

Original Research Article

Larvicidal efficacy of six medicinal plants on *Anopheles gambiae* s.l mosquito

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Abstract

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The larvicidal efficacy of some medicinal plants (*Ocimum gratissimum*, *Chromolaena odorata*, *Terminalia catappa*, *Carica papaya*, *Vernonia amygdalina* and *Cymbopogon itrates*) against the larvae of *Anopheles gambiae* mosquitoes was investigated in acute bioassay. Ten healthy laboratory bred larvae were tested using five different concentrations (200, 400, 600, 800 and 1000ppm) and a simultaneous control at 24, 48 and 72 hours of exposure. The results showed that all the plant extracts were seen to be effective at less than 1% (1000ppm), thus possessing good to moderate effect against the larvae; *C. citrates* has been the most effective with 451.8ppm followed very closely by *O. gratissimum* with 458ppm, *C. papaya* with 510.2ppm, *V. amygdalina* with 582.3ppm, *T. catappa* with 620.9ppm, the least was *C. odorata* with 744ppm at the end of the exposure time. The efficacy of the extracts as seen from this study can be used as potential substitutes of synthetic larvicides and local environment due to their availability. The effects of the extracts were seen to be largely independent on external factors, hence the need for further study on the characterization and profiling of the active ingredients, their effect against non-target species and the best time of application is highly recommended as they fit in as alternative larvicides in reducing *Anopheles* menace infestation.

Keywords: Medicinal plants, LC₅₀, Extracts potency, *Anopheles* larva

INTRODUCTION

In tropical and subtropical regions, mosquitoes serve as vectors of life threatening diseases such as malaria, lymphatic filariasis, dengue, yellow fever and various encephalitis, etc (Tehri and Singh, 2015). Anopheline mosquitoes have been the only vectors of malaria to man and members of the *Anopheles gambiae* complex are the principal and most efficient vectors in tropical Africa. Malaria results in 214million new cases and 438,000 deaths annually in the world with Africa accounting for 88% of the occurrence and death (WHO, 2015) The most profitable time of controlling them is as soon as the rain commences when the population are trying to establish and peak, (CDC, 2015). An understanding of their biology, itrates and ecology is very essential in

developing control strategies to these diseases, (CDC, 2010; Godfray, 2013).

Control of mosquitoes has been carried out by the use of physical manipulation, genetic, biological and chemical methods, the latter has made by use of synthetic chemicals which has resulted in adverse effects on the environment and human lives due to biomagnifications, resistance buildup. Plant sources are an important weapon in the storehouse of mosquito control (Ghosh *et al.*, 2012) with multiple activities such as anti-feedants, insecticides, repellants, growth inhibitors, oviposition deterrents, etc. (Tehri and Singh, 2015). These groups provide an advantage over synthetic insecticides as they are less toxic, less prone to development of insect

resistance and easily biodegradable (Shaalan *et al.*, 2005, Remia and Logaswany, 2010, Ghosh *et al.*, 2012, Gokularishnan *et al.*, 2013, Kishore *et al.*, 2013, Ofoegbu *et al.*, 2013, Unnikrishnan, 2014). The biological activities are majorly the manifestations of phytochemicals detected in crude extracts of the plants (Ayoola and Adeyeye, 2010, Ijeh *et al.*, 2010, Udochuckwu *et al.*, 2015).

The plants used in this study are known for their traditional and medicinal uses, and these include *Ocimum gratissimum*, *Chromolaena odorata*, *Terminalia catappa*, *Carica papaya*, *Vernonia amygdalina* and *Cymbopogon itrates* against *Anopheles gambiae* s.l larvae

METHODOLOGY

Study Area

This study was carried out at the Animal and Environmental Biology postgraduate laboratory, Animal house and the Pharmacognosy laboratory, University of Benin, Benin City, Nigeria.

Plant Collection, Preparation and Extraction

Leaves of the plants were collected from within the University and Evbuomore community; the leaves were rinsed, shade dried, pulverized and extracted by maceration.

Concentrate Preparation

Stock solution (10,000ppm) were prepared by dissolving 1000g in 100ml distilled water, the test concentration (200ppm, 400ppm, 600ppm, 800ppm and 1000ppm) were prepared by serially diluting the stock according to WHO protocol (WHO, 2005).

Mosquito Collection and Rearing

Larvae were collected from rain pools within the University, transported to the Laboratory, separated by instars into rearing pans, fed with yeast, stabilized to produce adult which were fed with 10%w/v sugar solution and bloodfed with guinea pig; the rearing environment was 27±5°C and 75-85%, the F2 generation were used for the assay.

Larval Assay

Assay was carried out according to WHO, 2005 with

slight modification, 10 healthy L3/L4 larvae were placed in each test bowl containing 100ml of distilled water with the appropriate concentration, mortality was observed in 24, 48 and 72 hours, respectively, the setup was made up of four replicates and a simultaneous control which contained 1ml of solvent and 100ml of water.

Data Analysis

Data were analyzed using Graphpad Prism and SPSS, version 21. Values less than 5% (P<0.05) were considered to be statistically significant.

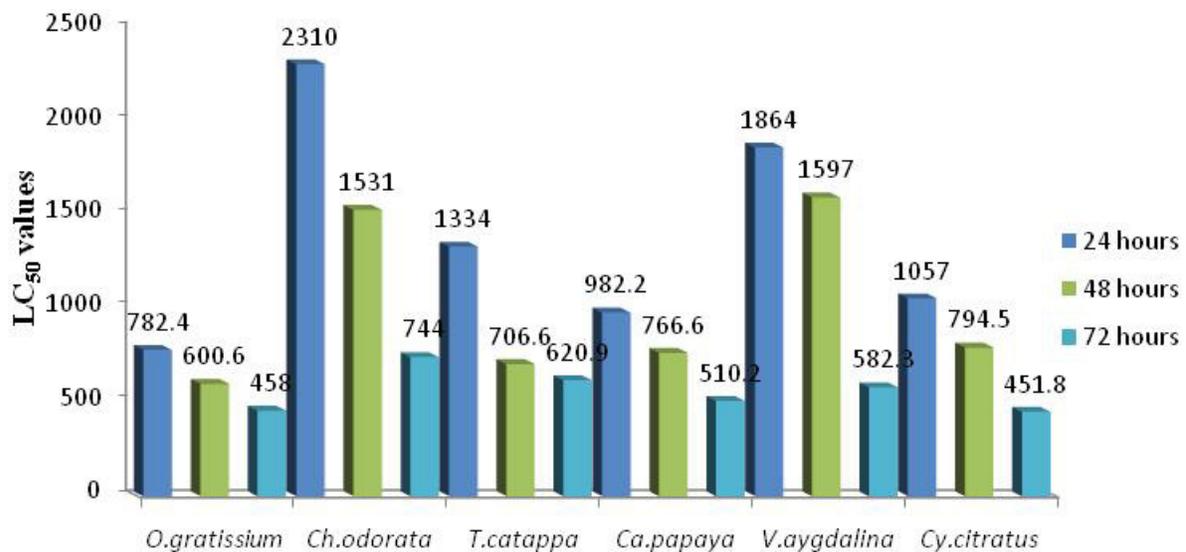
RESULT AND DISCUSSION

The result of the efficacy of *O. gratissimum*, *Ch. odorata*, *T. catappa*, *Ca. papaya*, *V. amygdalina* and *Cy. citratus* methanol extracts against *An. gambiae* larva for the different exposure and the regression equation for the plots is shown below in Table 1 while the figure 1 shows the variation of these efficacy, Figures 2-7 shows the regression plot of each plant extract against *An. gambiae* larvae.

In this study, lethal concentration values were calculated at three time periods (24hrs, 48hrs and 72hrs respectively). At the end of the exposure period, it was observed that the efficacy was in the order *Cy. citratus*<*O. gratissimum*<*Ca. papaya*<*V. amygdalina*<*T. catappa*<*Ch. odorata*; which were within acceptable limits for consideration of an extract as a larvicide according to WHO (2005) standards. These plants have been investigated by several researchers and the findings agreed with their reports. Mgbemena, (2010) reported the comparative effect of *Cy. citratus*, *O.gratissimum* and *A. indica* ethanol extracts on *Ae. aegypti* larvae and observed that *A. indica* was most effective followed by *O. gratissimum* and *Cy. citratus* at LC₅₀ values of 8.32mg/ml, 19.50mg/ml and 34.67mg/ml respectively. Chukwura and lhekumere, (2013) reported that *O. gratissimum* was the second most potent extract when compared with other extracts which is similar to this study, with a lower LC₅₀ value of 52ppm against *An. gambiae* larvae, this may be due to the solvent and extraction methodology used. Ofoegbu *et al.*, (2013) reported LC₅₀ values of 60.9mg/ml and 73.6mg/ml for ethanol and methanol extracts respectively, Pratheeba *et al.*, (2015) reported that the chloroform, acetone and hexane extracts produced LC₅₀ values of 280ppm, 305ppm and 354ppm respectively which is lower and more potent than this study. Unachukwu *et al.*, (2016) reported LC₅₀ value of 50mg/ml of *O. gratissimum* against *An.gambiae*. Rajmohan and Logankumar, (2011) reported an LC₅₀ value of 101.49ppm on *Ae. aegypti* larvae, Lee Marvin *et al.*, (2012) reported that *Ch. odorata* LC₅₀ values were not derived because the mortality was less than 50% at the

Table 1. Larval toxicity effect of the six plant extract against the larvae of the malaria vector, *An. Gambiae*

Extract	Time	LC ₅₀	LCI	UCL	Regression Equation	χ^2	p-Value	r-Value
<i>O.gratissimum</i>	24hrs	782.4	616.4	993.1	Y=-4.524+0.0682X	6.576	0.001	0.9496
	48hrs	600.6	408.2	883.8	Y=-3.333+0.0875X	7.638	0.0007	0.9583
	72hrs	458	259.2	809.5	Y=2.262+0.09214X	6.177	0.0002	0.9750
<i>Ch.odorata</i>	24hrs	2381	1285	4153	Y=-0.357+0.0257X	1.742	0.0002	0.9744
	48hrs	1531	1073	2186	Y=1.071+0.03786X	1.524	<0.0001	0.9908
	72hrs	744	521.8	1061	Y=0.8333+0.006X	3.979	0.0002	0.9755
<i>T.catappa</i>	24hrs	1334	1003	1774	Y=-2.50+0.0400X	2.739	0.0003	0.9739
	48hrs	706.6	558.5	894.1	Y=0.2381+0.0604X	3.264	0.001	0.9836
	72hrs	620.9	549.1	702	Y=1.071+0.0728X	1.524	<0.0001	0.9975
<i>Ca.papaya</i>	24hrs	982.2	750.6	1285	Y=-2.024+0.0532X	4.271	0.0005	0.9645
	48hrs	766.6	558.5	894.1	Y=2.738+0.0629X	6.473	0.0012	0.9429
	72hrs	510.2	382.3	680.7	Y=3.095+0.679X	6.894	0.0006	0.9589
<i>V.amygdalina</i>	24hrs	1864	1318	2638	Y=-1.310+0.0268X	1.967	0.0003	0.9701
	48hrs	1597	1009	2528	Y=-1.667+0.0375X	2.282	0.0002	0.9793
	72hrs	582.3	395.1	858.1	Y=0.008+0.0775X	6.971	0.0006	0.9613
<i>Cy.citratrus</i>	24hrs	1057	820.9	1362	Y=-3.095+0.0504X	3.712	0.0003	0.9699
	48hrs	794.5	756.4	834.6	Y=1.429+0.0628X	2.334	<0.0001	0.9922
	72hrs	451.8	316.7	644.5	Y=3.571+0.0879X	4.236	<0.0001	0.9869

**Figure 1.** Lethal concentration values of *O. gratissimum*, *Ch. odorata*, *T. catappa*, *Ca. papaya*, *V. amygdalina* and *Cy. citratrus* extracts at the different exposure time against *An. gambiae* larvae

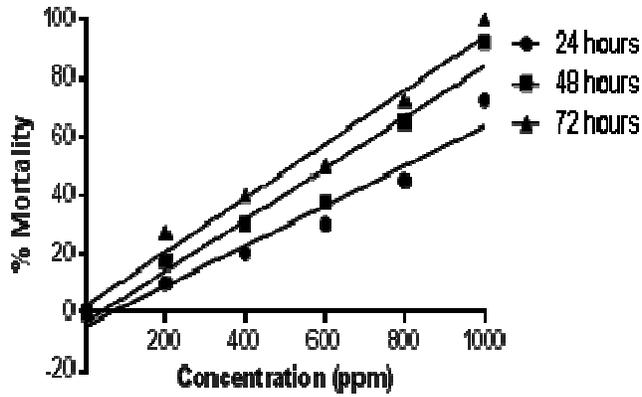


Figure 2. Regression plot of *O. gratissimum*

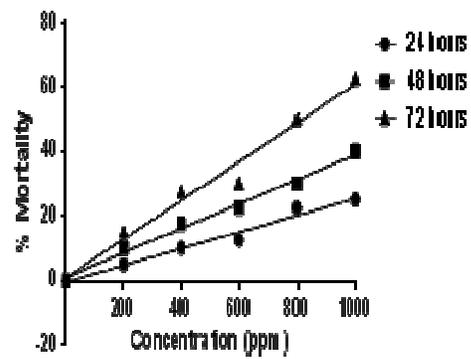


Figure 3. Regression plot of *C. odorata*

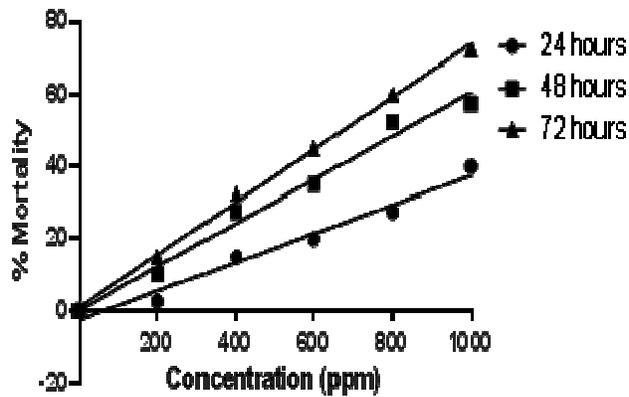


Figure 4. Regression plot of *T. catappa*

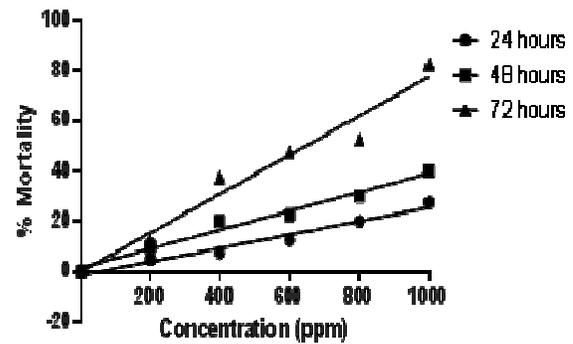


Figure 5. Regression plot of *Ca. papaya*

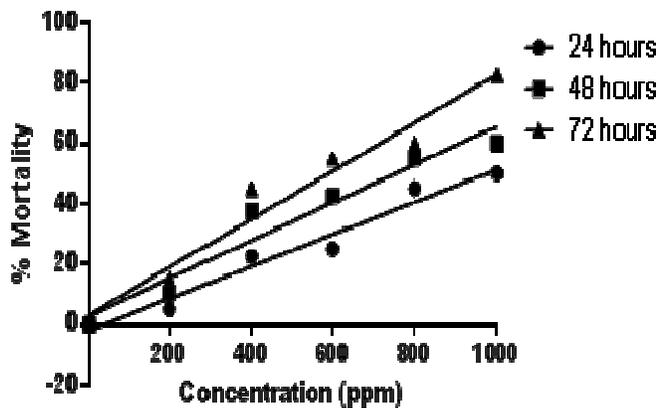


Figure 6. Regression plot of *V. amygdalina*

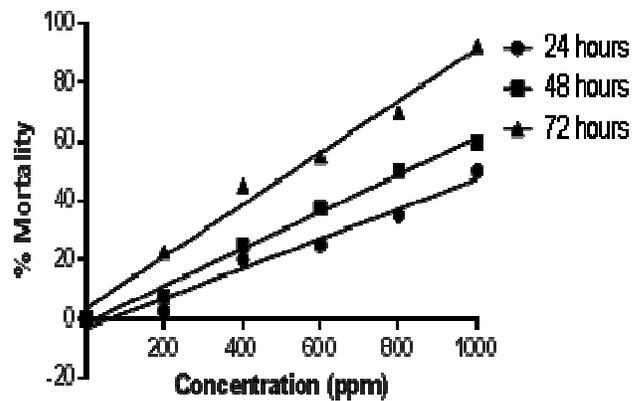


Figure 7. Regression plot of *C. citratus*

NB: $p < 0.05$ are significantly different, $p < 0.001$ are highly significantly different, while $p < 0.0001$ are very highly significantly different.

end of the exposure time, Sukhthankar, (2014) reported that LC₅₀ values for *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* were 43ppm, 138ppm and 1613ppm with *An. stephensi* values higher than this present finding, and could be attributed to the solvent used and the susceptibility of the vector species tested. Umar, (2015) reported 1.835ppm, 3.314ppm and 5.163ppm for n-hexane, ethanol and ethyl acetate fractionated extracts of *Ch. odorata* against *Ae. vittatus* larvae. Unnikrishnan, (2014) and Unithan and Unnikrishnan, (2015) and Dawuda, (2016) reported similar findings with *T. catappa* extracts against *Ae. aegypti* mosquito exhibiting lower LC₅₀ values than other tested plants, this could possibly be due to the Mosquito species. Rawani *et al.*, (2009) reported LC₅₀ values of 0.15%, 0.11%, 0.07% and 0.02% of aqueous extract of *Ca. papaya* against *Cx. quinquefasciatus* larvae. Ramanibai *et al.*, (2014) reported LC₅₀ value of 80.56ppm of acetone extract of *Ca. papaya* resulting in 61.6% mortality. Sesanti *et al.*, (2014) reported values of 422.311ppm with ethanol extracts of *Ca.papaya* on *An.gambiae* larvae. Rudi *et al.*, (2013), reported LC₅₀ values of 43.073ppm- 55.347ppm of on eight *Anopheles* species collected in South East Minahassa using three solvents for extraction. The difference between this present result and those from reported findings may be caused by the use of different raw material such as the solvent, plant varieties, method of extraction, mosquito species, as observed in reports of Sesantie *et al.*, (2014); Hayatie *et al.*, (2015) using seed producing species when compared to the non seed producing species of *Ca. papaya* used in this study. Reports on *V. amygdalina* against Mosquito vectors are rare especially with *Anopheles* species, while those for *T. catappa* have majorly been on *Aedes* species. Hence, more research on their efficacy especially on malaria species, characterization and profiling of the active ingredients is required.

CONCLUSION: Please provide

REFERENCES

- Ayoola PB, Adeyeye A (2010). Phytochemical and Nutrient evaluation of *Carica papaya* (Pawpaw) leaves. *IJRRAS* 5(3):325-328.
- Centre for Disease control, CDC (2010). *Anopheles* mosquitoes. Malaria. (04 September 2014).
- Centre for Disease Control, CDC (2015). Malaria- About Malaria - Biology of *Anopheles* mosquitoes. Malaria@cdc.gov. (27 June 2016).
- Chukwura EI, Iheukwumere I (2013). Larvicidal activity of *Ocimum gratissimum* and *Solenostemon monostachyus* leaves on *Anopheles gambiae*. *J. Sci. Industrial Res.* 72: 577-580.
- Dawuda KD (2016). Mosquito Larvicidal Prospects of *Terminalia catappa* (L.) and *Tamarindus indica* (L.) Seed Extracts in Laboratory and Field Bioassays. MSC Thesis, Ahmadu Bello University, Zaria, Nigeria. Department of Biological Sciences.
- Ghosh A, Chowdhury N, Chandra G (2012). Plant extracts as potential mosquito larvicides. *Indian J. Med. Res.* 135: 581-598.
- Godfray HGJ (2013). Mosquito Ecology and Control of Malaria. *Journal of Animal Ecology*; 82:15-25.
- Gokulakrishnan J, Elumalai K, Dhanasekaran S, Anandan A, Krishnappa K (2013). Pupicidal and repellent activities of *Pogostemon cablin* essential oil chemical compounds against medically important human vector mosquitoes. *Asian Pacific J. Trop. Dis.* 3(1): 26-31.
- Hayatie L, Biworo A, Suhartono E (2015). Aqueous extracts of seed and peel of *Carica papaya* against *Aedes aegypti*. *J. Med. Bioeng.* 4(5):417-421. Ijeh I.I, Omodamiro OD, Nwanna IJ (2005). Antimicrobial effects of aqueous and ethanolic fractions of two spices, *Ocimum gratissimum* and *Xylopiya aethiopicum*. *African Journal of Biotechnology*; 4: 953-956.
- Kishore N, Mishra BB, Tiwari VK, Tripathi VA (2011). A review on natural products with mosquitocidal potentials. In Tiwari VK, (Ed.). *Opportunity, challenge and scope of natural products in medicinal chemistry*. Kerala: Research Signpost. 335-365pp.
- Lee Marvin CD, Abantes MA, Asi MC, Balmeo NJC, Bustillo AMD, Calangi EM, Cruzado JR (2012). Larvicidal activity of four Philippine plants against Dengue virus vector *Aedes aegypti* (Linn). *THE STETH* 6: 14-28.
- Mgbemena IC (2010). Comparative evaluation of larvicidal potentials of three plant extracts on *Aedes aegypti*. *J. Ame. Sci.* 6: 435-440.
- Ofoegbu PU, Onyedineke NE, Essie NG, Isibor NG (2012). Laboratory evaluation of ethanolic and methanolic extracts of *Ocimum gratissimum* against larvae of *Anopheles gambiae* and non-target organisms. *Munis Entomology and Zoology.* 8(1):185-190.
- Pratheeba T, Rhaghavendran C, Nataragan D (2015). Larvicidal, pupacidal and adulticidal potential of *Ocimum gratissimum* plant leaf extract against filariasis inducing vector. *Int. J. Mosquito Res.* 2(2):01-08.
- Rajmohan D, Logankumar K (2011). Studies on the insecticidal properties of *Chromolaena odorata* (Asteraceae) against the life cycle of the mosquito, *Aedes aegypti* (Diptera: Culicidae). *J. Res. Biol.* 4:253-257.
- Ramanibai R, Deepika T, Madhavarani A (2014). Antimosquito Activity of leaf extract Of Neem (*Melia azedarach*) and Papaya (*Carica papaya*) detected against the larvae *Culex quinquefasciatus*. *Int. J. Innov. Res. Sci, Eng. Technol.* 3(4):11928-11935.
- Rawani A, Mallick HK, Ghosh A, Chandra G (2009). Larvicidal activities of three plants against filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitol Res* 105:1411-1417.
- Remia KM, Logaswamy P (2010). Larvicidal efficacy of leaf extract of two botanicals against the mosquito vector *Aedes aegypti* (Diptera: Culicidae) *Indian J. Nat. Products and Res.* 1 (2): 208-212.
- Rudi AR, Yermia SM, Rantje LW, Verawati IYR (2013). Malaria Mosquito Bionomic And Local Plant Extract Bioprospecting As Botanical Insecticide In Southeast Minahasa. *J. Nat. Sci. Res.* 3(14):39-50.
- Sesanti H, Arsunan AA, Hasanuddin I (2014). Potential test of Papaya leaf and seed extract (*Carica papaya*) as larvicides against *Anopheles* mosquito larvae mortality sp in Jayapura, Papua Indonesia. *Int. J. Sci. Res. Pub.* 4(6):1-7.
- Shalaan EA, Canyon D, Younes MWF, Abdel-Wahab H, Mansour A (2005). A review of botanical phytochemicals with mosquitocidal potential. *Environ. Int.*, 31: 1149-1166.
- Sukhthankar JH, Kumar H, Godinho MHS, Kumar A (2014). Larvicidal activity of methanolic leaf extract of the plant, *Chromolaena odorata* L. (Asteraceae) against vector mosquito. *Int. J. Mosquito Res.* 1(3):33-38.
- Udochukwu U, Omeje FI, Uloma IS, Oseiwe FD (2015). Phytochemical analysis of *Vernonia amygdalina* and *Ocimum gratissimum* extracts and their antibacterial activity on some drug resistant bacteria. *Ame. J. Res. Comm.* 3(5): 225-235
- Umar SA (2015). Larvicidal Potential of *Persea Americana* Seed and *Chromolaena odorata* Leaf against *Aedes vittatus* Mosquito. MSC Thesis, Ahmadu Bello University Zaria, Nigeria
- Unachukwu M, Okelue Q, Ozokonkwo O, Okolo S, Onah P, Okafor R (2016). Larvicidal Efficacy of *Vernonia amygdalina* and *Ocimum gratissimum* Extracts on Mosquito Larvae. *Asian J. Applied Sci.* 4(3):713-718.

Unithan AR, Unnikrishnan G (2015). Larvicidal Bioassay of Five Tropical Plants against *Aedes aegypti*. *World J. Pharmaceutical Res.* 4(10):2436-2446.

Unnikrishnan G (2014). Larvicidal and Pupicidal Activity of *Terminalia catappa* Leaf Extracts on *Aedes aegypti* Mosquito: A Vector Intervention. *IOSR J. Pharm. Biol. Sci.* (IOSR-JPBS) 9(2) II: 58-63

World Health Organization, WHO (2005). *Guidelines for laboratory and field testing of mosquito larvicides*. WHO/CDS/WHOPES/GCDPP/2005.13.

World Health Organization, WHO (2015) *Malaria*. World Health Organization, Geneva, Switzerland.