International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2016; 3(6): 20-25

Received: 04-09-2016
Accepted: 05-10-2016

Olorunfemi O Sunday
Department of Biology
Federal University of Technology, Akure, Ondo State, Nigeria

Akinwande Kayode L
Department of Biology
Federal University of Technology, Akure, Ondo State, Nigeria

Ashamo MO
Department of Biology
Federal University of Technology, Akure, Ondo State, Nigeria

Correspondence
Olorunfemi O Sunday
Department of Biology
Federal University of Technology, Akure, Ondo State, Nigeria

Laboratory review of sublethal effects of cypermethrin on oviposition, life span and egg development in *Culex quinquefasciatus*, Say (Diptera: Culicidae)

Olorunfemi O Sunday, Akinwande Kayode L and Ashamo MO

Abstract
Mosquitoes constitute the largest human disease vectors, which spend their adult life indoor and outdoor while the juvenile stages live in aquatic environment. This research reviews the sublethal effects of Cypermethrin on biology of *Culex quinquefasciatus* Say. Insects that survive lethal concentrations have been impacted with a sublethal dose which might not produce immediate mortality but have a prolonged effect on the biology and body biochemistry. Laboratory analyses was carried out with LD_{10}, LD_{20} and LD_{30} of cypermethrin at a temperature of 27-29 °C, relative humidity of 70-85% and photoperiod of 14:10 (L: D) and acetone was used as a chemical control. The mosquitoes were caged and provided with 10% sucrose syrup and blood meal. The percentage of adult emergence from the 4th instar larval of the mosquito were significantly different for all the sublethal concentrations and control ($F(3, 19) = 167.568$, $p = 0.000$). Mean rate of oviposition decreases from the Control = (107.20±3.02), LD_{10} = (104.40±2.82), LD_{20} = (93.67±2.69) and to LD_{30} = (87.00±2.19). The mean life span by the adult female decreases from 12.44+0.193 in the control to 7.20±0.109 at LD_{20} and the decrease were significantly different ($F(3, 19) = 226.149$, $p = 0.000$). In view of the environmental modifications that have impacted on our ecosystems and insect populations, there is a need to constantly review the lethal and sublethal effects of many insecticides as this will help to determine the dose response-status of insect vectors for prioritization and design of chemicals for control programmes.

Keywords: *Culex quinquefasciatus,* lethal, sublethal, oviposition, larva, adult, cypermethrin

1. Introduction
Mosquitoes are found in all continents of the World except in the Antarctica while approximately three-fourths of all the species inhabit the tropics and subtropics [1]. It is one of the most medically important insect species [2] which are vectors of disease pathogens such as malaria, filariasis, dengue fever and yellow fever, the diseases it transmits cause over hundred millions of clinical cases and millions of deaths annually [3] with subsequent reduction in economic growth in different region of the world [4]. *Aedes aegypti* is the vector of dengue fever and yellow fever, *Anopheles* species serves as the vector of malaria which is the leading cause of human mortality in developing countries [5]. *Culex* spp. particularly, *Culex quinquefasciatus* are vectors of urban and lymphatic filariasis which infects over 100 million people worldwide [5].

A lot of capital resources and human efforts have been channelled towards the reduction in the incidence and prevalence of the diseases transmitted by mosquitoes. Control efforts are programmed towards both adult and larval stages using several techniques: biological, chemical, cultural and integrated approach [6]. Among the conventional chemicals used for the control of mosquito's vectors, organophosphates and pyrethroids have been the most favourite mosquito control method worldwide. However, the indiscriminate use of the insecticides in domestic environments, as well as the specific indoor treatments has led to the development of resistance in the mosquito populations, impacting negative on the environment, human health and non- target organisms [7, 8].

Pyrethroids are among the most successful classes of insecticides. These insecticides are neurotoxic and are very similar in structure to the pyrethrins which are synthetic analogs of natural insecticides (pyrethrins) produced from *Chrysanthemum cinerariafolium* [4]. These chemicals are used to control mosquitoes and other medically important insect species in the

80's [9] and up till today, pyrethroids still emerged as a major class of highly active insecticides due to their high bio-efficacy and relatively low toxicity in comparison to organochlorine and organophosphate pesticides which have acute toxicity to mammals [10].

Frequent use of insecticides for control of insect vectors and pests induces various biological and physiological stresses on the insects systems and populations. We can classify these effects into chronic effects (resulting in high mortality), acute effect (less toxic with less mortality) and sub lethal effects. (Prolong toxic effect and with no immediate mortality). Sublethal effect is described as biological and behavioural changes characterised by the surviving insects following contact with a sub lethal dose of an insecticide [11], the chance of any insect receiving a lethal exposure from an insecticide deposit is a result of dynamic interactions between abiotic and biotic factors [12, 13]. The insects may contact the insecticide deposit only at sub lethal levels which may not kill them but alter their populations in different ways through changes in survivorship, reproductive capability and the genetic constitution of successive generations [11]. Sub lethal effects are expressed as physiological or behavioural changes of the insects that survive an exposure to a pesticide [14].

Daily application of pesticides and or insecticides in the control of agricultural pests and medical insect vectors is one of the precursors of environmental modifications taking place globally. This factor alone has created a lot of environmental stress which has impacted on the ecosystems and the fauna; insects are mostly impacted, through changes in its population, its biology, biochemistry and behaviour. Therefore, insects remain the major bio-indicators of these changes impacted by insecticides or pesticides responsible for environmental modifications. This study reviews the sub lethal effects of Cypermethrin on some key biology of *Culex* mosquito in order to update our knowledge on the current dose-response status of insect vectors and reassess the prioritization and design of the chemical for control programmes.

2. Methodology

Culex mosquitoes were reared under laboratory conditions by mass collection of eggs of the species in the field using water and yeast as bait attractants to adults that lay eggs. The eggs laid were transferred into a jar containing water with hay infusion and algae to aid its hatching and development into larvae, pupa and adults. The adults that emerged were kept in netted cages 35cm by 35cm by 35cm^3 at a temperature of 27-29 °C and relative humidity of 70-85%, and photoperiod of 14:10 (L: D). 10% sucrose syrup was provided daily as source of carbohydrate for the adults and in addition, blood meal was provided for the females from fur shaved white albino rats kept in the cage and human arms intruded for 10mins daily in the cage for the mosquitoes to suck blood. The experiment was carried from January to December, 2015, mosquito species used for the bioassays were identified as *Culex quinquefasciatus* by the Entomology Research Laboratory, Federal University of Technology, Akure.

2.1 Test chemicals

Commercial formulation of a Pyrethroid (Cypertex 10% EC), active ingredients containing Cypermethrin (Cypermethrin [CYM, (±) -cyano-3- phenoxybenzyl (±) cis, Trans-3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclop-ropanecarboxylate was used for the larval and adult bioassays. The insecticide is a contact poison, more resistant to degradation by light and air; and with low mammalian toxicity [15]. It was obtained on the counter from an agrochemical retailer shop in Akure, Ondo-State, Nigeria.

2.2 Preparation of dosages

A 1ppm stock solution of the commercial formulated products of the chemical (Cypertex 10% EC) was prepared using distilled water as solvent. For larval bioassay, serially diluted diagnostic concentrations of 0.1, 0.05, 0.025, 0.0125 and 0.00625 ppm were prepared through addition of aliquots of the serially diluted stock solution to water in measuring flask. The resulting diagnostic assay solutions in 1000 ml volume jars produced were stored at room temperature and later used for the bioassay. Similarly, for the adult bioassay, diagnostic concentrations of 25, 12.5, 6.25, 3.125, 1.563 and 0.781 ppm were also prepared through serial dilutions. For the adult bioassay, we impregnated the solutions on filter papers following WHO (1986) [16] protocol, Whatman filter papers of 4cm by 4cm size and area of 16cm^2 were treated with 1ml of each of the pre-determined doses for the adult bioassays. The filter papers were allowed dried for a day and wrapped in aluminium foils, stored at -20 ºC and later used. A control experiment was set up for both larval and adult bioassays using acetone, 1000ppm of stock solution of acetone was serially diluted to concentrations prepared in ppm for cypermethrin for both larva and adult assays. All the experiments were replicated four times. The diagnostic concentrations values used were predetermined from the dose instructions on the product label.

2.3 Determination of sublethal dosages

The mortality data obtained from larval and adult diagnostic bioassays were used to fix $LC_{10,}$ LC_{20} and LC_{30} of Cypermethrin from the extrapolations made on the probit and logit to obtain the log dose values for lethal treatments that recorded mortality of 10, 20 and 30%, the LC_{10}, LC_{20} and LC_{30} concentrations were used to assess the sublethal effects on the mosquito biology.

2.4 Test of sublethal dosage on adult emergence [16]

Twenty L_3 – L_4 of second filial generations larvae were released into each replicates of $LC_{10,}$ LC_{20} and LC_{30} dose concentrations of solutions prepared. The experiments were held at room temperature and humidity, the rate of growth and development of larvae was monitored till adults emergence and percentage emergence calculated [16].

2.5 Test of sublethal dosages on oviposition and egg development [17]

Twenty gravid females mosquitoes each were selected and exposed to each replicates treatments for $LC_{10,}$ LC_{20} and LC_{30} of Cypermethrin fixed from the probit and logit dose values in the primary bioassays. These treated gravid females in each dose replicates were returned to the breeding cages, fed with 10% sucrose syrup, supplied with jar of water and filter paper dipped in yeast, folded to form a cone to act as ovitrap. Eggs laid on the filter paper were counted under a dissecting microscope (S6 – BLED Stereo Zoom Dissecting Microscope, Swift MW). The eggs were transferred to the hatching trays

appropriately labelled; number of eggs that hatch to larvae were recorded as hatching rate. (Hatching rate (%) = number of eggs hatched / number of eggs that were laid.

2.6 Test of Sublethal dosages on life span of adult female mosquitoes [17]

The adult female mosquitoes both exposed and not exposed to the sub lethal doses of $LC_{10,}$ LC_{20} and LC_{30} of Cypermethrin were used to assess life span which is the mean number of days lived from adult emergence until mortality.

2.7 Data analysis

Data obtained from larvae and adult mortalities in the experiment were subjected to probit and logit analyses to fix sub-lethal doses. The means oviposition rate (OV), larva eclosion to adults, egg development and life span were compared in all treatments with control using Analysis of variance (ANOVA) by Duncan's multiple range test ($P < 0.05$). The statistical analyses were run using computer version software SPPS version 21.0.

3. Results

3.1 Determination of sublethal concentrations of cypermethrin on

3.10 4th Instar Larvae of *Culex* mosquitoes

The dose-response relationship of the 4th instar larvae of *Culex* mosquitoes to Cypermethrin at concentrations of 0.1, 0.05, 0.025, 0.0125 and 0.00625 ppm was estimated in percentage after 24hours. The straight line fitted by Probit analysis for the percentage mortality observed for each concentration of Cypermethrin tested had a linear equation y= 3.69 + 0.94*x. Extrapolations from the line of fits gave the sublethal concentrations values (LC_{10}, LC_{20}, and LC_{30}) which were estimated as 0.00318, 0.00798 and 0.0131ppm respectively (Table 1).

3.11 Adults *Culex* mosquitoes

Also, the dose response relationship of the adult of *Culex* mosquitoes to Cypermethrin at concentrations of 25, 12.5, 6.25, 3.125, 1.563 and 0.781ppm was estimated after 1hour. The straight line fitted by Probit analysis for the percentage mortality observed for each concentration of Cypermethrin tested had a linear equation y= -1.35+ 0.66*X. Extrapolations from the line of fits gave the sublethal concentrations values (LC_{10}, LC_{20}, and LC_{30}) which were estimated as 0.0944, 2.660 and 4.485ppm respectively (Table 2).

Table 1: Sublethal concentrations of cypermethrin on 4th instar larvae of *Culex* mosquitoes.

Sublethal concentration (ppm)	concentration (ppm)	Confidence limit at 95% Upper Bound – Lower Bound
LC_{10}	0.005	0.003- 0.007
LC_{20}	0.008	0.006-0.011
LC_{30}	0.012	0.09-0.017

Table 2: Sublethal concentrations of cypermethrin on adult *Culex* mosquitoes

Sublethal concentration (ppm)	Concentration (ppm	Confidence limit at 95% (Upper bound-Lower bound)
LC_{10}	1.110	(0.637–1.579)
LC_{20}	1.521	(1.521– 2.835)
LC_{30}	2.713	(2.713–4.544)

3.2 Effect of sublethal concentrations of Cypermethrin on 3.20 Adult Emergence

The percentage of adults that emerged from 4th instar larvae after exposure to sub lethal doses (LC_{10}, LC_{20}, and LC_{30}) of Cypermethrin test solutions were as shown in Figure 1. The percentages of adult emergence show significant decline from 96.00%±1.00 in the control to 90.00%±2.73, 53.00%±2.54, 39.00%±1.87 at LC_{10}, LC_{20} and LC30 respectively. The percentage adult emergence from the 4th instar larval of the Culex mosquito were significantly different for all the sublethal concentrations and control (F (3, 19) = 167.568, p = 0.000).

3.21 Oviposition and egg development

The oviposition and hatching of eggs in the *Culex* mosquitoes after treatment with sublethal doses (LC_{10}, LC_{20}, and LC_{30}) of Cypermethrin test solutions is as presented in figure 2. Number of eggs oviposited in the mosquitoes when exposed to the sublethal doses and the control were significantly different (F (3, 19) = 12.160, p = 0.000). The number of eggs oviposited in the Control = (107.20±3.02), LD_{10} = (104.40±2.82), LD_{20} = (93.67±2.69) and LD_{30} = (87.00±2.19). Similarly, the number of eggs hatched in the *Culex* mosquito exposed to sub-lethal concentrations of cypermethrin were significantly different from each other and from the control (F (3, 19) = 13.200, p = 0.000). It decreases significantly from the control to LD_{30} (Figure 2).

3.22 Life span of adult female *Culex* mosquitoes

There was a decrease in the life span of the adult female *Culex* mosquito after exposure to the sublethal doses (LC_{10}, LC_{20}, and LC_{30}) of cypermethrin compared to the acetone control. The number of days lived by adult female decreases from 12.44±0.193 in the control to 7.20±0.109 at LD_{20} (Figure 3). The decrease were significantly different (F (3, 19) = 226.149, p = 0.000).

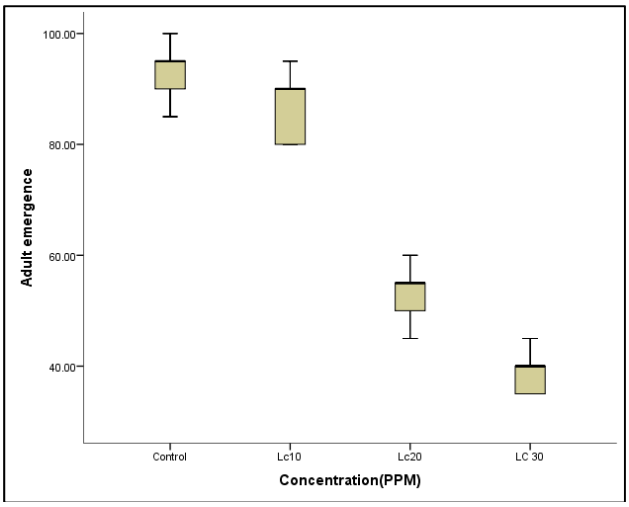

Fig 1: Number of adult emergence from 4th instar larval of *Culex* mosquitoes exposed to sublethal concentrations (in ppm) of cypermethrin

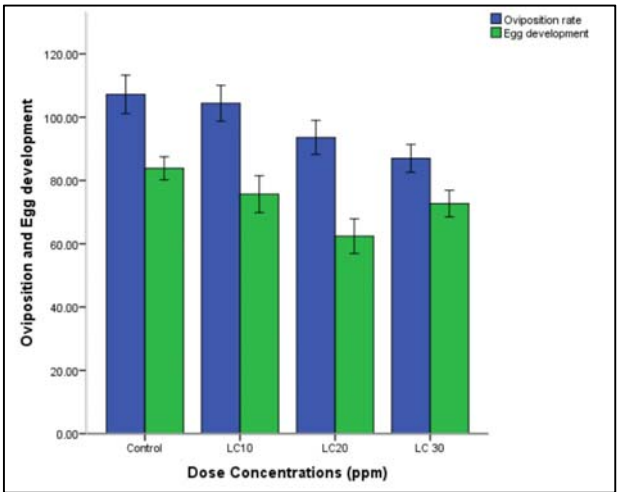

Fig 2: Number of eggs oviposited and developed to larva in *Culex* mosquitoes exposed to sublethal concentrations (in ppm) of cypermethrin

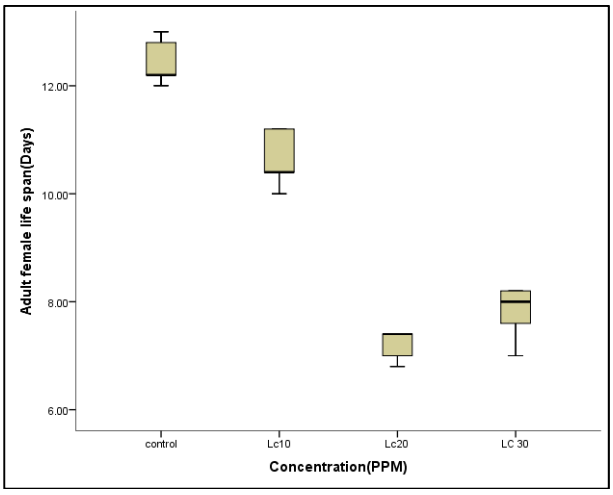

Fig 3: Life span (in days) of adult female *Culex* mosquitoes exposed to sublethal concentrations (in ppm) of cypermethrin

4. Discussion

In addition to direct mortality induced on mosquitoes when in contact with insecticides, those that survived the effect experience changes in their biology and biochemistry [14]. These changes are often more associated with pyrethroids insecticides, because it has a slower degradation and thus produce a higher degree of sublethal effects and resistance [18] other than immediate mortality.

The results obtained from both the larval and adult bioassays showed the two stages of *Culex* mosquitoes are susceptible to cypermethrin. Helson and Surgeoner [19] made a similar observation; that cypermethrin was highly toxic to mosquito larvae and pupae. The implications on any population of larvae and adults *Culex* mosquitoes species that survived a lethal concentration is an inducement with a sublethal dosage which can have a prolonged impact on its biological and biochemical parameters. *Aedes aegypti* and *Culex quinquefasciatus* have been found to be less susceptible to dichlovos but more susceptible to cypermethrin and dieldrin [20, 21]. The differences in the susceptibility of the larvae and adults could be attributed to intoxication which occurs at different levels of pharmacokinetic interactions, thus; penetration of barrier tissue, distribution, storage, metabolism in internal tissue, and molecular interaction with the target site [5, 22].

Sub lethal doses of Cypermethrin produce varying degree of changes in *Culex* species such as changes in the rate of adult emergence, life span, oviposition and number of eggs hatched. The decrease in percentage adult emergence on increase in sublethal dose agreed with observation made by Mark *et al*. [23] that dose response in *Culex* mosquitoes was characterized with decrease in percentage adult emergence with increase in concentration of sub lethal doses. This observation may probably be as a result of inability of the larvae to assimilate food, damage to the body wall and larval tissues [23]. The damage to the body wall of *Culex quinquefasciatus* resulted in decrease in percentage of emerging adults [24].

The confirmation from this study, that females exposed to sublethal doses of Cypermethrin lay small number of eggs agreed with observations made by Reyes-Villanueva *et al* [25], that different classes of insecticides cause a significant reduction in the number of eggs laid by insects on exposure, he made this statement from observation of exposure of *Aedes aegypti* to sub lethal concentrations/doses of Abate. The reduction observed in the number of eggs could indicate some adverse effects on mate locating instinct, courtship and associated physiological events such as spermatogenesis and sperm motility [26]. Similarly, the reduction in the mean egg production could be attributed to the reduced life span leading to early death of the treated female mosquitoes during or before commencement of oviposition. The decrease in the number of egg production with increasing concentration of the sub lethal doses would definitely have an effect on lifetime fecundity, which implies that sub lethal exposure to cypermethrin, will greatly; influence the populations of many filial generations. This is an added advantage in insecticidal control programmes that is targeted towards reducing the chemical application dosage for optimal result.

Contrary to our findings that life span of the *Culex* mosquito's decreases with exposure to sublethal dosages, Reyes-Villanueva *et al*. [25] found increased life span in adult female

Aedes aegypti mosquitoes when it was treated with temephos during larval stages. The exact cause of the significant decrease in the longevity of adult *Culex* mosquitoes due to treatment with sub lethal doses of Cypermethrin is likely to be a direct consequence of damage caused to the insect physiology by the disruption of the nervous system and associated aberrations due to abnormal hormone release and dehydration which may also occur. Determination of vectors life span is equivalent to determination of vector abundance and it is important for the life cycle in insects that serves as vectors [27]. Insect vectors like mosquitoes acquire pathogens from the infected individual through the blood meal for transmission of pathogens to an uninfected host. Mostly, the greater the life span of a particular vector, the greater it's vectorial capacity because the chance of vector-host contact is greater in long-lived vectors [27]. The significant reduction in adult life span can also be seen as an advantage, compounding the effect of the direct mortality due to the use of an insecticide, and should be taken into consideration when evaluating any control measures with pyrethroids.

5. Conclusion and Recommendations

Daily application of insecticides in the control of agricultural pests and medical insect vectors is one of the precursors of environmental changes that induce various biological and physiological stresses on the insects systems and populations. Insecticides are used indiscriminately to attain the ultimate objective of high mortality in the target insects. Whereas, cypermethrin when applied in a very low amount produces a sublethal effect with varying degree of changes in the insects that may not produce immediate mortality but gradual and prolong manifestations of disruptions in biological and biochemical parameters in the insects such as decrease in adult emergence, life span, reduction in oviposition and egg development. It is recommended that there should be regular review of toxicities of commonly used insecticides to prevail on the changes that are continuously mutating the insect populations through environmental stress. This will help in the prioritization and design of chemicals for mosquito control programmes.

6. Ethical Standard

This experiment was carried out in a controlled atmospheric laboratory conditions and there was no conflict with ethical standard.

7. References

1. Clements AN. The biology of mosquitoes: development, nutrition and reproduction. Chapman & Hall, London, UK. 1992, 1.
2. Weaver SC, Reisen WK. Present and Future Arboviral Threats. Antivirus Research. 2010; 85:328-345.
3. Snow RW, Craig M, Deichmann U, Marsh K. Estimating Mortality, Morbidity and Disability Due to Malaria among Africa's non-Pregnant Population. *B.* World Health Organ. 1999; **77:**624-640.
4. Killeen GF, Seyoum A, Knols BGJ. Rationalizing Historical Successes of Malaria Control in Africa in Terms of Mosquito Resource Availability Management. American Journal of Tropical Medicine and Hygiene. 2004; 71(2):87-93.
5. Curtis CF, Miller JE, Hodjati M, Kolaczinski JH, Kasumba I. Can anything be done to maintain the effectiveness of pyrethroid-impregnated bed nets against malaria vectors? Phil. Trans. R. Soc. London. 1998; (J5):1769-1777.
6. USEPA [Environmental Protection Agency]. Joint statement on mosquito control in the United States from the U.S. Environmental Protection Agency (EPA) and the U.S. Centers for Disease Control and Prevention (CDC): [Internet]. Available from: http://www.epa.gov/pesticides/health/mosquitoes 2007(Accessed September 20, 2016).
7. Hemingway SJ, Dunbar SJ, Monro AG. Pyrethroid resistance in German cockroaches (Dictyoptera, Blattellidae): resistance levels and underlying mechanisms. Journal of Economic Entomology. 1993; 86:1631-1638.
8. Hemingway J, Ranson H. Insecticidal resistance in insect vectors of human disease. Annual Review of Entomology. 2000; 45:371-391.
9. WHO. Vector Resistance to Pesticides. Fifteenth Report of the WHO Expert Committee on Vector Biology and Control. Techical Report. Series. 1992; 818, 1.
10. Wirtz K, Bala S, Amann A, Elbert A. A promise extended – future role of pyrethroids in agriculture. Bayer Crop Science Journal. 2009; 62(2):145-158.
11. Moriarty F. The sub lethal effects of synthetic insecticides on insects. Biology Review. 1969; 44:321-357.
12. Lee CY. Sub-lethal effects of insecticides on longevity, fecundity and behaviour of insect pests: a review. Journal of Biosciences. 2000; 11:107-112.
13. Lee CY, Yap HH, Chong NL. Sub lethal effects of deltarnethrin and propoxur on longevity and reproduction of German cockroaches, *Blattella germanica.* Entomology Experimental Application. 1998; 89:137-145.
14. Desneux N, Decourtye A, Delpuech JM. The sub lethal effects of pesticides on beneficial arthropods. Annual Review of Entomology. 2007; 52:81-106.
15. Kumar R. Insect pest control with special reference to African agriculture. Edward Arnold Publishers. 1984, 180.
16. WHO. Guidelines for laboratory and field testing of mosquito larvicides. WHO communicable disease control, prevention and eradication *WHO Pesticide Evaluation Scheme (*WHOPES). Geneva, Switzerland. 2005, 1-41.
17. Aiku AO, Yates A, Rowland M. Laboratory evaluation of pyriproxyfen treated bednets on mosquito fertility and fecundity: a preliminary study West African Journal of Medicine. 2006; 25:22-26.
18. Xu Q, Liu H, Zhang L, Liu N. Resistance in the mosquito, *Culex quinquefasciatus*, and possible mechanisms for resistance. Pest Management Science. 2005; 61(11):1096-1102.
19. Helson BV, Surgeoner GA. Efficacy of cypermethrin for the control of mosquito larvae and pupae, and impact on non-target organisms, including fish. J Am Mosquito Control Association. 1986; 2(3):269-75.
20. Campos J, Andrade Carlos FS. Larval susceptibility of *Aedes aegypti* and *Culex quinquefasciatus* populations to chemical insecticides**.** Rev. Saúde Pública. 2003;

37(4).
21. Umar A, Kela SL. Abdulrahman HT. Susceptibility of Mosquito larvae to conventional insecticides in a tropical arid ecosystem. Animal Research International. 2006; 3(1):407-409-407.
22. Narahashi T. Nature of the negative after-potential increased by the insecticide allethrin in cockroach giant axons. Journal of Cell Composition Physiology. 1962; 59:67-76.
23. Mark BH, Costas M, Christian J, Michael L, Gary D, Thompson WJ *et al*. A Review of Spinosad as a Natural Product for Larval Mosquito Control. Journal of the American Mosquito Control Association. 2010; 26(1):67-87.
24. Sharma P, Mohan L, Srivastava CN. Growth inhibitory nature of Artemisia annua extract against *Culex quinquefasciatus* Say. J Asia Pacific Entomology. 2006; 9:389-395.
25. Reyes-Villanueva F, Juarez-Eguia M, Flores-Leal A. Effects of sublethal dosages of abate upon adult fecundity and longevity of *Aedes aegypti*. Journal of the American Mosquito Control Association. 1990; 6(4):739-741.
26. Haynes KF. Sub lethal effects of neurotoxic insecticides on insect behaviour. Annual Review of Entomology. 1998; 33:149-168.
27. Evans AS, Kaslow RA. Viral Infections of humans: Epidemiology and control, 4th Edition. Plenum, NY. 1997, 300.