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New Mosquito Control Techniques as Countermeasures Against Insecticide Resistance

Hitoshi Kawada

*Institute of Tropical Medicine, Nagasaki University
Japan*

1. Introduction

Chemical substances, whether naturally occurring or artificially synthesized, continue to play an extremely beneficial role in human life. Life would be unsustainable without the benefits by products of chemical reactions, such as oxygen, water, and various types of nutrient substances. Drugs, foods, fibers, pesticides, etc. have been artificially designed and produced for protecting and improving the quality of human life. Nonetheless, chemical substances have been implicated in the deterioration of human environments and ecosystems. One of the main reasons for this unfair accusation might be inadequate regulations and information due to lack of scientific knowledge based on well founded ecotoxicological and pharmacological researches. Lack of information regarding chemicals has been the major factor that has led to undue credence being given to naturally occurring substances, and social movements, which are propagated by a handful of “fanatics,” for resisting artificial chemicals. Biorational and logical approaches by both users and suppliers of chemical substances are therefore required so that humans may continue to benefit from the correct use of chemicals.

Developmental research on pesticides of natural origin is believed to be one of the biorational approaches since it may reduce the adverse environmental impact of chemicals to the level of naturally occurring substances. One of the most successful events in the development of pesticide chemicals was the discovery of pyrethrum and the successful synthesis of pyrethroids. For example, one of the most classical synthesized pyrethroids allethrin (Schechter et al., 1948) continues to be used for preventing mosquito bites without any toxicological and operational problems. The use of pyrethroids for preventing mosquito bites is believed to be biorational because this chemical is safe for mammals. The most popular and long-standing formulations using pyrethroids are mosquito coils, mosquito mats, and liquid vaporizers. Pyrethroids belonging to the knockdown agent group, such as allethrin, pyrethrin, and prallethrin, are used in these formulations. In particular, *d*-allethrin still continues to be used in these types of formulations. Recently, a group of newly developed pyrethroids with high vapor pressure has come to open new era for pyrethroids. Metofluthrin is one of the above promising pyrethroids having high insecticidal activity and high vapor pressure (Ujihara et al., 2004). Metofluthrin belongs to the group of knockdown agents but has a unique

characteristic that none of the conventional pyrethroids possess. The most important unique characteristic of metofluthrin is its high vapor pressure. The vapor pressure of metofluthrin is >2 times and >100 times that of *d*-allethrin and permethrin, respectively, and it vaporizes at room temperature without heating, while other conventional pyrethroids require heating for vaporization. Another unique characteristic is its high efficacy against mosquitoes which is 28–79 times more effective than *d*-allethrin (Argueta et al., 2004). These unique characteristics of metofluthrin may lead to the development of new mosquito controlling devices that do not require any external energy for vaporization and have low cost and longer effective duration.

Pyrethroids belonging to the knockdown agent group have been successfully used worldwide for a long period as a spatial repellent. Spatial repellency will not induce any pyrethroid resistance since it has low lethal activity on the affected insects and causes less selection pressure on insect populations. The discovery of the phenoxybenzyl alcohol moiety accelerated the development of photostable pyrethroids that could be used for outdoor use, including agricultural purposes. These “second generation” pyrethroids have been used worldwide as good vector control agents with various application techniques, such as residual spraying, ULV spraying, and long lasting insecticide-treated net (LLITN). However, photostable and highly effective pyrethroids might accelerate the development of pyrethroid resistance in mosquito populations. Photostable pyrethroids consist of 2 structurally different types of chemicals according to the presence of α -cyano moiety, type I (permethrin, etofenprox, etc.) and type II (deltamethrin, lambda-cyhalothrin, cypermethrin, etc). Olyset® Net is one of the most promising LLITN. Olyset® Net is slow-releasing formulation composed of plastic fibers impregnated with permethrin—one of the most popular and safe type I pyrethroids. Recently, Siegert et al. (2009) reported that the Olyset® Net reduced landing attempts of mosquitoes and elevated their flight frequency, resulting in little mortality, while mosquito landing attempts on the PermaNet®, containing type II pyrethroid, deltamethrin, under the same conditions were sustained longer and caused greater mortality than the Olyset® Net. This appears to be important for an effective control of the mosquito population. The highly lethal pyrethroids with less excito-repellency appear to be most effective for reducing vector mosquito population. Such highly lethal pyrethroids, however, might accelerate the development of resistance. The excito-repellency of slow-released permethrin, on the contrary, might reduce the human–vector contact and blood feeding success. In fact, there was no difference between Olyset® Net and PermaNet® in the field efficacy as measured by blood feeding rate (Dabire et al., 2006). The positive use of excito-repellency of slow-released pyrethroids, therefore, might lead bio-rational vector control with the maximum reduction of mosquito biting and minimum risk of resistance.

Juvenile hormone mimics (JHMs), which have also been developed from natural sources, are among the most studied and effective chemicals, and are categorized as insect growth regulators (IGRs). These chemicals have a unique mode of action that is insect-specific, stage-specific, slow acting, and not neurotoxic (Miyamoto et al., 1992). Methoprene (Henrick et al., 1973) and pyriproxyfen (Hirano et al., 1998) are the most successful JHMs. Almost 40 years have passed since Williams (1967) suggested that JHMs could be the 3rd generation insecticides that will not adversely affect the ecosystem due to their target-specificity, and against which pests theoretically have no potential of developing resistance. However, the above beliefs have been proved incorrect or have changed during the 3 decades since the first successful JHMs methoprene and hydroprene were

commercialized. Insect resistance to JHMs has become common among agricultural and non-agricultural pests (Zhang et al., 1998; Cornel et al., 2002; Ishaaya et al., 2005), and several reports have demonstrated that JHMs may adversely affect the ecosystem if they are overdosed (Miyamoto et al., 1993; Trayler and Davis, 1996). Therefore, utmost care and high level expertise are a requisite for the biorational use of JHMs, and application of the minimum dose in the most effective manner will result in maximum benefits both to humans and the ecosystem.

In this chapter, several attempts to develop new mosquito control techniques with using a pyrethroid belonging to knockdown agent groups (metofluthrin) and a slow-released type I pyrethroid (permethrin) as a spatial and exito-repellent agent are introduced. The new biorational use of JHM (pyriproxyfen) as a “mosquito population growth regulator” is also discussed.

2. Field evaluation of spatial repellency of metofluthrin-impregnated plastic strips against vector mosquitoes

Metofluthrin (SumiOne®), 2,3,5,6-tetrafluoro-4-methoxymethylbenzyl(E:Z ≈1:8)(1*R*, 3*R*)-2,2-dimethyl-3-(prop-1-enyl)cyclopropanecarboxylate, is a newly synthesized pyrethroid. The high knockdown and lethal activity of metofluthrin against mosquitoes has been demonstrated previously. The high vapour pressure of metofluthrin (1.87×10^{-3} Pa at 25°C), which is 2-fold and 100-fold greater than those of *d*-allethrin and permethrin, respectively, enables vaporization at normal temperature in the absence of heating, while the other conventional pyrethroids require heating for evaporation. The unique characteristics of metofluthrin may lead to the development of novel mosquito-controlling devices that require no external energy for vaporization and those that provide long-term efficacy at low maintenance costs.

In preliminary studies, using a simple prototype device with metofluthrin-impregnated multilayer paper strips, the chemical showed promising spatial repellency against mosquitoes in both laboratory and field conditions (Kawada et al., 2004a; 2004b). Under simulated outdoor conditions, mosquitoes (*Anopheles* sp. and *Culex* sp.) were repelled by airborne metofluthrin vapours (Fig. 1, 2; Kawada et al, 2004a). The field tests suggested that metofluthrin may be a good candidate for the prevention of mosquito bites (*Anopheles sundaicus* (Rodenwaldt), *Anopheles balabacensis* (Baisas), and *Culex quinquefasciatus* (Say)) in shelters without walls (beruga), those used by people in Lombok Island, Indonesia and which are associated with high risk of malaria transmission (Kawada et al., 2004b). In order to increase the effectiveness of metofluthrin, Kawada et al. (2005a) manufactured a cylindrical slow-release plastic formulation that was impregnated with 1000 mg metofluthrin in a 20 g strip. By using this formulation, the authors obtained prolonged duration of activity (>14 weeks at the rate of 4 strips per beruga) in the beruga under outdoor conditions in Lombok (Fig. 3,4).

Further, spatial repellency of this plastic formulation against *Aedes aegypti* (L.) was achieved in the residential houses of Do Son, Hai Phong city, Vietnam (Kawada et al., 2005b). The above study has confirmed the long-lasting spatial repellent efficacy of metofluthrin-impregnated plastic strips against *Ae. aegypti* under indoor conditions; however, the effective duration (6 weeks at 1 strip per room) appeared insufficient for practical use (Fig. 5).

Fig. 1. Prototype Multilayer paper strip device impregnated with 200 mg of metofluthrin and outdoor human-baited collection with a double net (Strips were hung in a space between the inside and outside nets) (Kawada et al., 2004a).

Fig. 2. Changes in total number of mosquitoes collected per hour at indoor human-baited collection (HBI) and outdoor human-baited collection (HBO) after the treatment with multilayer paper strip device impregnated with metofluthrin (Kawada et al., 2004a).

Fig. 3. Metofluthrin-impregnated plastic strip for the trial and the field test scene with the strips in a beruga where Lombok people spend every evening before going to bed (Kawada et al., 2005a).

Fig. 4. Changes in the total number of mosquitoes collected per h during the trial for metofluthrin-impregnated plastic strips. Bars indicate the standard deviations (Kawada et al., 2005a).

Fig. 5. Treatment scene with a metofluthrin-impregnated plastic strip in a room, Do Son, Vietnam (Left photo) and changes in the number of mosquitoes collected in metofluthrin-treated and untreated houses (Right graphs). Upper graph shows the general density index based on the total number of mosquitoes collected (*Cx. quinquefasciatus* and *Ae. aegypti*) and lower graph for *Ae. aegypti* index (Kawada et al., 2005b).

Long-term effectiveness of these devices may be achieved by using the following methods: (1) designing devices of different shapes, (2) adopting formulations with different optimal compositions and densities of the plastic polymer in order to reduce the release rate of the active ingredient, (3) increasing the concentration of the active ingredient, and (4) increasing the number of strips per room. Accordingly, as the next step in the development of the devices, a new latticework plastic strip that was designed to reduce the release rate of metofluthrin to approximately 50% of that obtained in the previous plastic formulation was manufactured. The new latticework strips (approximately 600 mg metofluthrin per 12.3 g strip) at 1 strip per 2.6–5.5 m² were effective for at least 8 weeks against *Ae. aegypti* in the residential houses in My Tho city, Tien Giang province, Vietnam (Fig. 6,7; Kawada et al., 2006a).

Fig. 6. Metofluthrin-impregnated polyethylene latticework strip (left) and treatment scene of the strip in a room (right), My Tho city, Vietnam (Kawada et al., 2006a).

Fig. 7. Changes in the mosquito density index (female per house per day) of *Aedes aegypti* collected in metofluthrin-treated and untreated houses, My Tho city, Vietnam. Bars indicate 95% confidence limits (Kawada et al., 2006a).

The above new prototypes of metofluthrin-impregnated latticework plastic strips were evaluated against malaria vector, *Anopheles gambiae* Giles complex, in the Kongo villages of Bagamoyo district in coastal Tanzania (Kawada et al., 2008). The study using 20 houses, half intervention, half control, were conducted for 124-day period. Pyrethrum spray sheets collection and CDC light traps were used to sample mosquito population indices. The mosquito density indices of the intervention houses were observed to be significantly lower than those of the control houses when pyrethrum spray sheet collection was used (Fig. 8 and Table 1; $F = 4.61$, 1 df, $P = 0.038$; 98.7% reduction of total mosquito collection compared with that for the controls). These low indices were observed despite the large opening area of Bagamoyo houses that were considered to have a considerable negative effect on the spatial repellency of metofluthrin.

Fig. 8. Treatment scene of metofluthrin-impregnated latticework plastic strips in a room, Bagamoyo, Tanzania. CDC light traps were put inside rooms for collection of mosquitoes (Kawada et al., 2008).

Days after Intervention	Mosquito Density Index (<i>Anopheles gambiae</i> s. l.) ¹⁾			
	Intervention - (95%CI)		Control - (95%CI)	
20	0.2	(0.4)	2.0	(1.0)
34	0.2	(0.4)	11.4	(5.6)
61	0.0	(-)	8.0	(4.2)
89	0.0	(-)	7.2	(4.4)
124	0.0	(-)	2.4	(2.3)

¹⁾ Mosquito Density Index = No. of female mosquito / house / day

Table 1. Changes in the mosquito density index by pyrethrum spray sheet catch collection in the metofluthrin-intervention and control houses in Bagamoyo, Tanzania.

Table 2 lists the environmental factors and the effective duration of the metofluthrin-impregnated plastic strips in the present study as well as those of the intervention houses measured in My Tho city, Tien Giang, Vietnam, where a similar metofluthrin trial was conducted in the same season in the year 2005 (Kawada et al. 2006a). Variables including the average temperature and humidity were calculated on an hourly basis from June 20 to August 3, 2006 for Bagamoyo and from June 20 to September 4, 2005 for My Tho. The room temperature was lower and the humidity was higher in Bagamoyo houses compared to the corresponding conditions in the My Tho houses. Although the floor area and the volume were larger in the houses in My Tho compared to those in Bagamoyo, the corrected opening area per total average volume of the houses in Bagamoyo was almost twice that of houses in My Tho, thereby indicating that the Bagamoyo houses are more “open” than the My Tho houses (Kawada et al., 2008).

Environmental Factors	Trial Sites (Trial Year) - Target Mosquito			
	My Tho (2005) ²⁾		Bagamoyo (2006) ²⁾	
	<i>Ae. aegypti</i>		<i>An. gambiae</i> s.l.	
Average Temperature (°C) ¹⁾	29.1	(0.8)	24.8	(0.7)
Average Humidity (% RH) ¹⁾	70.1	(5.1)	75.3	(3.9)
Total Floor Area (m ²) / House	32.1	(10.5)	22.0	(14.1)
Total Volume (m ³) / House	129.3	(59.4)	58.7	(45.7)
Total Opening Area (m ²) / House	6.6	(5.0)	5.7	(4.3)
Corrected Opening Area / Volume	0.051		0.098	
No. of metofluthrin Strips / m ²	0.31		0.52	
Amount of metofluthrin (mg) / m ²	191		320	
Effective Duration (Weeks)	8		> 18	

1) June 20 - August 3, 2006 in Bagamoyo; June 20 - September 4, 2005 in My Tho
2) Figures in parenthesis are standard deviations

Table 2. Environmental factors of the intervention houses and effective duration of metofluthrin-impregnated plastic strips.

Metofluthrin-impregnated strips significantly reduced the density index of mosquitoes in the intervention houses in several different environmental conditions in Indonesia, Vietnam, and Tanzania. Kawada et al. (2004a, 2004b, 2005a, 2005b, 2006, 2008) reported that mosquitoes were repelled by airborne metofluthrin vapors due to the two main modes of pyrethroid action, i.e., knockdown activity and biting inhibition or disruption of orientation toward the host. Of these, the latter may be categorized as a sublethal and “delayed” effect that results from neural excitement, which appears to occur at an earlier stage of pyrethroid toxicity (MacIver 1964, Winney 1975, Birley et al. 1987). Kawada et al. (2006a) reported that both the increase in the average room temperature and the decrease in the opening area of the rooms treated with metofluthrin-impregnated strips exerted an increased spatial repellent effect. The increase in temperature might increase the evaporation rate, and the decrease in the opening area might retain the active ingredient inside the rooms thereby resulting in an increased concentration of metofluthrin in the air. The corrected opening area/volume in the houses in Bagamoyo was nearly twice as much as that of the houses in My Tho city (Table 2). A large opening area would potentially facilitate the entry of

endophilic and nocturnal mosquitoes, and the presence of large and numerous open eaves in the typical rural African houses are considered to be one of the most important entrances for invasion by *An. gambiae* during the night. Snow (1987) reported that the invasion by *An. gambiae*, *Anopheles melas* Theobald, and *Mansonia* sp. into the experimental huts was slightly affected by increasing the wall height. Lindsay et al. (2003) reported that the entry of *An. gambiae* into house was reduced by 37% subsequent to the closure of the eaves. Similarly, a significant contribution of open eaves to the increase in mosquito invasion was reported by Pålsson et al. (2004). We, therefore, argue that the large opening area of the houses in Bagamoyo might have negatively affected the spatial repellent efficacy of metofluthrin. The effective duration of repellency (>18 wk) is believed to be sufficient for the practical application of these devices considering the convenient replacement of the formulation. Further improvements related to the manufacturing of plastic strips, such as optimization of the composition and the density of the plastic polymer in order to reduce the loss of the active ingredient, may enable the development of an optimum formulation that would result in a longer effective duration and lower treatment cost.

3. Preventive effect of release controlled plastic net of permethrin (Olyset® Net) against vector mosquitoes

The use of insecticide-treated bed nets (ITNs) as a simple and inexpensive self-protection measure against malaria has been shown to reduce morbidity of children (< 5 years old) by 50% and global child mortality by 20%–30% (Binka et al., 1996; Lengeler et al., 1996; Nevil et al., 1996). Impregnation and the re-impregnation of ITNs, however, needed technical skills, materials, and human costs which may not always be available (Lines, 1996). The mosquito nets pre-treated with insecticide and with longer lasting effect (LLITNs) were one of the break-through measures to this problem (Guillet et al., 2001). Olyset® Net, made of polyethylene netting material (mesh 20 holes/cm²) with permethrin (2%) incorporated into the polymer before monofilament yarn extrusion, and the PermaNet®, made of polyester netting material (mesh 25 holes/cm²) with deltamethrin (55 mg ai/m²) incorporated in a resin coating of the fibers, are two successful products among the LLITNs which WHO had recommended.

Several attempts to apply the LLITNs to the other vectors, such as *Ae. aegypti* (Curtis et al., 1996; Igarashi, 1997; Kroeger et al., 2006; Jeyalakshmi et al., 2006) and *Phlebotomus* (Dinesh et al., 2008; Faiman et al., 2009; Emani et al., 2009; Kasili et al., 2010; Das et al., 2010), have been performed and trials to apply the LLITNs as the other controlling tools, such as curtains (Curtis et al., 1996; Igarashi, 1997; Kroeger et al., 2006; Vanlerberghe et al., 2011a; 2011b) and jar covers (Kroeger et al., 2006; Vanlerberghe et al., 2011a; 2011b), have been reported using Olyset® Net and/or PermaNet®. In this section, a new attempt for controlling *Ae. aegypti* using Olyset® Net as water jar covers is introduced and a new self-protection technique for preventing malaria vectors using Olyset® Net materials are proposed.

3.1 Effect of release controlled plastic net of permethrin (Olyset® Net) as water container cover on field populations of *Aedes aegypti* in Southern Vietnam

Dengue fever first appeared in Vietnam at Hanoi and Haiphong in 1959 and since then has become endemic throughout the whole country (Nam et al., 2000). Jars, tanks, and drums provide suitable breeding sites for *Ae. aegypti* in Vietnam (Phong and Nam, 1999; Nam et al., 2000; Tsuzuki et al., 2009) and these breeding sites are important targets for controlling

immature stages of *Ae. aegypti*. Insecticide treatment to such breeding sites with organophosphates such as temephos or insect growth regulator (IGRs) such as pyriproxyfen, both of which are recommended to treat in drinking water by WHO, seems to be best and most convenient measures. However, treatment of any insecticide to such breeding sites is legally prohibited in Vietnam, making the larval vector control more difficult. Preventing invasion of gravid female mosquitoes into the above breeding sites is also important as well as removing these habitats.

Tan Chanh, a commune of Long An province, located 30 km south from Ho Chi Minh City, Vietnam was selected as trial site. Release controlled plastic net of 2% permethrin (Olyset® Net) and EcoBio-Block® S, a novel release controlled system for the insect growth regulator, pyriproxyfen, composed of a porous volcanic rock and cement and which incorporated the aerobic bacteria groups of *Bacillus subtilis natto* as a water purifying agent (Kawada et al., 2006b) were used for the study. Residential colony of Tan Chanh was assorted into 20 adjacent clusters which contain each 50 houses. Among them, ten clusters were selected as trial site for intervention by Olyset® Net and EcoBio-Block® S and other 10 clusters as control. For trial site, all water jars were covered with lids equipped with Olyset® Net. Olyset® Net was cut in 30×150 cm and tied along the circumference of a lid (Fig. 9). Additionally, all the other breeding containers such as flower vase inside and peripheral of houses were treated with EcoBio-Block® S, crushed into small pieces (less than 1 cubic cm) and were put in the container at the rate of approximately 1g per 1 liter of water.

Fig. 9. Olyset® Net was cut in small pieces (Left Photo) and tied along the circumference of jar covers (Right Photo).

House index (Percentage of houses or premises positive for *Aedes* larvae) in Olyset® Net treated area was higher than that of control before intervention and the index sharply decreased during one month after intervention. The house index gradually increased from October, 2008 to February, 2009, while it kept lower value than that in untreated area. Container index (percentage of water holding containers positive for *Aedes* larvae) also decreased in the same way as in the house index in the Olyset® Net treated area and kept lower level at least for 5 months after intervention (Fig. 10).

Fig. 10. House index in Olyset® Net treated and untreated areas in Tan Chanh, Vietnam from August 2008 to February 2009. Intervention was carried out in September, 2008 (Tsunoda et al., 2011).

3.2 Small scale experiment of a house screening technique using Olyset® Net material in malaria endemic area in Kenya

Use of ITNs or LLITNs is effective against malaria vectors when the vector mosquitoes are endo-phagous and their feeding time corresponds to the time when people are sleeping inside bed net. Behavioral resistance, such as behavioral change in vector mosquitoes from endo-phagous to exo-phagous and/or shifting of biting time from midnight to dawn or dusk, may reduce the effectiveness of bed nets as well as physiological resistance to insecticides. Most important limitation for the effective use of bed net is that it is only effective when people are sleeping inside. Recently, Iwashita et al. (2010) reported that bed net use by children between five and 15 years of age in villages along the Lake Victoria, western Kenya was lower than that among the other age classes. Bed net use was strongly affected by sleeping arrangement and availability of suitable locations for hanging bed nets. The easiness of hanging a bed net is particularly important for children who often are sleeping in the other place such as living room where the net hanging is difficult. Daily hanging of bed nets in the above place might be troublesome for residents. Hence, the uses of bed net are sometimes limited to the persons sleeping in a bedroom (parents and babies) and the rest of family members (ex. children > 5 years old) are found to sleep in living room with no bed net, resulting in the high Pf positive case in these generations. Therefore, new devices which can substitute bed nets or new self-protection measures which are convenient and sustainable for residents is required for the more effective prevention of malaria vectors.

On the other hand, eaves, the gaps between the top of the wall and the roof, are one of the most common house structures in Africa and are thought to be the most important entrance for malaria vectors (Njie et al., 2009). Changes in house design may reduce human exposure to malaria vectors. Screening or closing eaves was reported to be effective (Lindsay et al., 2003). Restructuring of houses or physical closing the eaves, however, will require much cost and cause deterioration of living environment by blocking ventilation. Net screening of ceilings and eaves is likely to be well accepted and of greatest benefit to moderate disease transmission (Lindsey et al., 2003; Kirby et al., 2009). Use of nets with coarse mesh size will be most acceptable in considering the good ventilation.

Small scale trial using Olyset® Net materials were performed in Mbita, Nyanza province, western Kenya in 2010 and 2011. *Anopheles gambiae* s.s., *Anopheles arabiensis*, *Anopheles funestus* s.s. are main malaria vectors in this area. *Anopheles rivulorum*, which is one of the sibling species in *An. funestus* complex, is also minor vector in this area. The above three main vectors were recently reported to have developed multimodal pyrethroid resistance (Kawada et al., 2011a).

The Olyset® Net materials impregnated with 2% permethrin was used in the study. The net materials were cut and sewed into a 7 x 5 m sheet and ring bands were equipped on the diagonal position of the nets to ease the fixation of nets under ceiling (Fig. 11, 12). The study was performed in three houses (two houses for intervention, the other one house for control) in Nyandago village in Gembe East, Mbita Division in the Suba district of the Nyanza province, western Kenya. The shielding effect of the ceiling nets was evaluated by the number of indoor resting mosquitoes collected using the battery-powered aspirator.

Fig. 11. Ceiling net using Olyset® Net materials (Left) and outline sketch of the ceiling net installed in a house (Right) (Kawada et al., 2011b).

Fig. 12. Intervention scene of permethrin-impregnated ceiling net (Kawada et al., 2011b).

Olyset® Net experimentally used for covering the ceiling and closing eaves, in the present study, resulted in outstanding reduction of the number of resting mosquitoes inside houses. The number of mosquitoes drastically decreased 1 day after the intervention of ceiling nets and lower densities were kept for 9 months until the removal of the nets, while the mosquito density in the control house kept high level during the above period (Fig. 13). Lindsay et al. (2003) reported that little difference in the protecting effect of insecticide-treated and untreated screen nets. The present study, however, emphasizes the necessity of the presence of insecticide impregnated nets as a chemical barrier, which may partly be due to the coarse mesh size of Olyset® Net materials to ease the ventilation (Fig. 14). Screening of ceiling and closing eaves with insecticide-treated nets with coarse mesh size such as Olyset® Net will be acceptable way to residents and effective interfering measure for preventing the mosquito entering in houses with small cost and minimum environmental deterioration.

Fig. 13. Changes in the number of mosquitoes collected in the ceiling net intervention houses (NYAND 8, 11) and control house (NYAND 6). Red arrow indicates the day of intervention (Kawada et al., 2011b).

Fig. 14. Average number of mosquitos collected before intervention of permethrin-impregnated ceiling nets, after intervention, after removal of the permethrin-impregnated

ceiling nets, after intervention of permethrin-untreated ceiling nets, and after re-intervention with new permethrin-impregnated ceiling nets. Bars indicate 95% confidential limits. The same letters indicate no significant difference when square root of the ratio of the number of mosquitoes collected in the intervention house versus that collected in the control house was converted into Arcsin and the multiple comparison of the ratio was performed by Tukey's HSD test ($P = 0.05$) (Kawada et al., 2011b).

4. Effect of Juvenile Hormone Analogue (JHM), pyriproxyfen, as a mosquito population growth regulator

From a medical point of view, mosquitoes are thought to be the most important order of insects. Larviciding seems to be the most suitable measure for controlling mosquitoes, since larval habitats are often limited in small and/or local area. Most of the effect of JHMs is on the last instar larvae which becomes deformed or dies at pupal stage as a result of the treatment (Hirano et al., 1998). Pyriproxyfen was observed to cause vacuolation and inhibition of development of imaginal buds of *Ae. aegypti* larvae, and histolysis, such as disrupted mitochondria, abundant vacuoles and poorly-structured cytoplasmic organelles were also observed (Syafuruddin et al., 1990). Adult *Anopheles balabacensis*, which survived 48 hr of immersion in 0.005 ppb (one eighth of LC_{50}) of pyriproxyfen during their last larval instar, was found to show considerable reduction in sperm and egg production and also in blood feeding and mating activity (Iwanaga and Kanda, 1988).

JHMs act as “sterilant” when it was treated to adult insects. Methoprene was reported to affect ovarian development and adult longevity in *Ae. aegypti* (Judson and Lumen, 1976; Klowden and Chambers, 1989). The number of eggs/female and hatchability decrease with the application of pyriproxyfen to female *Ae. aegypti* (Kawada et al., 1993; Itoh et al., 1994). Additionally, when a blood-fed female of *Ae. aegypti* had been in contact with pyriproxyfen, a quantity of pyriproxyfen was transferred from her body to the water adjacent where she laid her eggs (Kawada et al., 1993; Itoh et al., 1994). Significant inhibition of emergence was observed in the field trial in Bangkok, where “resting traps” inside of which was treated with oil formulation of pyriproxyfen were placed in a room (Itoh, 1994). Recently, several semi-field trials on the control using the horizontal transfer of pyriproxyfen by *Ae. aegypti* females to their breeding sites were reported to be successful (Shihuinchu et al., 2005; Devine et al., 2009).

Ohba et al. (2011) reported the usefulness of pyriproxyfen-treated net for the control of *Aedes albopictus*. Two microcosm trials using the polyethylene net treated with 0.1% and 1% of pyriproxyfen were conducted (Fig. 15). Laboratory colony of *Ae. albopictus* was released into microcosms as shown in Fig. 15 and were allowed to feed on a mouse in a pyriproxyfen treated or untreated net cage ($50 \times 50 \times 50$ cm) on which small holes (ϕ 5 cm) were placed in order to the female mosquitoes could contact the nets when they flew into the cage for blood feeding. After experimental colony of *Aedes albopictus* was released into the microcosm containing pyriproxyfen treated nets, the number of eggs oviposited and the number of pupae were significantly lower compared with untreated controls (Fig. 16). Egg hatchability of the eggs oviposited by pyriproxyfen-exposed females was significantly suppressed and horizontal transfer of pyriproxyfen by females was also observed by bioassay using the water in which the above females oviposited.

Fig. 15. Diagram and photographs of the semi-field experiment. a, overall view of the greenhouse where the tents (simulated microcosm) were located; b, diagram of the microcosm. Larval container, eight ovitraps were put counting the number of eggs and pupae oviposited; Monitoring trap, two ovitraps were put for the observation of 1) egg hatchability, 2) larval bioassay for the emergence inhibition of pyriproxyfen transferred by adult females. Three microcosms were installed with pyriproxyfen-treated nets and the other three were for untreated nets (Ohba et al., 2011).

Fig. 16. Effect of pyriproxyfen on the number of females, number of eggs oviposited, and number of pupae in the microcosms in which *Ae. albopictus* females were allowed to contact pyriproxyfen-treated nets (* difference is significant at $P < 0.05$, one-way ANOVA) (Ohba et al., 2011).

5. Conclusion

Insecticides still provide the most promising countermeasures for controlling malaria, dengue hemorrhagic fever (DHF), and other arthropod-borne diseases. On an average, at the global level, > 500 tones of DDT, ca. 40 tones of organophosphates, ca. 20 tones of carbamates, and ca. 40 tones of pyrethroids are used as active ingredients annually for indoor residual spraying against malaria vectors (Zaim and Jambulingam, 2007). The average total amount of pyrethroids used annually as active ingredients between 2003 and 2005 at the global level was 161 tones, which is 16% of the total insecticide consumption and 36% of the total insecticide consumption if the amount of DDT, which is exclusively used in African countries, is excluded. Among pyrethroids that are used for vector control, 98.7% comprise photo-stable pyrethroids such as α -cypermethrin, bifenthrin, cyfluthrin, cypermethrin, deltamethrin, etofenprox, λ -cyhalothrin, and permethrin (Zaim and Jambulingam, 2007). Pyrethroid resistance will be a major problem for the vector control program, since at present, there are no suitable chemical substitutes for pyrethroids.

Vu et al. (2004) conducted WHO standard bioassay using adult *Ae. aegypti* collected in 22 places in 11 provinces and cities in four different regions of Vietnam and found that the mosquitoes were susceptible to pyrethroids in many places in the North and Centre regions but they were resistant in the South and Central Highlands in Vietnam. Kawada et al. (2009a) reported similar tendency in pyrethroid susceptibility in *Ae. aegypti* in Vietnam, and found that new *kdr* mutations on domain III were widely distributing in southern Vietnam (Kawada et al., 2009b). The above authors concluded this discrepancy in pyrethroid susceptibility in different regions to be due to the longer and extended use of pyrethroids in malaria and dengue fever control programs and in agriculture in the Southern and Central Highlands. Actually, a lot of pyrethroids have been treated as residual treatment inside houses and pyrethroid-impregnated bed nets for malaria control as a part of the National Malaria Control Program. The pyrethroid use for malaria control seems to be important factor in developing pyrethroid resistance in *Ae. aegypti* in highland region of Vietnam, since the DF/DHF cases are not serious and forest malaria continues to be endemic in this region as compared to the other regions and consequently the amount of pyrethroid treatment for dengue vector control in highland region is lower than the other regions. The pyrethroid treatment for malaria vector control appears to have been intensively conducted in the interior and along the periphery of human habitation areas, where incidentally, the breeding and resting sites of *Ae. aegypti* are located and this might account for the strong selection pressure toward *Ae. aegypti* (Kawada et al., 2009). In Vietnam, 24 tonnes of DDT was used for residual treatment against malaria vectors in 1993 and 1994. However, since the abandoning of DDT sprays in 1995, only pyrethroids (residual spraying of λ -cyhalothrin and α -cypermethrin and occasionally deltamethrin, and permethrin-impregnated bed nets) have been extensively used in large amounts, unlike in the other Asian countries (Nam et al., 2005; Zaim and Jambulingam, 2007). Although details regarding the amount of insecticides used for dengue control in Vietnam have not been published, 21,000 liters of photo-stable pyrethroid formulations such as λ -cyhalothrin, deltamethrin, and permethrin was reported to be used for dengue control in 20 southern provinces in 2007 (Epidemiological and virological vector surveillances for dengue control program in southern Vietnam, 2008, Pasteur Institute, Ho Chi Minh City). The extensive use of photo-stable pyrethroids, therefore, seems to have been very common in southern Vietnam.

On the other hand, in Kenya, the high allelic frequency of *kdr* mutations (L1014S) in both *An. gambiae* s.s. and *An. arabiensis* were reported to convergently distribute in western part including highland region as well as northern and southern coastal region of Lake Victoria (Kawada et al., 2011c). These regions are one of the focal points identified as a high vector transmission region in Kenya, and more than 50% of the population is exposed to $\geq 40\%$ PfPR₂₋₁₀ (*Plasmodium falciparum* parasite rate corrected to a standard age-range of 2 to less than 10 years old) (Noor et al., 2009) and accordingly high coverage of LLINs or ITNs has been accomplished. In fact, the percentages of households that have at least one LLIN in Nyanza and Western provinces ($>70\%$) were fairly higher than the other provinces ($<70\%$) (Kenya HDS Final Report, 2009). Moreover, high population density of *An. gambiae* s.s. and *An. arabiensis* in the above regions (Okara et al., 2010), as well as high human population density, might have increased the contact frequency of vector mosquitoes to LLITN/ITN, resulting in the high selection pressure with pyrethroids. Mathias et al. (2011) reported that the East African *kdr* allele (L1014S) coincidentally increased in frequency during the past decade in *An. gambiae* s.s. in western Kenya, most of which are homozygous *kdr* allele, as household ownership of insecticide-treated bed nets increased regionally.

Several factors are believed to play major roles in inducing pyrethroid resistance in mosquitoes. The most serious factor is the uncontrolled use of photo-stable pyrethroids. Photo-stable pyrethroids persist on substrates such as wall and floor surfaces for long periods and hence continue to kill insects that make contact with these substrates. This induces a strong selection pressure on the insect population resulting in a population of resistant offspring. In the past, the use of pyrethroids in aqueous environments was impossible since pyrethroids are highly toxic to aqueous organisms. However, recently, a new pyrethroid with low fish toxicity has been commercially produced and is widely used in aqueous environments such as paddy fields. This might cause considerable selection pressure on the mosquito larvae distributed in such environments. The most serious problem is that resistance to a single pyrethroid causes cross-resistance to all other pyrethroids, including knockdown agents. In fact, many reports concerning pyrethroid resistance have emerged after the successful application of pyrethroids as vector control agents. Therefore, the uncontrolled use of such pyrethroids might lead to the end of the golden age of pyrethroids.

Humans have invented insecticides to ensure comfort and to achieve ideal conditions. Good insecticides, therefore, should be as effective as possible so that the above mentioned goals are realized. However, the development and manufacturing costs of insecticides should be as low as possible (Kawada, 2009c). It is, therefore, our duty to use insecticides in the most effective and prudent manner possible in order to maintain their effectiveness and sustain their use. In order to effectively manage pyrethroid resistance, the establishment of a feasible insecticide management system and a regular monitoring system of pyrethroid susceptibility will be essential. Moreover, it is expected that new self-protection measures using exito-repellent type I pyrethroids are of great interest as substitutional or supplemental techniques for bio-rational vector control measures in the future, as well as reconsideration of the use of photo-unstable knockdown agents as spatial repellents, which effectively interfere with disease transmission without causing any selection pressure to insect populations.

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This book contains 30 Chapters divided into 5 Sections. Section A covers integrated pest management, alternative insect control strategies, ecological impact of insecticides as well as pesticides and drugs of forensic interest. Section B is dedicated to chemical control and health risks, applications for insecticides, metabolism of pesticides by human cytochrome p450, etc. Section C provides biochemical analyses of action of chlorfluazuron, pest control effects on seed yield, chemical ecology, quality control, development of ideal insecticide, insecticide resistance, etc. Section D reviews current analytical methods, electroanalysis of insecticides, insecticide activity and secondary metabolites. Section E provides data contributing to better understanding of biological control through *Bacillus sphaericus* and *B. thuringiensis*, entomopathogenic nematodes insecticides, vector-borne disease, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

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InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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