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*LARVICIDAL POTENTIAL OF STRAIN STREPTOMYCES INDIAENSIS (*ACTINOBACTERIA GROUP) AGAINST DENGUE MOSQUITO *AEDES AEGYPTI* (L.) (INSECTA:DIPTERA: CULICIDAE)

ARTI PRASAD AND ASHOK KUMAR*

Laboratory of Public Health Entomology, Department of Zoology, UCOS, MLSU, Udaipur, Rajasthan, India

*Corresponding author E-mail- ashokdagur198987@gmail.com

ABSTRACT

Mosquitoes are a source of great trouble to human beings and create a danger to public health, as vectors of diseases. Recently to manage vectors a biological control agent, bacteria has proved predictable insecticides, our present study was conducted to evaluate the mosquito larvicidal activity of *Streptomyces indiaensis* against dengue mosquito *Aedes aegypti* (L) as per the method recommended by World Health Organization (WHO, 2005). In the study highest larval per cent mortality was recorded at $3.5x14x10^6$ cfu/ml concentration as 94.44%. The Medan lethal concentration LC_{50} value for the third instar larvae of *Aedes aegypti* (L.) was 1.979 cfu/ml and LC_{90} value was 3.561cfu/ml. at 48 h of exposure time.

Keywords: *Dengue, Aedes aegypti (L.)*, Insecticide, percent mortality, *Streptomyces indiaensis* etc

INTRODUCTION

Mosquitoes are a source of great trouble to human beings and create a threat to public health (Boutayeb, 2006). Dengue is a mosquito-borne viral infection causing a flu-like illness and sometimes causing a potentially mortal difficulty called severe dengue. World Health Organization currently reported that the cases of dengue has increased 30-fold since the last 50 years 50–100 million cases of Dengue infections occur globally every year, putting almost half of the world's population at risk (WHO, 2017). In India dengue is widespread and prevalent in most major cities. Based on the data of National Vector Borne Disease Control Programme (NVBDCP), the number of cases reported in 2017 was about 157220 for dengue with 250 deaths in India (NVBDCP, 2017). *Aedes aegypti* (L.) and *Ae. albopictus* Skuse are the main vectors for dengue fever, dengue hemorrhagic fever and yellow fever (Jahan et. al., 2011; Service, 2004). Mosquito vectors control forms an essential component for the control of mosquito borne diseases. Although dengue and malaria vectors are effectively managed through larvicides and insecticides but there are many cases of insecticide resistance reported for Aedes, Anopheles species.

Vector control is recognized as an successful tool for controlling tropical diseases. In recent days as biological control agent Bacteria, have some important merits over predictable insecticides in mosquito control programs, besides being safe to non-target organisms including humans, also it is harmless to the environment. As *Bti* has been used operationally for the control of mosquitoes since last two decades, and its formulations are highly effective against *Aedes, Anopheles* and *Culex* mosquitoes species (Mahmood, 1998).

The bacterial strain *Streptomyces indiaensis* has been evaluated as a good larvicidal for Anopheles species in our laboratory by Dr. Priyansh Mathur, So our present study was conducted to evaluate the mosquito larvicidal activity of *Streptomyces indiaensis* against human dengue vector *Aedes aegypti* (L).

MATERIALS AND METHODS

Revival and identification of Bacterial strain *Streptomyces indiaensis*

The Lyophilized form of bacterial strain was obtained from Laboratory of Public Health Entomology, Department of Zoology, Mohan Lal Sukhadia University, Udaipur. For revival a loop full was inoculated 500 ml Erlenmeyer flask containing 250 ml of Nutrient broth medium, incubated at 30 ± 2°C up to growth. After 3 days, 1ml culture broth was inoculated on Starch casein agar and incubated at 30 ± 2°C for up to growth in aerobic conditions for pure culture. In next step, after obtaining pure culture the serially diluted culture was plated on Starch casein agar and again incubated at 30 ± 2oC for up to 7 days in aerobic conditions. Isolated

bacterial strains were identified on the basis of their morphological and biochemical characteristics, and confirmed according to Bergey's Manual of systematic Bacteriology 1& 2 (Palleroni, 1986; Sneath, 1986).

Test organisms

The mosquitoes *Aedes aegypti (L.)* were regularly maintained in the Insectary of Laboratory of Public Health Entomology Department of Zoology, under controlled temperature (28 ± 2°C) and relative humidity (75-80%) with 14:10-h light/dark photoperiod cycle. Mass rearing was maintained according to the established method of Limsuwan *et al*., with slight modifications (Limsuwan *et. al*., 1987). The larvae were reared in plastic/ porcelain trays at a density of about 450-500 larvae per 3 L of tap water and fed daily on finely ground dog biscuit and yeast powder in the 3:1 ratio until they became pupae. The pupae were transferred from the trays to plastic cups containing distilled water and placed in breeding cages (30×30×30 cm) for adult emergence. Adults were fed continually with 10 % sucrose soaked in cotton pads. A restrained albino rat/ rabbit was used periodically as a source of blood meal for females in egg production. The eggs, which were laid on filter paper in plastic cups, were kept for 3–4 days to air-dry, before submerging in tap water for hatching.

Larvicidal bioassay experiment

The larvicidal activity of the bacterial stain *Streptomyces indiaensis* was evaluated as per the method recommended by World Health Organization (WHO, 2005). Batches of 30 early third instar larvae were transferred to small disposable test beakers, each containing 100 ml of sterilized tap water without any chlorine were taken. The appropriate volume of dilution was added to 100 ml water in the beakers to obtain the desired target dosage, starting with the lowest concentration. Three replicates were set up for each concentration, and an equal number of controls were set up simultaneously using tap water. The control mortalities were corrected by using Abbott's formula (Abbott, 1925). The LC50 and LC90 were calculated after 24 and 48 h by Probit analysis (Finney, 1979).

$$\textbf{Corrected mortality} = \frac{\text{Mortality in treated} - \text{Mortality in Control}}{100 - \text{Mortality in Control}} \times 100$$

$$\textbf{Percent mortality} = \frac{\text{Number of Dead Larvae}}{\text{Number of Tested Larvae}} \times 100$$

Statistical analysis

The mortality observed (ml/l) was corrected using Abbott's formula during the observation of the larvicidal potentiality of the bacterial stain *Streptomyces indiaensis,* Statistical analysis of the experimental data was performed with MS EXCEL 2007 to find the LC50, Lc90.

Probit analysis for different doses of larval instars and different doses of pupae were done. The fiducial along with LC_{50} and LC_{90} values were calculated to find out the dose concentration for 50 percent and 90 percent mortality. Chi square test goodness of fit was also done to find whether the theoretical and observed values differ significantly or non significantly (Finney, 1979; Throne, 1995).

RESULTS AND DISCUSSION

The entomopathogenic bacterial strain *Streptomyces indiaensis,* of Actinobacteria group has been studied for use as ecofriendly and safe insecticide instead of eco enemy synthetic insecticide. The effect of larvicidal activity was demonstrated in the present study confirm their potential for control of larval population. Effect of *Streptomyces indiaensis,* on the third instar larvae increases with the increase in the dose concentration.

When different doses of *Streptomyces indiaensis* were applied on *Aedes aegypti (L.)* third instar larvae, significant increase in mean mortality with increase in dose as well as time was observed. Mean mortality was directly proportionate to time and doses. At lower doses of $0.5x14x10^6$ cfu/ml, 2.00 ± 1.63 and 5.33 ± 0.94 mean mortality and standard deviation were observed at 24 hours and 48 hours of time exposure respectively. At higher dose of 3.5 $x14x10^6$ cfu/ml, 16.33 ± 1.15 and 28.33 ± 2.08 mean mortality and standard deviation were calculated after 24 hours and 48 hours of time exposure respectively as compared to 0.33 ± 0.57 and 0.66 ± 0.57 in control respectively (Table 3).

Value of per cent mortality at lower dose concentration of $0.5x14x10^6$ cfu/ml was 17.77 after 48 hours but when dose level increased from 1.0,1.5, 2.0, 2.5, 3.0, $3.5\times14\times10^6$ cfu/ml percent mortality increased from 24.44, 37.77, 48.88, 61.11, 74.44 and 94.44 after maximum treatment time of 48 hours (Graph 1).

The probit equation for the per cent data was (Probit = -1.603 + 0.810 Dose) (Table 4). The Medan lethal concentration LC_{50} value for the third instar larvae of *Aedes aegypti* (L.) was 1.979 cfu/ml and LC_{90} value was 3.561cfu/ml (Table 6). When the Chi-square test conducted for the same data the value was (X^2= 4.917 at df = 6) non significant (Table 5).

Table 1. Morphological characteristics of *Streptomyces indiaensis, of Actinobacteria*

Colony Type	Size (mm)	Shape	Edge aspects	Consistence	Transparency/ Opacity	Color	Profile
S	2	Irregular	Lobate	Dry	Transparent	Grey	Convex

Table 2. Biochemical characteristics of *Streptomyces indiaensis, of Actinobacteria* colonies

Spore arrangement	Gram's staining	Acid-fast staining	Starch hydrolysis	Gelatin liquefaction	Casein hydrolysis
+ve	-ve	+ve	+ve	+ve	+ve

Table 3. Biochemical analysis of *Streptomyces indiaensis, of Actinobacteria* colonies

Indole	Methylene red	Vogas proskauer	Citrate	Urease	H_2S
–ve	+ve	+ve	+ve	–ve	–ve

Table 4. Carbohydrate fermentation test of *Streptomyces indiaensis, of Actinobacteria* colonies

Glucose	Lactose	Sucrose
+ve	+ve	+ve

Biotoxicity *Streptomyces indiaensis, of Actinobacteria group* on third instar larvae of *Aedes aegypti* (L.)

Table 5. Mean Mortality and Per cent Mortality

Dose (14×10^6 cfu/ml)	Mean Mortality ± Standard Deviation		Per cent Mortality	
	24 hrs	48 hrs	24 hrs	48 hrs
0.00	0.33 ± 0.57	0.66 ± 0.57	01.11	02.22
0.50	2.00 ± 1.63	5.33 ± 0.94	06.66	17.77
1.00	4.00 ± 1.00	7.33 ± 0.57	10.00	24.44
1.50	8.33 ± 1.52	11.33 ± 1.15	27.77	37.77
2.00	11.33 ± 1.52	14.66± 2.51	37.77	48.88
2.50	13.00 ± 1.73	18.33 ± 3.05	43.33	61.11
3.00	15.00± 1.00	22.33± 3.21	50.00	74.44
3.50	16.33 ± 1.15	28.33 ± 2.08	54.44	94.44

Table 6. Probit Analysis: Probit Equation

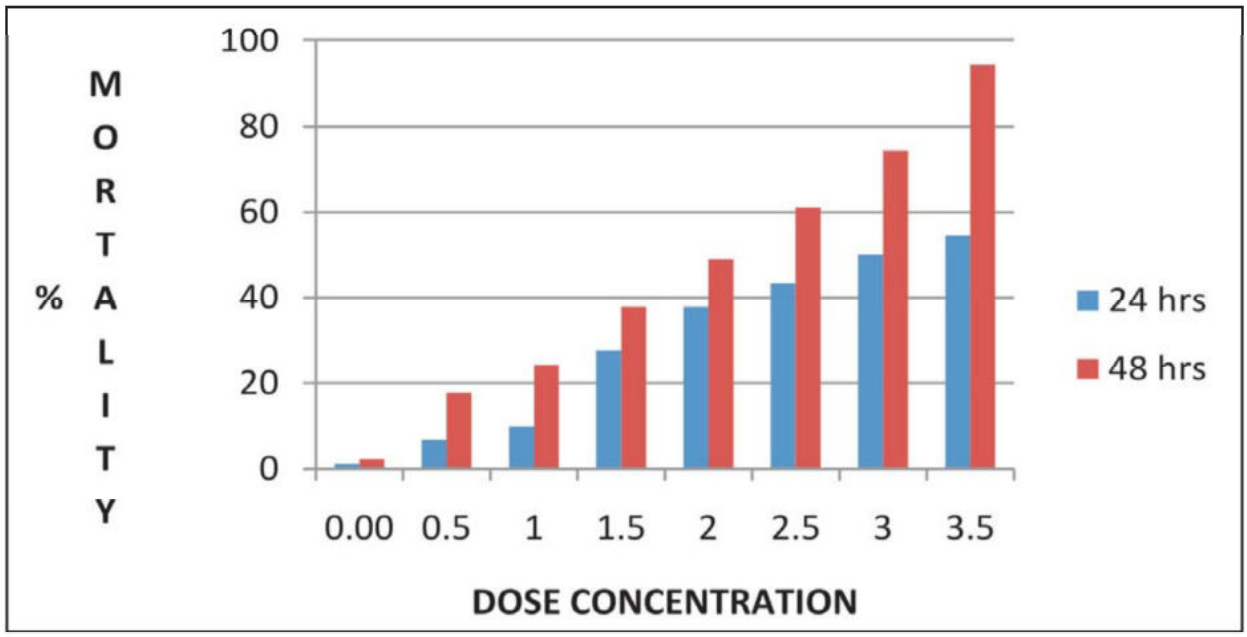
Streptomyces indiaensis	Probit=-1.603+0.810 (Dose)

Table 7. Chi Square

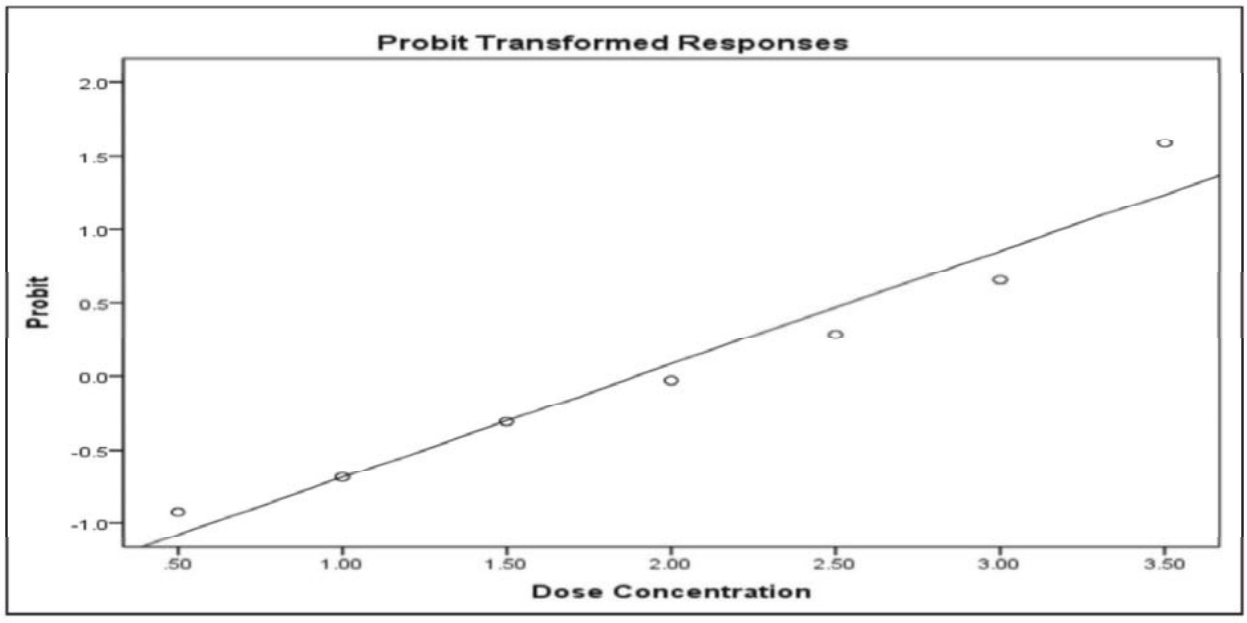
Chi-Square	df	Result
4.917	6	NS

Table 8. Lethal Concentrations

	Dose Concentration (14x106 cfu/ml)	95% CI	
		Lower Limit	Upper Limit
LC50	1.979	1.751	2.219
LC90	3.561	3.184	4.126



Graph 1. Graphical representation for biotoxicity of *Streptomyces indiaensis, of Actinobacteria group* on third instar larvae of *Aedes aegypti* (L.)



Graph 2. Probit transformed responses for concentrations v/s mortality against third instar larvae of *Aedes aegypti* (L) treated with *Streptomyces indiaensis, of Actinobacteria group.*

The mosquito larvae exposed to bacterial strain showed significant behavioural changes. The changes were observed within 30 min of exposure. The most prominent sign of *Aedes aegypti (*L.) was inability to come to the surface. The larvaes also show loss of equilibrium, restiveness and finally led to dealth. Morphological abnormalties were seen like totally damaged cephalothorax, poorely developed head, fragile abdominal segments and profuse anal hairs. These effects may be due to presence of neurotoxic effect of *Streptomyces indiaensis*. No such these types of behavioural changes were obtained in the control sets.

Onkarappa *et. al.,* (2010) evaluated the larvicidal activity of Streptomyces isolates with different- different butanol extract concentrations against the second instar

larvae of *Aedes aegyptii.* (L.) At a concentration of 5 mg/ml, both the isolates caused 100% mortality of the larvae. At concentrations of 1 and 2.5 mg/ml, isolate number 2 exhibited a stronger insecticidal or larvicidal activity than isolate1. Sundarapandian *et. al.,*(2002)reported that Actinomycetes play a significant role in the biological control of insects by developing insecticidal active compounds successfully proved against *Culex quinquefasciatus*. Ghazal *et. al.,* (2001) evaluated the larvicidal activity of fourty one Actinomycete soil isolates against 3rd larval instar of *Musca domestica*, and recorded that Streptomyces and Streptoverticillium were the most effective genera. Quinomycin a from the ethyl acetate extract of Streptomyces sp. KN-0647 was recorded as inhibitor og growth on the insects *Aphis glycines, Culex pipiens, Dendrolimus punctatus, Plutella xylostella* and *Spodoptera exigua.* (Lazzarini *et. al.,*2000 and Liu *et. al*., 2008). Xiong *et. al.,* (2004) reported that an Actinomycete, Streptomyces sp. 173 seawater isolated and sediments, were found as strong larvicidal activity against both brine shrimp and *H. armigera*, similar to that of avermectin B1.Kovendan *et. al.,* (2011) observed that *Streptomyces rochi* against the 1st to 4th instar larvae and pupae of *Anopheles stephensi* gave LC_{50} values for I II III and IV instar were 1.051, 1.157, 2.062, 2.166, respectively whereas it was 2.073 for the pupae.

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