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Bio-efficacy of *Ocimum gratissimum, Hyptis suaveolens* and *Bti* on the eggs and pupae of *Aedes aegypti*

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Abstract
A laboratory study was conducted to evaluate the bio-efficacy of *Ocimum gratissimum, Hyptis suaveolens* and *Bti* on the eggs and pupae of *Aedes aegypti*. The LC_{50} value of methanolic extract of *O.gratissimum* was 64.15 ppm and 74.49 ppm against the eggs and pupae. *H. suaveolens* caused LC_{50} for ovicidal and pupicidal effect at 72.13 and 99.87 ppm. *Bacillus thuringiensis israliensis* required 12.57ppm registered as LC_{50} value against the eggs. The synthetic insecticide chlorpyrifos at 11.68 and 48.16 ppm produced 50% mortality of eggs and pupae. Among the treatments *Bti* was efficient and ecofriendly ovicidal and pupicidal for *Aedes aegypti*.

Keywords: lc_{50}, ovicidal effect, pupicidal effect, *bacillus thruirngiensis isralensis*, *ocimum gratissimum*, *hyptis suaveolens*

1. Introduction
Mosquitoes are major vector for the transmission of several life threatening diseases. *Aedes aegypti* is generally known as a vector for an arbovirus responsible for dengue fever, which is endemic to Southeast Asi a, the Pacific Island area, Africa and the America [13]. Mosquito control has been becoming increasingly difficult because of the indiscriminate uses of synthetic chemical pesticides which have an adverse effect on the environment and imbalance the ecological equilibrium. Majority of the chemical pesticides are harmful to man, animals, some of which are not easily degradable and spreading toxic effects [8]. Eco-friendly and biodegradable natural insecticides of plant based have been receiving wide attention as an alternative measure for the control of insects, especially mosquitoes as several herbal extrusions are reported to have detrimental effect on insect pests.
More than 2000 plant species have already been screened as potent insecticides providing possiblities to replace synthetic chemical insecticides for controlling mosquito larvae and pupae [5, 2]. Plants from the families of asteraceae, lamiaceae, meliaceae and rutaceae are the most promising mosquito control agents of plant origin [15]. Considerable studies have emphasized for controlling mosquitoes [18,9]. A laboratory study was conducted the efficacy of *Ocimum gratissimum, Hyptis suaveolens and Bacillus thringiensis isralensis* against the eggs and larvae of *A. aegypti*.

2. Materials and Method
Stock Culture of *A.aegypti*
The eggs of *Aedes aegypti* was collected from National Institute of Communicable Diseases Centre, Coimbatore, Tamilnadu. The eggs were transferred to 24 x 18 x 4 cm size enamel coated tray containing 500 ml of water and maintained in the laboratory at 27±2º C and 85 % of relative humidity. Freshly hatched larvae were maintained in the tray and fed with the stock solution of dog biscuit and yeast at 3:1 ratio. Second, third and fourth instars larvae fed with the same powder till the larvae entered in to pupation. The pupae were collected from the culture tray and transferred to the glass beaker and kept in a mosquito cage (50 x 50x 50 cm) for adult emergence. The cage was made up of wooden frame and covered with fine mosquito net. The bottom of cage was fitted with strong card board. The cage door was fitted with muslin cloth to avoid escapes of adults. The adults were maintained at the laboratory condition 27±2º C and 75 to 85 % relative humidity, under 14 L:10 D photoperiod cycle. The adults fed with 10% sugar solution for a period of three days before they were provided an animal for blood feeding. The adult females were fed with the blood of chick. Ovitraps were placed inside the mosquito cage to allow them to lay eggs.

Preparation of botanical extracts
The leaves of *Ocimum gratissimum* and *Hyptis suaveolens* were washed in tap water and shade dried. Dried leaves were grinded with the help of mixer grinder. 100 gm of finely grinded powder of respective plants extracted with 300 ml of methanol with the help of soxhlet apparatus [19]. The extracted liquid was subjected to rotary evaporation in order to remove the chemicals. The dried residues dissolved in ethanol to prepare the stock solution. From the stock solution different concentrations of test solutions (50 to 800 ppm) were prepared as recommended by WHO [20].

Bioassay for ovicidal activity
Equal number of healthy adult mosquitoes (50 male and 50 females) were collected from the stock culture and introduced in separate cages with ovicups containing test solution. After 24 hours, the eggs were removed from the ovicups and introduced into the cups containing untreated water and maintained in the laboratory to find out the hatchability effect. The mortality percentage was corrected by using Abbott's formula [1]. Median lethal dose (LC_{50}) was calculated from the observed data through probit analysis [6].

Bioassay for pupicidal activity
For LC_{50} studies different concentrations of test solutions

were diluted in 150 ml of distilled water and poured into the glass beaker. 25 numbers of fresh, healthy pupae collected from the stock culture and introduced into the glass beaker containing test solution. Untreated distilled water with larvae is used as untreated control. Observations made on the mortality of larvae after 24 hours of release. The mortality percentage was corrected by using Abbott's formula [1]. Median lethal dose (LC_{50}) was calculated from the observed data through probit analysis [6].

3. Results and Discussion

The median lethal concentration of methanolic extract of *O. gratissimum* against the eggs of *A. aegypti* was 64.15ppm (Table-1). The LC90 was 115.53ppm. 72.13ppm was the LC_{50} value for the extract of *H. suaveolens*. These results correlate with the earlier research works. 41.0 and 21.8 % of ovicidal effect was observed when treated with *H. susveolens* from 500 to 1000ppm dose against *A.aegypti* [3]. The decrease in hatchability was dose dependent. The egg hatching rates varied significantly among the concentrations tested with an LC50 of 13.33ppm after 24 hours of exposure to *O. basilicum* essential oil [7]. Mortality of 100% with methanol extract of *Acalpha alnifolia* was exerted at 125 and 300 ppm against the eggs of *A. aegypti* [10].

The bio-control agent *Bacillus thuringiensis isralensis* offered the LC_{50} value of 12.57ppm with the fiducial limit of 13.05 and 12.02ppm. *Bti* concentrations from 0.5 to 2.0mg/l affected the number of eggs in each raft. Eggs exposed for 2 hours to 2.0 mg/l *Bti* had a hatch of 30% after 24 hours, the rate increasing to 57% after 72 hours [12]. Chorpyrifos exhibited 11.68ppm and lethal dose against the eggs.

The pupicidal effect of methanolic extract of *O. gratissimum* expressed as LC_{50} value of 74.49ppm. The fiducial upper and lower limit was 79.55 and 70.05ppm, respectively. LC_{90} value was 128.25ppm (Table - 2). *H. suaveolens* at 99.87ppm caused 50% pupal mortality. The LC_{90} value for *H. suaveolens* was 143.36ppm. The pupal mortality was 7.4 to 14.0% when treated with aqueous extracts of *H.suavelons* against the pupae of *A. aegypti* [3]. 2.6916 and 4.6521 mg/l as LC_{50} and LC_{90} value of chloroform extract of *O. gratissimum* against *Culex quinquefasciatus.* The acetone and hexane extracts offered the LC_{50} value of 3.1511 and 4.1407mg/l, respectively. The methanolic and water extracts registered 34.83 and 38.11mg/l as LC50 value [17]. The essential oil of *Plectranthus glandulosus and Callistemon rigidus* showed significant pupicidal potential against *A.aegypti* and the LC_{50} value was 2.66 and 27.22ppm, respectively [4]. Methanol extract of *Artemisia nilagirica* registered the LC_{50} value against the pupae was 542.11ppm and LC_{90} as 991.29ppm [14].

The pupal stages of *Anopheles gambiae* were less sensitive to *O. basilicum* essential oil comparing to the larval developmental stages. The minimal concentration to obtain 100% mortality was 100ppm after one hour and 24 hours exposure to *O.basilicum* essential oil [7]. For *Citrus sinensis* ethanol crude extract, the median lethal concentration values observed for the pupicidal activity against *A. aegypti* was 342.42ppm [11]. In the present study, *Bti* did not affect the pupae of *A. aegypti*. Chlorpyrifos at 48.16ppm caused fifty percent kill of pupae. Mosquito pupicidal activity of *Bacillus subtilis* in terms of LC_{50} and LC_{90} values against *A.aegypti* was 2.685 and 4.639µl/ml [16].

Table 1: Ovicidal effect of botanicals and bio control agent against of *Aedes aegypti*

Treatment	LC_{50} (ppm)	LC_{90} (ppm)	95% Fiducial limit (ppm)		$\bar{x}$	SD	SE	χ^2	Regression
			Upper	Lower					
O. gratissimum	64.15	115.53	69.54	59.62	59.73	27.21	1.95	0.029	Y=0.768x+2.133
H. suaveolens	72.13	120.32	77.59	67.36	52.8	29.41	6.04	9.76	Y=0.8213x-8.8
Bt. isralensis	12.57	23.76	13.05	12.02	49.66	20.46	1.95	3.54	Y=3.6533x+4
Chlorpyrifos	11.68	55.14	12.14	11.11	89.23	17.07	11.87	4.59	Y=0.5317x+56

Table 2: Pupicidal effect of botanicals, bio control agents against *Aedes aegypti*

Treatment	LC_{50} (ppm)	LC90 (ppm)	95% Fiducial limit (ppm)		$\bar{x}$	SD	SE	χ^2	Regression
			Upper	Lower					
O. gratissimum	74.49	128.25	79.55	70.05	50.66	25.48	1.44	3.34	Y=0.72x-3.33
H. suaveolens	99.87	143.36	103.68	95.02	36.80	19.16	3.19	7.95	Y=0.5387x-3.60
Chlorpyrifos	48.16	107.44	53.39	43.60	68.00	24.01	2.32	0.029	Y=0.6773x+17.2

4. Conclusion

Phyto-chemicals are advantageous due to their environmentally nature, target specificity, none or delayed development of resistance, higher acceptability and suitability for public health management. It was inferred that methanolic extract of *O. gratissimum* and *H. suaveolens* are effective on the eggs and pupae of *A.aegypti* and suitable to incorporate in the integrated vector management programme.

5. References

1. Abbott WS. A method of computing the effectiveness of insecticides. J. Econ. Entomol. 1925; 18:267-269.
2. Anupam Ghosh, Nandita Chowdhury, Goutam Chandra. Plant extracts as potential mosquito larvicides. Indian J. Med. Res. 2012; 135:581-598.
3. Arivoli S, Samuel T. Bioefficacy of *Citrullus colocynthis* (L.) Schrad (Cucurbitaceae) whole plant extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Inter. J. Current Res. 2011; 3(4):296-304.
4. Danga Yinyang Simon Pierre, Nukenine Elias Nchiwan, Esimone Charles Okechukwu, Younoussa Lame. Larvicidal and Pupicidal Toxicities of *Plectranthus glandulosus* and *Callistemon rigidus* Leaf Essential Oils against Three Mosquito Species. Journal of Mosquito Research, 2014, 4(2). doi: 10.5376/jmr.2014.04.0002.
5. Finney DJ. Probit Analysis 3rd edition. Cambridge, UK: Cambridge University Press, 1971.
6. Foko Dadji Gisele Aurelie, Nyegue Maximilienne Ascension, Tsila Henri Gabriel, Awono Ambene Parfait

Herman, Ndong Massouka Pauline, Tamesse Joseph Lebel. Chemical Composition and Ovicidal, Larvicidal and Pupicidal Activity of *Ocimum basilicum* Essential Oil against *Anopheles gambiae*. (Diptera: Culicidae). European Journal of Medicinal Plants. 2016; 16(3):1-13.
7. Ghosh GK. Biopesticide and integrated pest management. A.P.H. Publishing Corporation, New Delhi. 1991, 145-146.
8. Jeyabalan D, Arul N, Thangamathi P. Studies on effects of *Pelargonium citrosa* leaf extracts on malarial vector, *Anopheles stephensi* Liston. Bioresource Technology. 2003; 89:185-189.
9. Kovendan K, Murugan K, Mahesh Kumar P, Thiyagarajan P, John William S. Ovicidal, repellent, adulticidal and field evaluations of plant extract against dengue, malaria and filarial vectors. Parasitology Research. 2012; 112(3):1205-1219.
10. Murugan K, Mahesh Kumar P, Kovendan, Amerasan D, Subramaniam J, Jiang Shiou Hwang. Larvicidal, pupicidal, repellent and adulticidal activity of *Citrus sinensis* orange peel extract against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitology Research. 2012; 111(4):1757-1769.
11. Nayer S Zahiri, Mir S Mulla. Ovipositional and ovicidal effects of the microbial agent *Bacillus thuringiensis israelensis* on *Culex quinquefasciatus* Say (Diptera: Culicidae). Journal of Vector Ecology. 2005; 31(1):29-34.
12. Pancharoen C, Kulwichit W, Tantawichien T, Thisyakorn U, Thisyakorn C. Dengue infection: a global concern. Journal of Medical Association Thailand. 2002; 85:25-33.
13. Panneerselvam C, Murugan K, Kovendan K, Mahesh Kumar P. Mosquito larvicidal, pupicidal, adulticidal, and repellent activity of *Artemisia nilagirica* (Family: Compositae) against *Anopheles stephensi* and *Aedes aegypti*. Parasitology Researc. 2012; 111(6):2241-2251.
14. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: A review. J. American Mosquito Control Association. 1991; 7(2):210-237.
15. Sukumar K, Michael JP, Lewis RB. Botanical derivatives in mosquito control: a review, J. of the Am. Mosq. Control Assoc. 1991; 2(7): 210-237.
16. Sumitha KV. Thoppil. Larvicidal efficacy and chemical constituents of *O. gratissimum* L. (Lamiaceae) essential oil against *Aedes albopictus* Skuse (Diptera: Culicidae). Parasitology Research 2016; 115(2):673-680.
17. Tamilselvan Pratheeba, Chinnasamy Ragavendran and Devarajan Natarajan. Larvicidal, pupicidal and adulticidal potential of *Ocimum gratissimum* plant leaf extracts against filariasis inducing vector. Inter. J. Mosquito Res. 2015; 2(2):01-08.
18. Tsao R, Roman CFE, Peterson CJ, Coast JR. Plant growth regulatory effect and insecticidal activity of extracts of tree of heaven (*Ailanthus altissima*). BMC Ecology. 2002; 2:1-8.
19. Vogel. Text book of practical organic chemistry. The English language book society and Longman, London, 1978, 1368.
20. World Health Organization. Report of the WHO informal consultation on the evaluation on the testing of insecticides, CTD/WHO PES/IC/96.1. Geneva: WHO. 1996, 69.