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ACKNOWLEDGEMENTS I express my sincere thanks to R. Ramesh for his assistance in the isotopic measurements at P R L, Ahmedabad, and K. Gopalan for his encouragement and support to carry out this work The support and help provided by P. K. Banerjee in obtaining the core samples is thankfully acknowledged. I thank the referees of *Current Science* for their valuable comments. Thanks are also due to S Ramaswamy for providing me the samples of the core SK-72.

Received 15 February 1995, revised accepted 15 September 1995

Resistance to *Bacillus sphaericus* in *Culex quinquefasciatus* Say 1823

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Biological control of *Culex quinquefasciatus* using *Bacillus sphaericus* was considered a practical solution because of its specific and prolonged killing action against mosquito larvae. To study the feasibility of *B. sphaericus* ('Spherix') in mosquito control, multicentric trials were undertaken. Initially, *B. sphaericus* was very effective but within a year, after 20–25 rounds of application, field populations of *Cx. quinquefasciatus* developed resistance up to 150-fold. Genetic studies revealed that resistance was recessive, autosomal and controlled by more than one gene. This is the first report on nature and mode of inheritance of resistance against *B. sphaericus* in mosquitoes.

CULEX quinquefasciatus Say, 1823, is the major vector in the transmission of lymphatic filariasis caused by the nematode *Wuchereria bancrofti* in the tropics. To control *Cx quinquefasciatus*, *Bacillus sphaericus* is emerging as a promising larvicide[1,2]. Extensive multicentric field trials carried out by Malaria Research Centre in India showed that the spraying of 'Spherix' (a commercial preparation of *B. sphaericus* Russian strain B-101, Serotype H5a 5b;) at the rate of 1 g/m^2 provides control of *Cx. quinquefasciatus* breeding for 2–4 weeks[3,4]. After 20–25 rounds of continuous field application of *B. sphaericus* within a year, reduction in its efficacy against *Cx. quinquefasciatus* was noticed. In this communication we report the development of resistance against *B. sphaericus* in field populations of *Cx. quinquefasciatus* and its mode of inheritance in this species.

Adult mosquitoes from *B. sphaericus* sprayed and unsprayed areas of Farrukhabad and Ghaziabad (Uttar Pradesh), Madras (Tamil Nadu) and Panaji (Goa) were collected and transported to MRC laboratories in Delhi for colonization[5] and further studies. In addition to these field populations, two laboratory strains maintained since 1988, one each from Delhi state and Sonepat (Haryana) were also used in bioassay and genetic crosses. Bioassays were performed following the method of WHO[1] with necessary modifications[6]. One per cent stock suspension of Spherix in dechlorinated water was prepared fresh and was used immediately for the bioassays. Twenty-five late III/early IV instar larvae were placed in 250 ml of water containing the required dose of *B. sphaericus* for 24 h and scored for mortality. All the tests were carried out in two replicates at 27± 1°C. Dose–mortality responses of treated lines were analysed using probit regression method of Finney[7].

Dose–mortality regression of the field populations and two laboratory strains are given in Figure 1 and Table 1.

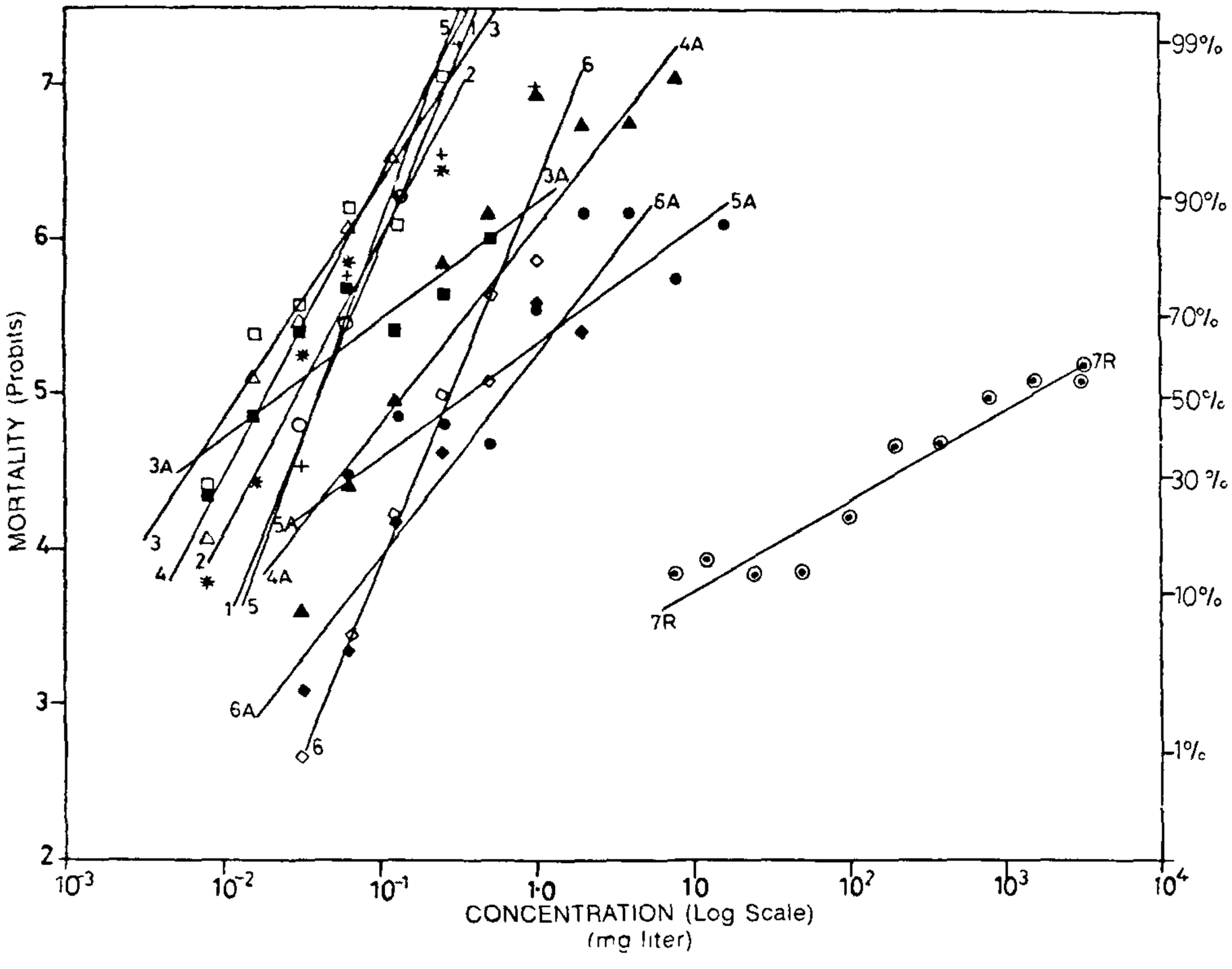

Figure 1. Dosage–mortality responses of susceptible laboratory strains from Delhi (1) and Sonepat (2) unsprayed (3–6) and sprayed (3A–6A) field populations Farrukhabad (3,3A), Ghaziabad (4,4A), Madras (5,5A), Panaji (6,6A), and of homozygous *B sphaericus*-resistant strain isolated from *B sphaericus*-sprayed area of Ghaziabad (7R). Each point represents observed mortality after 24 h of treatment. At each dose 2–4 replicates (25 larvae/replicate) were used.

The LC_{50} and LC_{90} values in mg/l for Delhi and Sonepat strains were 0.04 and 0.032, and 0.134 and 0.137 respectively. These values are considered as those of susceptible populations, since these lines were established from areas where *B. sphaericus* was never sprayed. From the dose–mortality response, a dose of 2 mg/l, which was approximately double the value of $LC_{99.9}$ of susceptible strains, was calculated and subsequently used as diagnostic dose in the present studies.

The LC_{50} and LC_{90} values of the unsprayed field populations were similar to the two susceptible strains except those of Panaji, which were higher (Table 1). The higher LC_{50} and LC_{90} values in Panaji unsprayed areas could be due to the possible vigour tolerance as indicated by nonsignificant chi-square values tested for heterogeneity of response and also by the slope values which were similar to other unsprayed areas (Table 1, Figure 1). However, the LC_{50} and LC_{90} values of populations from unsprayed areas were significantly different from those of the populations from the sprayed areas. This was supported by the nonoverlapping 95% fiducial limits of LC_{50} and LC_{90} values[8] as given in Table 1. Of the four *Cx. quinquefasciatus* populations collected from *B. sphaericus* sprayed areas, chi-square values of the dose–mortality responses of Madras and Ghaziabad populations were significant ($P < 0.05$), indicating the heterogeneous nature of these populations (Table 1).

Data on LC_{50}, LC_{90}, slope values and resistance ratios as given in Table 1 clearly indicate the selection of resistance to *B. sphaericus* in field population of *Cx. quinquefasciatus*. A homozygous *B. sphaericus*-resistant strain of *Cx. quinquefasciatus* was isolated from a population collected from the sprayed areas of Ghaziabad. This strain is being maintained in the laboratory without further selection pressure. The LC_{50}, LC_{90} and slope values of the selected resistant strain in the F_6 generation were 1663 mg/l, >3200 mg/l and 0.5794, respectively (Table 1). The LC_{50} value of this strain was ~52000-fold higher than the value of the susceptible Sonepat strain. The resistant strain continued to remain resistant in subsequent generations, indicating the selection of a pure homozygous-resistant *Cx. quinquefasciatus* line. This homozygous-resistant line was subsequently used in genetic crosses.

To establish the genetic basis of resistance, the homozygous-resistant strain was reciprocally crossed with susceptible Sonepat strain. The LC_{50} (0.031 and 0.032 mg/l), LC_{90} (0.12 and 0.14 mg/l) and slope (2.26

Table 1 Regression analysis of dose–mortality responses of laboratory and field-collected strains of *Culex quinquefasciatus* from unsprayed and *Bacillus sphaericus* sprayed areas to *Bacillus sphaericus*

Strain	LC_{50}(mg/l) (–)	RR	LC_{90}(mg/l) (–)	RR	Slope	X^2 (df)
Delhi. Lab-colonized	0.04 (0 03 – 0 05)	–	0 134 (0.1 – 0.177)	–	2.46	5.76 (4) n s.
Sonepat: Lab-colonized	0.032 (0 028 – 0 037)	–	0 137 (0.098 – 0.19)	–	2.03	7 45 (6) n.s.
Farrukhabad. Unsprayed	0.015 (0 011 – 0 021)		0 095 (0 069 – 0.132)		1.62	9.04 (5) n.s.
B s. sprayed	0.027 (0.016 – 0 046)	1 8×	1 028 (0 36 – 2.88)	10 8×	0 81	10 48 (5)**
Ghaziabad. Unsprayed	0 02 (0 017 – 0 026)		0.09 (0.06 – 0 12)		2 08	6.39 (6) n s
B s sprayed	0 14 (0.13 – 0.144)	7.0×	1 4 (0 97 – 2 03)	i5.5×	1.28	29.43 (8)*
Madras. Unsprayed	0.04 (0.03 – 0 048)		0.116 (0 09 – 0.151)		2 68	2.03 (4) n.s.
B s sprayed	0.33 (0 22 – 0.51)	8 25×	18 0 (8 4 – 38 6)	155.2×	0.74	19 82 (7)*
Panaji: Unsprayed	0.26 (0 24 – 0.3)		0 86 (0.7 – 1.06)		2.5	4.86 (5) n s.
B.s. sprayed	0.62 (0 5 – 0.76)	2 38×	5.97 (3 94 – 9 04)	6 94×	1 3	12.31 (6)**
Ghaziabad Homozygous resistant	1663 (820 – 3372)	52,000×	269277	–	0.5794	6 14(8) n s.

B s. : *Bacillus sphaericus*

$$\text{RR : Resistance ratio} = \frac{LC_{50} \text{ or } LC_{90} \text{ of } Cx \textit{ quinquefasciatus} \text{ from } B\ s\text{–sprayed area}}{LC_{50} \text{ or } LC_{90} \text{ of } Cx \textit{ quinquefasciatus} \text{ from unsprayed area}}$$

d.f. : Degrees of freedom (K–2).
n s. Not significant.
* : $P < 0\ 05$.
** : $0.1 > P > 0\ 05$.
(–)95% fiducial limits.

and 2) of F_1 progeny from the reciprocal crosses were almost similar to those of the susceptible strain and all the progeny died within 24 h at the diagnostic dose (2 mg/l), indicating the recessive nature of *B. sphaericus* resistance.

In backcrosses with susceptible strain, mortalities ranged between 55.68 and 74.4%, which are well below the expected 100% at the diagnostic dose; however, mortalities reached 90–97% when scored after 48 h (after 24 h of treatment, larvae were kept in normal water). Data from crosses of F_1 progeny with resistant strain and F_1 inbreeding are given in Table 2. In backcrosses with resistant strain (crosses 1, 2, 4 and 5), mortalities were significantly below the expected 50%, and in both the F_1 inbreeding (crosses 3 and 6), dead and alive larvae deviated significantly from the expected 3:1 ratio. In all the crosses delayed effect of the larvicide was noticed. It is not clear why there was delayed mortality in all crosses, when no delayed effect of *B. sphaericus* was observed in the homozygous-resistant line and 100% mortality was observed among F_1 and susceptible progeny within 24 h of treatment. Data from the above crosses do not fit with the hypothesis that resistance to *B. sphaericus* in *Cx. quinquefasciatus* is monofactorial.

Table 2. Results from backcrosses and F_1 inbred crosses of *Cx. quinquefasciatus* strains resistant and susceptible to *B sphaericus*

♀	♂	No. of larvae treated (no. rafts)	No. of larvae died (expected no.†)	X^2 value (d f. = 1)
R	R	975	426	14 50*
S	R	(7)	(487.5)	
R	R	1401	436	199.74*
R	S	(10)	(700 5)	
R	R	994	621	83 29*
S	S	(10)	(745 5)	
S	R	1431	448	200*
R	R	(10)	(715 5)	
R	S	1306	492	79 6*
R	R	(10)	(653)	
S	S	1663	1115	56 02*
R	R	(9)	(1247 25)	

R: Resistant strain isolated from Ghaziabad
S. Susceptible strain from Sonepat
†Based on the hypothesis that resistance is monofactorial, expected number of larvae that would die at the diagnostic dose of 2 mg/l for 24 h
*Significant at $P < 0\ 001$, ♀ = female ♂ = male

In both F_1 inbreeding and backcrosses, among the larvae that survived, males and females were in 1:1 ratio, which indicated the linkage of the resistant gene to autosomes. In this context it may be mentioned that in *Cx. quinquefasciatus*, sex karyotype of males and females is the same, and the sex is determined by a pair of alleles, *m* and *M*. Females are homozygous for *m* (*m'm*) and males heterozygous (*m/M*). Though the F_1 progeny from the reciprocal crosses exhibited similar responses, to rule out sex linkage, surviving larvae from backcrosses and F_1 inbreeding were examined for sex by dissecting the reproductive organs. If the gene is sex-linked in F_1 inbreeding and in backcrosses, where F_1 males are heterozygous for the resistant gene, depending upon whether the resistant gene is introduced into the crosses along with *M* or *m* gene, the sex of the surviving progeny would respectively be either male or female. However, the recombinants would include the other sex. As in all the crosses males and females are in 1:1 ratio, linkage of resistant gene to sex chromosomes is ruled out.

The study has thus established that the resistance to *B. sphaericus* in *Cx. quinquefasciatus* is recessive, linked to autosome/s and is controlled by more than one gene. No maternal effect was observed in the expression of resistance. Further, the homozygous resistant strain was found fully susceptible to *B. thuringiensis israelensis*, the other commonly used biolarvicide, the LC_{50} and LC_{90} values being similar to those of the susceptible strain.

It is well established that a rare dominant gene is selected more rapidly than a recessive gene[9]. Since the gene for resistance in *B. sphaericus* is recessive, theoretically it should have taken a long time to develop resistance in the field populations. However, resistance appeared shortly after a year of spraying (20–25 rounds) in all sprayed areas. The probable reason for this could be that during larviciding, large population of immatures are continuously exposed, increasing the selection pressure[10].

Contrary to the presumption that insects are unlikely to become resistant to microbial pesticides[11], resistance in lepidopterans to *B. thuringiensis*[12] and now resistance in the mosquito *Cx. quinquefasciatus* to *B. sphaericus* has developed.

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ACKNOWLEDGEMENTS. We are grateful to Drs I Kar (Madras), A Kumar (Panaji) and S. N Sharma (Farrukhabad) for sending field-collected mosquitoes. We express our sincere thanks to the following staff members for their excellent technical assistance Pritam Singh, K. B Masiwal, H D. Joshi, A K. Mukherjee, Uday Prakash, Krishan Gopal, Ram Kanwar and Bhagwan Das

Received 7 April 1995; revised accepted 4 September 1995

Lead impairs hepatic type I-5′-monodeiodinase activity and thyroid function in cockerels

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Chronic exposure of cockerels to 1.5 mg/bird/day of lead nitrate for a period of 30 days impaired thyroid function. Lead decreased serum triiodothyronine (T_3) concentrations, inhibited hepatic type I-5′-monodeiodinase enzyme activity and marginally increased serum thyroxine (T_4) concentrations. Our findings demonstrate the lead-induced inhibition of hepatic type I-5′-monodeiodinase enzyme activity in avian system, leading to decreased production of T_3, the most potent metabolic hormone.

TYPE I-5′-monodeiodinase (5′-D) is a microsomal membrane-bound enzyme[1]. The most potent thyroid hormone, triiodothyronine (T_3), is predominantly produced in extrathyroidal tissues by 5′-monodeiodination of phenolic ring of thyroxine (T_4), the hormone that is known to be the prohormone of the former[2]. Although 5′-D enzyme system is present in almost all tissues, liver and kidney are known to have the highest T_4 to T_3 conversion activity[3]. Regulation of the 5′-monodeiodination in pe-