



Larvicidal Activity of Solvent Extracts of *Nicotiana tabacum* L, and *Datura metel* L, (Solanales: Solanaceae) Against Larvae of Three Mosquito Species

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Abstract

Mosquitoes are insects that vector many of the life-threatening diseases. Control of these insects is one of the major problems of the world today. Leaves and seed kernel extracts of Tobacco; *Nicotiana tabacum* (L.) and Thorn-Apple; *Datura metel* (L.) were assayed for larvicidal activity against three mosquito species of *Aedes*, *Anopheles* and *Culex* respectively. Samples of the three species were collected from mosquito breeding sites and reared in the laboratory for continued source of larvae following standard procedures. 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 tested for larvicidal activity against 25 larvae of each genus in three replicates, alongside the controls over 12 to 72 hrs periods. The extracts that exhibited highest larvicidal activity were subjected to phytochemical screenings for searching active ingredients. Data were analyzed using Analysis of Variance, treatment means separated through DNMRT and lethal concentrations of the extracts were determined. Among the extracts tested, hexane extract of *D. metel* and methanol extract of *N. tabacum* caused highest larvicidal activity with more than 70% mortality on *Aedes* and *Anopheles* larvae when 4.0mg/mL was applied after 72 hrs (LC_{50} 2.94±0.1 and LC_{50} 2.58±0.1 mg/mL respectively). *Culex* larvae were less susceptible to all concentrations of the extracts. Though, larvicidal activity was observed to be directly 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.

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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 by WHO (2006) as the “king of diseases” puts more than 2.4 billion of the world’s population at risk. The annual incidence of malaria worldwide was estimated to be between 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 and sumitrin while others are used as larvicides against the larvae, like methoprene (an Insect Growth Regulator), Altosid, Abate, BVA oils and agnigue. 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 as insect toxicants such as repellents, feeding deterrents, attractants, sterilants and inhibitors of growth and development. The current study determined the efficacy and lethal formulations of *Nicotiana tabacum* and *Datura metel* on the larvae of *Aedes*, *Anopheles* and *Culex* mosquitoes as alternatives to synthetic insecticides against these insects. There are more than 3500 mosquito species in the world grouped into 41 genera and 135 subgenera (Reinert, 2000; CDC, 2004). Of these numbers, around 73 species occur in Nigeria (Philip, 1931; Service, 1963) with more prominent ones in the genus *Aedes*; 40 common species, *Anopheles*; one or more species found in each state and *Culex*; 29 species with 12 common ones (Gillette, 1972). Adults are capable of flying long distances, as much as 60 km during the day in search of a blood meal and become serious pests in residential communities (Robinson, 1995). *Aedes* mosquitoes are strong fliers known to fly many miles from their breeding sources; *Culex* and *Anopheles* are weak flies (Patric and Arwin 1983). However, the mosquito species associated with human environment, usually have a short flight range to find suitable larval breeding locations and suitable host for adult feeding within a distance of a few city blocks.

MATERIALS AND METHODS

Collection and extraction of plant materials

Seed samples of Thorn-Apple (*Datura metel*) and leaves of Tobacco (*Nicotiana tabacum*) were obtained from Dabagi farm of Usmanu Danfodiyo University, Sokoto (13° 00'N, 5° 14'E, alt 274 m) and identified by a plant taxonomist at the Herbarium of the Department of Botany, Usmanu Danfodiyo University, Sokoto. All the samples were air-dried under shade for 3 days. The leaves were pulverized into fine powder and sieved with 1 mm mesh. The seed coat of *D. metel* was removed manually using pestle and mortar. It was then winnowed and sieved to obtain whitish seed kernels (Ayinde *et al.*, 2020). The kernel was macerated using an electric blender (WARING MX-1100XT) and then shade-dried to obtain the powder. Crude extracts of each of hexane, chloroform and methanol were prepared from the powdered materials, filtered and evaporated in a drying cabinet overnight at 40°C. Each extract was subjected to fractionation with hexane, chloroform and methanol in the increasing order of polarity (Ogundare *et al.*, 2006). In this case, 300 grams of dried *N. tabacum* crude extract was reconstituted in 500 mL distilled water and equal volume of hexane was added in a separation funnel. This was mixed vigorously (manually) for about 40 minutes and the hexane fraction was carefully separated into a beaker. Equal volume of chloroform was added in the remaining aqueous fraction to obtain chloroform fraction and same process was repeated for methanol fraction. Each fraction was evaporated to dryness on a Buchi Rotavapor (Labortechnik, Switzerland). Four (4 g) gram sample was measured from each extract and made up to 1 L with distilled water to form 4000mg/L (4mg/mL) as stock. The *D. metel* extracts contain oil, thus, were

emulsified with 0.009 ml of polysorbate (Tween-80®) (Anyaele and Amusan, 2003) for each stock solution.

Collection and identification of mosquito larvae

Larvae of mosquitoes were sampled from three locations in Sokoto; 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). Breeding sites such as gutters and man-made ditches of less than 2 meters deep were searched for the larvae. Collection of the larvae was made using plastic dipper (300 ml, handle length: 11 cm) by simple scooping. The composite samples were sorted and identified to genus level in the laboratory using the keys of Harrison (2005) and pictorial guide of Michele and O'Meara (2008). Larvae belonging to three genera; *Aedes*, *Anopheles* and *Culex* mosquitoes were duly identified and used in the study.

Rearing of the larvae

The larvae were fed with brewer's yeast using WHO (2006) protocol in plastic trays inside wooden cum wire gauze cage (50 x 30 x 30 cm). The cage had internal demarcation of mesh netting (3 cm² 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 (shaved from the back and underside). In this procedure, two generations of the mosquitoes were maintained and used as larval stock for the study.

Larvicidal bioassay

Each extract was formulated into five concentrations which were informed by a preliminary test that found the lowest concentration that killed all the larvae and the highest concentration that killed none (Anyaele *et al.*, 2002). The concentrations

were 0.9, 1.6, 2.5, 3.4 and 4.0 mg/mL. Dilution formula was used to prepare the formulations and each was placed in particular 250 ml beaker capacity, also containing 5 mL of 12 mg albumin (1.3 g/100 mL H₂O) as larval food (Ima *et al.*, 2005), while the final volume adjusted to 100 mL. Into the beaker, 25 fourth instar larvae of *Aedes* mosquito from the larval stock were released. The treated larvae were maintained in the incubator held at $27 \pm 2^{\circ}\text{C}$ and $75 \pm 5\%$ RH (Pavela, 2015). A separate beaker containing same amount of larval food in 100mL distilled water was also provided with 25 fourth instar larvae of the same genus to serve as control. All treatments were replicated three times including the control. Observation on the mortality of both treated and untreated larvae was made at 12, 24, 48 and 72 hrs. by counting and recording the number of dead larvae in each case. Similar procedure was repeated when the larvae of *Anopheles* and *Culex* were treated. For *D. metel* control settings however, same amount of polysorbate 80® was added (0.009mL) in the beakers containing larvae, as in stock solution.

Phytochemical Analyses

Phytochemical screening of the most active extracts was carried out using standard procedures to search for bioactive compounds as described by Kumar and Maneemegalai (2008).

Analysis of Data

Data obtained from the dose-response assays were subjected to Analysis of Variance (ANOVA) procedure using SAS v.9.1 software and significant differences in the treatment means were separated with Duncan's New Multiple Range Test (DNMRT) at 5% level. The median and lethal concentrations (72 hr. LC₅₀, 90) of the

different extracts were determined along with other statistics using a computerized probit regression (license) developed as Statplus (2009).

RESULTS

Larvicidal Activity of the Extracts

The lowest concentration of *D. metel* hexane extract (0.9 mg/ml) administered to *Aedes* larvae for 12 hrs, caused a single death but slightly increased after 72 hrs. of exposure (Table 1). At 1.6 and 2.5 mg/mL, the mean larval mortality did not differ significantly ($p > 0.05$). Increase in concentrations of the extract to 3.4 mg/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 4.0 mg/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 when compared to mean death of larvae in the control. *Culex* larvae were observed for lowest mean death while *Anopheles* were the highest when treated with methanol extract of *N. tabacum* after 72 hrs. exposure to 4.0 mg/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)

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 (Table 7).

Analysis of Phytochemicals

Phytochemical compounds such as saponins, alkaloids, and steroids were observed to be highly present in *D. metel* and *N. tabacum* extracts while volatile oils were higher in *D. metel* and saponin glycosides higher in *N. tabacum* (Table 8). The quantity of some of the compounds in both the extracts was shown to be from trace amount to large amount (Table 9).

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 ±SD				Total mean death	Mortality (%)
			Exposure period (Hrs.)					
			12	24	48	72		
<i>Aedes</i> spp.	0.9	25	1.00±1.0	1.67±0.6	1.67±0.6	1.67±0.6	1.500 ^a	6.68
	1.6	25	1.00±0.0	2.00±1.0	2.33±0.6	2.33±0.6	1.917 ^a	9.32
	2.5	25	1.67±0.6	2.33±1.2	2.67±0.6	3.33±0.6	2.500 ^a	13.32
	3.4	25	2.00±1.0	2.67±0.6	3.00±1.0	18.00±2.0	6.417 ^b	72.00
	4.0	25	2.00±0.0	8.33±0.6	10.00±4.0	18.67±3.1	9.750 ^c	74.63
	Control	25	0.33±0.3	1.00±0.6	1.33±0.3	1.33±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±1.5	3.33±0.6	3.67±0.6	5.00±1.0	3.417 ^a	20.00
	1.6	25	2.33±1.5	3.33±1.2	3.67±0.6	5.67±0.6	3.750 ^a	22.68
	2.5	25	3.67±0.6	4.00±0.0	4.67±1.2	6.00±2.0	4.583 ^a	24.00
	3.4	25	3.67±2.1	6.67±1.2	7.33±1.2	16.00±4.6	8.417 ^b	64.00
	4.0	25	5.00±2.1	7.00±2.0	10.33±1.5	19.00±2.0	10.333 ^c	76.00
	Control	25	0.33±0.3	0.33±0.3	1.33±0.7	1.33±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±0.0	0.33±0.6	0.67±0.6	0.67±0.6	0.417 ^a	2.68
	1.6	25	0.00±0.0	0.33±0.6	1.33±0.6	1.67±0.6	0.588 ^a	6.68
	2.5	25	0.33±0.6	1.33±0.6	1.67±0.6	1.67±0.6	1.250 ^b	6.68
	3.4	25	0.00±0.0	2.00±1.0	2.67±0.6	3.67±1.2	2.083 ^c	14.68
	4.0	25	0.00±0.0	2.67±1.5	4.33±1.5	6.33±1.2	3.333 ^d	25.32
	Control	25	0.00±0.0	0.72±0.4	1.33±0.3	1.33±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) in the same row 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) in the same row 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) in the same row 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 ^{ae}	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
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}
	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) in the same row 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 ±SD				Total mean death	Mortality (%)
			Exposure period (Hrs.)					
			12	24	48	72		
<i>Aedes</i> spp.	0.9	25	0.00±0.0	0.67±1.2	1.67±0.6	1.67±0.6	1.000 ^{ad}	6.68
	1.6	25	0.67±0.6	0.67±0.6	1.67±1.2	2.00±1.0	1.250 ^a	8.00
	2.5	25	1.00±0.0	1.33±0.6	1.67±0.6	2.00±0.0	1.500 ^{ab}	8.00
	3.4	25	1.67±1.2	2.00±1.0	2.00±1.0	2.33±0.6	2.00 ^{bc}	9.32
	4.0	25	1.67±0.6	2.00±1.0	3.00±1.0	3.33±0.6	2.500 ^c	13.32
	Control	25	0.33±0.3	0.33±0.3	0.73±0.3	0.73±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±0.0	0.33±0.6	1.00±1.0	1.00±1.0	0.583 ^{ad}	4.00
	1.6	25	0.67±1.2	0.67±1.2	1.33±0.6	1.33±0.6	1.000 ^a	5.32
	2.5	25	0.67±0.6	1.67±0.6	1.67±0.6	2.67±0.6	1.667 ^b	10.68
	3.4	25	0.67±0.6	2.00±1.0	2.33±1.2	3.33±1.2	2.083 ^b	13.32
	4.0	25	2.33±0.6	2.67±0.6	3.67±0.6	4.67±1.2	3.333 ^c	18.68
	Control	25	0.00±0.0	0.00±0.0	0.73±0.3	0.73±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±0.0	0.00±0.0	0.67±1.2	1.00±1.0	0.417 ^{ad}	4.00
	1.6	25	0.00±0.0	0.33±0.6	0.67±0.6	0.67±0.6	0.417 ^{ad}	2.68
	2.5	25	0.67±0.6	1.00±0.0	1.00±0.0	1.00±0.0	0.917 ^{ab}	4.00
	3.4	25	1.00±1.0	1.33±0.6	1.33±0.6	1.67±0.6	1.333 ^b	6.68
	4.0	25	1.33±0.6	1.67±0.6	2.67±0.6	2.67±0.6	2.083 ^c	10.68
	Control	25	0.00±0.0	0.00±0.0	0.33±0.3	0.33±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) in the same row 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) in the same row 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.

DISCUSSION

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 larval mortality. *Culex* larvae were less susceptible to any of the extracts. Different mosquito species displayed different levels of susceptibility to the same extract. The mortality of *Cx. quinquefasciatus* was significantly lower than *An. stephensi* when exposed to Neemarine (Vatandoost and Vaziri, 2004). Toxicants in the extracts might be more

specific by affecting *Culex* species less rather than the other two species involved. It might also be possible that *Culex* mosquitoes have stronger detoxifying mechanisms against the toxins in the extract to warrant for lesser susceptibility. In addition, mortality of the larvae was observed to be directly proportional to concentrations and exposure periods. Chakkaravarthy *et al.* (2011) observed 72.50% and 87.0% mortality of *Culex quinquefasciatus* after 24 hrs exposures to 1000 ppm 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 quenufasciatus* larvae at concentration 70 ppm 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 2.5 mg/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), *Hyperium 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 trophane 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). Gopieshkanna and Kannabiran (2007) have observed the presence of carbohydrates, saponins, sphytosterols, phenols, flavonoids and tannins in the plant extracts having mosquito larvicidal activity. Alkaloids derived from a tropical vine; *Phyllum peltatum* was found to have larvicidal activity against malaria vector, *An. stephensi* (Thomas *et al.*, 2017). Ryanodine, an alkaloid from the tropical shrub, *Rafflesia 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 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 arundanasia*, *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 *Cx. 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 3.5 mg/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 saponnins, 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 directly proportional to concentration and exposure period. The lowest LC₅₀ values recorded for hexane extract of *D. metel* were against *Anopheles* and *Aedes* larvae (LC₅₀ 2.94±0.1 mg/mL and 3.13±0.1mg/mL respectively). Similarly, it was the methanol extract of *N. tabacum* that was observed for the lowest LC₅₀ value (LC₅₀ 2.58±0.1mg/mL) against *Anopheles* larvae, while the values for other extracts were greater than LC₅₀ 7.0mg/mL. The extracts could be used to control larval mosquitoes, offering an alternative to rather expensive and environmentally hazardous synthetic insecticides.

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