African Research Review

An International Multi-Disciplinary Journal, Ethiopia
Vol. 3 (3), April, 2009
ISSN 1994-9057 (Print) ISSN 2070-0083 (Online)

Repellent Action of Neem (*Azadirachta indica)* Seed Oil Cream against *Anopheles Gambiae* Mosquitoes

(Pp. 12-22)

***Aremu, O. I.* -** Dept of Phamaceutics and Pharmaceutical Technology, Faculty of Pharmacy Olabisi Onabanjo University, Shagamu solabank@yahoo.com.Tel-234-8033259890

Femi-Oyewo, M. N.
Dept of Phamaceutics and Pharmaceutical Technology, Faculty of Pharmacy Olabisi Onabanjo University, Shagamu

Popoola, K. O. K.
Dept of Zoology, Faculty of Science, University of Ibadan, Ibadan

Abstract
This paper conducted an evaluation of repellent effect of neem seed oil formulated in a vanishing type cream base against Anopheles gambiae mosquito under laboratory conditions using human volunteers. The seed oil was extracted and then prepared in five different concentrations of 0, 2.5, 5.0, 7.5 and 10.0% $^{w}/_{w}$ respectively in a vanishing cream base. A commercially available repellent Deet was used as control. The result shows that concentrations 2.5, 5.0, 7.5, and 10.0% $^{w}/_{w}$ and Deet (control) all repelled night-biting Anopheles gambiae mosquitoes at three consecutive 10mins interval for every 5-min exposure time. The duration of protection of various concentrations of neem seed oil cream and control (Deet) was of the order 10.0% > Deet> 7.5%> 5.0% >2.5%.The present study demonstrates potential of neem seed oil cream as mosquito repellent particularly at higher concentrations of 7.5 and 10.0%$^{w}/_{w}$ respectively. This finding may lead to

new and more effective strategies for protection from and control of mosquitoes.

Keywords: Neem, Mosquitoes, Human, Concentrations

Introduction

Many mosquito-borne diseases, such as malaria, dengue fever (DF), dengue hemorrahagic fever (DHF) and filariasis, are serious public health problems in tropical regions, especially in Africa and Asia. These diseases are transmitted to human beings through mosquito bite only since there is no effective vaccine available for the control of these diseases. Prevention of mosquito bites is one of the main strategies to control or minimize incidence of these diseases. The use of insect repellants can provide practical and economical means of preventing mosquito-borne diseases. It is important not only for local people in disease risk areas especially in tropical countries, but also for travellers who are vulnerable to diseases spread by mosquito vectors when they visit and seek leisure away from their home countries.

Although the most common mosquito repellent currently available on the market, deet (N,N-diethyl-3-methylbenzamide) has shown excellent protection from mosquito bites(Yap,1986;Walker *et al*,1996;Thavara *et al,*2001) and other biting insects(Coleman *et al,*1993) , there were reports of toxicity problems after application of deet, range from mild effects such as contact urticaria(Malbach and Johnson,1975) and skin eruption(Reuveni and Yagupsky,1982) , to severe reactions, such as toxic encephalopathy(Zadikoff,1979;Roland *et al,*1985;Edwards and Johnson,1987) . To overcome these adverse effects, attempts to find and develop repellents derived from plant extracts have been made by many workers. The development and use of locally available plants showing repellent activity avails an alternative strategy for the control or minimization of mosquito-borne diseases, especially in developing countries.

In the present study, neem Seed oil extracted from *Azadirachta indica* plant and formulated in Vanishing cream base was evaluated for repellent action against *Anopheles gambiae .Azadirachta indica* (A Juss) belongs to family meliaceae. The plant is indigenous to the Indo-Pakistan subcontinent (Vander *et al*,1987) , although it is now widely distributed in many countries of the world, it is believed that Indians migrating to African countries introduced it into that continent.

Materials and Method

Plant species: - Ripe seeds of neem plant were collected from Osogbo, a town in South Western part of Nigeria. The plant specie from which the seeds were collected was authenticated at Forest Research Institute of Nigeria (FRIN) with FHL No 107818.The seeds were sun dried until the moisture content was reduced to barest minimum. The seed kernels were later separated from the seed coat and stored in air tight containers.

Extraction of the oil: - The dried seed kernels were communited using blender model MX – 738 (Nakai, Japan). The blended materials were stored in air tight containers. Using the method of(Charmaine *et al,*2005) . Normal hexane was used as solvent for extraction at seed weight: solvent ratio1:10. The seeds were allowed to soak in the solvent for 8 days at room temperature. The solvent was then filtered through a whatman filter paper (No 1) to remove the coarse seed materials, into pre-weighed sterile containers. The containers were covered with filter paper and solvent was allowed to evaporate. The weight of the residue was calculated (Weight of the container plus extract minus the weight of the empty container) and the extracts were kept at room temperature.

Preparation of Repellant for Testing:- Five different concentrations of neem oil in Vanishing Cream base were made ($0\%^{w}/_{w}$, $2.5\%^{w}/_{w}$,$5.0\%^{w}/_{w}$,$7.5\%^{w}/_{w}$ and $10.0\%^{w}/_{w}$)

Test Mosquitoes:-The mosquitoes used in this study were disease free laboratory-reared female mosquitoes (age 8-11 days), *Anopheles gambiae* that were fed with $10\%^{w}/_{w}$ sugar solution. The repellency of the samples was assessed in the laboratory using human bait technique (Tawatsin *et al,*2006) . Ethical clearance was sorted from Olabisi Onabanjo University Teaching Hospital, Shagamu. Six volunteers (Age 23 – 50 years) participated in the laboratory tests as testing period lasted up to six or more hours depending on the efficacy of the repellent. Test timing was between 9pm- 3am since the mosquitoes are nights biting. Evaluation was carried out in replicates using 12(26 cubic centimeter) cages with five concentrations of the extract and Deet cream used as control.

To eliminate bias, samples were coded A – E prior to commencement of the experiment. A total of 240 female mosquitoes were used. 20 adult blood seeking female mosquitoes were placed in each cage and left for 24 hours

prior to the experiment to acclamitise. Mosquitoes were starved for nine hours before the experiment. Host seeking behaviour of the mosquitoes was tested prior to the experiment. This was done by placing a pre-cleaned hand (cleaned with alcohol) in each cage and counting the number of mosquitoes that alighted within 10 seconds. If at least five mosquitoes alighted on the hand, the mosquitoes inside such cage were regarded as host seeking and the repellency experiment continued. Then each volunteer will put the forearm that has been rubbed with the sample up to the wrist level and the number of mosquitoes that alighted or biting the treated area of each volunteers hand was recorded each minute (at 1, 2 and 3, 4, 5 minutes) for three consecutive 10 mins intervals to establish repellency. To determine the duration of protection of each concentration of the sample, the procedure was repeated every hour for six hours without a fresh application of the sample. Experiment was invariably terminated when it appears that none of the concentrations was capable of protection against the mosquitoes any longer particularly when there have been mosquito bite twice on two consecutive exposures.

Statistical Analysis seed oil cream **:-**Correlation between different concentrations of neem and mean duration of protection against Anopheles mosquitoes was established by calculating correlation coefficient .The significance of the relationship at 5% level was determined using student t-test statistical application application.

Result

$r= \{[\sum(x-x)\,(y-y)] / \sqrt{[\sum(x-x)^2 \sum(y-y)^2]}\}$,Where 'r' is correlation coefficient

$\sum x=25.0, \sum y=14.42, \sum xy=104.73, \sum x \sum y=72.1$

Therefore, $r=32.63/34.24 =0.95$

Value of correlation coefficient' r' indicates that there is close association between concentrations of neem oil cream and mean duration protection time against Anopheles mosquitoes.To test whether the observed correlation is due to chance or not ,astudent t- test is used to determine the significance at 5% level

$t=\{r\sqrt{[(n-2) / (1-r2)]}$, df= n-2

$t= \{0.95\sqrt{[3/(1-0.903)]}\} = 5.29$, df= 3

This is significant at 5% level, confirming the significance of the apparent association between concentrations of neem oil cream and mean duration protection time.

Discussion

The neem tree has long been recognized for its unique properties both against insects and in improving health. It is grown in most tropical and sub-tropical areas of the world for shade, raw materials for natural insecticides and medicines. Azadirachtin, a complex tetranorterpenoid limonoid compound from the neem seeds, is the main component responsible for the toxic effects in insects (Mordue and Nisbet, 2000).

In the previous study Hati et al, 1995 had reported that neem *(Azadirechta indica)* seeds oil in appropriate amount when smeared on the surface of the hand showed excellent repellent action against *Aedes aegypti* mosquitoes .They found out that the degree of repellency was in increasing order as the amount of oil increased.

The present work centers on focusing at the effect of neem (*Azadirachta indica)* seed oil in a formulated delivery design that will be cosmetically acceptable and at the same time effective as a repellent. The cream base used for the formulation is of vanishing type that has emollient property and is easily disappearing into the skin. It is non greasy and this makes it readily acceptable.

The neem seed kernel yields an acrid bitter greenish yellow to brown fixed oil. The calculated yield is between (19-25%) $^{w}/_{w}$. The experiment of the repellency was in two phases, first to determine the repellency properties of the various concentrations of neem oil cream 0.0 – 10.0%$^{w}/_{w}$ and the second phase was to establish the duration of protection against the insect bite.

Table 1 shows the result of repellency determination. As can be seen from the table, it appears that in the 5-minute exposure time i.e. (1, 2, 3, 4 and 5min) at 10 mins intervals, neem seed oil cream at concentrations (2.5%$^{w}/_{w}$ - 10.0%$^{w}/_{w}$) and control DEET repelled *Anopheles gambiae* mosquitoes. The alighting mosquitoes on the exposed part of the hand soon left without sucking blood. On the other hand the formulation without neem seed oil (0% concentration) could not protect the exposed hand from the mosquito bite.

This observation could be explained thus that as soon as alighting mosquitoes sense the discomfort or lethal effect of the chemical constituent of neem seed oil cream, they left

Also across the concentration range i.e. 2.5% - 10.0%$^{w}/_{w}$, the number of alighting mosquitoes appear reducing. There is a kind of linear relationship between the concentration of the formulation and the degree of repellency. This is in agreement with earlier work done by [14] on crude neem seed oil.There was also observed a decrease in the number of alighting mosquitoes in the 5-min exposure time across the three intervals for all concentrations except the one without neem seed oil.The more effectiveness across the intervals is probably due to the disappearing and even distribution of the neem oil cream in the dermis and epidermis as time goes on

The mean duration of protection time of various concentrations and control i.e. DEET against *Anopheles gambiae* as statistically analyzed with standard error of mean calculated is as presented in table II and plotted as fig I. As observed, there was no protection against mosquito bite at 0% concentration. Although it appears that at 5 – minute exposure time and hourly intervals, the no of alighting and biting mosquitoes generally was reducing. The reduction is probably due to the satisfaction of the mosquitoes being sufficiently fed as time and exposure goes on. The duration of protection for the other concentrations 2.5% - 10.0% follows thus 2.45±0.2, 2.47±0.2, 3.50±0.1 and 6.0±0.1 hrs respectively. While that of control (DEET_) is 4.58±0.1 hrs .The duration of protection is of increasing order with increase in the concentration of neem seed oil in the formulation.As statistically analysed the association between concentrations of the neem oil cream and mean duration protection time was established from computation of correlation coefficient r= 0.95.The significance of the association was further confirmed using't' test application ($p<0.05$). The repellency experiment was actually terminated at the time mosquitoes bite on two consecutive occasions. This is when it is concluded that the repellents potency has lapsed.

It is interesting to note that 10.0%$^{w}/_{W}$ neem seed oil cream was able to protect against mosquito bite longer than control (DEET)

It follows therefore that neem seed oil cream may be presenting great potentials against mosquito bite. Even at 7.5% concentration the formulation was able to protect for close to four hours which is the minimum acceptable

number of hours for protection against mosquitoes bite in Thailand (Tawatsin *et al* 2006).

Conclusion

Neem (*Azadirachta indica)* seed oil cream has proven to be very valuable as insect repellent particularly at higher concentrations of 7.5% and 10.0%$^{w}/_{w}$ respectively. This is veritable alternative to commonly used DEET that has been associated with certain skin reactions. It is therefore recommended that with a thorough stability and safety studies, neem seed oil cream formulations 7.5%$^{w}/_{w}$ or 10.0%$^{w}/_{w}$ could be submitted for regulatory scrutiny and subsequent availability commercially for human use.

References

Coleman R.E., Robert L.L., Roberts L.W.(1993) . Laboratory evaluation of repellents against four anopheline mosquitoes (Diptera: culicidae) and two phlebotomine sand flies (Diptera: Psychodidae). *J Med Entomolo. (30),499-502*

Charmaine Lioyd A.C., Menon .T., Umamaheshwari, K.(2005). Anticandidal activity of *Azadiracta indica.* Indian Journal of Pharmacology. (37),6,386-389

Edwards D.L., Johnson C.E.(1987) . Insect-repellent-induced toxic encephalopathy in a child. *Chin Pharm. (6),496-498.*

Hati A.K., Bhowmik ,K., Banerjee, A.(1995). Repellent action of neem (*Azadirachta indica)* seed oil-against *Aedes aegypti* mosquitoes. *Indian Journal of Dermatology. 40, (4),155-158*

Malbach, H.I., Johnson, H.L.(1975). Contact urticaria syndrome to diethyl-toluamide (immediate-type hypersensitivity). Arch Derma, (111) ,726-730.

Mordue A.J., Nisbet A.J.(2000).*Azadirachtin* from the neem tree *Azadirachta indica*: Its action against insects. An. Soc. Entomol. Bras 29,(4),1-19.

Reuveni, Y .H., Yagupsky, P.(1982). Diethyl toluamide containing insect repellent: adverse effects in worldwide use. Arch Derma, (118), 582-583

Roland, E.H., Jan, J.E., Rigg, J.M.(1985). Toxic encephalopathy in a child after a brief exposure to insect repellents. *Can Med Assoc.J,(132),155-156.*

Thawara,U.,Tawatsin,A.,Chompoosri,J.(2001).Laboratory and evaluation of the insect repellent 3535 (Ethyl butylacetylaminopropionate) and deet against the insect mosquito vectors in Thailand. *J. Am Mosquito Control Assoc. (17)* ,190 -195.

Tawatsin A, Thavara U, Chansang U.(2006). Field evaluation of Deet and three plant based essential oil repellent against mosquitoes, black flies (Diptera:Simulidae) and land leeches (Arhynchobdellida:Haemadipsidae) in Thailand. *J. Am Mosq Control Assoc.(22),313-360.*

Tawatsin A,Preecha A, Thavara U.(2006).Repellency of essential oils against four mosquito vectors. J.AM.Mosq. *Control Assoc.37,(5),915-931.*

Vander N.J, Vander W.G, Labaide R.P.(1987) . Immunomodulatory activity of an aqueous extract of *Azadiracta indica* stem bark. *J. Ethnopharmacol. (19), 125-131*

Walker T.W, Robert L.L, Copeland R.A .(1996). Field evaluation of arthropod repellents, Deet and piperidine compound against *Anopheles arabiensis* in Western Kenya. *J. Am mosquito Control Association. (12) ,172-176.*

Yap H.H.(1986). Effectiveness of soap formulations containing deet and permethria as personal protection against outdoor mosquitoes in Malaysia. *J. American Mosquitoes Control Association. (2), 63-67.*

Zadikoff C.M.(1979). Toxic encephalopathy associated with the use of insect repellent J. Pediat. (95), 140-142.

Table 1: Repellency Determination of various Concentrations against *Anopheles gambiae* at 10 mins intervals

Repellent concn (w/w)	Exposure Time(min)	No of Mosquitoes that alighted/ left			No of mosquitoes that alighted/bit		
		1st	2nd	3rd	1st	2nd	3rd
0%	1 2 3 4 5	NIL			10 9 8 8 7	11 8 6 5 6	10 6 7 5 6
2.5%	1 2 3 4 5	8 9 6 6 6	4 6 6 4 6	4 3 5	1 3 1	NIL	
5.0%	1 2 3 4 5	6 6 5 6 6	5 6 4 1 3	4 2	1 1	NIL	
7.5%	1 2 3 4 5	6 7 6 6 3	5 4 6 4			NIL	
10%	1 2 3 4 5	7 6 6 7 5		4 2 4 4 3		NIL	
DEET (control)	1 2 3 4 5	5 8 8 6 9	5 8 6 5 3	6 6 5 3 2		NIL	

Table 2: Duration of Protection of Repellant's Concentration against *Anopheles gambiae* at hourly intervals

Repellent concn (%/w)	Exposure Time(min)	Mean No of Mosquitoes Alighted/ left					Mean No of Mosquitoes Alighted/bit					Mean Duration in hours (± SE)
		1hr	2hrs	3hrs	4hrs	5hrs	1hr	2hrs	3hrs	4hrs	5hrs	
0%	1	NIL					4	3	2	2	2	NIL
	2						4	3	1	1	1	
	3						2	5	3	1	1	
	4						1	4	3	1	2	
	5						4	3	1	2	1	
2.5%	1	3	4		4	-	NIL	NIL	2	2	-	2.45±0.2
	2	3	5		3	-	NIL	NIL	3	1	-	
	3	1	2		3	-	NIL	NIL	4	4	-	
	4	3	82		3	-	NIL	NIL	2	2	-	
	5	5			3	-	NIL	NIL	1	2	-	
5.0	1	1	5	3	3	-	NIL	NIL	NIL	2	-	2.47±0.2
	2	2	5	5	2	-	NIL	NIL	NIL	2	-	
	3	2	4	4	6	-	NIL	NIL	4	3	-	
	4	2	5	6	5	-	NIL	NIL	3	4	-	
	5	4	6	3	6	-	NIL	NIL	4	2	-	
7.5%	1	2	4		3	2	NIL	NIL	NIL	1	1	3.50±0.1
	2	2	3		5	5	NIL	NIL	NIL	2	1	
	3	4	2		6	3	NIL	NIL	NIL	1	2	
	4	4	2	4	4	5	NIL	NIL	NIL	3	1	
	5	5	4	3	6	5	NIL	NIL	NIL	1	2	
10%	1	3	4		2	2	NIL	NIL	NIL	NIL	2	6.0±0.1
	2		3		1	3	NIL	NIL	NIL	NIL	2	
	3	2	2		3	1	NIL	NIL	NIL	NIL	1	
	4		2		1	1	NIL	NIL	NIL	NIL	1	
	5	2	1		1	1	NIL	NIL	NIL	NIL	2	
		2										
		1										
DEET	1	2	3	4	2	2	NIL	NIL	NIL	NIL	NIL	4.58±0.1
	2	3	2	3	2	2	NIL	NIL	NIL	NIL	NIL	
	3	2	3	3	4	2	NIL	NIL	NIL	NIL	NIL	
	4	4	3	2	3	3	NIL	NIL	NIL	NIL	2	
	5	3	2	1	3	1	NIL	NIL	NIL	NIL	1	

Table 2b: Analysis of association between neem oil concentrations and mean duration of protection.

Neem oil cream conc. in percentage(x)	Mean duration of protection in hrs (y)
0w/w	**0**
2.5w/w	2.45
5.0w/w	2.47
7.5w/w	3.5
10.0w/w	6.0
Total- 25.0	14.42

Fig 1: Duration of Protection of Neem Seed oil Cream Concentrations Against Anopheles Gambiae at Hourly Intervals