

BACILLUS SPHAERICUS AS MOSQUITO LARVICIDE

Pages with reference to book, From 199 To 201

Abdul Aziz, M.H. Qazi, Riaz, A. Pal (Department of Biological Sciences, Quaid-i-Azam University, Islamabad.)

Abstract

In Noorpur Shahan, a village in the outskirts of Islamabad, *Bacillus sphaericus* was tested to determine its efficacy against mosquito larvae. Since the creation of this new Islamabad district no mosquito control measure has been taken in the area and like so many other places in and around Islamabad, mosquito density is unusually high in this village. The efficacy of *Bacillus sphaericus* was studied upto seven weeks after its application and it gave good larval control (JPMA 39:199, 1989).

INTRODUCTION

In almost all countries, chemicals are used for mosquito control programmes. Continuous and indiscriminate use of these chemicals has adverse toxic effects on fishes and aquatic life, and other biotic flora and fauna is always under threat and the mosquitoes are also becoming resistant to these insecticides¹. Lack of proper knowledge of the man who is spraying the insecticide and of the residents where the insecticide is being sprayed, results in insecticide poisoning and mammalian toxicity. In 1976 alone 2500 cases of such poisoning were recorded in Pakistan, of which five were fatal², therefore, in the same Bulletin, WHO's recommendations for the safe use of pesticides were made². Considering the worldwide increase in insecticide resistance and other related hazards to biotic flora and fauna, the WHO Expert Committee on Vector Biology and Control discussed and suggested that counter-measures should be further investigated and encouraged³. Islamabad-Rawalpindi and its environs are heavily infested with different species of anopheline and culicine mosquitoes like *Culex tritaeniorhynchus*, *Culex fatigans*, *Aedes aegypti*, *Ae. albopictus*, *Anopheles culicifacies*, *An. fluviatilis*, *An. stephensi* and other lesser prevalent species. Apart from their role as vectors of different diseases, mosquitoes have always been a nuisance factor due to their anthropophilic habits. Therefore in the present studies *Bacillus sphaericus* which is harmful to mosquito larvae was tested under field conditions to investigate its efficacy against mosquito larvae. *Bacillus sphaericus* was isolated from mosquito larvae by Singer in 1973⁴. It is an endospore forming bacillus. Endospores are rod shaped with swollen terminals. Only highly toxic strains produce parasporal crystals resembling *Bacillus thuringiensis* other strains do not produce such crystals⁵. There are almost 30 strains of *Bacillus sphaericus*. Strain SSII-I was used in early days⁶⁻⁸. It was found to be unstable and ultimately the present day dry and powdered form was obtained in 1953 and is now being produced on a large scale. When the bacilli are ingested, along with other gut flora they are digested into the peritrophic membrane, crystal like bodies are dissolved, toxins released which penetrate the trophic membrane and the larvae die and then host tissue and posterior gut swells and deteriorates. The biological agents are all non-toxic to fish, food crops, animals and man when compared with the chemical insecticides.

MATERIAL AND METHODS

The study area for this project was selected by the availability of natural ditches serving as larval breeding places. Ideal places were found in Noorpur Shahan where 23 to 4sq. m. artificial ponds were made around a stream. The depth of the water was 8 to 10 centimeters. This slow moving stream has nearly clear and clean water with a gravel bed and serves as a good breeding place for both the culicine

and anopheline larvae throughout the year except during severe drought and in winter. The sides of the stream were entrapped along with water in the artificial ponds. Either 2 ml of *Bacillus sphaericus* was uniformly sprayed in these places by using a small spray gun. Each concentration was replicated twice and a control was also kept for comparison of the results. Observations were recorded at 2, 24, 48, 72 hours and then at weekly intervals up to 8 consecutive weeks after bacilli application. For taking observations larval dips were taken, the larvae were transferred to a dean cloth, counted quickly and then again transferred back to the same small pond. To confirm the death of the larvae due to microbes, 25 larvae from each ditch were picked randomly at the time of taking these observations. These larvae were torn with the help of a needle in the laboratory on slides, were dried, stained with Giemsa stain for 6 minutes and were then observed under oil immersion to see the presence of bacilli.

RESULTS

Before spraying *Bacillus sphaericus* at a volume of 1 ml per sq.m. the average population density was 52.1 larvae per dip and in the control site the average larval count was 44.8 larvae per dip. After 2 hours of microbial larvicide application the larval density in the treated site was found to be 21.8 larvae per dip count and in the control site as 45.4 larvae per dip count. The reduction in population density was 51.98 percent. 24 hours after application, the average population density in the treated site was zero larvae per dip count and in the control site was 43.6 larvae per dip count. There was thus a reduction in the population density of 100%, 48 hours after application, the larval density in the treated site was again zero larvae per dip count whereas 46 larvae in the control site were recorded. All the observations until four weeks after treatment gave 100% control of mosquito larvae whereas in the control site an average of plus/minus 50 larvae were collected during dip observations. In the fifth week after application, the results showed a larval densities of 5.8 and 51 larvae per dip count in the treated and control places respectively. There was an overall reduction in population density by 88.62 percent. Six weeks after treatment, the larval density in the treated place was 7.6 larvae per dip and 49 larvae per dip count in the control place, respectively. The reduction in population density was, therefore, 84.48 percent. After 7 weeks of pesticide application, the average larval dip count in the treated site was 12 larvae per dip count and in the control pond 50.8 larvae per dip count giving a population reduction of 76.38 percent. In the eighth week, due to a rise in temperature, natural death of the larvae occurred and the larval density in the control pond also decreased significantly and therefore the experiment was discontinued (Table-1).

TABLE I. Average Larval Dip Counts.

	<u>Bacillus sphaericus</u>		Control
	2 ml	2 ml	
Before spray	52.1	51.6	44.8
2hrs after	00	00	43.6
24hrs after	00	00	43.6
48hrs after	00	00	46.0
72hrs after	00	00	49.1
1 week after	00	00	51.2
2 weeks after	00	00	52.8
3 weeks after	00	00	50.8
4 weeks after	00	00	50.4
5 weeks after	5.8	00	51.0
6 weeks after	7.6	00	49.0
7 weeks after	12.0	5.0	50.8
8 weeks after	10.7	8.8	14.8

The site where two nil per sq.m. *B. sphaericus* was applied, gave 51.6 larvae per larval dip count before pesticide application. The larval density in the control site was 44.8 larvae per dip count. Three hours after pesticide application the larval dip count in the treated site was nil while it was 45.4 larvae in the control site. A hundred percent reduction in larval density had occurred. All the observations including 24,48 and 72 hours until six weeks after bacilli application gave complete freedom from mosquito larvae in the treated site whereas in controls, the average larval density ranged from 43.6 to 52.8 larvae per dip count. After seven weeks of treatment the average larval density in the treated and control sites was 5 and 50.8 larvae per dip count, respectively, the percent reduction in population density being 90.15%. In the eighth week, the experiment was discontinued because of rise in temperature and sudden abnormal death of larvae in both the treated and control sites (Table-II).

TABLE II. Percent reduction in Larval Density Bacillus Sphaericus.

	Larval dip count			Larval dip count in	
	Control	1ml	%reduction	2ml	%reduction
Before Spray	44.8	52.1	—	51.6	—
2 hrs after	45.4	51.98	51.98	00	100
24 hrs after	43.6	00	100	00	100
48 hrs after	46	00	100	00	100
72 hrs after	49.1	00	100	00	100
After 1 week	51.2	00	100	00	100
After 2 weeks	52.8	00	100	00	100
After 3 weeks	50.8	00	100	00	100
After 4 weeks	00.4	00	100	00	100
After 5 weeks	51	5.8	88.62	00	100
After 6 weeks	49	7.6	84.48	00	100
After 7 weeks	50.8	12	76.38	5	90.15
After 8 weeks	14.8	10.7	27.21	8	40.55

DISCUSSION

Bacillus sphaericus had good initial toxicity towards different species of anopheline and culicine larvae. Laojana and Boonluan⁹ used bacillus sphaericus against *Culex quinque fasciatus* and obtained a 90% mortality in clean water but according to them mortality in clean water lasted comparatively longer (35 weeks) than in polluted water. Bacillus sphaericus gave good residual toxicity which lasted for seven consecutive weeks. Although the population density of larvae in all the treated and control sites dropped abruptly due to rise in temperature and the experiment was discontinued but bacillus sphaericus was still killing the larvae and reducing their density. Two ml sq.m. was definitely better than 1 ml per sq. meter. It is, therefore, concluded that keeping in view the non toxic effects of microbial insecticides to biotic flora and fauna, chemicals may be replaced by biological pesticides. If a campaign is started for a period of 3 to 5 years with emphasis on establishing covered drainage systems in urban areas and the removal of breeding places in rural areas with the cooperation of the Ministry of Agriculture and Local Government and this campaign is supplemented with the use of bacillus

sphaericus and larvivorous fish in ponds and other selected breeding places, only then could we succeed in controlling malaria and the mosquito problem in the country. It is also concluded that further research in genetic engineering for mutually crossing the two bacilli, i.e., Bti and bacillus sphaericus should be conducted in the universities and research institutes with the aim of obtaining a better microbial pesticide.

REFERENCES

1. World Health Organization. Resistance of vectors and reservoirs of diseases of pesticides. WHO Tech. Rep. Ser., 1976; 585:88.
2. World Health Organization. Safe use of pesticides. WHO Tech. Rep. Set, 1979; 634:5.
3. World Health Organization. Resistance of vectors of disease to pesticides. WHO Tech. Rep. Ser., 1980; 655:63.
4. Singer, S. Insecticidal activity of recent bacterial isolates and their toxins against mosquito larvae. Nature (London), 1973; 244:110.
5. Davidson, E.W. and Myers, P. Small scale field trial of bacillus sphaericus. Fed. Eur. Microbiol. Soc. Microbiol. Lett., 1981; 10:261.
6. Singer, S. Entomogenous bacilli against mosquito larvae. Dev. Indus. Microbiol., 1974; 15:187.
7. Singer, S. Isolation and recognition of bacterial pathogens of Vectors, in biological regulations of vectors. Edited by J.D. Briggs. DHEW Publ. No. (NIH) 1987, p.77-1180,3-17.
8. Davidson, E.W. Pathogenesis of bacterial diseases of vectors in biological regulation of vectors. Edited by J.D. Briggs. DHEW Publ. No. (NIH) 1977, p. 77-1180.
9. Laojana, C. and Booluan, P. Small scale field trial of bacillus sphaericus against Culex quinquefasciatus. Mos. Borne Dis. Bull., 1987; 4:1.