Journal of Agricultural Technology 2010 Vol. 6(4): 703-719
Available online http://www.ijat-rmutto.com
ISSN 1686-9141

Larvicidal effects of *Azadirachta indica*, *Ocimum gratissimum and Hyptis suaveolens* against mosquito larvae

Okigbo, R.N.[1*], Okeke, J.J.[2] and Madu, N.C.[1]

[1]Department of Botany, Nnamdi Azikiwe University, Awka,Anambra Stat, Nigeria.
[2]Department of Zoology, Nnamdi Azikiwe University, Awka, Anambra Stat, Nigeria.

Okigbo, R.N., Okeke, J.J. and Madu, N.C. (2010). Larvicidal effects of *Azadirachta indica*, *Ocimum gratissimum and Hyptis suaveolens* against mosquito larvae. Journal of Agricultural Technology 6(4): 703-719.

The potency of petroleum ether leaf extracts of *A. indica* (Juss), *O. gratissimum* (L.) and *H. suavoelens* (L.) Poit as mosquito larvicides under laboratory conditions was investigated. Leaf extracts of test plants were extracted, distillated and evaporated using soxhlet apparatus. Twenty five larvae of *culex* mosquito species were exposed to various concentrations and observed over 24 hr for susceptibility. 100% mortality was achieved by *A. indica* at concentrations 40% and 35% and *O. gratissimum* at concentration of 50% after 24hrs while *H. suaveolens* at 60% which showed no significant effect as mosquito larvicide ($P \leq 0.05$). At concentration of 30% of each extract, mortality rate dropped to 96%, 99.2% and 1.34% for *A. indica*, *O. gratissimum* and *H. suaveolens* respectively. LD50 values of 14.3, 11.40 and 66.83 for *A. indica*, *O. gratissimum and H. suaveoleus* were obtained. Mortality rate over second and third hours were significantly lower ($p < 0.05$) and after 24hrs of exposure, no significant difference was observed between *A. indica* and *O. gratissimum. H. suaveolens* did not show any effective larvicidal activity at all concentrations and time. This present study provided evidence of larvicidal of *O. gratissimum* and *A. indica* petroleum ether extracts to have potential utilization as a larvicide to control *culex* mosquito and its borne diseases.

Key words: larvicide, ocimum, biopesticide, mosquito, leaf extracts

Introduction

Mosquitoes are among the well known group of insect vectors that transmit deleterious human diseases, which pose as the major public health challenges eroding development in the poorest countries of the world (Awad and Shimaila, 2003). Their medical importance as vectors for the transmission of serious diseases that cause morbidity, mortality, economic loss and social

[*]Corresponding author: Okigbo, R.N.; e-mail: okigborn17@yahoo.com

disruption such as malaria, filariasis, yellow fever, dengue and other viral diseases as well documented by (Becker *et al.*, 2003).

According to Bayer Environmental Sciences (2007) and Reinert (2000), there are about 3500 species of mosquitoes, grouped into 3 sub-families, Toxorhnychitinae, Anophelinae, and Culicinae. In Nigeria only, there are over 18 anophelines species Gillet (1972); female anopheles gambiae, *A. funestus*, *A. nili* and *A. melas* which transmit malaria and filariasis to man, *Aedes aegypt*, *A. africanus*, and *A. simpsoni* transmit yellow fever, while others species like *A. lentocephalus, A. irritans* etc transmit dengue and other viruses to man. *Culex nebulosus* transmit yellow fever and viral encephalisis to man (Bayer Environmental Science, 2007; Gillet, 1972). Thus one of the approaches for control of these mosquito-borne diseases is the interruption of the disease transmission, by killing or preventing mosquitoes from biting human being. As delineated by Awad and Shimaila (2003), the principal objective of vector control is the reduction in morbidity and mortality due to malaria and other diseases transmitted by mosquito, by reducing level of transmission. Larval habitat may be minimized especially in urban environment by sealing of drains and soakaways, removing receptacles containing water such as old tins, tyres etc. Where these physical measures are not possible, larvicides are usually applied.

Larvicides are chemical substances or group of insecticides used to stop mosquito larvae from maturing into biting adults that cause diseases. Larvicides usually applied are such as dichloro-diphenyl-trichloroethane (DDT), pyrethruim, heptachlor, diedrin and lindane have been used in the past to achieve this control measures.

The commonly and repeatedly used larvicides are fuel oils, kerosene and insecticide formulations (Truman *et al*., 1976). Synthetic organic larvicides, although very efficacious to target species such as mosquitoes can be detrimental to a variety of animal life including man. NICC (2003), reported that organophosphate temphos which is a larvicide usually used in breeding site, though slightly toxic to target organism, may cause headache, loss of memory and irritability to man. Besides, the incessant use of chemical insecticides has often led to the disruption of natural biological control system, and out break of insect species as noted by (Chaithong *et al.*, 2006). Moreover, these chemicals could be carcinogenic, mutagenic and teratogenic. WHO (2005), Brown (1986) and Lee *et al.* (2001), reported about the development of physiological resistance to these chemicals by mosquito species. Some mosquito species develop resistance to malathion Guneady *et al*. (1989), a conventional pesticide, and to deltamethrin Chen wen-mei (1990) like the adult *Culex pipens*.

An investigation into Larvicidal effects of neem plant (*Azudirachta indica*) Juss, scent plant (*Ocimum gratissimum*) (L.) and pignut weed (*Hyptis suavelolens*) (L.) (Poit) based on their ethno-botanical information as phytomedicinal plants have been used to control insects and diseases of man. Neem (*Azadirachta indica*) Juss is a tree in the mahogany family *Meliaceae,* one of the six species in the genus *Azadirachta*, and a native to India and Burma, growing in tropical and semi-tropical regions in Africa. In Nigeria it is popularly known as "Dogoyaro tree". It is an evergreen, fast growing tree of about 15-20 m of height, with an alternate, pinnate leaves arranged on a short petiole.

At the other hand scent plant (*Ocimum gratissimum*) (L.) is an indigenous perennial shrub in the family of *Labiatae*, belonging to the genus *Ocimum.* There are many species in the *ocimum* genus, *O. Canum, O. gratissimum, O. basilicum O. urticifolium, O. trichodon, and O. americanum* (Iwu, 1993). Amongst these *O. basilicum* and *O. gratissimum* are believed to have insecticidal effects (Iwu, 1993). *O. gratissimum* is popularly seen and known in Nigeria as Efinrin ajase in Yoruba, Ebavbokho in Bini, Aai doya to gida in Hausa and Nchonwu in Igbo (Owulade *et al.*, 2004).

Hyptis suaveolens (L.) Poit is a dicotyledonous, annual forb/sub shrub, which is a member of Laminaceae (mints family); belong to the genus *Hyptis* and it is distributed evenly in the tropic of West Africa. Base on its ethno-botanical evidence, people believed it is a strong mosquito repellent.

Toxic secondary metabolites from plants were extracted, tested and proved to affect insect nerve functions and behaviors. A considerable number of studies have emphasized the research and development of herbal substances for controlling mosquitoes (Tsao *et al.,* 2002; Sukurmar, 1991; Jeyabalan *et al.*, 2003).

Although results vary, natural plant extracts may be a possible alternative to synthetic organic insecticides, as they are effective and compatible with human and animal life and environment. These botanical extracts could also be used along with other insecticides under integrated vector control (ICMR, 2003). Plant products can be obtained either from the whole plant or from a specific part by extraction and evaporation with different types of solvent such as aqueous, methanol, acetone, petroleum ether, chloroform, hexane e.t.c depending on the polarity of the photochemicals. Studies carried out so far have shown that some photochemicals acts as general toxicant (insecticide/ Larvicide) both against adult as well as larval stage of mosquito while others interfere with reproduction (chemo-sterilant) or produce olfactory stimuli, thus acting as repellent (ICMR, 2003). Botanical extracts could have antifeedant,

ovipositional, ovicidal, adulticidal, larvicidal growth inhibition, chemosterilant and repellent effects on mosquito species.

There is needed for larvicides of natural origin environmentally safe, biodegradable and target specific to combat mosquito species as vectors of some human deleterious diseases (malaria, filariasis, encephalisis etc) which have posed a great threat to human existence at the larval stage. This study was to investigate the larvicidal effects of these plant extracts on larvae of culex mosquito, the mortality of these larvae caused by the various concentrations of the petroleum ether leaf extracts of these plants was compared. The influence of length of exposure (time) on the larvae cause by these plant extracts was determined.

Materials and methods

Collection, preparation and processing of leaf extracts of test plants

Twigs of the neem plant (*Azadirachta indica*) Juss, Scent plant (O*cimum gratissimum*) (L.) and pignut weed (*Hyptis suaveolens*) (L.) Poit were collected from St. John Girls Secondary School Awka, Eke-Awka Market and the forest of Nnamdi Azikiwe University Awka respectively an were brought to the Botany Department Laboratory, where they were properly identified by Professor C. N. Okeke, of the Department of Botany Nnamdi Azikiwe University Awka. The leaves were carefully washed and rinsed with tap water. Dead leaves were removed together with insect larvae from the twigs. Fresh leaves were separated from the twigs and were dried with tray in the laboratory under shade at room temperature 28±1ºc for 5 day (120 hrs) separately for the 3 test plants (*Azadirachta indica*, *Ocimum gratissimum* and *Hyptis suaveolens*).

Extraction of test plants leaves

The petroleum ether extraction of the test plants was carried out and soxhlet technique was applied using the apparatus. Petroleum ether and the ground samples of neem were used as solvent and solute respectively. Like wise scent plant (*Ocimum gratissimum*) and pignut weed (*Hyptis suaveolens*) were also used as individual solutes with the same solvent. 20 gm of each ground and sieved leaf samples were extracted with initial petroleum ether volume of 250 ml at a time monitored, respectively for each same extracted.

Procedure of the extraction of process

The completely dried leaves of the neem plant (*Azadirachita indica*) were ground with Binatone MX10 blender and sieved to get a fine powder of the

leaves. This was done to the rest of the test plant samples *(Ocimum gratissimum and Hyptis suaveolens)* separately. The petroleum ether leaf extracts from each ground and sieved leaf powder were obtained by using soxhlet apparatus. 20 gm of each ground and sieved leaf were weighed out using metler balance, wrapped in a plain white sheet of paper and then put in the timbel of the soxhlet apparatus compartment. Thereafter, the condenser was carefully and efficiently connected. An initial 250 ml volume of the solvent (Petroleum ether) was added with the aid of a funnel by passing it through the timbel containing the sample to the round bottom flask system of the soxhlet. Inlet and outlet of the condenser were connected to a hose respectively, for the recycling of the cold water during the extraction. The entire apparatus was supported by the frame work of retort stand and clamps. Finally, the hot plate (heat source) was switched on for the extraction. As the extraction continued, the yellow-green colour of the solution in the soxhlet body (timbel) becomes less coloured, while colour of the solution in the round bottom flask becomes brown. This was the same for *Ocimum gratissiumum* and *Hyptis suaveolens*. This trend continued until the colour in the soxhlet body becomes colourless while the solution in the flask becomes deep brown. The solvent (petroleum ether) has a boiling point of 80ºC. The extraction process lasted for 9 hrs. This procedure was diligently and carefully repeated for the rest of the test plants *Ocimum gratissimum* and *Hyptis suaveolens* in the same proportion of 20 gm of ground, sieved and dried sample. For *Ocimum,* time of extraction was the same as for neem (9 hrs) while *Hyptis suaveolens* lasted for (6 hrs). These extraction processes were conducted under room temperature of (28±1ºC) in the Botany Laboratory (Ahmed *et al.,* 2004).

Formulation of test materials (plant extracts)

Different concentrations were made for each of the plant extract used as shown below.

Formulation of petroleum-ether extract of (Azadirachta indica): 8 ml of petroleum either of the standard extract of Azadirachta indica was solubilized in 0.7 ml of acetone and then in distilled water of 19.3ml to obtain a percentage stock concentration of 40%. Serial dilution was made from this stock solution to obtain various percentage concentration of working solution of 35, 30, 25 and 30%.

Formulation of petroleum ether extract of (Ocimum gratissimum): 10 ml of petroleum ether of the standard extract of *Ocimum gratissimum* was solubilized in 0.7 ml of acetone and then in distilled water of 19.3 ml to obtain a percentage stock concentration of 50%. Serial dilution was from this stock concentration to obtain working solutions of 45, 40, 35 and 30%.

Formulation of petroleum ether extracts of (Hyptis suaveolens): 12 ml of Petroleum ether of the standard extract of *Hyptis suaveeolens* was solubilized in 0.7 ml of acetone and then in distilled water of 19.3 ml to obtain a stock percentage concentration of working solutions of 60%. Serial dilution was also made from stock concentration to obtain working dilutions of 50, 40, 30 and 20%.

Sources and rearing of test animal

Both eggs and larvae of *Culex* spp. were collected from small water receptacles like plastic bowls and buckets that were purposefully left outside the laboratory as traps for these insects. The receptacles were left outside for 7 days for proper trapping of the vector eggs and larvae. Larvae were properly identified by Mrs. C. E Nwankwo of the Department of Parasitology and Entomology Nnamdi Azikiwe University, Awka. The larvae collected from the receptacles were reared in the Botany Laboratory. The water containing both eggs and larvae were cleaned up into a plastic, bowl of 20cm in diameter and 15cm deep. Crumbs of biscuits and organic substances from top moist soil were supplied as feed for the newly hatched larvae. Water was changed everyday after the first 2 days to remove the scum for the subsequent larval stages. This technique was repeated several times to provide the needed instar for the larvicidal test. Besides, larvae were reared under room temperature of about 28±1°c (Ahmad, 2004).Larvae were visually and microscopically identified based on their various peculiar characteristics and morphological features *Culex* spp. are well known to breed in small receptacles containing water. Their larvae lie at an angle of 45°c to water surface with their heed downwards whole the tail is upward hence called bottom feeders. Anatomically larvae have long breathing siphon on the 8th segment. 3.5.

Bioassay of larvae with plant extract

In this present experiment, treatment of larvae with petroleum ether leaf extracts was conducted in accordance with WHO standard method (WHO, 1981). Twenty (20) larvae of culex mosquito were placed in a transparent plastic Petri dish of about 40 ml capacity. 15 ml of tap water was added into the Petri dish and was replicated three times. The larvae were then exposed to various concentrations of the required quantity of the percentage stock solutions of *A. indica*, *O. gratissimum* and *H. suaveolens*. Control medium was also maintained with 20 larvae in 15ml of tap water for each concentration in each plant extracts. Mortality rates were recorded three hourly over a 24 hr period at

a room temperature of 28±1ºC. Each of the test samples and standard were replicated 3 times. This method is modified from Ahmed *et al.* (2004).

Results

Extracts from test plant

The 20 g of tested plant e.g. *A. indica*, *O. gratissimum* and *H. suaveolens* yielded 16, 21, and 18 ml, respectively.

Larvicidal activity

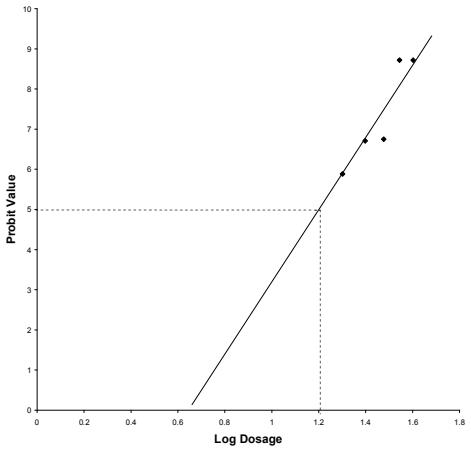
Petroleum ether extracts of leaves extracts of *A. indica* and *O. gratissimum* were observed and showed high mortality against *Culex* larvae at all concentration (Table 1, 2 and 3) after 24 hrs of exposure. With the first, second and third, 3-hour of bioassay, 100, 93.37, 90.28, 82.4 and 59.96% mortality were observed and recorded at concentration of 40, 35, 25, and 20% for *A. indica* and 96, 93.12, 85.68, 83.96 and 70.08% mortality were also observed and recorded for *Ocimum gratissimum* at different concentrations of 50, 45, 40, 30 and 20%. *Hyptis suaveolens* showed little or no larvicidal activity at 3.96, 3.96, 2.64, 1.34 and 0% (Table 4). Petroleum ether leaf extracts of *A. indica* compared with petroleum either leaf of *O. gratissimum* showed significant difference of level $P \leq 0.05$. *H. suaveolens* did not compare effectively as larvicide with *O.gratissimum* and *A. indica*. Therefore, the results showed a high significant difference at level $P \leq 0.01$.

Effects of concentration on culex larvae with respect to time

There was a gradual overall mortality rate decrease as concentration decrease in the petroleum ether leaf extracts of *A. indica*, *O. gratissimum* and *H. suaveolens* (Table 5). It was observed that at standard concentration of 40, 50 and 60% for *A. indica, O.gratissimum* and *H. suaveolens* respectively. The total mortality of 25 (100%), 25(100%), and 0.99 (3.96%) were obtained respectively after 24 hrs of exposure. Moreover at 30 and 20% concentrations of the extracts, 24 (96%) and 20.27(99.92%) and 23.11(92%) mortalities were equality recorded for *O. gratissimum* while with *H. suaveolens* 0.33 (1.32%) and 0.00 (0.00%) (Table 6). Therefore, there is a significant difference $P \leq 0.05$ with respect to decrease in mortalities caused by decrease in concentration.

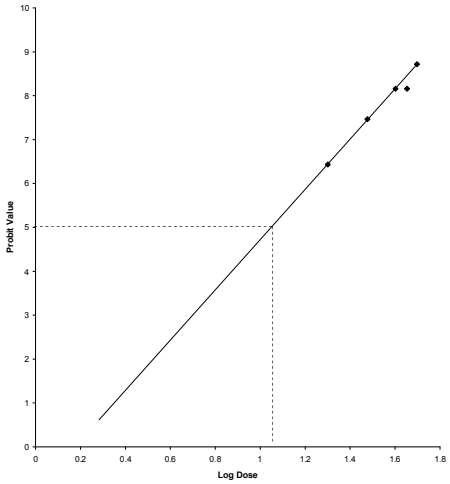
Lethal dosage Concentration LD_{50}

Using profit regression analysis log table, regression times were plotted for dose response to petroleum ether leaf extracts of *A. indica, O. gratissimum* and *H. Suaveolens* treatment of *Culex* larvae (Figs. 1, 2 and 3). For culex mosquito larvae, the Lethal Dosage to cause 50% mortality on the population (LD_{50}) was measured at 14.3, 11.40 and 66.83 ppm for *A indica*, O. *gratissimum* and *H. suaveolens* (Figs., 1, 2 and 3).



1.055=11.40 LD_{50}

Fig.1. Probit regression line for response of culex mosquito larvae to petroleum ether leaf extract of *Ocimum gratissimum* in laboratory test.

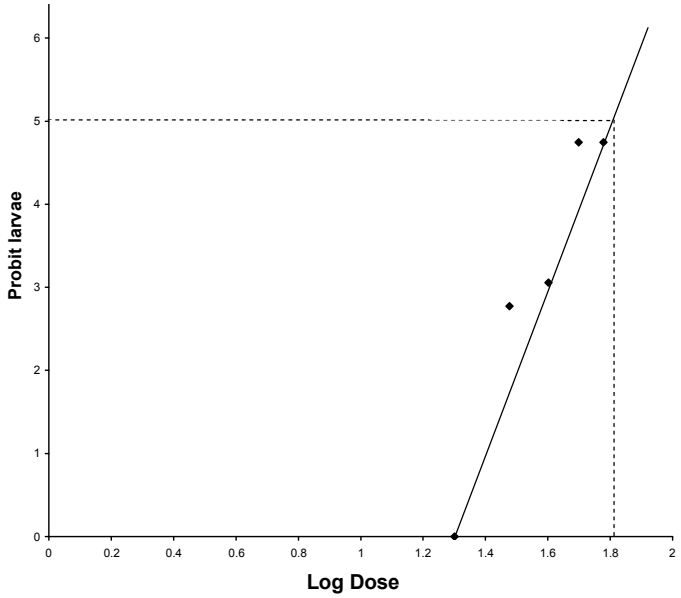


1.15=14.13 LD_{50}

Fig. 2. Probit regression line for response of culex mosquito larvae to petroleum ether leaf extract of *Azadirachta indica* in laboratory test.

Table 6. Pooled results of 1st, 2nd 3rd 3-hour mortality by *A. indica, O. gratissimum and H. suaveolens* against concentration of 40, 30 and 20%.

Larvicide concentration	**3hr**	**6hr**	**9hr**	
In percentage (%)	mortality by *A. indica*	Mortality *by O. gratissimum*	Mortality by *H. Suaveolens*	
40	25.00	24.98	00.66	
30	24.00	24.83	00.33	
20	20.57	72.92	00.99	143.48



1.825=66.40 LD_{50}

Fig. 3. Probit regression line for response of culex mosquito larvae to petroleum ether leaf extract of *Hyptis suaveolens* in laboratory test.

Discussion

The result of this present study showed that petroleum ether leaf extracts of *Azadirachta indica* (Juss) *Ocimum gratissimum* (L.) were effective as mosquito larvicides. This is in line with the results of the preliminary works done by Vatandoost and Vaziri (2004) using neem plant (*Azadirachta indica*) and Cavalcanti *et al.* (2004) using *O.gratissimum* and *H.suaveolens*. Extracts of *H. suaveolens* was not effective in the larvicidal bioassay and this corresponds with the work done by (Cavalcanti *et al.,* 2004).

There is difference between the bioactivities of the two test plant *A. indica and O. gratissimum* against *Culex* mosquito larvae at similar or equal concentrations. This could possibly due to of the age of the mosquito larvae, solvent used for extraction, concentrations made, chemical constituents and part of the plant used (Kumuda *et al*., 1991*). H. suaveolens* showed a wide observable range of differences when compared with *O. gratissimum* and *A. indica*. This could be resulted of the mode of its action or bioactivity on mosquito as insect. Cavalcanti *et al.,* (2004) as reported that *H. suaveolen*s can be act as having repellent activity (olfactory effect) instead of larvicidal just like *O.gratissium* and *A. indica*.

However there was a gradual overall mortality rate decreased as concentration decreasd in the extracts of *A. indica* and *O. gratissimum* and *H. suaveolens*. It was observed that there were significant differences between the low and higher concentrations of the extracts and higher mortality at higher concentration. This is consistent with the observation of Piyarat *et al*. (1974). Comparatively, *A. indica* compared favourably with *O. gratissimum* at higher concentration achieving 100% and 96% mortality after 24 hrs of exposure at significant level $P \leq 0.05$ respectively, while *H.suaveolens* showed a high significant difference at $P \leq 0.01$).

Moreover, mortality rate increased with increase in time irrespective of the concentrations, though there were still overall significant difference within the concentrations of the plant extracts in an increasing order of 8.86, 20.56 and 56 % for *A. indica* and 11.01, 34.61 and 40.22% for *O. gratissimum* and 0.553, 0.79 and 1.056% for *H. suaveolens,* depicting a slight slow rate of action, but this observation may not be considered a serious problem especially where length of time of exposure is not a critical factor. Additionally, petroleum ether leaf extracts of plants *O. gratissimum*, *A. indica* and *H. suaveolens* degrades rapidly being a plant product thereby reducing the possible accumulation of toxic residues in the environment. Mittal *et al*. (1995) stated that the advantage compared with the synthetic organic insecticides or WHO recommended larvicides like malathion. These observations are consistent with those of others who have worked with insecticides of plant origin.

Conclusively Extracts of *Azadirachta indica* and *O. gratissimum* may not be as quick acting within a very short time of 1-3 hrs on *culex* larvae, they could be used as potent larvicides as substitute for synthetic organic larvicides like malathion, fenthion, D. D. T., where time is not a critical factor having their peak bioactivity at the Second and third (6-hours) after application. This should be well appreciated in this study especially when efforts towards the use of insecticide/larvicides that are target specific and environmental friendly are needed.

Azadirachta indica and O*cimum gratissimum* extracts have added advantage of being less toxic to aquatic life, no health hazard and biodegradable in the environment.

References

Ahmed, H.H., Kamal, I., O.H and Ramky, R.M. (2004). Studies on the molluscidal and Larvicidal properties of *Solanum nignum* (L) leaves ethanol. Journal of the Egyptian Society of Parasitology 31:843.

Awad, O.M. and Shimaila, A., (2003). Operational use of neem oil as an alternative anopheline larvicide. Part A: Laboratory and field efficacy. Eastern Mediterranean Health Journal 9 (4): 637.

Bayer Environmental Science (2003). Mosquito the vector for malarial and Filaríais. www.ibl.gov./ERSP/generalinfo/contact.html.

Becker, N.D., Zgomba, M., Boase, C., Dahl, C., Lane, J., and Kaiser, A. (2003). Mosquitoes and their control New York: Kluwer Academic/Plenum Publishers. 325 pp.

Brown, A.W.A. (1986). Insecticide resistance in mosquitoes a pragmatic review. The Journal of the American Mosquito Control Association 2: 123-140.

Cavalcanti, E.S.B., Morris, S.M., Lima, M.A.A. and Santana, E.W.P. (2004). Larvicidal activity of essential oils from Brazilian plants against *Aedes aegyptic* (L.). Memorias do Instituto Oswaldo Cruz 99: 541-544.

Chaithong, U., Choochote, W., Kansuk, K., Jitpakdi, A., Tippawangkosol, P., Chaiyaist, D. Champakaew, D., Tuetun, B. and Pitasuwat, B. (2006). Larvicidal effects of pepper plants on *Aedes aegypti* (L.) (Diptera: Culicidae). Journal of vector ecology 31 (1): 138-144.

Chen-Wen-mei (1990). Selection of deltamethrin resistant Culex pipens (pallans) from depeterex resistant strain. Acta Entomologica Sinica 33(1): 14-20.

Gillet, J.D. (1972). Mosquitoes, Weiden field Nicholson London. 274 pp.

Guneady, A., Ebeid, A., Saleem, H. (1989). Development and reversion of malathion resistance in adult Culex pipens. Indian Journal Entomology 50(1): 45-54.

ICMR. (2003). Prospect of using herbal products in the control of mosquito vector Indian Council of Medical Research Bulletin 33(1): 10.

Iwu, M.M. (1993). Hand book of Africa Medicinal plant. CRC press Boca Roton 372 pp.

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

Kumuda, S., Michael, J.P. and Lewis, R.B. (1991). Botanical Derivative in mosquito control . A review journal of the American Mosquito Control Association 7(20): 210-237.

Lee, S.E., Kim, J.E. and Lee, H.S. (2001). Insecticide resistance in connecting interest. Agricultural Chemistry and Biotechnology 44:105-112.

Mittal, P.K., Adak, T. and Sharma, V.P. (1995). Bioefficacy of six neem (*Azadirachta indica)* products against mosquito larvae. Pesticide Research Journal 7: 35-39.

NICC-Comunicação e Saúde. (2003). Pesquisadores criticam o uso de produtos químicos no combate à doença. Available at: www.cpqam.fiocruz.br/nicc/comunic/jc200101.htm.

Owulade, M.O., Eghiankuwa, R.I. and Daramola, F.O. (2004) effects of aqueous extracts of *Hibiscus Sabdariffa* Calyces and *Ocimum gratissimum* leaves on intestinal transit in rats. African Journal of Biomedical Research 7(1): 31-33.

Piyarat, S.W., Fred, K. and Roy, S. (1974). Biologically active plant extracts control of mosquito. Journal of the American Mosquito Control Association 24(49): 398-402.

Reinert, J.F. (2000) New classification of composite genus Aedes. Journal of American Mosquito Control Association 16: 175-188.

Sukumar, K., Perich, M.J. and Boobar, L.R. (1991). Botanical derivatives in mosquito control: Journal of the American *Mosquito Control* Association 7: 210-237.

Truman, L. Beneth, G., Butts, S.W. (1976). Scientific guide to Pest Control Operations, 3rd ed. Cleveland Ohio. Harvest Publishing Company 207 pp.

Tsao, R., Roman, C.F.E., Peterson, C.J. and Coast, J. R. (2002). Plant growth regulatory effect and insecticidal activity of extracts of tree of heaven (*Ailanthus altissima*). BMC Ecology 2: 1-8.

Vatandoost, H. and Vaziri, V.M. (2004). Larvicidal activities of a neem tree extract (Neemarin) against mosquito larvae in the Islamic Republic of Iran. Journal of African Common diseases 38(1): 104-107.

World Health Organisation. (1981). Instructions for determining the susceptibility or mosquito larva to insecticide. WHO /VBC/81:807.

WHO (2005). Resistance of vectors and Reservoirs of Disease to pesticides. Technical Service Representative 737: 88 pp.

(Received 23 October 2009; accepted 20 August 2010)

Table 1. Effect of neem (*Azadirachta indica*) on *culex* larvae – Average mortality against concentration and time.

Concentration (%)	No.	Mean	No. of larvae dying						
	larvae exposed	3 hr	6 hr	9 hr	12 hr	15 hr	18 hr	21 hr	24 hr	Total Death after 24 hr
40	25	2±0.8	3.71±0.8	19.29±3	0	0	0	0	0	25
35	25	1.57±0.3	3.57±0.9	18.29±2	1.57±0.09	0.57±0.02	0	0	0	25
30	25	1.43±0.4	3.57±0.7	17.57±	1.14±0.07	0.28±0.03	0	0	0	24
25	25	3.14±0.9	8.57±0.9	8.89±0.5	3.00±0.9	1.00±0.1	1.14±0.08	0	0	20.14
20	25	2.71±0.7	6.28±1.2	6.00±0.9	2.57±0.6	1.71±0.2	1.00±0.09	0	0	20.27
control	25	0	0	0	0	0	0	0	0	00.00

Table 2. Effect of Petroleum ether Extract of *Ocimum gratissimum* on *Culex spp* – Average mortality rate against concentration and time.

Concentration (%)	No.	Mean	No. of larvae dying						
	larvae exposed	3 hr	6 hr	9 hr	12 hr	15 hr	18 hr	21 hr	24 hr	Total Death after (24)hr
50	25	2.71±0.7	11.86±2	9.43±1	0.43±0.02	0.43±0.01	0.14±0.09	0	0	25
45	25	3.14±0.3	10.14±2	10.00±2	0.83±0.04	0.28±0.023	0.57±0.1	0	0	24.98
40	25	2.57±0.9	8.28±1	10.57±2	1.57±0.08	0.85±0.01	1.14±0.2	0	0	24.98
30	25	2.71±0.6	7.14±1	11.14±3	1.14±0.09	1.28±0.08	1.42±0.1	0	0	24.83
20	25	2.71±0.9	5.85±1.1	9.14±1	1.42±0.08	0.57±0.03	0.57±0.1	1.71±0.08	1.14±0.09	23.11
Control	25	0	0	0	0	0	0	0	0	00.00

Table 3. Effect of petroleum ether extract of *Hyptis suaveolens* on Culex larvae – Average mortality against concentration and time

concentration (%)	No.	Mean	No. of larvae dying						
	larvae exposed	3 hr	6 hr	9 hr	12 hr	15 hr	18 hr	21 hr	24 hr	Total Death after 24 hr
60	25	0.33±0.01	0.33±0.01	0.33±0.01	0	0	0	0	0	0.99
50	25	0.33±0.01	0.33±0.01	0.33±0.01	0	0	0	0	0	0.99
40	25	0	0.33±0.01	0.33±0.01	0	0	0	0	0	0.66
30	25	0	0	0	0	0	0	0	0	0.33
20	25	0	0	0	0	0	0	0	0	0.00
Control	25	0	0	0	0	0	0	0	0	0.00

Table 4. Pooled results of average percentage mortality of mosquito larvae treated with extracts of *Azadirachta indica, Ocimum gratissimum* and *Hyptis suaveolens* in the first, second & third 3-hour.

No of larvae exposed	*A. indica*			*O. gratissimum*			*H. suaveolens*		
	concentration (%)	Total death after 9 hr	% mortality	Concentration (%)	Total death after 9 hr	% mortality	Concentration (%)	Total death after 9 hr	% mortality
25	40	25	100	50	24	96	60	0.99	3.96
25	35	23.43	93.37	45	23.28	93.12	50	0.99	3.96
25	30	22.57	90.28	40	21.42	85.68	40	0.66	2.64
25	25	20.6	82.14	30	19.42	83.96	30	0.33	1.32
25	20	14.99	59.96	20	17.42	70.08	20	0	0

Table 5. pooled results of total mortality after 24hrs of exposure to plant extracts of *Azadirachta indica, Ocimum gratissimum and Hyptis suaveolens*.

A. indica			*O. gratissimum*			*H. suavelons*		
concentration (%)	**Total death after 9 hr**	**% mortality**	**concentration (%)**	**Total death after 9 hr**	**% mortality**	**concentration (%)**	**Total death after 9 hr**	**% mortality**
40	25	100	50	25.00	100	60	0.99	3.96
35	25	100	45	24.98	99.92	50	0.99	3.96
30	24	96	40	24.98	99.92	40	0.66	2.64
25	23.14	92.56	30	24.83	99.30	30	0.33	1.34
20	20.27	82.28	20	23.11	92.44	20	0.0	%

Table 7. Probit log Table of concentrations of *Azadirachta indica, Ocimum gratissimum and Hyptis suaveolens*.

Azadirachta indica					*Ocimum gratissimum*					*Hyptis suaveolens*				
Dose (%)	Log dose	Observed mortality	correction mortality (%)	Probit value	Dose (%)	Log dose	Observed mortality	correction mortality (%)	Probit value	Dose (%)	Log dose	Observed mortality	correction mortality (%)	Probit value
40	1.6021	25.00	100	8.7190	50	1.6980	25	100	8.7190	60	1.7782	0.99	4.0	4.7467
35	1.5441	25.00	100	8.7190	45	1.6532	24.98	99.92	81.559	50	1.6990	0.99	4.0	4.7467
30	1.4771	24.0	96.0	6.7507	40	1.602	24.98	99.92	81.559	40	1.6021	0.66	2.6	3.0569
25	1.3979	23.14	92.0	6.7060	30	1.4711	24.83	99.32	7.4677	30	1.4771	0.33	1.32	2.7738
20	1.3010	20.27	81.10	5.8816	20	1.3010	23.11	92.44	6.4325	20	1.3010	0.00	0.00	0.00