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Larvicidal activity of three plants powders and aqueous extracts on *Anopheles* and *Culex* mosquito larvae (Diptera: Culicidae)

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Abstract
Anopheles and *Culex* (Diptera: Culicidae) mosquitoes are two of the major medically important pests in the Sudan. The present work objectives was to explore the larvicidal activity of local plants, *viz. Nerium oleander* L, *Calotropis procera* L. and *Ricinus communis* (khirwie) powders and aqueous-extracts of leaves and flowers of the first-two, and seeds of castor tested aganist 3rd and 4th instar larvae of *A. arabiensis* and *C. quinquefasciatus*, according to the standard methodes of WHO for rearing and assessment of mosquitoes susceptibility. Powder (3g) was suspended in 1L of tap water in a beaker (Replicate). Larvae (20/beaker) from both species were added separately to each replicate. Each treatment was replicated 3x, and the experiment was repeated twice. Mortality % was recoded after 24 hr. New set of 20 larvae was added to the same beaker every day after removing the previous group; the removal and addition was continued until 0% mortality was obtained. Regarding the aqueous-extracts, different concentrations were prepared from the tested morphological parts to determine the LD50 after 24 hr for both species. For *A. arabiensis* the effect of powders of *Nerium* leaves and flowers peresisted for 10 days with mean of 84% and 62%, respectively, while for *C. quinquefasciatus* the values were 10 days and 7 days, with means of 54% and 68%, respectively. Powders of *Ricinus* seeds peresisted for 8 days for *A. arabiensis* with mean 71% and for 8 days for *C. quinquefasciatus*, but with 58% mean.

Keywords: *Nerium oleander* L., *Calotropis procera* L., *Ricinus communis* (khirwie), *Anophels arabiensis*, *Culex quinquefasciatus*, mosquitoes, larvae, leaves, flowers, seeds, natural products, botanical insecticides

1. Introduction
Diptera (Culicidae) is of prime importance in medical entomology, containing blood sucking forms, disease vectors and various nuisances. The genus *Anopheles* includes over 400 species [1]. *A. gambiae* Giles complex is among the major vectors of malaria and filariasis [2]. *A. arabiensis* Patton is the principal malaria vector in the Gezira area, central Sudan [3]. *A. pharoensis* is an important pest species in the Gezira, where it causes a great deal of discomfort by biting humans [4].

Culex quinquefasciatus Say is generally regarded as a subspecies. It is a tropical vector of urban filariasis [5]. The species a worldwide pest, the most nuisance mosquito, and the tropical vector of arboviruses; it is widely distributed in the Gezira [6]. Mosquitoes are important vectors of several tropical diseases, including malaria, filariasis, and numerous viral diseases, such as dengue, Japanese encephalitis, yellow fever, Wuchereria bancrofti, Plasmodium (avian malaria), myxomatosis, and other diseases [7-9]. Mosquitoes alone transmit disease to > 700 million people annually [9, 10]. The only efficacious approach to minimizing the incidence of these diseases is to eradicate and control mosquito vectors, mainly by applying insecticides to larval habitants, and educating the public [11]. Synthetic insecticides are today at the forefront of mosquito controlling agents. Nevertheless, controlling the mosquitoes has become complicated because of their resistance to synthetic insecticides, as well as the toxicity of insecticides toworkers, inhabitants, deomestic animals, fish and other non-target organisms [12-14]. Moreover, the wide spread use of different types of pesticides has resulted in contamination of the environment [15].

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 [16]. *Nerium oleander* L**.** **(**Oleander: Apocynaceae) is a green shrub bears a thick, leathery, whorled, arrow like leaves and various colored flowers. The latex contains some glycosides, *viz*. neriin, oleanderin and folinerin. This plant is very toxic because of its cardioactive glycoside oleanderin [17]. Common names, geographical distribution, botanical description, parts used, chemical constituents, medicinal effects, cancer treatment and toxicology of *N. oleander* were well reviewed by [17, 18, 19, 20].

Calotropis procera Ait. (Asclepiadaceae) is the Giant milk weed (locally known as Usher) is a desert milky plant that bears large, simple and opposite arrangement leaves and a violet green flowers. The plant contains a caoutchouc substance. The latex contains a very toxic cardiac glycosides [21]. The active principles of the juice are calctin, calotropin, calotoxin and uscaridin, which are poisonous in nature [22]. It has been used against the toxins of snakes and scorpions in the Sudan [23].

Ricinus communis L. (Castor; 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 [24]. *R. communis* is known to either to stop the cell division proliferating tissues, resulting in sterility in insects, or to disrupt their physiological functions [24]. Seed oil contains a very toxic lectin, ricin and ricinine [25]. Mashlawi *et al* [26] stated that crude latex was more efficient than ethanolic leaves extract in on Culex larvae in the laboratory. The LC50 values of the crude latex and ethanolic leaves-extract were found to be 57.3 and 388.7 ppm, respectively. Also, a remarkable reduction in both the pupation percent and adult emergence was obtained. Moreover, the latex showed a highly delayed toxic effect on the pupae and adults resulted from treated larvae, where the pupal mortality was 100% at the highest concentrations used (75 and 150ppm).

2. Materials and Methods

2.1 Collection and Maintenance of Products

All plants were collected from different localities within Wad Medani Greater Locality, Gezira State, Central Sudan. Scientific names, English names, parts used (products) and abbreviations are as follows:

English	Scientific name	Product	Abbr-iviations
Oleander	*Nerium oleander* L.	Flowers	NF
		Leaves	NL
Giant milkweed	*Calotropis procera* Ait	Flowers	UF
		Leaves	UL
Castor	*Ricinus communis* L.	Seeds	RS

2.2 Collection and Maintenance of Mosquitoes

Larvae of *A. arabiensis* and *C. quinquefasciatus* were collected by the dipping technique. The collected larvae were transferred and reared in the laboratories of the Blue Nile National Institute for Communicable Diseases (BNNICD), University of Gezira, according to the standard method of rearing of mosquitoes [22]

2.3 Preparation of Aqueous -Extracts

The aqueous-extracts (aq-ex), i.e. the polar compounds, of the powders were prepared as was described by Balbaa [16].

2.4 Sustained Toxicity Tests

One liter of tap water in 1L beakers, and 20 larvae/ beaker (3rd or early 4th instar) *A. arabiensis* or *C. quinquefasciatus* were placed. This represented a replicate. Certain weight of each crushed dried (powder) product (3 g/L) was placed on the water surface of each beaker. Beakers were labelled and left under room conditions in the laboratory. Data was taken as dead larvae after 24 hr. Dead and a live larva were removed from the beaker after the 24 hr (acute toxicity). For investigating the persisence of the activity (toxicity), which is known by some scientist as residual effect, and by other as sustained toxicity (chronic), new sets of larvae were introduced to the same beakers. Tests were performed to at least for 7 days (with new set of lravae every day) on the same beakers, with the same previously added powder, and continued until obtaining 0% larval mortality. Each test was repeated 2-3 times and replicated 3x. Control patches (tap-water only) were consistently used so as to correct for the mortality using Abbott's formula [27].

2.5 Toxicity Tests of Aqueous -Extracts

A series of concentrations of the aq-ex was used in this test instead of the powder. The tests were conducted twice and each concentration was replicated 3x. Mortality data was recorded after 24 hr (acute) only. No new sets of larvae were substituted in this test as in (2.4) above.

2.6 Calculations and Statistics

2.6.1. Sustained toxicity data

Tables were drown 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 [27].

2.6.2 Sustained toxicity data

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 species or even between two products *via* simple t-test.

2.6.3. Aqueous-extract toxicity tests

Data was subjected to Probit analysis. Followed Kehail [28]. These appeared in the the sustained toxicity tables.

3. Results and Discussion

3. 1. Toxicity and Sustained Toxicity of *N. oleander*

3. 1.1 Flowers (NF)

Table (1) showed that, 100% mortality was recorded in the 6th day of the test against *Anopheles* larvae, while it was only 60% on those of *Culex* at that day. Mortality of *Anopheles* resulting from NF powder ranged between 0% - 100%. However, in *Culex* species the range was between 0% - 95%. No significant differences between susceptibilities of the two

species larvae (t_{cal} = 0.23, $t_{0.01}$ = 2.552) was detected.

When 3 g of NF powder was added to one liter of tap water, the expected maximum concentration was 670.5 mg/L (polar substances as water extract = 22.35 %). The LD50 of *Anopheles* larvae (281.54 ppm) was little higher 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 slightly lower homogeneity to the treatment than *Culex* larvae (R^2 = 0.987). Considerable mortalities were observed for 9 days in *Anopheles* larvae, while it was only for 7 days against *Culex* larvae.

3.1.2 Leaves (NL)

Table (1) also showed that mortality of *Anopheles* larvae, similar to that of the NF powder, ranged between 0% - 100%. However, the mortality of *Culex* larvae ranged between 0% - 92.5%; no significant differences between both materials. Considerable mortalities were observed for 10 days in *Anopheles* larvae, while the mortality 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 mixed with one liter tap water, the maximum expected concentration was 420.6 mg/L (polar substances or extract = 14.02%). The LD50 for *Anopheles* larvae (127.83 ppm) was less than that of *Culex* (144.49 ppm), and so was 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) to NL powder.

Table 1: Toxicity of 3 g of *N. oleander* leaves (LP) and flowers powder (FP) per 1-L tap-water on *A. arabiensis* and *C. quinquefasciatus* larvae.

Day no.	% Corrected Mortality			
	LP		FP	
	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
	10	10	10	7
	83.50	53.50	61.67	68.09
	379.44	936.39	884.17	238.38
	Y = -0.73 + 2.72X 127.83 mg/L 512.34 mg/L 0.842 0.78 1.359 0.589	Y = -0.27 + 2.44X 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

3.2 Toxicity of *C. procera* Powder

3.2.1 Flowers (UFP)

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

When 3 g of UFP was added to one L tap water, the maximum expected water-soluble fraction concentration was 692.4 mg/L (polar substances extracted = 23.08%). The LD50 for *Anopheles* larvae (328.8 ppm) was smaller than that for *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, UFP proved to be more potent against *Anopheles* larvae than towards *Culex* larvae. *Anopheles* larvae (R^2 = 0.842) revealed that it was more homogeneity than *Culex* larvae (R^2 = 0.790) towards this powder.

3.2.2 Leaves (ULP)

When 3 g of ULP was mixed with one L of tap water, the maximum expected concentration was 544.8 mg/L (18.16% polar extract). The LD50 of *Anopheles* larvae (166.7 ppm) was higher 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). The R^2 values for *Anopheles* larvae (0.925) exhibited a little more homogeneity to this powder 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.

Table 2: Toxicity of 3 g of *Calotropis procera* leaves and flowers powder /1L 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
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	10	10	7	5
mean	81.51	82.25	85.71	16.00
variance	1344.72	828.40	250.82	746.29
Aq-ex probit analysis				
Equation LD50 LD95 R^2 Slope SE-Y SE-X	Y = -4.31 + 4.19X 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

3.3 Toxicity of *Ricinus communis* Seeds

The effect of the 3g/L (137.4 ppm; 6.87% polar extract) 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 slightly lower homogeneity towards the treatment than their *Culex* counterparts (0.987).

Table 3: Toxicity of 3 g of *R. communis* seeds powder /1 L tap - water against *A. arabiensis* and *C. quinquefasciatus* larvae.

Day.	% 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	8	8
Mean	70.63	53.44
Variance	994.20	2144.53
Aq-ex Probit analysis		
Equation	Y = -9.25 + 7.62X	Y = -14.94 + 10.34X
LD50	74.14 mg/L	84.81 mg/L
LD95	121.70 mg/L	122.19 mg/L
R^2	0.958	0.987
Slope	3.27	3.48
SE-Y	1.527	1.599
SE-X	0.795	0.833

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