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An Intervention Study of Deltamethrin Impregnation of Lower Efficacy of Used Llins and Local Mosquito Nets in Ohnpinkone Ward, Nant Nhyin Village, Banmauk Township, Sagaing Region in Myanmar

Maung Maung Mya[1]; Myat Phone Kyaw[1]; Kyaw Thu Soe[2]; Pyae Linn Aung[2]; Than Tun[3]; Nwe New Aye[3]; Ye Kyaw Thu[2]; Swan Htet Aung[2]; Kyaw Kyaw Linn[4]

[1] *Department of Medical Research Yangon, MOGH*
[2] *ICEMR Project*
[3] *Vector Borne Diseases Control DOH*
[4] *Township Medical Officer Banmauk Township Sagaing Region*

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*Corresponding author:
Maung Maung Mya
E-mail:
dr.mgmgmya@gmail.com

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ABSTRACT

Long-Lasting Impregnated bed nets have been distributed in all villages in Myanmar. The insecticide efficacy should last for 3 years and re-distribution is necessary, but re-distribution was not covered to all townships in 2019. An intervention study was conducted in Ohnpinkone as test ward and Bwedarkone as control ward of Nant Nhyin village, Banmauk township, from December 2019 to October 2020. In the Ohnpinkone ward, all used LLINs with low efficacy of insecticide and traditional nets from households were impregnated with Deltamethrin at the rate of 55mg/meter square and compared with non-intervention ward Bwedarkone for 10 months. There were 10 *P. falciparum* cases in Ohnpinkone and 1 *P. falciparum* case in the Bwedarkone ward in the 2019 before intervention. After intervention in Ohnpinkone, the malaria cases were going down to 0. There were 3 malaria-positive cases were found in the control ward Bwedarkone, 66.67% higher up. Main vector *An. minimus* was found higher number in both Ohnpinkone (137) and Bwedarkone (109) wards by outdoor and indoor light trap catch methods. Moderate number of *An. Barbirostris* (126) and *An. Vagus* (104) were observed in the test ward and *An. vagus*, *An. philippinensis* in the control ward. After intervention, no malaria cases and vectors were caught up by indoor bedroom light traps in the test ward, showing 100% recovery till October, although malaria vectors were observed in the indoor light trap in the control ward. Therefore, Deltamethrin impregnation to used LLINs nets with expiry or lower efficacy, become higher bio-efficacy again. It is a very useful and cost-effective tool to control malaria transmission and man vector contact as well as reduced vector mosquitoes

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in malaria-endemic areas. The National Malaria Control Program may consider implementing this intervention in resources limited settings and or emergencies with supply chain shortages.

INTRODUCTION

Malaria is one of the major communicable disease-causing high mortality and morbidity among the population. Previously malaria is a main public health problem in Myanmar. Although now morbidity and mortality of malaria are reducing from 4% to 1% by 100000 population 2011 to 2018 (Health in Myanmar, 2019). Out of which, about half of the cases were *Plasmodium vivax* (*Pv*). *Plasmodium vivax* is gradually rising in Myanmar (Myint Oo et al., 1999). But malaria is endemic in hard-to-reach areas and border areas (MOHS, 2018). This disease is transmitted by biting the female *Anopheline* mosquito species. Out of 37 Anopheline species found in Myanmar, *An. dirus* and *An, minimus* are major vectors of malaria and *An. annularis* is a local vector of malaria in Rakhine State and *An. sundigus* is a vector of malaria in coastal areas in Myanmar (Khin Mung Kyi 1970). Although *An. annularis, An. maculatus, An. aconitus* and *An. philippinensis* are secondary vectors of malaria in Myanmar. In India the six species viz., *Anopheles culicifacies, An. stephensi, An. minimus, An. sundaicus, An. fluviatilis* and *An. dirus* are major vectors of malaria and three species viz., *An. annularis, An. philipinensis* (nivipus) and *An. varuna* are minor vectors of malaria (Sharma, 1999).

There has been a renewed emphasis on preventive measures at community and individual levels. Insecticide-treated nets (ITNs) are the most prominent malaria preventive measure for large-scale deployment in highly endemic areas (Schellenberg et al., 2002; Lengeler, 2004). Most research about the efficacy of net bed impregnation has been done in Africa and Asia (Kroeger et al., 1997). The study by Choi et al. (1995) showed a reduction of the malaria incidence rate by 50%. Also, the overall mortality and morbidity attributed to malaria in children aged 1-4 years were reduced by 63% and 70%, respectively, in areas in the Gambia where insecticide-treated nets were used (Alonso et al., 1991). The controlled trials carried out so far thus showed a reduction not only of malaria-related morbidity but also of child mortality (Lengeler, 2004).

In India on a pilot-scale study was done in 100 highly endemic districts under the Enhanced Malaria Control Programme. This is the first time ITNs have been incorporated into the malaria control programme to replace insecticide indoor residual spraying in India at Primary Health Centre (PHC)/district level (Jambulingam et al., 2008). In diverse social and epidemiological settings, the efficacy of the ITNs alone may not be the crucial determinant for the effective implementation of this community-based intervention measure (Binka and Adongo, 1997).

Nant Nhyin village is a remote and hard-to-reach area; it is 40-50 miles away from Banmauk City. There is a total of four wards, Ohnpinkone and Bwedarkone wards are included in Nant Nhyin village. The total population is about 5000 populations in the village. Most of the population are farmers remaining are health staff, school teacher and some are working in Gold mines. One monastery and one middle school are situated. The area of the Nant Nhyin is a hilly area and there are no pucker roads. One creek is across the village. So many creeks cross between Bunmouk and Nant Nhyin village. Malaria morbidity is found every month in this area. Therefore, there is a need to control malaria morbidity as well as the main vector. Long-lasting insecticide-treated nets have been distributed in all villages and

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insecticide efficacy should last for 3 years and re-distribution is necessary, but re-distribution was not covered to all townships in 2019. The study determined monthly malaria vectors occurrence in Ohnpinkone ward after impregnation of mosquito nets and Bwedarkone wards data was used as a control in Nant Nhyin villages because we have found 10 *Plasmodium falciparum* malaria cases in Ohnpinkone. Therefore, the study aims to determine the malaria prevalence rate and indoor malaria vector entrance rate after impregnation of Deltamethrin insecticide to mosquito nets.

MATERIALS AND METHODS

Study areas

The study was conducted in Ohnpinkone and Bwedarkone wards in Nant Nhyin village Banmauk Township Sagaing Region in Myanmar, where the morbidity of malaria is high.

Study period

The study period was one year, conducted from December 2019 to November 2020.

Study design

An intervention study design was used.

Malaria parasite detection

Sample collection: Before to after the intervention period, monthly finger-prick blood samples were taken from all the population in both Ohnpinkone and Bwedarkone words. Thick and thin blood films were made on greases free glass slides and dried in the air in shad in the room. Dried Thin films were fixed with absolute alcohol and dried at room temperature. Thick and thin blood slides were stained with 10 % Giemsa's stain for 5 minutes. Stained slides were washed with buffer water. Washed slides were dried at room temperature. Malaria parasites were diagnosed under an oil emersion lens (100X).

Identification of malaria parasites

Malaria parasites in thick and thin films were identified under an oil emersion lens. Malaria parasite was counted against 200 WBC.

Mosquitoes collection

Anopheles mosquitoes were collected by using CDC light traps such as Indoor light traps, Indoor bedroom light traps, and outdoor light traps collection methods from 18:00 hour to 00:06 hour in randomly selected households in before and after intervention period in both Ohnpinkone (Test) and Bwedakone (control) wards.

Larval surveys

Larval survey for breeding sites, susceptibility, and bio-efficacy tests: larval surveys were conducted in and around three kilometers away from the study villages. Water pools, domestic wells, streams, creeks, and pools, and all different types of water holding places were examined by 3 Dips /water holding places for larval detection (WHO, 1975). In and around the villages, water pockets, coconut shells, discarded tins and utensils, bamboo stumps including footprints of animals were examined. The captured larvae and pupae were put in labeled plastic bags and brought back to the laboratory for species identification and colonization.

Mosquito species identification

Collected *Anopheles* mosquitoes by light traps and adults emerged from larval survey were identified according to different identification keys (Harrison, 1980; Raid, 1967; Myo Paing, 1990b)

Insecticide susceptibility test

Insecticide susceptibility tests (WHO test kit): Collected F1 generation of adult female *Anopheles* mosquitoes from the larval survey were tested for measurement of insecticide susceptibility level using WHO test kits and standard procedures (WHO 1975). The efficacy of insecticides (which are commonly used for vector control in malaria-endemic areas) as Permethrin 0.75%, Cyfluthrin 0.15%, and Deltamethrin 0.05% impregnated

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paper with WHO test kits (WHO 1993) were provided to determine the susceptibility of mosquitoes and Bio-efficacy of deltamethrin impregnated nets.

Procedure

Ten F1 generations of *Anopheles* mosquitoes were introduced in WHO insecticide-impregnated paper attached plastic tube (WHO test kit) by sucking tube and exposed for 1 hour. After one hour of exposing the mosquitoes were then removed from the plastic tubes and placed in clean plastic tubes without paper with 10% glucose soak cotton and moisture was maintained by water soak damp towel. Percentage of knockdown was measured after 60 minutes of exposure and effective mortality was assessed after 24 hours exposure. Two replicate testing was done to confirm the susceptibility of mosquitoes. If the number of collected mosquito adults emerged from the larval survey was not more than ten while we used pool mosquito samples to test insecticides susceptibility. The susceptibility of mosquitoes was determined according to WHO (1993).

Bio-efficacy Test (Cone test)

Determination of insecticide persistence – Bioassays were carried out using the World Health Organization cone test method (WHO, 2013) after 3, 6, and 9 months of impregnation of mosquito nets in the Ohnpinkone wards to monitor the persistent effect of the insecticide treatment. Ten deltamethrin impregnated nets were randomly collected and bioassays were conducted. For comparison, tests were conducted in parallel on one untreated net obtained from the villagers. Ten sets of 10 F1 female *Anopheles* mosquito adults from the larval survey were exposed to the treated nets for 3 min and the mortality was recorded after 24 hours. *Anopheles minimus* and secondary vectors were used for the bioassay test. The same test was done for another randomly collected 20 ITNs for two days.

Data analysis

Data entry and collected monthly malaria and mosquito were analyzed by using Microsoft Excel software. Mosquito density, the main vector of indoor and outdoor light traps, mosquito susceptibility, and mortality, were calculated in percent.

RESULTS

Table 1 shows that 84 different types of mosquito nets were impregnated with deltamethrin at the rate of 55mg/meter squire. Of this used long-lasting insecticide nets (LLINs) (n=54), Cotton nets (n=6), Cylon nets (n=8), Traditional nets (n=16).

Table 1. Deltamethrin impregnation of different types of mosquito nets in Ohnpinkone ward.

Type of Mosquitoes nets	Number of nets	Good net	Tattered nets	% of good nets
Used LLINs nets	54	51	3	94.44
Cotton nets	6	6	0	100
Cylon nets	8	8	0	100
Traditional nets	16	16	0	100
Total nets	84	81	3	96.43

Table 2. shows that Indoor and Outdoor light traps collection in Ohnpinkone (Test ward) and Bwedarkone (control wards) and found that the highest number of vector *An. minimus* was found (n= 72) in indoor light traps collection followed by n=65 in the

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outdoor collection in Ohnpinkone ward. In Bwedarkone ward, the highest number of main vector *An. minimus* was found n=67 in outdoor light traps collection followed by n=36 in the indoor collection and lowest *An. minimus* was observed n=5 in bedroom Light traps catch. Although *An. barbirostris* was abundantly found n= 126 in Ohnpinkone and n=21 in Bwedarkone wards followed by *An. willmori* n=40 in Bwedarkone and n=21 in Ohnpinkone wards. Other secondary vectors such as *An. aconitus* (n= 40, 25)*, An annularis* (17, 14), *A culicifacies* (n=10*,* 2) *An. maculatus* (27, 9) and *An. kochi* (19, 9) were found in both areas. In bedroom light traps catch, has not collected any *Anopheles* mosquitoes in Test area of Ohnpinkone ward although in Bwedarkone wards n= 11 *Anopheles* mosquitoes were collected by bedroom light traps.

Table 2. *Anopheles* mosquitoes were collected by Light traps methods in two selected control and test wards in Nant Nhyin village.

Species	Ohnpinkone Ward (Test ward)				Bwedakone (Control ward)			
	Outdoor	Indoor house	Indoor bed room	Total	Outdoor	Indoor house	Indoor bed room	Total
An. kochi	5	14	0	19	3	6	0	9
An. barbirostris	42	84	0	126	10	11	0	21
An. hyrcanus	7	7	0	14	0	1	0	1
An. splendidus	31	10	0	41	28	10	0	38
An. minimus	65	72	0	137	36	67	5	109
An. varuna	11	40	0	51	11	18	1	30
An. maculatus	11	16	0	27	4	4	1	9
An. Jamesi	32	2	0	34	2	2	0	4
An. aconitus	16	24	0	40	10	15	0	25
An. stephensi	1	18	0	19	0	0	0	0
An. candidiensis	7	3	0	10	4	4	0	8
An. pallidus	2	10	0	12	1	0	1	2
An. theobaldi	0	0	0	0	0	0	0	0
An. Annularis	8	9	0	17	7	7	0	14
An. philippinensis	33	41	0	74	20	17	1	38
An. gigus	0	0	0	0	0	0	0	0
An. vagus	24	90	0	114	17	20	2	39
An. dirus	2	8	0	10	0	0	0	0
An. culicifacies	3	7	0	10	0	2	0	2
An. willmori	0	21	0	21	0	40	0	40
An. tesselatus	0	4	0	4	1	0	0	1
An. subpictus	1	4	0	5	0	1	0	1

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Total	301	484	0	785	154	225	11	390

Tabe 3 shows that detailed *Anopheles* larvae breeding site and abundances in study areas. The highest number of *Anopheles* larvae were collected from Rice fields n=1392(23.48%), followed by Sand pools and Creeks n=1171(19.75) and1156 (19.50%) and the lowest was observed in water well n=182(3.07%). *Anopheles minimus* larvae were abundantly found in. Rice fields (n=153), Sand pools (n=127), and Valley (n=129). Main vector *An. dirus* was found lowest number (n=22) in water wells in Ohnpinkone ward. The Secondary vector is *An. aconitus, An. maculatus, An. aconitus, An. annularis*, *An. philippinensis*, *An. culicifacies* and non-vectors *An. vagus, An.kochi, An. barbirostris* were abundant found in different water holding places.

Table 3. Different species of *Anopheles* larvae collected from different breeding places from Ohnpinkone and Bwedarkone wards

Species	16 Water well	8 sites of Valley	30 of Water pools	29 sites of Sand pool	45 sites of Rice field	38 sites of Creeks	Total	Density %
An. minimus	89	129	83	127	153	56	637	10.75
An. dirus	18	4					22	0.37
An. maculatus	32	79	89	105	168	99	572	9.65
An. aconitus	43	78	132	54	152	87	546	9.21
An. annularis		108	142	97	107	97	551	9.29
An. philippinensis		123	96	76	107	138	540	9.11
An. culicifacies		108	79	136	147	123	593	10.00
An. kochi		60	130	158	145	98	591	13.67
An. barbirostris		82	61	95	166	137	541	9.13
An varuna		67	59	98	69	78	371	6.26
An vagus		87	95	153	92	105	532	8.97
An willmori		65	71	72	86	138	432	7.29
Total	182	990	1037	1171	1392	1156	5928	100.00
Percent density %	3.07	16.70	17.49	19.75	23.48	19.50	100.00	

Red =primary vectors, Blue= Secondary vectors, Black= Non=vectors

Table 4. shows that all collected main vector *An. minimus* and *An. dirus*, Secondary vector *An. aconitus, An. varuna, An. annularis, An. philippinensis, An. maculatus, An, kochi. An. vagus* were found susceptible to WHO recommended insecticides as Deltamethrin 0.05%, Permethrin 0.75% and Cyfluthrin 0.15% impregnated paper.

Table 4. Susceptibility status of collected main and secondary vectors of Anopheles mosquitoes in test area

Species	Number of mosquitoes	WHO recommended insecticides impregnated paper	susceptible
An. minimus	30		susceptible

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An. dirus	9	Deltamethrin 0.05% Permethrin 0.75% Cyfuthrin 0.15%	"
An. aconitus	30		"
An. varuna	30		"
An. annularis	30		"
An. philippinensis	30		"
An. maculatus	30		"
An. kochi	30		"
An. vagus	30		"

Table 5. shows that bio-efficacy of before deltamethrin impregnation of mosquito nets in Ohnpinkone and Bwedarkone were found 6(20%) and 19(63.33%). Three months later, after impregnation of deltamethrin to mosquito nets in Ohnpinkone wards, the bio-efficacy was found 100% and in control ward Bwedarkone (non -impregnated with deltamethrin) the bio-efficacy was reduced from 63.33 to 43.33%. During intervention after 6 months later, the bio-efficacy of impregnated mosquito nets in Ohnpinkone and non-impregnated nets in Bwedarkone was found 100% sensitive in Ohnpinkone and 36.67% sensitive in Bwedarkone. After intervention in Ohnpinkone wards, the bio-efficacy of impregnated nets was found 96.67% sensitivity and in the control ward, the sensitivity of non-impregnated nets was found to be 16.67% in Bwedarkone ward.

Fig. 1. shows that before intervention 10 *P. falciparum* malaria cases were found in Ohnpinkone wards although the main vectors of *An. minimus* was collected in high number in March, April and July and 2 *An. dirus* was collected in June in indoor light traps catch. There were no malaria cases found during and after intervention period. But 1 *P. falciparum* positive cases was observed in pre-intervention months December and three malaria cases (2 Falciparum and 1 Vivax malaria cases) were found after intervention month in control ward Bwedarkone and main vector *An. minimus* was gradually increased from February to June and declined to October.

Table 5. Bio-efficacy of impregnated mosquito nets during and after intervention periods by the cone test method

Periods	(Ohnpinkone Test)		Bwedarkone (Control)	
	Tested nets	Bio-efficacy	Tested nets	Bio-efficacy
Pre-intervention (Before deltamethrin impregnation to nets)	30	6 (20%)	30	19 (63.33%)
During intervention (3 months later)	30	30(100%)	30	13 (43.33%)
During intervention (6 months later)	30	30(100%)	30	8 (36.67%)
After intervention (9 months later)	30	29(96.67%)	30	5 (16.67%)

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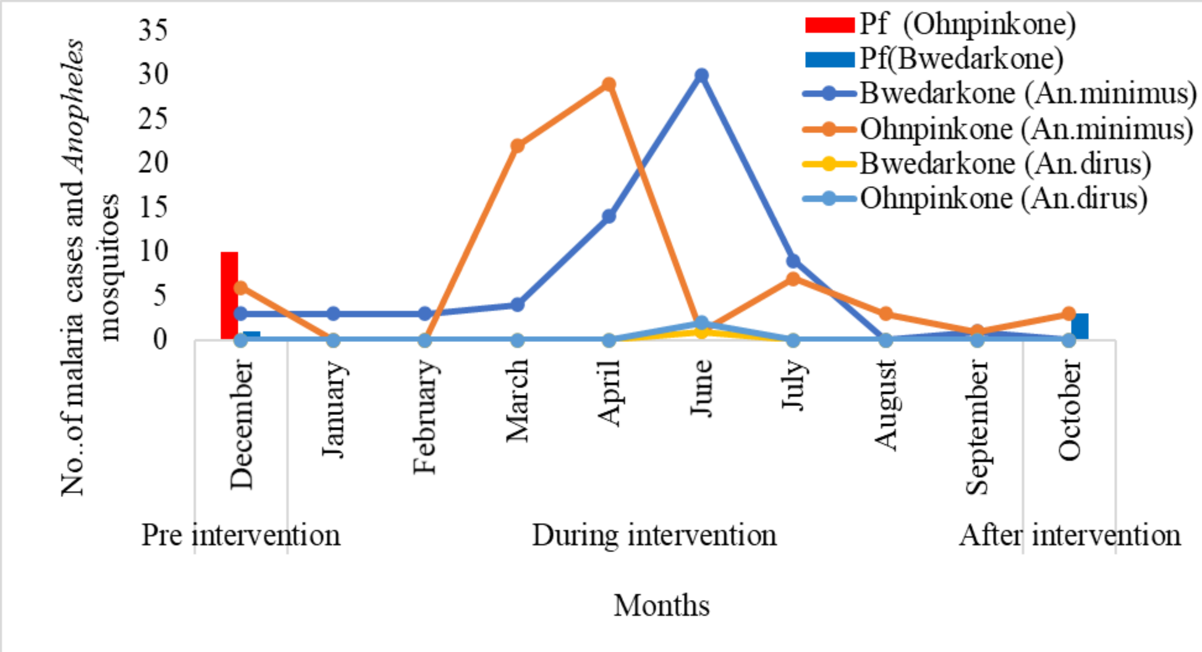

Fig. 1. Malaria cases and indoor main vectors collected by Light traps in study period.

Table 6. shows a 100% reduction of malaria cases were found after intervention periods. After deltamethrin impregnation of mosquito nets, the positive malaria cases were reduced to 0% during intervention month January to after intervention month (October). Although in the control ward as Bwedarkone was found, 66.67% of positive malaria cases were increased in the after-intervention period.

Table 6. Percentage reduction of malaria cases in after intervention period

Place	Pre-intervention	After intervention	Percentage
Ohnpinkone ward	10 malaria cases	O malaria cases	100% reduction
Bwedarkone ward	1 malaria cases	3 malaria cases	66.67 % increased

DISCUSSION

Malaria is a public health problem in Myanmar now due to the morbidity is high in hard-to-reach areas. Malaria morbidity is found every month in this area due to migrants, gold mine workers and rubber plantations (Maung Maung Mya et al., 2020a; Maung Maung Mya et al., 2014). *Plasmodium vivax* cases were found high prevalence in some areas and found Chloroquine resistance in Myanmar (Myint Oo et al., 1999, Myat Phone Kyaw et al., 1993). Therefore, there is a need to control malaria morbidity as well as man vector contact. We selected Ohnpinkone as the Test ward and determined monthly malaria morbidity and vectors occurrence in the Ohnpinkone ward after impregnation of mosquito nets. Bwedarkone wards data was used as a control because we have found 10 malaria cases in Ohnpinkone and one malaria case was found in the Bwedarkone ward in the preintervention period. Before the intervention period, a total of 84 used mosquito nets (Used LLINs nets 54, Cotton nets 6, Cylon nets 8, and Traditional nets 16) were collected and impregnated with Deltamethrin at the rate of 55mg/meter square. There are 4 kinds of mosquito nets in Ohnpinkone ward as LLINs nets, cotton nets, polyester nets and traditional nets; of this highest number of used LLINs nets were impregnated due to low bio-efficacy (20%). In the pre-intervention month, December 10, Falciparum malaria cases were found in Ohnpinkone wards and one *P. falciparum* case was found in Bwedarkone wards. During intervention months (January to September),

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no positive malaria cases were found till after intervention months (October) in the Ohnpinkone ward. In the control ward 3 malaria cases (2 *P. falciparum* and 1 *P. vivax*) were found after-intervention month (October). Other researchers revealed that malaria morbidity was high in Katinehtit village Kamamaung Township Kayin State, where Vivax malaria cases were found more prevalent (Maung Maung Mya et al., 2019b). In Bwedarkone and Ohnpinkone wards, a total of 785 and 390 *Anopheles* mosquitoes were collected within pre, during and post-intervention periods (December to October) of this n=484 by indoor and 301 by outdoor light traps were caught in Ohnpinkone wards and 386 *Anopheles* were collected by outdoor and 225 by indoor light traps were collected in Bwedarkone ward. A total of 22 *Anopheles* mosquito species were collected, *An. minimus* and *An. barbirostris* was found highest number in Ohnpinkone Ward and main vector *An. minimus* was found to be higher in indoor and outdoor than indoor and outdoor of Bwedarkone. In Ohnpinkone ward, *Anopheles* mosquitoes were had not collected in indoor bedroom catch. However, 11 *Anopheles* mosquitoes were collected by the indoor light trap method in Bwedarkone. *An. minimus* is a main vector of malaria in Myanmar (Khin Maung Kyi, 1970), and is abundantly found in Pyin Oo Lwin Township Mandalay Region (Maung Maung Mya et al., (2018). The very small number of *An. dirus* was observed from both study and control areas. *Anopheles dirus* is a main vector of malaria and found high density in Bago Yoma Mountain range, Mon State and Taninthayi Region (Tun Lin et al., 1995, Htay Aung et al., 1999). In India *An. dirus, An. minimus, An. culicifacies, An. stephensi, An. philippinensis* and *An. flivitellis* are the main vector of malaria (Sharma, 1999). In Thail and *An. dirus, An. minimus, An. maculatus* and *An. philippinensis* are the main vector of malaria (Tananchai et al., 2019). *Anopheles sinensis* is a main vector of malaria in China (Jia-Yun Pan et al., 2012) *Anopheles darlingi* has an endophilic behaviour and is the main vector of malaria in the Amazon region. The *Anophelines* exhibited a peak of activity in the evening and morning twilight and continued their activity throughout the night in this region (Suarez-Mutis et al., 2009). In Costa Marques, State of Rondonia, Brazil, a decreased number of anophelines collected intra-domiciliary was observed (Santos et al., 1999). In the present study, main vector *An. minimus* was found higher in indoor than outdoor catch by Light traps collection in both study areas.

Anopheles minimus larvae were abundantly collected from Rice field and valley followed by Sand pools and *An. dirus* were found in water wells and valley. *An. minimus*, *An. maculatus* and *An. aconitus* were found co-breeders of *An. dirus* in domestic water wells. The same result has been found in a water well in Ye Township, Mon State (Maung Maung Mya et al., 2020a). *An. minimus* larvae were abundantly found in slowly running water, foothill areas and rice field (Tun Lin et al., 1995; Pe Than Htun et al., 2005; Maung Maung Mya et al., 2019a). Only 2 *An. dirus* in Ohnpinkone and 1 *An. dirus* in Bwedarkone were collected by Indoor light traps. *An. dirus* is a main vector of malaria and abundantly found in Bago Yoma mountain range and larvae were found rock pools, domestic water wells in Mon, Kayin State and Tanintharyi Region (Tun Lin et al., 1995; Maung Maung Mya et al., 2018; Htay Aung et al., 1999).

In the present study F1 generation of *An. minimus* and *An. dirus*, were susceptible to WHO-recommended insecticides as Deltamethrin 0.05%, Permethrin 0.75% and Cyfluthrin 0.15% impregnated paper. All collected secondary vector *An. aconitus, An. varuna, An. annularis, An. philippinensis, An. maculatus, An. kochi* and *An. vagus* were found susceptible to above insecticides. The same result has been observed by other researchers in Taikkiy Township Yangon Region and other parts of Myanmar (Maung

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Maung Mya et al., 2002; Maung Maung Mya et al., 2019a).

Before deltamethrin impregnation, the bio-efficacy of mosquito nets was found very low sensitivity in Ohnpinkone and Bwedarkone was found over 60 % sensitivity. After deltamethrin impregnation for three and six months, the bio-efficacy test was found to be 100% sensitive. After 9 months later the bio-efficacy was slightly reduced to 96.67% sensitivity in the Ohnpinkone ward due to 10-15 times of washing with Fuji detergent cream. Although in control ward Bwedarkone, the bio-efficacy of ITN nets was gradually fallen down to 16.67% sensitivity because collected mosquito nets were washed 20 to 25 times washed and bio efficacy of all mosquito nets were expired their insecticide activity. The use of LLINs has been shown to be a highly cost-effective strategy for malaria prevention, and it has contributed to a significant reduction in disease morbidity and mortality in recent years (WHO, 2011). Same result has been found in the present study, deltamethrin impregnation to old, used and lower efficacy LLINs net were cost affected and gained higher bio-efficacy. All collected LLINs nets were more than 3 years duration of usage. Other researchers revealed that In Brazilian Amazon: Regarding the duration of use LLINs net, most participants in the interventional group used LLINs for≤5 years in all the surveyed years, whereas most participants in the control group used the LLINs for>10 years (Sousa et al., 2019). Some studies that specifically evaluated the use of mosquito nets have found between 15 and 50% of distributed LLINs remain unused (Baume and Marin 2007, Baume et al., 2009, Githinji et al., 2010). A present study found that both Ohnpinkone and Bwedarkone used their LLINs nets for 3 to 4 years; only 3 LLINs net have not been used and kept in showcase in Ohnpinkone.

In the present study, before intervention 10 *P. falciparum* malaria cases were found in the Ohnpinkone ward and one *P. falciparum* malaria case was found in Bwedarkone. After impregnation of used, old and low bio-efficacy LLINs nets, malaria cases were reduced to 0 cases till October it means that 100% reduction in Ohnpinkone was after intervention and very low number of main vector *An. minimus* was observed in January, February. Although main vectors of *An. minimus* was collected in high number in March, April and July and 2 *An. dirus* was collected in June in indoor light traps catch in Ohnpinkone ward. In Brazilian Amazon area, malaria is a seasonal disease with the highest number of cases occurring at the end of the rainy season when there is a greater presence of Anophelines (Sousa et al., 2019). There were no malaria cases found during and after the intervention period in the test ward. But 1 *P. falciparum* positive case was observed in pre-intervention months December and 3 malaria cases (2 Falciparum and 1 Vivax malaria cases) were found after intervention month in control ward Bwedarkone. Main vector *An. minimus* was gradually increased from February to June and declined to October in both test and control wards. The bedroom light traps result in Ohnpinkone were clearly inform that main vectors and other *Anopheles* were no entrance in the bedroom where ITN nets were used, it may be due to the fact that result could reflect the repellent action of the LLINs to vector mosquitoes. The same result has been found in different parts of Myanmar (Maung Maung Mya et al., 2020b). After intervention periods, 100% reduction of malaria cases were found in after deltamethrin impregnation of mosquito nets the positive malaria cases were reduced to 0% in during intervention month January to after intervention month (October). Although in the control ward, Bwedarkone was found 66.67% of positive malaria cases were increased in the after-intervention period. A study in Thanphyuzayet Township revealed that malaria parasite positive rates were significantly reduced when deltamethrin impregnation to cloths in Raber

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plantation workers. (Maung Maung Mya et al., 2014). A study in 2016 in sub-Saharan Africa, 54% of the at-risk population slept under an LLIN, which is a substantial increase from 30% in 2010 (WHO, 2017). It has already been demonstrated that LLINs are important for protecting all individuals in a community, including those who do not sleep under a mosquito net [Kilian et al., 2010].

CONCLUSION

Before intervention there were 10 *P. falciparum* cases in Ohnpinkone and 1 *P. falciparum* case in the Bwedarkone ward. After intervention in Ohnpinkone, the malaria cases were going down to 0. There were 3 malaria-positive cases were found in the control ward Bwedarkone, 66.67% higher up. Main vector *An. minimus* was found higher number in both Ohnpinkone (137) and Bwedarkone (109) wards by outdoor and indoor light trap catch methods. After intervention, no malaria cases and vectors were caught up by indoor bedroom light traps in the test ward, showing 100% recovery till October, although malaria vectors were observed in the indoor light trap in the control ward. Therefore, Deltamethrin impregnation to used LLINs nets with expiry or lower efficacy, become higher bio-efficacy again. Insecticide Treated Nets (ITN) are more effective and hygienic as well as they reduce the mosquito density in the bedroom and indoor as well as reduce the men vector contact. They could either complement or replace some of the preventive measures against the mosquito compare to other mosquito control techniques. Based on the present study, it is apparent that ITN based intervention technique is effective and cost-effective as well as appropriate for the control of malaria transmission and vector mosquitoes that they abundantly present in intervention and non-intervention areas. The bio-efficacy was a decrease of 20 % in Test Ward Ohnpinkone and efficacy persist over 60% in the use of these nets. The bio-efficacy results suggest a significant difference between Test and control wards. WHO revealed that below 80% of bioefficacy of LLINs nats need reimpregnation with insecticides. Therefore, it is necessary to impregnate used and low bio-efficacy mosquito nets if in short supply of new LLINs for the prevention of malaria transmission and the entrance of the main vector of *Anopheles* mosquitoes in the bedroom and in the indoor guest room in the test ward. Study suggested that the strategies used must be permanent in areas of high epidemiological risk and difficult geographical access, where people live at low socio-educational levels and that it is necessary to search for new intervention technues to ensure that the knowledge acquired results in a permanent modification of attitudes and behaviors.

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