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Research paper

Effect of Some Neem *(Azadirachta indica)* Organic Extracts Against Mosquitoes *Anopheles arabiensis* Patton

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ABSTRACT

Laboratory experiments were carried out at the National Malaria Centre, Sinnar State, Sudan, to investigate the effect 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 exhibited larvicidal properties against Anopheles arabiensis mosquito. However the seed hexane extract was superior over other ones, depicting minimum LC50 of 1998 mgℓ-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 apply with other conventional biological measures used in malaria vector control program after field verifications, keeping in mind the great concern raised about vector resistance and environmental hazards of conventional pesticides.

Keywords: *Anopheles arabiensis, Azadirachta indica, larvicides, mosquito, oviposition deterrency*

تأثير مستخلصات طبيعية من النيم (*Azadirachta indica* A.juss) على بعوض الانوفليس (*Anopheles arabiensis* Patton.)

فتح الرحمن ابراهيم الصديق

قسم وقاية النباتات، كلية الزراعة، جامعة سنار- السودان

تم اجراء تجارب معملية في المركز القومي للملاريا بولاية سنار- السودان، لمعرفة تأثير مستخلصات عضوية من اوراق وجنين بذور النيم (*Azadirachta indica* A. juss) على أطوار 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 الايثانول والهكسان للأجزاء المذكورة سابقاً من النيم، تبعاً لمقاييس منظمة الصحة العالمية. اوضحت النتائج ان جميع المستخلصات المختبرة من اجزاء النيم قد اظهرت تأثير قاتل ليرقات بعوض الانوفليس، وقد كان مستخلص الهكسان لجنين بذور النيم هو الاكثر تفوقاً على المستخلصات الاخرى معطياً اقل تركيز نصفي قاتل، بلغ 1998 ملجم/ لتر$^{-1}$. اظهرت المستخلصات تحت الاختبار وبكل التركيزات المستخدمة منها، خصائص مانعة لوضع البيض، فى حين انها لم تظهر تأثير قاتل يذكر للحشرة الكاملة للباعوض موضوع الدراسة. يمكن ان نخلص من هذا التقصي ان مستخلصات النيم تحت الدراسة يمكن ان تستخدم بتوافق مع الطرق البيولوجية التقليدية الاخرى في برامج مكافحة البعوض الناقل للملاريا وذلك بعد التقييم الحقلي، واضعين في الاعتبار الاهتمام العام بتنامي ظاهرة مقاومة النواقل للمبيدات والتأثيرات البيئية الضارة لها.

Introduction

Mosquito *Anopheles arabiensis* is one of the most important vectors of malaria in sub-saharan Africa, and it occurs in overlapping manner with other important species (Mabaso, 2004). Control of anopheline mosquito vectors of malaria by using synthetic insecticides has shown 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, 2003). In Sudan, although more recent studies indicated that resistant level has increased only marginally (Kamau and Valule, 2006). But there is concern that continued and/or increase use of insecticides may result in 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 number of plant extracts have been used against *Anopheles spp*. as control agents viz. *Calotropis procera* (Markouk *et al*., 2000); *Eucalyptus camaledulensis* (Yang and Ma, 2005) and *Ocimum basilicum* (Elsiddig, 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 arthropod pests of crops (Mansour and Salem, 2001; Sati *et al*., 2003; and Elsiddig, 2009). Nathan *et al*. (2005) explored the advantages of pure neem limonoids, and study the larvicidal, pupicidal, adulticidal and antiovipositional activity of neem limonoids. Azadirachtin, salannin and deacetylgedunin showed high bioactivity at all doses, while the rest of the neem limonoids were less active, and were only biologically active at high doses. Azadirachtin was the most potent in all experiments and produced almost 100% larval mortality at 1 ppm concentration. 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 varying degree of protection against different vector species (Moore *et al*., 2003).

The present study was carried to test the potentials of different neem organic extracts against *Anopheles arabiensis* larvae, and to evaluate their oviposition deterrency and mortality on adults.

Materials and Methods

Study area

Experiments were carried out at the National Malaria Centre, Sinnar- Sinnar State-Sudan.

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. However, ripe fruits of the plant were harvested from the same area and soaked in water 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 Rearing

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

Bioassay

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 one liter each. 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 WHO (1969). A group of third stage larvae of *Anopheles arabiensis* (twenty larvae) were placed in exposure bowls. The exposure period was 24 hours, during which no food was offered to the larvae. Mortality was recorded by counting the completely dead or moribund larvae together with the larvae that failed to reach the surface of the solution. Then data recorded was subjected to probit analysis using MSTAT-C package computer program (1991), 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 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. 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. 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 and Discussion

Results given in Table (1) demonstrated the crude hexane and ethanol neem extracts (leaves and seed kernels) at different concentrations depicted larvicideal effect against *Anopheles arabiensis* mosquito. These results agreed with Aliero (2003), who suggested that seed oil and leaf extract of neem *Azadirachta indica* had properties that could be developed and used in the control of Anopheles mosquitoes in the tropics. Moreover, it was observed that better mortality results were obtained by the neem seed kernel extract compared to neem leaves extract. The advantages of seeds over leaves was also confirmed by Grunwald *et al*. (1992), who concluded that the bioactive compounds in the neem were found throughout the tree, but those in the seed kernel were the most concentrated and accessible. Likewise, 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. Further, it was also observed that neem seed hexane extract exerted better mortality when compared to ethanol. Regarding this manner, 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, larvae and adults of certain pests.

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 $mg\ell^{-1}$.

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*adult from laying eggs. This result is agreed with Schmutterer (1990), who reported that neem based pesticides containing azadirachtin which is a

predominant active ingredient, having antifeedant, ovipositional deterrence repellency, and growth disruption against insects. Goektepe *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. However, "Neem Aura", 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 Segal (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 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 make little or no contact with the treated surface, and consequently mosquito intoxication does not occur.

Conclusion

This study clearly demonstrated that ethanol and hexane extracts of neem leaves and seed kernels exhibited 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 extracts, with negligible toxicity towards adult mosquitoes. The obtained results, after further field evaluation, will encourage the inclusion of these extracts in IPM programs for mosquito control with other natural and biological measures.

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

Concentration (mgℓ$^{-1}$)	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
	Water control		Solvent 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 *neem* organic extracts.

Parameter	Leaves extract		Seeds extract	
	Ethanol	**Hexane**	**Ethanol**	**Hexane**
Intercept	0.7817	1.5278	4.7710	8.0276
Variance of slope	0.0360	0.0288	0.0533	0.1215
Slope	1.446	1.907	2.816	4.154
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} (mgℓ$^{-1}$)	8282	4065	3380	1998

Table 3: Oviposition deterrency and adult mortality of *Anopheles arabiensis* resulting from different neem extracts.

Treatment	Mean No. of eggs	S.D	Oviposition activity index	Attractancy or deterrency	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

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