Global Journal of Science Frontier Research: C
Biological Science
Volume 19 Issue 1 Version 1.0 Year 2019
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

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By Rajasingh Raveen, Lokesh Kumar, Angeline Sugirtha, Nithya Karthikeyan, Samuel Tennyson, Subramanian Arivoli & Manickkam Jayakumar

Madras Christian College

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GJSFR-C Classification: *FOR Code: 060899*

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Rajasingh Raveen [α], Lokesh Kumar [σ], Angeline Sugirtha [ρ], Nithya Karthikeyan [ω], Samuel Tennyson [¥], Subramanian Arivoli [§] & Manickkam Jayakumar [χ]

Abstract- Botanicals have gained prominence as alternate to most artificial pesticides within the management of mosquitoes. Insecticides derived from botanical sources are natural products, are chiefly secondary metabolites and natural chemicals that have some advantages over their conventional counterparts in that they are highly degradable. In the present study, the phytoextracts of *Nicotiana tabacum* leaves was tested for larvicidal activity against the third instar larvae of the dengue vector, *Aedes aegypti* at concentrations of 0.0125, 0.025, 0.05 and 0.1% for 24, 48 and 72 hours of exposure. The petroleum ether extract exhibited the highest activity with respective LC_{50} values of 0.09, 0.03 and 0.01%. *Nicotiana tabacum* leaf extracts for the control of mosquito larva is well documented as many types of research have gone into the larvicidal activity of tobacco plant extracts for the control of vector mosquitoes, and the mode and mechanism of action of the phytochemical component nicotine responsible for the mortality of mosquito larvae is highlighted.

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I. Introduction

Among the blood-sucking insects that infuriate man and animals, mosquitoes are the foremost from a medical stance. Through evolution, nature has beautifully formed them that they may survive beneath the foremost adverse conditions and during a diversity of environments. If diversity of species, habits, numbers of individual and persistence in geological time are the measures, then mosquitoes without doubt are one of the "success groups among insects" of biological evolution (Samuel, 2010). Mosquitoes are particularly of high prevalence in more than 100 countries, infecting people every year globally (Akinkurolere *et al.*, 2011, Rahuman *et al.*, 2011). WHO has declared the mosquito as the "Public Enemy Number One," because mosquitoes are responsible for the transmission of various dreadful diseases (WHO, 1996). *Aedes aegypti*, the most efficient mosquito vector for arboviruses, responsible for transmission of dengue, chikungunya, urban yellow fever, and Zika virus fever (WHO, 2014, 2016) is highly anthropophilic and thrives near to humans preferring to live indoors. This species is domestic in their habits and is found breeding in the vicinity of dwellings where rain water stagnates in discarded tires, tins, bottles and also in water storage containers such as cisterns, barrels, pots, etc. (Jayakumar *et al.*, 2007).

One approach to decreasing the mosquito population is to interrupt the mosquito life cycle at the larval stage (Chowdhury *et al.*, 2008), since throughout the immature stages, mosquitoes are comparatively immobile, remaining in a more targeted space than they're in adults (Rutledge *et al.*, 2003). Although, the employment of artificial insecticides remained the foremost effective mode for mosquito control; myriads of problems associated with their usages like adverse environmental effects and physiological resistance, etc. has presented the necessity for an alternative method of mosquitocide which is economical, richly available, eco-friendly and biodegradable. Consequently, the use of botanicals has gained prominence as substitute to most artificial pesticides within the management of mosquitoes. Insecticides derived from botanical sources are natural products, are chiefly secondary metabolites and natural chemicals that have some advantages over their conventional counterparts in that they are highly degradable. Thus, the entomotoxic character of many botanicals within the management of various stages of several mosquito species have been extensively documented (Sukumar *et al.*, 1991; Shaalan *et al.*, 2005; Kishore *et al.*, 2011, 2014; Arivoli *et al.*, 2012a,b, 2015; Ghosh *et al.*, 2012; Samuel *et al.*, 2011, 2012a,b, 2016, 2018; Vargas, 2012; Raveen *et al.*, 2014, 2015, 2017a,b; Samuel and William, 2014; Benelli, 2015; Shaalan and Canyon, 2015; Jayakumar *et al.*, 2016; Kuppusamy *et al.*, 2016; Afzal *et al.*, 2018). Earlier, Samuel *et al.* (2012c) had evaluated the methanolic leaf extract of this plant species for its larvicidal activity against *Aedes aegypti*. Therefore, in continuation to the research carried by Samuel *et al.* (2012c), this work highlights on the mode and mechanism of action of the phytochemical component nicotine present in tobacco leaf extracts in addition to the testing of low, moderate and high polar solvent phytoextracts of *Nicotiana tabacum* leaves against the larvae of the dengue vector.

Author α σ ρ ω: Department of Zoology, Madras Christian College, Chennai 600 059, Tamil Nadu, India.
Author §: Department of Zoology, Thiruvalluvar University, Vellore 632 115, Tamil Nadu, India.
Author χ: Department of Zoology, University of Madras, Chennai 600 025, Tamil Nadu, India.
Author ¥: Assistant Professor, Department of Zoology, Madras Christian College, Chennai 600 059, Tamil Nadu, India.
e-mail: samtennyson@gmail.com

II. Materials and Methods

a) Plant collection and preparation of extracts

Mature and healthy *Nicotiana tabacum* leaves collected from Chennai, Tamil Nadu, India was taxonomically identified and confirmed at Department of Plant Biology and Plant Biotechnology, Madras Christian College, Chennai, Tamil Nadu, India. The leaves were then brought to the laboratory, washed in dechlorinated water, shade dried and small-grained with the aid of an electric mixer. The powdered leaves (1Kg) were extracted with different solvents (3L) each (low-polar: hexane, petroleum ether, dichloromethane; mid-polar: chloroform, ethyl acetate, acetone, methanol; and high_polar: distilled water) in a Soxhlet apparatus with minor modifications (Vogel, 1978) and air-dried to obtain the crude extracts. The crude extracts thus obtained were stored in airtight amber colored bottles at 4°C for bioassays.

b) Test mosquitoes

The larvae of *Aedes aegypti* which were obtained from Entomology Research Institute, Loyola College, Chennai, Tamil Nadu, India were free of exposure to insecticides. Cyclic generations of the above-mentioned vector mosquitoes were maintained separately in mosquito cages (2'x2'x2') in an insectary with a mean room temperature of 27±2°C and a relative humidity of 70-80%.The adult mosquitoes was fed on ten percent glucose solution in water. The eggs which were laid in ovitraps placed inside the mosquito cages was transferred to enamel larval trays maintained in the larval rearing chamber. The larvae was fed with larval food (dog biscuits and yeast in the ratio 3:1). The larvae on becoming pupae were collected, transferred to plastic bowls and kept inside another mosquito cage for adult emergence.

c) Larvicidal bioassay

The larvicidal bioassay was carried out as per the guidelines of the World Health Organization (WHO, 2005) with minor modifications. Larvicidal activity at test concentrations of 0.0125, 0.025, 0.05 and 0.1% of each crude leaf extract were assessed. The required test concentrations and quantity of test solution was prepared by serially diluting one percent stock solution of the crude extract. Twenty early third instar larvae from laboratory colonized *Aedes aegypti* of F_1 generation was introduced into glass beakers (250mL) containing 200mL of distilled water and test concentration. Untreated control (distilled water) and treated control (Tween 80 + distilled water) were maintained separately and run simultaneously. Mortality was observed 24, 48 and 72 hours after treatment. Moribund larvae was scored dead when they showed no signs of movement when probed by a needle at their respiratory siphon. A total of five replicates per trial and a total of three trials for each concentration were carried out.

d) Statistical analyses

The percent larval mortality was calculated using the formula (1) and corrections for control mortality (5-20%) when necessary was done using formula (2) of Abbott (Abbott, 1925). Statistical analyses of all mortality data of larvicidal activity was subject to probit analysis (SPSS, 2010). One-way Analysis of Variance (ANOVA) and Tukey HSD post-hoc tests were used to determine (i) if the mortality in treated bioassays significantly differed from that of the controls and at which doses in particular; and (ii) if there were significant differences in response between extracts of the plant. For the latter, analysis excluded control mortalities from the data. The differences was considered as significant at $P \leq 0.05$ level. All statistics was conducted in IBM SPSS Statistics v22 with significance set at 95% confidence (SPSS, 2010).

Percent larval mortality (1):

$$\frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

Corrected percentage of control mortality (2):

$$\frac{1 - n \text{ in T after treatment}}{n \text{ in C after treatment}} \times 100$$

Where, n is the number of larvae, T: treated and C: control.

III. Results and Discussion

No larval mortality was noted in the treated and untreated control. The crude leaf extracts of *Nicotiana tabacum* when tested on the larvae of vector mosquitoes, showed that the petroleum ether extract exhibited the highest activity against the larvae of *Aedes aegypti* with respective LC_{50} values of 0.09, 0.03 and 0.01% after 24, 48 and 72 hours of exposure. Data between the control (untreated and treated) and treated larvae was found be significant. Likewise, the data between the concentrations for each extract and the data between the extracts for each concentration was also found to be statistically significant except for the petroleum ether extract at 0.1% concentration (Fig. 1; Table 1). Quirino (2010) reported that *Aedes aegypti* larvae subjected to *Nicotiana tabacum* extracts showed LC_{50} values of 0.45 and 0.12%; and LC_{90} of 0.98 and 0.25% after 24 and 48 hours respectively whose LC_{50} values are ten times higher than the results of the present study. However, the aqueous, acetone, chloroform and methanol extracts of *Nicotiana tabacum* caused 100% mortality against the larvae of *Culex quinquefasciatus* after 24 hours of exposure at 1000ppm with LC_{50} values of 163.81, 76.27, 105.85 and 83.38ppm respectively and their LC_{50} values are lower than the present study pertaining to different vector species (Rahuman *et al.*, 2009).

The world of plants comprises a rich untapped pool of phytochemicals that will be widely employed in place of artificial pesticides within the mosquito management programme. The search is ongoing to seek newer pesticides which can be potent, safe, economical and readily available. A wide choice of trees and shrubs has been found to contain phytochemicals that will be of use within the management of mosquitoes. Plants and plant components provide a rich supply of novel drug compounds, as plant-derived drugs have made the greatest contribution to human health. The employment of plant extracts, as well as other alternative forms of medical treatment, is relishing great popularity in the late nineteen nineties. Kishore *et al*. (2011, 2014) reviewed the efficacy of phytochemicals against mosquito larvae according to their chemical nature. Since the botanicals are less likely to cause ecological damage, they could be utilized as insecticides against vector mosquitoes.

Nicotiana tabacum leaf extracts for the control of mosquito larva is well documented as many types of research have gone into the larvicidal activity of tobacco plant extracts for the control of vector mosquitoes, therefore the discussion section of this paper highlights on the mode and mechanism of action of the vital phytochemical components, especially nicotine present in tobacco leaf extracts responsible for the death of mosquito larvae. Mittal *et al*. (2003) discovered that tobacco contains the alkaloid nicotine, tar and carbon monoxide as the main components which vary in concentration in different parts of the plant. Of these components, nicotine has been projected as an effective insecticide because it is fully biodegradable and effective in controlling insects (Philipson, 2001). The concentration of nicotine usually increases with the age of the plant with the mature plant having about 64% nicotine in leaves, 18% in the stem, 13% in the root, 5% in flowers and 0% in seeds (Olofintoye *et al*., 2011). The elevated concentration of the alkaloid nicotine in the leaves of *Nicotiana tabacum* may be responsible for the death of *Aedes aegypti* larvae. Nicotine (IUPAC nomenclature (S)-3-(1- methyl pyrrolidine-2-il) pyridine) is a pyrrolidine alkaloid produced in large quantities acts as a defense against herbivores and is an excellent neurotoxin, in particular against insects (Devi *et al*., 2012). The nicotine alkaloids are characteristic compounds in the chemistry of the *Nicotiana* genus (Sisson, 1990) and alkaloids are the active metabolites in this plant (Tso, 1962). Liu *et al*. (2012) considered alkaloids among the active molecules against mosquito larvae. Alkaloids are nitrogenous compounds that show insecticidal properties at low concentration, and therefore the mode of action on insect vectors varies with the structure of their molecules, however many are reported to affect acetylcholinesterase (AChE) or sodium channels as inhibition of AChE activity is responsible for terminating the nerve impulse transmission through the synaptic pathway (Rattan, 2010). Alkaloids work by constricting blood vessels and depressing autonomic nervous system activity thereby conducive to the insecticide's effectiveness in killing the larvae of mosquitoes and disrupting the life cycle of the mosquito (Simon-Oke *et al*., 2015).

Nicotine and the related alkaloids nornicotine and anabasine obtained from the extract of tobacco foliage induces highly insecticidal effects as they are synaptic poisons that mimic the neurotransmitter acetylcholine. Therefore, they cause symptoms of poisoning similar to those seen with organophosphate and carbamate insecticides (Regnault-Roger and Philogène, 2008). About its mode of action, nicotine is an extremely fast acting nerve toxin. It competes with acetylcholine, the dominant neurotransmitter, by attaching to acetylcholine receptors at nerve synapses and causing uncontrolled nerve firing. This disruption of normal nerve impulse activity results in rapid failure of those body systems that depend on nervous input for proper functioning (El-Wakeil, 2013). Nicotine causes hyperactivity and death in insects and worms as the nicotinic acetylcholinesterase receptors (nAChRs) is located in the post-synaptic dendrites of all neurons in the brain, spinal cord, ganglia, and muscular junctions. In insects, although nAChRs is not expressed at the neuromuscular junction (where synaptic transmission is glutamatergic), acetylcholine is the major excitatory neurotransmitter in insect brain (Breer and Sattelle, 1987) and nAChRs play a central role in rapid cholinergic synaptic transmission (Sattelle, 1980; Sattelle and Breer, 1990). However, their extreme toxicity to insects contrasts with their low toxicity to all vertebrate taxa, and this selectivity is due to a different kind of nAChRs found in vertebrates (Matsuda *et al*., 2001; Tomizawa and Casida, 2003).

References Références Referencias

1. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology. 1925; 18: 265-267.
2. Afzal S, Shah SS, Ghaffar S, Azam S, Aril F. Review on activity of medicinal plant extracts against mosquito genera *Anopheles* and *Culex*. International Journal of Entomology Research. 2018; 3(6): 8-14.
3. Akinkurolere RO, Adedire CO, Odeyemi OO, Raji O, Owoeye JA. Bioefficacy of extracts of some indigenous Nigerian plants on the developmental stages of mosquito (*Anopheles gambiae*). Jordan Journal of Biological Science. 2011; 4(4): 237-242.
4. Arivoli S, Raveen R, Samuel T, Sakthivadivel M. Adult emergence inhibition activity of *Cleistanthus collinus* (Roxb.) (Euphorbiaceae) leaf extracts against *Aedes aegypti* (L.), *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Diptera:

Culicidae). International Journal of Mosquito Research 2015; 2(1): 24 28.
5. Arivoli S, Ravindran KJ, Raveen R, Samuel T. Larvicidal activity of botanicals against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). International Journal of Research in Zoology. 2012b; 2(1): 13-17.
6. Arivoli S, Ravindran KJ, Samuel T. Larvicidal efficacy of plant extracts against the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). World Journal of Medical Sciences. 2012a; 7(2): 77-80.
7. Benelli G. Plant-borne ovicides in the fight against mosquito vectors of medical and veterinary importance: A systematic review. Parasitology Research. 2015; 114:3201-3212.
8. Breer H, Sattelle DB. Molecular properties and functions of insect acetylcholine receptors. Journal of Insect Physiology. 1987; 33: 771-790.
9. Chowdhury N, Ghosh A, Chandra G. Mosquito larvicidal activities of *Solanum villosum* berry extract against the dengue vector *Stegomyia aegypti*. BMC Complementary and Alternative Medicine. 2008; 8: 1-8.
10. Devi S, Gupta AK, Singh M. Ethano-medicinal use of plants belonging to families Fabaceae and Solanaceae, Hamirpur district (H.P.). International Journal of Scientific Research Publications. 2012; 2(11): 2250-3153.
11. El-Wakeil NE. Botanical pesticides and their mode of action. Gesunde Pflanzen. 2013; 65:125-149.
12. Ghosh, A, Chowdhury N, Chandra G. Plant extracts as potential larvicides. Indian Journal of Medical Research. 2012; 135: 581-598.
13. Jayakumar M, Arivoli S, Raveen R, Samuel T. Larvicidal and pupicidal efficacy of plant oils against *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae). Journal of Entomology and Zoology Studies. 2016; 4(5): 449-456.
14. Jayakumar M, Samuel T, William SJ. *Aedes*: An ever dangerous enemy in our society. In: Defeating the public enemy, the mosquito: A real challenge, William SJ. (Ed), Loyola College, Chennai, Tamil Nadu, India. 2007, 205-221.
15. Kishore N, Mishra BB, Tiwari VK, Tripathi V, Lall N. Natural products as leads to potential mosquitocides. Phytochemistry Reviews. 2014; 13: 587-627.
16. Kishore N, Mishra BB, Tiwari VK, Tripathi V. A review on natural products with mosquitocidal potentials. In: Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry. (Ed.) Tiwari VK. Kerala: Research Signpost, 2011, 335-365.
17. Kuppusamy E, Dhamodharan KI, Jayakumar S. Role of plants and plant based products towards the control of insect pests and vectors: A novel review. Journal of Coastal Life Medicine. 2016; 4(11): 902 917.
18. Liu ZL, Liu QZ, du SS, Deng ZW. Mosquito larvicidal activity of alkaloids and limonoids derived from *Evodia rutaecarpa* unripe fruits against *Aedes albopictus* (Diptera: Culicidae). Parasitology Research. 2012; 111(3): 991-996.
19. Matsuda K, Buckingham SD, Kleier D, Rauh JJ, Grauso M, Sattelle DB. Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. Trends in Pharmacological Sciences. 2001; 22(11): 573-580.
20. Mittal PK, Subbarao SK. Prospects of using herbal products in the control of mosquito vectors. ICMR Bulletin. 2003; 33(1):1-10.
21. Olofintoye FK, Simon-Oke IA, Omoregie OB. Larvicidal properties of *Datura stramonium* (jimson weed) and *Nicotiana tabaccum* (tobacco) extracts against the larvae of (*Anopheles* and *Culex*) mosquitoes. International Multi Disciplinary Journal. 2011; 5(2): 337-344.
22. Philipson JD. Phytochemistry and medicinal plants. Phytochemistry. 2001; 56: 237-243.
23. Quirino T F. 2010. Avaliação do potencial inseticida de solução de *Nicotiana tabacum* L. (Solanacea) para o controle de *Aedes aegypti* (L.) (Diptera: Culicidae). Trabalho de Conclusão de Curso (Graduação em Ciências Biológicas)- Universidade Estadual da Paraíba, Campina Grande. 2010, 54.
24. Rahuman AA, Bagavan A, Kamaraj C, Vadivelu M, Zahir AA, Elango G, Pandiyan G. Evaluation of indigenous plant extracts against larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitology Research. 2009; 104: 637-643.
25. Rahuman A A. Efficacies of medicinal plant extracts against blood-sucking parasites. Parasitology Research. Monographs. 2011; 1: 19-53.
26. Rattan SR. Mechanism of action of insecticidal secondary metabolites of plant origin. Crop Protection 2010; 29: 913-920.
27. Raveen R, Fauzia A, Pandeeswari M, Reegan D, Samuel T, Arivoli S, Jayakumar M. Laboratory evaluation of a few plant extracts for their ovicidal, larvicidal and pupicidal activity against medically important human dengue, chikungunya and Zika virus vector, *Aedes aegypti* Linnaeus 1762 (Diptera: Culicidae). International Journal of Mosquito Research. 2017; 4(4): 17-28.
28. Raveen R, Kamakshi KT, Deepa M, Arivoli S, Samuel T. Larvicidal activity of *Nerium oleander* L. (Apocynaceae) flower extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). International Journal of Mosquito Research. 2014; 1(1): 36-40.
29. Raveen R, Pandeeswari M, Ahmed F, Reegan D, Samuel T, Arivoli S, Jayakumar M. Bioefficacy of

Nerium oleander Linnaeus (Apocynaceae) floral extracts on the larva of three vector mosquitoes of medical importance. International Journal of Mosquito Research. 2017b; 4(6): 65-77.
30. Raveen R, Samuel T, Arivoli S, Madhanagopal R. Evaluation of mosquito larvicidal activity of *Jasminum* species (Oleaceae) crude extracts against the filarial vector *Culex quinquefasciatus* Say (Diptera:Culicidae). American Journal of Essential Oils and Natural Products. 2015; 2(4):24-28.
31. Regnault-Roger C, Philogène BJR. Past and current prospects for the use of botanicals and plant allelochemicals in integrated pest management. Pharmaceutical Biology. 2008; 46: 41-52.
32. Rutledge CR, Clarke F, Curtis A, Sackett S. Larval mosquito control. Technical Bulletin of the Florida Mosquito Control Association. 2003; 4: 16-19.
33. Samuel T, Arivoli S, Raveen R, Bobby M, Dhinamala K. Larvicidal activity of *Arecca catechu*, *Nicotiana tabacum* and *Piper betle* leaf extracts against the dengue vector *Aedes aegypti* (L.) (Diptera: Culicidae). International Journal of Research in Biological Sciences. 2012; 2(4): 157-160.
34. Samuel T, Arivoli S, Raveen R, Selvakumar S, Jayakumar M, Lokesh K. Bioefficacy of *Catharanthus roseus* (L.) G. Don (Apocyanaceae) and *Hyptis suaveolens* (L.) Poit (Lamiaceae) ethanolic aerial extracts on the larval instars of the dengue and chikungunya vector *Aedes aegypti* Linnaeus 1762 (Diptera: Culicidae). International Journal of Mosquito Research 2018; 5(4): 7-18.
35. Samuel T, Arivoli S, William J. Phytonanopesticides in vector control: A review. Journal of Applied Zoological Researches. 2016; 6(2): 1-11.
36. Samuel T, Ravindran KJ, Arivoli S. Bioefficacy of botanical insecticides against the dengue and chikungunya vector *Aedes aegypti* (L.) (Diptera: Culicidae). Asian Pacific Journal of Tropical Biomedicine. 2012a; 2: S1842-S1844.
37. Samuel T, Ravindran KJ, Arivoli S. Screening of plant extracts for ovicidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae). Applied Botany. 2011; 40: 5456-5460.
38. Samuel T, Ravindran KJ, Arivoli S. Screening of twenty five plant extracts for larvicidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae). Asian Pacific Journal of Tropical Biomedicine. 2012b; 2: S1130-S1134.
39. Samuel T, William SJ. Potentiality of botanicals in sustainable control of mosquitoes (Diptera: Culicidae). In: Achieving Sustainable Development: Our Vision and Mission, (Ed.) William, S.J. Loyola College, Chennai, Tamil Nadu, India, 2014, 204-227.
40. Samuel, T. 2010. Studies on the mosquitocidal activity of *Ageratum houstonianum* Mill. (Asteraceae) against vector mosquitoes. Ph.D. Thesis, University of Madras, Chennai, Tamil Nadu, India.
41. Sattelle DB, Breer H. Cholinergic nerve terminals in the central nervous system of insects: molecular aspects of structure, function and regulation. Journal of Neuroendocrinology. 1990; 2: 241-156.
42. Sattelle DB. Acetylcholine receptors of insects. Advances in Insect Physiology. 1980; 15: 115–215.
43. Shaalan EA, Canyon DV. A review on mosquitocidal activity of botanical seed derivatives. Current Bioactive Compounds. 2015; 11: 78-90.
44. Shaalan EAS, Canyon D, Younes MWF, Wahab HA, Mansour AH. A review of botanical phytochemicals with mosquitocidal potential. Environmental International. 2005; 31:1149-1166.
45. Simon-Oke LA, Afolabi OJ, Ajayi OT. Larvicidal activity of a perennial herb, *Solanum xanthocarpum* against the larvae of culicine species. Futa Journal of Research in Sciences 2015; 1: 152-156.
46. Sisson VA. Alkaloid composition of the *Nicotiana* species. Beitrage zur Tabakforschung International. 1990; 14(6): 327-339.
47. SPSS. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. 2010.
48. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: A review. Journal of the American Mosquito Control Association. 1991; 7:210-237.
49. Tomizawa M, Casida JE. Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Annual Review of Entomology. 2003; 48: 339-364.
50. Tso TC. Some novel concepts on the biosynthesis and biogenesis of tobacco alkaloids. Botanical Bulletin- Academia Sinica Taipei. 1962; 3: 61-71.
51. Vargas MV. An update on published literature (period 1992-2010) and botanical categories on plant essential oils with effects on mosquitoes (Diptera: Culicidae). Boletin de Malariologia Y Salud Ambiental. 2012; 2(2):143-193.
52. Vogel AL. Text Book of Practical-Organic Chemistry. The English Language Book Society and Longman London. 1978, 1363.
53. W.H.O. "Zika virus", Fact sheet, World Health Organization. 2016.
54. W.H.O. A global brief on vector-borne diseases [Document number: WHO/DCO/WHD/2014.1]. Geneva, Switzerland. 2014.
55. W.H.O. Guidelines for laboratory and field testing of mosquito larvicides. WHO, Geneva, WHO/CDS/WHOPES/GCDPP/13. 2005.
56. W.H.O. The World Health Report, WHO. Geneva. 1996.

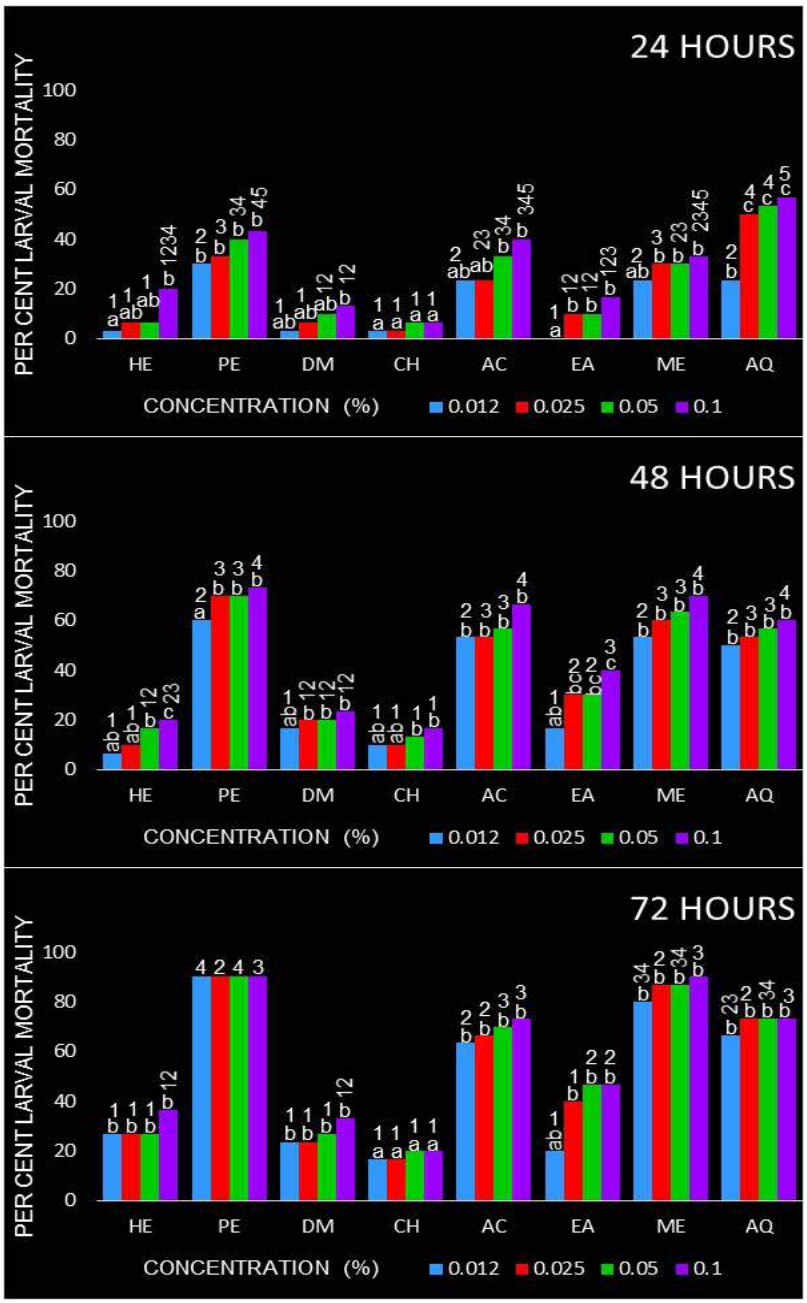

Fig. 1: Percent larval mortality of *Aedes aegypti* on exposure to tobacco leaf extracts.

HE: Hexane; PE: Petroleum ether; DM: Dichloromethane; CH: Chloroform; AC: Acetone; EA: Ethyl acetate; ME: Methanol; AQ: Aqueous. Different alphabets on the bar indicate statistical significant difference between concentrations and different numericals on the bar indicate statistical significant difference between the extracts. All differences were statistically significant at P≤0.05 level by One-way ANOVA followed by Tukey's test performed.

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Table 1: Probit analysis of tobacco leaf extracts against *Aedes aegypti* larvae.

Solvents	LC_{50} (%)			LC_{90} (%)		
	24h	48h	72h	24h	48h	72h
Hexane	0.15	0.11	.011	0.24	0.19	0.23
Petroleum ether	0.09	0.03	0.01	0.19	0.10	0.06
Dichloromethane	0.19	0.15	0.12	0.32	0.29	0.23
Chloroform	0.32	0.19	0.17	0.51	0.32	0.32
Acetone	0.10	0.05	0.03	0.20	0.13	0.10
Ethyl acetate	0.16	0.10	0.08	0.25	0.19	0.16
Methanol	0.11	0.04	0.02	0.23	0.11	0.06
Aqueous	0.06	0.05	0.03	0.14	0.14	0.10