International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2016; 3(6): 26-30

Received: 05-09-2016
Accepted: 06-10-2016

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Evaluation of larvicidal action of natural extracts on mosquito larvae of *Aedes aegypti* (Diptera: Culicidae)

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Abstract
Mosquitoes are known to cause more diseases than any other group of arthropods and affect millions of people throughout the world. The current study was aimed to investigate larvicidal potential of three natural extracts such as *aloe vera* and onion, alcohol and cloves, marigold and garlic against the dengue vector, *Aedes aegypti*. Insecticidal susceptibility tests were carried out using WHO standard method and the mortality was observed after 24-h exposure. All the tested extracts showed moderate to good larvicidal activities. However, the maximum larval mortality was observed in the extract of the alcohol and cloves that showed high efficacy on the Aedes mosquito larva. The findings of the present studies suggested that the use of *aloe vera*, onion and garlic has a strong effect in acting as a mosquito larvicide and the natural plant extracts can be prepared at home and used at any cost of time which is feasible for the masses.

Keywords: Larvicidal potential. natural extracts, dengue vector, *Aedes aegypti*, mortality

1. Introduction
Mosquitoes transmits a number of diseases than any other group of arthropods and affect millions of people throughout the world. WHO has declared the mosquitoes as "public enemy number one" [1]. Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting over 700,000,000 people every year globally and 40,000,000 of the Indian population [2]. Among the mosquito borne diseases, dengue fever dengue haemorrhagic fever, yellow fever and chikungunya are endemic in Southeast Asia and Africa and it is transmitted by *Aedes aegypti* (Linn.) [3].

The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds [3]. Different insecticides are used for the chemical control of mosquitoes. But these insecticides and larvicides are very expensive. They also cause pollution and toxicity to man, crop, plants, domestic animals, wild life and also kill the other desirable fauna by introducing the toxicant in food chain. The mosquitoes are becoming resistant to a wide range of pesticides [4]. This makes room to consider some other larvicides or bio insecticides which must be cheap and appropriate and could safely be used for vector control.

In recent years, use of many of the synthetic insecticides in mosquito control programme has been limited [5]. It is due to the high cost, concern for environmental sustainability, higher rate of biological magnification through ecosystem and increasing insecticide resistance on a global scale [6]. These factors have resulted in an urge to look for environment friendly, cost-effective, biodegradable and target specific insecticides against mosquito species. Considering these, the application of eco-friendly alternatives such as biological control of vectors has become the central focus of the control programmme in lieu of the chemical insecticide [7, 8].

Identifying efficient bio-insecticides as being suitable and adaptive to ecological conditions, is imperative for continued effective vector control management [9, 10]. Botanicals have widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and in future may act as suitable alternative product to fight against mosquito borne diseases [11, 12]. In this regard, natural products of plant origin with the insecticidal property have been tried in the recent past for the control of various insect pests and vectors. In the light of the above knowledge, a few local plants like *aloe vera*, onion, garlic, cloves and marigold were screened and their natural extracts selected for bioassay in the

present investigation. Thus the aims and objectives of the present work is to develop a simple inexpensive and nontoxic method for the control of mosquito larvae of *Aedes aegypti* by natural extracts which can be applied easily by the ordinary man without use of costly spraying equipment.

2. Materials and Methods

2.1Test organism

The test organism *Aedes aegypti* was reared and the larvae were fed a diet of powdered dog biscuits in the ratio of 3:1, kept at 27 ± 2 ºC and 75%-85% relative humidity (RH), with a photoperiod of 14:10 LD for the larval growth. Larval instars were checked for its attainment of fourth stage. At this final stage the larva were collected and tested for its mortality using the natural extracts.

2.2 Preparation of larvicidal natural extracts

Natural extracts were prepared using insect repellent plants such as *aloe vera* and onion, alcohol and cloves, marigold and garlic. The extracts prepared are diluted with distilled water for 3 different concentrations 5%, 10% and 15% separately taken in spray bottles. The larvicidal action of the natural extracts was tested on the larval forms of the *Aedes aegypti* mosquitoes. This is done by spraying the different extracts on the larval forms taken in 3 different petri plates. The mortality rate of the larva using different extracts were observed and noted for its mortality.

2.3 Preparation of *aloe vera* and onion extract

The *aloe vera* leaflets were collected from the garden and the spicules along with the skin was cut into pieces to get the gelatine (gel) and onion numbered 10-15 were collected from the kitchen, the skin was peeled off and finely grounded by using the pestle and mortar. The condensate is referred to as the onion extract. The gel of *aloe vera* and the onion extract was mixed together and the distillate was filtered by using a clean cotton cloth. The filtered distillate was collected in the beaker of 200ml capacity. Thus, the *aloe vera*- onion extract was prepared at 5%, 10% and 15% concentrations.

2.4 Preparation of alcohol and cloves extract

The alcohol (ethyl alcohol) acquired from the laboratory was taken in a beaker of 50ml capacity. The cloves used in the culinary works numbered 20 was collected and soaked in the beaker containing the ethyl alcohol. The beaker was left undisturbed for about 4-5days. The essence of the cloves in alcohol was noted for its larvicidal repellency. The filtrate was thus collected in a beaker of 200 ml capacity and 2-3drops of almond oil was added to it which marks the high repellency activity. Thus the extract collected was prepared at 5%, 10% and 15% concentrations.

2.5 Preparation of marigold and garlic extract

Marigold flowers were collected from the bouquet market and the petals were crushed in the pestle and mortar to get the extract. Similarly garlic cloves numbered 8-10 were collected from the kitchen and grounded finely by using pestle and mortar. Both the extract was mixed and filtered using a clean white cotton cloth in a beaker of 200ml capacity. Thus the extract collected was prepared at 5%, 10% and 15% concentration. Three different spray bottles holds the extract of these concentrations.

2.6 Larvicidal bioassay

The larvicidal activity of crude extracts of the selected plants was assessed by the protocol of WHO. For the bioassay in a container 30 fourth instar larvae were kept in 249 ml of distilled water with 1 ml of extracts (400 ppm) in DMSO. Tween-80 was used as an emulsifier at concentration of 0.02% (v/v). After 24 hours exposures the dead larvae were counted and the percentage mortality was recorded from the average of six replicates.

2.7 Spray test

The extract was sprayed on the *Aedes* larva taken in 3 petriplates. 10 sprays for 25 seconds were handled in each petriplates followed by one another. Likewise the other two extracts alcohol and cloves extract, marigold and garlic extract were also sprayed and the results were observed and recorded.

3. Results & Discussion

The results of larvicidal efficacy of different natural extracts of the selected plants are shown in Fig 1, 2 and 3. All the plant extracts showed good to moderate effect on fourth instar larvae of *Ae. aegypti* after 24 h of exposure at increasing concentration. The highest mortality (100%) was observed in the extract of cloves and alcohol followed by *Aloe vera* and Onion extract which showed moderate larvicidal efficacy (Table- 2). The marigold and garlic extract show the low rate of its efficacy in acting as a larvicide.

When 15% of the natural extract of cloves and alcohol were applied, all the larvae exhibited 100% mortality (Table-3). Decreasing the amount of the extract to 10 and 5% reduced the mortality to 93 and 73%, respectively. For the *Aloe vera* and onion extract (Table 2), 90% mortality was recorded when the larvae were treated with 15% extract, while 60% mortality was recorded with 15% of marigold and garlic extracts, respectively(Table-1). The highest mortality observed for 15ml alcohol-clove extract was found to be 100%. This work demonstrates the potency of alcohol and clove extract in the control of *Aedes* larvae. Clove and alcohol extract appeared as the most lethal among the various extracts tested. The high mortality recorded for clove-alcohol extract might be attributed to deficiency of dissolved oxygen in the water. Previous studies with *Ambrosia maritima* have shown that extracts of *Ambrosia* plant parts do possess insecticidal effects to achieve result similar to that of leaf extract [13, 14].

The intention of this investigation on *Aedes aegypti* larva aims at killing the larva in the more organic way. The synthetic larvicides has adverse effects on the environment, which leads to the contamination of the chemicals and their combined effect. The chemicals used may also alter the physiological features of the mosquito larva that it may cause some vigorous changes and lead to the invasion of such larva.

It is evident from the result that the various concentrations of the three plant extracts were the main cause of mortality in *A. aegypti* larvae. Sukumar *et al.,* (1991) have reported 3 species of family Sapindaceae, namely *Koelreuteria paniculata* (extracts of seeds and leaves), *Paullinia fuscescens* (extracts of seeds and fruits) and *Sapindus saponaria* (extracts of seeds and fruits) were found to be effective against mosquito larvae [15]. Surendran *et al*. (2009) have stated that the fruit extract of *Sapindus emarginatus* against *A. aegypti* revealed the LC_{50} values of 92.9 ppm and found extract positive for the presence

of saponin [16]. Various authors have evaluated larvicidal activity of *Cestrum* species on mosquitoes; where they found a steroidal bioactive compounds responsible for mosquitocidal activity [17]. Jawale *et al.*, (2010) have reported *Cestrum nocturnum* as larvicide against *Aedes aegypti* mosquito where methanol extract outstand as highly active larvicide, achieving 100% larval mortality in 24 hours when tested in the concentration of 45 µg/mL (soxhlet) and 25 µg/mL (percolation) [18]. Wiesman and Chapagain (2006) have reported a strong correlation between saponin content and mortality for *Aedes aegypti* (yellow fever mosquito) larvae exposed to extracts of *Balanites aegyptiaca*. 0.0014% (v/w) of the most active fraction proved to be sufficient to kill 50% of the larvae before formation of adults [19].

Table 1: Mortality rate of marigold and garlic extract on *Aedes aegypti* larva

Conc. of natural extracts (%)	Time exposure (mins)													
	1	2	3	4	5	6	7	8	9	10	11	12	Total	% mortality
15	2	2	1	2	2	1	1	1	2	1	1	2	18	60
10	1	1	0	1	1	1	1	1	1	1	1	2	12	40
5	2	0	0	1	0	0	1	1	1	0	0	0	6	20
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2: Mortality rate of *Aloe vera* and onion extract on *Aedes aegypti* larva

Conc. of natural extract s (%)	Time exposure (mins)													
	1	2	3	4	5	6	7	8	9	10	11	12	Total	% mortality
15	2	4	2	1	3	4	2	2	2	1	1	3	27	90
10	1	2	1	2	3	3	2	2	1	2	1	1	21	70
5	1	0	0	0	2	1	1	3	0	2	0	1	11	36
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3: Mortality rate of alcohol and clove onion extract on *Aedes aegypti* larva

Conc. of natural extracts (%)	Time exposure (mins)													
	1	2	3	4	5	6	7	8	9	10	11	12	Total	% mortality
15	3	2	3	2	3	3	4	3	2	2	1	2	30	100
10	2	3	2	1	3	2	2	4	2	2	2	3	28	93
5	2	2	2	3	1	3	2	1	1	1	2	2	22	73
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Figures

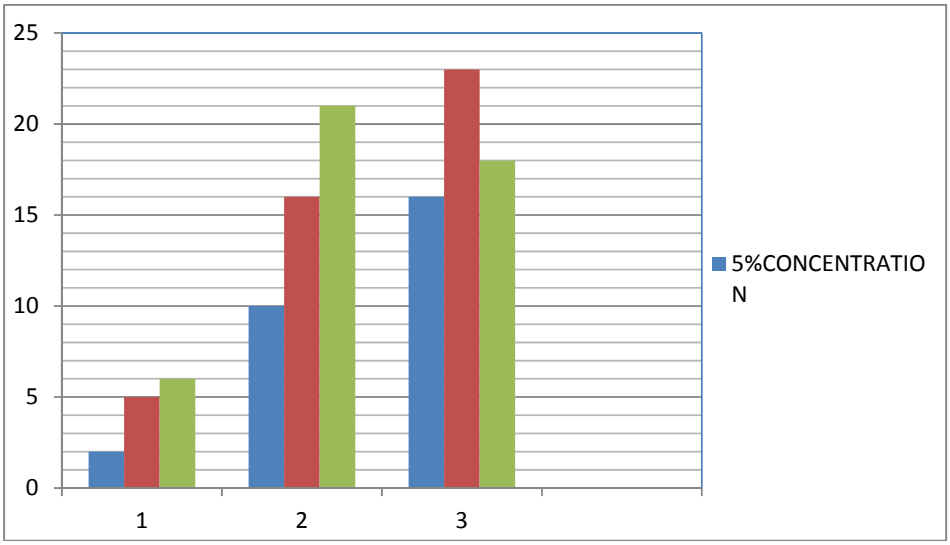

Fig 1: Larvicidal Activity of *Aedes* mosquito larva after the treatment of *Aloe vera* and onion extract

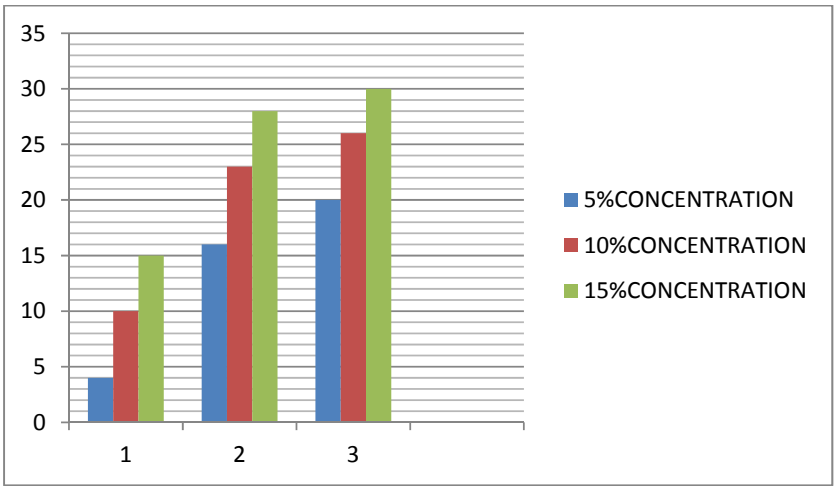

Fig 2: Larvicidal Activity of *Aedes* mosquito larva after the treatment of alcohol and clove extract

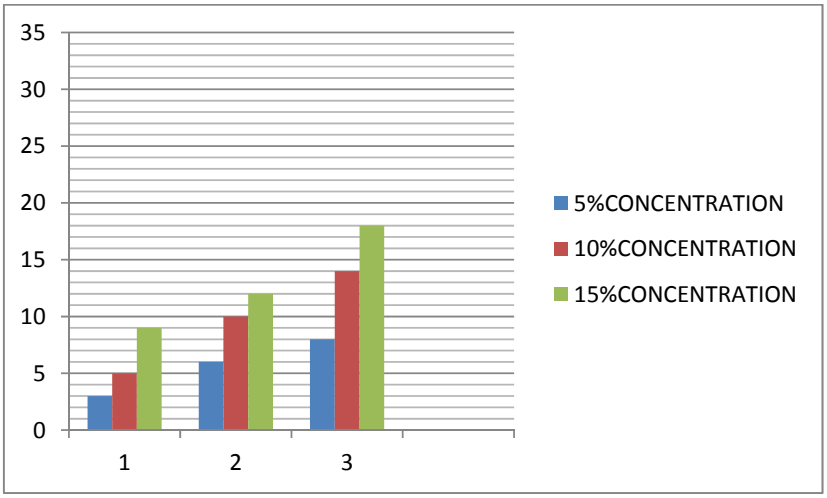

Fig 3: Larvicidal Activity of *Aedes* mosquito larva after the treatment of marigold and garlic extract

Plates

Plate 1: Natural Extracts

Plate 2: Larvicidal bioassay

Plate 3: *Aedes* mosquito larva before and after treatment

4. Conclusions

The outcome of present results concluded that mosquitocidal property of the natural extracts against *Aedes aegypti*. Among them, alcohol-clove extract exhibits highest mortality rate compared with other extracts. Thus plants could be an alternative source for mosquito larvicides because they constitute a potential source of bioactive chemicals and generally free from harmful effects. Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution [20]. Further analysis is required to isolate the active principles and its mode of action in inhibiting the developmental stages in *Aedes aegypti*. The phytochemicals of alcohol and clove extracts can be well utilized for preparing biocides or insecticidal formulation.

5. References

1. Kalyanasundaram M, Das PK. Larvicidal and synergistic activity of plant extracts for mosquito control. Ind J. Med. Res, 1985; 82-85.
2. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. Ind J. Med. Res, 2012; 135:581-98.
3. Amer H Mehlhorn. Larvicidal effects of various essential oils against *Aedes, Anopheles*, and *Culex* larvae (Diptera: Culicidae). Parasitol. Res, 2006; 99:466–472.
4. Kovendan K, Murugan K. Effect of medicinal plants on the mosquito vectors from the different agroclimatic regions of Tamil Nadu, India. Advances in Env. Bio, 2011; 5(2):335-344.
5. Upadhyay SK, Singh PK. Receptors of garlic (*Allium sativum*) lectins and their role in insecticidal action. Protein J, 2012; 31:439–446.
6. Amer A, Mehlhorn H. Larvicidal effects of various essential natural extracts against Aedes, Anopheles, and Culex larvae (Diptera: Culicidae) Parasitol. Res. 2008; 99:466–472.
7. Bagavan A, Rahuman AA, Kamaraj C, Geetha K. Larvicidal activity of Onion extract against *Aedes aegypti* and *Culex quinquefasciatus*. Parasitol. Res. 2008; 103:223–229.
8. Barnard DR, Rui De Xue. Laboratory evaluation of mosquito repellent against Aedes *albopictus, Culex nigripalpus* and *Ochlerotatus triseriatus* (Diptera: Culicidae) J. Med. Entomol. 2004; 41:726–730.
9. Cheng SS, Liu JY, Chang ST, Tsai KH, Chen WJ. Bioactivity of selected plant essential extracts against the yellow fever mosquito *Aedes aegypti* larvae. Bioresour. Technol. 2003, 89: 99-102.
10. Ahmed AS. In vitro effects of aqueous extracts of garlic (*Allium sativum*) and onion (*Allium cepa*) on *Aedes, Anopheles* and *Culex* sp. Parasitol Un J (PUJ), 2010; 3(1 & 2):45–54.
11. Shaalan EA, Canyon DV, Younes MW, Abdel-Wahab H, Mansour AH. Effects of sub-lethal concentrations of synthetic insecticides and *Callitris glaucophylla* extracts on the development of *Aedes aegypti*. J. Vector Eco. 2005; 30:295-298.
12. Azima LH, Ho TM, Vishalani VN, Ahmad TY. Laboratory evaluation of six crude plant extracts as repellents against larval mosquitoes of *Culex* and *Aedes aegypti* sp. Asian Pacif J of Trop Biomed S. 2012; 257-259.
13. Amusan A, Idowu A, Arowolo F. Comparative toxicity effect of bush tea leaves (*Hyptis suaveolens*) and orange peel (*Citrus sinensis*) oil extract on larvae of the yellow fever mosquito *Aedes aegypti*. Tanzania J. Health Res, 2005; 7:174-178.
14. Norashiqin M, Sallehudin S. The Repellent Activity of Piper aduncum Linn (Family: Piperaceae) Essential Oil against Aedes *aegypti* Using Human Volunteers. J. Trop. Med. Parasitol, 2008; 31:639.
15. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: a review. J. American Mosq. Control Asso, 1991; 7:210–237.
16. Surendran SN, Kumaran V, Sivarajah R, Krishnarajah S R, Srikaran R, Raghavendra K. A note on the larvicidal efficacy of saponin constituted crude extracts of plant and animal origin against *Aedes aegypti* L. J. Natn. Sci. Foundation Sri Lanka. 2009; 37(3): 215-217.
17. Kamaraj C, Bagavan A, Elango G, Zahir AA, Rajkumar G, Mariamuthu S. Larvicidal activity of medicinal plant extracts against *Anopheles stephensi* and *Culex tritaeniorhynchus*. Indian J. Med. Res. 2010; 134:101-106.
18. Jawale C, Kirdak R, Dama L. Larvicidal activity of *Cestrum nocturnum* on *Aedes aegypti*. Bangladesh J. Pharmaco. 2011; 5(1): 39-40.
19. Wiesman Z, Chapagain BP. Larvicidal activity of saponin containing extracts and fractions of fruit mesocarp of *Balanites aegyptiaca*. Fitoterapia.2006; 77:420-424.
20. Prajapati VA, Tripathi K, Aggarwal KK, Khanuja SPS. Insecticidal, Repellent and Oviposition-De- terrent Activity of Selected Essential Oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*, Bioresour. Technol, 2005; 96(16):1749-1757.