JJBS

Volume 4, Number 4, December 2011
ISSN 1995-6673
Pages 237 - 242

Jordan Journal of Biological Sciences

Bioefficacy of Extracts of some Indigenous Nigerian Plants on the developmental stages of mosquito (*Anopheles gambiae*)

Rotimi O. Akinkurolere*, Chris O. Adedire, Olusola O. Odeyemi, Joshua Raji and Josiah A. Owoeye

Biology Department, Federal University of Technology P.M.B. 704, Akure, Nigeria.

Received 7 June 2011; received in revised form 29 July 2011; accepted 1 August 2011

Abstract

The bioactivity of hexane extract from the nuts of *Anacardium occidentale* (Linnaeus), ethanol extracts from the bark of *Myrianthus arboreus* (P. Beauv) and fruits of *Xylopia aethiopica* (Dunal), were studied at five concentration levels (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) against the larvae, pupae and adults of *Anopheles gambiae* (Giles). Results indicated that *X. aethiopica* caused significantly ($P < 0.05$) higher mortality of larvae, pupae and adult mosquitoes than other plant extracts tested. It caused 100%, 57.50% and 92.50% larva, pupa and adult mortality, respectively at 0.5% concentration. Also, based on the lethal concentration average (LC_{50}) results, *X. aethiopica* was the most effective, with LC_{50} values of 0.23, 0.40 and 0.29 µg/ml on the larvae, pupae and adults *An. gambiae*, respectively, followed by *A. occidentale* (LC_{50} 0.28, 0.45 and 0.34 µg/ml), then *M. arboreus* (LC_{50} 0.32, 0.64 and 0.36 µg/ml). The results of our findings were discussed in line with use of biorationals as an affordable, readily accessible, and environmentally friendly alternative means of reducing malaria disease in Nigeria, by controlling *An. gambiae* mosquito, a major vector of malaria pathogen.

Keywords: *Anacardium occidentale*, *Anopheles gambiae*, fumigant toxicity, mosquito, *Myrianthus arboreus*, *Xylopia aethiopica*.

1. Introduction

Mosquitoes transmit diseases to more than 700 million people annually in Africa, South America, Central America, and much of Asia, with Africa being the most affected continent. Nigeria, being the most populous country in Africa, its citizens residing in the country, is at the greatest risk of malaria disease. Malaria is the most prevalent of mosquito borne diseases; being endemic in about 109 countries, affecting 190-330 million people and causing about one million deaths every year. *Anopheles* mosquito is the insect vector responsible for the transmission of the causative pathogen (plasmodium) of malaria (WHO, 1996; Akinkurolere and Zhang, 2007; WHO, 2010; RBM, 2011).

Over the last 50 years, insect pests control has mainly been with synthetic chemical insecticides such as organochlorines, organophosphates, carbamates, and pyrethoids, of which organophosphates and carbamates are the major classes in use today. However, problems due to pesticides resistance, negative effect on non-target organisms (including humans and the environment) (Rembold, 1984; FAO, 1992; Franzen, 1993) have been associated with use of synthetic chemical pesticides. In addition, these pesticides are expensive, more hazardous to handle, leave toxic residues in food products, and are not easily biodegradable. Thus, attention is fast shifting to alternative pest management strategies.

Before organochlorine and organophosphate insecticides were discovered in the late 1930s and early 1940s, botanical insecticides were important products for pest management even among the industrialized countries (Isman, 1997). Small holder farmers and researchers have often claimed successful use of plant products in insect pest control. Plant materials such as spices, vegetable oils, extracts, powders or ash (Ofuya, 1986; Ajayi *et al.*, 1987; Lale, 1992; Lajide *et al.*, 1998; Keita *et al.*, 2001; Akinkurolere *et al.*, 2006; Adedire *et al.*, 2011) have been reported for their insecticidal efficacy. And unlike synthetic chemical insecticides that kill both pests and non target organisms, natural insecticides including botanicals are relatively target specific (Isman, 1997). They are also biodegradable, environmentally friendly, and can also be used in insecticide resistance management programmes (Saxena, 1987). Hence, could serve as good alternatives to chemical insecticides.

WHO (2010) reported that the primary public health intervention for reducing malaria transmission at the community level is through vector control. It is the only intervention that can reduce malaria transmission from very high levels to close to zero. Therefore, in a resolve to align with the efforts of the federal government of Nigeria to eradicate malaria within its sovereignty, researchers have routinely screened botanicals for their potency against mosquitoes. The present study was therefore carried out to determine the bioactivity of three indigenous Nigerian plants namely: *Anacardium occidentale*

* Corresponding author: roakinkurolere@yahoo.com

(Linnaeus), *Myrianthus arboreus* (P. Beauv) and *Xylopia aethiopica* (Dunal) A. Rich against the larvae, pupae and adults of *Anopheles gambiae* (Giles). The toxicity of these plants against some insects had earlier been reported (Asawalam *et al.*, 2006; Oparaeke and Bunmi, 2006; Adedire *et al.*, 2011).

2. Materials and Methods

2.1. Collection of plant materials

The fruits of *X. aethiopica* and seeds of *A.occidentale* were purchased from Oba market, Akure, while the barks of *M. arboreus* were collected from the forest reserve at the Federal University of Technology, Akure (FUTA), Nigeria. The plant materials were identified in the department of Forestry and Wood Technology, FUTA.

2.2. Extraction of plant materials

The nuts of *A. occidentale* were sundried for three weeks to allow for easy cracking and to prevent the kernels from crushing. Thereafter, the nuts were mechanically cracked to obtain the kernel. Clean dried kernels were pulverized into fine powder using an electric blender (Binatone® Model BLG400) (Adedire *et al.*, 2011). Amount of 200 g was soaked in 2 litres of hexane and heated in water bath at 60°C for 1 h and then decanted. The solvent was separated from the extract by vacuum evaporation. The crude extract (semi-solid paste) was kept in a dark bottle, labeled and preserved in the refrigerator till further use.

The dried fruits of *X. aethiopica* were pulverized as described earlier. Amount of 200 g of the *X. aethiopica* powder was soaked in 100 ml of ethanol in a conical flask. The flask was capped with rubber cork and left for 24 h undisturbed. Afterward, the mixture was filtered with sterile filter paper (Whatman no. 1) into a fresh conical flask. The filtrate was transferred into the sample holder of the rotary evaporator where the ethanol solvent was evaporated at its boiling temperature of 70°C. The crude extract obtained was stored in the refrigerator (Aina *et al*., 2009).

The bark of *M. arboreus* was first chopped into small pieces before air-drying. When they were crisp dry, the bark was ground into powder with the aid of a blender as described above. Amount of 2 litres of ethanol was added to 1000 g of the plant powder and soaked for 48 h. Thereafter, it was filtered, and the filtrate was concentrated on rotary evaporator. The extract was also stored as above until further use.

2.3. Collection and rearing of mosquito

Mosquito baits, consisting of shallow containers with a large surface area, were established under a partial shade outside. The container was filled with rain water in order to mimic mosquito natural breeding environment and to attract adults for oviposition. Small quantity of industrial yeast was sprinkled on the surface of the water and allowed to decompose slowly; this was added to nourish the developing larvae. Wild mosquitoes were allowed to freely visit the bait and to lay eggs. Afterward, the containers bearing mosquito eggs/larvae were transferred to the laboratory, identified and maintained at temperature of 28 ± 2°C, 75 ± 5% RH and 14:10 L:D. Soon after the pupae emerged, they were transferred to a screened cage with dimension 20 × 20 × 20cm, where the adults emerged. After emergence, female mosquitoes obtained blood meal from caged immobilized albino rat; this is to make their eggs fertile, while male mosquitoes were fed on a 10% sucrose solution. Then egg-mass were kept to continue the next generation.

2.4. Effect of plant extracts on larvae and pupae of An. gambiae

Larvicidal and pupacidal activity of the plant extracts was carried out at different concentration by preparing the required stock solutions following the standard procedure (WHO, 1996). The desired concentrations were achieved by adding 1.0 µg of crude extract of any of the three plant materials to 100 ml of de-chlorinated water. From this, five concentrations (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) of each plant extract were prepared. The extracts were mixed with water in a beaker at the desired concentration in the presence of small amount of yeast powder to serve as food source for the larvae. Then 10 larvae or pupae of *An. gambiae* were introduced into the beaker. Control (water only) beakers were similarly infested. There were three replicates for each concentration and the control. Mortality was observed over 24 h, after which the larva or pupae were introduced into distilled water to notice recovery. A recovery time of 5 minutes was allowed (WHO, 1996). The larval mortality in treatments was corrected for the controls (Abbott, 1925). Larvae or pupae were counted as dead when they were not coming to the surface for respiration and were insensitive to probe (Sivagnaname and Kalyanasundaram, 2004).

2.5. Fumigant effect of plant extracts on adult An. gambiae

The fumigant property of the plant extracts was used to assess their efficacy against the adults of *An. gambiae*. Ten adults were placed inside a test-tube and plugged with cotton wool. Strips of filter papers (3cm × 3cm) were soaked in varying concentrations of extracts and then suspended in the test-tube. Each treatment and the control were replicated three times. Mortality was recorded after 3 h of application.

2.6. Data analysis

For each treatment in 2.4 and 2.5 above, there were more than five trials from which data were pooled together and their averages (means) were determined. Data were subjected to analysis of variance (ANOVA), and means were separated using Tukey's test. The ANOVA and LD_{50} were performed with SPSS 11.0 software (SPSS, Inc., 2007). Because percentage mortality of *An. gambiae* in treatments was not normally distributed, data were first normalized by arcsine transformation before analysis. After analysis, back-transformed data were used in the tables.

3. Results

Tables 1 to 5 show the mean percentage mortality of larvae, pupa and adults of *An. gambiae* at 24 h after exposure to varying concentration levels (0.1%, 0.2%, 0.3%, 0.4%, and 0.5%) of *X. aethiopica*, *A. occidentale* and *M. arboreus* plant extracts. At the lowest concentration (0.1%) tested, plant extracts had significant

effect on larval ($F_{3, 8}$ = 1.56; P = 0.002) and adult ($F_{3, 8}$ = 21.88; P = 0.019) mortality, but not on pupal mortality ($F_{3, 8}$ = 15.63; P = 0.116). *X. aethiopica* caused significantly (P < 0.05) higher mortality of larvae and adult mosquitoes than other plant extracts tested (Table 1).

Table 1. Percentage mortality (mean ± standard error) of *Anopheles gambiae* at 24 h post treatment with 0.1% concentration of extracts of *Xylopia aethiopica*, *Anacardium occidentale* and *Myrianthus arboreus*

	Developmental stages		
Plant extracts	**Adults**	**Pupae**	**Larvae**
Control	0.00 ± 0.00 [a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
X. aethiopica	15.00 ± 0.20[c]	7.50 ± 0.20[a]	5.00 ± 0.37[b]
A. occidentale	7.50 ± 0.15[ab]	5.00 ± 0.15[a]	0.00 ± 0.00[a]
M. arboreus	2.50 ± 0.10[b]	0.00 ± 0.00[a]	0.00 ± 0.00[a]

Means within the same column followed by the same letter(s) are not significantly different at P>0.05 using Tukey's test.

At 0.2%concentation, plant extracts had significant effect on the mortality of larvae ($F_{3, 8}$ = 82.81; P = 0.002), pupae ($F_{3, 8}$ = 3.13; P < 0.001) and adults ($F_{3, 8}$ = 3.19; P < 0.001) of *An. gambiae* (Table 2).

Table 2. Percentage mortality (mean ± standard error) of *Anopheles gambiae* at 24 h post treatment with 0.2% concentration of extracts of *Xylopia aethiopica*, *Anacardium occidentale* and *Myrianthus arboreus*

	Developmental stages		
Plant extracts	**Adults**	**Pupae**	**Larvae**
Control	0.00 ± 0.00 [a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
X. aethiopica	27.50 ± 0.25 [c]	22.50 ± 0.25 [c]	40.00 ± 0.25 [b]
A. occidentale	22.50 ± 0.34 [b]	12.50 ± 0.34 [b]	37.50 ± 0.34 [b]
M. arboreus	2.50 ± 0.10 [a]	0.00 ± 0.00 [a]	30.00 ± 0.37 [b]

Means within the same column followed by the same letter(s) are not significantly different at P>0.05 using Tukey's test.

A similar trend of results was observed across the other plant extracts concentration further examined (Tables 3, 4 and 5). In all cases, larval mortality was the highest, while the lowest mortalities were observed in the pupa stages of the mosquito. And at 0.4% concentration, *X. aethiopica* evoked 100% mortality on *An. gambiae* larva (Table 4). The plant extract also caused 57.50% and 92.50% pupa and adult mortality, respectively at 0.5% concentration (Table 5). The efficacy of the plant extracts against *An. gambiae* was in the following order: *X. aethiopica* > *A. occidentale* > *M. arboreus*.

Table 3. Percentage mortality (mean ± standard error) of *Anopheles gambiae* at 24 h post treatment with 0.3% concentration of extracts of *Xylopia aethiopica*, *Anacardium occidentale* and *Myrianthus arboreus*

	Developmental stages		
Plant extracts	**Adults**	**Pupae**	**Larvae**
Control	0.00 ± 0.00 [a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
X. aethiopica	42.50 ± 0.49[c]	37.50 ± 0.49[c]	80.00 ± 0.49[c]
A. occidentale	37.50 ± 0.53[c]	30.00 ± 0.53[bc]	67.30 ± 0.53[bc]
M. arboreus	12.50 ± 0.57[b]	5.00 ± 0.57[ab]	42.50 ± 0.57[b]

Means within the same column followed by the same letter(s) are not significantly different at P>0.05 using Tukey's test.

Log probit analysis of the mortality data (Table 6) further revealed that *X. aethiopica* was the most effective with LC_{50} values of 0.23, 0.40 and 0.29 µg/ml on the larvae, pupae and adults of *An. gambiae*, respectively, followed by *A. occidentale* (LC_{50} 0.28, 0.45 and 0.34 µg/ml) then *M. arboreus* (LC_{50} 0.32, 0.64 and 0.36 µg/ml).

Table 4. Percentage mortality (mean ± standard error) of *Anopheles gambiae* at 24 h post treatment with 0.4% concentration of extracts of *Xylopia aethiopica*, *Anacardium occidentale* and *Myrianthus arboreus*

	Developmental stages		
Plant extracts	**Adults**	**Pupae**	**Larvae**
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
X. aethiopica	70.00 ± 0.47^{c}	52.50 ± 0.47^{d}	100.00 ± 0.00^{d}
A. occidentale	67.50 ± 0.67^{c}	33.00 ± 0.67^{c}	82.50 ± 0.00^{c}
M. arboreus	27.50 ± 0.73^{b}	15.50 ± 0.73^{b}	60.00 ± 0.73^{b}

Means within the same column followed by the same letter(s) are not significantly different at $P>0.05$ using Tukey's test.

Table 5. Percentage mortality (mean ± standard error) of *Anopheles gambiae* at 24 h post treatment with 0.5% concentration of extracts of *Xylopia aethiopica*, *Anacardium occidentale* and *Myrianthus arboreus*

	Developmental stages		
Plant extracts	**Adults**	**Pupae**	**Larvae**
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
X. aethiopica	92.50 ± 0.41^{d}	57.50 ± 0.41^{d}	100.00 ± 0.00^{c}
A. occidentale	75.30 ± 0.44^{c}	47.50 ± 0.44^{c}	100.00 ± 0.00^{c}
M. arboreus	52.50 ± 0.76^{b}	25.50 ± 0.76^{b}	92.50 ± 0.76^{b}

Means within the same column followed by the same letter(s) are not significantly different at P>0.05 using Tukey's test.

Table 6. LC_{50} (µg/ml) of *Xylopia aethiopica*, *Anacardium occidentale* and *Myrianthus arboreus* obtained from the mortality test on *Anopheles gambiae*

			Confidence limit			
Developmental stage	**Plants**	**LD_{50}**	**lower**	**upper**	**DF**	***P***
Larva	***X. aethiopica***	0.23	0.15	0.29	3	<0.0001
	A. occidentale	0.28	0.21	0.32	3	<0.0001
	M. arboreus	0.32	0.27	0.37	3	<0.0001
Pupa	***X. aethiopica***	0.40	0.34	0.46	3	<0.0001
	A. occidentale	0.45	0.39	0.48	3	<0.0001
	M. arboreus	0.64	0.59	0.72	3	<0.0001
Adults	***X. aethiopica***	0.29	0.25	0.37	3	<0.0001
	A. occidentale	0.34	0.28	0.43	3	<0.0001
	M. arboreus	0.36	0.32	0.45	3	<0.0001

4. Discussion

The toxicities of phytochemical compounds on target species vary depending on the plant part from which they have been extracted (Tuetun *et al.*, 2004). Other variations are due to responses of species and their developmental stages to the specific extract, solvent of extraction, geographical origin of the plant, phytosensitivity of compounds in the extract, effect on growth and reproduction, etc. (Jeyabalan *et al.*, 2003).

It has been widely reported that crude or partially purified plant extracts are less expensive and highly efficient for the control of mosquitoes, rather than the purified compounds (Cavalcanti *et al.*, 2004; Jenson *et al*., 2006).

All the plant extracts evaluated in this study effectively reduced the population of larvae, pupae, and adults of *An. gambiae* that were confined to simulated shallow water in the laboratory. The mortality effect, however, varied according to the plant and concentration of the extract.

The results of the present study show that *X. aethiopica* was the most potent of the three extracts. The markedly high toxicity of *X. aethiopica* against the larvae, pupae and adults of *An. gambiae* could be due to its strong pungent odour. A number of plants with high pungency have been reported for their bioactivity against insect pests (Dupriez and De Leener, 1998; Akinkurolere *et al*., 2006; Aina *et al*., 2009). The essential oil of *X. aethiopica* contains secondary plant compounds such as Alpha pinene, Betaphellandiene, Betapinene, 1- 8 Cineode, Gamma terpinene, Lindalyl acetate, Pinanol, Verbenene, Pinocarvone, L-carveol, Terpinene-4-ol, Myrtenal, Myrtenol, Cuminal, and Phellandral1 (Asawalam *et al.*, 2006). Since most of these compounds had been implicated in insect mortality through stomach poisoning,

contact toxicity etc., the high toxicity of *X. aethiopica* against the mosquito could therefore be due to the effect of one or more, or a combination of these compounds (Philipson and Wright, 1991).

A. occidentale and *M. arboreus* also exerted high toxicity against *An. gambiae*, though not as high as observed with *X. aethiopica* extracts. *A. occidentale* contain anacardic acid and cardinol (Rehm and Espig, 1991), quercetin and kaempferol glycosides (Oliver-Bever, 1986), triacylglycerols, fatty acids, several unsaponifiable compounds, triterpene, alcohols, sterols and tocopherols. The roots of *M. arboreus* contain several pentacyclic triterpenoids. Euscaphic acid, myrianthinic acid, tormentic acid, ursolic acid, a derivative of ursonic acid and triterpene acids have been isolated from stems. The bark contain tormentic and euscaphic acids, myriaboric acid, myrianthinic acid, and arboreic acid. The wood also contains myrianthiphyllin and lignin cinnamate (Burkill, 1985; Ngounou *et al.*, 1988; Tamboue Deffo and Nekam, 1993). These secondary plant compounds are similar to those found in *X. aethiopica*, some of which had been reported for their immunomodulatory, haemolytic, allelopathic and insecticidal activities (Echendu, 1991; Golob *et al.*, 1999; Adedire *et al.*, 2011).

The larvae of *An. gambiae* was the most susceptible developmental stage to plant extract treatment (with 100% mortality at 0.4% of *X. aethiopica* within 24 h), followed by the adults, then pupae. Other researchers have also made similar observations (Amusan and Okorie, 2002; Promsiri *et al*., 2006; Aina *et al.*, 2009). The mosquito larvae feed actively, by so doing, doses of plant active components could be ingested, thereby leading to stomach poisoning. Furthermore, *Anopheles* larvae lack a respiratory siphon, they breathe through spiracles located on the 8th abdominal segment and therefore must come to the surface frequently (Kaufmann and Briegel, 2004) to breathe. The plant extracts used in this study are oily; hence, the oils could block the spiracles, resulting in asphyxiation and death of the larvae (Akinkurolere *et al*., 2006; Adedire *et al.*, 2011). The pupae do not feed and are not active thus, reducing their chances of pesticide uptake cum susceptibility.

Results from this study are in accord with previous findings, where varying degrees of efficacy of plant materials against mosquito species were reported (Al Dakhil and Morsy, 1999; Amusan and Okorie, 2002; El-Bokl, 2003; Nathan *et al.*, 2005; Promsiri *et al*., 2006; Singh *et al.*, 2006).

Nigeria is located in malaria risk zones of sub Saharan Africa. And being a highly populated country, her populace are at the highest risk globally of deaths as a result of malaria (a mosquito borne disease), which kills more than one million people annually (WHO, 2010; RBM, 2011). Many of these deaths are as a result of poverty, because several poor Africans may not be able to afford the cost of medication or good accommodation to screen out mosquitoes. Therefore, in line with Nigeria governments' continued effort to eradicate malaria, researchers have routinely screened readily available plants for their cure for malaria or bioefficacy against the vector of malaria pathogen, the *Anopheles* mosquito. Here we conclude that the three plant materials used in this investigation, especially *X. aethiopica* was highly effective against *An. gambiae* and could be included in the management strategies of mosquitoes. In addition to their environmental friendliness, these plants are readily available, cheap, and would be affordable to the resource-poor persons in Nigeria.

Acknowledgements

The authors would like to thank the technologists at Biology Department, Federal University of Technology, Akure for their assistance during mosquito rearing and preparation of plant extracts. The comments by two anonymous reviewers towards improving the quality of this paper are acknowledged.

References

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *J Econ Entomol*, **18**: 265-267.

Adedire CO, Obembe OM, Akinkurolere RO and Oduleye SO. 2011. Response of *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae: Bruchinae) to extracts of cashew kernels. *J Plant Diseases Protect*, **118(2):** 75-79.

Aina SA, Banjo AD, Lawal OA and Jonathan K. 2009. The efficacy of some plant extracts on *Anopheles gambiae* mosquito larvae. *Acad J Entomol*, **2 (1):** 31-35.

Akinkurolere RO, Adedire CO and Odeyemi OO. 2006. Laboratory evaluation of the toxic properties of forest Anchomanes, *Anchomanes difformis* against pulse beetles *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Insect Sci.*, **13**: 25-29.

Akinkurolere RO and Hongyu Z. 2007. Symbiosis in mosquitoes and its role in vector control. *Int. J. Med. Parasitic Dis*, **34(1):** 1-7.

Al Dakhil MA and Morsy TA. 1999. The larvicidal activities of the peel oils of three citrus fruits against *C. pipiens*. *J Egypt Soc Parasitol*, **29**: 347-352.

Amusan AAS and Okorie TG. 2002. The use of piper fruits oil as protectant of dried fish against *Dermestes maculates*. *Global J. Pure and Applied Sci*., **8(2):** 197-201.

Asawalam EF, Emosairue SO and Hassanali A. 2006. Bioactivity of *Xylopia aetiopica* (Dunal) A. Rich essential oil constituents on maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). *Electronic J Environ Agric Food Chem*, **5(1):** 1195-1204.

Burkill HN. 1985. Useful Plants of Tropical West Africa. Pergamum Press, London. pp 231-237.

Cavalcanti ES, Morais SM, Lima MA and Santana EW. 2004. Larvicidal activity of essential oils from Brazilian plants against *Ae. Aegypti*. L. Mem. *Inst Oswaldo Cruz*, **99**: 541 – 544.

Dupriez H and De Leener P. 1998. **African Gardens and Orchards-Growing Vegetables and Fruits**. Macmillan Publishers Ltd, London. pp 333.

Echendu TNC. 1991. Ginger, cashew and neem as surface protectants of cowpeas against infestation and damage by *Callosobruchus maculatus* (F.). *Trop Sci*, **31**: 209-211.

El-Bokl MM. 2003. Latent toxicity of azadirachtin treatment on *C. pipiens* (Diptera: Culicidae). *J Egypt Acad Soc Environ Develop*, **3:** 63-74.

FAO (Food Agricultural Organization). 1992. **Pesticide Residues in Food**. Report number 116: Rome.

Franzen H. 1993. Need for development of new strategies for locust control. In: Renbold H, (Eds.) **New Control for Locust Control**, Bona. pp 89.

Golob P, Moss C, Dales M, Fidgen A, Evans J and Gudrups I. 1999. The use of spices and medicinal as bioactive protectants for grains. FAO Agricultural Services Bullettin #137. Natural Resources Institute Chatham Maritime Chatham, Kent United Kingdom. Page 16.

Isman MB. 1997. Neem and other botanical insecticide: barriers to commercialization. *Phytoparasitica*, **25**: 339-344.

Jenson TG, Palsson K and Borg-Karlson AK. 2006. Evaluation of extracts and oils of mosquito (Diptera: Culicidae) repellent plants from Sweden and Guinea-Bissau. *J Med Entomol*, **43:** 113-119.

Jeyabalan D, Arul N and Thangamathi P. 2003. Studies on effects of *Pelargonium citrosa* leaf extracts on malarial vector, *Anopheles stephensi*. *Liston Bioresour. Technol*, **89:** 185-189.

Kaufmann C and Briegel H. 2004. Flight performance of the malaria vectors *Anopheles gambiae* and *Anopheles atroparous* (CPDF). *J Vector Ecol*, **29(1):** 140-153.

Keita SM, Vincent C, Schmit J, Arnason JT and Belanger A. 2001. Efficacy of oil of *Ocimum basilicum* L. and *O. gratissimum* L. applied as an insecticidal fumigant and powder to control *Callosobruchus maculatus* (Fabr.). *J Stored Prod Res*, **37:** 339–349.

Nathan SS, Kalaivani K and Murugan K. 2005. Effects of neem limonoids on the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Act Trop*, **96:** 47-55.

Ngounou FN, Lontsi D and Sondengam BL. 1988. A pentacyclic triterpene diacid from *Myrianthus arboreus. Phytochemistry*, **27(7):** 2287-2289.

Oliver-Bever B. 1986. **Medicinal Plants in Tropical West Africa**. Cambridge University Press, Cambridge, United Kingdom.

Oparaeke AM and Bunmi OJ. 2006. Insecticide potential of cashew, *Anacardium occidentale* L. for the control of the beetle, *Callosobruchus subinnotatus* (Pic) (Coleoptera: Bruchidae) on bambarra groundnut, *Voandzeia subtrranea* (L.) Verde. *Archives of Phytopathol Plant Prot*, **39(4):** 247-251.

Philipson JD and Wright CW. 1991. Antiprotozoal compounds from plants sources. *Planta Medica*, **57**:553–559.

Promsiri S, Naksathit A, Kruatrachue M and Thavara U. 2006. Evaluations of larvicidal activity of medicinal plant extracts to *Aedes aegypti* (Diptera: Culicidae) and other effects on a non target fish. *Insect Sci*, **13:** 179-188.

Rehm S and Espig G. 1991. **The Cultivated Plants of the Tropics and Subtropics**. Verlag Josef Margraf, Weikersheim, Germany.

Renbold H. 1984. Secondary plant compounds in insect control with special reference to azadirachtins. *Adv Invertebr Reproduct*, **3**: 481-491.

Roll Back Malaria, Malaria key facts, January 2011, (Accessed May 25th, 2011 at http://www.rollbackmalaria.org/keyfacts.html)

Saxena RC. 1987. Antifeedants in tropical pest management. *Insect Sci. Applic*, **8**: 731-736.

Singh RK, Dhiman RC and Mittal PK. 2006. Mosquito larvicidal properties of *Momordica charantia* Linn (Family: Cucurbitaceae). *J Vect Borne Dis*, **43**: 88–91.

Sivagnaname N and Kalyanasundaram M. 2004. Laboratory evaluation of methanolic extract of *Atlantia monophylla* (family: Rutacea) against immature stages of mosquitoes and non-target organisms. Mem. *Inst Oswaldo Cruz, Rio de Janeiro*, **99(1):** 115-118.

SPSS, Inc. **Statistical Package for Social Sciences**. SPSS, Inc., Chicago, IL. 2007.

Tamboue Deffo E and Nekam P. 1993. Chemical study of some anti-diarrhoea plants used in the central province of Cameroon. *Revue de Medecines et Pharmacopees Africaines*,**7(2):** 97-107.

Tuetun B, Choochote W, Rattanachanpichai E, Chaithong U, Jitpakdi A, Tippawangkosol P, Riyong D and Pitasawat B. 2004. Mosquito repellency of the seeds of celery (*Apium graveolens*). *Ann Trop Med Parasitol*, **98:** 407-417.

WHO. 1996. World Health Organization. **Instruction for Determining the Susceptibility and Resistance of Mosquito Larvae to Insecticides**. WHO/VBC/75.583, mimeographed document.

WHO. 2010. World Health Organization, **Malaria: fact sheets** No. 94, April 2010, (Accessed May 25th, 2011 at http://www.who.int/mediacentre/factsheets/fs094/en/)