Effect of organic extracts of *Cafure* leaves (*Eucalyptus camaldulensis* Dehn.) on mosquitoes *(Anopheles arabiensis* Patton.)

Fathelrahman. I. Elsiddig

Plant Protection Department, Faculty of Agriculture, Sinnar University, Sudan,
e-mail: elsiiddig@yahoo.com.

ABSTRACT

Laboratory experiments were conducted at the National Malaria Centre, Sinnar State, Sudan to investigate the effects of organic extracts of *Cafure* leaves (*Eucalyptus camaledulensis* Dehn.) against malaria vector in Sudan (*Anopheles arabiensis*) Patton (Diptera:Culicidae). The larvicidal activities of different concentrations of ethanol and hexane extracts were measured according to the WHO standards for testing susceptibility of mosquito larvae to insecticides. The effects of extracts on repellency, oviposition deterrency and mortality of adult insects were also measured. Results indicated that hexane extract showed the best larvicidal effect with LC_{50} of 127.9 mg ℓ^{-1}. It proved superiority over the standard larvicide Temphos® in the initial levels, meanwhile, ethanol extract exerted poor larvicidal effect (LC_{50} 8276 mg ℓ^{-1}). Results showed that hexane extract at 10% concentration repelled mosquitoes, for two and a half hours. However, ethanol concentrations depicted no repellency. All the tested extracts exhibited oviposition deterrency properties but had little insecticidal activities.

INTRODUCTION

Mosquitoes transmit many serious diseases to man, such as malaria, yellow fever, falariasis and encephalitis. Their attack on farm animals lead to losses in weight and reduction in milk production. Members of *A. gambiae* complex are regarded as the most efficient vectors of human malaria throughout the African continent. However, *A. arabiensis* is the major malaria vector in the Sudan (Dukeen, 1981). Control measures of malaria in the Sudan are largely based on using different groups of larvicides and adulticides (Himedan, 2000). However, resistance of vectors to chemical insecticides is one of the major problems causing failure of malaria control programs in the world, in addition to the increasing cost of synthetic chemicals and the serious hazards they impart to the environment. This has necessitated the need for development of friendly environmental, biodegradable and low cost methods for mosquito control (Babiker, 1991).

Phytochemicals obtained from plants with proven mosquitoes control potential can be used as an alternative to synthetic insecticides or along with them under an integrated control programme. Mosquito larvae of different species display different susceptibilities to phytochemicals. In general, *Aedes* larvae are more susceptible to insecticides and plant extracts than *Culex* larvae. The generalization made with *Aedes* and *Culex* larvae does not always hold true with *Anopheles* species. The susceptibility of *Anopheles* larvae vary since they can be more or less susceptible than *Culex* and *Aedes* larvae to plant extracts (Shaalan *et al*., 2003). Large numbers of plant extracts have been used against *Anopheles* spp. as control agents *viz*: *Azadirachta indica*, *Ocimum* spp.(Ascher,1993), *corus calamus* (Ranaweera,1996), *Calophylum inphylum* (Pushpalatha and Muthukrishnan,1999) and *Calotropis procera* (Markouk *et al*., 2000). Fresh leaves of *Eucalyptus* contain large amounts of volatile terpenes and essential oils which are mainly composed of cineol (eucalyptol) that has been reported to display pesticidal qualities against some pests *viz*: store pests (*Ryzopertha dominica*, *Sitotroga cereallela*, *Sitophilus oryzae*, and *Tribolium confusum*),root-knot nematodes (*Meloidogynae arenaria*), termites and tree locust (Mahamoud, 2003). Different species of *Eucalyptus* have been reported to posses biological activities against mosquito *viz*. *E. globus* labill, *E. erreticinis* L. and *E. camaldulensis* Dehn. The latter was only reported in Sudan (Mohagir, 2000). Therefore, the objectives of this study were to test the repelling, oviposition deterring, and larvicidal effects of organic extracts of *cafure* leaves against *A. arabiensis* mosquitoes.

MATERALS AND METHODS

Preparation and extraction of the plant material

Fresh leaves of *E. camaldulensis* were collected from Shambat campus, University of Science and Technology, Sudan, dried under shade for 15 days, then powdered to a uniform mesh. Extraction was done at the Department of Pesticides Alternatives of the Environmental and Natural Research Institute, using soxhlet extractor, firstly with hexane and then with ethanol (98%). The solvents were removed by means of rotary evaporator.

Mosquito culture

A. arabiensis mosquitoes were reared at the insectory of the National Malaria Centre, Sinnar State-Sudan, using the method described by Zarroug *et al*. (1988).

Tests on larvae

Twenty percent solutions from each of ethanol and hexane extracts were prepared using tap water. Serial dilutions were made to give the concentrations of 500, 1000, 3000, 5000, and 10000mgℓ^{-1} in a

final volume of one liter each. The concentrations of the standard larvicide Temphos® (50% E.C) ranged from 0.001 to 0.625mgℓ^{-1}. Water and solvents controls were prepared with the same final volumes, and all treatments were replicated four times. These treatments were then evaluated for mosquito larvicidal activity according to the method of the WHO (1969). Mortality was recorded and subjected to probit analysis using M Stat-C package computer program, to calculate LC_{50} values.

Tests on adult

The method adopted was the excito-repellency test recommended by the WHO (1979). Solutions of 20% from each of the ethanol and hexane extracts were 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 poured on five filter papers (24 cm diameter) until wetting, and then were embedded in the internal part of the main box. Two petri dishes lined with a piece of wetted cotton and covered with filter paper were prepared; one was placed in the main box and the other in the trap box to serve as an egg laying sites. The standard insecticide
Deltamethrin® (25% EC.) was used as a standard at the same dose used for mosquito nets impregnation, using 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 formula of Leonard and Ehermon (1970) (A= (N0-Nb)/Nt) where: A= repellency or attractancy, N0= number of insects in the treated sector, Nb= number of insects in the untreated sector, and Nt= number of insects in both sectors. Ovipositon activity index (OAI) was determined after 24 hours using the formula of Kramer and Mulla (1979) *viz*. OAI= (Nt-Nc)/ (Nt+Nc).Where OAI= oviposition activity index, Nt= number of eggs in the treatment and Nc= number of eggs in the control. OAI values +1 indicate an attractive effect, while OAI values -1 indicate deterrency activity of the material tested. Adult mortality was recorded after 24 hours and presented in percentage.

RESULTS

Results in Table 1 shows that all *cafure* (*E. camaldulensis*) leaf extracts exhibited mortality to *A. arabiensis* larvae, compared to that of the two controls, i.e. water and solvent. The mortality rate of using the ethanol extract ranged from 5% in the lower concentration (500) to 62.5% in the higher one (10000mgℓ^{-1}). However for the hexane extract, it ranged from 82.5% in the lower concentration to 100% in the higher. The standard larvicide Temphos ®depicted larval mortality that ranged from 7.5% in the lower concentration (0.001mgℓ^{-1}) to 100% in 0.625mgℓ^{-1}. Table 2 Showed that the LC_{50} of *cafure* leaves ethanol, and hexane extracts and the standard larvicide Temphos® to *A. arabiensis* larvae were 8276, 127.9, and 0.003 mgℓ^{-1}, respectively.

Table 3 shows that the 10% of the *cafure* hexane extract was the only treatment that depicted repellency to *A. arabiensis* adult. The negative result reflects the repellency, which started to decline after two and half hours. Table 4 demonstrated that all rates of *cafure* extracts exhibited

deterrency effect to *Anopheles* adult. The percentages of mortality are shown in Table 4. They were in the order of 3.3%, 12%, and 23.3% for ethanol concentrations of 1%, 5%, and 10%, respectively, and 5.3, 14, and 27.3 % for hexane concentrations of 1, 5, and 10%, respectively. The standard insecticide depicted 34.7 % mortality.

Table 1. Mortality percentage caused by different *Cafure* leaf organic extracts to *Anopheles arabiensis* larvae.

	Cafure leaves ethanol extract (CLE)				
Concentration (mgℓ^{-1})	500	1000	3000	5000	10000
Mortality (%)	05.0	11.3	18.8	25	62.5
S.E (±)	0.35	0.22	0.22	0.61	0.29
	Cafure leaf hexane extract (CLH)				
Concentration (mgℓ^{-1})	500	1000	3000	5000	10000
Mortality (%)	82.5	96.3	97.5	100	100
S.E (±)	0.25	0.41	0.25	0.00	0.00
	Standard synthetic larvicide (Temphos®)				
Concentration (mgℓ^{-1})	0.001	0.005	0.025	0.125	0.625
Mortality (%)	7.5	71.3	100	100	100
S.E (±)	0.43	0.22	0.00	0.00	0.00
	Water control		Solvents control		
Mortality (%)	0.00		0.00		
S.E (±)	0.00		0.00		

Table 2. Probit regression line parameters of response of *Anopheles arabiensis* larvae to different *Cafure* organic extracts.

Parameter	*Cafure* leaf ethanol extract	*Cafure* leaf hexane extract	Standard synthetic larvicide
Intercept	0.6427	1.4330	12.4425
Variance of slope	0.0338	0.1681	0.1058
Chi-square	14.9360	10.8699	2.8984
Probability	0.6663	0.8997	0.9999
Degrees of freedom	18	18	18
Logarithm LC_{50}	3.9674	2.1067	2.5028
Variance of logarithm LC_{50}	5.5631	0.0439	1.6290
LC_{50} ($mg\ell^{-1}$)	8276	127.9	0.003

Table 3. Repellency resulting from different *Cafure* organic extracts to *Anopheles arabiensis* adults using Leonard and Ehermon (1970) formula.

Formula: A= (N0-Nb) Nt

Conc.	Time/min	*Cafure* leaf ethanol extract Repellency factor		*Cafure* leaves hexane extract Repellency factor		Deltamethrin ®	
1%	30	+0.60	Attractancy	+0.44	Attractancy	-0.41	Attractancy
	60	+0.63	"	+0.47	"	-0.41	"
	90	+0.71	"	+0.49	"	-0.41	"
	120	+0.76	"	+0.55	"	-0.44	"
	150	+0.79	"	+0.55	"	-0.44	"
	180	+0.84	"	+0.55	"	-0.45	"
5%	30	+0.36	"	+0.20	"		
	60	+0.36	"	+0.20	"		
	90	+0.36	"	+0.20	"		
	120	+0.39	"	+0.28	"		
	150	+0.49	"	+0.33	"		
	180	+0.52	"	+0.33	"		
10%	30	+0.17	"	-0.17	Repellency		
	60	+0.17	"	-0.17	"		
	90	+0.17	"	-0.17	"		
	120	+0.17	"	-0.17	"		
	150	+0.31	"	-0.12	"		
	180	+0.31	"	-0.06	"		

Table 4. Ovipositon deterrency and adult mortality resulting from different *Cafure* organic extracts to *Anopheles arabiensis* adults.

Treat.	Number of eggs	SD	Oviposition activity index	Attractancy or deterrency	Adult mortality (%)
CLE (%)					
1	31.00	1.73	-0.7220	Deterrency	03.33
5	29.67	4.16	-0.7308	"	12.00
10	00.00	0.00	-1.0000	"	23.33
CLH					
1	20.67	8.50	-0.7443	Deterrency	05.33
5	00.00	0.00	-1.0000	"	14.00
10	00.00	0.00	-1.0000	"	27.33
SSI	41.67	1.53	-0.7125	Deterrency	34.66

*CLE= *Cafure* leaves ethanol extract
*CLH= *Cafure* leaves hexane extract
* SSI = Standard synthetic insecticide
* SD = Standard deviation

DISCUSSION

Larval mortality due to *cafure* leaf extracts revealed that the performance of hexane extract was better than that of ethanol, since it exhibits high mortality rates with minimum concentrations (Table 1). The main active component of *E. camaldulensis* was the cinoel (eucalyptol) oil obtained through hexane extraction only. This active ingredient has been reported to be used as a mosquito larvicide (Corbet, 1995). The same trend was obtained with the LC_{50} results, when the hexane extract depicted LC_{50} of 127.9 mgL^{-1} while the ethanol extract showed LC_{50} of 8276 mgℓ^{-1} (Table 2). On the other hand, the two lower concentrations of hexane (500 and 1000 mgℓ^{-1}) extract showed higher efficacy than their counterparts of the standard larvicide in causing larval mortality. These findings were in conformity with the results of Burfield and Reekie (2005) who stated that the initial level of activity of the cinoel oil was greater than the commercial larvicide.

Repellency to *A. arabiensis* adults (Table 3) showed that the hexane extract at 10% concentration was the only treatment that exhibited repellency, which continued up to two and a half hours. In a laboratory test in South Africa, Trigg and Hill (2000) concluded that *Eucalyptus* based insect repellent gave satisfactory levels of personal protection against *A. arabiensis*, for 5 hours. In China, Yang and Ma (2005) test *Eucalyptus* solution of 15% and 30% for human protection against bites of *Aedes albopictus*. They found that duration of protection reached up to 5 hours.

The negative results of oviposition activity index (Table 4) proved that all *E. camaldulensis* leaf extracts deterred *A. arabiensis* females from laying eggs and the deterrency was concentration dependent. Moreover, the performance of the extracts on egg laying was more effective than that of the standard insecticide. This result could be attributed to the irritant effect caused by hexane extract to the *Anopheles* females which lead to confusion in egg laying.

The mortality results illustrated that all *E. camaldulensis* extracts exerted little adulticide action to *A. arabiensis*; which was inferior than that of the standard insecticide. However, the hexane extract at the rate of 10 % gave 27.2 % mortality rate which could be monitored to accomplish better results (Table 4). This result agreed with that of Senthi (2006) who reported that essential oils extracted from *E. tereticonis* suppressed the adult activity of *A. stephensi* at higher doses and could serve as a natural mosquitocide.

CONCLUSION

Cafure leaf hexane extracts gave reliable and competitive results with the synthetic chemicals in depicting larval mortality, repellency, oviposition deterrency, and adult mortality to *Anopheles arabiensis* mosquitoes. This encourages the inclusion of this product in the IPM programs with other control measures.

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تاثير مستحلصات عضوية من الكافور (*Eucalyptus camaldulensis* Dehn.) على بعوض الانوفليس (*Anopheles arabiensis* Patton).

فتح الرحمن ابراهيم الصديق

قسم وقاية النباتات، كلية الزراعة، جامعة سنار،سنار، السودان.

الخلاصة

تم اجراء تجارب معملية فى المركز القومى للملاريا بولاية سنار لمعرفة تاثير مستخلصات عضوية من اوراق الكافور على البعوض الناقل للملاريا بالسودان (*Anopheles arabiensis* Patton). تم قياس النشاط القاتل لليرقات وذلك لتركيزات مختلفة من مستخلصات الايثانول والهكسان تبعاً لمقاييس منظمة الصحة العالمية المستخدمة لاختبار حساسية يرقات البعوض للمبيدات. تم اختبار المستخلصات ضد الحشرة الكاملة للانوفليس وذلك بحساب التاثير الطارد كل نصف ساعة، والتاثير المانع لوضع البيض والقاتل للحشرة الكاملة بعد 24 ساعة من التعرض. اوضحت النتائج ان مستخلص اوراق الكافور الهكسانى اعطى اعلى تاثير قاتل لليرقات وذلك بتركيز نصفى قاتل 127,9 مجم/لتر-1، مع تفوق على المبيد القياسى تمفوس فى المستويات الاولى للتركيزات. اظهرت النتائج ايضاً ان المستخلص الهكسانى بتركيز 10% هو المعاملة الوحيدة التى ادت لطرد الحشرة الكاملة وذلك لفترة زمنية امتدت لساعتين. اظهرت المستخلصات تحت الاختبار خصائص مانعة لوضع البيض مع قليل من المميزات القاتلة للحشرة الكاملة.