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Efficacy of different Neem *Azadirachta indica* organic extracts on mosquitoes *Anopheles arabiensis* Patton

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Abstract: Laboratory experiments were carried out at the National Malaria Centre, Sinnar State-Sudan, to evaluate the effects of organic extracts of leaves and seed kernels of neem *Azadirachta indica* A.juss. against larvae and adults of the main malaria vector in Sudan *Anopheles arabiensis* Patton. Larvicidal activity, oviposition deterrency, and adult mortality after 24 hours exposure were measured according to the WHO standards, using ethanol and hexane extracts of the mentioned neem parts. Results indicated that all tested extracts exhibit larvicidal properties against *Anopheles arabiensis* mosquito. However, the seed hexane extract was superior to other ones, depicting minimum LC_{50} of 1998 $mg\ell^{-1}$. Oviposition deterrency to Anopheles adult was noticed from all tested extracts, with their different concentrations. Meanwhile the extracts showed negligible insecticidal characteristics to the tested mosquito. It can be concluded from the present investigation, that the tested neem extracts could be compatible to be applied with other conventional biological measures used in malaria vector control program after field verifications. Keeping in view the great concern raised about vector resistance and environmental hazards of conventional insecticides.

Key words: *Azadirachta indica*, mosquito Oviposition deterrency, Larvicides, *Anopheles arabiensis.*

INTRODUCTION

Mosquito *Anopheles arabiensis* is one of the most important vectors of malaria in sub-Saharan Africa, and it occurs in an overlapping manner with other important species (Mabaso *et al.*, 2004). Control of anopheline mosquito vectors of malaria by using synthetic insecticides has shown a greater impact on morbidity and mortality caused by this disease. Regarding that insecticide resistance is widely spread in Africa where it has been associated with the use of insecticides in public health for mosquito control and in agriculture for pest control(Kristan *et al.,* 2003). In Sudan, although more recent studies indicated that resistant level had increased only marginally (Kamau and Vulule 2006), there is a concern that continued and/or increasing the use of insecticides may result in an increased resistance that would threaten the sustainability of the vector control strategies(Maharaj *et al.,* 2005).

Phytochemicals obtained from plants with proven mosquito control potentials can be used as an alternative to synthetic insecticides or along with them under integrated control programmes. Large numbers of plant extracts have been used against *Anopheles* spp. as control agents viz. *Calophylum inphylum* (Pushpalatha and Muthukrishnan 1995); *calotropis procera* (Markouk *et al.,* 2000); *Eucalyptus camaledulensi* (Yang and Ma 2005) and *Ocimum basilicum* (Elsiddig and Khei 2007). In Sudan neem *Azadirachta indica* tree is widely spread and it is found almost in every part of the country. A number of workers studied the effect of the different parts of neem tree on different

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arthropod pests of crops, viz. (Mansour and Salem, 2001; Satti *et al.,* 2003 ; and Elsiddig, 2009). However neem oil and other commercial preparations of neem have been found as potential mosquito larvicides,(Mittal *et al.,* 1995, Batra *et al.,* 1998) reported that neem oil emulsion in water was found to control breeding of *Culex quinquefasciatus***,** *Anopheles stephensi***,** and *Aedes egypti* in pools, basement tanks, and desert coolers. Topical application of 2% neem oil mixed with coconut oil produced various degree of protection against different vector species (Moore *et al.,* 2002). The present study designed to test the potentials of different neem organic preparations against *Anopheles arabiensis* larvae, as well as evaluating their oviposition deterrency and mortality on adults.

MATERIALS AND METHODS

Preparation and extraction of the plant material

Fresh leaves of neem *Azadirachta indica* were collected from Shambat campus, Sudan University of Science and Technology, dried under shade for 10 days, and then powdered to a uniform mesh. Ripe fruits of the plant were harvested from the same area and soaked in a water container to remove pulps. The obtained seeds were dried under shade for 10 days. The well-dried seeds were decorticated to obtain the kernel separately, which powdered to a uniform mesh. Extraction was done for the two prepared parts at the Department of Pesticides Alternatives of the Environmental and Natural Research Institute-Sudan, 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 10000 $mg\ell^{-1}$ in a final volume of each one liter. 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. These volumes of each concentration were 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 a filter paper were prepared; one was placed in the main box, and the other in the trap box to serve as egg-laying sites. 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. Oviposition 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 deterrence activity of the material tested. Adult mortality was recorded after 24 hours and presented in percentage.

ABRIVATIONS:NLE= Neem Leaves Ethanol Extract, NLH= Neem Leaves Hexane Extract, NSE= Neem Seed Ethanol Extract, NSH= Neem Seed Hexane Extract, OAI= Oviposition Activity Index, S.D= Standard Deviation, S.E= Standard Error, WHO= World Health Organization, IPM= Integrated Pest Management, LC= Lethal Concentration.

RESULTS AND DISCUSSION

The results given in Table (1) demonstrated that the crude neem extracts (leaves and seed kernels) with their different solvents depicted larvicide effect against *Anopheles arabiensis* mosquito. This agreed with(Aliero 2003), who suggested that seed oil and leaf extract of neem *Azadirachta indica* contain properties that could be developed and used in the control of mosquitoes in the tropics, as a result of his studies on the larvicidal effect of neem on Anopheles mosquitoes. In the same table, better mortality result was obtained by the neem seed kernel extract compared to neem leaves extract. The advantages of seeds over leaves could be justified by the fact mentioned by (Grunwald *et al*., 1992), that the bioactive compounds in the neem were found throughout the tree, but those in the seed kernel were the most concentrated and accessible. Results in Table (1) also showed that neem seed hexane extract exerted better mortality when compared to ethanol. Regarding this manner, the hexane solvent was well known to remove the oil from the seed (non-polar), and this oil was an interesting material that could be used to kill eggs and larvae of certain pests.(Aliero 2003) concluded that seed oil appeared as the most lethal among various parts tested against *Anopheles spp*. He attributed this to deficiency of dissolved oxygen in the water.

Table (1). Mortality percentage caused by different neem organic extracts to *Anopheles arabiensis* larvae.

Concentration ($mg\ell^{-1}$)	500	1000	3000	5000	10000
	Neem Leaves Ethanol Extract (NLE)				
Mortality %	7.5	10	15	16.25	67.5
S.E (±)	0.22	0.0	0.35	0.22	0.50
	Neem Leaves Hexane Extract (NLH)				
Mortality %	11.25	18.75	55	61.25	92.5
S.E (±)	0.65	0.41	0.87	1.14	0.83
	Neem Seeds Ethanol Extract (NSE)				
Mortality %	5	12.5	25	81.25	100
S.E (±)	0.35	0.56	0.0	0.54	0.0
	Neem Seeds Hexane Extract (NSH)				
Mortality %	5	25	93.75	98.75	100
S.E (±)	0.35	0.0	0.41	0.22	0.0

Table (2) showed results of probit regression analysis which demonstrated the LC_{50} of different plant extracts. It depicted the same trend of the mortality results in table (1) when the neem hexane extracts of the tested parts exerted lower LC_{50}. However, the seed extract was the best treatment compared to the other ones, with LC_{50} of 1998 $mg\ell^{-1}$.

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

Parameter	Leaves ethanol extract	Leaves hexane extract	Seeds ethanol extract	Seeds hexane extract
Intercept	0.7817	1.5278	4.7710	8.0276
Variance of slope	0.0360	0.0288	0.0533	0.1215
Chi-square	28.999	23.742	47.825	10.158
Probability	0.0483	0.1636	0.0001	0.9266
Degrees of freedom	18	18	18	18
Logarithm LC_{50}	3.9181	3.6090	3.4694	3.3005
Variance of logarithm LC_{50}	0.0059	0.0001	0.0008	0.0008
LC_{50} ($mg\ell^{-1}$)	8282	4065	3380	1998

The negative results of Oviposition Activity Index (OAI) presented in table (3) Demonstrated that the different neem part organic extracts with their different concentrations had the ability to deter *Anopheles arabiensis* adults from laying eggs. This result agreed with(Schmutterer 1990), who reported that neem based pesticides containing azadirachtin, which is a predominant active ingredient, have antifeedant, ovipositional deterrence repellency, and growth disruption against insects. (Goktepe *et al.*, 2004) confirmed the previous conclusions and continued reporting that they are relatively safe towards non-target biota, with minimum risk of direct adverse effects and contamination of water bodies. Neem Aura^{R}, a commercial botanical product containing neem ingredients, was proved to be highly effective oviposition deterrent to *Aedes albopictus*, it reduced oviposition by 76%(Xue *et al.*, 2001).

Adult mortality presented in table (3) revealed that lower mortality percentages were induced by

different tested parts when applied as paper impregnation. This result agreed with that of (Sagar and Sehgal 1996) who stated that, though neem products show high larvicidal activity, they do not show adulticidal action. However, (Khan and Ahmed 2000) revealed the toxicity of crude neem extract and commercial eucalyptus against the adult housefly Musca *domestica* when measured as a topical application. From the result of oviposition deterrency, it could be assumed that while neem extract had the ability to deter adult from laying eggs, the mosquito makes little or no contact with the treated surface and consequently mosquito intoxication does not occur.

Table (3). Ovipositon deterrency and adult mortality of *Anopheles arabiensis* resulting

Treatment	Number of eggs	S.D	Oviposition activity index	Attractancy ordeterrency	Adult mortality (%)
NLE 1%	29.67	4.16	-0.7308	Deterrency	00.00
5%	17.33	2.08	-0.8344	"	00.00
10%	15.00	0.00	-0.8551	"	02.00
NLH 1%	55.67	0.58	-0.4339	Deterrency	00.00
5%	08.33	3.51	-0.8884	"	00.00
10%	00.00	0.00	-1.0000	"	04.67
NSE 1%	48.00	1.00	-0.6000	Deterrency	00.00
5%	23.67	1.18	-0.7790	"	03.33
10%	13.67	2.08	-0.8671	"	07.33
NSH 1%	33.33	1.15	-0.6176	Deterrency	02.67
5%	33.33	0.58	-0.6176	"	15.33
10%	00.00	0.00	-1.0000	"	26.00

from different *neem* organic extracts

CONCLUSION

This study clearly demonstrated that ethanol and hexane extracts of neem leaves and seed kernels exhibited a larvicidal effect on *Anopheles arabiensis* mosquito, with the superiority of the seed hexane extract. However, oviposition deterrency properties were observed from all concentrations of the tested extract with negligible toxicity characteristics to adult mosquitoes. The obtained results will still encourage the inclusion of these extracts in IPM programs for mosquito control with other natural and biological measures.

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تأثير مستخلصات عضوية من النيم (*Azadirachta indica* A.juss) على بعوض الأنوفليس العربي (*Anopheles arabiensis* Patton)

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المستخلص: تم إجراء تجارب معملية في المركز القومي للملاريا بولاية سنار – السودان، لمعرفة تأثير مستخلصات عضوية من أوراق وجنين بذور النيم (*Azadirachta indica* A.juss) على طور اليرقة والحشرة الكاملة للبعوض الناقل للملاريا بالسودان (*Anopheles arabiensis* Patton.). تم قياس النشاط القاتل لليرقات، التأثير المانع لوضع البيض، والقاتل للحشرة الكاملة بعد 24 ساعة من التعرض وذلك باستعمال مستخلصات من الإيثانول والهكسان للأجزاء المذكورة سابقاً من النيم، تبعاً لمقاييس منظمة الصحة العالمية. أوضحت النتائج أن جميع المستخلصات المختبرة من أجزاء النيم قد أظهرت تأثيراً قاتلاً ليرقات بعوض الأنوفليس العربي. وقد كان مستخلص الهكسان لجنين بذور النيم هو الأكثر تفوقاً على المستخلصات الأخرى معطياً أقل تركيز نصفي قاتل، بلغ 1998 ملجم/ 1000 لتر. أظهرت المستخلصات تحت الاختبار وبكل التركيزات المستخدمة منها، خصائص مانعة لوضع البيض، في حين أنها لم تظهر تأثيراً قاتلاً يذكر للحشرة الكاملة للبعوض موضوع الدراسة. يمكن أن نخلص من هذا التقصي إلى أن مستخلصات النيم تحت الدراسة يمكن أن تستخدم بتوافق مع الطرق البيولوجية التقليدية الأخرى في برامج مكافحة البعوض الناقل للملاريا وذلك بعد التقييم الحقلي، واضعين في الاعتبار الاهتمام العام بتنامي ظاهرة مقاومة النواقل للمبيدات التقليدية والتأثيرات البيئية الضارة لها.

الكلمات المفتاحية: *Azadirachta indica*، البعوض، *Oviposition deterrency*، مبيد اليرقات، Anopheles *arabiensis*.

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