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The larvicidal activity of powders and aqueous-extracts different parts of four plant species fruits against *Anopheles arabiensis* Paton (Diptera: Culicidae) larvae from Wad Medani, Central Sudan

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Abstract
According to WHO, populations of *Anopheles arabiensis* Paton (Diptera: Culicidae) from the Gezira state, central Sudan, produced strains showing resistance to a wide range of insecticides. 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. In the present work, the larvicidal potentialies of four different local plants fruits were investigated using different morphological parts (seeds, whole fruit, pericarp) and in the forms of powders or aqueous -extracts. These plants were: orange *(Citrus sinensis* L.**)**, Pomegranate ***(****Punica granatum* L.), bitter apple (*Citrullus colocynthis* Schrad**)**. and pepper ***(****Capsicum annuum* L.**)**.The standard methods of WHO for rearing and assesment of mosquitoes susceptibility were carefully followed. The results revealed that the LD50 for bitter apple fruit powder was 144.2 mg/L, for pepper fruit 165.3 mg/, for orange seeds powder 255.9 mg/L, for pomegranate seeds powder 718.7 mg/L, for orange pericarp powder (980.7 mg/L and, lastly for pomegranate pericarp powder was 1266.9 mg/L. The effects of the treatments on the larvae showed significant differences in their persistence in water. Pepper fruit powder killed 50-80% within the first 3 days, whereas orange seeds powder killed 35-100% within the first 6 days. The bitter apple powder effect persited for 2 days only (85 and 55% for days 1 and 2, respectively) and, then drastically declined (15 and 10% for days 3 and 4, respectively). More investigations are needed to isolate the active ingredients of each powder and subject them to bioassay.

Keywords: *Anopheles arabiensis*, Botanicals, larvicides, orange, pepper, omegranate, Colocynth

1. Introduction

After years of selection pressure by insecticides, whether for public health or agricultural purposes, many vector populations became resistant to the insecticides used. By the end of 1985, a total of 50 anopheline species were recorded as resistant to one or more insecticides [1]. Populations of *Anopheles arabiensis* Paton from the Gezira State, Central Sudan, produced strains showing resistance to a wide range of insecticides [2]. 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 [3].

Punica granatum L. (Pomegranate : Punicaceae) fruits are fleshy with a leathery thick coat. Seeds are polygonal and endosperm-free. The most important reported substances are pelletierine alkaloid and gallotonnic acid [4, 5, 6, 7]. *Capsicum annuum* L. (Capsicum: Solanaceae fruits are horn-like. It contains a volatile with burning sensation alkaloid capsaicin [4]. Ann *et al*. [8] stated that Female *Manduca Sexta* moths (Lepidoptera: Sphingidae) exhibit upwind orientation and egg laying-related behavior when exposed to volatiles released by the *C. annuum* as a host-plant.

Citrus sinensis L. (Orange: Rutaceae**)** fruits are spherical yellow to orange in color. Citrus flavonoids are widely distributed group of polyphenolic compounds with health-related properties, which are based on their antioxidant activity. Flavanones, flavones and flavonols are the flavonoids present in *Citrus* Spp. These compounds are powerful antioxidants and can be used directly as repellents or toxins [9].

The common names, geographical distribution and some valuable information were adequately reviewed [10, 11, 12, 13, 14]. *Citrullus colocynthis* Schrad. (Colocynth, Handal, bitter apple: Cucurbitaceae) fruit contains a very bitter glycosides colocynthin and cucurbitacin. Also, the fruit contains some resins, pectins and saponins [5]. Several authors [1, 15, 16] reviewed the characteristics and chemical composition of *C. colocynthis* and reported that the fruit possess a molluscicidal, nematocidal and rodenticidal activity [17, 18, 19, 20]. Some studies showed it can be used in the malarial vector *Anopheles stephensi* control [21]. The seeds are inodorous and intensely bitter [22]. Detailed studies revealed the presence of cucurbitacin B and E spinasterol and others, alkanes, aliphatic (eleatrin), colocynthin, sterols alcohols alkaloids, and choline base. The seed contains about 16% fixed oil almost odorless, with a very bitter taste [23]. Saponins and glycoside were also found in this plant possessing a hypoglycemic effect on rabbits [24].

The present work aims to investigate the potentiality of different parts of fruits of four plants as powders (P) or aqueous-extracts (aq-E) as larvicides to be used againest *A. arabiensis* larvae. The idea is to avail to the community or the municipalities natural productsthat are when ecologically-sound, efficient, safe, low-cost, unhazardous, and simple applicable control methods for *A. arabiensis* larvae is required

2. Materials and Methods

2.1 The Study Area

The location of 14º 22- 14º26 of Wad Medani town occupies the central parts of the Sudan Gezira area. Different sites within Wad Medani Greater Locality were selected for sampling mosquitoes and collecting the natural products.

2.2 Collected Plant Material

Plants scientific names, local or Arabic names, English names, parts used (products) and abbreviations were as follows:

English name	Scientific name	Product
Pomegranate	*Punica granatum* L.	Pericarps (PP)
		Seeds (PS)
pepper	*Capsicum annuum* L.	Fruits (PPF)
Orange	*Citrus sinensis* L.	Pericarps (OP)
		Seeds (OS)
Colocynth	*Citrullus colocynthis* Schrad.	Fruits (CF)

2.3 Collection and Maintenance of Mosquitoes

Larvae of *A. arabiensis* were collected by means of dipping. Laboratory hatched larvae were reared and treated according to the standard methods of WHO [2].

2.4 Preparation of Aqueous Extracts

Aqueous extractions were prepared following the recommended methods of Balbaa [5].

2.5 Sustained Toxicity Tests

One liter of tap water was kept in 1L beakers, and to each beaker 20 larvae (3rd or early 4th instar) were placed. This represented one replicate. Certain weight of each crushed dried product (2 to 4 g/L) was placed on the water surface of each beaker. Data was taken as dead larvae after 24 hr. New sets of larvae were replaced to the same jars. Each test was repeated 2-3x. Control patches, in similar jars, were consistently used so as to perform a required corrections.

2.6 Toxicity Tests of Aqueous Extracts

To new sets of beakers with different concentrations of the aq-E, 20 L3 or L4 larvae were added. Mortality data was recorded after 24 hr. No new sets of larvae were substituted in this test. Tests were replicated 2-3x. The control tests were done by adding tap water only.

2.7 Statistics of Sustained toxicity data

Tables were drown to illustrate the sustained toxicity effect of product against the 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 [25]. Data was subjected to a normal descriptive statistics (size, variance and mean). Descriptive statistics were used to represent a descriptive view for the data, and also to conclude the significant differences in the susceptibilities between any two products *via* simple t-test.

2.8 Statistics of Aq-E toxicity tests

Concentrations of the aqq-E were transferred to log-conc. *i.e.* log dose, while the corresponding corrected mortalities were transformed as Probits. Log-dose and the corresponding Probit were subjected to the Probit analysis as was done in Kehail [26].

3. Results and Discussion

3.1 Toxicity of *Capsicum annuum* (Pepper) Fruits (PPF)

The 2 g/L resulted in the first-day in 80% mortality. Starting the 2nd -day, the effect was decreased to 70, 50, 25, 0, and 5%, respectively, for days 2, 3, 4, 5 and 6 (Table 1). The LD50 was 165.3 mg/L and the LD95 was 1123.9 mg/L.

Table 1: Toxicity of 3 g of *C. annuum* fruits powder /1-L tap water on *A. arabiensis* 3rd instar larvae.

Day no.	% Corrected Mortality
1	80
2	70
3	50
4	25
5	20
6	5
7	0
8	0
Summary descriptive statistics	
N	6
Mean	41.66
Variance	886.67
Aq-ex probit analysis	
Equation	Y = 0.63 + 1.97X
LD50	165.30 mg/L *(163.41 mg/L)
LD95	1123.99 mg/L *(978.41 mg/L)
R^2	0.875
Slope	0.87

3.2 Toxicity of *C. sinensis* (orange)

3.2.1 Pericarps Powder (OP)

The results of the OP are not encouraging in controlling the larval populations (Table 2). Mortalities of 35% and lower were registered for the first 2 days only. The 3 g of OP were expected to yield 975.6 mg/L (32.52%). The exrapolated LD50 for *Anopheles* was expected to be *ca*. 980.3 ppm, and the LD95 of 1882 ppm. Consequently, concentration higher than 3g/L is required to be used as a larvicide.

3.2.2 Seeds Powder (OS)

A different picture was given by the performane of OS. The powder was effective for 6 successive days; days 2-to-5 resulted in 100% mortality. Days 1 and 6 resulted in 35 and 70% mortality, respectively (Table 2). The mean of the 6 days was 72.1%, and an LD50 of 255.9 ppm (higher than thePPF), while the LD95 was 596.7 ppm (lower than PPF). There is a significant difference between potentialities of seed and pericarp powders ($t_{cal} = 2.58$, $t_{0.05} = 1.771$).

The expected concentration from the 3 g of OS in 1L tap water was 656.7 mg/L (21.89%). This concentration cover the range required for the LD50 and the LD95. Consequently, OS is more effective against *Anopheles* larvae at 3g/L. Benavente-Garcia *et.al* [9], supported these results as he reported that: Citrus flavonoids can be used directly as repellents or toxins. Seeds powder is more effective than pericarps powder.

Table 2: Toxicity of 3 g of *C. sinensis* pericarps and seeds powder /1L tap water on *A. arabiensis* 3rd instar larvae.

Day no.	% Corrected Mortality	
	Pericarps (OP)	**Seeds (OS)**
1	30	35
2	35	100
3	0	100
4	0	100
5	0	100
6	0	70
7	0	0
8	0	0
Summary descriptive statistics		
N	2	6
Mean	32.50	84.16
Variance	12.50	724.17
Aq-ex probit analysis		
Equation	Y = -12.32 + 5.79X	Y = -5.74 + 4.46X
LD50	980.72 mg/L	255.90 mg/L
LD95	1881.96 mg/L	596.74 mg/L
R^2	0.881	0.797
Slope	1.07	2.36

3.3 Toxicity of *C. colocynthis* (Bitter Apple) Fruits (CF)

Table (3) showed that the mortality of the 3rd instar larvae ranged between 0 - 85%. Considerable mortalities were observed for 2 days. When 3 g of CF powder was added 1L tap water, the maximum concentration expected was 239.4 mg/L (polar substances = 7.98%). The LD50 was 144.26 ppm, i.e. smaller than PPF, and the LD95 was 353.75 ppm; i.e. the smallest among all treatments. This plant contains a very bitter glycosides colocynthin and cucurbitacin. Also it contains some resins, pectins and saponins [4]. The plant exerted acceptable results against the larvae.

Table 3: The effect of 3 g *C. colocynthis* fruits powder per 1-L tap water on *A. arabiensis* 3rd instar larvae.

Day no.	% Corrected Mortality
1	85
2	55
3	15
4	10
5	0
6	0
7	0
Summary descriptive statistics	
N	4
Mean	41.25
Variance	1256.25
Aq-ex probit analysis	
Equation	Y = -4.09 + 4.21X
LD50	144.26 mg/L
LD95	353.75 mg/L
R^2	0.984
Slope	0.97

3.4 Toxicity of *P. granatum* (Pomegranate)

3.4.1 Pericarps Powder (PPP)

Extremely high concenrations of the PPP are required to cause very low levels of mortalities. Moreover, their effects did not persist for more than three days. The LD50 and the LD95 were 1,266.9 and 5,636.2 mg/L (Table 4). Therefore, it was obvious that the PPP of this plant is not a candidate for larval control. When 3 g/L of PPP is expected to end up with >1,500 mg/L (52.8%), which is more than that required for causing a significant level of toxicity.

3.4.2 Seeds (PS)

The results of the PS were not better than those of the PPP; the solubility did not exceed 13.3%; the effects did not exceed three days also (Table 4). Again, the seeds of this plant are of no value for this species larval control. The most important ingredients reported in the literature are pelletierine alkaloid and gallotonnic acid [4].

Table 4: Toxicity of 3 g of *P. granatum* pericarps and seeds powder per 1- L tap water on *A. arabiensis* larvae.

Day no.	% Corrected Mortality	
	Pericarps	**Seeds**
1	15.0	10.0
2	0.0	17.5
3	42.5	25.0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
Summary descriptive statistics		
N	2	3
Mean	28.75	17.51
Variance	378.13	65.25
Aq-ex probit analysis		
Equation	Y = -2.85 + 2.53X	Y = -1.97 + 2.44X
LD50	1266.97 mg/L	718.72 mg/L
LD95	5636.22 mg/L	3378.22 mg/L
R^2	0.889	0.995
Slope	0.75	1.28
SE-Y	1.668	0262
SE-X	0.516	0.980

4. Conclusion

The results revealed that the products (powders) tested can be potential natural products for controlling mosquito larvae by the public in the breeding sites. They are available, easy to apply and can cause no harm to the environment. Moreover, the active ingredients of some of them can be isolated in the laboratories for structure- activity relationship studies and formulation, if possible.

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