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Larvicidal, adult emergence inhibition and oviposition deterrent effects of foliage extract from *Ricinus communis* L. against *Anopheles arabiensis* and *Culex quinquefasciatus* in Sudan

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Abstract. Malaria and filariases are prevalent in Sudan and their control depends largely on preventive measures against mosquito vectors. The present work aimed to investigate the larvicidal, adults emergence inhibition and oviposition deterrent effects of aqueous extracts from leaves of *Ricinus communis* L. against the mosquitoes, *Anopheles arabiensis* and *Culex quinquefasciatus* as a biological control means. The larval mortality was observed after 24 hours. The LC50 values calculated were 403.65, 445.66 and 498.88ppm against 2nd, 3rd and 4th instar larvae of *An. arabiensis* and 1091.44, 1364.58 and 1445.44ppm against 2nd, 3rd and 4th larval instars of *Cx. quinquefasciatus*. 50% of adult emergence inhibition (EI50) were 374.97 and 1180.32ppm against 3rd instar larvae of *An. arabiensis* and *Cx. quinquefasciatus*. The extract showed oviposition deterrent effect against both species. Results reveal that the crude extract of *R. communis* possesses remarkable larvicidal, adult emergence inhibition and oviposition deterrent properties against both the tested species and can be used as biological control means.

INTRODUCTION

Malaria and filariasis rank amongst the world's the most prevalent tropical vector-borne communicable diseases. An estimated 300-500 million people are infected with malaria annually, resulting in 1.5-3 million deaths (WHO, 2000). Malaria remains a major health problem in Sudan. About 20-40% of out patient clinic visits and approximately 30% of total hospital admissions are due to malaria (WHO and UNICEF, 2005). As for lymphatic filariasis (LF), a highly debilitating infection, about 30% of the global population at risk is estimated to be in the LF-endemic countries of the African region (WHO, 2006). Sudan is included in the Afro- tropical endemic region; the population at risk is about 12.2 million, and the disease is endemic in the southern states of the country (Satti & Abdel Nur, 1974; Elsetouhy & Ramzy, 2003; WHO, 2005b).

One of the methods to control these diseases is to control the vectors for bringing about interruption in disease transmission. The control of mosquitoes in larval stage is considered an efficient way in the integrated vector management (Rutledge *et al.*, 2003). Since the discovery of DDT, mosquito control approach has been almost completely based on synthetic organic insecticides. The appearance of insecticide resistance in major vector species, environmental pollution and increased costs of insecticides, prevent their extensive use. To combat some of these problems, various studies on natural plant products against mosquito vectors have been indicated as a possible alternative to synthetic insecticides (Mittal & Subbarao, 2003; Rajkumar & Jebanesan, 2005; Promsiri *et al.*, 2006). *Ricinus communis* L., (Euphorbiaceae), probably native to Africa, is growing naturally in many parts of Sudan. The aim of this work is to investigate the effect of crude aqueous extract from leaves of *R. communis* against the larval stages of *Anopheles arabienses* (the main malaria vector in Sudan) and *Culex quinquefasciatus* (the vector of filariasis) with respect to adult emergence inhibition, and oviposition deterrence. Since it is considered environmentally safe, less expensive and economical, as well as practical in application with minimum care by individuals and communities.

MATERIALS AND METHODS

The study area

The selected area for this study (Shambat Village) lies in the western part of Khartoum North town, on eastern bank of the River Nile between latitude 15.40N and longitude 32.32E. The period of the study was from June 2005 to September 2007.

Collection and rearing of Mosquitoes

Larvae of mosquitoes were collected from breeding sites within the study area, and reared under laboratory conditions at 25-28°C. The larvae were fed on powdered yeast. Pupae were collected daily in small bowls containing clean water and transferred to the cage measuring 30x30x30 cm covered with mosquito net for adult emergence. Adult mosquitoes were provided with cotton soaked with a 10% sugar solution and they were periodically blood fed on pigeons. Petri-dishes provided with moist cotton or filter papers were placed at the bottom of each cage for oviposition.

Plant collection and extraction

Leaves of *R. communis* L., were collected during the flowering season from plants growing in the study area, dried under shade and finely powdered. Five grams from the powdered leaves was soaked in a separate bottle (500ml) containing 250ml distilled water. The solution was allowed to stand for 24 hours with vigorous occasional shaking; the suspension was filtered with filter paper (Whatman No. 1). The marc was washed several times with distilled water and filtered. The final volume was adjusted to 500ml by adding distilled water to prepare stock solution of 1%. The stock solution was then serially diluted with water to prepare the test concentrations required.

Larvicidal effect

Larvicidal effects of the extract were determined by following the WHO standard procedure (WHO, 2005b). Twenty-five laboratory reared 2nd, 3rd and 4th instar larvae of each mosquito species were transferred to test cups, each containing 100ml of the required concentration. Four replicates were set up for each test concentration, with four replicates of control. The experiment was performed under laboratory conditions at 25-28°C. Mortality in larvae was recorded 24 hours post-treatment.

Adult emergence inhibition (EI) effect

The inhibition of adult emergence was evaluated by following the WHO standard procedure for testing insect growth regulators (WHO, 2005b). Only late 3rd instar larvae were used. The method of the larvicidal activity was followed. Because of the long duration of the test the larvae were fed with yeast at two days' intervals until mortality count was made. Mortality of the larvae and pupae were recorded at 24 hr interval. At the end of observation period, the impact is expressed as EI% based on the number of larvae that do not develop successfully into viable adults. The experiments stopped when all the larvae or pupae in the controls have died or emerged as adults.

Statistical analysis

Data were subjected to probit analysis. The regression equation (Y = a + bx), lethal concentration that killed 50% and 90% of the population (LC50 - LC90), fiducial limit (F.

L.) with 95% confidence limit (C. L.) were calculated.

Oviposition deterrent effect

The oviposition deterrent test was performed using the method of Xue *et al.*, (2001) and Rajkumar & Jebanesan (2005). Five cages were designed and placed side by side as A, B, C, D and E for each bioassay. Fifteen gravid females of *An. arabiensis* and *Cx. quinquefaciatus* were transferred to each cage. A 10% sucrose solution was available at all times. Three concentrations of the extract which showed high, moderate and low mortality in the larvicidal activity were prepared. From each concentration 100 ml were taken and put in the test cup in the cage A, B and C. Three test cups each containing 100 ml of dechlorinated tap water were prepared as controls and placed in opposite corners in the cage. In cage D all the experimental concentrations were placed without control. While in cage E two cups of control were placed without any treated cup. Three replicates for each concentration were run. After 48 hours, the number of eggs laid in treated and control cups were recorded. In the case of *An. arabiensis* the test cup was replaced by Petri dishes with filter paper.

The percent effective repellency for each concentration was calculated using the following formula:

$$ER\ (\%) = \frac{NC - NT}{NC} \times 100$$

Where ER = percent effective repellency; NC = number of eggs in control; and NT = number of eggs in treatment.

RESULTS AND DISCUSSIONS

The toxicity of crude extract of *R. communis* against 2nd, 3rd and 4th instar larvae of *An. arabiensis* and *Cx. quinquefasciatus* was evaluated. The data were recorded and statistical data calculated as presented in Table 1 and Figures 1-3, as LC50, LC90, 95% fiducial limit, regression coefficient (r^2) and regression equation. The 50% mortality (LC50 values) were 403.65, 445.66 and 498.88ppm against 2nd, 3rd and 4th instar larvae of *An. arabiensis* and 1091.44, 1364.58 and 1445.44ppm against 2nd, 3rd and 4th instar larvae of *Cx. quinquefasciatus*, respectively.

In the present study 2nd instar larvae were more susceptible than those of the 3rd instar and the latter was more susceptible than the 4th instar larvae of both tested species. Also *An. arabiensis* was found more susceptible than *Cx. quinquefasciatus* to the extract. The results reveal that LC50 value of the extract was very low against both tested mosquitoes as compared to the other plants extracts. The leaf extracts of *Pavonia zeylanica* and *Acacia ferrugginea* showed larval mortality at LC50 of 2214.7 and 5362.6ppm respectively against the third instars larvae of *Culex quinquefasciatus* after 24 hours treatment (Vahitha *et al.*, 2002). The leaf extract of fives species of Cucurbitacious plants, *Momordica charntia*,

Table1. Larvicidal effects of leaf extract of *R. communis* L. against 2nd, 3rd and 4th instars larvae of *An. arabiensis* and *Culex quinquefasciatus*

Mosquito species	Instar	LC50 ppm	LC90 ppm	Regression equation	F. L. with 95% C. L.	r^2
An. arabiensis	2nd	403.65	920.45	Y = 3.572X – 4.308	±2.373	0.957
	3rd	445.66	1114.29	Y = 3.217X – 3.521	±2.373	0.972
	4th	498.88	1364.58	Y = 2.935X – 2.920	±2.373	0.980
Cx. quinquefasciatus	2nd	1091.44	1753.88	Y = 6.208X – 13.858	±2.024	0.971
	3rd	1364.58	2046.44	Y = 7.308X – 17.914	±2.020	0.981
	4th	1445.44	2187.76	Y = 7.101X – 17.436	±2.020	0.984

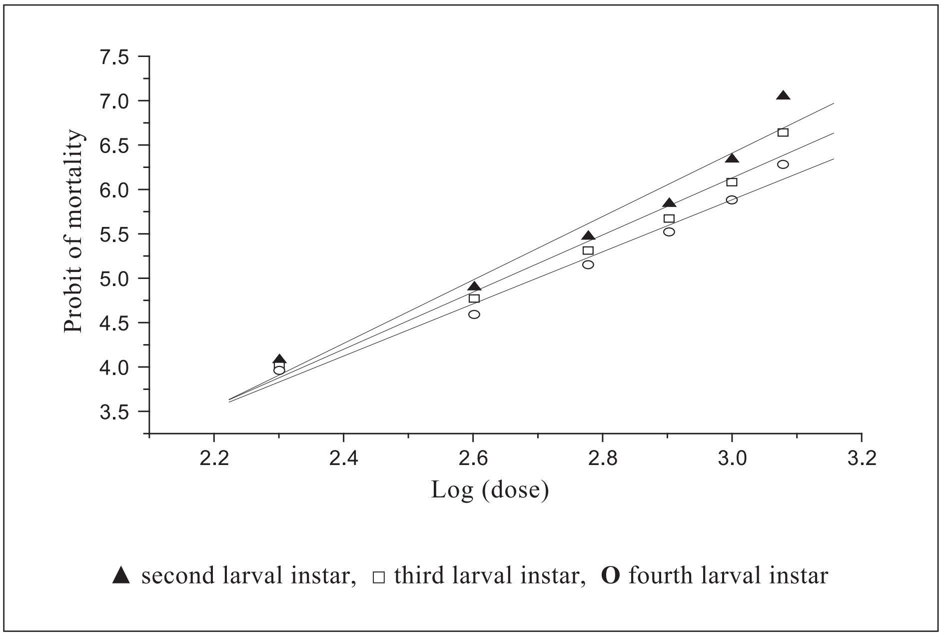

Figure 1. Larvicidal effects of leaf extract of *Ricinus communis* L. against 2nd, 3rd and 4th instars larvae of *Anopheles arabiensis* expressed as linear regression.

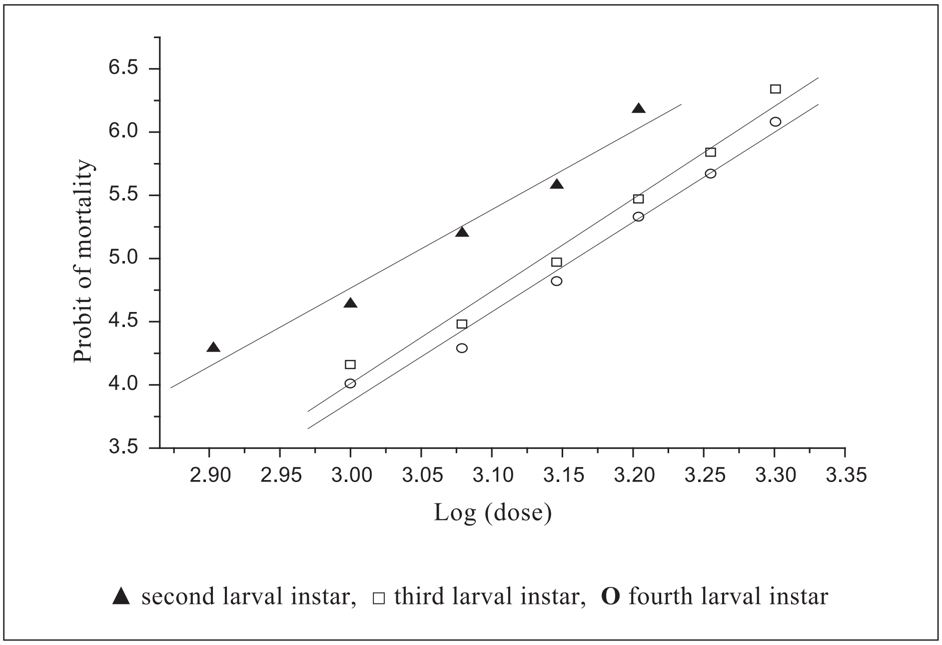

Figure 2. Larvicidal effects of leaf extract of *Ricinus communis* L. against 2nd, 3rd and 4th instars larvae of *Culex quinquefasciatus* expressed as linear regressions.

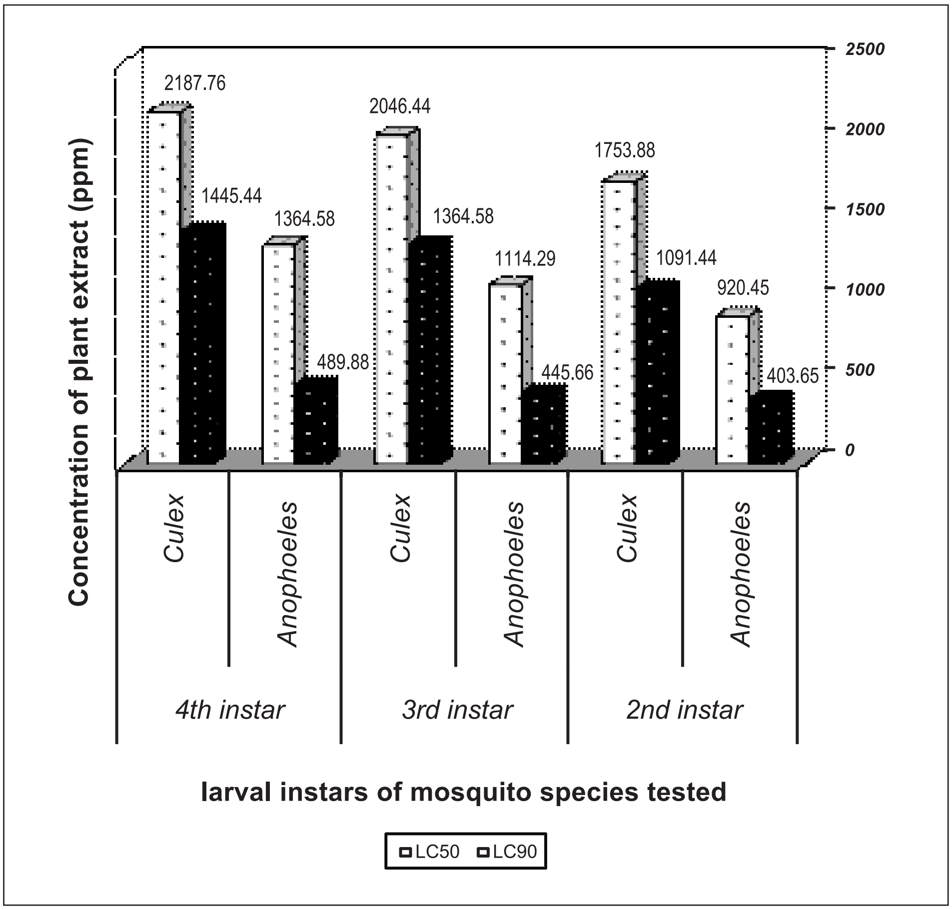

Figure 3. Larvicidal effects of leaf extract of *Ricinus communis* against 2nd, 3rd and 4th instars larvae of *Anopheles arabiensis* and *Culex quinquefasciatus* expressed as LC50 and LC90.

Trichosanthes anguina, *Luffa acutangula*, *Benincasa cerifera* and *Citrullus vulgaris* showed larvicidal activity at LC50 of 465.85, 567.81, 839.81, 1189.30 and 1636.04ppm, respectively (after 24 hours treatment) against the 3rd instar larvae of *Cx. quinquefasciatus* (Prabakar & Jebanesan, 2004). Also the result agree with the finding of Pushpanathan *et al.* (2006) since they had reported that 2nd instar larvae of *Cx. quinquefasciatus* were more susceptible than 3rd instar, and the latter was more susceptible than 4th instar larvae to the essential oil extracted from *Cymbopogon citratus*, with LC50 – LC90 of 144.54 – 284.27, 165.70 – 318.48 and 184.18 – 359.01ppm for 2nd, 3rd and 4th instar larvae, respectively. The varying susceptibility levels of both tested species of mosquitoes are probably due to differences in the physiological characteristics of the two species. This agree with previous results that found the four mosquitoes, namely, *Culex tritaeniorhynchus*, *Anopheles stephensi*, *Aedes aegypti* and *Cx. quinquefasciatus* whose larvae showed different susceptibility to the oil extract of *Ipomoea cairica* Linn., in which case higher concentration was required for *Cx. quinquefasciatus* followed by *Ae. agypti*, *An. stephensi* and lower concentration for *Cx. tritaeniorhynchus* (Thekkevilayil *et al.*, 2004). Mosquito larvae of different species display different susceptibilities to the same phytochemical. The susceptibility of *Anopheles* larvae can vary since they can be more or less susceptible than *Culex* and *Aedes* larvae to botanical derivatives and insecticides (Shaalan *et al.*, 2005) Preliminary evaluation of larvicidal activity of aqueous extracts from *R. communis* showed strong toxic activity against 4th instar larvae of four mosquito species *Culex pipiens*(L.), *Aedes caspius*, *Culiseta longiareolata* and *Anopheles maculipennis* with LC50 of 600, 270, 200 and 1090mg/L respectively (Brahim *et al.*, 2006). The bioactivity of photochemical against mosquito larvae can vary significantly depending on plant species, plant part, age of plant part, solvent used in extraction and mosquito species (Shaalan *et al.*, 2005). The statistical data of the adult emergence inhibition (EI) activity of extract against *An. arabiensis* and *Cx. quinquefsciatus* presented in Table 2, Figures 4 and 5. EI50 - EI90 were 374.97–979.49ppm and 1180.32–1849.27ppm, respectively.

Table 3 shows the effect of different concentrations of aqueous extract on the oviposition deterrence against gravid females of *An. arabiensis*. In the cage A, 350 eggs were laid in the control cup, while in the corresponding treated cup (1200ppm) no eggs were laid. A similar observation was shown in cage B and C, where 410 and 505 eggs, respectively, were laid in the control cup kept in the cage B and C. No eggs were laid in the corresponding treated cup in both cages. In the cage D where choice of control was not found, maximum of eggs (290 eggs) were laid in the lowest concentration (200ppm), and minimum of eggs (60 eggs) were laid in the highest concentration (1200ppm), while in the moderate concentration (600ppm), 140 eggs were laid, suggesting that the gravid mosquito have chemo sensation which enable it to detect volatile chemical emanating from breeding site and choose the least concentration for its eggs laying. In cage E where only control was found about 650 eggs were laid.

The crude aqueous extract of *R. communis* at different concentrations (1200, 600 and 200ppm) showed (90–100%) oviposition deterrence and effective repellence against *An. arabiensis* when the extract is to be used as material of choice (treated – control). However, when the three concentrations were found without control (choice) the avoidance of eggs laying was not shown, and the maximum of eggs were laid in the low concentration (200ppm). A similar observation was shown on *Cx. quinquefasciatus*, at concentrations of 1600, 1200 and 600ppm (Table 4), suggesting that the extract produce volatiles compounds act as a repellent against mosquitoes. Our results agree with some previous studies, such as in laboratory the leaf extract of *Solanum trilobatum* greatly reduced the number of eggs deposited by gravid *An. stephensi* at several concentrations. At the highest concentrations (1-0.075%) the extract reduced eggs laying by 90 - 99%. Lower concentrations (0.01%) also had deterrent activity of 18.4% (Rajkumar & Jebanesan, 2005). The isolated flavonoids from *R. communis* L leaves showed potential insecticidal, ovicidal and oviposition deterrent activities against *Callosobruchus chinensis* L (Coleoptera: Bruchidae) (Upasani *et al.*, 2003). One plant species may possess substances with a wide range of activities, e.g. Neem (*Azadirachta indica*) products showed antifeedant, oviposition deterrence, repellency, growth disruption, sterility and larvicidal action against a variety of insects (Schmutterrer, 1990; Mulla & Su, 1999). Mosquitoes are known to perceive visual, thermal and olfactory stimuli which enable them to detect light source, odour and several other volatile chemicals emanating from the skin, breath and waste products of their hosts (Takken, 1991; Davis & Bowen, 1994). *Anopheles gambiae* preferred to oviposit on unmodified substrates from natural larval habitats containing live microorganism rather than sterilized ones, suggesting that microbial populations in breeding sites produce volatiles that serve as semiochemicals for gravid *An. gambiae*. These signals in conjunction with other (non-olfactory) chemical and physical cues, may be used by the female to assess the suitability of potential larval habitats in order to maximize the fitness of her offspring (Sumba *et al.*, 2004). The Afrotropical *Cx. quinquefasciatus* respond significantly better to trap baited with carbon dioxide than to either octenol, acetone or butyric

Table 2. The adult emergence inhibition effects of leaf extract of *Ricinus communis* L. against 3rd instars larvae of *Anopheles arabiensis* and *Culex quinquefasciatus*.

Mosquito Species	EI50 ppm	EI90 ppm	Regression equation	F. L. with 95% C. L.	r^2
An. arabiensis	374.97	979.49	Y = 3.071X – 2.904	± 2.335	0.949
Cx. quinquifasciatus	1180.32	1849.27	Y = 6.578X – 15.209	± 2.006	0.976

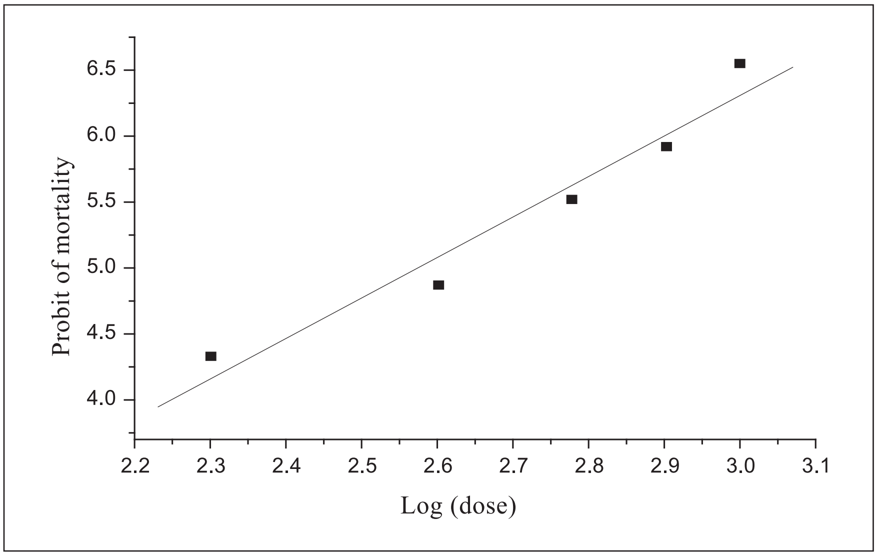

Figure 4. The adult emergence inhibition effects of leaf extract of *Ricinus communis* against 3rd instars larvae of *Anopheles arabiensis*, expressed as linear regression.

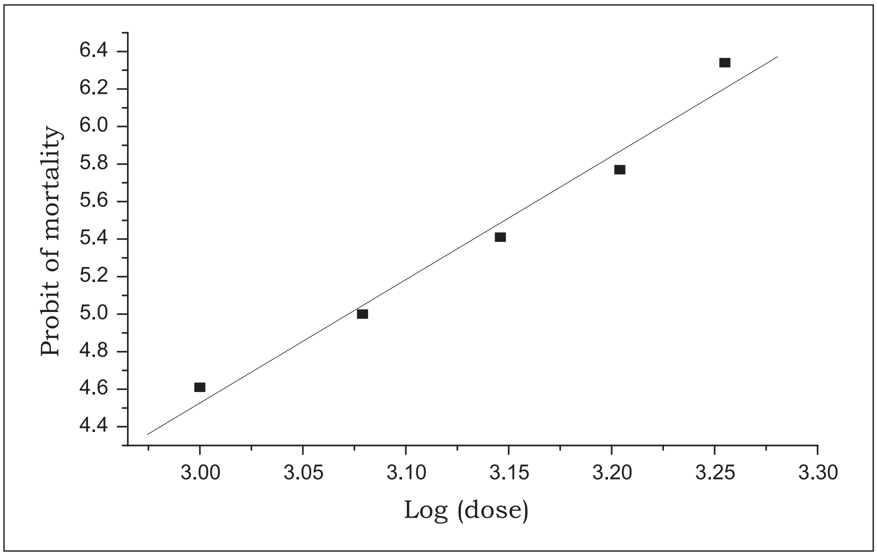

Figure 5. The adult emergence inhibition effects of leaf extract of *Ricinus communis* against 3rd instar larvae of *Culex quinquefsciatus*, expressed as linear regression.

Table 3. Oviposition deterrent effects of leaf extract of *Ricinus communis* L. against gravid, female *Anopheles arabiensis*

Cage	A		B		C		D			E	
Dose ppm	C	1200	C	600	C	200	1200	600	200	C	C
No. of eggs laid	350	0	410	0	505	25	60	140	290	310	340
ER %	100		100		90.5						

Table 4. Oviposition deterrent effects of leaf extract of *Ricinus communis* L. against gravid, female *Culex quinquefasciatus*

Cage	A		B		C		D			E	
Dose ppm	C	1600	C	1200	C	600	1600	1200	600	C	C
No. of eggs laid	345	0	425	0	490	40	45	165	385	350	330
ER %	100		100		90.1						

C = Control.
ER = Effective repellency.

acid, and that human foot odour contain stimuli to which *Cx. quinquefasciatus* is attracted (Mboera *et al.*, 2000). In this study the biological activity of the extract may be due to various compounds, including phenolics, terpenoides, flavonoids and alkaloids which may jointly or independently contribute to produce larvicidal, adult emergence inhibition, and/or oviposition deterrent effects against both tested species of mosquitoes.

We conclude that the aqueous extracts from leaves of *R. communis* possess remarkable larvicidal, adult emergence inhibition, and oviposition deterrent effects against *An. arabiensis* and *Cx. quinquefasciatus* mosquitoes. This plant might be used as a source of natural biocides, for many reasons; first, ecologically acceptable (natural products); secondly, economically (actually free and practically easy) the plant is well known and available in many areas of Sudan, and thirdly the active ingredients located in leaves and extractable with water. Further studies are needed to investigate the toxicity of this plant against wide range of non-target organisms, e.g. *Gambusia affinis*, *Orechromis niloticus*, tadpole and higher organisms such as mice.

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