



Larvicidal Activity and Lethal Concentrations of *Nicotiana tabacum* L. and *Datura metel* L. (Solanales: Solanaceae) on Mosquito Larvae

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ABSTRACT

Leaves and seed kernel extracts of *Nicotiana tabacum* (L.) were assayed for larvicidal activity against 4th instar larvae of *Aedes*, *Anopheles* and *Culex* mosquitoes. Mosquito larvae of those genera were collected from breeding site and reared in the laboratory for continued source of larvae. The plants parts used were extracted with hexane, chloroform and methanol in increasing order of polarity. Each extract was formulated into 0.9, 1.6, 2.4, 3.5 and 4.0mg/mL concentrations and then tested against 25 larvae of each genus alongside control setting over 12 to 72 hrs periods. Phytochemical screenings of the most active extracts were carried out for searching the active ingredients. Data was analyzed through Analysis of Variance and lethal concentrations of the extracts were determined. The hexane extract of *D. metel* (LC₅₀ 2.58 ±0.1mg/mL) exhibited the highest larvicidal activity with more than 70% mortality on *Aedes* and *Anopheles* larvae when 4.0mg/mL was applied after 72hrs. *Culex* larvae were less susceptible to all concentrations of the extracts. Though larvicidal activity was observed to be indirectly proportional to concentrations and larval exposure periods, significant differences (p <0.05) in the means of larval death were observed. The active extracts were found to contain alkaloids, saponins, steroids, flavonoids and glycosides from moderate to high quantity which could be responsible for larvicidal activity. Future formulation studies, toxicity profile and possible mechanism of action may lead to the development of potential insecticidal products.

Keywords: Larvicidal activity, mosquito larvae, phytochemicals, plant extracts

INTRODUCTION

Mosquito control is an important and basic public health function. Of all insects that transmit disease, mosquito represents by far the greatest menace (Mdoe *et al.*, 2014). The World Health Organization (WHO) (2006) has declared mosquito "Public Enemy Number One". They act as a vector for most of the life threatening diseases like malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, West Nile virus infection, *etc.*, in many other parts of the world. Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting over 700,000,000 people every year (Ghosh *et al.*, 2012). Malaria for instance recognized as the "king of diseases" puts more than 2400 million of the world's population at risk (WHO, 2006). Its annual incidence worldwide is estimated to be 300-500 million cases killing 1.1 to 2.7 million people (Thomas *et al.*, 2017). Disease transmission can occur upon the bites from infected female mosquito but the disease organism must reproduce in the mosquito, or at least survives long enough to infect the salivary glands (Conti *et al.*, 2013).

Control efforts can be justified when a mosquito poses a nuisance or is an economic or health-related pest or vector. Most of the widely used vector interruption methods are synthetic insecticide-based. Many of them target the adult mosquitoes as adulticides such as DDT, BHC, pyrethrins, pyrethroids, chlorpyrifos, malathion, carbaryl, sumitrin *etc.* while others are used as larvicides against the larvae, like methoprene (IGR), Altosid, Abate, BVA oils, agnigüe *etc.*

Though, synthetic insecticides remain to be the major chemical weapons against vectors, large-scale use for the control of mosquitoes has led to the development of resistance in populations of insects, non-biodegradability, harmful effects on natural enemies, and environmental pollution (Shakeel *et al.*, 2017; Ahmed *et al.*, 2018). Resistance to pesticides was not serious before the early 1940s when raw plant materials were the principal agents of pest control (Bakkali *et al.*, 2008). In recent years, the search for newer products and alternatives for mosquito control that are environmentally safe, target-specific and easily degradable is on the rise (Ohia and Ana, 2015). A wide variety of plant metabolites have been identified to be effective not only as insect toxicants but also as repellents, feeding deterrents, attractants, sterilants, inhibitors of growth and development. The current study determined the lethal formulations of *N. tabacum* and *D. metel* on the larvae of *Aedes*, *Anopheles* and *Culex* mosquitoes as alternatives to synthetic insecticides against these insects.

MATERIALS AND METHODS

Extraction of Plant Materials

Seed samples of *Datura metel* and leaves of *Nicotiana tabacum* were obtained from Dabagi farm of Usmanu Danfodiyo University, Sokoto (13° 00'N, 5° 14'E, alt 274m) and identified by a plant taxonomist. All the samples were pulverized into powder and prepared into crude extracts then each fractionated with hexane, chloroform and methanol to obtain

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separate fractions (Ogundare *et al.*, 2006). Each was evaporated to dryness and then made up to 1 L to form 4000 mg/L (4 mg/mL) as stock solution. The *D. metel* extracts were later emulsified with 0.009mL of Tween-80® (Anyaele and Amusan, 2003) for each stock solution.

Collection of Mosquito Larvae

Larvae of mosquitoes were sampled from breeding sites in three locations of Sokoto Town; Gidan Igwai, (13° 08' N, 5° 12' E), Illela Garage (13° 04' N, 5° 16' E) and Tudun Wada (13° 03' N, 5° 14' E) using plastic dipper (300 mL: 11cm length) with simple scooping. The samples were sorted and identified to generic level; *Aedes*, *Anopheles* and *Culex* using keys of Harrison (2005) and pictorial guide of Michele and O'Meara (2008).

Larval Culture

The larvae were fed with brewer's yeast as per WHO (2006) protocol in plastic trays inside wooden cum wire gauze cage (50 x 30 x 30cm). The cage had internal demarcation of mesh netting (3cm² per hole), served as pigeon box. Adults that emerged out were fed on 10% sucrose solution but females blood-fed in addition with restrained three – weeks old chick to maintained larval culture.

Bioassay

Five concentrations for each extract were formulated (0.9, 1.6, 2.5, 3.4 and 4.0 mg/mL) separately in 250mL beaker capacity also containing 5mL of 12mg albumin as larval food (Ima *et al.*, 2005). Into each beaker, 25 fourth instar larvae of the mosquitoes from the larval stocks were released and maintained in the incubator held at 27 ± 2°C and 75 ± 5 % RH (Pavela, 2015). All treatments were replicated three times including the control. Mortality was recorded for both treated and untreated larvae at 12, 24, 48 and 72hrs.

Phytochemical Analyses

Phytochemical screening of the most active extracts was made using standard procedures to search for bioactive compounds (Kumar and Maneemegalai, 2008).

Analysis of Data

Dose – response assays were subjected to ANOVA using SAS v.9.1 software, significant differences in the treatment means were separated with DNMR at 5% level while 72hr. LC_{50, 90} were determined through probit regression analysis.

RESULTS

Larvicidal Activity of the Extracts

Mortality recorded among mosquito larvae exposed to different concentrations of *D. metel* hexane extract over periods of 12 to 72 hrs, is presented in Table 1. The lowest concentration of the extract (0.9 mg/mL) administered to *Aedes* larvae for 12 hrs, caused a single death but slightly increased after 72hrs of exposure. At concentrations 1.6 and 2.5 mg/mL, the means of larval death did not differ significantly ($p > 0.05$). Increase in concentrations of the extract to 3.4mg/mL and 4.0mg/mL caused highly significant ($p < 0.05$) death of the larvae. On *Anopheles* larvae, the lethal activity was also related to increase in concentration and exposure period. The highest mean death of *Culex* larvae was observed for 4.0mg/mL of the extract

after 72 hrs of exposure, but mortality was very low. The mortality values observed for all the larvae treated with *N. tabacum* hexane extract, *D. metel* and *N. tabacum* chloroform extracts, and *D. metel* methanol extract in Tables 2- 5 respectively, were very low, but there were significant differences ($p < 0.05$) in the means of larval death caused by the treatments compared to control mean death of larvae. *Culex* larvae were observed for lowest mean death while *Anopheles* highest when treated with methanol extract of *N. tabacum* after 72 hrs exposure to 4.0mg/mL (Table 6). Significant differences ($p < 0.05$) among treatment means and with control were observed. The highest mortality among the larvae was 80.0% (*Anopheles*) and the lowest was 4.0% (*Culex*)

Lethal concentrations determined (LC_{50/90} values)

Lethal concentrations of the extracts on the larvae of *Aedes*, *Anopheles* and *Culex* 72 hrs post-exposure are shown in Table 7. The methanol extract of *N. tabacum* exhibited the lowest LC₅₀ value (2.58±0.1 mg/mL) followed by hexane extract of *D. metel* (3.13±0.1 mg/mL) against *Anopheles* larvae which indicated marked larvicidal activity. Except for the corresponding LC₉₀ observed against *Aedes* and *Anopheles* larvae, the remaining values were more than two times fold compared to highest concentration applied.

Analysis of Phytochemicals

Qualitative and quantitative constituents of the most active larvicidal extracts are presented in Tables 8 and 9 respectively.

DISCUSSION

In this study, it was established that, the larvae of *Aedes* and *Anopheles* were more sensitive to the hexane extract of *D. metel* and methanol extracts of *N. tabacum* respectively with significantly ($p < 0.05$) higher means of larval death. *Culex* larvae were less susceptible to any of the extracts. In addition, mortality of the larvae was observed to be indirectly proportional to concentrations and exposure periods. Chakkaravarthy *et al.* (2011) observed 72.50% and 87.0% mortality of *C. quinquefasciatus* after 24 hrs exposures to 1000ppm concentration of hexane and chloroform extracts of *Azadirachta indica* respectively. The observed mortality of *Anopheles stephensi* exposed to 4, 6, and 8ppm concentrations of methanol extract of *D. metel* were 26, 73 and 78% respectively (Kumar and Murugan, 2011). Similarly, hexanolic extract of *Ricinus communis* caused 93% mortality on *Culex quinquefasciatus* larvae at concentration 70ppm within 48 hrs (Yahaya and Ruqayya, 2013). When the ethyl acetate, chloroform and methanol extracts of aerial parts of *Tagetes minuta* were tested against the larvae of *An. stephensi* at 2.5mg/mL, it was the chloroform extract that had the highest efficacy with 64% mortality on the larvae (Hadjiakhoodi *et al.*, 2005). Some other plants whose parts were extracted with the same solvents used in this study that recorded similar effect on mortality of different mosquito larvae include *Lantana camara* (Kumar and Maneemegalai, 2008), *D. metel*, *Cocculus hirsutum* and *Cassia occidentalis* (Kumar and Murugan, 2011), *Hypericum polyanthemum* (Riberiro *et al.*, 2007), *Margaritaria discodea* (Habeeb, 2010), *Allium*

sativum, *Jatropha curcas*, *Mikania schenkii*, *Pionciana regia* and *Spathodea campanulata* (Consoli *et al.*, 1989).

The larvicidal activity of *D. metel* hexane extract and *N. tabacum* methanol extract observed in this study could be attributed to the alkaloids and saponins contents in the extracts. Presence of alkaloids and saponins in *Datura* is in agreement with the report of Berkov and Zayed (2004), that *D. metel* is known to contain 36 tropane alkaloids, saponins, tannins, sugars, proteins, free fatty acids and nitrate. According to Rizvi *et al.* (2018) alkaloids are available as the main constituents of different parts of the plant and vary in concentration depending on the specific part and the species. They are the main depressant of the central nervous system producing most effect on the hypothalamus (Kutama *et al.*, 2010). The percentages of saponins and alkaloids in *D. metel* and *N. tabacum* were however moderate, but the compounds were reported to be toxic to animals even in low concentration (Kovendan *et al.*, 2012). Gopieshkhanna and Kannabiran (2007) have observed the presence of carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins in the plant extracts having mosquito larvicidal activity. Alkaloids derived from a tropical vine; *Phyllium peltatum* was found to have larvicidal activity against malaria vector, *An. stephensi* (Thomas *et al.*, 2017). Ryanodine, an alkaloid from the tropical shrub, *Raffesia speciosa* has been used as a commercial insecticide against European corn borer (Klock, 1987). Furthermore, physostigmine, an alkaloid from *Physostigma venenosum* was the compound upon which, carbamate insecticides were designed (Rice, 1983). Saponins were also reported toxic to cold blooded animals and insects at certain concentrations. Those derived from *Balanites aegyptiaca* exhibited maximum larvicidal activity against *Aedes aegypti* larvae (Wiesman and Chapagan, 2006). In addition, Medlock *et al.* (2012) reported that dipterous insects are unable to hydrolyze saponins. Pelah *et al.* (2002) reported the use of commercial saponins from *Quillaja saponaria* bark as a natural larvicide against *Ae. aegypti* and *Cx. pipiens*.

Variation in the larvicidal activity of the extracts against the larvae could result from variations in extraction solvent and or the mosquito species. Probably, different types of chemicals were extracted from the same parts of the plant in these solvents that resulted in variable activity when tested against the larvae. It is known that different mosquito species displayed different levels of susceptibility to the same extract. Sujatha *et al.* (1988) reported variations in toxicological efficacy with three mosquito species to petroleum ether extract of *Aconus calamus*, *Ageratum conyzoides*, *Annona squamosa*, *Bambusa arundanacia*, *Madhuca longifolia* and *Citrus medica* to which *Ae. aegypti*, *An. gambiae* were completely susceptible while *Cx. quinquefasciatus* was relatively susceptible.

Both methanol and ethanol flower extracts of *L. camara* were found to have higher rate of larvicidal activity against *Ae. aegypti*, whereas in the *C. quinquefasciatus* species, the concentrations of the extracts have to be increased to achieve similar effect on larval mortality. Insects respond to increasing doses of a toxicant or insecticide by increased mortality thus, the mortality effect of a toxicant on its host can be expressed as an LC₅₀ value. The LC₅₀ values obtained in this study were closer to the range of LC₅₀ values of the extracts from the aerial parts of *Tagetes minuta* (1.5mg/mL to 3.5mg/mL) on the larvae of *An. fluviatilis* as reported by Macedo *et al.* (1999). Also, similar to those reported by Kumar *et al.* (2014) who observed LC₅₀ values between 2.35 and 18.88 mg/mL against *An. stephensi* upon exposure to the extracts of the same plant within 48 hours. Kumar and Murugan (2011) reported LC₅₀ values of 5.89ppm and 6.43ppm from the extract of *D. metel* against *An. stephensi* and *Cx. quinquefasciatus* respectively. Variations observed could be attributed to mosquito species used, environmental factors, type of solvents used and selective action of toxicants in the extracts. The observations made presently for mosquito larvicidal activity of *D. metel* and *N. tabacum* extracts may support the claim that many members of the Solanaceae plant family to which both plants belong are poisonous to insects.

CONCLUSION

Larvicidal activity of *D. metel* and *N. tabacum* extracts on mosquito larvae that could be associated with the presence of alkaloids and saponins, was established in this study. The extracts of hexane and methanol of *D. metel* and *N. tabacum* respectively were the most effective against *Aedes* and *Anopheles* larvae, while the *Culex* larvae were less susceptible to both. However, the activity of the extracts against mosquito larvae was indirectly proportional to concentration and exposure period. Lethal concentrations of the extracts were determined (log probit analysis) and compared. The extracts could be used to control larval mosquitoes, offering an alternative to rather expensive and environmentally hazardous synthetic insecticides.

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Table 1: Larvicidal activity of hexane extract of *D. metel* against 4th instar larvae of mosquitoes

Mosquito larvae tested	Concentration (mg/100mL)	Number of larvae treated	Mean death \pm SD				Total mean death	Mortality (%)
			Exposure period (Hrs.)					
			12	24	48	72		
<i>Aedes</i> spp.	0.9	25	1.00 \pm 1.0	1.67 \pm 0.6	1.67 \pm 0.6	1.67 \pm 0.6	1.500 ^a	6.68
	1.6	25	1.00 \pm 0.0	2.00 \pm 1.0	2.33 \pm 0.6	2.33 \pm 0.6	1.917 ^a	9.32
	2.5	25	1.67 \pm 0.6	2.33 \pm 1.2	2.67 \pm 0.6	3.33 \pm 0.6	2.500 ^a	13.32
	3.4	25	2.00 \pm 1.0	2.67 \pm 0.6	3.00 \pm 1.0	18.00 \pm 2.0	6.417 ^b	72.00
	4.0	25	2.00 \pm 0.0	8.33 \pm 0.6	10.00 \pm 4.0	18.67 \pm 3.1	9.750 ^c	74.63
	Control	25	0.33 \pm 0.3	1.00 \pm 0.6	1.33 \pm 0.3	1.33 \pm 0.3	0.997 ^d	5.32
	Effect over time		1.533 ^a	3.400 ^b	3.933 ^b	8.800 ^c		
<i>Anopheles</i> spp.	0.9	25	1.67 \pm 1.5	3.33 \pm 0.6	3.67 \pm 0.6	5.00 \pm 1.0	3.417 ^a	20.00
	1.6	25	2.33 \pm 1.5	3.33 \pm 1.2	3.67 \pm 0.6	5.67 \pm 0.6	3.750 ^a	22.68
	2.5	25	3.67 \pm 0.6	4.00 \pm 0.0	4.67 \pm 1.2	6.00 \pm 2.0	4.583 ^a	24.00
	3.4	25	3.67 \pm 2.1	6.67 \pm 1.2	7.33 \pm 1.2	16.00 \pm 4.6	8.417 ^b	64.00
	4.0	25	5.00 \pm 2.1	7.00 \pm 2.0	10.33 \pm 1.5	19.00 \pm 2.0	10.333 ^c	76.00
	Control	25	0.33 \pm 0.3	0.33 \pm 0.3	1.33 \pm 0.7	1.33 \pm 0.7	0.833 ^d	5.32
	Effect over time		3.267 ^a	4.867 ^b	5.933 ^b	10.33 ^c		
<i>Culex</i> spp.	0.9	25	0.00 \pm 0.0	0.33 \pm 0.6	0.67 \pm 0.6	0.67 \pm 0.6	0.417 ^a	2.68
	1.6	25	0.00 \pm 0.0	0.33 \pm 0.6	1.33 \pm 0.6	1.67 \pm 0.6	0.588 ^a	6.68
	2.5	25	0.33 \pm 0.6	1.33 \pm 0.6	1.67 \pm 0.6	1.67 \pm 0.6	1.250 ^b	6.68
	3.4	25	0.00 \pm 0.0	2.00 \pm 1.0	2.67 \pm 0.6	3.67 \pm 1.2	2.083 ^c	14.68
	4.0	25	0.00 \pm 0.0	2.67 \pm 1.5	4.33 \pm 1.5	6.33 \pm 1.2	3.333 ^d	25.32
	Control	25	0.00 \pm 0.0	0.72 \pm 0.4	1.33 \pm 0.3	1.33 \pm 0.3	0.845 ^a	5.32
	Effect over time		0.067 ^a	1.333 ^b	2.133 ^c	2.600 ^c		

Means followed by the same letter(s) are not significantly different ($p > 0.05$) with DNMR Test

Table 2: Larvicidal activity of hexane extract of *N. tabacum* against 4th instar larvae of mosquitoes

Mosquito larvae tested	Concentration (mg/100mL)	Number of larvae treated	Mean death \pm SD				Total mean death	Mortality (%)
			Exposure period (Hrs.)					
			12	24	48	72		
<i>Aedes</i> spp.	0.9	25	0.00 \pm 0.0	0.67 \pm 1.2	1.33 \pm 0.6	2.33 \pm 1.5	1.083 ^a	9.32
	1.6	25	0.67 \pm 1.2	1.00 \pm 1.0	1.33 \pm 0.6	2.67 \pm 0.6	1.417 ^{ab}	10.68
	2.5	25	1.00 \pm 1.0	1.67 \pm 0.6	2.00 \pm 1.0	3.33 \pm 0.6	2.00 ^{bc}	13.32
	3.4	25	2.00 \pm 0.0	2.00 \pm 0.0	2.33 \pm 0.6	4.00 \pm 1.0	2.583 ^c	16.00
	4.0	25	2.67 \pm 1.2	2.67 \pm 1.2	3.00 \pm 1.0	5.33 \pm 1.2	3.417 ^d	21.32
	Control	25	0.00 \pm 0.0	0.33 \pm 0.3	0.72 \pm 0.3	1.33 \pm 0.3	0.595 ^a	5.32
	Effect over time			1.267 ^a	1.600 ^a	2.000 ^a	3.533 ^b	
<i>Anopheles</i> spp.	0.9	25	0.67 \pm 1.2	1.67 \pm 0.6	1.67 \pm 0.6	1.67 \pm 0.6	1.417 ^a	6.68
	1.6	25	1.00 \pm 1.0	2.00 \pm 1.0	2.00 \pm 1.0	2.33 \pm 0.6	1.833 ^b	9.32
	2.5	25	1.00 \pm 1.0	2.67 \pm 1.2	3.33 \pm 1.2	3.67 \pm 0.6	2.667 ^b	14.68
	3.4	25	2.00 \pm 1.0	2.67 \pm 0.6	3.33 \pm 0.6	4.00 \pm 1.0	3.000 ^c	16.00
	4.0	25	3.00 \pm 0.0	3.33 \pm 0.6	4.33 \pm 0.6	5.67 \pm 0.6	4.083 ^c	22.68
	Control	25	0.33 \pm 0.3	0.33 \pm 0.3	0.73 \pm 0.3	1.33 \pm 0.3	0.880 ^d	5.32
	Effect over time			1.533 ^a	2.467 ^b	2.933 ^{bc}	3.467 ^c	
<i>Culex</i> spp.	0.9	25	0.00 \pm 0.0	0.67 \pm 1.2	1.33 \pm 0.6	1.33 \pm 0.6	0.833 ^a	5.32
	1.6	25	0.00 \pm 0.0	1.33 \pm 0.6	1.00 \pm 1.0	1.33 \pm 1.2	0.917 ^a	5.32
	2.5	25	1.00 \pm 1.0	1.33 \pm 1.2	2.00 \pm 0.0	2.00 \pm 0.0	1.583 ^b	8.00
	3.4	25	1.67 \pm 0.6	2.00 \pm 0.0	2.67 \pm 1.2	2.67 \pm 1.2	2.250 ^c	10.68
	4.0	25	1.67 \pm 0.6	2.00 \pm 0.0	2.67 \pm 0.6	3.33 \pm 0.6	2.417 ^c	13.32
	Control	25	0.33 \pm 0.3	0.33 \pm 0.3	1.00 \pm 0.0	1.00 \pm 0.0	0.665 ^a	4.0
	Effect over time			0.867 ^a	1.467 ^b	1.933 ^{bc}	2.133 ^c	

Means followed by the same letter(s) are not significantly different ($p > 0.05$) with DNMR Test

Table 3: Larvicidal activity of chloroform extract of *D. metel* against 4th instar larvae of mosquitoes

Mosquito larvae tested	Concentrations (mg/100mL)	Number of larvae treated	Mean death \pm SD				Total mean death	Mortality (%)
			Exposure period (Hrs.)					
			12	24	48	72		
<i>Aedes</i> spp.	0.9	25	0.00 \pm 0.0	0.67 \pm 0.6	1.00 \pm 0.0	1.67 \pm 0.6	0.833 ^{ad}	6.68
	1.6	25	0.67 \pm 0.6	1.33 \pm 0.6	1.33 \pm 0.6	1.67 \pm 0.6	1.250 ^a	6.68
	2.5	25	0.67 \pm 0.6	1.33 \pm 0.6	2.00 \pm 1.0	2.33 \pm 1.5	1.583 ^{ab}	9.32
	3.4	25	1.33 \pm 0.6	1.67 \pm 1.2	2.33 \pm 1.5	3.00 \pm 1.7	2.083 ^b	12.00
	4.0	25	2.00 \pm 0.0	2.00 \pm 0.0	3.33 \pm 1.5	4.33 \pm 0.6	2.916 ^c	17.32
	Control	25	0.16 \pm 0.4	0.44 \pm 0.6	0.83 \pm 0.6	1.17 \pm 0.6	0.653 ^d	4.68
	Effect over time		0.933 ^a	1.400 ^{ab}	2.000 ^{bc}	2.600 ^c		
<i>Anopheles</i> spp.	0.9	25	0.33 \pm 0.6	0.33 \pm 0.6	0.33 \pm 0.6	0.67 \pm 0.6	0.416 ^a	2.68
	1.6	25	0.67 \pm 1.2	1.00 \pm 1.0	1.00 \pm 1.0	1.33 \pm 1.5	1.000 ^a	5.32
	2.5	25	1.67 \pm 1.6	1.67 \pm 0.6	1.67 \pm 0.6	2.67 \pm 0.6	1.916 ^b	10.68
	3.4	25	2.33 \pm 0.6	2.67 \pm 0.6	2.67 \pm 0.6	4.00 \pm 2.0	2.916 ^c	16.00
	4.0	25	2.33 \pm 1.6	3.33 \pm 1.2	4.33 \pm 0.6	5.33 \pm 1.5	3.833 ^d	21.32
	Control	25	0.28 \pm 0.5	0.45 \pm 0.5	0.83 \pm 0.6	1.2 \pm 0.6	0.691 ^a	4.80
	Effect over time		1.466 ^a	1.800 ^a	2.000 ^a	2.800 ^b		
<i>Culex</i> spp.	0.9	25	0.00 \pm 0.0	0.00 \pm 0.0	0.00 \pm 0.0	0.67 \pm 0.6	0.166 ^a	2.68
	1.6	25	0.33 \pm 0.6	0.33 \pm 0.6	0.33 \pm 0.6	1.67 \pm 1.5	0.666 ^{ab}	6.68
	2.5	25	0.67 \pm 0.6	0.67 \pm 0.6	1.00 \pm 0.0	2.33 \pm 1.5	1.166 ^b	9.32
	3.4	25	1.33 \pm 0.6	1.33 \pm 0.6	1.67 \pm 1.5	3.72 \pm 1.8	1.833 ^c	14.88
	4.0	25	2.67 \pm 0.6	2.67 \pm 0.6	2.81 \pm 0.7	3.89 \pm 1.8	2.750 ^d	15.56
	Control	25	0.16 \pm 0.4	0.44 \pm 0.6	0.88 \pm 0.5	0.88 \pm 0.5	0.591 ^a	3.52
	Effect over time		1.000 ^a	1.000 ^a	1.066 ^a	2.200 ^b		

Means followed by the same letter(s) are not significantly different ($p > 0.05$) with DNMR Test

Table 4: Larvicidal activity of chloroform extract of *N. tabacum* against 4th instar larvae of mosquitoes

Mosquito larvae tested	Concentrations (mg/100mL)	Number of larvae treated	Mean death \pm SD				Total mean death	Mortality (%)
			Exposure period (Hrs.)					
			12	24	48	72		
<i>Aedes</i> spp.	0.9	25	0.00 \pm 0.0	0.00 \pm 0.0	1.00 \pm 1.0	1.33 \pm 0.6	0.583 ^{ac}	5.32
	1.6	25	0.00 \pm 0.0	1.00 \pm 1.0	1.33 \pm 0.6	2.33 \pm 0.6	1.167 ^{ab}	9.32
	2.5	25	0.33 \pm 0.6	1.33 \pm 0.6	1.67 \pm 0.6	2.33 \pm 0.6	1.417 ^b	9.32
	3.4	25	1.67 \pm 1.2	2.00 \pm 1.0	2.67 \pm 0.6	3.00 \pm 1.0	2.333 ^c	12.00
	4.0	25	3.00 \pm 1.7	3.33 \pm 1.5	4.00 \pm 1.0	4.33 \pm 1.5	3.667 ^d	17.32
	Control	25	0.00 \pm 0.0	0.00 \pm 0.0	0.71 \pm 0.3	1.00 \pm 0.0	0.427 ^e	4.00
	Effect over time			1.000 ^a	1.533 ^{ab}	2.133 ^{bc}	2.667 ^c	
<i>Anopheles</i> spp.	0.9	25	0.00 \pm 0.0	0.33 \pm 0.6	1.67 \pm 2.1	3.00 \pm 2.0	1.250 ^a	12.00
	1.6	25	0.33 \pm 0.6	0.67 \pm 0.5	1.33 \pm 0.6	2.67 \pm 1.5	1.250 ^a	10.68
	2.5	25	1.33 \pm 1.2	2.00 \pm 0.0	2.67 \pm 1.2	3.00 \pm 1.0	2.250 ^{ab}	12.00
	3.4	25	0.67 \pm 1.2	3.00 \pm 1.0	4.00 \pm 1.7	4.33 \pm 1.5	3.000 ^b	17.32
	4.0	25	3.33 \pm 1.5	4.00 \pm 1.7	4.33 \pm 1.5	5.67 \pm 0.6	4.333 ^c	22.68
	Control	25	0.00 \pm 0.0	0.33 \pm 0.3	0.73 \pm 0.3	1.33 \pm 0.3	0.427 ^d	5.32
	Effect over time			1.133 ^a	2.000 ^{ab}	2.800 ^b	3.733 ^c	
<i>Culex</i> spp.	0.9	25	0.33 \pm 0.6	1.00 \pm 1.0	1.33 \pm 0.6	1.33 \pm 0.6	1.000 ^{ac}	5.32
	1.6	25	1.00 \pm 1.0	1.33 \pm 0.6	1.67 \pm 0.6	2.33 \pm 1.5	1.583 ^a	9.32
	2.5	25	1.67 \pm 2.1	2.67 \pm 0.6	2.67 \pm 0.6	3.33 \pm 1.5	2.583 ^a	13.32
	3.4	25	0.67 \pm 2.1	2.67 \pm 1.2	3.33 \pm 0.6	3.67 \pm 0.6	3.083 ^b	14.68
	4.0	25	1.67 \pm 0.6	3.33 \pm 1.2	3.67 \pm 0.6	3.67 \pm 0.6	3.083 ^b	14.68
	Control	25	0.33 \pm 0.3	0.71 \pm 0.3	0.71 \pm 0.3	0.71 \pm 0.3	0.615 ^c	2.84
	Effect over time			1.067 ^a	2.200 ^b	2.533 ^b	2.867 ^b	

Means followed by the same letter(s) are not significantly different ($p > 0.05$) with DNMR Test

Table 5: Larvicidal activity of methanol extract of *D. metel* against 4th instar larvae of mosquitoes

Mosquito larvae tested	Concentration (mg/100mL)	Number of larvae treated	Mean death \pm SD				Total mean death	Mortality (%)
			Exposure period (Hrs.)					
			12	24	48	72		
<i>Aedes</i> spp.	0.9	25	0.00 \pm 0.0	0.67 \pm 1.2	1.67 \pm 0.6	1.67 \pm 0.6	1.000 ^{ad}	6.68
	1.6	25	0.67 \pm 0.6	0.67 \pm 0.6	1.67 \pm 1.2	2.00 \pm 1.0	1.250 ^a	8.00
	2.5	25	1.00 \pm 0.0	1.33 \pm 0.6	1.67 \pm 0.6	2.00 \pm 0.0	1.500 ^{ab}	8.00
	3.4	25	1.67 \pm 1.2	2.00 \pm 1.0	2.00 \pm 1.0	2.33 \pm 0.6	2.00 ^{bc}	9.32
	4.0	25	1.67 \pm 0.6	2.00 \pm 1.0	3.00 \pm 1.0	3.33 \pm 0.6	2.500 ^c	13.32
	Control	25	0.33 \pm 0.3	0.33 \pm 0.3	0.73 \pm 0.3	0.73 \pm 0.3	0.533 ^d	2.92
	Effect over time		1.000 ^a	1.333 ^a	2.000 ^b	2.267 ^b		
<i>Anopheles</i> spp.	0.9	25	0.00 \pm 0.0	0.33 \pm 0.6	1.00 \pm 1.0	1.00 \pm 1.0	0.583 ^{ad}	4.00
	1.6	25	0.67 \pm 1.2	0.67 \pm 1.2	1.33 \pm 0.6	1.33 \pm 0.6	1.000 ^a	5.32
	2.5	25	0.67 \pm 0.6	1.67 \pm 0.6	1.67 \pm 0.6	2.67 \pm 0.6	1.667 ^b	10.68
	3.4	25	0.67 \pm 0.6	2.00 \pm 1.0	2.33 \pm 1.2	3.33 \pm 1.2	2.083 ^b	13.32
	4.0	25	2.33 \pm 0.6	2.67 \pm 0.6	3.67 \pm 0.6	4.67 \pm 1.2	3.333 ^c	18.68
	Control	25	0.00 \pm 0.0	0.00 \pm 0.0	0.73 \pm 0.3	0.73 \pm 0.3	0.365 ^d	2.92
	Effect over time		0.867 ^a	1.467 ^b	2.000 ^b	2.600 ^c		
<i>Culex</i> spp.	0.9	25	0.00 \pm 0.0	0.00 \pm 0.0	0.67 \pm 1.2	1.00 \pm 1.0	0.417 ^{ad}	4.00
	1.6	25	0.00 \pm 0.0	0.33 \pm 0.6	0.67 \pm 0.6	0.67 \pm 0.6	0.417 ^{ad}	2.68
	2.5	25	0.67 \pm 0.6	1.00 \pm 0.0	1.00 \pm 0.0	1.00 \pm 0.0	0.917 ^{ab}	4.00
	3.4	25	1.00 \pm 1.0	1.33 \pm 0.6	1.33 \pm 0.6	1.67 \pm 0.6	1.333 ^b	6.68
	4.0	25	1.33 \pm 0.6	1.67 \pm 0.6	2.67 \pm 0.6	2.67 \pm 0.6	2.083 ^c	10.68
	Control	25	0.00 \pm 0.0	0.00 \pm 0.0	0.33 \pm 0.3	0.33 \pm 0.3	0.165 ^d	1.32
	Effect over time		0.600 ^a	0.867 ^{ab}	1.267 ^{bc}	1.400 ^c		

Means followed by the same letter(s) are not significantly different ($p > 0.05$) with DNMR Test

Table 6: Larvicidal activity of methanol extract of *N. tabacum* against 4th instar larvae of mosquitoes

Mosquito larvae tested	Concentration (mg/100mL)	Number of larvae treated	Mean death \pm SD				Total mean death	Mortality (%)
			Exposure period (Hrs.)					
			12	24	48	72		
<i>Aedes</i> spp.	0.9	25	0.67 \pm 1.2	1.00 \pm 1.0	2.67 \pm 1.6	2.67 \pm 1.6	1.750 ^a	10.68
	1.6	25	1.00 \pm 1.0	2.67 \pm 1.6	3.33 \pm 1.2	3.67 \pm 0.6	2.667 ^{ab}	14.68
	2.5	25	2.33 \pm 1.5	3.00 \pm 1.0	4.00 \pm 1.0	4.67 \pm 0.6	3.500 ^b	18.68
	3.4	25	2.67 \pm 1.2	4.67 \pm 1.5	5.00 \pm 1.0	5.67 \pm 1.2	4.500 ^c	22.68
	4.0	25	4.67 \pm 1.2	4.67 \pm 1.2	5.67 \pm 1.2	6.33 \pm 1.2	5.333 ^c	25.32
	Control	25	0.00 \pm 0.0	0.73 \pm 0.3	0.73 \pm 0.3	1.00 \pm 0.3	0.615 ^d	4.00
	Effect over time		2.267 ^a	3.200 ^b	4.133 ^c	4.600 ^c		
<i>Anopheles</i> spp.	0.9	25	0.00 \pm 0.0	3.33 \pm 1.5	4.67 \pm 1.5	5.67 \pm 1.5	3.417 ^a	22.68
	1.6	25	0.00 \pm 0.0	4.67 \pm 2.5	6.00 \pm 2.0	6.67 \pm 1.2	4.333 ^a	26.68
	2.5	25	2.33 \pm 0.6	6.67 \pm 2.1	8.67 \pm 1.5	9.67 \pm 2.5	6.833 ^b	38.68
	3.4	25	4.00 \pm 2.0	9.67 \pm 3.1	10.33 \pm 2.5	17.67 \pm 2.5	10.417 ^c	70.68
	4.0	25	6.33 \pm 2.1	15.67 \pm 2.5	18.67 \pm 2.5	20.33 \pm 2.1	15.250 ^d	80.00
	Control	25	0.00 \pm 0.0	0.33 \pm 0.3	0.33 \pm 0.3	0.73 \pm 0.3	0.348 ^e	2.92
	Effect over time		2.533 ^a	8.000 ^b	9.667 ^c	12.000 ^d		
<i>Culex</i> spp.	0.9	25	0.00 \pm 0.0	0.33 \pm 0.6	1.00 \pm 1.0	1.00 \pm 1.0	0.583 ^a	4.00
	1.6	25	0.67 \pm 1.2	1.33 \pm 0.6	1.33 \pm 0.6	2.00 \pm 0.0	1.333 ^{ab}	8.00
	2.5	25	1.00 \pm 1.7	1.67 \pm 1.2	2.33 \pm 0.6	2.67 \pm 0.6	1.917 ^{bc}	10.68
	3.4	25	1.00 \pm 1.0	2.33 \pm 0.6	2.67 \pm 0.6	3.33 \pm 1.5	2.333 ^c	13.32
	4.0	25	1.0 \pm 0.0	3.00 \pm 2.0	3.33 \pm 1.5	3.67 \pm 2.1	2.750 ^c	14.68
	Control	25	0.00 \pm 0.0	0.33 \pm 0.3	1.00 \pm 0.0	1.00 \pm 0.0	0.582 ^a	4.00
	Effect over time		0.733 ^a	1.733 ^b	2.133 ^b	2.533 ^b		

Means followed by the same letter(s) are not significantly different ($p > 0.05$) with DNMR Test

Table 7: Lethal concentrations (72 hrs.) of *D. metel* and *N. tabacum* extract against 4th instar larvae of mosquitoes

<i>D. metel</i> extract	Mosquito spp.	No of larvae treated	Concentrations		95% confidence limit (mg/mL)		X ²
			LC ₅₀ (mg/mL)	LC ₉₀ (mg/mL)	LCL	UCL	
Hexane	<i>Aedes</i> spp.	25	3.13±0.1	4.66	2.93	3.32	8.25
	<i>Anopheles</i> spp.	25	2.94±0.1	5.35	2.67	3.21	14.43
	<i>Culex</i> spp.	25	5.60±0.3	8.43	5.10	6.11	3.98
Chloroform	<i>Aedes</i> spp.	25	9.35±0.5	16.20	8.36	10.34	1.09
	<i>Anopheles</i> spp.	25	6.22±0.3	9.88	5.69	6.75	0.23
	<i>Culex</i> spp.	25	8.60±0.4	14.18	7.78	9.40	0.06
Methanol	<i>Aedes</i> spp.	25	14.62±0.7	26.19	13.17	16.07	0.59
	<i>Anopheles</i> spp.	25	7.26±0.3	11.91	6.59	7.93	0.55
	<i>Culex</i> spp.	25	11.01±0.9	17.76	9.29	12.72	2.04
<i>N. tabacum</i> extract							
Hexane	<i>Aedes</i> spp.	25	9.12±0.4	16.90	8.25	9.99	0.61
	<i>Anopheles</i> spp.	25	7.46±0.4	13.14	6.74	8.17	0.49
	<i>Culex</i> spp.	25	10.45±0.5	17.77	9.39	11.51	0.55
Chloroform	<i>Aedes</i> spp.	25	9.46±0.5	16.51	8.58	10.35	0.74
	<i>Anopheles</i> spp.	25	9.56±0.5	18.20	8.60	10.53	1.84
	<i>Culex</i> spp.	25	10.10±0.5	18.11	9.10	11.10	0.24
Methanol	<i>Aedes</i> spp.	25	7.58±0.4	14.70	6.79	8.38	0.04
	<i>Anopheles</i> spp.	25	2.58±0.1	4.87	2.32	2.84	8.29
	<i>Culex</i> spp.	25	11.03±0.6	19.73	9.94	12.12	0.002

LC₅₀ = lethal concentration that kills 50% of the exposed larvae, LC₉₀= lethal concentration that kills 90% of the exposed larvae
 LCL = lower confidence limit, UCL = upper confidence limit, X² =chi-square values

Table 8: Qualitative analysis of phytochemical contents of the most active extracts

Phytochemical compounds	Plant extracts	
	<i>D. metel</i>	<i>N. tabacum</i>
Flavonoids	+	ND
Tannins	ND	ND
Saponins	+++	+++
Alkaloids	+++	+++
Cardiac Glycosides	++	++
Saponin Glycosides	ND	+++
Volatile oils	+++	+
Glycosides	++	++
Steroids	+++	+++
Anthraquinones	ND	ND

Key: ND = Not Detected, + = Present in trace amount, ++ = Moderately present, +++ = Highly present

Table 9: Quantitative Phytochemical analysis of most active extracts of the plants (in g% W/V) used

Plants extracts	Compounds (per-cent)			
	Saponins	Alkaloids	Flavonoids	Total glycosides
<i>D. metel</i> hexane extract	2.48	2.89	6.81	9.16
<i>N. tabacum</i> methanol extract	2.88	3.2	-	7.03

Key: 0.01-2g% = Trace amount, 2-3g% present, 3-above present in large amount

REFERENCES

- Ahmed, S.M.; Saeed, M.; Nawaz, A.; Usman, M.; Shoukat, R.F.; Li, S.; Zhang, Y.; Zeng, L.; Zafar, J.; Akash, A. (2018). Monitoring of quantitative and qualitative losses by Lepidopteran, and Homopteran pests in different crop production systems of *Brassica oleracea* L. *Journal of Entomology and Zoology Studies*, 6: 06–12.
- Anyaele, O.O. and Amusan, A.A.S. (2003). Toxicity of hexanolic extract of *Dennettia Tripetala* (G.Baxer) on larvae of *Aedes aegypti* (L). *African Journal of Biomedical Research*, 6: 49 – 53.
- Anyanwu, I.N., Agbeck, R.I.S., Ajanusi, O.J. and Uma, J.U. (1991) A survey of culicids (Mosquitoes) in a Northern Guinea savana town of Zaria Kaduna state. *Nigeria. Journal of Parasitology*, 2: 137 – 148.
- Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. (2008). Biological effects of essential oils—A review. *Food Chemistry Toxicology*, 46: 446–475.
- Berkov, S. and Zayed, R. (2004). Comparison of Trophane alkaloid spectra between *Datura innoxia* grown in Egypt and Bulgaria. *Zeitschrift for Nature Forschung*, 89: 184-186.
- Chakkaravarthy, V.M., Ambrose, T., Vincent, S., Arunachalam, R., Paulraj, M.G., Ignacimuth, S. and Annadurai, G. (2011). Bioefficacy of *Azadirachta indica* (A.Juss) and *Datura metel* (Linn.) leaves extracts in controlling *Culex quinquefasciatus* (Diptera: Culicidae). *Journal of Entomology*, 2: 1-7.
- Conti, B.; Leonardi, M.; Pistelli, L.; Profeti, R.; Ouerghemmi, I. and Benelli, G. (2013). Larvicidal and repellent activity of essential oils from wild and cultivated *Ruta chalepensis* L. (Rutaceae) against *Aedes albopictus* Skuse (Diptera: Culicidae), an arbovirus vector. *Parasitology Research*, 112: 991–999.
- Ghosh, A., Chowdhury, N. and Chandra, G. (2012). Plant extracts as potential mosquito larvicides. *Indian Journal of Medical Research*, 135: 581-598.
- Gopieskhanna, V. K., and Kannabiran, K. (2007). Larvicidal effect of *Hemidesmus indicus*, *Gymnema Sylvestre*, and *Eclipta prostrate* against *Culex quinquefasciatus* mosquito larvae. *African Journal of Biotechnology*, 6(3): 307 – 311.
- Habeeb, S.M. (2010). Ethno- Veterinary and medical knowledge of crude plant extracts and its methods of application (Traditional and Modern) for tick control. *World Applied Sciences Journal*, 11(9): 1047-1054.

- Hadjiakhoondi, A., Vatandoost, H., Khanavi, M. and Reza, M.A. (2005). Biochemical investigation of different extracts and larvicidal activity of *Targets minuta* L. on *Anopheles stephensi* larvae. *Iranian Journal of Pharmaceutical Sciences*, 1(2): 81-84.
- Harrison, B.A. (2005). Field identification of adult and larval mosquitoes. Available on line at http://www-rciorutgers.edu/2_insects/mosq/life.htm. (accessed August 2017).
- Ima, A. B., Cicero, B.M. Alexandre, A.P. and Denise, V. (2005). Evaluation of methoprene effect on *Aedes aegypti* (Diptera: Culicidae) development in laboratory conditions. *Memorias do Instituto Oswaldo Cruz*, 100 (4): 1 – 17.
- Klock, J.A. (1987). Natural plant compounds useful in insect control. *American Chemical Society*, 330: 396-415.
- Kovendan, K., Murugan, K. and Vincent, S. (2012). Evaluation of larvicidal activity of *Acalypha alnifolia* Kleinex Willd. (Euphorbiaceae) leaf extract against the malarial vector, *Anopheles stephensi*, dengue vector, *Aedes aegypti* and Bancroftian filariasis vector, *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitology Research*, 110: 571-81.
- Kumar, A.N. and Murugan, K. (2011). Antimalarial and antivectoral activities of traditionally used indigenous medicinal plants. *Advances in environmental Biology*, 5(4): 407-412.
- Kumar, M.S. and Maneemegalai, S. (2008). Evaluation of larvicidal effect of *Lantara camara* Linn. Against mosquito species *Aedes aegypti* and *Culex quinquefasciatus*. *Advances in Biological Research*, 2 (3-4): 39-43.
- Kumar, S.; Mishra, M.; Wahab, N.; Warikoo, R. (2014). Larvicidal, repellent, and irritant potential of the seed-derived essential oil of *Apium graveolens* against dengue vector, *Aedes aegypti* L. (Diptera: Culicidae) *Front. Public Health*, 2: 147-152.
- Kutama, A.D. Mohammed, A.S. and Kiyawa, A.S. (2010). Hallucinogenic effect of *Datura metal* L. leaf extract in albino rats. *Bioscience Research Communications*, 22(4): 215-220.
- Macedo, M.E., Rotraut, A.G.B. and Consoli (1997). Screening of Asteraceae (Compositae) plant extracts for larvicidal activity against *Aedes fluviatilis* (Diptera: Culicidae). *Memoes. Institude. Oswaldo Cruz. Rio de Janeiro*, 92(4): 565-570.
- Mdoe, F.P.; Cheng, S.S.; Lyaruu, L.; Nkwengulila, G.; Chang, S.T.; Kweka, E.J. (2014). Larvicidal efficacy of *Cryptomeria japonica* leaf essential oils against *Anopheles gambiae*. *Parasite Vector*, 7: 426.
- Medlock, J.M., Hansford, K.M., Schaner, F., Versteirt, V., Hendrickx, G., Zeller, H. and Bortel, W.V. (2012). A review of the invasive mosquitoes in Europe: Ecology, public health risks, and control options. *Vector-Borne Zoonotic Disease*, 12: 435–447.
- Michele, M.C. and O'Meara, G.F. (2008). *Quick Guid to Mosquito Genera* (Larva-Adult). Florida Medical Entomology Laboratory, University of Florida. 1-83pp.
- Ogundare, A.O. Adetuyi, F.C. and Akinyosoye, F.A. (2006). Antimicrobial activity of *Vernonia tenoreana*. *African Journal of Biotechnology*, 5(18):1663-1668.
- Ohia, C.M.D. and Ana, G.R.E.E. (2015). Bio-insecticides: the one- health response to mosquito borne diseases of public health importance. *Journal of Biology, Agriculture and Healthcare*, 5: 22–26.
- Pavela, R. (2015). Acute toxicity and synergistic and antagonistic effects of the aromatic compounds of some essential oils against *Culex quinquefasciatus* Say larvae. *Parasitology Research*, 114: 3835–3853.
- Pelah D., Abramovich, Z., Markus, A. and Wiesman, Z. (2002). The use of commercial saponin from *Quillaja saponaria* bark as a natural larvicidal agent against *Aedes aegypti* and *Culex pipiens*. *Journal of Ethnopharmacology*, 81(3): 407-409.
- Rice, E. L. (1983). Pest control with nature's chemicals. University of Oklahoma Press, Norman Ok. 136-137.pp.
- Rizvi, S.A.H.; Tao, L.; Zeng, X. (2018). Chemical composition of essential oil obtained from *Artemisia absinthium* L. grown under the climatic condition of Skardu Baltistan of Pakistan. *Pakistan Journal of Botany*, 50: 599–604.
- Shakeel, M.; Farooq, M.; Nasim, W.; Akram, W.; Khan, F.Z.A.; Jaleel, W.; Zhu, X.; Yin, H.; Li, S.; Fahad, S. (2017). Environment polluting conventional chemical control compared to an environmentally friendly IPM approach for control of diamondback moth, *Plutella xylostella* (L.), in China: A review. *Environmental Science Pollution Research*, 24: 14537–14550.
- Sujathar C.H Vasuki, V., Mariappan, T., Kalyanasundaran, M. and Das, P.K. (1988). Evaluation of Plant extracts for biological activity against mosquitoes. *International Pest Control*, 30: 122-124.
- Thomas, A.; Mazigo, H.D.; Manjurano, A.; Morona, D.; Kweka, E.J. (2017). Evaluation of active ingredients and larvicidal activity of clove and cinnamon essential oils against *Anopheles gambiae* (sensu lato). *Parasite Vector*, 10, 411 – 416
- World Health Organization (2006). Malaria Entomology and Vector Control. *Communicable Diseases Cluster WHO*, 48-90.
- Wiesman, Z. and Chapagan, B.P (2006). Larvicidal activity of the fruit mesocarp extract of *Balanitis aegyptiaca* and its saponin fraction against *Aedes aegypti*. *Dengue Bulletin*, 29: 203-207.
- Yahaya, M.M. and Ruqayya, I.I. (2013). Evaluation of hexanolic extracts of *Ricinus communis* (L.) kernel against the larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae). *International Journal of Applied Research and Technology*, 2: 172 – 179.