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MICROBIAL CONTROL OF MALARIA VECTORS IN HUBEI PROVINCE, PEOPLE'S REPUBLIC OF CHINA

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ABSTRACT: In the Province of Hubei, more than 20 million people on both sides of the Yangtze River are threatened by the malaria agent *Plasmodium vivax*. In 1985, 81,718 cases of malaria were reported, which corresponds to an annual incidence of 16.8 cases/10,000 people. The main vector is *Anopheles sinensis*. In a cooperative program between the Province of Hubei and the State of Baden-Württemberg, Germany, laboratory and field experiments as well as large-scale field trials and routine treatments with different preparations of *Bacillus thuringiensis* var. *israelensis*, *Bacillus thuringiensis* 187 (local strain), and *Bacillus sphaericus* (strains C3-41 and 2362) were carried out in Hubei Province. During the last few years, approximately 10 tons of B.t. 187 and 14 tons of B.s. strain C3-41 (local strains) have been produced each year using natural resources in Hubei Province to treat 12,000 hectares of mosquito breeding sites. The application of microbial control agents alone has reduced the incidence of malaria in the test area from 5.6 cases/10,000 people in 1986 before routine treatments to 0.8 cases/10,000 people in 1989.

INTRODUCTION

The Hubei Province is located on the Yangtze River in the subtropical monsoon zone with an average rainfall of 750-1,600 mm each year. The annual mean temperature is 13-18°C. The lakes along the Yangtze River, together with the high ground-water level and drainage system, provide not only optimal conditions for growing rice but also ideal breeding conditions for mosquitoes.

Plasmodium falciparum and *Plasmodium malariae* no longer occur in Hubei due to the successful control of the major vectors, *Anopheles minimus* (Theobald) and *Anopheles anthropophagus* (Xu and Feng), through the application of DDT since the early sixties. However, more than 20 million people on both sides of the Yangtze River are still threatened by the malaria agent *Plasmodium vivax*. In 1985, 81,718 cases of malaria were reported, which corresponds to an annual incidence of about 16.8 cases/10,000 people for the whole Province. In some highly infested areas, the incidence may be as high as 37.1 cases/10,000 people. However, control of the main vector, *Anopheles sinensis* (Wiedemann), has recently become more and more difficult with the insecticides used to date because of developing resistance and the ecological and toxicological risks.

Other diseases transmitted by mosquitoes are Japanese-B encephalitis (main vector: *Culex tritaeniorhynchus* [Giles]), and Brugian and Bancroftian filariasis (vectors: *An. sinensis* and *Culex quinquefasciatus* [Say]). In 1985, the prevalence of Brugian filariasis, which occurs mainly in the flatlands, was 50 cases/10,000 people, whereas, Bancroftian filariasis, dominating in the mountain area, had a prevalence of 10 cases/10,000 people. Dengue is occasionally transmitted by *Aedes albopictus* (Skuse).

BIOLOGY AND ECOLOGY OF THE VECTOR SPECIES

Anopheles sinensis

The larval density in rice fields and in densely vegetated, less polluted ditches and ponds is usually high at the end of May and the end of August/early September. The adults usually appear in the first half of May, with a peak in early June and a second peak in early September. They hibernate in underground caves, cowsheds, hay-lofts, and other sites protected from frost and wind.

Anopheles anthropophagus

The adults appear (later than those of *An. sinensis*) in early June with a peak in late September/early October. The larvae prefer unpolluted and shaded water-bodies, e.g., densely vegetated rice fields before the rice harvest in autumn. The adults disappear in late October and only the embryonated eggs hibernate until the following year.

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Culex quinquefasciatus

This is the most abundant species in cities and villages. It appears in May with a first peak in the middle of June and a second peak in late September. The adult population decreases the middle of October. The main breeding sites are heavily polluted sewage water ditches in and close to the communities. Usually the adults hibernate, but larvae can also survive the winter, even in water covered with ice for a short time.

Culex tritaeniorhynchus

The adults appear in high densities in late May, however, only in rural areas where rice fields are commonly the most favoured breeding sites. Hibernation sites are unknown.

Aedes albopictus

The adults appear in June and disappear during October. They usually occur in low densities except in areas where bamboo and artificial containers, such as clay jars or tires are abundant as breeding sites.

CONVENTIONAL MOSQUITO CONTROL MEASURES

Mosquito control in Hubei started in the early 1950's with the investigation of the biology and distribution of the native mosquito populations as the basis for successful control of major vector species (Li et al. 1985). For more than 30 years chemical insecticides, such as DDT, malathion, sumithion, and DDVP for adult control and the organophosphate dipterex for larval control achieved a relatively simple and economically acceptable control of almost all vector species.

Today, about 40 tons of DDT are still used each year in Hubei for residual wall spraying, as well as three tons of deltamethrin for bednet impregnation, an investment of about 100,000 U.S. dollars each year. Although the yearly budget for chemicals is increasing, the effect on the major malaria vector, *An. sinensis,* is very limited due to the onset of resistance against these chemicals.

ORGANIZATION AND BUDGET OF MOSQUITO CONTROL

Mosquito control is carried out by members of the Health Care System under the leadership of the Provincial Bureau of Health in Hubei. The Institute of Parasitic Diseases (IPD) is responsible for the scientific and technical training of the staff employed by the Health Care Systems, such as epidemiological and entomological studies, selection of the most effective and environmentally safe methods, and training of field workers.

In each county and the major cities, an Anti-Epidemic Station is responsible for mosquito control. Each station employs at least two to three medical doctors, four to six technicians, and about 30 to 50 field workers. There are 72 Anti-Epidemic Stations distributed throughout the Province, and more than 3,000 people work within these stations at the municipal and county levels. The annual budget for all the Anti-Epidemic Stations amounts to approximately one million U.S. dollars.

MICROBIAL CONTROL OF MOSQUITOES

The IPD was early to investigate microbial control measures for the management of mosquito-borne diseases because of the development of insecticide resistance, the high costs, and environmental risks of chemicals. *Bacillus thuringiensis* var. *israelensis* (B.t. H-14) and *Bacillus sphaericus* (B.s.) have proved to be efficient and environmentally safe microbial agents (Li 1989, Wang and Yu 1989, Wang et al. 1989, Wang et al. 1990, Xia et al. 1989, Zhang et al. 1989, and Zhen 1987).

In addition to their efficiency and ecological safety, an important advantage of these microbial larvicides is the control of the vector's developing stages, which are concentrated in defined breeding sites, whereas, a much larger area must be treated for the chemical control of dispersed adults. The IPD therefore started to work not only with B.t.i. but also with other, local microbial agents. In 1980, *Bacillus thuringienesis* 187 was isolated from a soil sample from the shore of Lake Dong Ting, in the Hunan Province in southern China. The Department of Virology, Hubei Academy of Science, isolated a new, very potent strain of *B. sphaericus* in 1986, named B.s. C3-41. The characteristics of B.t. 187 are similar to those of B.t.i. Both belong to the same serotype H-14, but some differences exist in the biochemistry and in the size of the vegetative cells (Zhang et al. 1984).

Supported by the World Health Organization, the IPD began to carry out the first small field tests with B.t.i. as well as with the local strain B.t. 187 in 1983 and with B.s. (strain 2362) in 1986. In 1987, the first large field tests with B.t. 187 against *An. sinensis* and with B.s. strain C3-41 against *Cx. quinquefasciatus* were carried out. In a cooperative program between the Province of Hubei and the State of Baden-Württemberg, Germany, the scientific and organizational framework for the large-scale application of microbial agents is being improved (Becker and Xu 1990). The objectives are to carry out detailed investigations on the biology and ecology of the most important mosquito vectors in order to provide the basis for successful control by microbial means, to improve the measures and techniques for routine control operations in both countries, and to develop low-cost and efficient formulations of B.t.i., B.t. 187, and B.s. Various formulations of B.t.i. and B.t. 187 as well as B.s. (strains 2362 and C3-41) were tested in the laboratory and in the field.

1. Laboratory tests with fluid and powder formulations.

   Two B.t.i. powder formulations (ABG-6164, activity: 10,000 ITU/mg, and Bactimos, activity: 6,000 AAU/mg), one B.t. 187 formulation (a fluid concentrate; activity: 400 ITU/mg), two B.s. powder formulations (ABG-6184 and BSP-2, strain 2362), and a B.s. C3-41 formulation (fluid concentrate, activity: 270 ITU/ml) were tested against larvae of *Cx. quinquefasciatus, An. sinensis*, and *Ae. albopictus* in the laboratory. The tests were conducted according to the World Health Organization standard scheme (WHO 1981).

   While the B.t. H-14 preparations are very effective against larvae of *Ae. albopictus*, they are less active against *Cx. quinquefasciatus* larvae (TABLE. 1). On the other hand, a relatively low dosage of *B. sphaericus* is enough to kill *Culex* larvae, whereas, *Aedes* larvae are less sensitive. However, none of the microbial preparations tested provided satisfactory control of *An. sinensis* larvae. Furthermore, the local strain B.s. C3-41 is about five times as active against *Cx. quinquefasciatus* as the local B.t. strain.

2. Field Tests.

   B.t. H-14 (Bactimos, activity: 6,000 AAU/mg and ABG-6164, activity: 10,000 ITU/mg) and B.s. formulations (ABG-6184 TP; BSP 2 TP; BSP 2 FC) were tested against *Cx. quinquefasciatus* in nine drainage channels and ponds. Once before and 1, 2, 3, and 7 days after treatment the density of the larval populations was determined by counting the number of larvae in ten 1-liter dips. The concentrations tested were 0.5 and 1 ppm. Depending on the size of the breeding site and the desired dosage, the required amount of the formulation was mixed with 1 liter of spring water and applied evenly on the water surface with a 1-liter hand sprayer.

TABLE 1. Activity (LC_{50} in ppm) of various preparations of *Bacillus thuringiensis* and *Bacillus sphaericus* against fourth-instar larvae of *Culex quinquefasciatus*, *Aedes albopictus*, and *Anopheles sinensis* in the laboratory.

		Species		
Bacillus	Product	*Culex quinque.*	*Aedes albop.*	*Anopheles sinensis*
B.t. H-14	ABG-6164 WP 10,000 ITU/mg	0.037	0.025	2.4
B.t. H-14	Bactimos WP 6,000 AAU/mg	0.28	0.058	6.4
B.t.-187	Emulsion 400 ITU/mg	0.58	0.60	2.2
B.s.	ABG-6184 TP Strain 2362	0.009	0.72	1.9
B.s.	BSP-2 TP Strain 2362	0.001	0.54	10.0
B.s. C3-41	Emulsion 270 ITU/mg	0.1	-	-

The BSP 2 (B.s. powder formulation) in particular proved to be very effective at concentrations of 0.5 and 1 ppm (TABLE 2). Few to none of the living *Culex* larvae were present one day and seven days after application. Even newly hatching larvae died in water bodies seven days after treatment with high concentrations. The B.s. fluid concentrates were less effective, which could be the result of a lower content of the active ingredient. In the application of B.t.i. WP, a concentration of at least 1 ppm had to be used in order to achieve a satisfactory mortality rate. Bactimos showed a good immediate effect (99.6% mortality rate after one day), but the mortality rate dropped rapidly during the next two days.

3. Laboratory and field tests with different granules.

Taking into account the feeding behavior of *Anopheles* and *Culex* larvae as surface and suspension feeders, respectively, granule formulations were tested. Surface-active B.t.i. sand granules were produced using sand (grain size: 1-2 mm), vegetable oil (binder), egg-lecithin (spreading agent), wheat flour (phagostimulant), and B.t.i. WP (6,000 AAU/mg). The formulation was mixed in different combinations:

a) 37.5 kg sand, 1.6 liter vegetable oil, 0.4 liter egg-lecithin, 1.5 kg B.t.i. WP (without phagostimulant).
b) 75 kg sand, 2.1 liter vegetable oil, 1 kg wheat flour, 1.5 kg B.t.i. WP (without spreading agent).
c) 37.5 kg sand, 1.5 liter vegetable oil, 1.5 kg B.t.i. WP (without phagostimulant or spreading agent).

TABLE 2. Field evaluation of various wettable powder and fluid formulations of *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* against larvae of *Culex quinquefasciatus*.

		Number of Larvae/10 Dips Pre- and Post-Treatment				
Product	Dose (ppm)	Pre-Treatment	1	2	3	7(days)
ABG-6184 TP B.s.	0.5	232	11 (95.3)	28 (87.9)	72 (69)	272 (0)
ABG-6184 TP B.s.	0.5	256	15 (94.1)	29 (88.7)	24 (90.6)	114 (55.5)
BSP 2 TP strain 2362	0.5	366	12 (96.7)	189 (48.4)	0 (100)	0 (100)
BSP 2 TP strain 2362	1	112	1 (99)	0 (100)	4 (96.4)	0 (100)
BSP 2 Fluid strain 2362	0.5	554	300 (45.8)	33 (94)	84 (84.8)	91 (83.6)
BSP 2 Fluid strain 2362	1	790	104 (86.8)	20 (97.5)	106 (86.6)	35 (95.6)
ABG-6164 TP B.t.i.	1	86	13 (84.9)	29 (66.3)	12 (86)	15 (82.6)
Bactimos 6.000 AAU/mg	1	220	1 (99.5)	69 (68.6)	69 (68.6)	—

( ) = percent reduction; L_1-L_4 = larval instar.

For the field tests, mass breeding sites of *An. sinensis* and *Cx. tritaeniorhynchus* were selected and treated with the different combinations of sand granules such that the B.t.i. concentration always remained 1.5 kg Bactimos WP/ha. One pond (50 m x 20 m; depth 0.5 m) was treated with 4.1 kg of formulation a, one ditch (27 m x 2 m; depth 0.3 m) with 0.43 kg of formulation b, and a second ditch (17 m x 2 m; depth 0.3 m) with 0.138 kg of formulation c. Once before and 1, 2, and 3 days after treatment, the density of the larval populations was determined by counting the number of larvae of *An. sinensis* and *Cx. tritaeniorhynchus* in ten 1-liter dips. The granules were equally distributed on the surface by ground application.

Slow-release floating granules were produced using organic particles as a carrier, elm bark (adherent compound), wheat bran (inducer), and B.t. 187 culture broths (active ingredient) in a 1:1:1:1-mixture. In a series of tests, the tailor-made B.t. 187 granules were tested at different dosages. Samples of 25 third/fourth-instar larvae were placed in containers with 150 ml of deionized water, and granules were added at dosages of 0.125, 0.25, 0.375, 0.5, 0.625 g/m^2. Each dosage was tested in three replicates, and three containers were left untreated. The temperature was 25±2°C. Every 24 hours the mortality rate was assessed by removing the dead and still living larvae and another 25 larvae were added until the mortality rate was less than 60 percent. In addition, field tests with B.t. 187 were conducted in a rice-field (30 m x 20 m; 0.20 m) against *An. sinensis*. The application rate was 6 kg/ha and the evaluation of the results took place according to the above mentioned procedure until third-instar larvae occurred and the mortality rate was less than 80 percent.

The Bactimos sand granules yielded mortality rates of 88.6 percent (formulation c), 94.7 percent (formulation a), and 98.4 percent (formulation b) against *An. sinensis* as well as 63.5 percent (formulation c), 89.3 percent (formulation a) and 93.8 percent (formulation b) against *Cx. tritaeniorhynchus* within 24 hours after application (TABLE. 3). However, the long-term effect was not satisfactory since the number of larvae increased in all test sites three days after application.

At an application rate of 5 to 6.25 kg/ha, the tailor-made B.t. 187 granules yielded mortality rates of more than 80 percent for more than 15 days (Fig. 1). In rice fields, B.t. 187 granules applied at a dosage of 6 kg/ha were effective for about 11 days (TABLE. 4).

Similar results were achieved in preliminary tests against *Cx. quinquefasciatus* in sewage water ditches. Granules produced with *B. sphaericus* C3-41 culture broth using the same ingredients as for the B.t. 187 granules yielded sufficient control for two weeks at a dosage of 2 kg/ha.

4. Routine Treatment.

Before the local B.t. 187 and B.s. C3-41 strains were applied in routine treatments in certain counties of Hubei (e.g. Shashi), safety and selectivity tests were conducted. No adverse effects on nontarget organisms were found. In routine treatments, the fluid formulations are applied in *An. sinensis* and *Cx. quinquefasciatus* breeding sites inside and near the villages and towns using high-pressure sprayers attached to 600-litre tanks pulled by minitractors. Two litres of fluid formulation of either B.t. 187 or B.s. C3-41 are mixed with each 600-litre tank full of spring water, which can be applied at a dosage of 3-5 ppm. According to experience in the field, the treatments were necessary every seven days during summertime or every ten days during autumn, depending on temperature. Twenty-four treatments are usually needed during the control season. In the last few years, approximately 10 tons of B.t. 187 and 14 tons of B.s. C3-41 have been produced each year in Hubei Province by using natural resources, which was enough to treat about 12,000 ha of mosquito breeding sites. One litre of B.t. 187 with a potency of 400 ITU/mg costs about one U.S. dollar, and B.s. C3-41 with a potency of 270 ITU/mg, 1.20 U.S. dollar.

In 1989, in the first large-scale treatments against *An. sinensis* in rice fields, about 200 kg of the new floating granules were used to treat an area of about 30 ha (dosage: 7 kg/ha). Because of their long-term effects, the granules only needed to be applied every two to three weeks, thus, lowering the manpower and material costs.

IMPACT ON THE MOSQUITO POPULATION AND DISEASE OCCURRENCE

The impact of the treatments was recorded by measuring both the adult mosquito population density and the incidence of malaria both before and after the campaign. The mosquito population density was measured by counting the number of mosquitoes invading houses. At different selected areas of the City of Shashi (total area: about 20 km^2), one window (1 x 1.2 m) in each of ten single-room houses was opened between 7:30 in the evening and 6:30 in the morning. A

TABLE 3. Field evaluation of various B.t.i. (Bactimos) sand granules against larvae of *Anopheles sinensis* and *Culex tritaeniorhynchus* in the People's Republic of China.

Formulation	Treatment Rate	Habitat Area/Depth	Species	Number of Larvae/10 dips Pre- and Posttreatment: Pretreatment	1	2	3 (days)
Formulation A	41 kg/ha	pond 1000 m^2/0.5 m	*A. sinensis* (L1-L4)	38	2 (94.7)	7 (81.6)	16 (57.9)
Formulation A	41 kg/ha	pond 1000 m^2/0.5 m	*Cx. tritaeniorh.* (L1-L4)	224	24 (89.3)	31 (86.2)	23 (89.7)
Formulation B	79.6 kg/ha	ditch 54 m^2/0.3 m	*A. sinensis* (L1-P)	126	2 (98.4)	22 (82.5)	26 (79.4)
Formulation B	79.6 kg/ha	ditch 54 m^2/0.3 m	*Cx. tritaeniorh.* (L1-L4)	192	12 (93.8)	0 (100)	20 (89.6)
Formulation C	40.5 kg/ha	ditch 34 m^2/0.3 m	*A. sinensis* (L1-L4)	70	8 (88.6)	20 (71.4)	30 (57.1)
Formulation C	40.5 kg/ha	ditch 34 m^2/0.3 m	*Cx. tritaeniorh.* (L1-L4)	74	27 (63.5)	24 (67.6)	30 (59.5)

FORMULATION A: 37.5 kg sand + 1.6 liter vegetable oil + 0.4 liter egg lecithin + 1.5 kg B.t.i.

FORMULATION B: 75.0 kg sand + 2.1 liter vegetable oil + 1 kg wheat flour + 1.5 kg B.t.i.

FORMULATION C: 37.5 kg sand + 1.5 liter vegetable oil + 1.5 kg B.t.i.

( ) = percent reduction

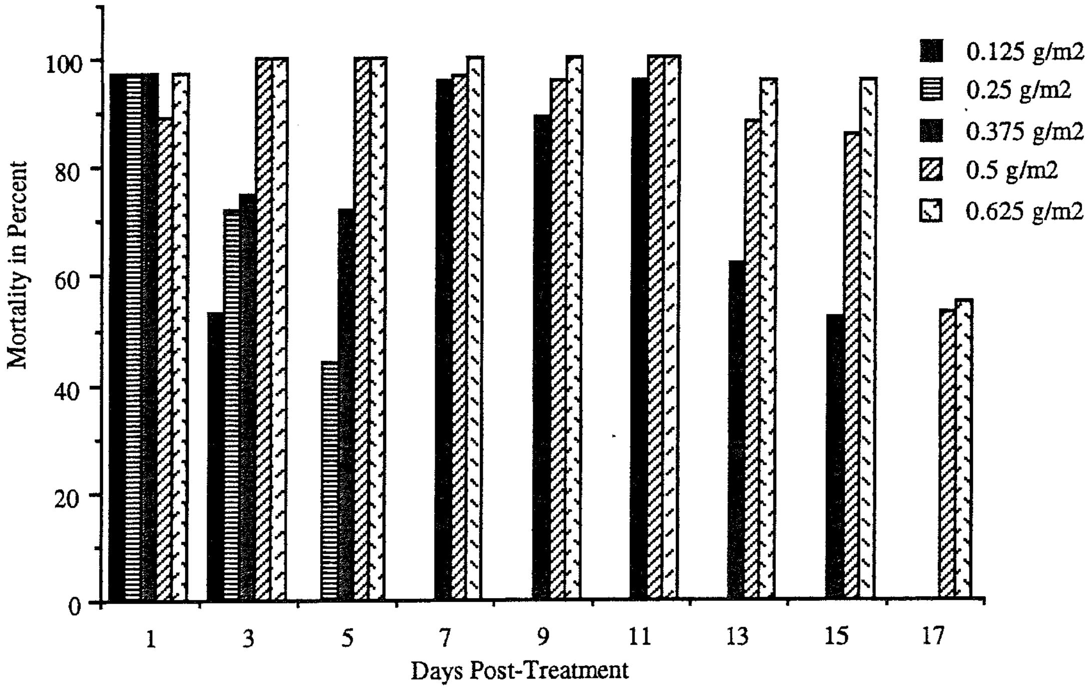

Figure 1. Efficacy of B.t. 187 granules against fourth-instar larvae of *Anopheles sinensis* in bioassays.

person sleeping under a mosquito net served as bait. In the morning the mosquitoes were collected for one hour, always by the same person. The attack rate per person per night was calculated, and the average number from all sites per month was determined. The dominant species were *Cx. quinquefasciatus* and *An. sinensis*. These counts were always made in the same houses twice a month between April and October, one year before and during the years with large-scale applications of B.t. 187 and B.s. C3-41.

The incidence was determined by evaluating the number of malaria cases reported by hospitals, clinics, and village doctors. Blood-smear tests were taken from each person with a temperature above 37.5°C. Every

TABLE 4. Efficacy of B.t. 187 granules at a dosage of 0.6 g/m^2 against *Anopheles sinensis* larvae in a rice field.

Instars	Number of Larvae/5 Dips Pre- and Post-treatment						
	Pretreatment	1	2	3	5	7	11 (days)
Instar I	4	0	0	1	1	4	0
Instar II	9	1	0	0	2	4	2
Instar III	2	1	0	0	0	0	2
Instar IV	3	0	0	0	0	0	0
Total	18	2	0	1	3	8	4
Control area	18	20	14	13	9	6	13
Mortality rate in %		89	100	95	83	56	78

ten days, the number of cases was reported to the Anti-Epidemic-Station in Shashi. Additionally, the reduction in numbers of malaria cases was proven by blood-smear tests, which were taken randomly. Each year between 12,000 (1986) and 14,800 (1989) blood-smears were taken to determine the parasite-positive rate in Shashi City.

In 1986, before application of the microbial control agents between April and October, an average of 9.5 mosquitoes/person/night could be determined with a peak of 21.2 mosquitoes/person/night in June and a second peak of 16.5 mosquitoes/person/night in September. After application, the densities were significantly reduced, the seasonal averages (April until October) being 4.1, 1.8, and 2.2 mosquitoes/person/night in 1987, 1988, and 1989, respectively (Fig. 2). This figure also shows not only that the absolute number of mosquitoes during the seasons was reduced, but also that the peaks, which are important for the transmission of malaria, were depressed.

Solely through the application of microbial agents, the incidence of malaria in Shashi was significantly reduced, from 5.6 cases per 10,000 people in 1986 before treatment to 2.14, 1.6, and 0.8 cases/10,000 people after treatment in 1987, 1988, and 1989, respectively (Fig. 3). In 1986, before application of the microbial control agents, the positive rate in fever cases was 1.60 percent. However, in 1989 after large-scale routine treatments, the positive rate was only 0.24 percent (TABLE 5).

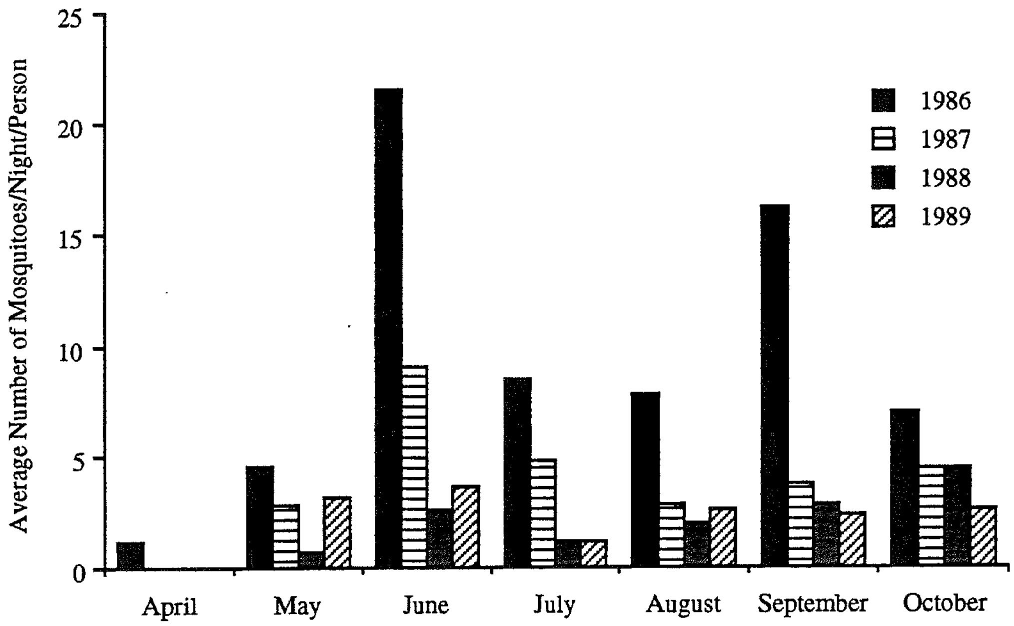

Figure 2. Comparison of the mosquito attack rates before (1986) and after B.t. 187 and B.s. C3-41 applications in Shashi.

CONCLUSION

The application of microbial control agents in integrated malaria control programs can replace chemicals when resistance to them becomes a problem. Formulations of microbial agents based on available, local resources are not only easily produced but can also be very cost-effective compared with the common chemicals. In addition to the well-organized operational use of microbial control agents, epidemiological and entomological studies are vital prerequisites for assessing the impact of the treatments on the mosquito-borne disease.

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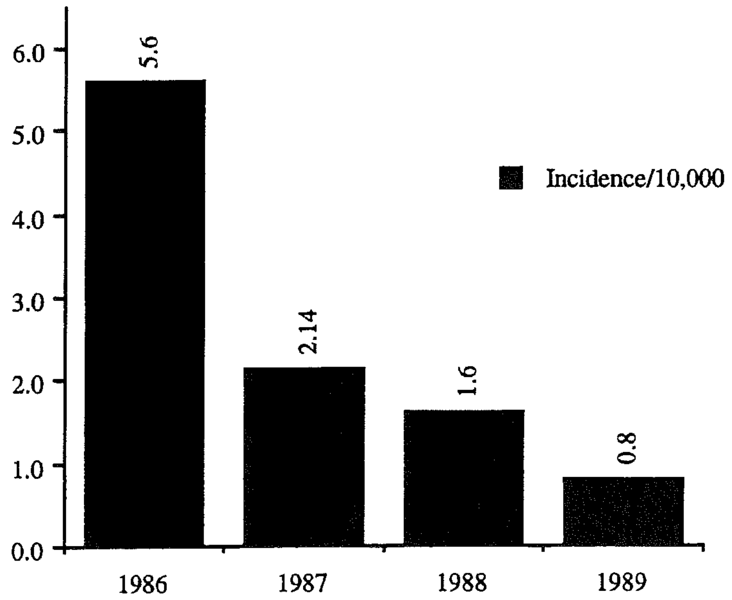

Figure 3. Incidence of malaria in the test area (Shashi) before (1986) and after B.t. 187 and B.s. C3-41 treatments.

TABLE 5. Parasitaemia rate in fever cases before (1986) and after B.t. 187 and B.s. C3-41 applications in the City of Shashi.

Year	No. of Smears Exam.	Positive	Positive Rate (%)
1986	12,043	193	1.60
1987	13,741	75	0.55
1988	13,279	58	0.44
1989	14,800	36	0.24

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