

## AN INDIGENOUS VIRULENT STRAIN OF *BACILLUS THURINGIENSIS*, HIGHLY PATHOGENIC AND SPECIFIC TO MOSQUITOES

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THE Vector Control Research Centre (VCRC) has been trying to isolate indigenous strains of microbial pathogens of mosquito larvae, and several agents have been isolated<sup>1-2</sup>. One strain of *Bacillus thuringiensis* (VCRC B-17) was isolated from soil sample collected from paddy fields about 10 km away from Pondicherry, which was purified and mass-cultured following standard methods<sup>3</sup>.

In the laboratory, the entomotoxicity of this strain was evaluated against fourth instar larvae of *Culex quinquefasciatus*, *C. tritaeniorhynchus*; and *Anopheles culicifacies* and *A. stephensi* (vectors of filariasis, Japanese encephalitis and malaria, respectively) and its efficacy was compared with *B.t.* var. *israelensis* which has been advocated for use as mosquito larvicide by the World Health Organization<sup>4-5</sup>. Safety tests were conducted with silkworms, honey bees and larvivorous fishes.

The strain VCRC B-17 kills mosquito larvae within 24 hr and was found more virulent than *B.t.* var. *israelensis* (Table I). When applied in highly polluted water with heavy breeding of *C. quinquefasciatus*, mortality ranging from 94.6 to 97.5% was observed

in different instars, 24 hr after treatment at a dose of  $16 \times 10^6$  viable spores per litre. Decrease in pupal production was only 43.4% after 24 hr, but 97.4% reduction was observed after 48 hr (Table II). There was no mortality observed in silkworms, honeybees or larvivorous fishes tested indicating the safety of the agent for non-target organisms, with a dose ten times that is necessary to kill mosquito larvae.

Microbial pesticides are being increasingly advocated to control vectors of communicable diseases because of the limitations for the use of chemical insecticides and the resultant environmental pollution. The isolation of this strain of *B. thuringiensis* is a significant development in the search for new tools for vector control. The serotype of this new strain

TABLE I

*LD<sub>50</sub> doses of B. thuringiensis (B-17) and B.t. var. israelensis for different species of mosquito vectors\**

Vector species	Pathogen	
	<i>B. thuringiensis</i> (B-17)	<i>B.t. var.</i> <i>israelensis</i>
<i>Culex quinquefasciatus</i>	$14 \times 10^4$	$34 \times 10^4$
<i>Culex tritaeniorhynchus</i>	$11 \times 10^4$	$13 \times 10^4$
<i>Anopheles culicifacies</i>	$144 \times 10^4$	$344 \times 10^4$
<i>Anopheles stephensi</i>	$83 \times 10^4$	$198 \times 10^4$

\* Refer to viable cell count per 250 ml of water with 50 larvae.

TABLE II

*Efficacy of indigenous strain of B. thuringiensis (B-17) against immatures of filariasis vector in situ*  
(Figures in parentheses represent percentage reduction or increase over pre-treatment reading)

Hours after treatment	Number of immatures or pupae per dip*									
	I Instar		II Instar		III Instar		IV Instar		Pupae	
	C	T	C	T	C	T	C	T	C	T
0	22.9	22.4	20.6	20.1	19.6	17.6	27.9	20.5	10.9	7.6
24	23.8 (+3.9)	1.2 (-94.6)	20.0 (-2.9)	0.5 (-97.5)	14.4 (-26.5)	0.6 (-96.6)	18.1 (-35.1)	1.0 (-95.1)	7.0 (-35.8)	4.3 (-34.4)
48	20.6 (-10.1)	2.3 (-89.7)	16.3 (-20.9)	0.7 (-96.5)	21.7 (+10.7)	0.8 (-95.5)	16.0 (-42.7)	2.8 (-86.3)	8.3 (-23.9)	0.2 (-97.4)

\* Mean of eleven replicates

C : Control      T : Treated

needs to be ascertained and steps are being taken for further identification of the serotype through the World Health Organization.

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**LABORATORY STUDIES ON THE LONGEVITY, INSTAR DURATION AND GROWTH OF THE MALE OF *DAPHNIA LUMHOLTZI* SARS (CLADOCERA: DAPHNIDAE)**

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Most descriptions of Cladocera are based on the study of parthenogenetic females. Males are rare, diminutive and occur for brief periods in field populations. Thus earlier studies on the biology of temperate and tropical forms were almost exclusively confined to females. A review of earlier literature indicated only one report; this dealt with the male of *Daphnia magna*<sup>1</sup>. A comparison was made between male and female life spans at different temperatures (8°, 10°, 18° and 28° C). It was pointed out that the male life span was almost equal to that of the female at low temperatures (8° and 10° C) and markedly less at higher temperatures (18° and 28° C). In the absence of any other report, the present study of *Daphnia lumholtzi* Sars (Cladocera: Daphnidae) is of interest.

The material for this study was collected from Ward's Lake, Shillong (Lat. 25° 5' N; Long. 91° 9' E) with the help of a plankton net of bolting silk (No. 25). Primiparous females were isolated and reared individually in small petri dishes containing filtered lake water. Of the neonatae, which emerged on embryogenesis, healthy juveniles were separated out. Even at the first stage, male instars could be distinguished. About 20 neonatae were reared separately in the filtered lake water in small petri dishes. Daily length increments in different instars were recorded. A

replicate with another set of 20 neonatae was also studied. The data, thus obtained, provided information on longevity, instar duration and growth (Table I). During the course of this study, the laboratory temperature varied between 12–18° C and pH of the lake water between 6.5–6.9.

TABLE I

Shows the number of instars, mean instar length, duration and cumulative duration of the different instars of male *Daphnia lumholtzi*

Instar Number	Mean instar length (mm)	Mean instar duration (hours)	Cumulative duration (hours)
1.	20.505 ± 0.082	41.33 ± 6.136	41.33
2.	0.627 ± 0.084	42.95 ± 6.087	84.28
3.	0.817 ± 0.117	45.88 ± 4.715	130.16
4.	0.863 ± 0.031	48.00 ± ..	178.15
5.	0.879 ± 0.119	52.66 ± 6.020	230.82
6.	0.916 ± 0.099	54.35 ± 7.491	285.17
7.	1.007 ± 0.123	56.57 ± 7.335	341.74
8.	1.061 ± 0.102	57.75 ± 7.861	399.49
9.	1.102 ± 0.109	63.69 ± 5.764	463.18
10.	1.144 ± 0.054	64.61 ± 6.076	527.79
11.	1.176 ± 0.159	66.54 ± 6.267	594.33
12.	1.214 ± 0.043	63.00 ± 5.555	657.33
13.	1.235 ± 0.025	70.50 ± 4.242	727.83

The results of this study indicate that the average life span of male of *Daphnia lumholtzi* is 36.33 days as compared with 42.1 days<sup>2</sup> of the parthenogenetic female. This difference in the longevity of the two sexes almost corresponds to that recorded in *Daphnia magna* at 18° C.

During its life span, the male of *D. lumholtzi* underwent 12 moults. The first male instar measured 0.505 mm in length and the maximum length of 1.235 mm was attained at penultimate instar. There was gradual increase in the duration of the different instars throughout the life span. The length increment of different instars did not follow any trend, although the maximum growth was observed between second and third instars.

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