SUSCEPTIBILITY OF *Aedes aegypti* PUPAE TO NEEM SEED KERNEL EXTRACTS

[1]UMAR, Abba., [2]KELA, Santa Larit and [2]OGIDI, Jonathan Asadabe
[1]Department of Biological Sciences, University of Maiduguri, PMB 1069, Maiduguri, Borno State, Nigeria
[2]Biological Sciences programme, Abubakar Tafawa Balewa University, PMB 0248, Bauchi, Bauchi State, Nigeria

Corresponding Author: UMAR, Abba, Department of Biological Sciences, University of Maiduguri, PMB 1069, Maiduguri, Borno State, Email: Aumar66.ng@Hotmail.com

ABSTRACT

Laboratory bioassays were conducted to evaluate the pupicidal activity of neem (Azadirachta indica) seed kernel extracts (NSKE) on Aedes aegypti. The neem seed kernel powder was sequentially extracted with hexane, benzene, ethyl acetate, acetone, DMSO, 2-propanol, ethanol, methanol and distilled water. Ten concentrations (0.0, 0.25, 0.5, 1.0, 2.0, 4.0, 5.0, 10.0, 15.0 and 20.0%) of the neem extracts were used for the bioassays. Each treatment was replicated five times. Twenty-laboratory strains of Aedes aegypti pupae were exposed to each concentration. Pupae were not fed during the exposure periods. Pupal mortality was assessed after 1 and 24 hours of exposure. The results of the effects of 1h exposure indicated decreased pupicidal mortality with decreasing extracts toxicity thus: ethyl acetate (LC_{50} = 0.06%) > acetone > (LC_{50} = 0.29%) > benzene (LC_{50} = 0.82%) > hexane (LC_{50} = 3.13%) and propanol (LC_{50} = 7.63%). No pupal mortality was observed with extracts from Dimethyl sulfoxide (DMSO), ethanol, methanol and distilled water. The results of the effect of extract for 24h exposure indicated pupicidal mortality in 2-propanol (LC_{50} = 0.67%) and ethanol (LC_{50} = 1.70%). No pupicidal mortality was observed with hexane, benzene, ethyl acetate, acetone, Dimethyl sulfoxide (DMSO), methanol and distilled water extracts. The ability of some neem extracts to kill Aedes pupae at relatively low concentrations presents an alternative to the use of synthetic pesticides for control of mosquitoes. This technique is environmental friendly, biodegradable, less expensive, and locally available in mosquito endemic area. Potentials for adoption in mosquito management programmes cannot be overemphasized.

Keywords: *Azadirachta indica*, *Aedes aegypti*, Pupicidal

INTRODUCTION

Aedes aegypti Linn is a prevalent mosquito in the sahel savanna regions of Nigeria (Molineaux and Gramiccia 1980).They are established vector of yellow, dengue and other haemorrhagic viral fevers (Gubler, 1997). The epidemics of these diseases have been reported in Nigeria (Nasidi *et al.,* 1989).

Both private and public health mosquito control in Nigeria is largely base on the conventional synthetic insecticides (Don-Pedro and Adegbite, 1985). These conventional insecticides are associated with high costs (Jackai, 1993), persistent development of resistance in many of the mosquito species (Brown, 2002), adverse effects on non-target organisms (Hennessey *et al.,* 1992), human toxicity reactions (Liu *et al.* 2003) and non-suitability in integrated pest management programmes (Schmutterer, 1990).

The problems associated with synthetic insecticides necessitated investigations in to phytochemicals for mosquito control, since they are environmental friendly, biodegradable, less expensive, and locally available in mosquito endemic area (Novak, 1985). Phytochemicals with diverse mode of actions may be effective against resistant vector species and can be easily integrated with other mosquito control measures in both private and public mosquito control programmes (Sivagnaname and Kalyanasundaram, 2004).

Neem, *Azadirachta indica* (Family: Meliaceae) have met these requirements (Ipek *et al.,* 2004) and can play a vital role in mosquito control measures (Sukumar *et al.,* 1991). Although intensive work on neem as natural insecticides in Nigeria began in 1981 in the crop protection Unit of the Department of Agricultural Extension Services, University of Ibadan (Ivbijaro, 1987), little attention has been given to the potential of neem in mosquito control in Nigeria (Aleiro, 2003).

This study investigates the effects of neem seed kernel extracts as bio-insecticides against *Aedes aegypti* for possible usage in integrated mosquito control programmes in North Eastern Nigeria and other mosquito endemic areas of the third world.

MATERIALS AND METHODS

Insect Culture: The eggs of *Aedes aegpti* were obtained from the National Vector and Malaria Control Unit, Yaba, Lagos. They were reared inside cages (60 × 60 × 60 cm) in the insectry of the Department of Biological Sciences, University of Maiduguri. The adults of both sexes were fed with 10 % glucose solution (Sneller and Dadd, 1977). In addition, females were fed on blood meal twice a week from restrain chicken with shaved abdominal

feathers (Azmi *et al.,* 1998). A 250 ml glass beaker containing 150 ml of distilled water with a filter paper smoothly adhered to the inner wall serves as oviposition sites. The larvae were held in plastic containers and were daily fed on a pinch of finely powdered liver and brewer's yeast mixed at the ratio of 3: 2 (wt\wt) (Roberts, 1998). When not needed for studies, the eggs were stored in a laboratory shelf at 37 ± 5^0C.

Neem Seed Kernel Extraction of Active Ingredient: Neem seeds were collected from mature disease-free trees in University of Maiduguri in October 2004. The neem seeds were air dried and stored in the laboratory. The dried neem seeds were decorticate to remove the kernels and air dried for 5 days before pulverization with an electric blender and sieved with 40 mesh screen to obtain a fine powder. 500 g of neem seed kernel powder was sequentially extracted either with 2 liters of hexane or benzene or ethyl acetate or acetone or Dimethyl sulfoxide (DMSO) or 2-propanol or ethanol or methanol or distilled water for 48 hours at room temperature ($37 \pm 5\ ^0$C). The mixture was stirred and filtered through Whatman number one filter paper and the filtrates were evaporated to dryness in a water bath (40°C). The residues were air dried by placing the container near an electric fan (Ascher, 1981). The powder or liquid obtained from each extraction was stored in a refrigerator at 4 ^{0}C separately in labeled specimen bottles for bioassay.

Pupal Susceptibility Tests: The pupicidal effects of the neem seed kernel extracts (NSKE) were determined using standard procedures (WHO, 1970). The bioassay was conducted under varying laboratory conditions (37 ± 5^0C Temperature, 80 ± 5% Relative Humidity and 12:12 Light: Dark Photoperiods). Ten concentrations (0.0, 0.25, 0.5, 1.0, 2.0, 4.0, 5.0, 10.0, 15.0 and 20.0 %) of each organic solvent and distilled water extracted neem seed kernel were used for the bioassay. Each treatment was replicated five times. Twenty-laboratory bred Aedes *aegypti* pupae were used per replicate. The pupae were siphon with eyedropper and immediately exposed in to 100 ml of each concentration of NSKE or distilled water (control). The exposure containers (250 ml glass beakers) were covered with mesh screen to prevent the escape of emerging adults. The pupal mortalities were recorded 1 and 24 hours of exposure. The pupae were probe with needle and moribund pupae were counted as dead (Azmi *et al* 1998). The results were analyzed using StatsDirect Statistical Software Version 4.2 (StatsDirect, 2005) to obtain the probit values.

RESULTS

The results of the pupicidal effects of 1h exposure of *A. aegypti* pupae to neem seed kernel extracts are presented in Table 1. The result indicated pupicidal effects with decreasing toxicity in ethyl acetate (LC_{50} = 0.06 %) > acetone > (LC_{50} = 0.29 %) > benzene (LC_{50} = 0.82 %) > hexane (LC_{50} = 3.13 %) and propanol (LC_{50} = 7.63 %) extracts.

Table 1: Effects of 1h exposure of neem seed kernel extracts on laboratory strain *Aedes aegypti* pupae

Extracts	*Lethal concentration values*	
	LC_{50} (%)	**LC_{90} (%)**
Hexane	3.13 (1.50 -23.28) *	374.03 (35.91 -196.67)
Benzene	0.82 (00.60 -1.94)	6.41 (2.34 -9.05)
Ethyl acetate	0.06 (7.45 - 0.20)	0.42 (0.01 -0.67)
Acetone	0.29 (0.07- 0.74)	1.84 (0.81 -6.28)
Dimethyl sulfoxide (DMSO)	0.00 (0.00-0.00)	0.00 (0.00-0.00)
2-propanol	7.63 (3.18 -847.23)	169.44 (20.57- 328.93)
Ethanol	0.00 (0.00-0.00)	0.00 (0.00-0.00)
Methanol	0.00 (0.00-0.00)	0.00 (0.00-0.00)
Distilled water	0.00 (0.00-0.00)	0.00 (0.00-0.00)

** Numbers in parentheses are 95% confidence limits*

However, no pupal mortalities were observed with extracts from DMSO, ethanol, methanol and distilled water. The results of the pupicidal effects of 24h exposure of *A. aegypti* pupae to neem seed kernel extracts are presented in Table 2.

Table 2: Effects of 24 h exposure of neem seed kernel extracts on laboratory strain *Aedes aegypti* pupae

Extracts	*Lethal concentration values*	
	LC_{50} (%)	**LC_{90} (%)**
Hexane	0.00 (0.00-0.00)*	0.00 (0.00-0.00)
Benzene	0.00 (0.00-0.00)	0.00 (0.00-0.00)
Ethyl acetate	0.00 (0.00-0.00)	0.00 (0.00-0.00)
Acetone	0.00 (0.00-0.00)	0.00 (0.00-0.00)
Dimethyl sulfoxide (DMSO)	0.00 (0.00-0.00)	0.00 (0.00-0.00)
2-propanol	0.67 (0.37- 0.96)	1.67 (3.83 -83.08)
Ethanol	1.70 (1.03 -526.91)	19.53 (3.69 -2027.47)
Methanol	0.00 (0.00-0.00)	0.00 (0.00-0.00)
Distilled water	0.00 (0.00-0.00)	0.00 (0.00-0.00)

** Numbers in parentheses are 95% confidence limits*

The result indicated pupal mortalities with decreasing toxicity in extracts from 2-propanol (LC_{50} = 0.67 %) and ethanol (LC_{50} = 1.70 %). However, hexane, benzene, ethyl acetate, acetone, DMSO, methanol and distilled water extracts of NSK had no pupicidal effects.

DISCUSSION

The toxicities of neem seed kernel extracts to mosquito pupae under laboratory conditions were studied. The findings of this report will serve as base line data for mosquito control in northeastern Nigeria. The present investigation revealed pupal mortalities in ethyl acetate, acetone, benzene, hexane, 2-propanol and ethanol extracts. These results corroborate earlier investigations, that neem seed extracts that are effective against insects were extracted with hexane, ethyl ether, acetone, ethanol, and methanol (Jacobson, 1981). Schmutterer (1990) reported that neem seed extracted with hexane, pentane, ethanol, methanol, esters and dichloromethane as well mixtures of any of these solvents with water possessed insecticidal activities.

The results also showed that the toxicity of neem extracts decreased with polarity of extraction solvent. This contradicts the results of Zebitz (1984) that revealed the toxicity of neem extracts increased with the polarity of the extraction solvents. Schaver and Schmutterer (1981) revealed that nonpolar solvents are more effective than polar ones in extracting substances active against mites from neem kernels.

Although the present findings showed that methanolic extracts had no pupicidal effects on *A. aegypti* pupae, the results of Sivagnaname and Kalyanasundaram (2004) showed that methanolic extracts of the leaves of A. indica was effective against *Culex quinquefasciatus* and A. *aegypti* pupae. These authors revealed that the extract was less effective against the larvae of *Anopheles stephensi, but* was more effective against the pupae of *A. stephensi* compared to other species. However, these authors did not use sequential extraction technique and the preceding solvents might have extracted most active components. The potency of the methanolic extract reported by Sivagnaname and Kalyanasundaram (2004) could be due to the ability of methanol to extract a great amount of polar compounds and its effectiveness in eluting salanin, desacetyl-nimbin and nimbin (Feuerhake, 1984).

The result of present Rao *et al.* (1992) experimented under field conditions showed that application of neem cake powder at a dose of 500 kg/ha alone or coated on urea resulted in drastic reduction in the late instars larvae and pupae of culicine mosquitoes. In another studies Rao *et al.* (1995) showed that lipid rich fractions of neem was effective in control of the breeding of culicine vectors of Japanese encephalitis and equally produced significant reduction in populations of anopline pupae.

The pupal mortality observed in the hexane extracts could be due to the effects of the oil fractions on their respiratory system. In earlier studies, Corbet *et al.* (1995) observed susceptibility of mosquito larvae and pupae to surface materials entering their tracheal system and reported that essential oils increased the tendency to tracheal flooding and chemical toxicity. It has been established that pupal mortality was due to the effects of azadirachtin, the most biologically active substance from neem that modifies the insect's physiology by influencing hormonal systems especially that of ecdysone (Schmutterer, 1990). Mordue and Blackwell (1993) had earlier reported that azadirachtin prevented ecdysis and apolysis and caused pupal death before and during molting. The results showed that the susceptibility of *A. aegypti* pupae to neem seed kernel extracts were lesser then that of 4th instar larvae confirming earlier results (Boschitz and Grunewald, 1994) that revealed that sensitivity of *A. aegypti* towards Neemzal decreased with increasing age of the larvae.

Further research on the potentials of these extracts in field conditions become imperative for practical implementations of mosquito control programmes to protect human populations from the scourge of mosquito-borne diseases in the northeastern Nigeria and other mosquito endemics areas of the world.

REFERENCES

ALIERO, B. L. (2003). Larvicidal effects of aqueous extracts of Azadirachtin indica (neem) on the larvae of Anopheles mosquitoes. *African Journal of Biotechnology, 2(9):* 325 – 327.

ASCHER, K. R. S. (1981). Some physical (solubility) properties and biological sterilant for *Epilachna varivestis* females effects of a dried methanolic neem (*Azadirachtin indica)* seed kernel extract. Pages 63 – 74. *In*: SCHMUTTERER, H. and ASCHER, K. R. S. (Eds). *Natural pesticides from the neem tree (Azadirachtin indica A. Juss) and other tropical plants. Proceedings of 1st International Neem Conference*, Rauischholzhausen, Federal Republic of Germany, 16 – 18 June, 1980.

AZMI, M. A., NAQVI, S. N. H., AHMAD, I., TABASSUM, M. R. and ANBREEN, B. (1998). Toxicity of neem leaves extract (NLX) compared with Malathion (57 E.C.) against late 3rd instar larvae of *Culex fatigans* (wild strain) by WHO method. Tropical Journal of Zoology, *22:* 213 – 218.

BOSCHITZ, C. and GRUNEWALD, J. (1994).The effects of Neemzal on Aedes aegypti (Diptera :Culicidae). *Journal of Applied Parasitology*, *35(4):* 251 – 256.

BROWN, A. W. A. (1986). Insecticide resistance in mosquitoes: a pragmatic review. *Journal of the American Mosquito Control Association*, *2:* 123 – 139.

CORBET, S. A., DANAHAR C. W., KING V., CHALMERS, C. L. and TILEY, C. F. (1995). Surfactant-enhanced essential oils as mosquito larvicides. *Journal of Experimental and Applied Entomology, 75:* 229 – 236.

DON-PEDRO, K. N. and ADEGBITE, T. O. (1985). Nuvan resistance in a strain of *Aedes aegypti* in Lagos, Nigeria. *Environmental Pollution, 38:* 19 – 24.

FEUERHAKE, K. J. (1984). Effectiveness of technical solvents for the extraction of neem seed components with insecticidal activity. Page 103 – 113. *In:* Schmutterer, H. and Ascher, K. R. S.

(Eds.). *Natural pesticides from the neem tree (Azadirachtin indica A. Juss) and other tropical plants*. *Proceedings of 2nd international Neem Conference,* Rauischholzhausen, Federal Republic of Germany, 25 – 28 May, 1983.

GUBLER, D. J. (1997). Epidemic Dengue/Dengue Haemorrhagic Fever: A Global Public Health Problem in the 21st Century. *Dengue Bulletin*. *21:* 21 – 31.

HENNESSEY, M. K., NIGG, H. N. and HABECK, D. H. (1992). Mosquito (Diptera: Culicide) adulticide drift into wildlife refuges of the Florida keys. *Journal of Environmental Entomology, 21(4):* 714 – 721.

IPEK, G. RALP, P. and MOHAMMED, A. (2004). Ecological risk assessment of neem-based pesticides. *Journal of Environmental Science and Health, Part B- Pesticides, Food Contaminants and Agricultural Wastes*, *39(2):* 311 – 321.

IVBIJARO, M. F. (1987). Prospects for neem in Nigeria agriculture. Pages 525 – 533. *In*: SCHMUTTERER, H. and ASCHER, K. R. S. (Eds) Natural pesticides from the neem tree (*Azadirachtin indica* A. Juss) and other tropical plants. *Proceedings of 3rd International Neem Conference,* Nairobi, Kenya, 10 - 15 July, 1986.

JACKAI, L. E. N. (1993). The use of neem in controlling cowpea pests. *International Institute of Tropical Agriculture Research Bulletin*, *7:* 5 – 11.

JACOBSON, M. (1981). Neem Research in the US Department of Agriculture: Chemical, biological and cultural aspects. Pages 33 – 42. *In:* SCHMUTTERER, H. and ASCHER, K. R. S. (Eds) Natural pesticides from the neem tree (*Azadirachtin indica* A. Juss) and other tropical plants. *Proceedings of 1st International Neem Conference,* Rauischholzhausen, Federal republic of Germany, 16 – 18 June, 1980.

LIU, W., ZHANG, L., JAMAL, H. H., JULIANA, J., ZAILINI, H. and BERNANRD, G. D. (2003). Mosquito coils Emissions and health implications. *Environmental Health Perspectives, 111(12):* 1430 – 1437.

MOLINEAUX, L. and GRAMICCIA, G. (1980). *The Gaarki project research on the epidemiology and control of malaria in Sudan savannah of West Africa.* World Health Organization, Geneva. 406 pp.

MORDUE, A. J. and BLACKWELL, A. (1993). Azadirachtin: An update. *Journal of Insect Physiology. 39:* 903 – 924.

NASIDI, A., MONATH, T. P., DEOCK, K., TOMORI, O., CORDELLIER, R., OLALEYE, O. D., HARRY, T. O., ADENIYI, J. A., SORUNGBE, A. O., AJOSE-COKER, A. O., VAN DER LAAN, G. and OYEDIRAN, A. B. O. 1989). Urban yellow fever epidemic in western Nigeria, 1987. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 83(3):* 401 – 406.

NOVAK, D. (1985). Non-chemical approaches to mosquito control in Czechoslovakia. Pages 185 – 196. *In:* LAIRD, M. and MILES, J. W. (Eds). *Integrated mosquito control methodologies.* Volume 2. Academic Press, Dan Diego.

RAO, D. R., REUBEN, R., VENUGOPAL, M. S., NAGASAMPAGI, B. A. and SCHMUTTERER, H. (1992). Evaluation of neem, *Azadirachta indica*, with and without water management, for the control of culicine mosquito larvae in rice-fields. *Journal of Medical and Veterinary Entomology. 6(4):* 318 – 324.

RAO, D. R., REUBEN, R. and NAGASAMPAGI, B. A. (1995). Development of combined use of neem (*Azadirachta indica)* with and without water management, for the control of culicine mosquito larvae in rice-fields. *Journal of Medical and Veterinary Entomology, 9(1):* 25 – 33.

ROBERTS, D. (1998). Overcrowding of *Culex sitiens* (Diptera: Culicidae) larvae: population regulation by chemical factors or mechanical interference. *Journal of Medical Entomology, 35(5):* 665 – 669.

SCHAUER, M and SCHMUTTERE, H. (1981). Effects of neem seed kernel extracts on the two-spotted spider mite, *Tetranychus urticae.* Pages 259 – 266. *In:* SCHMUTTERER, H. and ASCHER, K. R. S. (Eds) Natural pesticides from the neem tree (*Azadirachtin indica* A. Juss) and other tropical plants. *Proceedings of 1st International Neem Conference,* Rauischholzhausen, Federal republic of Germany, 16 – 18 June, 1980.

SCHUMTHERER, H. (1990). Properties and potential Natural pesticides from the Neem tree, *Azadirachtin indica*. *Annual Review of Entomology, 35:* 271 – 279.

SIVAGNANAME, N. and KALYANASUNDARAM, M. (2004). Laboratory Evaluation of methanolic extract of A*tlantia monoplyla* (Family: Rutacaeae) against immature stages of mosquitoes and non-target organisms. *Memorias do Instituto Oswaldo Cruz, Rio de-Janeiro, 99(1):* 115 – 118.

SNELLER, V. P. and DADD, R. H. (1977). Requirement for sugar in a chemically defined diet for *Aedes aegypti* larvae. *Journal of Experimental and Applied Entomology, 14:* 387 – 392.

STATSDIRECT (2005). StatsDirect Ltd.uk Version 4.2, 11 Gresham Way Cheshire, M33 3UY UK.

SUKUMAR, K., PERICH, M. J. and BOOBAR, L. R (1991). Botanical derivatives in Mosquito control: A review. *Journal of the American Mosquito control Association.* 7: 210-217.

WHO (1970) Expert committee on insecticides, 17th Reports, *World Health Organization Technical Report Series No. 433 (Annexes 2A and 2B),* Geneva.

ZEBTIZ, C. P. W. (1984). Effect of some crude and azadirachtin enriched neem (*Azadirachtin indica*) seed kernel extracts on larvae of Aedes *aegypti. Journal of Experimental and Applied Entomology.* 35: 11 – 16.