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Original Article

Effect of Metofluthrin-Impregnated Spatial Repellent Devices Combined with New Long-Lasting Insecticidal Nets (Olyset® Plus) on Pyrethroid-Resistant Malaria Vectors and Malaria Prevalence: Field Trial in South-Eastern Malawi

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SUMMARY: Long-lasting insecticidal nets (LLINs) experience some operational problems that reduce their effectiveness, such as limited spaces for hanging, biting of mosquitoes outdoors, a shift of key biting time from midnight to dawn or dusk, and development of pyrethroid resistance in mosquitoes. The concept of spatial repellency may be a countermeasure to overcome the above issues. The effect of the combined use of metofluthrin-impregnated spatial repellent devices (MSRDs) and LLINs (Olyset® Plus) on malaria prevalence and vector mosquitoes were examined in malaria endemic villages in south-eastern Malawi. The intervention reduced the infection rate in children as well as the number of pyrethroid-resistant vector mosquitoes. To achieve effective malaria control, continued intervention using MSRDs with 2 strips per 10 m^2 at 3-month intervals to reduce the density of malaria mosquitoes is recommended.

INTRODUCTION

Several studies have shown the effectiveness of insecticide-treated nets (ITNs) for malaria control (1, 2). However, ITNs and Long-lasting insecticidal nets (LLINs) have several operational limitations (3). ITNs or LLINs effectively protect people from mosquito blood sucking only while they sleep in their bed nets; however, outside of the nets, they are not protected (3-6). Sleeping arrangements and availability of locations for hanging the nets affect bed net use (4). The use of bed nets is sometimes limited to parents and babies, and remaining family members have to sleep without bed nets (6). A major obstacle to successful malaria vector control program is pyrethroid resistance of mosquitoes. Pyrethroid resistance is high in *Anopheles gambiae* s.l. in West and Central Africa (7). Pyrethroid resistance in *An. funestus* has also been reported in South Africa, Mozambique (8), and Malawi (9). New, convenient, and sustainable self-protection measures substituting or complementing LLINs are required for more effective malaria control (3).

Metofluthrin (SumiOne®) belongs to a new pyrethroid group that has unique characteristics (10). Metofluthrin has a high vapor pressure (1.96×10^{-3} Pa at 25°C) and can be vaporized without heating. Another unique characteristic is its high insecticidal and spatial repellent activity against mosquitoes. The spatial repellency of metofluthrin-impregnated spatial repellent devices (MSRDs) has been evaluated against *An. gambiae* in Tanzania (11) and western Kenya (Kawada *et al*. unpublished) and the possibility of its effective use against pyrethroid-resistant mosquitoes was demonstrated. Airborne metofluthrin vapor repelled mosquitoes through two main modes of action, namely, knockdown and excito-repellency (11-15).

In the present study, we demonstrated the effectiveness of MSRDs as a possible, novel, self-protection measure. The effects of the devices in combination with LLINs on the density of pyrethroid-resistant mosquitoes and malaria prevalence in children are discussed based on the results of field trials in south-eastern Malawi.

MATERIALS AND METHODS

Study site: The study was performed in three adjacent villages, Chiliko, Chilore, and Lamusi, in Likangala, an eastern area of Zomba district, in the south-eastern region of Malawi (Fig. 1). Zomba district belongs to a malaria endemic area with high malaria prevalence (16). Malaria is one of the leading causes of morbidity in the district, accounting for 66.8% of the total deaths (Zomba District Health office, 2009).

Insecticide susceptibility tests for mosquitoes found at the study sites: *An. arabiensis* larvae were collected from paddy fields and water pools between February and May 2015. Larvae were reared until the emergence of adults for susceptibility testing. *An. funestus* s.s. adult mosquitoes were collected by aspiration with a battery-powered aspirator (BioQuip Products, Rancho Dominguez, CA) from February to March 2018.

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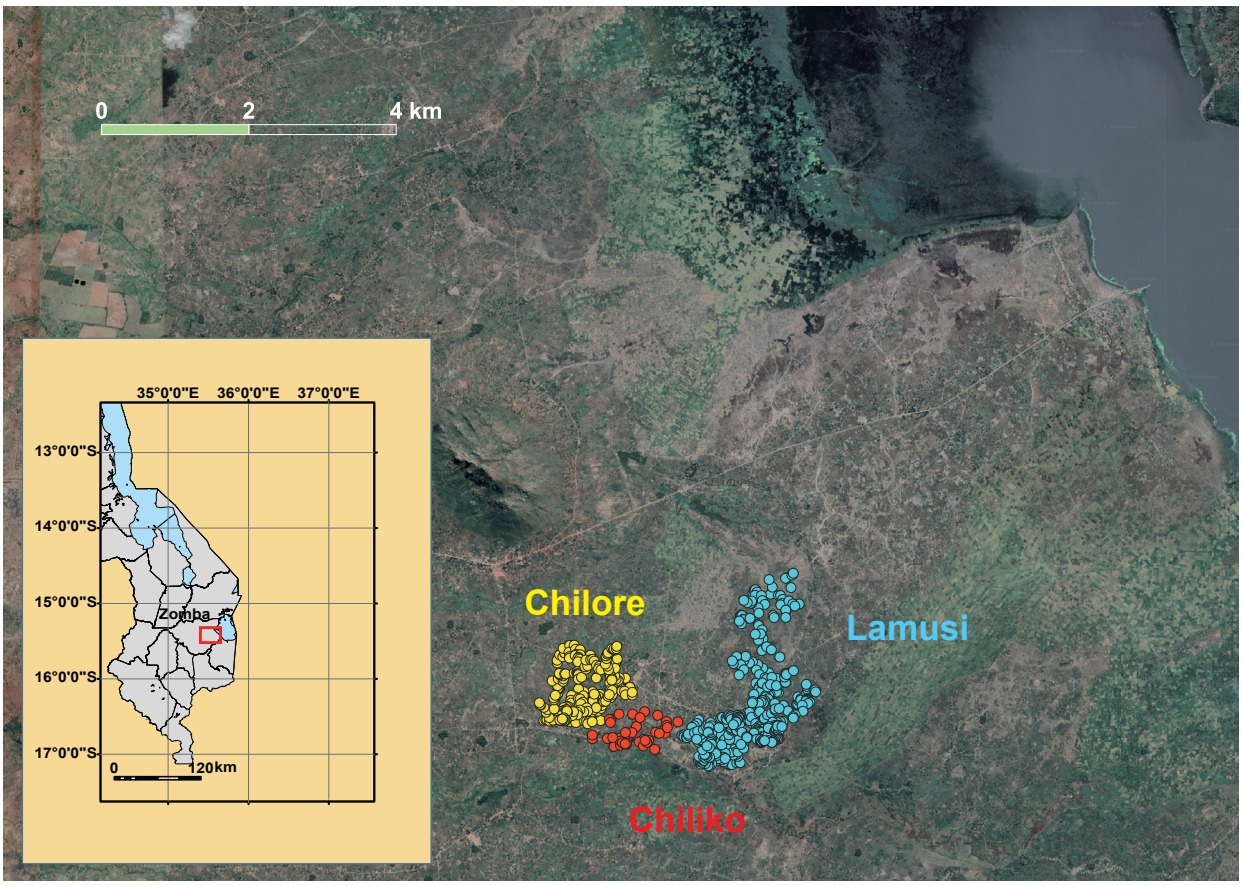

Fig 1. (Color online) Map of the study sites. QGIS 3.6 (https://qgis.org/ja/site/) and its plugin "Google Map Satellite" (https://satellites.pro) were used. Circles indicate the houses used for the study.

Insecticide susceptibility tests were performed using World Health Organization test tube kits (WHO/CDS/CPC/MAL/98.12). Papers impregnated with 0.75% permethrin, 0.05% deltamethrin, 0.5% etofenprox, 4% DDT, 0.1% propoxur, and 1.0% fenitrothion were used. Cytochrome P450 monooxygenase inhibitor, piperonyl butoxide (PBO; 98%, WAKO Pure Chemical Industries, Osaka, Japan), glutathione S-transferase inhibitor, DEM (diethyl maleate; ≥96%, Sigma-Aldrich, Saint Louis, MO, USA), and esterase inhibitor, DEF (Tribuphos; 97%, WAKO Pure Chemical Industries, Ltd, Japan) were used as synergists. Papers impregnated with permethrin/PBO (0.75/0.75% and 0.25/0.25%), permethrin/DEM (0.75/0.75% and 0.25/0.25%), and permethrin/DEF (0.75/0.75% and 0.25/0.25%) were used. One to three-day-old blood-unfed females of *An. arabiensis* and field-collected, blood-fed and blood-unfed females of *An. funestus* s.s. were used for the tests. Knockdown was assessed during 60 min and mortality was determined after 24 h. Knockdown of *An. funestus* was observed in a mixed group consisting of blood-fed and unfed mosquitoes and mortality was recorded separately. Three to six replications using approximately 10 mosquitoes were performed for each insecticide.

Species identification: Mosquitoes were identified microscopically as *An. gambiae* s.l. and *An. funestus* s.l. based on the identification keys (17). DNA was extracted using the RedExtract-N-Amp Tissue PCR kit (Sigma-Aldrich Japan, Tokyo, Japan) and species identification was performed using multiplex PCR (18, 19).

Detection of point mutations in the voltage-gated sodium channels of *An. arabiensis* and *An. funestus* s.s.: To verify the presence of point mutations at L1014 in *An. arabiensis* and *An. funestus* s.s., PCR and direct DNA sequencing was conducted according to the procedures described by Kawada et al. (20).

Preliminary small-scale field survey to determine the number of MSRDs required for each house: A preliminary small-scale trial comprising 40 houses of Chiliko village was performed between January and April 2015.

Intervention using Olyset® Plus LLINs and MSRDs: Olyset® Plus (2% permethrin and 1% w/w PBO, Sumitomo Chemical Co., Ltd., Tokyo, Japan) nets were delivered to 29 houses after removing the old bed nets and 11 houses were left unchanged as controls (The control houses used Olyset® Net with 2% w/w permethrin). Olyset® Plus nets were supplied in sufficient numbers to cover all the residents. Of the 29 houses, 20 were treated with MSRDs (polyethylene net materials of 8 × 15 cm impregnated with 10% w/w metofluthrin, Sumitomo Chemical Co., Ltd., Tokyo, Japan) at 2 different dose regimens; 10 houses were treated with 2 strips per 10 m^2 and the other 10 houses with 3 strips per 10 m^2. The house residents were informed about the study and their written consent was obtained before conducting the interventions. The intervention was performed on January 22 and 23, 2015. Four months after hanging the MSRDs, all strips were collected on May 18, 2015, and the remaining

quantities of active ingredients were analyzed using gas chromatography.

Mosquito collection: Mosquitoes were collected from the 40 houses after pyrethrum was sprayed. Pre-intervention collections were performed on January 10 and 15, 2015, and post-intervention collections were conducted after 1 week (January 27, 31, and February 1, 2015), 1 month (February 24 and 25, 2015), 2 months (March 28 and 31, 2015), and 3 months (April 24 and 27, 2015).

Large-scale field trial involving all residential houses in 2 villages: Large-scale trial was performed to investigate the effect of the MSRD intervention combined with Olyset® Plus against the malaria infection rate in children, between May 2015 and June 2016.

Intervention using Olyset® Plus nets and MSRDs: All houses in Chilore (215 houses) and Lamusi (408 houses) were part of the study. Sufficient numbers of Olyset® Plus nets to cover all residents were delivered to all houses after removing the old bed nets. MSRDs were used in two different intervention regimens; a single treatment at the rate of 2 strips per 10 m^2 and a two-time treatment at the same dosage with a 3-month interval. The house residents were informed about the study and their written consents were obtained before conducting the interventions. Olyset® Plus nets were delivered on December 9 and 10, 2015. The first intervention of MSRDs in the 2 villages was implemented from January 6 to 12, 2016, and the 2nd MSRD intervention in Chilore was conducted on April 22 and 23, 2016.

Mosquito collection: Twenty houses each in Chilore and Lamusi were randomly selected for mosquito collections. Pyrethrum spray collection for the 40 houses were performed. Pre-intervention collections were performed on December 3 and 7, 2015, and post-intervention collections were conducted after 1 month (February 12 and 21, 2016), 2 months (March 7 and 8, 2016), 3 months (April 12, 13, 16 and 17, 2016), and 4 months (May 10 and 11, 2016). The collected mosquitoes were identified according to the procedures described above.

Blood collection and detection of malaria parasites: Pre-intervention blood collections for children (under 10 years old) were conducted from May 5 to 8, 2015 with 74 children in Chilore and 112 children in Lamusi. Post-intervention blood collections were conducted on May 2 and 3, 2016 with 50 children in Chilore and 105 children in Lamusi. A finger-prick blood sample (5 µL) was obtained from each child. The presence of malaria parasites was determined using a rapid diagnostic test (RDT) for detecting histidine-rich protein II antigen of *Plasmodium falciparum* and *Plasmodium* lactate dehydrogenase of *P. vivax* (RDT, SD Bioline Malaria Rapid Diagnostic Test Kits, Standard Diagnostics, Inc, Yongin, Korea). Another 100 µL of the blood was placed on a filter paper for PCR analysis. Clinical malaria was defined by an axillary temperature of 37.5°C or higher and diagnosis of parasitemia by RDT. Malaria positive children were treated by administering a combination of artemether/lumefantrine syrup. DNA extraction from the above dried blood was performed using a QIAamp DNA Blood Mini Kit (Qiagen, Tokyo, Japan). Nested-PCR was performed according to the method described by Snounou *et al*. (21) with the primers for outer IrPLU5(5′-CCT GTT GTT GCC TTA AAC TTC-3′) and rPLU6(5′-TTA AAA TTG TTG CAG TTA AAACG-3′), for inner rFAL1 (5′-TTA AAC TGG TTT GGG AAA ACC AAA TAT ATT-3′) and rFAL2 (5′-ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC-3′) for *P. falciparum* and rVIV1 (5′-CGC TTC TAG CTT AAT CCA CAT AAC TGA TAC-3′) and rVIV2 (5′-AAG GAA AGA AAG TCC TTA-3′) for *P. vivax*. A GeneAmp PCR (Tokyo, Japan) was used for performing all PCR analyses. The above procedures were repeated at least three times for the RDT-positive samples and the same procedures were repeated for the PCR-positive samples.

Detection of malaria parasites from mosquitoes: DNA was extracted from the head-thorax regions of the mosquitoes, and nested-PCR was performed in the same manner as described above.

Data analysis: A generalized linear mixed model (GLMM) with Poisson distribution was used to analyze the variation in the number of mosquitoes collected in the different types of interventions. The date of collection was considered a random factor. GLMM with binomial distribution was used to analyze the variation in *P. falciparum* parasitemia in children belonging to houses wherein different types of interventions were implemented. The family (house) of the children was considered a random factor. GLMMs were conducted using the lme4 package in R software (http://www.R-project.org). Tukey's multiple comparison tests were performed using the multcomp package for R software.

Ethics: The protocol for the study was reviewed and approved by the Chancellor College Research and Ethics Committee, University of Malawi, and the Ethical Committee of Institute of Tropical Medicine, Nagasaki University (Approval No. 140605123-2).

RESULTS

Insecticidal susceptibility of *An. arabiensis*, and *An. funestus* s.s.: *An. arabiensis* collected from the study site showed high resistance to the following diagnostic levels of pyrethroids: 0.75% permethrin, 0.05% deltamethrin, and 0.5% etofenprox. The mortality rate was 60.5%, 82.8%, and 87.2%, respectively. High resistance was also observed for 4% DDT. In contrast, 0.1% propoxur and 1.0% fenitrothion showed high mortality (both 100%) (Fig. 2-A). Among the synergists examined in combination with permethrin, PBO was the most active, enhancing both mortality and knockdown in *An. arabiensis* (Fig. 2-B).

An. funestus s.s. showed resistance to permethrin, DDT, and propoxur, but showed a high mortality rate (100%) with 1.0% fenitrothion. As observed in *An. arabiensis*, PBO was also an effective synergist, enhancing both the mortality and knockdown (Fig. 3). Not a single point mutation at the voltage-gated sodium channel (L1014F or L1014S) was detected among 62 and 28 female adult *An. arabiensis* and *An. funestus* s.s. mosquitoes, respectively

Preliminary small-scale field survey to determine the number of MSRDs required per house: A total of 232 *An. arabiensis* and 82 *An. funestus* s.s. female mosquitoes were collected from the 40 houses in Chiliko. More *An. arabiensis* (157 females) were collected in the beginning and middle of the rainy

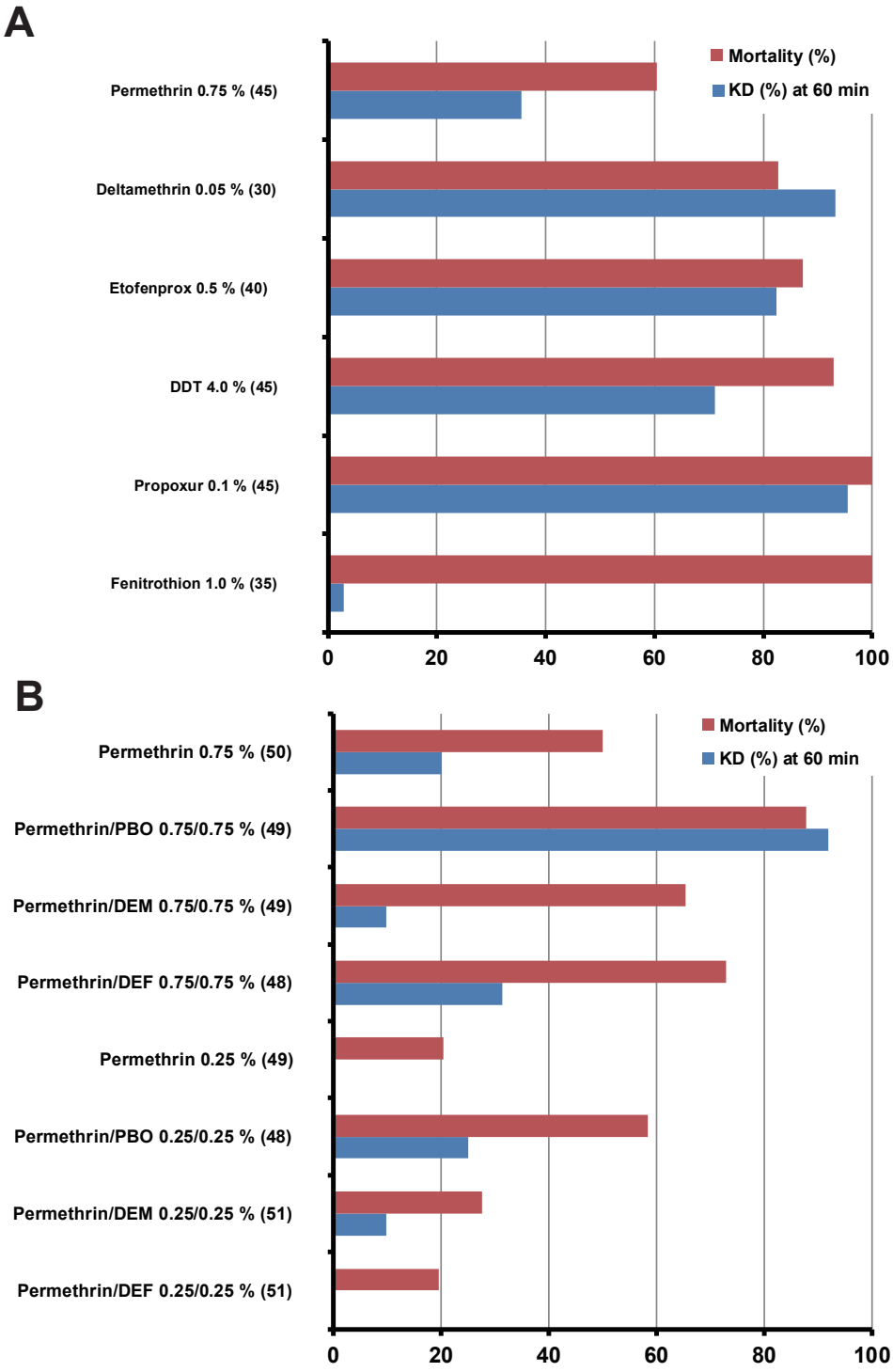

Fig 2. (Color online) Insecticide susceptibility of *Anopheles arabiensis* females (A) and synergistic activity of PBO, DEF, and DEM on permethrin (B) by WHO tube test. Figures in parenthesis are the number of insects used.

season (January to February 2015) than that of *An. funestus* s.s. (17 females). In contrast, the number of *An. funestus* s.s. (65 females) increased to as many as that of *An. arabiensis* (75 females) at the end of the rainy season (March and April 2015).

The differences in the number of mosquitoes collected among the 4 intervention types were significant at the day before the intervention, 1 week, 1 month, 2 months, and 3 months after intervention ($P < 0.001$). The reduction in the number of mosquitoes was highest in the houses treated with Olyset® Plus + MSRDs (both, at 2 strips per 10 m^2 and 3 strips per 10 m^2). Significant reduction in the number of mosquitoes compared to the control houses was observed at 3 months after the abovementioned intervention regimes were implemented, although the mosquito numbers slightly resurged after 3 months of intervention (Fig. 4). The residual amount of active ingredient in the MSRDs (total 124 strips from 20 houses, initially impregnated with 10% w/w) 4 months after intervention was 2.75 ± 0.34%

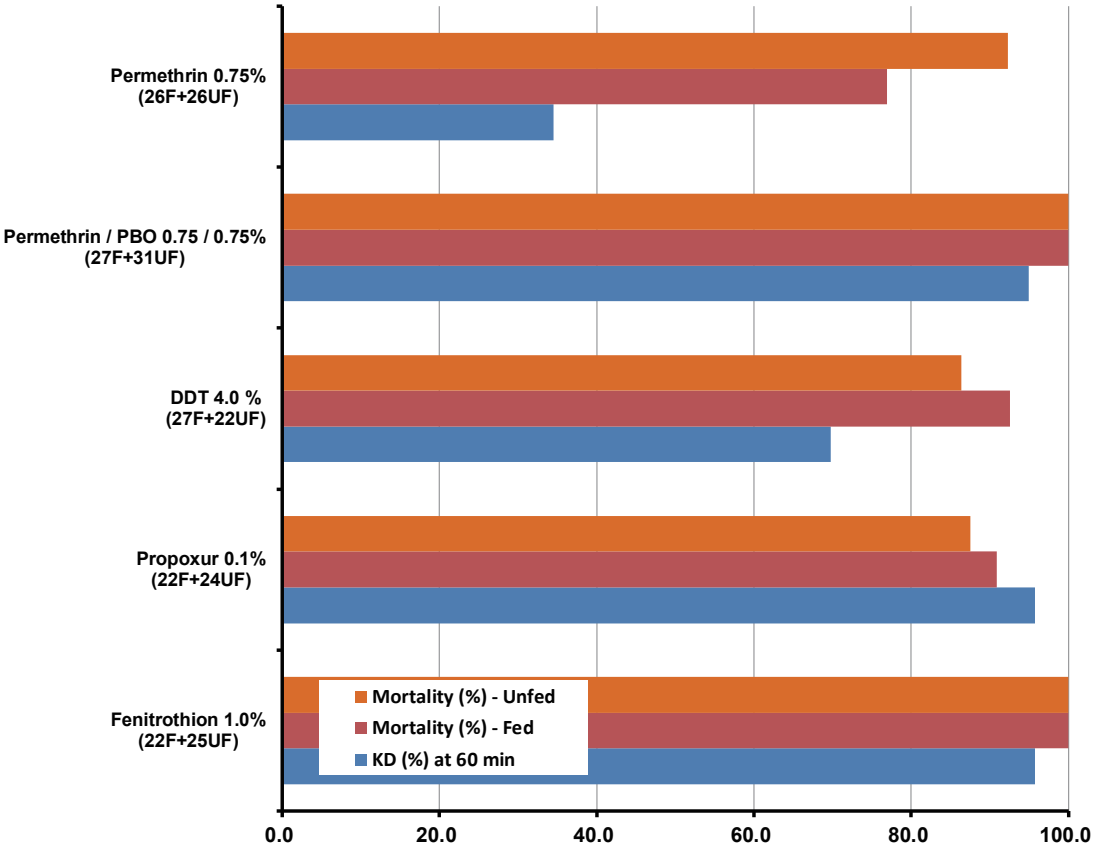

Fig 3. (Color online) Insecticide susceptibility of *Anopheles funestus* females by WHO tube test. Figures in parenthesis are the number of insects used in the bioassay (F, Blood-fed female; UF, Blood-unfed female).

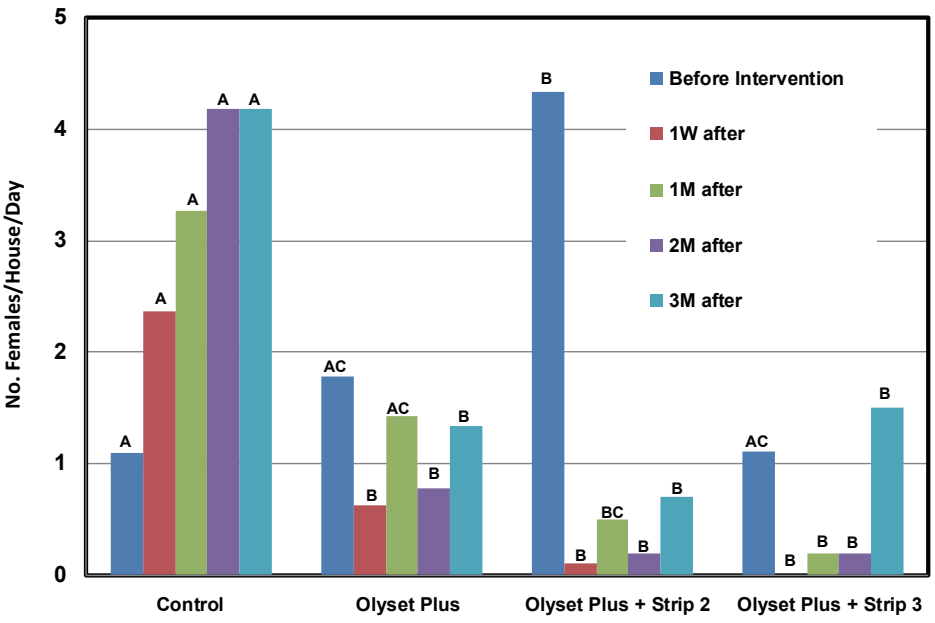

Fig 4. (Color online) Changes in the number of female *An. gambiae* s.l. and *An. funestus* s.s. before and after the intervention. The different letters in the same color bar indicate significant difference ($P < 0.01$) by GLMM using the Poisson distribution with dates of collection as random effect.

(w/w).

Large-scale field trial including all residential houses in two villages: The total number of female mosquitoes collected from the randomly selected 40 houses included 187 *An gambiae* s.l. (185 *An. arabiensis* and 2 *An. gambiae* s.s.) and 77 *An. funestus* s.l. (71 *An. funestus* s.s., 2 *An. rivulorum* Leeson, and 4 *An. parensis* Gillies). The number of mosquitoes collected in the pre-intervention survey (December 2015, at the beginning of the rainy season) was small for both

Table 1. Changes in the number of female Anopheline mosquitoes per house (*Anopheles gambiae* s.l., and *Anopheles funestus* s.l.) before the intervention, and 1, 2, 3, and 4 months after the intervention of MSRD (2 strips/10 m^2) + Olyset® Plus

	Pre-intervention	1 month after first intervention	2 months after first intervention	3 months after first intervention	1 month after 2nd intervention
Chilore village (MSRD 2 times treatment)[1)]					
Anopheles gambiae s.l.[2)]	0.75	1.8	0.17	0.33	0
Anopheles funestus s.l.[3)]	0	0.67	0.44	0.94	0.35
Total	0.75[4)]	2.5[5)]	0.61[6)]	1.28[7)]	0.35[8)]
Lamusi village (MSRD single treatment)[1)]					
Anopheles gambiae s.l.[2)]	0.25	2.0	0.41	0.21	0.94
Anopheles funestus s.l.[3)]	0	0	0.12	0.58	0.56
Total	0.45[4)]	1.95[5)]	0.53[6)]	0.79[7)]	1.5[8)]

[1)]: The first intervention of the strips in Chilore and Lamusi villages was performed from January 6–12, 2016, and the 2nd intervention in Chilore was performed on April 22–23, 2016. Olyset® Plus nets were delivered to all houses in Chilore and Lamusi on December 9 and 10, 2015.
[2)]: Contains 185 *Anopheles arabiensis* and 2 *Anopheles gambiae* s.s..
[3)]: Contains 77 *Anopheles funestus* s.s., 2 *Anopheles rivulorum*, and 4 *Anopheles parensis*.
[4)]: $\chi^2 = 1.52$, $df = 1$, $P = 0.22$.
[5)]: $\chi^2 = 1.27$, $df = 1$, $P = 0.26$.
[6)]: $\chi^2 = 0.10$, $df = 1$, $P = 0.75$.
[7)]: $\chi^2 = 1.63$, $df = 1$, $P = 0.20$.
[8)]: $\chi^2 = 10.0$, $df = 1$, $P = 0.0014$.

Table 2. Number of children infected with parasites before and after the intervention ofMSRD with combination of Olyset® Plus

Chilore Village (MSRD 2 times intervention)

Intervention	RDT		PCR	
	Total	Positive %	Total	Positive %
Before				
No. examined	74	51.4[1)]	74	33.8[2)]
Plasmodium falciparum positive	38		25	
After				
No. examined	50	34.0[1)]	50	24.0[2)]
Plasmodium falciparum positive	17		12	

Lamusi Village (MSRD single intervention)

Intervention	RDT		PCR	
	Total	Positive %	Total	Positive %
Before				
No. examined	112	28.6[3)]	112	24.1[4)]
Plasmodium falciparum positive	32		27	
After				
No. examined	105	24.8[3)]	105	15.2[4)]
Plasmodium falciparum positive	26		16	

[1)]: $\chi^2 = 3.68$, $df = 1$, $P = 0.055$; [2)] $\chi^2 = 1.30$, $df = 1$, $P = 0.25$; [3)] $\chi^2 = 0.40$, $df = 1$, $P = 0.53$; [4)] $\chi^2 = 2.33$, $df = 1$, $P = 0.13$.

An. gambiae s.l. (20 females) and *An. funestus* s.l. (4 females). A greater number of *An. gambiae* s.l. (134 females) mosquitoes were collected in the middle of the rainy season (February and March 2016) than that of *An. funestus* s.l. (23 females). In contrast, the dominance was reversed at the end of the rainy season (April and May 2016), during which the number of *An. funestus* s.l. (50 females) was greater than that of *An. gambiae* s.l. (33 females). Average *P. falciparum* sporozoite positive rates during the trial in *An. arabiensis* and *An. funestus* s.s.

were 16.4% (Chilore) and 5.7% (Lamusi), and 20.5% (Chilore) and 11.1% (Lamusi), respectively.

The differences in the number of mosquitoes between the 2 intervention types were not significant before intervention and 1, 2, and 3 months after intervention (P = 0.22, 0.26, 0.75, and 0.86, respectively), while the difference after 4 months (1 month after 2nd intervention of the strips was implemented in Chilore) was significant (P = 0.016), indicating decreasing spatial repellency with the single treatment of MSRD after 4 months but continued high spatial repellency by the 2nd intervention of MSRD (Table 1).

Parasitemia in blood samples collected from children: *P. falciparum* infection rates were reduced after intervention in both the villages as compared to those before the intervention, although these differences were not statistically significant (Table 2). No significant difference in body temperature after the intervention was observed between parasite positive and parasite negative children [35.9 ± 0.8°C and 35.4 ± 0.9°C in Chilore (P = 0.40), and 36.3 ± 1.0°C and 36.0 ± 1.0°C in Lamusi (P = 0.60), respectively].

DISCUSSION

Two major malaria vectors, *An. arabiensis* and *An. funestus* s.s., obtained from the study site, showed high resistance to pyrethroids. Some mixed function oxidase (MFO)-related oxidative mechanisms were thought to be the major factors responsible for metabolic resistance.

In the preliminary field study, significant reduction in the number of mosquitoes was observed in the houses treated with MSRDs in combination with Olyset® Plus compared to that of the control houses. The effective duration of the single treatment of MSRD is thought to be less than 4 months with 2 strips per 10 m^2, since the mosquito numbers slightly resurged after 3 months of intervention. Given the results described above and in view of reducing the practical cost, 2 strips per 10 m^2 of MSRD is recommended as the intervention dosage for a house. In the study site, the rainy and mosquito breeding season normally starts in November and lasts until early May. Therefore, two-time interventions of MSRDs would be required to cover this season (6 months) for effective reduction of mosquitoes in the study sites.

In the large-scale trial, a reduction in *P. falciparum* infection rates was observed in children in Chilore and Lamusi, 4 months after the first intervention of MSRD (1 month after the second intervention in Chilore). As the body temperature of parasite positive children was within the normal range, these children were thought to be asymptomatic carriers of the parasite along with most of the adults in the villages. The infection rate did not decrease sharply possibly because of the presence of these asymptomatic carriers who harbored parasites that might not be directly affected by MSRD and LLIN interventions and were supplying sources of gametocytes to mosquitoes (22, 23). A decrease in mosquito blood feeding frequency does not only indicate a reduction in the frequency of sporozoite inoculation but also a reduction in the rate of gametocyte transfer from humans to mosquitoes, resulting in a gradual decrease in infection rates. Although parasites infecting the inhabitants may not disappear immediately, prevention of mosquito blood feeding by the continued intervention using MSRDs will be a convenient and effective measure to maintain the contact frequency with vector mosquitoes at low levels.

Although the mechanism underlying the effectiveness of metofluthrin against pyrethroid-resistant mosquitoes has not yet been elucidated, it might be explained by the difference in chemical structure between the phenoxybenzyl alcohol-based pyrethroids, such as permethrin and deltamethrin, and tetrafluorobenzyl alcohol-based pyrethroids, such as transfluthrin and metofluthrin. Transfluthrin has the potential to control pyrethroid-resistant mosquitoes with P450-mediated enhanced metabolic factor, since the P450-detoxifying enzymes preferably bind at the phenoxybenzyl alcohol moiety, but not at the tetrafluorobenzyl moiety (24). Similarly, the resistance ratio of transfluthrin and metofluthrin was much smaller than that of permethrin against permethrin-resistant wild colonies of *Culex quinquefasciatus* Say (25).

The mode of action of airborne metofluthrin against mosquitoes is also of interest. In a previous study, *Aedes aegypti* (L.) exposed to metofluthrin vapor were inhibited from biting humans mainly due to quick knockdown and high killing activity of metofluthrin but not due to repellency (26). Most of the *Ae. aegypti* adult mosquitoes present in the metofluthrin-treated room, died, or were knocked down without escaping (27). Contrastingly, significant excito-repellency of *An. gambiae* s.s. was reported in the artificial huts treated with metofluthrin (28). The above inconsistency may be caused by the difference in mosquito species with different host preferences. Therefore, the spatial repellency of metofluthrin is the combined result of multiple functions, such as, killing and knock down effects, excito-repellency, and the declining effect of host-seeking eagerness. Studies on the mode of action of metofluthrin in pyrethroid-resistant mosquitoes and further verification of the spatial repellency in field with MSRDs might provide breakthroughs in possible measures for malaria control in endemic areas.

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Conflict of interest None to declare.

REFERRENCES

1. Takken W. Do insecticide-treated bednets have an effect on malaria vectors? Trop Med Int Health. 2002;7:1022-1030.
2. Russell TL, Lwetoijera DW, Maliti D, et al. Impact of promoting longer-lasting insecticide treatment of bed nets upon malaria transmission in a rural Tanzanian setting with pre-existing high coverage of untreated nets. Mal J. 2010;9:187.
3. Kawada H. Potential control measures for pyrethroid-resistant malaria vectors. Acta Hortic. 2017;1169:59-72.
4. Iwashita H, Dida G, Futami K, et al. Sleeping arrangement and house structure affect bed net use in villages along Lake Victoria. Mal J. 2010;9:176.
5. Kawada H, Ohashi K, Dida GO, et al. Preventive effect of permethrin-impregnated long-lasting insecticidal nets on the blood feeding of three major pyrethroid-resistant malaria vectors in western Kenya. Parasit Vect. 2014;7:383.

6. Minakawa N, Kongere JO, Dida GO, et al. Sleeping on the floor decreases insecticide treated bed net use and increases risk of malaria in children under 5 years of age in Mbita District, Kenya. Parasitol. 2015;142:1516-27.
7. Corbel V, N'guessan R. (2015) Distribution, mechanisms, impact and management of insecticide resistance in malaria vectors: A pragmatic review. In: Spooner J, editor. Anopheles Mosquitoes: A Detailed Research. New Jersey, USA: Foster Academics; 2015. p. 92-149.
8. Ranson H, N'guessan R, Lines J, et al. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends in Parasitology. 2011;27:91-8.
9. Wondji CS, Coleman M, Kleinschmidt I, et al. Impact of pyrethroid resistance on operational malaria control in Malawi. PNAS. 2012;109:19063-70.
10. Matsuo N, Ujihara K, Shono Y, et al. Discovery and development of a novel pyrethroid insecticide 'Metofluthrin (SumiOne, Eminence). Sumitomo Kagaku. 2005;2005-II:1-15. <https://www.sumitomo-chem.co.jp/english/rd/report/files/docs/20050200_imi.pdf>
11. Kawada H, Temu EA, Minjas JN, et al. Field evaluation of spatial repellency of metofluthrin-impregnated plastic strips against *Anopheles gambiae* complex in Bagamoyo, coastal Tanzania. J Am Mosq Control Assoc. 2008;24:404-9.
12. Kawada H, Maekawa Y, Tsuda Y, et al. Trial of spatial repellency of metofluthrin-impregnated paper strip against *Anopheles* and *Culex* in shelters without walls in Lombok, Indonesia. J Am Mosq Control Assoc. 2004;20:434-7.
13. Kawada H, Maekawa Y, Tsuda Y, et al. Laboratory and field evaluation of spatial repellency with metofluthrin-impregnated paper strip against mosquitoes in Lombok Island, Indonesia. J Am Mosq Control Assoc. 2004;20:292-8.
14. Kawada H, Maekawa Y, Takagi, M. Field trial of the spatial repellency of metofluthrin-impregnated plastic strip against mosquitoes in shelters without walls (Beruga) in Lombok, Indonesia. J Vect Ecol. 2005;30:181-5.
15. Kawada H, Iwasaki T, Loan LL, et al. Field evaluation of spatial repellency of metofluthrin-impregnated latticework plastic strips against *Aedes aegypti* (L.) and analysis of environmental factors affecting its efficacy in My Tho city, Tien Giang, Vietnam. Am J Trop Med Hyg. 2006;75:1153-7.
16. Kleinschmidt I, Sharp B, Mueller I, et al. Rise in malaria incidence rates in South Africa: small area spatial analysis of variation in time trends. Am J Epidemiol. 2002;155:257-64.
17. Gillies MT, Coetzee M. A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical region). South African Institute for Medical Research. 1987;No. 55.
18. Scott JA, Brogdon WG, Collins FH. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. Am J Trop Med Hyg. 1993;49:520-9.
19. Koekemoer LL, Kamau L, Hunt RH, et al. A cocktail polymerase chain reaction (PCR) assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. Am J Trop Med Hyg. 2002;66:804-11.
20. Kawada H, Dida GO, Ohashi K, et al. (2011): Multimodal pyrethroid resistance in malaria vectors *Anopheles gambiae* s.s., Anopheles arabiensis, and *Anopheles funestus* s.s. in western Kenya. PLoS One 2011;6:e22574.
21. Snounou G, Viriyakosol S, Zhu XP, et al. High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Mol Biochem Parasitol. 1993;61:315-20.
22. Maeno Y, Nakazawa S, Dao le D, et al. A dried blood sample on filter paper is suitable for detecting *Plasmodium falciparum* gametocytes by reverse transcription polymerase chain reaction. Acta Trop. 2008;107:121-7.
23. Nakazawa S, Culleton R, Maeno Y. In vivo and in vitro gametocyte production of *Plasmodium falciparum* isolates from Northern Thailand. Int J Parasitol. 2011;41:317-23.
24. Horstmann S, Sonneck R. Contact bioassays with phenoxybenzyl and tetrafluorobenzyl pyrethroids against target-site and metabolic resistant mosquitoes. PLoS One. 2016;11:e0149738.
25. Argueta TBO, Kawada H, Sugano M, et al. Comparative insecticidal efficacy of a new pyrethroid, metofluthrin, against colonies of Asian *Culex quinquefasciatus* and *Culex pipiens pallens*. Med Entomol Zool. 2004;55:289-94.
26. Ritchie SA, Devine GJ. Confusion, knock-down and kill of *Aedes aegypti* using metofluthrin in domestic settings: a powerful tool to prevent dengue transmission? Parasit Vect. 2013;11:262.
27. Rapley LP, Russell RC, Montgomery BL, et al. The effects of sustained release metofluthrin on the biting, movement, and mortality of *Aedes aegypti* in a domestic setting. Am J Trop Med Hyg. 2009;81:94-9.
28. Stevenson JC, Simubali L, Mudenda T, et al. Controlled release spatial repellent devices (CRDs) as novel tools against malaria transmission: a semi-field study in Macha, Zambia. Mal J. 2018;17:437.