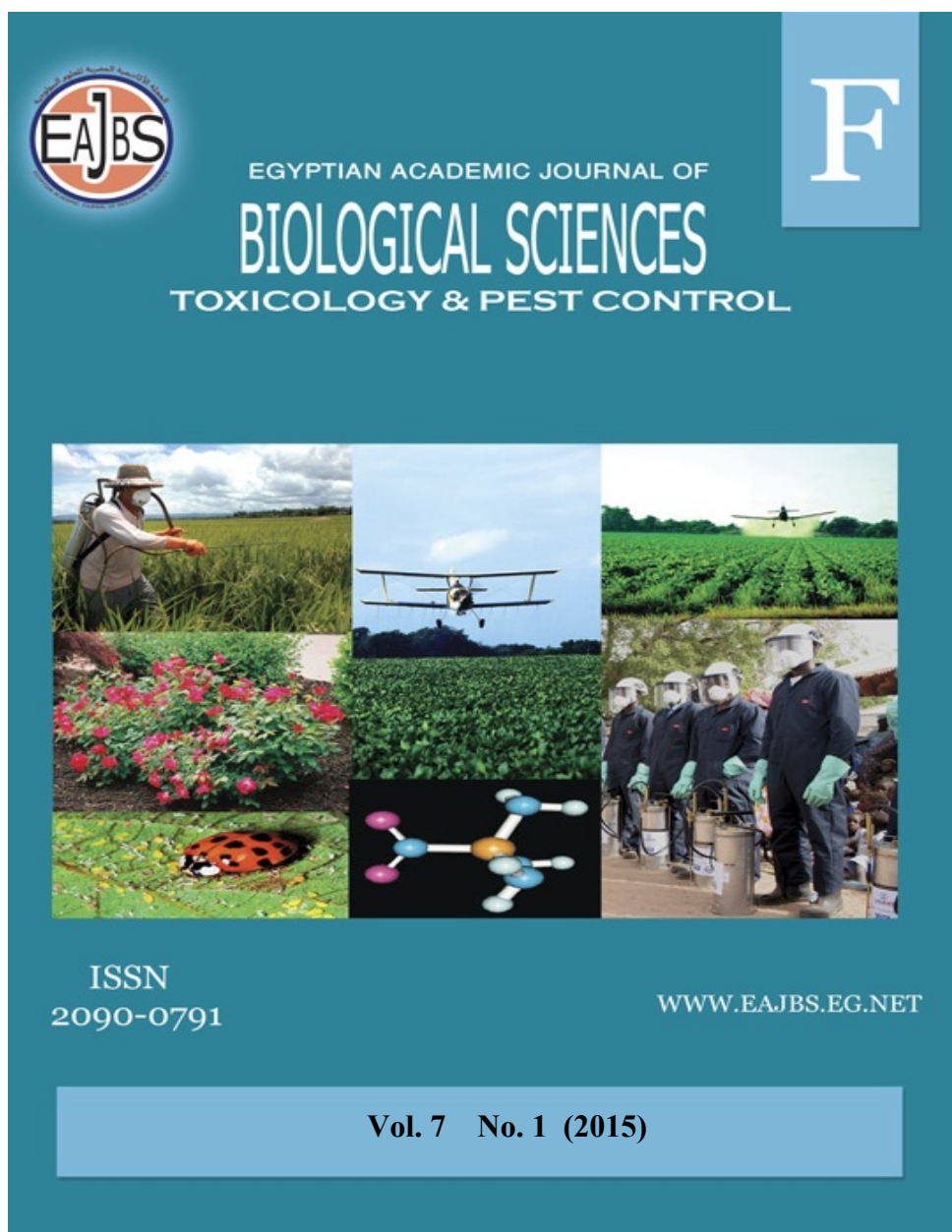


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Response of mosquito (*Anopheles arabiensis* patton) adult to leaves hexane extract of Rehan (*Ocimum basilicum* L.)

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ABSTRACT

Laboratory experiments were conducted at the National Malaria Centre, Sinnar State, Sudan, to test the efficacy of hexane extracts of rehan leaves (*Ocimum basilicum*) used as paper impregnation, against adult mosquito *Anopheles arabiensis* Patton. Repellency every 30 minutes, oviposition deterrency and adult mortality after 24 hours exposure were calculated using an excito-repellency box recommended by the WHO. Results indicated that rehan hexane extract at 10% concentration exhibited repellency property to the tested mosquitoes up to 2 hours, and it was superior to the standard insecticide. However all tested concentrations (1%, 5%, 10%) depicted oviposition deterrency to the insect, but had a little insecticidal effect compared to the standard.

INTRODUCTION

Perhaps the most medically important blood sucking arthropod world-wide is the mosquito, which transmits diseases such as malarias, dengue and yellow fever. More, over they annoy man and farm animal (Harwood and James, 1969). *Anopheles arabiensis* (*A. arabiensis*) mosquitoes possess the major malaria vector in the Sudan (Dukeen, 1981). Different strategies were adopted to control this insect which largely depend on chemical means using synthetic insecticides (Himedan, 2000), meanwhile *Anopheles* mosquitoes have become resistant to these chemicals, because of heavy and frequent application which leads to the problems of toxic residues contaminating the environment and adversely affecting non-target organisms (Sina and Adultman, 2001). This dictates the need to develop safe, less expensive, and preferably locally available materials for the control of this insect.

Large number of plant extracts have been used against *Anopheles spp.* as control agents viz. *Azadirachta indica* (Ascher, 1993); *Acorus calamus* (Ranaweera, 1996); *Calophyllum inphylum* (Pushpalatha and Muthukrishnan, 1999); *calotropis procera* (Markouk *et al.*, 2000) and *Eucalyptus camaledulensis* (Yang and Ma, 2005). Essential oils of Rehan (*Ocimum basilicum*) and two species of *Ocimum sanctum* possesses some insecticidal action against flies and mosquitoes (Chopra *et al.*, 1942). Guenther (1961) reported that *Ocimum viridae* was used as mosquito repellent.

However Watt and Breyer (1962) revealed that there is a practice of burning *Ocimum americanum* in rooms to repel mosquitoes in West Africa.

The present study was aimed to test the hexane extract of *Ocimum basilicum* leaves for its efficacy as repellent, oviposition deterrent, and adulticide against *A. arabiensis* mosquito.

MATERIALS AND METHODS

Location of the study:

The experiments were carried out at the National Malaria Centre, Sinnar city, about 300 km south Khartoum, Sudan.

Preparation and extraction of the plant materials:

Fresh leaves of *Ocimum basilicum* were collected from Shamb at campus, Sudan University of Science and Technology (SUOST), dried under shade for 10 days, then powdered to a uniform mesh. Extraction with hexane solvent was done to the obtained material at the Department of Pesticides Alternatives of the Environmental and Natural Research Institute (ENRI), using soxhlet extractor. Then, the solvent was removed by means of rotary evaporator.

Mosquito Rearing:

Mosquito *A. arabiensis* was reared at the insectory of the National Malaria Centre, sinnar state-Sudan, using the method described by Zarroug *et al.* (1988). This method is briefly described as follows: eggs of *A. arabiensis* were collected in 5 inches enamel bowls. The bowls were lined with cotton wool on the top of which wetted filter paper placed. After hatching, the larvae were transferred to rearing bowls 15 inches in diameter and 6 inches deep. Then the bowls were filled with tap water to a depth of 3 inches. 300-400 larvae were reared in each bowls, and Small amount of baby food (Riri) was added to the bowls daily as a source of food until 4th stage was reached. After pupation, the

pupae were strained off, washed with clean water, placed in small bowls and transferred to the appropriate cages until adult emergent. The males were supplied with 10 % glucose solution, while the females were twice a week fed on rabbits blood. The rearing temperature ranged between 26-30^oC, and the relative humidity was between 70- 80%.

Experiment:

The method adopted in this experiment was the excito-repellency test which is recommended by the WHO (1979). It used an excito-repellency box that was composed of a demountable plywood box of 50 X 50 X 50 cm, attached to another small trap box 30 X 30 X 30 cm. The opening in the wall of the main box corresponds to an opening in the trap, so adult mosquito could move freely between the two boxes. This opening was closed with a sliding door that could be changed at desired intervals to count the mosquitoes inside.

Solution of 20% from leaves hexane extracts of the tested plant was prepared, and dilutions were made to form concentrations of 1%, 5%, and 10% in a final volume of 50 ml. This volume of each concentration was distributed on five filter papers (24 cm diameter) until wetting, and then the wetted papers embedded in the internal part of the main box. Two Petri dishes lined with piece of wetted cotton, covered with filter paper were placed, one in the main box and the other in the trap box to serve as an egg laying media. The standard insecticide Deltamethrin was tested using the same dose as that used for mosquito nets impregnation, adopting the same previous procedure. All treatments were replicated three times with water and solvents controls for comparison.

Fifty gravid *A. arabiensis* mosquitoes were then released inside the main box. Repellency was calculated every half hour using the Leonard and Ehermon formula (1970) {A= (N0-

Nb)/Nt} where: (A= Repellency or Attractancy, N0= number of the insects in the treated sector, Nb= number of insects in the untreated sector, Nt= number of insects in both sectors). Oviposition Activity Index (OAI) was determined after 24 hours using the Kramer and Mulla formula (1979) viz: $OAI = (Nt - Nc) / (Nt + Nc)$ where: (OAI= Oviposition Activity Index, Nt= number of eggs in the treatment, Nc= number of eggs in the control). OAI values +1 indicates an attractive effect, while OAI values -1 indicates deterrency activity of the material tested. Adult mortality was

recorded after 24 hours and presented in percentage.

RESULTS

Result in Table (1) showed repellency resulted from different concentrations of rehan leaves hexane extract to *A. arabiensis* adult using Leonard and Ehermon formula (1977). In this result the third concentration 10% was the only plant treatment that exhibit repellency, which declined after the forth duration time (120 minutes). However, the standard synthetic insecticide Kothrin^R showed repellency properties also, but it deteriorated after the third duration time (90 minutes).

Table 1: Repellency resulted from Rehan leaves hexane extract to *Anopheles arabiensis* adult using Leonard and Ehermon formula.

Formula: $A = (N0 - Nb) / Nt$

Conc.	Time/min	Rehan leaf hexane extract		Controls and standard		
		Repellency factor		Repellency factor		
1%	30	+0.31	Attractancy	solvent	+0.81	Attractancy
	60	+0.36	"		+0.84	"
	90	+0.36	"		+0.92	"
	120	+0.39	"		+0.92	"
	150	+0.39	"		+0.92	"
	180	+0.41	"		+0.92	"
5%	30	+0.15	"	water	+0.92	"
	60	+0.15	"		+0.92	"
	90	+0.15	"		+0.92	"
	120	+0.23	"		+0.92	"
	150	+0.28	"		+0.92	"
	180	+0.28	"		+0.92	"
10%	30	-0.33	Repellency	Standard	-0.41	Repellency
	60	-0.33	"		-0.41	"
	90	-0.33	"		-0.41	"
	120	-0.33	"		-0.44	"
	150	-0.28	"		-0.44	"
	180	-0.28	"		-0.45	"

The data given in Table (2) demonstrated the Oviposition deterrency measured to the eggs laid by *A. arabiensis* adult subjected to different treatments according to Kramer & Mulla

(1977). The Oviposition Activity Index calculated in this result showed that all rehan concentrations and the standard insecticide cause deterrent effect to the tested insect.

Table 2: Oviposition deterrency resulted from Rehan leaves hexane extracts to *Anopheles arabiensis* adult using Kramer and Mulla formula.

Treatment	Average Eggs Number	S.D	Oviposition Activity Index (O.A.I)	Attractancy or deterrency	Means
RLH (1%)	00.00	0.00	-1.0000	Deterrency	0.710
RLH (5%)	00.00	0.00	-1.0000	"	0.710
RLH (10%)	00.00	0.00	-1.0000	"	0.710
SSI	41.67	1.53	-0.7125	Deterrency	

Result in Fig. (1), showed the mortality percentage in *A. arabiensis* adult exposed to different treatments. It depicted the following mortalities: (2.67,

3.3, and 11.33 %) for the concentrations 1%, 5% and 10% respectively. However, the standard insecticide showed 34.66% mortality.

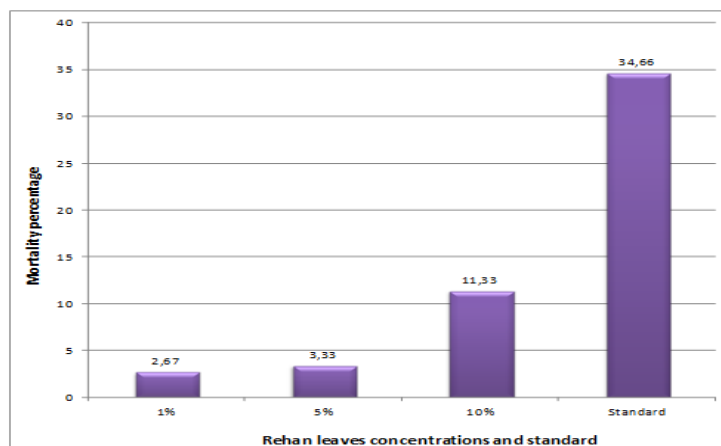


Fig. 1: Mortality percentage resulted from different Rehan leaves hexane extracts to *Anopheles arabiensis* adult.

DISCUSSION

Results of repellency demonstrated in Table (1) revealed that the higher concentration of rehan hexane extract was the treatment that showed repellency as the standard insecticide did, since the author classified the treatment as repellent or attractant. This result agreed with that of Seyoum (2002), who revealed that when testing different plants as repellents against *A. gambiae* in an experimental huts at western Kenya, *Ocimum basilicum* leaves was the most effective plant. Moreover, Bernard (1999), stated that various plant extracts such as basil oil (*Ocimum basilicum* L.; *Ocimum gratissimum* L.; *Ocimum americanum* L.; and *Ocimum tenuiflorum* L.) have been recorded as mosquito repellents. Regarding time factor, it was cleared that the rehan concentration (10%) was superior to the synthetic insecticide, as it sustained repellency up to 120 minutes, compared to 90 minutes protection exhibited by the standard insecticide. However, Tawatsin *et al.* (2001) evaluated the repellency of several plant oils against *A. Dirus* using human bait technique. They found that

hairy basil *Ocimum basilicum* repelled the mosquitoes for up to 8 hours under cage condition.

The negative results of OAI showed in Table (2) demonstrated that the different concentrations of rehan hexane extract had the ability to deter *A. arabiensis* adult from laying eggs. The standard insecticide could do the same, but the plant extract treated media showed no egg lay, in comparison with some noticed in the synthetic insecticide. This agreed with the previous result of repellency, and could be justified with that rehan extract exert convincing irritant effect, which caused confusion to the mosquito female from laying egg. This result came in line with the findings of Yarnell and Abascal (2005). They found that *Ocimum scantum* leaf extract was effective in inhibiting egg laying by *A. stephensi* female mosquitoes.

The literature offers little or no information on the toxicity of natural plant products against adult mosquito species (Elsiddig, 2007). Result of this study illustrated in general that in comparison with the synthetic insecticide, lower mortality percentages

were induced by different rehan concentrations when applied as paper impregnations. From this result it was assumed that while rehan hexane extracts caused repellency to mosquito female, it makes little or no contact with the treated surface. Though, intoxication dose not occur. Xue and Ali (2003) confirm this argue when reported that contact with aerosols of many commercial mosquito repellents resulted in knock-down and death in female mosquitoes. They continued that man-made chemical repellents acts more quickly in this regard than natural products-based repellents.

CONCLUSION

This study concluded that Rehan leaves hexane extract could repel adult *A. arabiensis* mosquito up to 2 hours. Instead of this accomplished by using higher rate (10%), although it was still performing better than the standard insecticide K-othrin. Meanwhile, the previously mentioned extract was highly effective in deterring the tested species, since no egg oviposition was noticed by using low concentrations. On the other hand, a negligible adult toxicity was exhibited compared to the standard.

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ARABIC SUMMERY

استجابة الطور الكامل لبعوض الانوفليس العربي *Anopheles arablensis* لمستخلص الهكسان من أوراق الريحان *Ocimum basilicum*

فتح الرحمن الصديج

قسم وقاية النبات - كلية الزراعة - جامعة سنار - السودان

استهدفت الدراسة اختبار فعالية مستخلص الهكسان لاوراق الريحان بعد تشبييع اوراق الترشيح بها، وذلك على الحشرة الكاملة لبعوض الانوفليس العربي باجراء تجارب معملية فى المركز القومى للملاريا بولاية سنار - السودان.

تم حساب التأثير الطارد كل نصف ساعة، بالاضافة للتاثير المانع لوضع البيض والتاثير القاتل للحشرة بعد ٢٤ ساعة من التعرض. تم ذلك باستخدام صندوق الطرد الموصى به من قبل منظمة الصحة العالمية. دلت النتائج على ان مستخلص الهكسان لاوراق الريحان بتركيز ١٠% قد اظهر تاثير طارد للحشرة الكاملة المختبرة وذلك لفترة تصل لساعتين، مع تفوقه على المبيد القياسى. بينما اظهرت كل التركيزات المستخدمة من المستخلص تحت الدراسة (١%، ٥%، و ١٠%) تاثير مانع لوضع البيض على انثى الحشرة، فى حين اظهرت كل التركيزات المذكورة تاثير قاتل قليل بالمقارنة مع المبيد القياسى.