Int. Res. J. of Science & Engineering, 2020; Special Issue A7: 546-552 SJIF Impact Factor 6.68
ISSN: 2322-0015

RESEARCH ARTICLE **OPEN ACCESS**

Neem Oil (*Azadirachta indica*) as a Herbal control formulation against mosquitoes.

Thote Jayashri G

Research Scholar Sevadal Mahila Mahavidhyalay, Nagpur.
Email: jayashri.thote@gmail.com

Manuscript Details
Available online on http://www.irjse.in
ISSN: 2322-0015

Cite this article as:
Thote Jayashri G. Neem Oil (Azadirachta indica) as a Herbal control formulation against mosquitoes., *Int. Res. Journal of Science & Engineering*, February, 2020. Special Issue A7: 546-552.

ABSTRACT

Mosquitoes transmit serious human diseases like malaria, dengue. JE, Chikungunya causing millions of deaths every year. Use of synthetic insecticides to control vector mosquitoes has caused physiological resistance and adverse environmental effects in addition to high operational cost. Insecticides of botanical origin have been reported as useful for control of mosquitoes. *Azadirachta indica* (Meliaceae) and its derived products have shown a variety of insecticidal properties. The present paper discusses the larvicidal activity of neem-based biopesticide for the control of mosquitoes.

Keywords: *Azadirachta indica* (Meliaceae), neem-based biopesticide, of mosquitoes, Chikungunya.

INTRODUCTION

Mosquitoes transmit serious human diseases like malaria, filariasis, Japanese encephalitis, dengue haemorrhagic fever and yellow fever causing millions of deaths every year [1]. Extensive use of chemical insecticides for control of vector borne diseases has created problems related to physiological resistance to vectors, adverse environmental effects, high operational cost and community acceptance [2]. Numerous plant products have been reported either as insecticides for killing larvae or adult mosquitoes or as repellents for mosquito biting and are one of the best alternatives for mosquito control [2, 3].

Neem trees, (*Azadirachta indica*) native of India, belonging to family Meliaceae are fast growing evergreen trees ranging in height from 12 – 24 m.

They are widespread in tropical and subtropical regions of the world, including semi-arid and wet-tropical regions [4]. Neem seeds contain approximately 99 biologically active compounds of which azadirachtin, nimbin, nimbidin and nimbolides are major molecules. Many of these derived products have antifeedancy, ovicidal activity, fecundity suppression besides insect growth regulation and repellency against insects [5–10]. Neem products have low toxicity to birds, fish and mammals and are less likely to induce resistance due to their multiple mode of action on insects. In addition to this, insect growth regulatory activity of neem weakens the cuticle defence system of the larvae causing easy penetration of pathogenic organisms into insect system. Azadirachtin, a biologically active compound has been promoted as a new insecticide that is considered more eco- friendly than synthetic insecticides. The pesticidal efficacy, environmental safety and public acceptability of neem and its products for control of crop pests has led to its adoption into various mosquito control programmes [8, 11].

The present study was aimed to determine the larvicidal potential of the emulsified neem oil formulation against different mosquito genera under natural field conditions in India.

METHODOLOGY

Larvicidal efficacy of an emulsified concentrate of neem oil which was derived from neem tree found in India. *Azadirachta indica* was derived from the Nimboni of neem tree. This formulation was evaluated against late 3rd and early 4th instar larvae and pupal stage of different genera of mosquitoes. The larvae were exposed to different concentrations (0.5-5.0 ppm) of the formulation along with untreated control. Larvicidal activity of the formulation was also evaluated in field against Anopheles, Culex, and Aedes mosquitoes. The formulation was diluted with equal volumes of water and applied @ 140 mg a.i./m(2) to different mosquito breeding sites with the help of pre calibrated knapsack sprayer. Larval density was determined at pre and post application of the formulation using a standard dipper.

RESULTS AND DISCUSSION

Median lethal concentration (LC(50)) of the formulation against Anopheles stephensi, Culex quinquefasciatus and Aedes aegypti was found to be 1.6, 1.8 and 1.7 ppm respectively. LC(50) values of the formulation stored at 26 degrees C, 40 degrees C and 45 degrees C for 48 hours against Ae. aegypti were 1.7, 1.7, 1.8 ppm while LC(90) values were 3.7, 3.7 and 3.8 ppm respectively. An application of the formulation at the rate of 140 mg a.i./m(2) in different breeding sites under natural field conditions provided 98.1% reduction of Anopheles larvae on day 1; thereafter 100% reduction was recorded up to week 1 and more than 80% reduction up to week 3, while percent reduction against Culex larvae was 95.5% on day 1, and thereafter 80% reduction was achieved up to week 3. The formulation also showed 95.1% and, 99.7% reduction of Aedes larvae on day 1 and day 2 respectively; thereafter 100% larval control was observed up to day 7.

1 Larvicidal Bioassay

Larvicidal bioassay of the formulation was performed on late 3rd and early 4th instar larvae of *Anopheles stephensi*, a primary vector of urban malaria,*Culex quinquefasciatus* a common vector of filariasis, and *Aedes aegypti* a common vector of dengue, dengue haemorrhagic fever and yellow fever. The larvae were obtained from Nagpur urban area. [12]. Twenty-five larvae were released into 500 ml glass beakers containing 250 ml distilled water. The larvae were provided a mixture of dog biscuit and yeast powder in a 3:2 ratio as nutrients and supplemented with different concentrations (0.5 to 5.0 ppm) of the formulation. The experiments were carried out at 30°C ± 2°C. Five replicates of each concentration were run under the same microclimatic conditions along with untreated control. Mortality of larvae was monitored at 24 hours. The percent corrected mortality was calculated using Abbott's formula [13] and Log probit analysis was used to determine the median lethal concentration (LC_{50})/90% lethal concentration (LC_{90}) of the formulation.

2 Laboratory Study

Mean LC_{50} and LC_{90} values (95% confidence limits) of the neem oil formulation against *An. stephensi*, *Cx. quinquefasciatus* and *Ae, aegypti* are given in Table 1.

Mean LC_{50} values of the formulation were 1.6, 1.8 and 1.7 ppm while LC_{90} were 3.4, 3.5 and 3.7 ppm against *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* respectively.[14]

4.3 Field evaluation of larvicidal activity

Before start of the study, a preliminary survey of various breeding sites of mosquitoes was carried out. In all 73 breeding sites were surveyed inside the Scrap, tyre, Plastic container, construction sites, mud pot and household containers out of which 67 sites were found positive (91.8%) for *Aedes* larvae. Larvae collected from these breeding sites were identified as *Ae. aegypti* and *Ae. albopictus*. A survey of different breeding sites such as tanks, pits, drains were also carried at Nagpur urban area for *Culex* and *Anopheles* breeding. *Culex* and *Anopheles* larvae collected from these sites were identified as *Cx. quinquefasciatus, An. Stephensi* respectively.

Mean percent reduction of larval density against *Cx. quinquefasciatus* and anophelines in different breeding habitats are given in Table 1 & 2 respectively. In pits, percent reduction of *Culex* larvae was 95.9, 90.2, 87.2 on days 1, 2, 3 respectively of post application, while more than 70% reduction was observed up to week 3. In tanks 91.6% – 92.4% reduction of *Culex* larvae was observed up to day 7 of post application thereafter 80.7% reduction was noted up to week 3, while in drains there was more than 90% larval control up to day 7 and remained above 75% up to week 3.

Table 1: Larvicidal activity of neem oil formulation against mosquito in laboratory

Species	Larvicidal activity (ppm)	
	LC_{50} (Mean ± sd)	**LC_{90} (Mean ± sd**
Anopheles stephensi	1.6 ± 0.4 (1.1 – 2.5)*	3.4 ± 0.5 (2.7 – 4.0)
Culex quinquefasciatus	1.8 ± 0.5 (1.2 – 2.6)	3.5 ± 0.6 (2.8 – 4.2)
Aedes aegypti	1.7 ± 0.3 (1.3 – 2.1)	3.7 ± 0.5 (3.1 – 4.3)

Table 2: Larvicidal activity of neem oil formulation against culex mosquitoes larvae in field

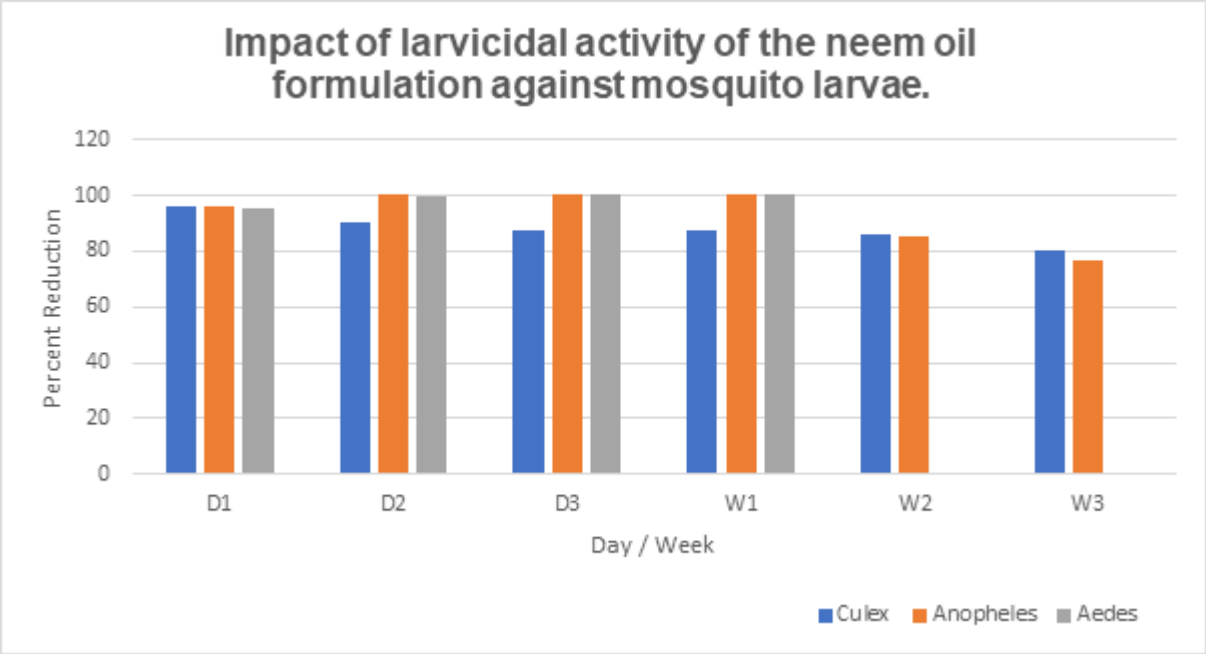
Mosquito species	**Breeding sites**	**Pre treatment density**	**Percent reduction of larval density (mean ± sd)**					
			Day-1	**Day-2**	**Day-3**	**Week-1**	**Week-2**	**Week-3**
Culex	Pits	28.9 ± 10.6	95.9 ± 3.5	90.2 ± 6.9	87.2 ± 11.0	87.5 ± 8.2	85.9 ± 8.0	80.5 ± 7.3
	Tanks	26.8 ± 11.5	91.9 ± 5.8	93.2 ± 3.2	97.7 ± 1.9	92.4 ± 8.0	86.2 ± 8.2	80.7 ± 9.2
	Drains	115.7 ± 64.6	99.4 ± 0.6	98.8 ± 1.2	98.6 ± 1.4	84.9 ± 4.6	85.0 ± 11.8	77.8 ± 11.0
			95.5 ± 4.1	**94.1 ± 4.3**	**94.5 ± 5.5**	**89.9 ± 2.5**	**85.7 ± 0.8**	**79.7 ± 1.6**

Table 3: Larvicidal activity of neem oil formulation against anopheles mosquitoes larvae in field

Mosquito species	**Breeding sites**	**Pre treatment density**	**Percent reduction of larval density (mean ± sd)**					
			Day-1	**Day-2**	**Day-3**	**Week-1**	**Week-2**	**Week-3**
Anopheles	Pits	13.5 ± 7.5	96.2 ± 4.5	100	100	100	85.4 ± 14.1	76.6 ± 9.6
	Tanks	10.4 ± 5.7	98.2 ± 1.8	100	100	100	87.0 ± 9.7	77.7 ± 10.0
	Construction Sites	13.0 ± 6.7	100	100	100	100	98.7 ± 1.3	97.0 ± 3.0
			98.1 ± 1.9	**100**	**100**	**100**	**90.4 ± 7.2**	**83.8 ± 11.5**

Table 4: Larvicidal activity of neem oil formulation against *Aedes* mosquitoes in field

Breeding sites	Pre treatment larval density	pH	Percent reduction of larval density (Mean ± sd)			
			Day-1	Day-2	Day-3	Day-7
Tyres	10.3 ± 4.1	8.0–9.0	94.3 ± 4.5	98.6 ± 1.4	100	100
Machinery scraps	14.5 ± 8.6	8.0–9.0	96.0 ± 3.0	100	100	100
Iron container	19.2 ± 5.7	8.5	98.1 ± 1.5	100	100	100
Iron box	11.0 ± 6.0	8.0–8.5	96.9 ± 2.0	100	100	100
Iron tanks	9.0 ± 2.6	8.0–8.5	85.2 ± 6.5	100	100	100
Plastic scrap	6.0	8.5	100	100	100	100
			95.1 ± 5.2	**99.7 ± 0.3**	**100**	**100**



An effective control of late instars anopheline larvae in tanks was observed with 98.2% reduction on day 1, followed by 100% reduction up to day 7 and more than 75% reduction till week 3. In pits, 96.2% control was recorded on day 1, 100% up to day 7 and more than 75% reduction up to week 3. The mean percent reduction of *Culex* larvae was 89.9–95.5% up to day 7 followed by 79.7–85.7% up to week 3, while for *Anopheles* larvae mean percent reduction was 90.4–100% up to day 7 followed by 83.8–90.4% up to week 3 (Table 2). Larvicidal activity of the formulation against aedes larvae in different breeding sites is given in Table 3. There was 85.2% to 98.1% reduction of *Aedes* larvae on day 1 of post application of the neem oil formulation, thereafter 99.7% to 100% reduction was recorded up to day 7.

Discussion

Neem trees are found throughout India with a myriad of uses in medicine, as well as pest control [4]. Neem-based pesticides are now extensively used in agriculture practices all over the world. It contains azadirachtin, which is a predominant insecticidal active ingredient, having antefeedent, ovipositional deterrence repellency, growth disruption, sterility and larvicidal action against insects [6]. There are various reports of control of mosquito breeding under field conditions. An emulsion of neem oil in water was

found to be effective in controlling breeding of *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti* in pools, tanks and coolers up to 2 to 3 weeks [15], whereas an application of neem cake powder resulted in drastic reduction in the late instar larvae and pupae of culicine mosquitoes in paddy field [16].

Mean LC_{50} values were 1.6, 1.8 and 1.7 ppm against *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*, while LC_{90} were 3.4, 3.5 and 3.7 ppm respectively. LC_{50} of the formulation stored at 26°C, 40°C and 45°C for 48 hours were 1.7, 1.7, 1.8 ppm while LC_{90} values were 3.7, 3.7 and 3.8 ppm respectively which revealed that there was no difference in the biopestcidal activity of the neem oil formulation at different storage temperatures. No significant difference of larvicidal activity of the formulation was also observed during 18 months storage period at room temperature. In the present study an application of the formulation at the rate of 140 mg *a.i.*/m^2 in pits, tanks and drains provided above 90% reduction of *Culex* larvae up to week 1 and thereafter 80% reduction up to week 3, whereas 100% reduction was observed in *Anopheles* larvae up to week 1, after that more than 80% reduction was recorded up to week 3.

Dhar *et al* [17] demonstrated the inhibitory effect of neem oil volatiles on gonotropic cycle in *An. stephensi* and *An. culicifacies*. A neem oil formulation containing 32% neem seed oil (an equivalent of 0.03% azadirachtin), an emulsifier (5%) and 63% iso propanol (solvent) was investigated for its larvicidal activities against *An. gambiae* [18]. It was toxic to mosquito larvae with LC_{50} value of 11 ppm and also reported to possess insect growth regulators. Gianotti and co workers [19] used powdered seeds of neem trees and applied twice a week to known breeding sites for *An. gambiae* at the rate of 10 gm/m^2 of pool surface area for effective larval control. Azadirachtin acts as anti-ecdysteroid and kills larvae by growth inhibition effect [20]. In the present investigation, neem oil formulation was found effective to control mosquito larvae in different breeding habitats under natural field conditions and more than 80% reduction of *Anopheles*, *Culex* and *Aedes* larvae was observed up to three weeks of post application.

Neem-based biopesticides and neem extracts have a wide range of effects against insect pests including repellence, feeding, toxicity, sterility and growth regulator activity and are relatively safe towards non-target biota with only minimal risk of direct adverse effects on aquatic biota from contamination of water bodies [21, 22]. Allelochemicals such as azadirachtin, nimbin, nimbidin, nimbolides, nimolic acid, salannin, melianttriol, azadirachtol present in neem affect the biochemical and physiological processes of insect system and nullify the insect detoxification mechanism thereby not allowing the pest to develop resistance. As an emulsifiable concentrate, the neem oil formulation had greatly reduced sized particles and evenly mixed within the water column with a few suspended particles on the water surface. The spread of these fine particles probably increased the efficacy of formulation.

Control of mosquito larvae becomes a very pertinent issue in controlling the rapid replication of mosquitoes in management of vector- borne diseases. In the present study, neem oil formulation showed promising larvicidal activity against important vectors of malaria, filaria, dengue, dengue haemorrhagic fever, yellow fever and chikungunya. Development of resistance in temephos and *Bacillus thuringiensis* [22,23] is a matter of concern for operational use as larvicides. Although the present formulation may be more costly than other larvicidal agents, such as temephos and *B. thuringiensis*, it has the advantage of being eco-friendly, effective and ability to prevent the development of pest resistance.

CONCLUSION

The neem oil formulation is a herbal product. This was found effective in controlling mosquito larvae in different breeding sites under natural field conditions. As neem trees are widely distributed in India, their formulations may prove to be an effective and eco-friendly larvicide, which could be used as an alternative for malaria control.

Conflicts of interest: The authors stated that no conflicts of interest.

REFERENCES

1. Mittal PK, Subbarao SK: Prospects of using herbal products in the control of mosquito vectors. ICMR Bull. 2003, 33: 1-10.
2. Brown AWA: Insecticide resistance in mosquitoes; a pragmatic review. J Am Mosq Control Assoc. 1986, 2: 123-140.
3. Sukumar K, Perich MJ, Boobar LR: Botanical derivative in mosquito control: A Review. J Am Mosq Control Assoc. 1991, 7: 210-237.
4. National Research Council: Neem: a tree for solving global problems. Report of an adhoc panel of the Board on Science and Technology for International Development. 1992, National Academy Press, Washington, DC
5. Isman MB: Botanical insecticides, deterrent and repellents in modern agriculture and an increasingly regulated world. Ann Rev Entomic. 2006, 51: 45-66. 10.1146/annurev.ento.51.110104.151146.
6. Schmutterer H: Properties of natural pesticides from the neem tree, *Azadirachta indica*. Ann Rev Entomol. 1990, 35: 271-297. 10.1146/annurev.en.35.010190.001415.
7. Locantoni L, Guisti F, Cristofaro M, Pasqualini L, Esposito F, Lupetti P, Habluetzel A: Effect of neem extract on blood feeding oviposition and oocyte ultra structure in *Anopheles stephensi* Liston (Diptera: Culicidae). Tissue Cell. 2006, 38: 361-371. 10.1016/j.tice.2006.08.005.
8. Su T, Mulla MS: Antifeedancy of neem products containing Azadirachtin against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). J Vector Ecol. 1998, 23: 114-122.
9. Sharma VP, Dhiman RC: Neem oil as a sand fly (Diptera: Psychodidae) repellent. J Am Mosq Control Assoc. 1993, 9: 364-366.
10. Schmutterer H: The neem tree (*Azadirachta indica*) and other Meliceous plants. Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and other porposes. 2002, Mumbai: Neem Foundation, 1
11. Su T, Mulla MS: Ovicidal activity of neem products (azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera; Culicidae). J Am Mosq Control Assoc. 1998, 14: 204-209.
12. World Health Organization: Instruction for determining the susceptibility or resistance of mosquito larvae. 1981
13. Abbott WS: A method of computing the effectiveness of an insecticide. J Econ Entomol. 1925, 18: 265-266.
14. World Health Organization: Chemical methods for the control of vector and pests of public health importance. Edited by: Chavassee DC, Yap NH. 1997, WHO
15. Batra CP, Mittal PK, Adak T, Sharma VP: Efficacy of neem-water emulsion against mosquito immatures. Indian J Malariol. 1998, 35: 15-21.
16. Rao DR, Reuben R, Venugopal MS, Nagasampgi BA, Schmutterer H: Evaluation of neem – *Azadirachta indica* with and without water management for the control of culicine mosquito larvae in rice field. Med Vet Entomol. 1992, 6: 318-324. 10.1111/j.1365-2915.1992.tb00627.x.
17. Dhar R, Dawar H, Garg SS, Basir F, Talwar GP: Effect of volatiles from neem and other natural products on gonotropic cycle and oviposition of *Anopheles stephensi* and *An. culicifacies*. J Med Entomol. 1996, 33: 195-201.
18. Okumu FO, Knols BGJ, Fillinger U: Larvicidal effects of a neem (*Azadirachta indica*) oil formulation on the malaria vector *Anopheles gambiae*. Malar J. 2007, 6: 63-10.1186/1475-2875-6-63.
19. Gianotti RL, Bomblies A, Dafalla M, Issa-Arzika I, Duchemin JB, Eltahir EAB: Efficacy of local neem extracts for sustainable malaria vector control in an African village. Malar J. 2008, 7: 138-10.1186/1475-2875-7-138.
20. Zebit CPW: Effect of some crude and *Azadirachta*-enriched neem (*Azadirachta indica*) seed kernel extracts of larvae of *Aedes aegypti*. Entomol Exp Appl. 1984, 35: 11-16. 10.1007/BF00343450.
21. Kreutzweiser DP: Non-target effects of neem based insecticides on aquatic invertebrates. Ecotoxicol Env Safety. 1997, 36: 109-117. 10.1006/eesa.1996.1485.
22. Goektepe I, Portier R, Ahmedna M: Ecological risk assessment of neem based pesticides. J Env Sci Hlth B. 2004, 39: 311-320. 10.1081/PFC-12003024
23. DiogoFernandesBellinato,1PriscilaFernandesVian a-Medeiros,1

SimoneCostaAraújo,2,3,4AdemirJ.Martins,2,3,5 JoséBentoPereiraLima,2,3 andDeniseValle1,5, Resistance Status to the Insecticides Temephos, Deltamethrin, and Diflubenzuron in Brazilian Aedes aegypti Populations. Volume 2016, Article ID 8603263, 12 pages

24. Andre ´ Luiz de Almeida Melo, Vanete Thomaz Soccol, and Carlos Ricardo Soccol Bacillus thuringiensis: mechanism of action, resistance, and new applications: a review. Article in Critical Reviews in Biotechnology · September 2014 DOI:10.3109 /07388551.2014.960793 ·Source: PubMed