SPACE SPRAYING OF BACTERIAL AND CHEMICAL INSECTICIDES AGAINST *ANOPHELES BALABACENSIS* BAISAS FOR THE CONTROL OF MALARIA IN SABAH, EAST MALAYSIA

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Abstract. A pilot study was undertaken to determine the effectiveness of space application of insecticides for the control of malaria in Ranau, a district in Sabah. A village each was treated monthly: with chemical adulticide - alpha cypermethrin (Fendona SC^R/10SC^R) at 2 g a.i./10,000 m^2 in Pahu; with biological larvicides - *Bacillus thuringiensis israelensis* (Vectobac 12AS^R) at 500 ml/10,000 m^2 or *B. sphaericus* (Vectolex WG^R) at 500 g/10,000 m^2 in Pinawantai; and with a mixture of chemical adulticide and biological larvicide in Togop Laut. All sprayings were conducted using a portable mist blower. During the study period all villages, including Tarawas the untreated village, received the conventional malaria control measures. Entomological and epidemiological surveillance was used to measure the effectiveness of the space application. The entomological surveillance indicated that the *An. balabacensis* population was significantly reduced by alpha cypermethrin in Pahu and Togop Laut and *B. sphaericus* in Pinawantai; but was not reduced by *B.t.i.* in Pinawantai. There was a significant reduction in the number of malaria cases and in the slide positivity rate in the treated villages during the study period. The pilot study does indicate that space application of larvicides/adulticides or a mixture of both is able to reduce the malaria vector population and the malaria transmission. A larger scale study needs to be undertaken in a malarious village/province to determine whether space application of insecticides together with other malaria control measures will be able to eradicate malaria.

INTRODUCTION

The state of Sabah, situated in the north of Borneo Island, annually contributes to ≥ 50% of malaria cases in Malaysia (VBDCP, 2001). The confirmed malaria vectors in Sabah are *Anopheles balabacensis*, *An. sundaicus* and *An. flavirostris* (Chooi, 1985). *An. balabacensis* has been incriminated as the principle malaria vector in this state and also in other neighboring countries in Southeast Asia (Reid, 1968); it is also a vector of simian malaria (Cheong *et al,* 1965) and transmits simian malaria to humans (Reid, 1968).

In the malaria control program, vector control is employed to interrupt the malaria parasite transmission by vectors. The two most widely used vector control methods are indoor residual spraying and insecticide impregnated mosquito nets. In Sabah, indoor residual spraying with DDT

was initiated in 1955 with the implementation of the Malaria Eradication Program and it was sprayed at 2 g/m^2 for two cycles per year (Chow, 1970). *An. balabacensis* was first found to show tolerance to DDT after 27 cycles of DDT spraying. Since then, susceptibility tests have shown a steady increase in the level of DDT tolerance in the mosquito vector (Hii, 1984). *An. balabacensis* is able to enter, bite, and to a relatively great extent, survive in recently sprayed walled huts. This situation was made worse in poorly constructed structures with incomplete walls or structures without any walls.

In Sabah, the *An. balabacensis* also exhibits a refractory habit, *ie* the female mosquitos avoid walls treated with DDT for a period of three to four months after treatment (Colless, 1953; Scanlon and Sandhinand, 1965).

In 1993, the use of insecticide impregnated mosquito nets was actively promoted in Sabah to supplement indoor residual spraying. The mosquito nets are mainly used indoors as a form of

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protection against biting mosquitos. Initially, permethrin was the insecticide of choice and this was replaced in the late 1990s with lambda-cyhalothrin and deltamethrin (Chooi and Tanrang, 2000). The incidence rate of malaria was reduced by 11% in Sabah with the introduction of insecticide impregnated mosquito nets (Zainal, 1998). Nevertheless, the impregnated mosquito net is not able to provide complete protection to villagers who are still tending to outdoor activities at the early biting hours of *An. balabacensis*, *ie* 18.00 hour, and it also does not provide protection to villagers who refuse to use the nets during the peak biting hours (Zainal, 1998). The impregnated mosquito net is also not able to provide complete protection from *An. balabacensis* which exhibits exophilic and exophagic behavior, *ie* in a recent study in Sabah by Tanrang *et al* (1999) the *An. balabacensis* was observed to bite 27 times more outdoors than indoors.

There is also a possibility of the vector, *An. balabacensis* has developed resistance to permethrin. A preliminary insecticide susceptibility status was determined on all mosquitos caught during the entomological surveillance in March 2000. The susceptibility status of the mosquitos towards insecticide was detected by using the WHO Bioassay Test Kit and the Rapid Insecticide Resistance Detection Kit (R_Est Kit™); it was observed that the anopheline mosquitos have developed resistance to 4% DDT and 0.75% permethrin; were moderately susceptible to 0.05% lambda cyhalothrin; and susceptible to 5% malathion and 0.15% cyfluthrin. The results of the Insecticide Resistance Detection Kit (R_Est Kit™) correlated with the WHO Bioassay Test Kit results (Nazni, personal communication).

The increased tolerance of *An. balabacensis* to DDT, its refractory habit to DDT, its early man-biting hours, its exophilic and exophagic behavior, together with the total incomplete protection given by impregnated mosquito nets necessitates additional adult mosquito control measure(s) such as space spraying of adulticides to further reduce or eliminate malaria in Sabah.

In Sabah, the *An. balabacensis* larvae were found breeding in shallow muddy pools formed by human or animal footprints, within the vicinity of the villages (Rohani *et al,* 1999). The ex-

tensiveness of its breeding habitats together with the rugged terrain and scattered human population in the malarious villages have not been amenable to malaria control through direct application of temephos or anti-malarial oil in the anopheline breeding sites or by source reduction (Tham, 1994). Therefore, alternative larviciding measures, such as space spraying of larvicides, need to be implemented to further reduce or eliminate malaria in Sabah.

The following study was undertaken to study the effectiveness of space spraying a chemical adulticide : alpha cypermethrin (Fendona SC[R] or Fendona 10SC[R]); and biological larvicides : *Bacillus thuringiensis israelensis (B.t.i.)* (Vectobac 12AS[R]) and *Bacillus sphaericus* (*B.sp*) (Vectolex WG[R]) for malaria control in Sabah.

MATERIALS AND METHODS

The study was conducted in the Ranau District (05º58′N, 116º42′E), Sabah. The district is located 160 km northeast from Kota Kinabalu, the capital of Sabah. It is classified under the highest priority for health status among the 24 districts in Sabah, and it is endemic to malaria (Ranau District Health Office, Sabah, 2000). This district, which is 549 m above sea level, has a tropical climate with an average temperature of 32ºC and a relative humidity of 85-95%. The annual rainfall in this district is about 2,500 mm, with a peak rainfall of about 1,000 mm in the 1st quarter of the year. Sabah had an unusual dry period from November 1997 to the 1st quarter of 1998, where Ranau only had 138.4 mm rainfall from January-April 1998 (Malaysian Meteorological Service, 2002).

Field site

Four villages were selected for the field study in Timbua, a sub-district of Ranau in Sabah. Timbua has 22 villages with a population of 3,553 people. Four villages, Pinawantai, Pahu, Togop Laut and Tarawas, were chosen based on a high malaria incidence rate, a high vector population, epidemiological and entomological studies having been conducted in these villages in recent years, and the villages were accessible at all times of the year (Rohani *et al*, 1999). The villages were adjacent to one another with a separating distance

of at least 3.5 to 6.0 km.

Pinawantai, a village of 11×10^4 m^2 with 31 houses and 119 residents, was sprayed with *Bacillus thuringiensis israelensis (B.t.i.)* (Vectobac 12AS^R) or *Bacillus sphaericus* (*B.sp*) (Vectolex WG^R). Pahu, a village of 36×10^4 m^2 with 30 houses and 178 residents, was sprayed with alpha cypermethrin (Fendona SC^R or Fendona 10SC^R). Togop Laut, 25×10^4 m^2 with 28 houses and 107 residents, was sprayed with either a mixture of *B.t.i* and alpha cypermethrin or *B.sp* and alpha cypermethrin. Tarawas, 20×10^4 m^2 with 59 houses and 216 residents, was used as the control (untreated) village in this study. The usual malaria control measures, *ie* the indoor residual spraying and the use of insecticide impregnated mosquito nets were in operation in the 4 villages during the trial period.

Insecticides

The study was conducted using the following insecticides: a commercial aqueous suspension of *Bacillus thuringiensis israelensis (B.t.i.)* (Vectobac12AS^R, Abbott Laboratories - containing 1,200 ITU/mg against *Aedes aegypti* larvae); a commercial wettable granule formulation of *Bacillus sphaericus* (*B.sp*), (Vectolex WG^R, Abbott Laboratories - containing 650 BsITU/mg against *Culex quinquefasciatus* larvae); and alphacypermethrin (Fendona SC^R or Fendona 10SC^R-BASF). The dosages of the insecticides used were: Vectobac 12AS^R - 500 ml/10^4 x m^2; Vectolex WG^R-500 g/10^4 x m^2; and Fendona SC^R/ Fendona 10SC^R-2 g a.i./10^4 x m^2.

The recommended dosage for alphacypermethrin at 2 g a.i./10^4 x m^2 was achieved in the spray area by mixing Fendona SC/Fendona 10SC^R with sieved stream water. The *B.t.i.* formulation, Vectobac12AS^R was a viscous formulation, and it was diluted with stream water to a ratio of 1:1 to enable the formulation to flow smoothly without clogging the mist blower nozzle. For *B.sp*, 500 g of Vectolex WG^R required 2.5 l of the clear stream water to give a homogenous suspension that flowed smoothly through the sprayer nozzle. All insecticide formulations were prepared at the field site prior to their application.

Spraying trials

The spraying trials were initiated in November 1998 with *B.t.i.* and alphacypermethrin being sprayed monthly until December 1999. *B.sp* and alphacypermethrin were sprayed monthly for 6 months from March 2000 to August 2000. All sprayings were conducted using a portable mist-blower (Mist Blower MD300, Maruyama MfgCo, Inc,^{TM}). Sprayings were not conducted in January 1999 and October 1999 due to unavailability of *B.t.i.* In November 1999, two sprayings were conducted within a span of 12 days upon the availability of *B.t.i.* The biological larvicides were sprayed in Pinawantai either in the cool hours of early morning or the late afternoon; and the chemical adulticide or the mixture of the chemical adulticide and biological larvicide were sprayed between 18.00 to 21.00 hour in Pahu and Togop Laut, respectively.

During spraying operations, the volume switch dial of the mist-blower was maintained at 1, with the throttle lever at 4. This ensured that the spray formulations flowed smoothly without clogging the sprayer nozzle and the spray men could spray the entire village at a convenient walking speed. The spray men covered the entire village in a systematic manner where each knew their spray route. After each spray the insecticide tank was washed with chlorhexidine gluconate solution (4% w/v) to ensure that there was no cross contamination of spray insecticides between villages.

The effectiveness of the spraying trials was evaluated by larval mortality, spray droplet analysis together with epidemiological and entomological surveillance.

Larval mortality and spray droplet analysis

Before spraying, 10 containers with 200 ml water, together with 10 magnesium oxide coated (MgO) slides were placed randomly amongst vegetation at 10 different points in each village. The containers and the MgO slides were collected 30 minutes after spraying and were analysed at the Division of Medical Entomology, Institute for Medical Research, Kuala Lumpur.

The larvicidal activity of the sprayed bacterial insecticides was monitored by introducing 20 laboratory bred mosquito larvae (L3/L4) into each container, *Ae. aegypti* larvae were used to analyse the *B.t.i.* spraying and mortality was recorded 24

hours post exposure, and *Cx. quinquefasciatus* larvae were used to analyse the *B.sp* spraying, and mortality was recorded 48 hours post exposure.

The distribution and size of sprayed particles were monitored through the use of MgO coated slides. Droplet diameter was measured for an average of 30 droplets for each MgO coated slide using a calibrated micrometer. The data were analysed using the droplet analysis program of Sofield and Kent (1984).

The larval mortality and droplet analysis were only evaluated for a limited number of spraying trials due to logistics.

Entomological surveillance

In November 1998 and in March 2000, entomological surveillance was conducted before the sprayings of *B.t.i* with alphacypermethrin and *B.sp* with alphacypermethrin were initiated, respectively consecutively. The surveillance was conducted 2 days before spraying, *ie*, about 4 weeks after the previous spray. For the months of June to August 2000, the surveillance was conducted 2 weeks after the previous spray, not 4 weeks. The surveillance schedule was changed from 4 to 2 weeks post spray as we wanted to correlate the effectiveness of the spraying to the mosquito's life cycle, which is 10-14 days.

Mosquitos were caught outdoors using the bare leg catch (BLC) technique from 18.00 to 24.00 hr in all 4 villages. All caught mosquitos were identified. The parity of the female anophelines was determined by examination of the ovaries and the salivary glands were examined for sporozoites.

Epidemiological surveillance

The epidemiological surveillance data was obtained from the Ranau Health Office, Sabah. The malaria incidence rate was monitored by monthly mass blood surveys (MBS) and passive case detection (PCD) in the village clinics and hospitals. MBS covered about 70% of the local population.

RESULTS

Droplet analysis

Droplets were observed on all MgO coated slides that were placed randomly in the villages during spraying. This indicates that the spray equipment, *ie* the portable mist-blower, was able to disperse the 3 different insecticide formulations, without clogging the spray nozzle. It also indicates that the portable mist-blower was able to disperse the insecticide droplets into the targeted sites. The volume median diameter (vmd) of the sprayed particles for all the treatments was in the range of 111.0 μm to 191.0 μm, indicating that the particles were of fine spray (Matthews, 1985). The vmd of the sprayed *B.t.i.* (Vectobac 12AS[R]) droplets, 111.18 ± 8.75 μm, was significantly smaller in size than the *B.sp* (Vectolex WG[R]) droplets, 164.07 ± 9.67 μm ($p < 0.05$). The disparity in the droplet size could be due to Vectobac 12AS[R], an aqueous suspension formulation prepared by the manufacturer; while Vectolex WG[R] is a water dispersible granular formulation which is suspended in water just prior to spraying by the spray men, producing a preparation of less uniform suspension and of larger droplets than Vectobac 12AS[R].

Larval mortality

Larvicidal activity was observed in all the containers placed in the 3 treated villages. In Pinawantai, *B.t.i.* achieved a 68.9 ± 7.3% *Ae. aegypti* larval mortality on 24 hours exposure and *B.sp*. achieved a 85.5 ± 9.0% *Cx. quinquefasciatus* larval mortality on 48 hours exposure. Larval mortality was also achieved in test samples from Togop Laut, where a mixture of bacterial and chemical insecticides was sprayed, *ie* 85.8 ± 7.3% for *B.t.i.* and alphacypermethrin and 100% for *B.sp*. and alphacypermethrin. The larval mortality results indicate that the sprayed bacterial insecticides do exhibit mosquito larvicidal activity and alphacypermethrin is not antagonistic to the bacterial larvicides.

Entomological surveillance

The mosquitos caught outdoors using the bare leg catch (BLC) technique from 18.00 to 24.00 hr in all the 4 villages were identified and the anopheline mosquitos were dissected to determine parity and malaria infection. The landing rate, *ie* number of mosquitos caught per person per night, was only determined for *Anopheles balabacensis*, the principal malaria vector in Sabah. The landing rate is as shown in Figs 1 and 2. In November 1998 and in March 2000 surveil-
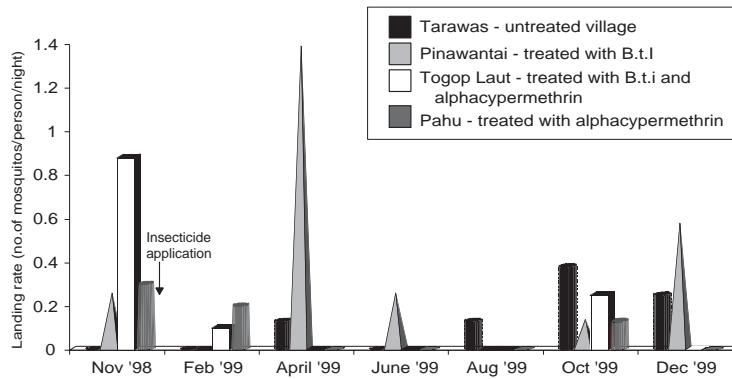
Fig 1–Entomological surveillance - landing rate of *Anopheles balabacensis,* the principal malaria vector in the study villages during the trial period, November 1998-December 1999.
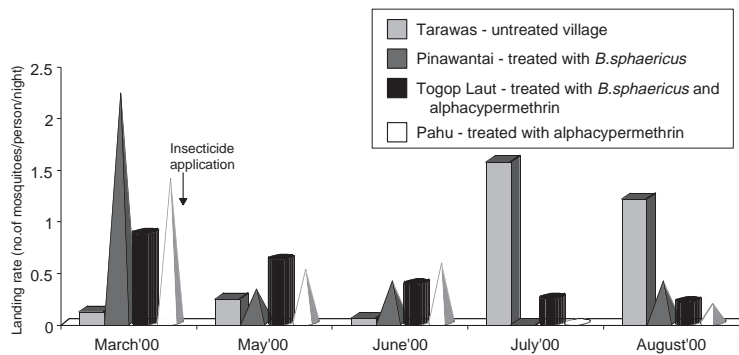

Fig 2–Entomological surveillance - landing rate of *Anopheles balabacensis,* the principal malaria vector in the study villages during the trial period, March-August 2000.

Togop Laut and Pahu. In the untreated village, Tarawas, a landing rate of 0-0.38 was observed during the study period with most months having a higher landing rate than the villages treated with the chemical adulticide or a mixture of the chemical and bacterial insecticides.

The parity rates of *An. balabacensis* in the treated villages, 81.82-100% were not significantly different from the untreated village (p >0.05). Infected *An.balabacensis* were not detected.

B.sphaericus and alphacypermethrin (Fig 2)

The 3 treated villages, Pinawantai, Togop Laut and Pahu, had their highest landing rates of 2.21, 0.88 and 1.38 mosquito/man/night respectively, in March 2000, just before the initiation of spraying. After the spraying was initiated landing rates decreased in all the treated villages and it was maintained at 0-0.6. In the untreated village, Tarawas, an increase in the landing rate was observed over the study period with the highest landing rate of 1.6 in July 2000.

The parity rates of *An. balabacensis* in the treated villages were 44.44-73.68%, not significantly different from the untreated village (p >0.05). Infected *An. balabacensis* were not detected.

Epidemiological surveillance

The number of reported malaria cases in the study villages together with the other villages in the sub district of Timbua is shown in Table 1. The number of reported malaria cases in Kaingaran and Malinsou, the subdistricts adjacent to Timbua is also shown in Table 1. In 1998, with residual spraying and impregnated mosquito nets as malaria control measures, the 3 villages, Pinawantai, Togop Laut, and Pahu, contributed to 40% of the malaria cases

lance was conducted before the sprayings of *B.t.i.* with alphacypermethrin and *B.sp* with alphacypermethrin were initiated, respectively.

B.thuringiensis israelensis and alphacypermethrin (Fig 1)

Togop Laut and Pahu had their highest landing rates of 0.88 and 0.30 mosquito/man/night, respectively in November 1998, before the spraying was initiated. After the spraying was initiated, the landing rates significantly decreased in both these villages with most months having a null landing rate. In Pinawantai, where *B.t.i.* was sprayed, the highest landing rate was after the 4th spray, followed by another peak in December 1999. As for the other months, Pinawantai did not have an obvious landing rate reduction as did

Table 1

Number of reported malaria cases in study villages and other villages in Timbua subdistrict[a].

Village	1998	1999	2000
Pinawantai (treated with *B.t.i./B.sp*)	14	0	0
Pahu (treated with alphacypermethrin)	9	1	0
Togop Laut			
(treated with *B.t.i./B.sp* and alphacypermethrin)	1	1	0
Tarawas (untreated village)	6	5	5
Other untreated villages in Timbua subdistrict[b]	30	1	13
Total	60	8	18
Kaingaran subdistrict[c]	186	112	115
Malinsou subdistrict[c]	158	27	26

[a]Data obtained from Ranau Health Office, Sabah.
[b]Other villages are the other 17 villages in Timbua subdistrict.
[c]Kaingaran and Malinsou subdistricts are situated adjacent to Timbua subdistrict.

in the Timbua subdistrict. In 1999, when *B.t.i*. and/ or alphacypermethrin was sprayed, the 3 treated villages contributed to 25% of the malaria cases in Timbua, while Tarawas, the untreated village, contributed to 63% of the reported malaria cases. The 3 treated villages had a significant reduction in malaria cases in 1999 compared to 1998 (p < 0.05).
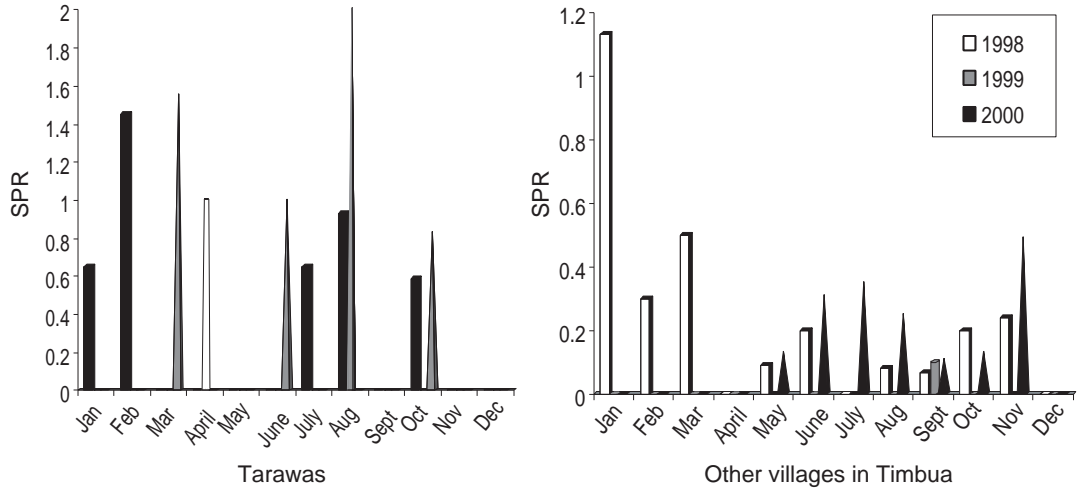
In 2000, when *B.sp* and/or alphacypermethrin were sprayed, there were no reports of malaria cases in any of the treated villages. Tarawas, and the other 17 untreated villages, contributed to 100% of the malaria cases in Timbua, with 83.33% of these cases being reported between March to August 2000, when Pinawantai, Togop Laut, and Pahu were treated with *B.sp* and/ or alphacypermethrin. The untreated villages had significantly higher numbers in comparison with the treated villages (p < 0.05).

The slide positivity rate (SPR) for the 4 study villages together with the other 17 villages in Timbua is shown in Fig 3a and Fig 3b. The treated villages had a SPR of 0.46-3.72 in 1998 (pre-treatment), with Pinawantai having the highest SPR. Since November 1998, when the spraying of insecticides was initiated, slide positivity was only observed in Pinawantai at a SPR of 0.62 (July 1999) and 0.66 (May 2000). Pahu and Togop Laut had a negative SPR. Tarawas, the untreated study village, together with the other 17 untreated villages in Timbua, had an unchanging SPR pattern throughout the study period.
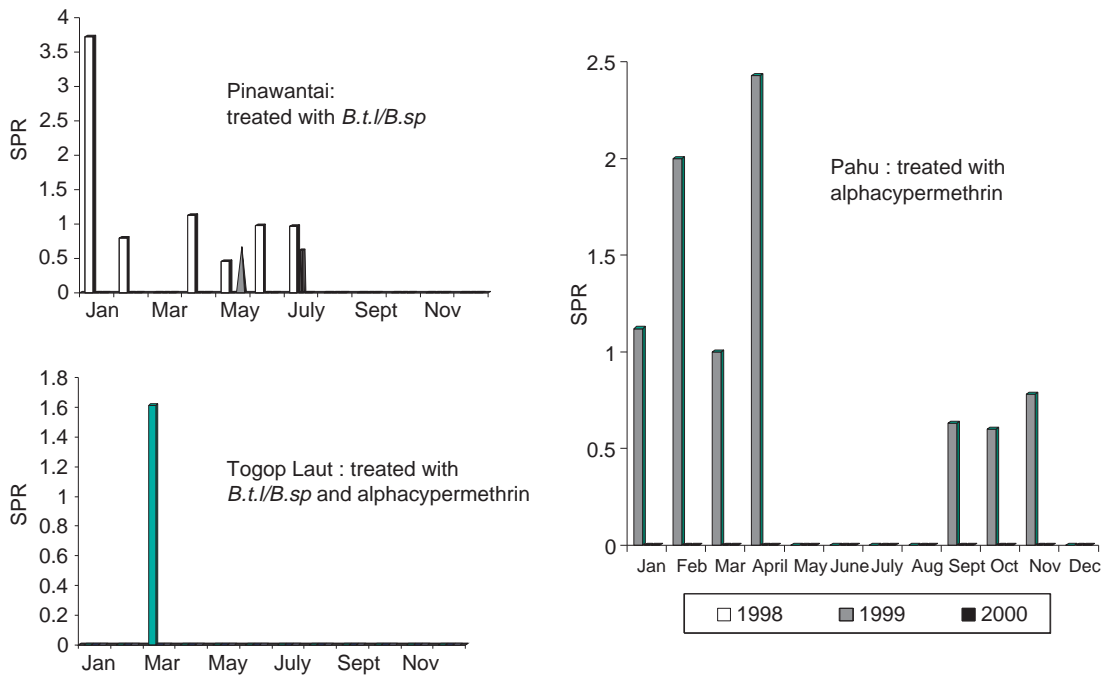
DISCUSSION

The present malaria vector control program in Malaysia depends on the use of chemical insecticides, *ie* residual spraying of deltamethrin, permethrin impregnated mosquito nets and larviciding with temephos. The reliance on synthetic chemical insecticides and the pressure of chemical insecticides on the environment can be decreased markedly by increasing the usage of biological insecticides for mosquito vector control. Field trials with *B. thuringiensis israelensis* (*B.t.i.*) and *B. sphaericus* (*B.sp*) have achieved measurable malaria control in Madagascar (Romi *et al,* 1993), India (Kumar *et al,* 1994; 1998) and Cameroon (Barbazan *et al,* 1998). In these field trials *B.t.i.* and *B.sp* were introduced directly into the larval breeding habitats. In Ranau, Sabah the larval breeding habitats of *An. balabacensis* are too extensive for direct application of bacterial insecticides into the breeding sites. This study measured the effectiveness of dispersing non chemical based environmentally friendly bacterial insecticides by space application.

The villages were space sprayed monthly with *B.t.i* (Vectobac 12AS[R]) and alpha cypermethrin (Fendona 10SC[R]) from November 1998 to December 1999, followed by *B.sp* (Vectolex WG[R]) and alpha cypermethrin (Fendona SC[R]) from March to August 2000. The potency of the products, Vectobac 12AS[R] and

Fig 3a–Slide positivity rate (SPR) in untreated villages in Timbua*.

Fig 3b–Slide positivity rate (SPR) in treated villages in Timbua.

Vectolex WG[R], were determined by bioassaying against laboratory bred *Aedes aegypti* and *Culex quinquefasciatus* larvae, respectively. The bioassay method was as described by CTD/WHOPES (1996). For the trial Vectobac 12AS[R] was purchased in 2 consignments, in December 1998 and August 1999. The first consignment gave a LC50 of 0.0052 mg/l, while the second consignment had a LC50 of 0.074 mg/l. The potency of the first consignment was further reduced on storage under field conditions to 0.094 mg/l (LC50). Throughout the study period the *B.t.i.* consign-

ments were used at the dosage of 500 ml/10,000 m² irrespective of the decreasing product potency. The decreasing product potency is believed to have contributed to inconsistent mosquito control in the field as observed in the landing rate of *An. balabacensis* in Pinawantai, the village which was sprayed with Vectobac 12AS[R] (Fig 1). The village had the highest landing rate after the 4th Vectobac 12AS[R] spray (April 1999) and another surge in December 1999. Togop Laut and Pahu had their highest landing rates before spraying was initiated. After spraying was initiated, the landing rates significantly decreased in both these villages with most months having a null landing rate. The significant reduction of the mosquito population in Togop Laut and Pahu is most probably contributed by alpha cypermethrin. The decreasing product potency of Vectobac 12AS [R] could be due to the aqueous formulation of the product. On the other hand, Vectolex WG[R] is marketed in a dry form and is suspended in water just prior to use. Thus, the potency of the product remained stable throughout the study period with a LC50 of 0.02 mg/l against *Cx. quinquefascitus* larvae. It has also been observed that the potency of Vectolex WG[R] remains stable for a minimum of 2 years for products stored at room temperature of 28º-32ºC (Seleena, unpublished data). The potency stable product maintained a significantly reduced mosquito landing rate of 0-0.4 in Pinawantai throughout the 6 month study period (Fig 2). Although *B.t.i.* and *B. sp* have been proven to be effective mosquito control agents in the field, the formulated product must be used at its registered potency. It is essential that the mosquito vector control agency acquires the appropriate bacterial formulation which can be stored in the field until use at its registered potency.

In Ranau, Vectobac 12AS[R] was sprayed at a dosage of 500 ml/10,000 m². This dosage was proven to give a complete mortality in the *Ae.aegypti* population for 7 days post-treatment on spraying indoors with a portable mist blower attached with an ULV nozzle, Maruyama Mfg Co, Inc™ (Seleena *et al,* 1999). This same dosage did not produce similar *An. balabacensis* control results when sprayed outdoors in Pinawantai. In the central highlands of Madagascar, complete larval control of *An. arabiensis* was achieved in the

rice fields with direct application of Vectobac 12AS[R] at 600 ml/10,000 m² (Romi *et al*, 1993). In Ranau, a more efficient and sustainable control could have been achieved if a higher Vectobac 12AS[R] dosage had been used for outdoor space spraying, *ie* at 1.0 l/10,000 m².

Vectolex WG[R] sprayed at a dosage of 500 g/10,000 m² gave a significant mosquito landing rate reduction in comparison to Vectobac 12AS[R]. This could be due to *B.sp* having more advantages than *B.t.i.* in controlling *An. balabacensis* which are found breeding in temporary ground pools, buffalo wallows and animal hoof prints which harbor an organic rich environment. *B.sp* is known to give extended residual control, especially in organically rich aquatic environments (Davidson *et al*, 1984). The endotoxin of *B.sp* persists in the water column and is available for larvae to feed on longer than *B.t.i* toxins (Lacey *et al,* 1984), and the larvae that are infected and paralyzed by *B.sp* toxins accumulate on the water surface. Fresh spores and toxins are released from larval cadavers into the aquatic environment for reinfection (Charles and Nicolas, 1986).

The spraying of bacterial and chemical insecticides did not eradicate the mosquito population in the treated villages but reduced the mosquito population in comparison to the pretreatment period. The insecticides reduced the mosquito population to the threshold that would cause malaria, as cases were not reported in any of the treated villages since the initiation of insecticide spraying, except for one case that was reported in Pinawantai in August 1999. Malaria cases continued to be reported in the untreated village, Tarawas, in an unchanging pattern throughout the study period.

Since the initiation of insecticide spraying, the SPR in the treated villages remained negative, just as the number of reported malaria cases (Fig 3b). Null SPR and null cases could have been contributed by the reduced mosquito population in the treated villages and also by the reduced vectorial capacity of *An. balabacensis* for the malarial parasite. The vectorial capacity has been reported to decrease in adults emerging from *B.t.i* and *B.sp* treated larvae. The adults are less likely to carry occysts and those that were oocyst posi-

tive carried significantly fewer oocysts than the untreated controls. Consequently, the proportion of mosquitos with sporozoites was also significantly lower in the treated group, indicating mosquitos surviving bacterial treatment did not fully support parasite development. The oocyst rate and density were significantly lower in *B.sp* treated *An.stephensi* which were infected with *Plasmodium yoelii* (Noireau and Karch, 1983), in *B.sp* treated *An. quadrimaculatus* infected with *P.berghei* (Young *et al*, 1990); and in *B.t.i.* treated *Ae.aegypti* infected with *P. gallinaceum* (Kala and Gunasekaran, 1999). The bacterial toxins disrupt the larval midgut epithelium, therefore it seems possible that it also disrupts the successful attachment and invasion of the epithelial cells by the ookinetes. Anopheline mosquitos having fewer oocysts infect a much lower human proportion (Macdonald, 1957). In the treated villages in Timbua, the space sprayed *B.t.i.* and *B.sp*, in addition to causing larval mortality, could have also inhibited the development of malarial parasites in adult *An. balabacensis* mosquitos emerging from larvae surviving the treatment, leading to a lower sporozoite rate. The transmission potential of the vector could have been reduced, leading to a negative SPR and no malaria cases in the bacteria treated villages. The reduced vectorial capacity phenomena could also explain the significant malaria control in Pinawantai in 1999 when it was treated with a sub-lethal dosage of *B.t.i* (500 ml/10,000 m^2) from a product of decreasing potency from November 1998 to December 1999. During this period the vector population remained relatively high in comparison to the pre *B.t.i* treatment period and to other treated villages, but there were no reported malaria cases and the SPR was significantly reduced in 1999 in comparison to 1998 ($p < 0.05$).

Chemical insecticides are not known to reduce the vectorial capacity of mosquitos for pathogens. The vectorial capacity did not reduce for *An.stephensi*, treated with sub-lethal doses of deltamethrin and cypermethrin at larval and adult stages, to *P. berghei* (Chunina *et al,* 1990); *Ae. aegypti*, treated with insect development inhibitor at the larval stage, to *Brugia pahangi* (Gaaboub and Busvine, 1976); anopheline mosquitos treated with DDT, malathion or dimilin, to

P. yoelii nigeriensis (Prasittisuk and Curtis, 1982); and *Ae.aegypti* treated with sub-lethal doses of malathion to dengue virus (Argubano, 1996) . The space sprayed alphacypermethrin in Pahu and in Togop Laut interrupted the malaria transmission by suppressing the vector population *via* adult mortality. This was proven in a study that was conducted in Melinsou, Ranau a malarious village of 12x10,000 m^2 in April 2001 (Seleena and Lee, 2001). The adulticidal activity of alphacypermethrin was evaluated by placing 10 cages, of blood-fed field collected anopheline mosquitos, randomly throughout the spray village. One of the cages held 18 *An. balabacensis* mosquitos. A complete adult mortality was achieved in the *An. balabacensis* population together with other field collected anophelines within 1 hour post-spray.

This pilot study covered a rugged terrain of only 92x10,000 m^2, without any replicate villages, because of logistics and limited funds. The preliminary data show that malaria transmission can be completely interrupted in malarious villages, which have *An. balabacensis* or similar anophelines as the malaria vector, with space spraying of chemical or/and biological insecticides with backpack sprayers. There is a need for a larger scale study to further confirm that malaria transmission can be interrupted with space application of insecticides. In 1980, it was reported that space ULV application of sumithion gave a sustained *An. balabacensis* control in Sabah for 2-3 weeks (Hii, 1980). All ASEAN countries either have *An. balabacensis* or other anopheline species with similar breeding habitats or/and biting behavior to *An. balabacensis* as their malaria vector (Harinasuta and Reynolds, 1984). It is not realistic to totally rely on residual spraying and impregnated mosquito nets to control exophagic and exophilic anopheline species. The present malaria control program in ASEAN countries needs to be reviewed to include outdoor control measures, such as space application of insecticides. A routine application of *B.t.i.* or *B.sp* insecticides is advocated over chemical insecticides as bacteria are: target specific, environmentally more friendly; to date there is no resistance development in mosquitos to *B.t.i.*, and it will maintain the anopheline population at a level that will not be a nuisance or a human vector. It is advis-

able not to use chemical insecticides, especially a pyrethroid based insecticide, over a continuous period of time as the anopheline mosquitos in malarious areas have usually been exposed to surfaces sprayed with permethrin based insecticides and permethrin impregnated mosquito nets. Anopheline mosquitos in Ranau, Sabah have also shown to have developed tolerance/resistance to 0.75% permethrin (Nazni, pernonal communication). It is advisable to spray a chemical adulticide simultaneously with a bacterial larvicide during a malaria outbreak. Fendona SC[R], can be sprayed simultaneously with Vectobac 12AS[R] or Vectolex WG[R] as the larvicidal toxins of Vectobac 12AS[R] and Vectolex WG[R] are not degraded in the presence of Fendona SC[R] and the bacterial formulations are not antagonistic to the adulticidal activity of Fendona SC[R] (Seleena *et al*, 1999; Seleena and Lee, 2001).

The mosquito vector control program managers have expressed their concern on whether the ground staff are able to implement space application in remote villages as this has never been a method of choice for malaria vector control. The 2-year study in Ranau, Sabah has shown otherwise. The spray team headed by a local public health inspector together with the villagers showed their capabilities to conduct this study without any major hitches. Therefore, space application of insecticides is a practical inexpensive technique which can be implemented in all ASEAN countries with proper staff training to eradicate malaria.

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