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LETHAL EFFECT OF BOTANICALS AND BIO-CONTROL AGENTS ON THE LARVAE OF *AEDES AEGYPTI*

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ABSTRACT

The present study is aimed to evaluate the median lethal dose of botanical extracts of *Ocimum gratissimum*, *Hyptis suaveolens*, bio control agent *Bacillus thuringensis isralensis* against the larvae of *Aedes aegypti*. The LC_{50} value of *O.gratissimum* was 55.28 and 59.62 ppm against third and fourth instar larvae. *H. suaveolens* required 59.07 and 79.63 ppm to produce fifty percent larval mortality during the age of third and fourth instar. 2.18 and 3.17 ppm of *Bacillus thuringensis isralensis* registered as LC_{50} value on second and third instar larvae. The synthetic chemical pesticide chloripyrifos achieved fifty percent kill at 5.79 and 6.41 ppm. Among the treatments, *Bacillus thuringensis isralensis* required least dose to kill the larvae after 24 hours of treatment.

KEYWORDS: Median Lethal Dose (LC_{50}), Larvicidal Effect, Botanical Extracts, *Bacillus thuringensis isralensis.*

INTRODUCTION

Mosquitoes are the most important in public health importance, which transmit a number of diseases like malaria, filariasis, dengue, Japanese encephalitis, etc., causing millions of deaths every year (Arivoli and Samuel, 2011: Das *et. al*., 2007). During the past several decades, many synthetic organic insecticides have been developed and effectively used to eliminate mosquitoes. Most insecticides developed after DDT generally have been synthetic, non-selective in nature. Although they effectively controlled pest species, their extensive use has

led to serious social and environmental repercussions (Anyack and Amusan, 2003). Presently the mosquito control programme has been limited due to lack of novel insecticides, high cost of insecticides, concern for environmental sustainability, harmful effect on human health and other non-target organisms, their non- biodegradable nature, higher rate of bio-magnification and increasing insecticide resistance on a global scale (Brown, 1986; Russel and Skilleter, 2009).

In recent times, chemicals derived from plants (Botanicals) have been used as pest management tool as they are shown to ecologically friendly. Plant based bioproducts are mostly non-toxic to humans and other mammals and have high degree of biodegradation (Rahuman *et. al*., 2009; Tripathi *et. al*., 2002). Kishore *et. al*., (2011) reviewed the efficacy of phytochemicals against mosquito larvae based on their chemical nature and mosquito larvicidal potentiality of several plant derived secondary metabolites. Oleanolic acid, a bioactive component present in the leaves of *Ocimum gratissmum* was effective against the larvae of *A. aegypti* (Njoku *et. al*., 2008). The application of entamopathogen such as bacteria, virus and fungi are the other alternative and safe pest management tool. *Bacillus thringensis isralensis* (Bti) and its toxins are extensively used for pest control purpose in agriculture, forestry and public health programme since 1930s. (Hilbeck and Schmidt, 2006). *Bti* are highly infective and toxic to dipteran larvae and useful for bio larvicides in mosquito eradication programme (Goldberg and Margaliat, 1977). Tanya *et. al*., (2003) conducted laboratory bioassay of water dispersible granule formulation of *Bti* (3000 ltu/mg) against third instar larvae of six common Australian mosquito species and found that it was for treatment of freshwater larval habits. Hence, a study was conducted to evaluate the lethal concentration of botanical extracts of *O. gratissimum, Hyptis suaveolens, Bacillus thuringensis isralensis* (*Bti*) and chloripyrifos against third and fourth instar larvae of *A. Aegypti*.

MATERIALS AND METHODS

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±2ºC 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 (Vogel, 1978). 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, 1996.

Bioassay for larvicidal activity

Larvicidal bioassay followed the WHO standard's (WHO, 2005). For LC50 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 third and fourth instar larvae separately 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 (Abbott, 1925). Median lethal dose (LC_{50}) was calculated from the observed data through probit analysis (Finney's, 1971).

RESULTS AND DISCUSSION

The LC_{50} value of botanical extracts, bio-control agents and synthetic chemical pesticide chlorpyrifos against third instar larvae of *A. aegypti* is presented in Table - 1. The LC_{50} value of *Ocimum gratissimum* against the third instar larvae of *Aedes aegypti* was 55.28 ppm with the fiducial limit of 60.02 and 51.07ppm. The LC_{90} was 110.05 ppm. This correlates with the earlier findings of Rathy *et.al.*, (2015). They found aqueous extracts prepared from *O. gratissimum* has strong Larvicidal activity against third instar larvae of *A. albopictus* and reported 100 percent mortality at 96 hours of treatment at the dosage of 8ml. The LC50 value

of 21.83 µg/ml for the leaf extract of *O. gratissimum* against *Culex gelidus*. (Kamaraj and Rahuman, 2010). Mgbemena (2010) found that 19.50µg/ml of *O. gratissimum* was the lethal dose against *A.aegypti*.

Table - 1. Lethal concentration (LC_{50}) of botanicals, bio control agents against third larval instar 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	55.28	110.05	60.02	51.07	64.53	26.10	3.02	0.119	Y=0.736x+9.33
H. suaveolens	59.07	99.86	64.52	55.20	54.33	27.05	2.78	1.09	Y=0.9653x-6.00
Bt. isralensis	2.18	11.52	2.64	1.75	87.50	19.38	7.11	1.10	Y= 3.3487x+45.65
Chlorpyrifos	5.79	9.46	6.27	5.23	42.20	25.05	1.26	3.32	Y= 11.2x-14

In the present study, *H. suaveolens* offered the median lethal dose (LC_{50}) of 59.07 with the upper and lower limit of 64.52 and 55.20 ppm, respectively. This finding corroborates with earlier observations of Okigbo *et.al*., (2010). They noted 100% mortality was achieved by *O. gratissimum* at the concentration of 50% after 24 hours, while *H. suaveolens* at 60% showed no significant effect as mosquito larvicide. The LD_{50} values of petroleum ether leaf extracts of *O. gratissimum* and *H.suaveolans* was 11.40 and 66.83.

This experiment revealed that the bio-control agent *Bacillus thuringiensis israelensis* (*Bti*) exhibited 2.18 ppm as median lethal dose when treated against third instar larvae. The median lethal dose of *Bti* ranged between the limits of 2.64 and 1.75 ppm. The LC_{90} was 11.52 ppm. The LC_{50} of chlorpyrifos was 5.79ppm. This observation comparable with the research works of Tanya *et. al*., (2003). They observed the water dispersible granule formulation of *Bti* at 3000 ltu/mg significantly effective against third instar larvae of six common Australian mosquito species found in freshwater larval habits. Bela and Swapnil (2015) reported that the secondary metabolites isolated from *Bacillus* caused 100% mortality at a concentration of 100ppm.

The median lethal dose of *O. gratissimum* against the fourth instar larvae was 59.62 ppm. The fiducial limit ranged between 55.23 and 64.54ppm. The LC_{90} was 114.94 ppm. Fifty percent mortality was observed after 24 hours of treatment with *H. sauveolens* at 76.63ppm (Table - 2). These findings correlate with the earlier findings of Pratheeba *et. al*., (2015). They found that hexane, chloroform, ethyl acetate, acetone, methanol and water extracts of *O. gratissimum* registered the LC_{50} value against the larvae of *A.aegypti* was 39.86mg/l,

37.21mg/l, 24.57mg/l,28.70mg/l, 30.38mg/l and 52.56mg/l. Acetone, hexane and chloroform extracts of *O. gratissimum* registered 3.0511mg/l, 3.5407mg/l and 2.8916mg/l as median lethal dose against the larvae of *C. quinquefasciatus.* Sosan *et.al*., (2001) reported the larvicidal activities of essential oils obtained from *O. gratissimum* against *A.aegypti* as they achieved 100% mortality in 120ppm concentration.

Table - 2. Lethal concentration (LC50) of botanicals, bio control agents against fourth larval instar 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	59.62	114.94	64.54	55.23	61.06	25.98	6.61	1.08	Y=0.7253x+6.67
H. suaveolens	79.63	134.28	84.46	75.33	46.66	27.94	5.05	2.17	Y=0.784x-12.13
Bt. isralensis	3.17	18.75	3.73	2.68	73.66	14.52	1.95	4.62	Y= 2.5864+41.33
Chlorpyrifos	6.41	10.79	6.94	5.98	36.66	21.19	1.57	2.83	Y= 9.4667x-10.67

The experiment revealed that *Bt. israelensis* offered 3.17 and 18.75 ppm as LC_{50} and LC_{90}value against the fourth instar larvae of *A. aegyoti*. 6.41ppm of chlorpyrifos exhibited the larval mortality after 24 hours of release. This observation confirms the findings of Chandrakan *et.al*., (2016). They found that *Bti* standard strains offered the LC50 value of 1.635ppm and *Bt* SV2 (Isolate) registered 1.891ppm as median lethal dose against the fourth instar larvae after 24 hours of treatment. *Bt* positive control exhibits lethality at an early hour of inoculation having the highest larvicidal potency with LC_{50} value of 0.36 CFU/ml at 24 hours and 0.57 CFU/ml at 48 hours. Among the different *Bt* isolates tested for toxicity and lethality of the bacterial isolates was only observable after 48 hours. Isolates from the garden soil exhibits the highest larvicidal potency with LC_{50} values of 0.85, 0.97, 0.13 and 0.32 CFU/ml. These results indicated that *Bt* isolated from garden soil is promising as larvicide against *A. aegypti* (Jing and Franco, 2011). Frank and Stephen (1980) found the LD50 of *Bt. israelensis* was 0.166 (Fiducial limit was 0.157 - 0.175ppm) after 24 hours of treatment. They also reported that the susceptibility of *A. aegypti* to *Bt*. I was depend on the age of larvae. They registered 0.122ppm and 0.77ppm as LC_{50} value of the 24 hour old larvae when exposed to 24 and 48 hours. Similarly, the LD_{50} value of *Bti* was 0.421 and 0.394ppm when treated against the 96 hour old larvae for 24 and 48 hours.

CONCLUSION

The present research study revealed that the plant based extracts and the bio-control agent proved their larvicidal activity against the larvae of *A. aegeypti.* Among the botanicals, *O.*

gratissimum was potent larvicide. The synthetic chemical pesticide chlorpyrifos is also showed larvicidal property. *Bti* was highly effective mosquito larvicide than all other treatments. The limitations of use of synthetic chemical pesticides can overcome by the *O. gratissimum* extract and *Bt. israelensis* are the suitable green larvicide for mosquito management.

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