				
Equation LD50 LD95 R^2 Slope SE-Y SE-X	Y=-4.31+0.19X 166.71 mg/L 410.55 mg/L 0.925 1.91 1.629 0.689	Y = 0.70 + 2.06X 122.29 mg/L 764.71 mg/L 0. 995 0.70 0.270 0.117	Y= -3.91 + 3.54X 328.81 mg/L 955.49 mg/L 0.917 2.48 2.953 1.087	Y= -5.27 + 3.54X 415.57 mg/L 2314.23 mg/L 0.889 0.75 2.972 1.055

Table (3): Toxicity of 3 g of *R. communis* seeds powder per 1.00 L tap water against *A. arabiensis* and *C. quinquefasciatus* larvae.

Day no.	**% Corrected Mortality**	
	Anopheles	Culex
1	65.0	7.5
2	55.0	5.0
3	85.0	5.0
4	100.0	95.0
5	100.0	100.0
6	95.0	100.0
7	57.5	90.0
8	7.5	25.0
9	0	0
10	0	0
Summary descriptive statistics		
N Mean Variance	8 70.63 994.20	8 53.44 2144.53

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Aq-ex probit analysis		
Equation LD50 LD95 R^2 Slope SE-Y SE-X	Y = -9.25 + 7.62X 74.14 mg/L 121.70 mg/L 0.958 3.27 1.527 0.795	Y = -14.94 + 10.34X 84.81 mg/L 122.19 mg/L 0.987 3.48 1.599 0.833

Discussion:

The toxic effects of *C. procera*, *N. oleander* and *R. communis* are worldwide proved against various insects. Abdalla *et al*., (2009) studied the efficacy of leaves extract of *C procera* in controlling *A arabiensis* and *C quinquefasciatus* mosquitoes, while Bgum *et al*., (2011) evaluated its efficacy on *Musca domestica*.

The toxic effects of *Nerium oleander* on larvae of the desert locust *Schistocerca gregaria* was reported by Bagari *et al*., (2013). Fouad *et al*., (2015) and Shaurub and Ghada, (2016), reported the larvicidal activity of *N oleander* against *Culex pipiens* Mosquito.

Alonso and Santos, (2013), documented the toxicity of *R communis* seed oil against the ant *Atta sexdens* rubropilosa, while Sabina *et al*., (2014), documented its toxicity against the malaria vector *Anopheles gambiae*, whereas Rizzardo *et al*., (2012) proved that, *Apis mellifera* pollination improves agronomic productivity of *R. communis*.

Conclusions:

According to the LD50 values, *Anopheles* larvae were more susceptible to all tested products than *Culex* larvae. The products can be ranked from the more potent toward the Anopheles larvae as follows: *RS*, *NL*, *UL*, *NF* and lastly *UF*, which is relatively similar to that against *Culex* larvae.

Recommendations:

The study recommends adding these natural products in *Anopheles* and *Culex* larval control, and also running more sensitivity tests specially on human and on the aquatic predators.

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