

# LARGE-SCALE EVALUATION OF *BACILLUS SPHAERICUS* C3-41 LOCAL PRODUCTS FOR CONTROLLING *CULEX QUINQUEFASCIATUS* IN URBAN AREA

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During June to October, 1993, the large-scale field trial of *Bacillus sphaericus* c3-41 local products against *Culex quinquefasciatus* was carried out in Wuhan city. Large breeding sites (more than 2m<sup>2</sup>) including ponds, ditches, troughs were treated with 3ml/m<sup>2</sup> liquid formulation once a ten-day interval, and small containers and soak pits were treated with 8g/m<sup>2</sup> briquette formulation once a month. The results showed 70%~100% reduction of larval density and successful control of seasonal adult density peaks.

**Key words** *Bacillus sphaericus* Local products *Culex quinquefasciatus*

A desire to minimize environmental impact of insecticides as well as to combat insecticide resistance has increased in utilization of microbial insecticides in world. In the past 10 years, some *Bacillus sphaericus* strains showed to be efficacious microbial control agents to mosquitoes. Although it has a narrower spectrum of activity than *Bacillus thuringiensis* (H-14), it appears to be more active against larvae of certain genera of culicine species (Hertlein et al, 1981; Lacey and Singer 1982). Though several strains of *Bacillus sphaericus* were reported to exhibit high activity against larvae of several mosquito species in the laboratory and under field condition (Mulla et al 1988; Lacey and singer 1982), there are few reports on real impact of *Bacillus sphaericus* on adult mosquitoes. The large-scale field tests

were initiated in Shui Guohu district of Wuhan city to evaluate the influence of local products of *Bacillus sphaericus* C3-41 in urban area on adults of *Culex quinquefasciatus*.

## MATERIALS AND METHODS

**Background of the trial area** The field tests were conducted in the Shui Guohu district of Wuhan city with a population of 12000, covering a 9-square kilometer area, 2 kilometer away from control area. In test area *Culex quinquefasciatus* was the dominant mosquito species and the breeding sites included ponds, drains, soak pits, ditches, water jars and water troughs. The characteristics of distribution of breeding sites are that major parts of breeding sites concentrated in the gardens and plant parks, where the jars and troughs kept water for a whole year. They are important breeding sites of *Culex quinquefasciatus*.

**Formulations** The products of 93-BF briquette (160IUT/mg) formulations of *Bacillus sphaericus* C3-41 used were manufactured by Department of Mosquito Control, Institute of parasitic Diseases, Hubei Academy of Medical Sciences. Liquid formulation (200IUT/mg) was produced by Microbialplant of Hubei Academy of Agriculture.

**Laboratory assay** Before field tests, all of the formulations used were evaluated in the

laboratory against 3rd and 4th instar larvae of laboratory strain of *Culex quinquefasciatus*. The bioassay was carried out with the standard procedure recommended by WHO TDR/SHAERICUS/85. 3. In brief, stock suspension of each preparation (W/V) was made with tap water, and serial dilutions in tap water were prepared as needed. The required amounts of the proper strength dilution were added into 500ml enamel bowl, containing 25 3rd or 4th—instar larvae of *Culex quinquefasciatus* in 250 ml tap water. Each formulation was tested at 5 concentrations each with 3 replications. Along with each test, 3 cups were left untreated as controls. All tested organisms were kept at temperature of 27°C. After 48h of exposure, mortality readings were taken, and data obtained were subjected to Log probit regression analysis to obtain the LC50 and LC90 estimates (mg/liter) and 95% confidence limits. With these formulations, the international standard *Bacillus sphaericus* strain 1593 (rb80) provided by The Institute of Pasteur, Paris, France, was also tested and used as a standard.

**Field tests** Field tests was conducted during June to October 1993. The test area was divided into four small treated areas on personal responsibility of treatment. Based on investigation of breeding sites in 1992, all six different ecological types of breeding sites were mapped and numbered. Application of larvicides was started in all breeding sites in the early June this year. The large breeding sites (more than 2m<sup>2</sup>) were treated with liquid formulation at a dose of 3ml/m<sup>2</sup> once a 10—day interval; the small containens and small breeding sites (less than 2m<sup>2</sup>) were treated with briquette formulation at a dose of 8g/m<sup>2</sup> once a month.

### Assessment efficacy

**Larvae:** In the test area, the larval collections were conducted in all the numbered breeding sites before and after treatment. 5 or 10 dips were taken with a 500ml dipper per breeding site 2 days before and 2 days after treatment, then per 10 days. In control areas, the larval collections were done in six selected plots with the same sampling technique.

**Adults:** Adult density data were obtained by three human—bait and one to three light—trap catches in each trial and control area. The human—bait catches were carried out for 2 hours at selected houses three times per month. Mosquitoes were caught by persons sitting with legs and foot bared to the knee from 19 : 30 to 21 : 30. The miniature CDC light traps each with a standard 6v, 100mA bulb were set outside houses and hung about 1.5 meter high against the floor. The light—trap catches were carried out for whole night from 19 : 30 to 06 : 00 next morning, three times a month.

**Data analysis** The data of larval collections from both of trial and control areas were calculated by the formula of Mulla et al (1971) to obtain percent reduction of larval population and the relative population index (R. P. I.) was calculated by the formula of Li Xiao—Pong for assessment of adult mosquitoes in treated area.

## RESULTS

**Laboratory assay** The data on susceptibility of *Culex quinquefasciatus* larvae of laboratory strain to *B. sphaericus* C3-41 products in laboratory are presented in Table 1. *Culex quinquefasciatus* larvae were susceptible to liquid and briquette formulations with LD<sub>50</sub> values of 0. 010 and 0. 0402 mg/L, respectively

after 48h exposure.

**Table 1. Laboratory bioassay of two local products of *Bacillus sphaericus* C3-41 against 3rd and 4th instar larvae of *Culex quinquefasciatus* lab. strain**

formulation	time (h)	LD <sub>50</sub> (mg/L)	95% conf. limits (mg/L)	r	regression equation (Y=a+bx)
liquid	48	0.0100	0.0052~0.0319	1.00	y=2.400+2.600x
briquette	48	0.0402	0.0384~0.0526	0.984	y=3.718+2.123x

**Field trials**

**Larvae:** Using liquid formulation to treat large breeding sites at dose of 3ml/m<sup>2</sup> in an interval of 10 days and using briquette to treat containers and small breeding sites at dose of 8g/m<sup>2</sup> in a month interval resulted in 74%~100%, 95%~100%, 94.7%~100% and 70%~98.5% reductions in test areas 1, 2, 3 and 4, respectively during the three-month observation period. It was observed that *B. sphaericus* C3-41 showed no any efficacy against *Aedes albopictus* at a dose of 6~9ml/m<sup>2</sup> liquid formulation (Table 2).

**Adults:** A significant decrease of the adult mosquito population in the operational area was observed in comparison with untreated control area. The mean of human-bait catches in both of operational and control areas

were 4.91 and 15.68, the mean of light-trap catches were 1.14 and 11.8, respectively. The average value of relative population index (R. P. I.) obtained by sampling of human-bait and light-traps was 25.8 and 20.9 respectively, showing a highly effective control of adult mosquitoes (Table 3). It was also noted that the seasonal density peaks of *Culex quinquefasciatus* adult mosquitoes did not occur in treated area.

**DISCUSSION**

In recent ten years, most of the field trials on formulations of *Bacillus sphaericus* have been conducted only on the efficacy of mosquito larvae. In order to evaluate impact on the adults by treatment with suitable formulation of *B. sphaericus* C3-41 local products in the varied breeding sites, the large-scale field trials were carried out in urban area. The results showed that in urban area, using liquid formulation to treat large open breeding sites at dose of 3ml/m<sup>2</sup> once ten days and briquette formulation to treat containers and small breeding sites at dose of 8 g / m<sup>2</sup> per month

**Table 2. Comparison of larval density of *Culex quinquefasciatus* in both of treated and control areas**

pretreatment mean density (larvac/dipper)	mean density of larvae/dip and reduction of larvae(%) (month, day)												
	6.10	6.20	6.30	7.10	7.20	7.30	8.10	8.20	8.30	9.10	9.20	9.30	
<b>treated</b>													
1	18.47	0.24 (98.3)	0.18 (98.2)	0 (100.0)	0.26 (95.0)	0.23 (95.3)	0.34 (90.0)	0.1 (92.4)	0.18 (74.0)	0.06 (98.5)	0.04 (95.5)	0.20 (99.3)	0.06 (99.2)
2	23.40	0.12 (99.5)	0.20 (99.6)	0.40 (95.1)	0.30 (95.1)	1.24 (98.2)	0.22 (99.1)	0.50 (93.7)	0.14 (99.6)	0.20 (95.1)	0.32 (98.6)	0.18 (97.0)	0 (100.0)
3	66.80	0 (100.0)	0.10 (99.7)	0.12 (99.1)	0.90 (98.0)	0.26 (99.7)	0.22 (96.0)	0.14 (98.8)	0.18 (98.9)	0.18 (99.4)			
4	25.50	0.36 (98.5)	0.70 (94.3)	0.02 (97.5)	0.40 (81.7)	0.12 (95.4)	0.10 (95.4)	0.70 (78.4)	0.18 (98.5)	0.34 (93.4)	0.16 (92.5)	0.24 (93.2)	
<b>control</b>													
1	22.60	17.60	12.60	7.30	6.30	5.70	4.00	1.30	0.70	5.00	1.00	39.70	92.00
2	113.30	136.70	29.30	39.00	30.00	65.30	128.00	38.70	41.60	20.00	23.30	30.00	43.30
3	40.00	27.00	61.30	28.00	28.30	52.00	34.60	17.30	2.30	3.30	7.00	10.30	19.30
4	288.30	230.00	111.00	7.30	19.70	23.70	20.30	29.30	115.30	20.70	46.70	19.30	32.00
$\bar{x}_{\text{treated}} = 0.2490$ $\bar{x}_{\text{check}} = 37.17$ $t = 5.95$ $P < 0.001$ $n' = 48 + 48 - 2 = 94$													

Table 3. The results of adult densities in human-bait and light-trap catches of *Culex quinquefasciatus* in both of treated and control areas

time (month, day)	check area			treatmental area							
	human bait		light traps	human bait				light traps			
	1	2	1	1	2	3	R. P. I.	1	2	3	R. P. I.
pretreatment	13	11	9	12	9	12		10	7	6	
6. 20	13	20	10	10	4	4	59.5	7	0	1	31.5
6. 30	26	15	9	4	5	2	19.2	5	0	3	35.0
7. 10	41	33	23	10	5	5	19.7	8	0	2	16.8
7. 20	33	38	13	10	3	5	18.4	9	0	2	33.2
7. 30	18	22	14	5	7	6	32.7	0	1	0	2.5
8. 09	8	15	10	6	2	4	45.9	2	0	1	11.6
8. 30	5	6	4	2	0	1	10.3	0	0	3	29.2
9. 10	5	16	3	1	2	0	12.8	0	0	0	39.0
9. 20	9	8	5	1	1	2	16.6	0	0	0	23.0
9. 30	2	12	27	3	2	1	31.6	0	0	0	4.3
10. 10	2	13	18	0	1	0	4.4	0	0	0	6.4
means	15.3	17.75	12.58	5.6	3.5	3.6	25.8	3.6	0.66	1	20.9
$\bar{x}_{\text{check}}=15.68$ $\bar{x}_{\text{treated}}=4.86$ $t=4.91$ $P<0.001$ $n'=24+36-2=58$											

resulted in reduction of larval population varied from 70% to 100%. It is evident from this study that local products of *B. sphaericus* C3-41 are effective to reduce adult population of *Culex quinquefasciatus*, compared with control area. The seasonal density peaks of adults were under control in the test year.

The characteristics of breeding site distribution in urban area of Wuhan city is that most of breeding sites are containers and small soak pits, these breeding sites concentrate in the gardens and plant parks and these are important breeding sites in urban area of this city. Developing long-acting formulations for treating those places mentioned above is more economical than short-acting formulation. It was reported that liquid formulation (BSP-2) of *B. sphaericus* strain 2362 produced good initial control at rate of 0.56 kg/ha and lasted for 7 to 14 days. However, some granular for-

mulations (ABG-6185) yielded excellent initial and persistent control for 14~21 days at dose of 5.6 kg/ha (Mulla et al, 1988). In 1992, our field trials in the similar ecological area also demonstrated that sustained release of briquette formulation showed excellent persistent control. Under field condition, the residual efficiency of briquette in water jars could last for over two months, much longer than that of liquid. Unfortunately, the briquette did not showed satisfactory control in large and open breeding sites. In present study, we treated breeding sites with suitable formulations according to breeding types and obtained satisfactory control.

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## 球形芽孢杆菌 C3-41 地方制剂大面积防制 城市致倦库蚊的现场效果评价

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**摘要** 本文报告 1993 年 6~10 月,在武汉市水果湖地区应用球形芽孢杆菌 C3-41 地方制剂大规模防制致倦库蚊的现场研究。用 3mg/m<sup>2</sup> 乳剂处理大面积孳生地(大于 2m<sup>2</sup>),每 10d 一次,包括水塘、水沟、水槽等;用 8g/m<sup>2</sup> 的块剂处理小型容器和积水坑,一月一次。结果表明,幼虫密度下降了 70%~100%,并能成功的控制成蚊季节性高峰。

**关键词** 球形芽孢杆菌 地方制剂 致倦库蚊

## 成武县 1988~1992 年丝虫病流行病学监测

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成武县历史上是山东省班氏丝虫病流行较重的地区。70 年代始,经大面积普查普治和重点病区全民服药,微丝蚴阳性率从 1972 年的 3.04% 降至 1983 年的 0.01%,同年经卫生部确认达到了基本消灭丝虫病标准。于 1988~1992 年全面展开了丝虫病后期监测。

### 一、纵向监测

选择原丝虫病流行严重的九女乡 2 个行政村为监测点进行监测。1988 年血检 809 人,阳性 1 人,阳性率为 0.12%。1989~1992 年血检 2 344 人,结果均为阴性。1990 年血清荧光抗体试验 201 人份,阳性 9 人,阳性率为 4.48%。1991 年检测 8~11 岁儿童 125 人份,

经省寄生虫病防治研究所 IFAT 检测均为阴性。1988~1992 年在监测点病家周围于流行季节共捕获淡色库蚊 8 562 只,均未发现阳性。

### 二、横向监测

对九女、伯乐、天官 3 个乡镇 5 个重点行政村监测。累计血检 2 355 人,检出微丝蚴血症 3 人,阳性率为 0.13%。1992 年自然人群及 1~10 岁儿童分别血检 844、520 人,IFAT 检测均为阴性。1988 年复查 1980 年以来 29 例微丝蚴阳性者,仍阳性者 1 人,加上纵向村 1 人,共 2 例微丝蚴血症阳性者;1990~1992 年血检均为 0。