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Full Length Research Paper

Applications of natural products in the control of mosquito-transmitted diseases

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Mosquito-transmitted diseases remain one of the most significant causes of mortality in the African continent, despite successes in controlling these diseases in other regions of the world. The disproportionate impact in areas of poverty suggests a need for control that is efficient and does not require complex technological control strategies. Focusing on the vectors of disease, the mosquito, there are many alternatives to synthetic, chemical pesticides that await discovery and development. Although some natural products have been described, there is still a need for continuing research that incorporates endogenous knowledge in the selection process for potential vector control candidates. Recent experiments using natural products are summarized. Ultimately, a paradigm shift in research that evaluates natural products in a comparative manner will help to produce new materials for effective and efficient control of vectors and thereby achieve sustainable reduction of the impacts from the diseases they carry.

Key words: Mosquito, candidates, endogenous, diseases, disproportionate.

INTRODUCTION

Despite centuries of efforts to control mosquitoes as a part of disease prevention strategies, many regions of the world are still struggling to overcome the burdens of vector-transmitted diseases. Malaria infects over 300 million people per year, striking disproportionately in areas of poverty and lower economic growth (Sachs and Malaney, 2002) and is regarded as one of the top three causes of communicable illness worldwide. It has been estimated that 1 million children in sub-Saharan Africa die each year from malaria (Tolle, 2009). Although recently on the decline, mosquito-transmitted filariasis has infected over 120 million people globally (Michael et al., 1996), imparting serious quality of life issues. Dengue virus and the resulting severe form of dengue hemorrhagic fever, has experienced an expanding geographic range in recent decades as the *Aedes aegypti* vector has returned to regions where it was once eliminated (Gubler, 1998). This bears troubling concerns for the management of *Aedes*-associated infections such as yellow fever as well as for the spread of chikungunya virus (Chretien et al., 2007). Arboviral encephalitis viruses (including West Nile, St. Louis encephalitis, and Japanese encephalitis) have been found in North America in the past decade, most notably with the spread of West Nile through North and Central America (Mackenzie et al., 2004).

In terms of affecting humanity, malaria alone is estimated to cause 100 million incidences and the associated complications in children younger than 5 years in sub-Saharan Africa each year (Greenwood et al., 2005). The impact upon this demographic group must be considered astounding. In addition to outright health effects, the economic disparities have been calculated in terms of average gross domestic product (GDP) to be five times lower and yield 2% less annual average growth of GDP in areas of endemic malaria (Sachs and Malaney, 2002). By contrast, most of the wealthy, developed nations outside of Africa have had good success in eliminating malaria from within their borders.

In the historic campaigns to eliminate mosquito-borne disease, using malaria as an example, alterations to watershed systems on small and large scales have provided some success, especially when combined with community and individually based interventions such as insecticide treated bed nets and quinine medications (Utzinger et al., 2001). In consideration with other aspects

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of a multidisciplinary approach, vector control is one of the key components of the Roll Back Malaria Initiative, started by the WHO in 1998 to reduce the global malarial burden by 2010. It has also been the inspiration for the Bite The Bug! Campaign of Islamic Relief and United Against Malaria's efforts in advance of the 2010 World Cup in South Africa.

CONTROL OF VECTORS

Vector control has experienced a paradigm shift over time as public health officials have come to better appreciate the potential applications of natural products in the mission of disease control. Certain attributes of insecticides can be described to better define what would make an effective control agent, chiefly the toxicity and the latency (or residual capacity). In general, these two attributes are typically represented as the main performance indices for insecticide evaluation: namely, the LD_{50} and the test period, respectively. Repellents typically provide a low toxicity, although preventing a bite by a mosquito is a worthy goal in itself, but the duration of effectiveness is markedly short. Products that have high toxicity and can kill the mosquito are often highly desirable but can come with associated non-target costs. It has been argued that both increased toxicity and prolonged latency in combination would provide the most competitive approach to mosquito control (Shaalan et al., 2005), but for reasons of resistance evolution (see below), this may not always be the case. In practice, it has been noted that the synergistic effects of compounds through using mixtures has been effective both in efficacy and resistance (Isman, 1997). Pyrethroid chemicals, which were originally utilized as compounds extracted from the flowers of *Chrysanthemum coccineum* L. and *C. cinerariifolium* (Trev.) Bocc., are now available as synthetic chemicals, for example, permethrin. However, the zeal for a reductionist approach that indentifies the primary active agents and attempts to synthesize a chemical isomer may miss the potential for control strategies that are functional and available to a broader contingent of society.

MODES OF ACTION AGAINST SPECIFIC LIFE STAGES

Adult

Because the adult female mosquito is biting the host in search of blood when transmitting these diseases of concern, personal protection through this route has long been considered an important concern for the individual. Plant essential oils have been indicated to function through repellency of adult females, thereby preventing the biting activity that spreads disease. Unfortunately, many of these types of natural repellents are irritating to the skin, have unacceptable odors, or generally provide a limited duration of effectiveness (Barnard, 1999). The most durable and protective repellent compound available on the market, N,N-diethyl-3-methylbenzamide (DEET), has proven effective over the past several decades of commercial use although misuse can impart undesirable side-effects (Fradin, 1998; Fradin and Day, 2002). Due to the need for repeated applications as repellents lose effectiveness over time, these do not provide sustainable approaches for the global considerations of control or rolling back of mosquito-borne diseases. Far more cost effective in reducing the incidence and transmission of malaria by adult mosquitoes are the insecticide-treated bed nets that play a prominent part of the strategies for Roll Back Malaria (Tolle, 2009). Permethrin-treated bed netting serves as a cornerstone of prevention in current strategies, luring mosquitoes to the individual sleeping under the net and killing the vector with contact insecticide. These approaches serve both the community and the individual when deployed on a broad basis. It is not common, however, to find effective products that work against all life stages, probably due to the high degree of separation in the life cycle between the mobile, nectar- and blood-feeding adults and the aquatic, filter-feeding larvae.

Larval

Focusing mosquito reduction efforts on the larval stage has the advantage of controlling the vector prior to dispersal or acquisition of the disease and interrupting the life cycle before it can cause harm. Although new synthetic chemicals have not yet impacted the market, there are a number of chemicals available to target mosquito larvae, including organophophates such as temephos and insect growth regulators (IGRs) like methoprene (Rose, 2001), although resistance has been found to each of these in the field (Dame et al., 1998; Raymond et al., 2001).

Biological control of mosquito larvae has been managed through the use of vertebrate predators in the example of the mosquito fish, *Gambusia affinis*, which have met with a range of results. There have been studies indicating both positive and negative results from mosquito fish, with intraguild predation occurring as the exotic fish consume native aquatic predators of the mosquito and thereby fail to achieve desired control (Bence, 1988). Other biological control methods are reviewed in Legner (1995), although some recent work on invertebrate predatory beetles (Culler and Lamp, 2009) has focused on utilizing conservation biological control efforts that enhance the impacts of native species. Use of bacterial toxins in the form of *Bacillus sphaericus* and *Bacillus thuringiensis* var. *israelensis* have also proved efficacious against larvae (Fillinger et al., 2003), while relatively non-toxic to the surrounding environment.

NATURAL PRODUCTS WITH NOTED LARVICIDAL ACTIVITY

Neem

Extracts from the seed oil of the neem tree *Azadiracta indica*, A. Juss. have a number of biologically active compounds that have been shown to be effective against mosquitoes as well as other insects. Neem oil has a known function as an antifeedant, preventing feeding activities via chemoreception likely due to volatile, organic components (Schmutterer, 1990) and also as a disruptor of endocrine function, acting as an insect growth regulator and thereby causing developmental aberrations (Mordue (Luntz) and Blackwell, 1993). The active compound azadirachtin A is a triterpenoid (Ley et al., 1993), although synergistic effects of other compounds should not be overlooked. Extraction of azadirachtin for the purposes of an insecticide typically utilize the seed kernel, although it is often necessary to blend the product with additional emulsifiers to formulate for dispersal in water. Neem oil formulations combined with polyoxyethylene ether, sorbitan dioleate and epichlorohydran were shown to be effective against 3rd and 4th stage larvae in field studies undertaken in India (Dua et al., 2009). Reports on neem usage in Central Northern Nigeria have indicated the utility of this natural product against a number of pests, including weevils, scale insects and root disease agents (Salako et al., 2008). Non-target effects against aquatic invertebrates such as mayflies have been reported to be minimal for short terms exposures at the defined environmental exposure concentrations used for evaluation of forest insect pests (Kreutzweiser, 1997).

Efficient production methods have presented a challenge to the widespread adoption of neem, however there exists evidence that low cost, local production methods might be feasible for larval control in villages (Gianotti et al., 2008). Ultimately, the development of neem extracts against mosquito vectors of human disease will depend on the simplification of current technological constraints that address the issues of production and distribution of this compound (Isman, 1997).

Essential oils and other plant extracts

The use of plant extracts to control parasitic infections has been a strong interest of researchers around the globe, carrying the potential for development of alternative control strategies. In selecting plants to test as agents against mosquitoes, it may be useful to include endogenous knowledge about plant resources. Including plants which have been used as traditional medicines allows researchers to incorporate the observations of a wider variety of experiences. While plant selection should draw from a broader inventory, standard testing regimens for potential insecticides should be utilized by researchers in order to produce results that facilitate comparative analyses (CTD/WHO PES/IC, 1996; WHO CDS/ WHO PES, 2005). It is important that these efforts include careful analysis of the solvents used in the extraction or re-suspension process to ensure that adequate controls exist for comparison between the active compounds and the solvent (Zahir et al., 2009). Utilizing endogenous knowledge concerning plants with traditional medicinal value has proven fruitful in identifying potential sources of phyto-extracts with insecticidal activity (Rahuman et al., 2009b), There have been many attempts to assay the activity of particular plant extracts against vectors of human disease, in particular through the utilization of plants for which such knowledge exists (Mathew et al., 2009). Recent studies using these approaches have yielded some promising plants with larvicidal activity (Table 1). Essential oils that have indicated adult repellency have also been shown to function as larvicides, indicating a cross-functional potential for utilizing these natural products (Zhu et al., 2008) (Table 2).

Products have been created from combinations of various plant extracts including aromatic extracts, edible oils and other essential oils that have shown some viability as a larvicide against *Culex* mosquitoes, e.g. Akse Bio2 (Cetin et al., 2004). When specific solvents are revealed through preliminary screenings to extract biologically active compounds, it is important to recognize that complex mixtures of plant extracts may provide the activity observed (Elango et al., 2009). These screening studies can provide new resources for more complete examinations, once the appropriate sources and solvent extraction techniques have been identified (Yang et al., 2004).

The diversity of plant resources that exists in the tropics indicates the need to continue the search for effective control agents derived from natural products. The cross-species interactions that occur in these regions promote diversity (Dobzhansky, 1950) that may be useful to harness in controlling diseases that now persist primarily in those same regions. This is not to say that the solutions would necessarily exist contemporaneously to the problems, indeed certain natural reservoirs may be continents away from the diseases they might help control. It is the potential for biotic interactions to exceed abiotic pressures in directing the evolution of specific biochemical pathways and products that might be useful when harnessed against disease carrying pests.

EVOLUTION OF RESISTANCE

The finding of field populations exhibiting high levels of resistance to pyrethroid, carbamate and organophosphorus insecticides creates concern for the sustainability of current control practices and threatens the attempts to further increase vector control. The consequences of mosquito resistance to pesticides can include resurgence

Table 1. A review of natural products recently evaluated for activity in controlling mosquito larvae.

Natural product examined	Preparation source, solvent	Active amount for LC_{50} ± SE (ppm)	Larval species tested	Reference source
Achyranthes aspera L. (Amarantaceae)	Leaf extract, ethyl acetate	48.83 ± 3.43	*Aedes subpictus*	(Zahir et al., 2009)
Anisomeles malabarica (L.) Sims. (Lamiaceae)	Leaf extract, chloroform	135.36 ± 8.77	*A. subpictus*	(Zahir et al., 2009)
Gloriosa superba L. (Liliaceae)	Flower extract, methanol	106.77 ± 7.30	*A. subpictus*	(Zahir et al., 2009)
Ricinus communis L. (Euphorbiaceae)	Leaf extract, methanol	102.71 ± 7.27	*A. subpictus*	(Zahir et al., 2009)
Aegle marmelos (Linn.) Correa ex Roxb (Rutaceae)	Leaf extract, ethyl acetate	167 ± 10.39	*A. subpictus*	(Elango et al., 2009)
Andrographis paniculata (Burm.f.) Wall ex. Nees. (Acanthaceae)	Leaf extract, hexane	67.24 ± 5.13	*A. subpictus*	(Elango et al., 2009)
Cocculus hirsutus (L.) Diels (Menispermaceae)	Leaf extract, methanol	142.83 ± 10.96	*A. subpictus*	(Elango et al., 2009)
Eclipta prostrata L. (Asteraceae)	Leaf extract, ethyl acetate	78.28 ± 5.49	*A. subpictus*	(Elango et al., 2009)
Clitoria ternatea L. (Fabaceae)	Seed extract, methanol	116.8	*Anopheles stephensi*	(Mathew et al., 2009)
C. ternatea	seed extract, methanol	148.2	*Culex quinquefaciatus*	(Mathew et al., 2009)
C. ternatea	Seed extract, methanol	195.0	*Aedes aegypti*	(Mathew et al., 2009)
Cedrus deodara Roxb. Loud (Pinaceae)	Stem bark, methanol	95.19 ± 8.25	*C. quinquefaciatus*	(Rahuman et al., 2009b)
N. tabacum L. Solanaceae	Leaf extract, hot water	76.27 ± 5.24	*C. quinquefaciatus*	(Rahuman et al., 2009b)
Canna indica L. (Cannaceae)	Leaf extract, methanol	69.76 ± 4.91	*C. quinquefaciatus*	(Rahuman et al., 2009a)
Ipomea carnea Jacq. spp. *Fistulosa* Choisy (Convolvulaceae)	Leaf extract, hot water	41.07	*C. quinquefaciatus*	(Rahuman et al., 2009a)

of disease in areas where success had been achieved (Pimentel et al., 1992) and serve to highlight the concern for finding alternative control technologies. Results that indicate the delay of resistance evolution when compounds are mixed as compared to single, purified compounds (Feng and Isman, 1995), shows that natural products might be favorable to reducing resistance evolution. Metabolic mediation of insecticide effects has been known at the cellular level from a number of studies. One of the earliest types of known resistance development was shown in resistance to organo-phosphates that had once been effective in mosquito control, via acetylcholinesterase insensitivity (Takahashi and Yasutomi, 1987). Cytochrome P450-dependent monoxygenases have been implicated as a mechanism of resistance to pyrethroids, with measurements showing over-expression of certain of these genes in highly resistant field populations (Müller et al., 2008). This indicates that more caution is warranted in the widespread application of synthetic pesticides. Although there are indications that mutations in resistance genes are generally rare within populations, there are concerns about the extent to which these genes can be spread into new populations as well as the selection pressures exerted by overuse of pesticides (Pasteur and Raymond, 1996).

In addition to risks of pesticide resistance, overuse of insecticides can introduce both acute and chronic health risks to applicators, alter the composition of native predators and impair the productivity of agriculture (Pimentel et al., 1992). Anti-mosquito campaigns in history have had success in altering watershed characteristics, including draining swamps, modification of river boundaries and vegetation clearing, but there are other environmental costs to the freshwater ecosystem associated with these types of alterations to the landscape. Given the importance of the global productivity of freshwater ecosystems, estimated at over $6 trillion/year (Dudgeon et al., 2006), appropriate consideration to both positive and negative impacts of such approaches should be considered. For these reasons it would be beneficial to explore the potential variety of natural and synthetic control technologies that can be utilized in reducing vector prevalence with a mind to limiting the impacts of disease.

RESISTANCE BY THE PARASITE TO DRUGS

Emergence of multi-drug resistance by the *Plasmodium* parasite has prompted demand for alternative treatment methods using artemisinin-

Table 2. A summary of essential oils recently evaluated for activity in controlling mosquito larvae.

Essential oil source in plant	Larvicidal activity for LT_{50} @ 200 ppm (h)	Larval species tested	Reference
Curcuma longa L. (Zingiberaceae)	6.32 ± 0.75 9.28 ± 2.93 0.90 ± 0.02	*Aedes aegypti* *Aedes albopictus* *Culex pipiens*	(Zhu et al., 2008)
Eucalyptus citriodora Hook. (Myrtaceae)	1.12 ± 0.39 0.85 ± 0.39 0.12 ± 0.04	*A. aegypti* *A. albopictus* *C. pipiens*	(Zhu et al., 2008)
Santalum album L. (Santalaceae)	1.06 ± 0.11 1.82 ± 0.06 1.55 ± 0.07	*A. aegypti* *A. albopictus* *C. pipiens*	(Zhu et al., 2008)
Cinnamomum cassia L. (Lauraceae)	1.09 ± 0.11 11.04 ± 3.28 3.12 ± 0.18	*A. aegypti* *A. albopictus* *C. pipiens*	(Zhu et al., 2008)

based drugs. Manufactured from the annual wormwood (*Artemisia annua* L.), or also semi-synthetically derived, this class of drugs has been highly efficacious against drug-resistant malaria parasites (Brisibe et al., 2008). Natural products may also provide novel methods to control the parasite within the human body, including through dietary strategies that alter the interaction of malaria with hemoglobin (Jackson, 1990).

CONCLUSIONS

The use of natural product chemistry that reduces mosquito populations at the larval stage can provide many associated benefits to vector control. The selection of natural products that limit the environmental impacts of pesticides due to shorter latency may be beneficial in preventing the evolution of resistance. In addition to addressing the needs for treatment of water near human habitation, natural products utilized as mosquito insecticides may provide a useful tool to promote localized control of persistent vector-borne diseases.

REFERENCES

Barnard DR (1999). Repellency of essential oils to mosquitoes (Diptera: Culicidae). J. Med. Entomol. 36: 625-629.

Bence JR (1988). Indirect Effects and Biological Control of Mosquitoes by Mosquitofish. J. Appl. Ecol. 25: 505-521.

Brisibe EA, Uyoh EA, Brisibi F, Pedro MM, Ferreira JFS (2008). Building a golden triangle for the production and use of artemisinin derivatives against falciparum malaria in Africa. Afr. J. Biotechnol. 7: 4884-4896.

Cetin H, Erler F, Yanikoglu A (2004) Larvicidal activity of a botanical natural product, AkseBio2, against *Culex pipiens*. Fitoterapia, 75: 724-728.

Chretien J, Anyamba A, Bedno SL, Breiman RF, Sang R, Sergon K, Powers AM, Onyango C, Small J, Tucker C, Linthicum K (2007) Drought-associated chikungunya emergence along coastal East Africa. Am. J. Trop. Med. Hyg. 76: 405-407.

CTD/WHO PES/IC (1996). Report of the WHO Informal Consultation on the evaluation and testing of insecticides.

Culler LE, Lamp WO (2009). Selective predation by larval Agabus (Coleoptera: Dytiscidae) on mosquitoes: support for conservation-based mosquito suppression in constructed wetlands. Freshwater Biol. 54: 2003-2014.

Dame D, Wichterman G, Hornby J (1998). Mosquito (*Aedes taeniorhynchus*) resistance to methoprene in an isolated habitat. J. Am. Mosquito Control Assoc. 14: 200-203.

Dobzhansky T (1950). Evolution in the Tropics. Am. Scientist, 38: 209-221.

Dua V, Pandey A, Raghavendra K, Gupta A, Sharma T, Dash A (2009) Larvicidal activity of neem oil (*Azadirachta indica*) formulation against mosquitoes. Malaria J. 8(124).

Dudgeon D, Arthington A, Gessner M, Kawabata Z, Knowler D, Leveque C, Naiman R, Prieur-Richard A, Soto D, Stiassny M, Sullivan C (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. 81: 163-182.

Elango G, Rahuman A, Bagavan A, Kamaraj C, Zahir A, Venkatesan C (2009) Laboratory study on larvicidal activity of indigenous plant extracts against *Anopheles subpictus* and *Culex tritaeniorhynchus*. Parasitol. Res. 104: 1381-1388.

Feng R, Isman MB (1995). Selection for resistance to azadirachtin in the green peach aphid,Myzus persicae. Cellular and Molecular Life Sci. 51: 831-833.

Fillinger U, Knols BGJ, Becker N (2003). Efficacy and efficiency of new *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* formulations against Afrotropical anophelines in Western Kenya. Trop. Med. Int. Health, 8: 37-47.

Fradin MS, Day JF (2002). Comparative Efficacy of Insect Repellents against Mosquito Bites. N Engl. J. Med. 347: 13-18.

Fradin M (1998). Mosquitoes and mosquito repellents: A clinician's guide. Ann. Internal Med. 128: 931-940.

Gianotti R, Bomblies A, Dafalla M, Issa-Arzika I, Duchemin J, Eltahir E (2008). Efficacy of local neem extracts for sustainable malaria vector control in an African village. Malaria J. 7(138).

Greenwood BM, Bojang K, Whitty CJ, Targett GA (2005). Malaria. Lancet, 365: 1487-1498.

Gubler DJ (1998). Dengue and Dengue Hemorrhagic Fever. Clin. Microbiol. Rev. 11: 480-496.

Isman M (1997). Neem and other Botanical insecticides: Barriers to commercialization. Phytoparasitica, 25: 339-344.

Jackson FLC (1990). Two evolutionary models for the interactions of dietary organic cyanogens, hemoglobins, and falciparum malaria. Am. J. Hum. Biol. 2: 521-532

Kreutzweiser DP (1997). Nontarget Effects of Neem-Based Insecticides on Aquatic Invertebrates. Ecotoxicol. Environ. Saf. 36: 109-117.

Ley SV, Denholm AA, Wood A (1993). The chemistry of azadirachtin.

Nat. Prod. Rep. 10: 109-157.
Mackenzie JS, Gubler DJ, Petersen LR (2004). Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. Nat. Med. 10: S98-S109.
Mathew N, Anitha M, Bala T, Sivakumar S, Narmadha R, Kalyanasundaram M (2009). Larvicidal activity of *Saraca indica*, Nyctanthes arbor-tristis, and *Clitoria ternatea* extracts against three mosquito vector species. Parasitol. Res. 104: 1017-1025.
Michael E, Bundy DA, Grenfell BT (1996). Re-assessing the global prevalence and distribution of lymphatic filariasis. Parasitology, 112(Pt 4): 409-428.
Mordue (Luntz) A, Blackwell A (1993). Azadirachtin: an update. J. Insect Physiol. 39: 903-924.
Müller P, Warr E, Stevenson BJ, Pignatelli PM, Morgan JC, Steven A, Yawson AE, Mitchell SN, Ranson H, Hemingway J, Paine MJI, Donnelly MJ (2008). Field-Caught Permethrin-Resistant *Anopheles gambiae* Overexpress CYP6P3, a P450 That Metabolises Pyrethroids. PLoS Genet. 4: e1000286
Pasteur N, Raymond M (1996). Insecticide resistance genes in mosquitoes: Their mutations, migration, and selection in field populations. J. Hered. 87: 444-449.
Pimentel D, Acquay H, Biltonen M, Rice P, Silva M, Nelson J, Lipner V, Giordano S, Horowitz A, D'Amore M (1992). Environmental and Economic Costs of Pesticide Use. BioScience, 42: 750-760.
Rahuman A, Bagavan A, Kamaraj C, Saravanan E, Zahir A, Elango G (2009a) Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitology Research 104:1365-1372.
Rahuman A, Bagavan A, Kamaraj C, Vadivelu M, Zahir A, Elango G, Pandiyan G (2009b). Evaluation of indigenous plant extracts against larvae of Culex quinquefasciatus Say (Diptera: Culicidae). Parasitol. Res. 104: 637-643
Raymond M, Berticat C, Weill M, Pasteur N, Chevillon C (2001). Insecticide resistance in the mosquito *Culex pipiens*: what have we learned about adaptation? Genetica 112: 287-296.
Rose RI (2001). Pesticides and public health: Integrated methods of mosquito management. Emerging Infect. Dis. 7: 17-23.
Sachs J, Malaney P (2002). The economic and social burden of malaria. Nature, 415: 680-685
Salako EA, Anjorin ST, Garba, CD, Omolohunnu EB (2008). A review of neem biopesticide utilization and challenges in Central Northern Nigeria. Afr. J. Biotechnol. 7: 4758-4764.
Schmutterer H (1990). Properties and Potential of Natural Pesticides from the Neem Tree, *Azadirachta Indica*. Annu. Rev. Entomol. 35: 271-297.
Shaalan EA, Canyon D, Younes MWF, Abdel-Wahab H, Mansour A (2005) A review of botanical phytochemicals with mosquitocidal potential. Environ. Int. 31: 1149-1166.
Takahashi M, Yasutomi K (1987). Insecticidal resistance of *Culex tritaeniorhynchus* (Diptera: Culicidae) in Japan: genetics and mechanisms of resistance to organophosphorus insecticides. J. Med. Entomol. 24: 595-603.
Tolle MA (2009). Mosquito-borne Diseases. Current Problems in Pediatric and Adolescent Health Care, 39: 97-140.
Utzinger J, Tozan Y, Singer BH (2001). Efficacy and cost-effectiveness of environmental management for malaria control. Trop. Med. Int. Health, 6: 677-687.
WHO CDS/WHO PES (2005). Guidelines for laboratory and field testing of mosquito larvicides.
Yang Y, Park I, Kim E, Lee H, Ahn Y (2004). Larvicidal Activity of Medicinal Plant Extracts Against *Aedes aegypti*, Ochlerotatus togoi, and *Culex pipiens* pallens (Diptera: Culicidae). J. Asia-Pacific Entomol. 7: 227-232.
Zahir A, Rahuman A, Kamaraj C, Bagavan A, Elango G, Sangaran A, Kumar B (2009) Laboratory determination of efficacy of indigenous plant extracts for parasites control. Parasitol. Res. 105: 453-461.
Zhu J, Zeng X, O'Neal M, Schultz G, Tucker B, Coats J, Bartholomay L, Xue R (2008). Mosquito larvicidal activity of botanical-based mosquito repellents. J. Am. Mosquito Control Assoc. 24: 161-168.