BACKGROUND

Malaria is a mosquito-borne dangerous infectious disease of human caused by parasitic protozoans of the *Plasmodium* (P.). Annually malaria disease infects millions of people and kills hundreds of thousand of them. Malaria parasites are transmitted from the infected people to the healthy ones by the mosquitoes *Anopheles* (An.). Malaria is distributed from latitude 64° north to 32 latitude south, more concentrated in African, South American and Pacific-Asian countries.

In Vietnam, in spite of the constant efforts made by the National Malaria Control Program (NMCP), the malaria control achievements are instable. Malaria is still a big challenge and threat for the people living in the mountainous, especially the remote, less developed areas inhabited by the ethnic minority people who have a habit of hill farming practice in the swidden fields, overnight sleeping in the milpa huts.

In Binh Thuan province the number of malaria patients in 2010 compared to that of 2006 was reduced by just 6.22 %. The malaria index 2010 as compared to 2006 was increased by 18.24 %. The malaria malaria laboratory confirmed cases were and patients mainly concentrated in the 5 hyper endemic communes (according to the 2009 epi-stratification for malaria intervention. In 2010, the number of malaria patients in the district of Bac Binh alone made up 45% of that in the whole province (323/720). Malaria prevalence was the highest among the people who have a habit of hill farming practice in the swidden fields, overnight sleeping in the milpa huts. The number of malaria patients in two communes of Phan Son and Phan Tien (district of Bac Binh) in 2010 made up 52.4 % as compared to that in the 5 hyper endemic communes (121/231) or 17.8 % compared to that in the whole province (121/678). Although malaria vector control measures were constantly made over many years, density of the primary malaria vector An .dirus indoor biting and outdoor resting was found high.

As shown by the study findings, in the hyper endemic areas, a high density of indoor biting and outdoor resting rate of two primary vectors of *Anopheles dirus* and *An. minimus*, causing a reduction of effectiveness of residual house spraying. The local *An. dirus* and *An. minimus* were found active biting in early evening when people were still outside the mosquito nets. The insecticide treated nets (ITN) were therefore had low protective effects. To overcome these problems, a combination of long lasting and multiply washable nets and repellent cream was recommended for the forest goers and overnighters.

From the above mentioned point of view, we designed and conducted a thesis "Assessment of malaria situation in Binh Thuan province (1991-2010) and investigation into effectiveness of the combination of the repellent cream Soffell with long lasting insecticidal mosquito net (Permanet 2.0) in some hypper-endemic malaria sites" to

- 1. evaluate the exact malaria situation in Binh Thuan province,
- 2. evaluate effectiveness of the repellent cream Soffell and residual effect of insecticide on the Permanet 2.0, and
- 3. investigate into the compliance of the local people with two above mentioned subjects.

SCIENTIFIC, NOUVEAU AND REALISTIC SIGNIFCANCE OF THE THESIS

The nouveau contribution of the thesis

With this thesis, malaria in Binh Thuan province over the past 20 years (1991 - 2010) including the situation of forest going and field hut overnight sleeping has the first time been evaluated. The challenges and difficulties in the application of vector control for those people were also indicated.

An additional vector control measure using a combination of repellent cream and the long-lasting chemical treated nets Permanet 2.0 has been first time investigated. Permanet 2.0 was found to reduce density of indoor biting behavior of *An. dirus* over night with the protection ability of 80%, which of Soffell against human biting of *An. dirus* was 89% for a period of 6 - 7 hours. The protection of combined repellent cream Soffell and Permanet 2.0 against human biting *An. dirus* was 92%.

Scientific significance

The thesis has inclusively reviewed and summed up the malaria situation in Binh Thuan province over the period 1991-2010 as well as identified the objective and technical factors which helped sharply but unsustainably reduce malaria in the province. As a result, some additional active malaria control measures including the vector control for the hyper endemic areas have been recommended. The high scientific and realistic significance and considerable contribution to the control and roll back malaria in the province of these new applications have been proven.

Reallistic significance

The thesis has shown the concrete data of the malaria situation in the province including the malaria prevalence, malaria vector. On the basis of this database, the appropriate vector control measures have been recommended and undertaken in the endemic and hyper-endemic areas (areas with migrant population, common habit of forest farming practice and overnight milpa sleeping; the remote and less developed area) of Binh Thuan province. These vector control measures have resulted in effective vector control in these areas and contributed significantly to NMCP.

THE THESIS STRUCTURE

The thesis comprises of 137 pages, 33 tables and 23 graphs with the following content: Background (3 pages), Scientific, Nouveau and Realistic Significance (1 page), Overview of Data (43 pages), Material and Method (15 pages), Results (35 pages), Discussion (36 pages), Conclusion and Recommendation (3 pages), References (1 page). Results (34 pages), Discussion (36 pages), Conclusion and Recommendation (3 pages). The references included 175 (78 Vietnamese and 97 English references).

Chapter 1. OVERVIEW OF DATA 1.1. Malaria and vector control in the world

1.1.1. Malaria in the world

In 2010, malaria was the cause of morbidity with 207 million infections and mortality with 627 deaths. It is estimated that malaria still threats 3.4 million people throughout the world (mainly in Africa and Southeast Asia). WHO, therefore warns that the target of malaria eradication by 2015 can be infeasible.

1.1.1.1 Studies of Anopheles

So far, 481 species of *Anopheles* have been found, of which more than 70 have been indentified as the malaria vectors. Their composition, distribution, vectorial role are varied from one to another species. By chromosome, electrophoresis enzyme and PCR, some previous studies made in the last decades have defined that *An. dirus* was a complex species and temporarily named them (A, B, C, D, E, F): *An. dirus* A, (Peyton & Harrrison 1979); *An. dirus* B, (Hii, 1982); *An. dirus* C, *An. dirus* D, (Baimai *et al*, 1988). Harbach et al (2007) have also identified Minimus with two species officially named *An. minimus* (species A) and *An. harrisoni* (species C) and a species unofficially named *An. minimus* E. Chiristophers S. R. (1911) studied the development of eggs in

mosquito body. Beklemishev (1940) studying geology of *Anopheles* discovered three stages of reproduction cycle. Rusell (1946) and Carneval (1978) studied host behavior and attractive factors of mosquitoes. Polodova (1949) studied and defined biological ages of mosquitoes.

In 1992, WHO announced 72 chemical resistant mosquito species, of which 69 DDT resistant, 38 organic phosphorus resistant, 17 species resistant to all three mentioned ones. The increasing number of species have been found to be resistant to chemical, that by the year 2000 about 100 mosquitoes including 50 species of *Anopheles* have been found chemical resistant. In 1880, Laveran the first time discovered and described malaria parasite in the human blood cell in Algeria. In 1897, Ronald Ross, Grass, Bigmani and Bastianelli described the full cycle of development of malaria parasite in human and *Anopheles*.

1.1.2. Vector control studies in the world

1.1.2.1. Studies of insecticides

In 1934, Paul Miller developed DDT (Zedler compounded in 1874). The first pesticide of pyrethroid group was descovered by Standinger and it was natural pyrethrine (biollethrine) in 1924. In 1973, Elliott and Onwaris successfully compounded permethrine. At the end of 70s and early 80s, the first application of wall residual spray of ICON, detamethrine; mosquito nets treated with detamethrine, permethrine, Fendona were confirmed preliminarily effective.

1.1.2.2. Malaria vector control

In Brazil, Cavalcante (1996), Xavier (1986) carried out an experiment trial of deltamethrin treated curtance (25 mg active/m²) covering the huts of miners. Kroeger et al. (1995) investigated lambda – cyhalothrin (10 – 30 mg/m²) treated mosquito nets in Colombia. Sexton (1994) experimentally studied hammocks covered with permethrin (500 mg active/m²) nets in the communities which had the habit of using hammocks. Moore et al (2007) investigated DEET 15 % and found its repellent effect of 95% during 6 hours after application in Guatemala and in Peru

1.2. Malaria and malaria vector control in Vietnam

1.2.1. Malaria situation in Vietnam

The Malaria Eradication Program (MEP) was carried out between 1976 and 1990. Since 1976 malaria fight had been deteriorated due to the war consequences and other factors as shortage of man power, downgrade of the health care network, unorganized population migration. In addition, the malaria situation was aggravated by the technical difficulties such as drug resistant parasite, outdoor resting malaria mosquitoes...). Malaria therefore was increased in many places. In 1980, malaria resurgence, an onset of malaria rage was spread in the mountainous and coastal areas, which infected 511,557 and killed 1,138 people nationwide.

In 1991, the MEP was shifted to the National Malaria Control Program (NMCP) with a great State investment and joint efforts of all sectors. As a result, the considerable achievements were obtained. After 10 years (1991 – 2000) of launching the new program, malaria infection was reduced by 73.1% as compared to that of 1991 (1,091,251 infections). Mortality was reduced by 98.5% as compared to that in 1991 (4,641 cases. In 2012, eight cases of death due to malaria were reported, 42.9% lower compared to that in 2011. The number of malaria patients was 43.717 reduced by 4.1% compared to that of 2011). The reduction, however was recorded in only 5/7 zones. In the other two zones (Central coastal and Highland) it remained increasing by 7% và 12%, respectively. The increase, however was recognized as consequences of the local human problems such as unorganized population movement, border-crossing business, forest farming practice and milpa hut overnight sleeping. These challenges hindered effectiveness of the malaria control measures and contributed to the deterioration of malaria situation.

1.2.2. Studies of Anopheles

Laveran's studies of malaria mosquitoes in the South of Vietnam were published (1901 – 1904). Galliard and Đang Van Ngu (1946) developed a list of mosquito species which comprises of 22 species. In 1987, Institute of Malaria, Parasitology and Entomology published "Table of *Anopheles* in Vietnam" (mosquitoes, larva). In 1996, Tran Đuc Hinh announced the identification of 58 species, sub-species and genus of *Anopheles*, and described a *sp*. In 2005, Ho Đinh Trung reported 59 species of *Anopheles*, not including some complexes with numerous members as *An. maculatus* with at least 10 members, *An. minimus* with two members. Ngo Thi Huong et al (2004, 2007) identified a complex of Minimus including *An. minimus* and *An. harrisoni* found distributed widely in the Center and South of Vietnam. Meanwhile, the complex of Dirus was found with the presence of only An. *dirus* (= *An. dirus* A).

Ho Đinh Trung, Vu Đuc Chinh (2010) evaluated susceptibility to the insecticide of pyrethroid groups in 36 sites from North to South, Highland and Eastern South and found that mosquitoes *An. minimus* in less 50 % study sites were still susceptible to insecticides. Some populations of An. *minimus* were found to be resistant to all three insecticides of alpha – cypermethrin, lambda – cyhalothrin and permethrin. So far, in Vietnam as well in the world there have been no repots on *dirus* resistant to the insecticides used in the malaria control.

According to Ho Đinh Trung (2003), three primary malaria vectors, six secondary and five suspicious ones have been identified in Vietnam as following: Primary vectors: *An. dirus* distributed in the jungle, edges of jungle, thin forest from latitude 200 north (Thanh Hoa province southwards); *An. minimus* is distributed in jungle edges, thin forest and hilly areas throughout the country; *An. epiroticus* is distributed predominantly in the brackish water from Phan Thiet southwards. Secondary vectors: *An. aconitus*, *An. jeyporiensis*, *An. maculatus*, *An. sinensis*, *An. Vagus a*re distributed in all mountainous areas of the country; *An. subpictus* are found in the coastal areas.

* Malaria vector control

Nguyen Tuyen Quang et al (2001), studied malaria vector in the Khanh Phu commune, Khanh Vinh district, Khanh Hoa province, confirmed that the An.minimus population in this commune had came to a very low density after three-year application of insecticide treated mosquito nets (ITN). Trương Van Co et al (2007) evaluated effectiveness of the bed net treated with Icon 2.5 CS in the Iacor commune, Chur Sê district, Gia Lai province realized a considerable density reduction of Anopheles caught by light traps prior and after ITN application (from 15.5 mosquito/trap/night (m/t/n) to 1.75 m/t/n. Truong Van Co, Nguyen Thi Duyen et al (2010) conducted an evaluation of Fendona 10 SC for residual spraying (RS) and ICON 2.5 CS for bed net impregnation in Vinh Kim commune, Vinh Thanh district and found a general effect of preventing An. dirus from coming into the house by RS and ITN was 65 %. A trial of curtaince treated with Fendona 10 SC at the dose of 25mg/m^2 by Nguyen Anh Tuan et al (2011) showed an effect of reduction of mosquitoes attacking the latex workers in the rubber plantation in Gia Lai from 3.07 % to 0.32 %. Ho Dinh Trung (2008), investigated into the hammocks covered with Permanet 2.0 to protect the forest goers and milpa hut overnighters proved that 4 month after application in Ea Kar district, Đak Lak province the malaria prevalence in the study group was 0.51% compared to 4.44% before the intervention (P < 0.001). Nguyen Tuyen Quang et al (2005), tested a repellent cream containing DEET 13 % and apprised an ability of 85 % reduction of *An. dirus* attacking human in the forest in Khanh Phu commune.

1.3. Malaria and malaria vector control in Binh Thuan province **1.3.1.** Malaria situation in Binh Thuan

Binh Thuan is 1 of 15 provinces of Central-Highland. Malaria in Binh Thuan is ranked tenth compared to that over 15 provinces. Risk of malaria in Binh Thuan, however is high as the province borders with the others with complicated malaria situation as Ninh Thuan, Binh Phươc and Lam Đong. In general, malaria in Binh Thuan is found to have decreased (in 2010, the morbidity and mortality were decreased by 89,58% and 99%, respectively as compared to that in 1991). This reduction was unstable according to the provincial annual malaria reports. Since 2008 onward, malaria tends to come back and go up. Remarkably, in 2009 the infection cases increased by 60% compared to those of 2008 (720 /450 cases in 2008). In 2010, malaria decreased by a small level (by 5.83%), of which three cases of severe malaria and one death were recorded. More seriously, over three first months of 2011 alone, the number of infection cases in the whole province raised by 27.33% compared to the same time of the previous year and made up nearly 1/3 of all infection cases of 2010. In 2012, in the whole province 746 malaria patients were reported (increased by 3.6 % compared to that in 2011), mainly concentrated among the forest goers and milpa hut overnighters.

1.3.2. Vector control in Binh Thuan

Studies conducted on the effectiveness of appropriatness of the malaria control measures applied in two hyper-endemic communes of Dong Giang and La Da), Ham Thuan Bac district, Binh Thuan province during the period between 1998 and 2000 showed that distribution of anti-malarial drugs CV8 permethrin treated nets for the forest goers and overnighters helped reduce infection rate by 4.6 time and malaria laboratory confirmed cases by 4.3 times. Ho Van Hoang et al (2014) studied the factors of malaria transmission among the moving people in Ham Can commune, Ham Tan district showed the findings the malaria infection rate among the migrant people was 8.42%, much higher compared to that of non-migrant ones (1.18%) (P<0,001).

Chapter 2. OBJECTS AND METHODS

2.1. Objects and materials

2.1.1. Study objects

2.1.1.1. Anopheles mosquitoes of vector capacity

Primary vectors as *An. dirus* and other vectors as *An. maculatus* in the areas of milpa huts.

2.1.1.2. People who often go to their milpa stay overnight in the milpa huts

People aged 15 year old and older who must stay overnight in the milpa huts at least three nights or once/month.

2.1.2. Study materials

2.1.2.1. Long lasting treated nets Permanet 2.0

Permanet 2.0 is long lasting deltamethrin treated net produced by Vestergaard Frandsen (Switzerland). The net is made of polyester fiber treated with deltamethrin at a dose of 55 mg/m² (=1.4 g/kg fiber/100 denier, 1.8g/kg fiber 75 – denier). Deltamethrin is protected by a plastic layer to be durable to washing. Permanet 2.0 was temporaily recommended by WHOPES in 2004. WHO officially admitted its technical specification and recommended international use in December 2009.

2.1.2.2. Repellent cream Soffell and the use indication

Soffell is a soothing lotion for mosquito bites with the active elements of Diethyltoluamide (DEET) 13 %, chemical formula: $C_{12}H_{12}NO$, Youth Pte Herlia Indah, Indonesia produces under the authority of Fountain of Youth Pte Ltd. Soffell is the lotion for mosquito bites bottled 70ml with aromatic odour of orange or lemon, skin damping element and anti-mosquito agent DEET which can repel mosquito for 6 – 10 hours. The cream is attested by Pasteur institute Ho Chi Minh as "instimulative agent" and is certified by the Ministry of Health as family and health care lotion No. VNDP – HC – 691 – 08 – 13

Indication use: even application on skin of neck, face, hand, feet, or clothes with a thin layer.

2.2. Study site

Endemic communes in the whole province of Bình Thuận

Two communes of Phan Tien and Phan Son of Bac Binh district (selected as the study site to evaluate the effects of combination of repellent cream Soffell and Permanet 2.0 and residual effects of Permanet 2.0).

2.3. Study time

From 1991 to 2010: collection of retrospective data of malaria and the general malaria situation of Binh Thuan province.

From 2011 to 2013: Evaluate the effects of Soffell combined with Permanet 2.0 and residual effect of chemical of Permanet 2.0.

2.4. Study method

2.4.1. Study design

Retrospective analysis of data on malaria epidemiology and vector control measures collected from the various malaria information and the annual provincial malaria reports or periodical malaria reports by the Provincial Center for Malaria and Goiter Control of Binh Thuan province.

Intervention study with control

2.4.2. Data collection

Data collection of malaria and Anopheles information between 1991 - 2010

+ Malaria situation (malaria patients, prevalence/1000 population, death due to malaria, mortality/100.000 population, laboratory confirmed malaria cases).

+ Mosquito *Anopheles* (species composition and distribution per epi-stratification zones, density of primary vector *An. dirus* and *An. minimus* by different collection methods.

+ Residual spraying and bednet impregnation with the insecticides used in NMCP in Vietnam.

- Base line survey before the intervention

The frequent forest goers and milpa hut overnighters are targeted to be involved in the study. The local residents aged 15 year and older were interviewed using a designed questionnaire to be sampled. Their behavior of bed-net using and other anti-mosquito activities were also questioned.

- Post-intervention survey

The survey and analysis techniques of Anopheles mosquitoes followed the WHO and NIMPE specifications. Mosquitoes were caught in the milpa hut places at the forest edge of the communes of Phan Tien and Phan Son. Two milpa huts with a distance 3 km from each other from each commune were selected to evaluate the density of *Anopheles*: One was used to measure the effect of Permanet 2.0 alone (by indoor human bait trapping), the other to the combination of the repellent cream and Permanet 2.0 by indoor human bait trapping). The huts must be at a

distance 3 km from each other. On the other hand, to evaluate the effect of repellent cream alone, *Anopheles* was collected by outdoor human baiting that must take place at least 200m from the house with the combination trial of Soffell and Permanet 2.0.

+ *Anopheles* density was calculated to evaluate the effects of Permanet 2.0 alone.

A house was selected for over night human baiting (from 6pm to 6am next day). Two men baiting in turn, one from 6 to 12pm, the other from 12pm to 6 am next day. It was repeated every month, four consecutive nights each (during 8 months).

Night 1: A non-treated polyester net was hung (as a control net). The bait man sit 50cm from the net to catch mosquitoes.

Night 2: A Permanet 2.0 was hung and the bait man sit 50cm from the net to catch mosquitoes .

Night 3: A non-treated polyester net was hung (as a control net). The bait man sit 50cm from the net to catch mosquitoes

Night 4: A Permanet 2.0 was hung and the bait man sit 50cm from the net to catch mosquitoes

+ *Anopheles* density was calculated to evaluate the effect of the repellent cream and Permanet 2.0.

A house was selected for over night human baiting (from 6pm to 6am next day). Two men baiting in turn, one from 6pm to 6am next day, the other from 12pm to 6am next day. It was repeated every month, four consecutive nights each (during 8 months).

Night 1: A non-cream applied man (as control). The bait man sit 50cm from the non-treated net to catch mosquitoes.

Night 2: A bait man applied cream on the open skin (neck, face, hand, feet) and sit 50cm from the net to catch mosquitoes .

Night 3: A non-cream treated bait man (as a control net) sit 50cm from the net to catch mosquitoes (as control).

Night 4: A bait man applied cream on the open skin (neck, face, hand, feet) and sit 50cm from the net to catch mosquitoes

+ *Anopheles* density was calculated to evaluate the effect of the repellent cream Soffell alone.

Density of *Anopheles* (m/h/m) was calculated by over-night outdoor human baiting (from 6pm to 6am) Each night two bait men: one from 6pm to 12pm, the other form 12pm to 6am next day. Once a month with four consecutive nights each during 8 months.

Night 1: A non-cream applied man (as control).

Night 2: A bait man applied cream on the open skin (neck, face, hand, feet at 6pm .

Night 3: A non-cream treated bait man (as a control).

Night 4: A bait man applied cream on the open skin (neck, face, hand, feet) at 6pm.

The surveys were conducted in July, August, September, November and December of 2013 and January, March of 2014. The collected mosquitoes were recorded per the catching hour, night. Taxonomy was made following the NIMPE classification (2008) and reserved in plastic tube with anti-moisture gels.

- Evaluation of community compliance with the use of Soffell and Permanet 2.0

+ Survey of (by interviewing) 450 residents who were provided with Permanet 2.0 and repellent cream Soffell on their compliance.

+ Interviewing 100 residents using Permanet 2.0 (according to the annex 4) and 100 residents using the repellent cream Soffell (according to the annex 5) 10 days after they started using the nets and creams. The interviewees were randomly selected from the list of the forest goers and overnighters who had received Permanet 2.0 and Soffell. They were supervised for a safe use, and side effects of the products if any (rash, eye stimulus, sneeze, headache, nausea, drizzle, diarrhea, stomachache)

- The field trial Permanet 2.0 were collected and brought to NIMPE for the bio-test to evaluate the residual effects of chemical on the Permanet 2.0.

2.4.3. Sampling and sample size

Targeted residents aged 15 years and old (overnight in the milpa huts at least tree nights or once a month were sampled. The sample size was calculated according to the number of Permanet 2.0 provision using the sample size formula for a community intervention. To collect enough 450 forest frequent goers and overnighters, a random selection from the list of residents living in the five hamlets of two communes was made as following: hamlet KaLip: 105 residents, hamlet Bonthop: 105 residents, hamlet Tamon: 105 residents, hamlet Tien Thanh: 105 residents, hamlet Tien Đat: 30 residents. Each person was provided with 1 Permanet 2.0 and 1 Soffell 70 ml. They were followed up their compliance and any side effects.

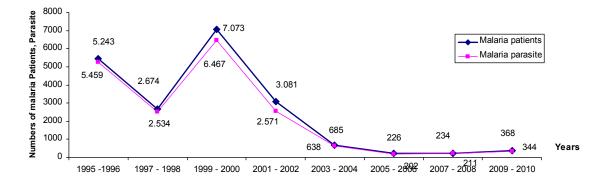
2.4.5. Data analysis: Database was entered by Epidata 3.1 and processed by SPSS 15.

2.4.6. Ethical clearance: The study was approved the Ethical Committee of NIMPE.

Chapter 3. RESULTS 3.1. Malaria situation in Binh Thuan (1991 – 2010) 3.1.1. Indexes of morbidity and mortality (1991 – 2010)

The 20-year (1991 - 2010) intervention of malaria control in Binh Thuan had resulted in a remarkable reduction of malaria infection. The number of malaria patients in 2010 was reduced by 89.58 % as compared to that in 1991 (678/6.506); the prevalence/1000 population was reduced by 92.75% (from 8.00 down to 0.58). However, in 2009 the number of malaria infection increased by 60% compared to that in 2008 (720/450 cases of 2008). In 2010, the infection cases were reduced inconsiderably compared to that in 2009 (by 5.83%), among them three cases of severe malaria and one death were reported. In average, annually 12.3 death cases/99.2 severe and complicated were recorded.

According to the malaria epi-stratification, in 2009, the five communes in Binh Thuan are located in the hyper-endemic zones, including: Phan Son, Phan Tien, Phan Lam, My Thanh and La Ngau. The great efforts of intervention including the investment of man resource, technical measure were made in the hyper-endemic zones to reduce the morbidity, mortality and prevention of outbreak.



Graph 3.1. Malaria patients and laboratory-confirmed cases in five hyper-endemic communes in (1995 – 2010)

Malaria patients and laboratory-confirmed cases were highest during two years 1999 - 2000 (7,073 malaria patients and 6,467 laboratory-confirmed cases). They were sharply reduced during the following years of 2003 - 2004 (685 malaria patients and 638 laboratory-confirmed cases), and of 2005 - 2006 ((226 malaria patients and 202 laboratory-confirmed cases). However, since 2009 - 2010 malaria patients and laboratory-confirmed cases trend to go up (368

malaria patients and 344 laboratory-confirmed cases) detected predominantly among the forest goers and milpa hut overnighters (Graph 3.1).

3.1.2. Mosquitoes Anopheles in Binh Thuan province (1991-2010)

In two years 1991 – 2010, *Anopheles* surveys were conducted in the three epi-stratification zones: Zone III (hypoendemic), zone IV (medium-endemic), zone V (hyperendemic); In 18 study sites (528 catching times, 48,795 hours) a total of 36.159 *Anopheles* mosquitoes belonging 28 genus (Table 3.1).

Table 3.1. Species of Anopheles their distribution ratecollected per epi-stratification zones in Bình Thuận (1991 – 2000)

TT	Species		III (1)	Zone		Zone		To	tal
	1	No.		No.	Rate	No.	Rate	No.	Rate
			%		(%)		(%)		(%)
1	An. aconitus	0	0	1.475	16,71	2.917	15,07	4.392	12,16
2	An. argyropus	0	0	11	0,13	20	0,10	31	0,06
3	An. aitkeni	0	0	6	0,07	11	0,06	17	0,05
4	An. annularis	0	0	8	0,09	15	0,08	23	0,06
5	An. barbirostris	8	0,1	62	0,7	123	0,64	193	0,54
6	An. barbumbrosus	0	0	13	0,15	35	0,18	48	0,13
7	An. campestris	0	0	0	0	4	0,02	4	0,01
8	An. crawfordi	0	0	21	0,24	27	0,14	48	0,13
9	An. dirus	0	0	188	2,13	2.093	10,81	2.281	6,32
10	An. indefinitus	5	0,06	24	0,27	12	0,06	41	0,11
11	An. kochi	0	0	87	0,98	106	0,55	193	0,53
12	An. karwari	0	0	78	0,88	87	0,45	165	0,46
13	An. jamesi	0	0	0	0	7	0,04	7	0,02
14	An. jeyporiensis	0	0	0	0	88	0,45	88	0,24
15	An. maculatus	0	0	842	9,55	6.575	33,97	7.417	20,52
16	An. minimus	2.300	28,84	455	5,15	375	1,94	3.130	8,66
17	An. nivipes	0	0	78	0,88	160	0,83	238	0,66
18	An. nigerimus	0	0	4	0,04	10	0,05	14	0,04
19	An. pediteaniatus	746	9,35	513	5,81	478	2,47	1.737	4,81
20	An. pampanai	345	4,32	23	0,26	295	1,52	663	1,83
21	An. philipinensis	0	0	684	7,76	953	4,92	1.637	4,53
22	An. splendidus	0	0	758	8,59	802	4,14	1.560	4,31
23	An. sinensis	86	1,08	578	6,55	462	2,39	1.126	3,11
24	An. tessellatus	32	0,40	196	2,22	415	2,14	643	1,78
25	An. subpictus	69	0,87	0	0	0	0	69	0,19
26	An. umbrosus	0	0	8	0,09	0	0	8	0,02
27	An. vagus	4.386	54,98	2.713	30,75	3.247	16,77	10.346	28,61
28	An. varuna	0	0	0	0	40	0,21	40	0,11
Tot	tal caught number	7.977	100	8.825	100	19.357	100	36.159	100
	Total)	2		2			

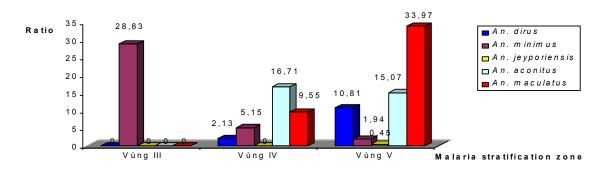
Note: (1) Survey made in 3.172 hours, 58 times, 3 sites;

(2) Survey made in 13,000 hours, 185 times, 5 sites;

(3) Survey made in 32.623 hours, 285 times, 10 sites.

In Zone III 7,977 individual mosquitoes of nine species of *Anopheles* have been collected (made up 23.68%). Only one primary vector of *An. minimus* was caught (2.300 individual mosquitoes or 28.83%). They were distributed mainly in Binh Thanh commune, Tuy Phong district. This commune is located in the Zone III (according to epi-stratification 2003) and according to epi-stratification and intervention 2009 the commune was at risk of malaria resurgence. Secondary vectors of *An. maculatus, An. aconitus* và *An. Jeyporiensis* have not been found.

In Zone IV: 8,825 individual mosquitoes of 23 species of *Anopheles* have been collected (made up 24.4%) Two primary vector of *An. dirus* (188 individual mosquitoes or 2.13%) and *An. minimus* (455 individual mosquitoes or 5.15%) were present. In addition, some other secondary vectors as *An. aconitus* (1.475 individual mosquitoes or 16.71%) and An. *maculatus* (842 individual mosquitoes or 9.55%).



Graph 3.2. Rate (%) of primary and secondary malaria vectors per epi-stratification and intervention

Zone V: More number of *Anopheles* collected as compared to that in the Zone III (17,291/7,977) about 2.2 times and that in the Zone IV (19.357/8.825) approximately 2.19 times. Two primary vectors of *An. dirus* (10.81%) and *An. minimus* (1.94%) were present. In addition, 3 secondary vectors of *An. jeyporiensis* (0.45%), *An. aconitus* (15.07%) and *An. maculatus* (3.97%). were caught

Primary vector *An. dirus* was found in both Zone IV and Zone V. *An. minimus* was caught in all three zones with the decreasing caught number as in the zone III (2,300 individuals), zone IV (455 individuals) and zone V (375 individuals).

The total average density of the caught number of *An. dirus* by indoor light trap (by 3.6 times) compared to outdoor light (13.6 ± 6.27 m/t/m compared to 3.81 ± 1.84 m/t/m). A higher density *An. minimus* was caught by indoor light trap (1.3 times higher) compared to that by

outdoor light trap (7.55 \pm 4.12 m/t/n compared to 5.59 \pm 3.75 m/t.m) (Table 3.2).

The average density of *An. minimus* collected by indoor human baiting reduced from 0.38 ± 0.06 m/h/n (1991 – 1995) to 0.22 ± 0.15 m/h/m (2006 – 2010) and by outdoor human baiting reduced from 0.08 ± 0.05 m/h/m (1991 – 1995) to 0.06 ± 0.04 m/h/n (2006 – 2010).

	Collection methods						
Periods	Outdoor human bait (m/h/m) (No. Mos./hour/man)	Indorr human bait (m/h/m)	Indoor light trap (m/l/n) (No.mos./light/night)	Outdoor light trap (m/l/n)	SNN (m/h) (No.mos./hour)		
An. dirus; TB	\pm SD						
1991 - 1995	$0,28 \pm 0,19$	$0,12 \pm 0,05$	$3,44 \pm 1,47$	$2,25 \pm 1,00$	0		
1996 - 2000	$0,2 \pm 0,11$	$0,12 \pm 0,06$	$5,42 \pm 0,98$	$0,16 \pm 0,12$	0		
2001 - 2005	$0,16 \pm 0,12$	$0,05 \pm 0,03$	$1,38 \pm 1,26$	$0,4 \pm 0,28$	0		
2006 - 2010	$0,6 \pm 0,23$	$0,08 \pm 0,09$	$3,36 \pm 2,56$	$1,0 \pm 0,44$	0		
Tổng cộng TB ± SD	1,24 ± 0,65	$0,\!37\pm0,\!23$	13,6 ± 6,27	3,81 ± 1,84	0		
An. minimus;	$TB \pm SD$						
1991 - 1995	$0,38 \pm 0,06$	$0,08 \pm 0,05$	$4,93 \pm 2,72$	$4,\!45 \pm 2,\!97$	$0,\!29\pm0,\!32$		
1996 - 2000	$0,\!97\pm0,\!37$	$0,37 \pm 0,4$	$0,1 \pm 0,09$	$0,33 \pm 0,24$	$3,05 \pm 1,47$		
2001 - 2005	$0,34 \pm 0,09$	$0,05 \pm 0,02$	$0,63 \pm 0,34$	$0,73 \pm 0,47$	$1,01 \pm 0,92$		
2006 - 2010	$0,22 \pm 0,15$	$0,06 \pm 0,04$	$1,89 \pm 0,97$	$0{,}08\pm0{,}07$	$0,03 \pm 0,02$		
Tổng cộng TB ± SD	1,91 ± 0,67	0,56 ± 0,51	7,55 ± 4,12	5,59 ± 3,75	4,38 ± 2,73		

 Table 3.2. Density of An. dirus and An. mimimus measured by different collection methods and catching times in Binh Thuan province

The average desity of indoor human bait catching *An. dirus* was found reduced from 0.12 ± 0.05 m/h/m (1991 – 1995) to 0.05 ± 0.03 c/g/ng m/h/m (2001 – 2005). The indoor human bait catching showed a reduction of average density of *An. dirus* from 0.28 ± 0.19 m/h/m (1991 – 1995) to 0.16 ± 0.12 m/h/m (2001 – 2005). During the period between 2006 and 2010 density of *An. dirus* by indoor human bait catching was 0.6 ± 0.23 m/h/m, collected mainly from the milpa huts (Table 3.2).

3.1.3. Effectiveness of the vector control measures (1991-2010)

Annually, house residual spraying and bednet impregnation were periodically conducted in the communes in the medium endemic (zone IV) and hyperendemic (zone V) following the annual plan of IMPE Quy Nhon with the exact zones indications for residual spraying and bednet impregnation. A total of 3,089,328 people were protected by the house residual spraying and bednet impregnation, of which 419,683 people were protected by house residual spraying and 2,669,645 by bednet impregnation. The number of people protected by house residual spraying was gradually reduced (in 2005: 43,625 people; in 2010: 22,796 people) as replaced by wider protection by bednet impregnation from 1000 residents in 1991 up to 66,350 - 126,255 people (between 1993 - 1995) and reached 163,299 - 197,173 people (during 1999 - 2010).

3.1.4. The exact situation of malaria infection among the milpa goers and overnighters

Table 3.3. The correlation between malaria infection and forest going and milpa hut sleeping (2007 – 2009) in three communes of Phan Son, Phan Lam

		Malaria	Malaria infection		
		Yes	No		
Forest going	Yes	366	3.760	4.126	
and milpa hut sleeping	No	49	2.532	2.581	
Cộng		415	6,292	6,707	

(OR = 5,03; P < 0,001)

It was found that the forest goers and overnighters have an infection risk 5.03 times higher than that of those who do not (P < 0.001).

Table 3.4. Correlation of malaria infection and the habit of using the mosquitonets of the milpa people

nets of the milpu people						
		Malaia	Total			
		Yes	No			
Sleeping inside the	Yes	38	206	244		
inside the bednet	No	11	160	171		
Total		49	366	415		

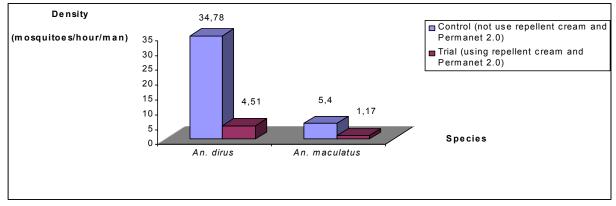
(OR = 2.68; P < 0.05)

The table 3.4 shows that the milpa practice people who did not sleep inside the mosquito nets were infected with malaria at a rate 2.68 times higher than those who slept inside the bednet when they were on the milpa huts with (P < 0.05).

3.2. Effect of the repellent cream Soffell combined with Permanet **2.0** and residual effect of insecticide on Permanet **2.0**

In the study sites, primary vector *An. dirus* and secondary vector *An.maculatus* were caught, of which *An. dirus* was of highest density of 39.29 m/h/m (34.78 m/h/m at control and 4.51 at the trial sites). No

An.minimus was caught in the study sites. The density of An.dirus and An.maculatus at the control was higher in the trial sites (Graph 3.3).



Graph 3.3. Density *An. dirus* và *An.maculatus* caught in the trial and control Table 3.5. Susceptibility test of laboratory-rearing *An. dirus*

_	to	deltamethrin	0.0)5%	

Test time	-	coes exposed to	Control mosquitoes		
	deltamet	hrin 0.05%			
	No. testing	Mortality (%)	No. testing	Mortality (%)	
	mosquitoes		mosquitoes		
June/2011	100	100	20	0	
November/2011	100	100	20	0	
June/2013	100	100	20	0	

Trial site: NIMPE

Climatic condition: temperature:26^oC - 28^oC, humidity 75 - 80%

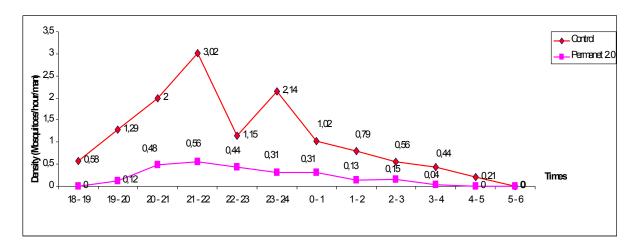
The mean *An. dirus* mortality over three trials was found to be 100% showing that this species was still fully susceptible to the testing insecticide.

Table 3.6. The results of evaluation of the residual effect of unwashedPermanet 2.0 after 7 month's use.

Mosquitoes	No. mosquitoes exposed to Permanet 2.0		Control mosquitoes				
NIMPE laboratory-	Number	Mortality after 24 hours	Number	Mortality after 24 hours			
rearing An. dirus	200	100	50	0			
Field caught <i>An. dirus</i> in the trial site of Phan Tiến commune, Bac Binh district.	50	100	20	0			

Trial condition: Temperature 25.8^oC, humidity 78 %

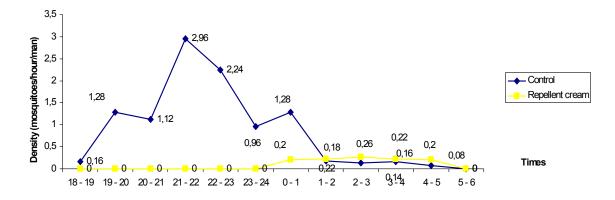
A 100% mortality of labo-rearing and field collected *An. dirus* exposed to the unwashed Permanet 2.0 after seven month's use was found.



Graph 3.4. Night biting of *An. dirus* in the trial of permanet 2.0 and the control.

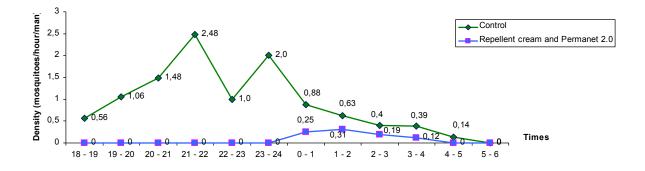
The repellent cream was obviously protective if compared to the control. When combined with Permanet 2.0 the increased effects were found. Permanet 2.0 was found to reduce the number of biting mosquitoes which, however were still able to contact people early from 7pm (density 0.12 m/n/m) gradually reduced toward the dawn (Graph 3.4) while at the control *An.dirus* started biting from 6-7pm (0.58 m/h/m) and got to a peak at 9-10pm (3.02 m/h/m).

The evaluation of Permanet 2.0 effects compared with the control was calculated following the formula of Hederson Tilton and Kaplan – Meier. Permanet 2.0 effects against *An. dirus* was 80 %.



Graph 3.5. Density of night biting *An. dirus* caught in the trial and control of cream Soffell.

The repellent cream can repel and prevent mosquitoes from outdoor human contacting within 6-7 hours after the application. Before 12pm no the cream applied parts), but they were found biting from 12pm onwards the morning. (The total density biting from 12pm to 5am was 1.1 m/h/m). The evaluation of Permanet 20 effects was calculated following the formula of Hederson Tilton and Kaplan – Meier. Soffell effects against *An. dirus* was 89 % during 6-7 hours.



Graph 36. Density of night time human biting *An. dirus* caught in the control and trial of Soffell combined with Permanet 20

At the control site, *An. dirus* started biting very early (0.56 m/h/m) from 6-7pm with two peaks of biting (2.48 for one hour (9-10pm) and 2.0 m/h/m from 11 to 12pm and started to reduce towards the dawn. When the repellent cream was used with Permante 2.0, *An. dirus* was not found biting before 12pm, but started biting from 1am at a density of 0.25 m/h/m which gradually reduced towards 3-4am next day. The evaluation of effects of Permanet combined with Soffell was calculated following the formula of Hederson Tilton and Kaplan – Meier. Soffell effects combined with Permanet 20 against *An. dirus* was 92 %.

3.3. Community compliance with the use of combined Permanet 2.0 and repellent cream Soffell Side effects caused by Permanet 2.0 and repellent cream Soffell

The residents involved in the study were interviewed to investigate into their compliance with the use of Permanet 2.0 six months after the trial started. Of 382 interviewed (April 2013) residents, 82.0% were found to use Permanet 2.0 when overnight sleeping in the milpa huts, and only 1.0% said they used them for siesta there. Over the first three months of using, 16.8% (63/374) nets were washed and 41.7% (156/374) were washed after six months.

A total of 450 residents were interviewed ten days after they received Soffell finding that 367 residents used this repellent cream (81.5%) of which 71.1% of them had night time use while only 9.3% had day time use in the milpa huts and 6.3% had home use at night time.

The light side effects were found with the use of Permanet 2.0 such as rash (3 %), eye stimulus (6 %).

Interviewing of 100 residents using repellent cream as night human bait catchers showed no side effects.

Chapter 4. DISCUSSION

4.1. Malaria situation in Binh Thuan (1991-2010)

4.1.1. Indexes for morbidity and mortality

Binh Thuan is 1 of 15 provinces of Central-Highland. Malaria prevalence in Binh Thuan was ranked tenth compared to that over 15 provinces. Since 1991, the NMCP has launched in the whole country in general as well as in Binh Thuan province in particular with the great material and technical investment, efforts and technical guidelines from NIMPE and Provincial Health Department. As a result, malaria in Binh Thuan was year after year decreased, but this reduction was unstable and a big threat of malaria resurgence is still present. Since 2008 onward, malaria tends to come back and go up. Remarkably, in 2009 the infection cases increased by 60% compared to those of 2008 (720 /450 cases in 2008). In 2010, malaria decreased to a small level (by 5.83%). The situation analysis showed that malaria infection mainly occurred among the forest goers and milpa hut overnighters. In addition, the unorganized population migration of the local residents and their low malaria perception was the challenges for the malaria control.

4.1.2. Anopheles in Binh Thuan province

Mosquito surveys on malaria vector were conducted. *An. dirus* was found to be present mainly in the zone V (hyper endemic zone) where 2,093 mosquitoes were caught. *An. minimus* was found predominantly present in the zone III, where 2,300 mosquitoes were collected. (or 28,84% of the total caught mosquitoes) (Table 3.1). The density of indoor human biting *An. dirus* was 3.35 times higher compared to that by outdoor human biting (Table 3.2). This figure suggested that *An. dirus* had a habit of coming into the house for blood sucking at night. According to the studies of Nguyen Xuan Quang (2012) the density of *An. dirus* biting near the forest at the National Park Chur Mom Ray in Kom Tum province was 5 times higher (0.05 m/h/m) compared to that far from the forest (0.01 m/h/m). A similar result was also found in Kon Ka Kinh, Gia Lai province where the density of *An. dirus* human

biting near the forest (0.13 m/h/m) was 4 times higher compared to that far from the forest (0.03 m/h/m).

4.1.3. Malaria infection among the forest goers and overnighters

Almost all the communes in the hyper endemic areas are inhabited by the ethnic minority communities who have a common habit of milpa practice and overnight. Malaria infection, therefore occur predominantly among these residents. Table 3.3 shows that the forest goers and overnighters were 5.03 times higher vulnerable to malaria infection compared to the ones who do not (P<0,001). These findings suggest that the risk factors for malaria infection can be significantly changed if appropriate solutions of vector control for them were applied. The findings of this study showed a much higher risk of malaria infection (by 1.6 times) for the forest goers (by the study of Ta Thi Tinh in two communes of Khanh Vinh and Khanh Trung, 2003) compared to that for the people who do not. A rate of 45.6% of the malaria infection cases were found among the forest goers and overnighters.

4.2. Evaluation of effects of Soffell combined with Permanet 2.0 and the residual effects of chemical on Permanet 2.0

4.2.1. Composition of *Anopheles* caught in the trial sites

An. dirus, the primary malaria vectors was predominant among the three caught species of mosquitoes with the highest density (39.29 m/h/m). An. minimus was not found during the survey (Table 3.3) suggested that the landscape of the collection sites was conducive habitat for An. dirus development only and was a high risk factor for malaria transmission if appropriate malaria control solutions were not conducted.

4.2.2. Susceptibility of laboratory-rearing *An. dirus* to deltamethrin 0.05%

A 100% mortality 24-hour after trial of *An. dirus* showed that this vector species was still fully susceptible to the used insecticide (Table 3.5). The study of Ho Đinh Trung (2010) determined that in Vietnam and another countries with *An. dirus presence* there have been no reports about resistance of this species to the insecticides used in the malaria control so far.

4.2.3. Night biting activities of An. dirus

Protective effect of the repellent cream used in the trial against mosquitoes especially when combined with Permanet 2.0 was evaluated when compared to the control. Mosquitoes were hampered from coming to the house. Permanet 2.0 was found not to keep away all mosquitoes from flying in for bloodsucking (Table3.4).

Between 6-12pm, the density of caught *An. dirus* in the trial site of Soffell and Permanet 2.0 was 14.4 times lower (27.5 m/h/m /1.91 m/h/m) compared to that in the control. It is suggested the time from 6 to 12pm is mosquito biting peak at night. Between 12pm - 5am number of caught *An.dirus* was declined in both trial and control and no more were caught afterward (Table 3.4). We found that as flying in, *An. dirus* as hampered by the repellent cream and Permanet 2.0 descend to rest on the wall and wait until they are not hampered by the chemical from biting. It was observed that both bloodfull and hungery *An. dirus* mosquitoes resting on the house walls at control night but almost all mosquitoes were hungry at night when Soffell and Parmanet 2.0 were used. The number of wall resting mosquitoes was almost not changed toward the dawn.

4.2.4. Effect of repellent cream combined with Permanet 2.0

Repellent effect of Permanet 2.0 was not optimal as it can not keep away all *An. dirus* from flying in. It was found to reduce the number of biting mosquitoes over night but not fully prevent them from human biting. The evidence was that mosquitoes still approach and bite early, from 7pm. The protective ability of Parmanet 2.0 against *An. dirus* is 80% (Table 3.4).

The repellent cream Soffell was found to have outdoor protective effect for 6-7 hours after the application with the protective ability of 89%. Before 12pm no *An. dirus* was found to attack people (no mosquitoes descended on the Soffell application sites) (Table 3.5). On the other hand, *An. dirus* was observed to still sting the human baits at the non-applied parts of body (biting through shorts, shirts...) but at a low rate. It is, therefore suggested that a daily double application of Soffell is needed for the forest goers and milpa overnighters to increase the protective effect. Application must be on both skin and clothes. The protective effect can be much increased if Soffell is combined with Permanet 2.0. (Protective effect against *An. dirus* was 92%). The protective time can also be prolonged lasting over night (Table 3.7). The combination of Sofell and Permanet 2.0 as a protective tool for the frequent forest goers and overnighters can be an appropriate solution for malaria vector control.

4.3. Community compliance with the use of the repellent cream and Permanet 2.0.

All the 382 people involved in the study were home interviewed to evaluate their compliance with the use of Soffell and Permanet 2.0. A total of 82.0 % of the Permanet users were found to use the nets properly (sleeping inside the net when overnight in the milpa huts). Only 1.0% used the net for daily hut sleeping. The finding showed that Permanet 2.0 received a good compliance from the community. This result accorded with the study findings by Ho Đinh Trung (2008) determining that the chemical coated mosquito nets Permanet 2.0 was widely accepted by the surveyed communities. Nearly 90% of the study net users were found to routinely use the nets (day and night sleeping in the milpa huts) and about 72% properly used the net for malaria prevention. (night sleeping inside)

In term of Sofell use, 81.5 % of interviewees affirmed that they used the cream during 10 days after they received, of which 71.1% properly used applying on the skin when they were in the forest milpa at night; 6.3% had day use when in the forest, and 9.3% used in their Permanet house in the village. The cream users said if the cream was applied on the skin, they saw no mosquitoes bite but fly near and around the applied sites. On the other hand, the interviewees said they used the repellent cream as they saw lots of mosquitoes in their working places daytime (especially when cutting wood, bamboo for house construction, growing manioc, picking bamboo shoots...) Moreover, the local residents also bought Soffell from the free market for forest job practice as very well realized the effect of this repellent cream. According to WHO (2003), although impregnated with deltamethrin at a dose 55 mg/m² Permanet 2.0 does not cause side effects higher as compared to that of deltamethrin treated nets at the routine dose of 25 mg/m². The interview showed inconsiderable side effects with the use of Permanet 2.0 such as rash (3.0%) and eye stimulus (6.0%). These side effects were light and disappeared automatically without any medical treatment.

One hundred of the cream users were interviewed ten days after the application. None of them complained about any of the above mentioned side effects when using the Soffell. In addition, they satisfied with aroma of orange, lemon of the product. The study findings of Nguyen Tuyen Quang et al (2005) determined that the repellent cream was highly appreciated by the forest goers for its higher realistic convenience and safety than the insecticide treated nets.

CONCLUSION

1. Malaria situation in Binh Thuan (1991-2010)

- After 20-year prevention with malaria control program, the malaria prevalence had reduced from 8,000/1,000 pop. (1991) to 0.58/1,000 pop. (2010). the mean annual mortality from 1.44/1,000pop. (from 1991 to 2010). The highest malaria mortality was recorded in 1991 with 106 deaths compared to one in 2010.

-The primary malaria *An.dirus* vectors were present in both zones IV and V. *An.minimus* was found in all three zone III, IV and IV but with reducing number from zone III (2,300) to zone 4 (455) and zone V (375). People with the habit of forest farming practice and milpa sleeping are at risk of malaria infection 5.03 times higher than the other ones.

2. Effectiveness of Soffell combined with Permanet 2.0 and residual effects of chemical treated Permanet.

- In the study sites, *An. dirus* was predominant with the density of 39m/h/m. Field unwashed chemical treated net Permanet 2.0 seven month after treatment was found to be still effective.

- Permanet 2.0 can reduce the number human biting *An.dirus* (the repellent effect was 80%). Soffell (13% DEET) can prevent 89% of *An.dirus* from biting for 6-7 hours. A combination of Permanet 2.0 and Soffell was found to increase the repellent effect to 92%.

3. Community compliance with the use of the repellent cream and Permanet 2.0.

- The residents involved in the study showed a good compliance with the cream and nets. Interviewing revealed a rate of 87.8% proper use of Permanet 2.0 in spite of a low rate of side effects of rash (3%) and eye stimulus (6%). A similar rate (81.5%) of Soffell use with no side effects was also recorded.

RECOMMENDATION

The study must further be conducted in a wider scale to more precisely evaluate the vector control effects of the combined repellent cream Soffell and Permanet 2.0 to protect the people in the hyperendemic areas.

Repellent cream containing DEET must be produced at a low price, more convenient use for the local consumption. The forest goers and milpa overnighters must be helped with a State subsidice for a wide and routine use of repellent cream for malaria vector control.