ISSN 2201-2796

Mosquito Repellant Property of *Azadirachta Indica* Extract (Fruit Bark and Seed Kernel)

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***Abstract*- Malaria is a serious public health problem in the tropics. This study, investigated the mosquito repellent effect of the plant *Azadirachta indica* extracts of the seed kernel and fruit bark on human volunteers. Two different mosquito species were used, female anopheles and culex . It was observed that the fruit bark exhibited slight repellent activity, significant at $p<0.05$ for the number of bites between 0-60 minutes by culex mosquitoes. The seed kernel extract exhibited significant repellent activity, significant at $p<0.01$ and also the extract was more effective on the female anopheles than on the culex mosquito species.**

***Index Terms*— Malaria, Azadirachta indica, Mosquito, female anopheles, culex.**

I. INTRODUCTION

Malaria is a mosquito-borne infectious disease of humans and animals caused by eukaryotic protest of the genus *Plasmodium*. The disease results from the multiplication of *Plasmodium* parasites with the red blood cell causing symptoms that typically include fever, headache and severe weakness progressing to coma or death. It is widespread in tropical and sub-tropical regions including most of sub-saharan Africa, Asia and America. Malaria is largely caused by *Plasmodium falciparum* while Plasmodium *vivax*, *Plasmodium ovale* account for few cases (Sutherland *et al*., 2010) and *Plasmodium malariae* is generally a milder disease that is rarely fatal. *Plasmodium knowlesi* is a zoonosis that causes malaria in macaques but can also infect human (Singh *et al*., 2004).*Plasmodium falciparum* is the most severe form of malaria which is responsible for the vast majority of death associated with the disease (Snow *et al*., 2005).

Azadirachta indica is a fast growing, long-live tree with unpleasant smelling wood that's about 12 meters in height. It has evergreen pinnate leaves and small fragrant yellow-white flowers, followed by green-yellow berries (Zillur and Shamim, 1993). Its name is derived from the Persian word *azadiddiraki* which means noble tree and this plant is native to India and Pakistan. *Azadirachta indica* also known as neem, is a member of the miliaceae family and a botanical cousin of mahogany (Zillur *et al*., 1996). This plant is reputed to be responsible for the pesticidal, larvicidal, antifeedant or repellant action on various insects (Premila, 2006). The Azadirachtine is found in all parts of the plants but in a higher concentration in the seed

(Premila, 2006). Studies have shown that neem is a natural mosquito repellant plant (Kilonzo *et al*., 2007). Traditionally, *Azadirachta indica* is used as oil cake obtained from seeds, used as fertilizer and manure (Birendra, 2012). The Green twigs are used as tooth brushes and as prophylactic mouth and teeth compliance.

The leaves are kept in suit cases to repel insects and to preserve woollens. Seeds yield famous magose oil of disagreeable garlic like flavor. The oil is said to be effective in treatment of leprosy and skin diseases. It is also used as a cure for management of dog disease. The leaves in poultice are used for healing of wounds. The oil from neem seed provides significant protection from various mosquitoes (Chatter *et al*., 1994). Research has proven that besides azadirachitin, salanim, gedunin, azadinone, nimbin, nimbidine, nimbicidine, nimitinol, are also important liminods which act as an excellent effect on insects and pest (Su *et al*., 1998).

This study is to evaluate the mosquito repellant action of the fruit bark and seed kernel of the plant on female anopheles mosquito and culex mosquito species.

II. METHODS

Breeding and Harvesting Of Mosquitoes

The female anopheles and culex mosquito eggs and larvae were obtained at Zoology Department University of Jos. These eggs and larvae were transferred with water from the pond into the 4 litres transparent buckets and covered. The eggs and larvae in the transparent buckets were observed daily for development into pupa. As the pupae emerged, it was scooped and put into an open transparent plate, kept at different positions in the mosquito cages. Yeast was added into the plate and at each corner on top of the mosquito cage, a cotton wool soaked with sugar solution (which serves as glucose) for the matured mosquitoes that would emerge to feed on. At an interval of 3-4 days the mosquitoes were fed with human blood and observed daily till used for the study.

Plant Collection, Identification and Extraction

Azadirachta indica fresh fruits were collected in April 2012 from Langtang L.G.A Plateau state. The fresh fruits were identified and authenticated by Mr Ikechukwu Chijioke of Federal College of Forestry, Jos. The fresh fruits were sun dried for 2 weeks and the seeds were separated from the fruit bark by breaking open the fruit. The seed kernel and the fruit bark were further dried for another two weeks, after which was pounded to granules form using a mortar and pestle. Each granulated samples were weighed and extracted. The two different extracts gave a yield of 7%w/v for seed kernel and 8%w/v for fruit bark extract.

Mosquito Repellant Activity

25 female anopheles mosquitoes were used for this experiment. Arms (3) of human volunteers were coated with a non-fragrance-vaseline (normal saline), which served as negative control for the study. The arms were inserted into each of the mosquito cages at the same time, and observed for the following: the number of mosquito that parched and bite the exposed arm**.** After the time of duration of 10 minutes, the arms were removed from the mosquito cages; each mosquito was observed and checked for any behavioral change. The subjects were allowed to rest for 30 minutes, and then re-exposure is done without reapplication of the sample. The experiment was repeated for 2 hours. Same treatment was repeated with seed kernel, fruit bark and Odomos (standard repellant). The procedures (above) were repeated using culex mosquito species**.**

Statistical Analysis

The results were expressed as mean ± standard error of mean. Statistical significance was determined by one way ANOVA followed by Durinett multiple comparison test and values of $P < 0.05$ were considered significant.

III. RESULTS

There was no repellant activity exhibited by the standard drug (Odomos) as well as the extracts on the number of bites between 0-60 minutes and 90-120 minutes by the culex mosquitoes as shown in table 1.

Table 1; Number of bites between 0-120 minutes by culex mosquitoes

Treatment	**Mean**	
	0 – 60 minutes	90 – 120 minutes
Normal Saline	13.67± 6.89	11.67± 5.84
Odomos (standard)	13.67± 1.76	0.67± 0.67
$Extract_1$ (fruit bark)	4.00± 1.53	3.00± 1.73
$Extract_2$ (seed kernel)	3.00± 1.00	2.33 ± 1.45

Values expressed as mean±SEM

Table 2 shows that the extract exhibited repellent activity when compared with the standard, significant at $p<0.05$ within the duration of 0-60 minutes while the extracts and the standard showed no repellent activity on the number of bites between 90-120 minutes.

Table 2: Number of bites between 0-120 minutes by culex mosquitoes

Treatment	**Mean**	
	0 – 60 minutes	90 – 120 minutes
Normal Saline	7.00± 0.58	3.33± 1.76
Odomos (standard)	1.67± 1.20*	0.33± 0.33
$Extract_1$ (fruit bark)	2.00± 1.53*	1.67±0.88
$Extract_2$ (seed kernel)	2.00±0.38*	1.00±0.58

Values expressed as mean±SEM *($p<0.05$)

In Table 3, analysis on the number of perches between 0-60 minutes, the extract exhibited significant repellent activity against the number of perches by the female anopheles mosquitoes at 0-60 minutes. The repellent activity of the extract was similar to that of the standard, on the number of perches between 90-120 minutes which is significant at (p<0, 01)

Table 3: Number of perches between 0-120 minutes by female anopheles mosquitoes

Treatment	Mean	
	– 60 minutes	90 – 120 minutes
Normal Saline	18.62± 1.20	13.00±1.00
Odomos (standard)	3.67±2.03**	3.67± 0.67**
$Extract_1$ (fruit bark)	4.67± 2.03**	5.33± 1.03**
$Extract_2$ (seed kernel)	4.35± 2.52**	3.33±1.76**

Values expressed as mean ±SEM ** (P<0.01)

The standard and extracts showed repellent activity against the number of bites by female anopheles mosquitoes within 0-60 minutes which is significant at (p<0.01) while on the number of bites between 90-120 minutes the extract and standard showed no repellent activity against the number of bites by the female anopheles mosquitoes as shown in Table 4

Table 4: Number of bites between 0-120 minutes by female anopheles mosquitoes

Treatment	Mean	
	0 – 60 minutes	90 – 120 minutes
Normal Saline	14.00± 1.00	9.00± 4.59
Odomos (standard)	3.33± 1.67**	1.33±0.88
$Extract_1$ (fruit bark)	3.67± 1.230**	2.00± 1.15
$Extract_2$ (seed kernel)	2.33± 1.20 **	3.00± 1.72

Values expressed as mean ±SEM ** (P< 0.01)

IV. DISCUSSION AND CONCLUSION

Most mosquito repellants are designed to make the surfaces unpleasant or unattractive to mosquitoes. They typically contain active ingredient (12%N, N-diethyl-3-methyl benzamide) or (12% N, N-diethyl-benzamide) that repel mosquito (Anne, 2014). Azadirachta, is a powerful insect antifeedant that disrupts metamorphosis as moth larvae at extremely low concentration (Van, 2004). A number of studies have shown that Neem compounds are more effective insect repellent than the widely used synthetic chemical N,N-diethyl-m- tolumide (Farahna *et al*,2010).

The mechanism of action of mosquito repellants is that carbondioxide, excretory products and lactic acid present in sweat of warm blooded animals' acts as an attractive substance for female mosquitoes. The perception of the odor is through chemoreceptors present in the antennae of mosquitoes. The repellents block the lactic acid receptors thus destroying upwind flight and as a result, the mosquito lose contact with the host (Elissa, 2004: Patel , 2012) usually mosquito repellants work by masking human scent or by using a scent which insects naturally avoid. The presence of the odor in the neem seed and kernel oil must have contributed to the repellent action seen in the mosquitoes under study.

Taste molecules also are involved; this suggested that the repellent deters biting and feeding because it activates taste cells that are present on the insect's tongue, legs and wing margin. When mosquito lands, it tastes the skin of the host with it gustatory receptors before it bites, thus there is avoidance responses not only through the sense of smell but also through the sense of taste. Neem seed and kernel have a punget smell which is similar to garlic and it contain sulfurous compound as reported by Akin,(2010), this attribute to its reduce plaque and act as mosquito repellent. In this study, the repellent action of the extract can be attributed to the odor possessed by the extracts and the taste of the extracts.

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). 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. Similarly, Mishra,(2010) evaluated that there was mosquito repellent action of neem oil at various concentration.

The activeness, after application of the extracts (fruit bark) for the anopheles was less and decreased with time. Alyssa,(2010) observed similar effect of the Neem oil reduces as time passes. *Culex* mosquitoes are known to be stronger and live longer than female *anopheles* mosquitoes (Afshin, 2003). The number of bite was more with female *anopheles* mosquitoes and less with culex mosquitoes. Female *anopheles* mosquitoes feed on human blood as a source of providing food and protection for their eggs (Degennaro, 2013) while most *culex* species are partial to biting birds rather than human (Richard, 1977). Similarly, female *anopheles* species are known to be weaker, having a shorter-life span and hardly survive in cold environment and bites more when compared to the *culex* mosquitoes species (Williams, 1983) . Degennaro, (2013) stated that there are many different species of mosquitoes and each species has its favorite host(s). The mosquito species that like human blood are *Aedes aegypt* and

Anopheles mosquitoes. However, this study showed that the extracts (seed kernel) exhibited a repellent efficacy with the *culex* mosquito species but more with the female anopheles species.

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