THE EFFICACY OF LONG LASTING INSECTICIDAL NETS (LLINS) AND INSECTICIDE TREATED NETS AGAINST *Anopheles* MOSQUITOES

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ABSTRACT

Field bio-efficacy of 0, 5, 10, 15 and 20 times washed PermaNet 2.0, Icon LLIN, Deltamethrin and Icon treated cotton and Nylon nets (ITN) against wild caught *Anopheles* mosquitoes were tested with WHO cone test Kit method in Myaebinthar village Myothit Township, Magwe Region. Mosquitoes were exposed in cones for 5 min and 15 min fixed exposure period under room temperature in raining and cold season. Knockdown effect was measured after 60 min of exposure. In raining and cold season, a total of 221 and 196 mosquitoes, consist of 8 and 6 species of *Anopheles* adults were collected. Maximum number of *An. culicifacies* was caught followed by *An vagus* in cattle bait k net, human bait and lowest was collected in morning resting collection. In both season maximum number of main vector *An. minimus* was caught on human and cattle bait collection between 21:00 and 24:00 hours. The bio efficacy of 0 and 5 washed all PermaNet 2.0, Icon and deltamethrin LLINs and ITNs found 100% knockdown ability with all caught mosquitoes. *An.minimus* and *An.culicifacies* found to be 80-100% knockdown effect against 10 washes with PermaNet 2.0 and Icon LLINs in both season. Knockdown efficacy was significantly declined between 5 and 20 washes, 100-60% knockdown on LLINs and 90-20% for Deltamethrin and Icon treated nets in both season. PermaNet 2.0, Icon LLINs were found higher wash resistance ability till 15 washes than deltamethrin and Icon treated cotton net but nylon net was found lowest wash resistance needs repeated treatment.

Contribution/Originality: This study contributes to existing literature to develop a testing method for investigation the bioefficacy of ITN nets and LLINs nets.

1. INTRODUCTION

Malaria is a tropical human disease caused by protozoan parasite belonging to genus *Plasmodium*, is one of the most important infectious diseases in the World. Its global burden and economic cost are still enormous, and it caused about 225 million cases resulting 781 000 deaths in 2009 [1]. Malaria is considered to be the most prevalent vector-borne disease worldwide. At present 1326 million people or 83% of population in South East Asia (SEA) are at risk of malaria [2]. The prevalence of malaria has not changed significantly in the malaria prone areas. In Myanmar, malaria has been a main priority publication health problem and there are 600,000 malaria cases annually. Morbidity rate in 2006 is 9.1/1000 and mortality rate is 2.97/100000 population [3]. In Myanmar 71% of 55 million populations are residing in malaria risk areas of various hilly, coastal and plain areas of the country. The
poor brae the highest burden of malaria, they are at a higher risk of becoming infected with malaria, because they live in dwellings that offer little protection from mosquitoes yet they may not afford protection methods like insecticide-treated treated nets (ITNs) from mosquitoes [4]. The two main methods: insecticide-treated mosquito nets (ITNs) and residual spraying are known to be highly effective and current evidence suggests that they are very similar in their relative impact on malaria incidence [5]. ITNs are being implemented as part of national malaria control programs around the world [6]. ITNs lead to a reduction of human-vector contact and diminish mosquito population [7, 8] and also provide a physical barrier with high coverage levels that benefit the whole community [9]. There are two categories of ITNs: conventionally treated net and long-lasting insecticidal nets (LLINs). A conventionally treated net is a mosquito net that has been treated by dipping in a World Health Organization (WHO) recommended insecticide. To ensure its continued insecticidal effect, the net should be re-treated regularly, usually after about three washes or at least once a year. LLIN is a factory-treated mosquito net made with netting material that has insecticide incorporated within or bound around the fibers. The net must retain its effective biological activity without re-treatment for at least 20 WHO standard washed under laboratory conditions and three years under fields conditions [10]. Pyrethroids (alpha-cypermethrin, cyfluthrin, deltamethrin, etofenprox, lambda-cyhalothrin and permethrin) are the insecticides that are currently recommended by the WHO Pesticide Evaluation Scheme (WHOPES) for applying on mosquito nets [11, 12]. WHO recommended three LLINs brands are now commercially available: Gloria, et al. [13] and Yorkool LLIN [14].

In Myanmar ParmaNet 2.0 Icon Treated LLINs are commercially available and distributed to community by government and NGOs to prevent mosquitoes in risk areas. Wide spread use of insecticides treated nets and LLINs accelerated insecticide resistance in arthropods. Therefore it is necessary to determine the levels of susceptibility and provide the information needed to decide whether a particular insecticide should continue in use or be replaced by an alternative insecticide. The current study investigates the knock down effect and mortality rate in adult Anopheles mosquitoes after exposure to unwashed and washed Insecticide impregnated nets (ITNs) and Long lasting insecticidal nets (LLINs) for wild Anopheles mosquitoes.

2. MATERIALS AND METHODS

PermaNet 2.0 (LLIN) (160x180x150cm) net is a 100% polyester net (mesh: 156/inch² = 25/cm²) coated with a deltamethrin-containing (55mg/m²), (Receive a full WHO recommendation in 2008), is a registered trademark of DCT S.A. Switzerland, made by Vestergaard Frandsen disease control Textiles.

Deltamethrin treated cotton net and Nylon net were treated with deltamethrin (55mg/m²) according to WHO dipping method.

Icon LLIN (160x180x150cm) net (mesh: 156/inch² = 25/cm²) is a 100% polyethylene net in which Icon is coated (50mg/m²), made by Thailand.

Icon treated cotton net and Nylon net were treated with Icon (50mg/m²) according to WHO dipping method.

Deltamethrin and Icon were parched from local market.

2.1. Study Area

Myaypyinthar village, Myothit Township Magwe Region was selected for the collection of blood fed wild adult Anopheles mosquitoes to test efficacy of long lasting insecticidal nets and insecticide treated nets. Myaypyinthar village is situated beside Magwe Naypyidaw road. The village is 6 kilometer away from Soon dam. Myaypyinthar Myothit car road is across the village. The population is about 2000, 90% of the people are plantation workers, remaining are government workers, and hunters. One primary school and one rural health center are situated in the village.
2.2. Collection of Anopheles Mosquitoes

Anopheles mosquitoes were collected in Myaypyinthar village, Myothit Township Magwe Division. Two corners of the village areas were selected for mosquito collection by WHO sucking tube and in cattle by a big mosquitoes net (330x330x180cm), human bait indoor, outdoor, cow shit collection and morning resting collection were done. The collection of mosquitoes were done for 3 days and three nights volunteers for mosquitoes collection were given mefloquine prophylaxis regimen for a week as a prophylactic measure. Larvae of mosquitoes were collected within 3 km radius of the village for three days and sample of larvae from different sites were brought to DMR laboratory for mosquito species identification and further study.

2.3. Identification of Collected Mosquitoes

Collected Anopheles mosquitoes from different methods (cattle human, indoor and outdoor and morning resting collection) and adult from larva survey were identified by morphological methods according to Barraud [15]; Raid [16]; Harrison and Scanlon [17]; Harrison [18] and Myo, et al. [19].

2.4. Bioassays Test

Insecticide susceptibility of collected some wild life mosquitoes were tested with WHO corn test method [10] with 0, 5, 10, 15, and 20 times washed PermaNet 2.0 LLINs, Icon LLINs, Deltamethrin treated nets and Icon treated nets for 5min and 15min exposure times under room temperature for raining and cold seasons. In this test, three cones each were fixed horizontally on each net, and five wild collected adult Anopheles females were exposed to each cone for 5mins and 15mins. The mosquitoes were then removed from cones and placed in paper cups then provide water and sugar as food. The percentage of knock down (KD) was recorded after 60 min after exposure. Each test was done three time using 5 mosquitoes per cone/ net.

2.5. Statistical Analysis

Field data were analyzed by using Microsoft EXCEL. Percentage Knockdown (KD) vs number of washes were calculated.

3. RESULTS

3.1. Adult Mosquitoes Collection

1. A total of 221 and 196 Anopheles adult mosquitoes were collected in raining and cold season by different catching methods and only 8 species in raining and 6 pieces in cold season were found in this area Table 1 and Table 2. Maximum number of An. culicifacies was caught followed by An. vagus in cattle bate k net and human bait collection as well as very small number was collected in morning resting collection. Maximum number of the main vector of An. minimus was caught in cattle and human bait collection between 21:00 and 24:00 hours periods in both seasons.

3.2. Larva Survey

Table 2 shows the mosquitoes emerged from 74 anopheles larvae collected from different breeding sites. An culicifacies larvae were found in maximum number followed by An.vagus, An maculatus, very few number of An minimus larvae were collected in water pools and creek beside the village.

3.3. Insecticide Susceptibility Test (5min Exposure)

Collected main vector of An. minimus and secondary vectors of An. annularis, An. culicifacies, An. tersellatus, An. maculatus as well as non-vector of An. barbirostris were tested for susceptibility on different washed PermaNet 2.0, Icon LLINs and Deltamethrin and Icon treated cotton and nylon nets under different exposure time.
3.4. PermaNet 2.0 LLIN and Deltamethrin Treated Cotton and Nylon Net ITN

![Figure 1](image1.png)

**Figure 1.** Knockdown effect of *Anopheles* mosquitoes against different washing frequencies of LLINs and Deltamethrin insecticide treated nets under 5 min exposure time in rainy season.

**Source:** Myaypyinthar village, Myothit Township.

Figure 1 and Table 1 shows that PermaNet 2.0 LLIN: After testing 5 min exposure, Knockdown effect of mosquitoes on unwashed PermaNet 2.0 was found 100% in all collected main and secondary vectors of *Anopheles* mosquitoes within one hour. And also same 100% knockdown effects were found with unwashed deltamethrin treated cotton and nylon nets. After 5 washed 100% knockdown were found in *An. minimus*, *An. culicifacies*, *An. aconitus*, *An. tersellatus*, and *An. maculatus* and 93.33% were found in *An. annularis*, *An. vagus* and *An. barbirostris* against PermaNet 2.0. After 5 washes the percentages of knockdown were 80-86% for both cotton and nylon net.

After 10 washes of PermaNet 2.0 LLIN, percentage of knockdown was observed 80 to 93.33% in all tested main and secondary vectors of malaria. After 10 washes of deltamethrin treated cotton and nylon nets found that 80% knockdown in *An. minimus*, 73.33% in *An. annularis* and *An. aconitus* and remaining were found 60-66.67% against cotton net and 10 washes nylon net found to be 40-46.67% knockdown in all tested *Anopheles* mosquitoes.

After 15 washes of PermaNet 2.0 LLIN the percentage of knockdown for the washed net was found 80% in *An.minimus* and *An.culicifacies* and remaining *Anopheles* mosquitoes were found between 66-73% knockdown. After 15 washes of deltamethrin treated cotton net, 66.67% knockdown was observed in main vector of *An. minimus* remaining mosquitoes were found to be 53-60% knockdown. After 15 washes of deltamethrin treated nylon nets found to be 13-20% knockdown in all tested *Anopheles* mosquitoes.

After 20 washes, the efficacy of the PermaNet 2.0 LLIN decreased to 60-66.67 % knockdown in all mosquitoes.

Although after 20 washes of Cotton net and nylon net found to be 13-20% and 0-6% knockdown effect with all tested mosquitoes.

3.5. Icon LLIN and Icon Treated Cotton and Nylon Nets in Raining Season

Figure 2 and Table 2 shows that Icon LLIN: After testing 5 min exposure, knockdown effect of mosquitoes on unwashed Icon was found 100% in all collected main and secondary vectors of *Anopheles* mosquitoes within one hour. And also same 100% knockdown effects of primary and secondary vectors were found with unwashed Icon treated cotton net and 93-100% knockdown effects of primary and secondary vectors were found with unwashed nylon nets. After 5 washed 93.33% knockdown were found in all tested mosquitoes except 86.67% was found in *An. barbirostris* against Icon LLIN net and after 5 washes of Icon treated cotton net but 5 washes nylon net, 60% knockdown were found in *An minimus*, *An.culicifacies*, *An. annularis*, *An aconitus* and *An. tersellatus* and remaining were found 46-53%.
After 10 washes of Icon LLIN net the knockdown effect of all mosquitoes were found to be 73-86% knockdown. After 10 washes of Icon treated cotton net found 66-80% knockdown of all primary and secondary vectors of Anopheles mosquitoes and with nylon nets found to be 26-33.33% knockdown in all tested mosquitoes except An. maculatus observed 40% knockdown.

After 15 washes of Icon LLIN showed that, the knockdown was ranging from 60-66.67% in all Anopheles mosquitoes but 40-46.67% with Icon treated cotton net. The knockdown affect after 15 washes of Icon treated nylon net was decreased to 13-26% in all mosquitoes.

After 20 washes of Icon LLIN found 30% to 40% knockdown in all mosquitoes and decreased knockdown efficacy after 20 washes of Icon treated cotton net and nylon net i.e. ranging between 6-20% and 0-13% respectively.

3.6. Permanet 2.0 LLIN and Deltamethrin Treated Cotton and Nylon Net ITN in Cold Season

Table 3 and Figure 3 shows that the main vector of An. minunus and secondary vectors of An. annularis, An. culicifacies, An. tersellatus and An. maculatus and non vector of An barbirostris were tested for susceptibility to different washed PermaNet 2.0, Icon LLIN and deltamethrin and Icon treated cotton and nylon nets under 5 min exposure time in cold season.
PermaNet 2.0 LLIN: After testing 5 min exposure, knockdown effect of mosquitoes on unwashed PermaNet 2.0 was found 100% in all collected main and secondary vectors of *Anopheles* mosquitoes within one hour. And also same 100% knockdown effects were found with unwashed Deltamethrin treated cotton and nylon nets. After 5 washes 86-93% knockdown were found in all tested anopheles mosquitoes against PermaNet 2.0. After 5 washes the percentage of knockdown were 80-86% for both cotton and nylon net.

After 10 washes of PermaNet 2.0 LLIN, percentage of knockdown was observed 80% in all tested main and secondary vectors of malaria. After 10 washes of Deltamethrin treated cotton nets found that 80% knockdown in main vector of *An. minimus* and secondary vectors of *An. annularis, An. culicifacies, An. tersellatus* and *An. maculatus* and 10 washes nylon net found to be 40-53% knockdown in all tested *Anopheles* mosquitoes.

After 15 washes of PermaNet 2.0 LLIN the percentage of knockdown for the washed net was found 73.33% knockdown in all tested mosquitoes. After 15 washes of Deltamethrin treated cotton net, 46-60% knockdown was observed in main vector of *An. minimus* and secondary vectors of mosquitoes. After 15 washes of Deltamethrin treated nylon nets found to be 13-26% knockdown in all tested *Anopheles* mosquitoes.

After 20 washes of PermaNet 2.0 LLIN the percentage of knockdown for the washed net was found 53-60% knockdown in all tested mosquitoes. Cotton and nylon nets were found lower efficacy than PermaNet 2.0 LLIN nets.

### 3.7. Icon LLIN and Icon Treated Cotton and Nylon Nets in Cold Season

![Figure 4](image)

**Figure 4.** Knockdown effect of *Anopheles* mosquitoes against different washing frequencies of Icon LLIN and Icon treated nets under 5 min exposure time in Cold season.

**Source:** Myaypyinthar village, Myothit Township.

Table 4 and Figure 4 shows that Icon LLIN: After testing 5 min exposure, knockdown effect of mosquitoes on unwashed Icon was found 93 to 100% in all collected main and secondary vectors of *Anopheles* mosquitoes within one hour. And also same 93-100% knockdown effects of primary and secondary vectors were found with unwashed Icon treated cotton and 86-100% knockdown effects of primary and secondary vectors were found with unwashed nylon nets. Knockdown effect of 5 washes of Icon LLIN, Icon treated cotton net and Icon treated Nylon nets were ranging between 86-93.33%, 80-86.67% and 73-86.67% respectively in all tested *Anopheles* mosquitoes.

After 10 washes of Icon LLIN net the knockdown effect of all mosquitoes were found to be 80-86% knockdown. After 10 washes of Icon treated cotton net found 60-77% knockdown of all primary and secondary vectors of *Anopheles* mosquitoes and with nylon nets found to be 33.33-46.67% knockdown in all tested mosquitoes.
After 15 washes of Icon LLIN showed the knockdown ranging from 60-66.67% in all Anopheles mosquitoes except An. barbirostris and 46-60% with Icon treated cotton net. After 15 washes of Icon treated nylon net the knockdown effect was decreased to 13-20% in all mosquitoes.

After 20 washes of Icon LLIN found 40 to 48% knockdown in all mosquitoes and knockdown efficacy after 20 washes of Icon treated cotton net and nylon net were ranging between 6-20% and 0-13% respectively.

3.8. Insecticide Susceptibility Test (15min Exposure) in Raining Season

The main vector of An. minunus and secondary vectors of An. annularis, An. culicifacies, An. tersellatus and An. maculatus and non vector of An barbirostris were tested for susceptibility to different washed PermaNet 2.0, Icon LLIN and Deltamethrin and Icon treated cotton and nylon nets under 15min exposure time in raining season.

3.9. PermaNet 2.0 LLIN And Deltamethrin Treated Cotton and Nylon Net ITN

Table 5 and Figure 5 shows that PermaNet 2.0 LLIN, Deltamethrin treated cotton net and Deltamethrin treated nylon net: After testing 15 min exposure, Knockdown effect of all anopholes mosquitoes on unwashed PermaNet 2.0, cotton ITN and Nylon ITN were found 100% knockdown in all collected main and secondary vectors of Anopheles mosquitoes within one hour. After 5 washes 100% knockdown were found all tested Anopheles mosquitoes against PermaNet 2.0. After 5 washes of both cotton and nylon nets were observed 86-100% knockdown effect.

After 10 washes of PermaNet 2.0 LLIN net, the knockdown effect of all mosquitoes was found to be range between 93-100% knockdown. After 10 washes of Deltamethrin treated cotton net and nylon net were found 80-86.67% and 66-80% knockdown of all primary and secondary vectors of Anopheles mosquitoes.

After 15 washes of Permanet 2.0 LLIN net, cotton and nylon net were found to be ranges between 73-86.67%, 53-60% and 40-46.67% knockdown respectively.

After 20 washes of all tested mosquito nets were found decrease knockdown efficacy ranging between 66-73.33% with PermaNet2.0, 33-46.67% with Cotton ITN and 20-26.67% with Nylon ITN against Anopheles mosquitoes respectively.
3.10. Icon LLIN and Icon Treated Cotton and Nylon Nets (15min Exposure) in Raining Season

Table 6 and Figure 6 shows that after testing of 15 min exposure with unwashed Icon LLIN, Icon treated cotton and Nylon net were found 100% knockdown effect of all collected primary and secondary vectors of Anopheles mosquitoes within one hour. After 5 washes of Icon LLIN, Icon treated Cotton net and Nylon net were found 93-100%, 86-100% and 80-100% knockdown respectively. After 10 washes of tested mosquitoes nets, the knockdown effect of mosquitoes were found 86.67% with Icon LLIN, 80-86% with Icon treated Cotton net and 53-60% with Nylon net respectively. After 15 washes of Icon LLIN net, cotton and nylon net were found to be ranges between 66-73%, 46-53% and 26-40% knockdown respectively. After 20 washes of all tested mosquito nets were observed decrease knockdown efficacy ranging between 46-60% with Icon LLIN, 26-33% with Cotton ITN and 13-20% with Nylon ITN against Anopheles mosquitoes respectively.

3.11. Insecticide Susceptibility Test (15min Exposure) in Cold Season

The main vector of An. minimus and secondary vectors of An. annularis, An. culicifacies, An. tersellatus, An. maculatus and non malaria vector of An barbirostris were tested for susceptibility to different washed PermaNet 2.0, Icon LLIN and Deltamethrin and Icon treated cotton and nylon nets under 15min exposure time in cold season.

3.12. Permanet 2.0 LLIN and Deltamethrin Treated Cotton and Nylon Net ITN

Table 6 and Figure 6 shows that after testing of 15 min exposure with unwashed Icon LLIN, Icon treated cotton and Nylon net were found 100% knockdown effect of all collected primary and secondary vectors of Anopheles mosquitoes within one hour. After 5 washes of Icon LLIN, Icon treated Cotton net and Nylon net were found 93-100%, 86-100% and 80-100% knockdown respectively. After 10 washes of tested mosquitoes nets, the knockdown effect of mosquitoes were found 86.67% with Icon LLIN, 80-86% with Icon treated Cotton net and 53-60% with Nylon net respectively. After 15 washes of Icon LLIN net, cotton and nylon net were found to be ranges between 66-73%, 46-53% and 26-40% knockdown respectively. After 20 washes of all tested mosquito nets were observed decrease knockdown efficacy ranging between 46-60% with Icon LLIN, 26-33% with Cotton ITN and 13-20% with Nylon ITN against Anopheles mosquitoes respectively.
Table 7 and Figure 7 shows that after testing 15 min exposure of all anopheles mosquitoes on unwashed and 5 washes PermaNet 2.0, Cotton ITN and Nylon ITN were found 100% knockdown in all collected main vector and secondary vectors of *Anopheles* mosquitoes within one hour.

After 10 washes of Permanet 2.0 LLIN net, cotton net and Nylon net were found 93.33%, 86.67% and 73-80% knockdown effect of all tested mosquitoes respectively.

After 15 washes of Permanet 2.0 LLIN net, cotton net and Nylon net were found 86-93.33%, 73-80% and 46-66.67% knockdown effect of all tested mosquitoes respectively.

After 20 washes of PermaNet 2.0, Deltamethrin treated cotton net and nylon net were found to be 80-86%, 53-60% and 33-40% knockdown of all primary and secondary vectors of tested *Anopheles* mosquitoes.

### 3.13. Icon LLIN and Icon Treated Cotton and Nylon Nets (15min Exposure Time in Cold Season)

![Figure 8. Knockdown effect of Anopheles mosquitoes against different washing frequencies of Icon LLIN, Icon treated Cotton and Nylon nets under 15 min exposure time in cold season. Source: Myaypyinthar village, Myothit Township.](image)

Table 8 and Figure 8 after testing of 15 min exposure with unwashed Icon LLIN, Icon treated cotton and Nylon net were found 100% knockdown effect of all collected primary and secondary vectors of *Anopheles* mosquitoes within one hour. After 5 washes of Icon LLIN, Icon treated Cotton net and Nylon net were found 93-100%, 86-100% and 86-100% knockdown respectively.

After 10 washes of tested mosquitoes nets, the knockdown effect of mosquitoes were found 80% with Icon LLIN, 73-80% with Icon treated Cotton net and 46-53% with Nylon net respectively.

After 15 washes of Icon LLIN net, cotton and nylon net were found to be ranges between 66.67%, 40-53% and 26-33.33% knockdown respectively.

After 20 washes of all tested mosquito nets were found decrease the knockdown efficacy range between 46-53% with Icon LLIN, 33-40% with Cotton ITN and 13-20% with Nylon ITN against *Anopheles* mosquitoes respectively.

### 4. DISCUSSION

New types of insecticide treated bed nets ITN and LLIN are being made available in the current, worldwide campaign against malaria. The current study evaluated the efficacy of unwashed and washed PermaNet 2.0, Icon LLIN, Deltamethrin treated Cotton net and Nylon net, Icon treated Cotton and Nylon net under field with wild collected primary malaria vector of *An. minimus*, and secondary vectors of *An. annularis*, *An. culicifacies*, *An. aconitus*, *An. vagus* *An. tersellatus* and *An. maculatus* and non vector of *An. barbirostris* were tested for susceptibility of the mosquitoes in raining and cold season for 5 and 15 min exposure on net by Cone [10]. Knockdown effect was measured after 60 minute. Pyrethroid insecticides are neurotoxins and neurophysiological studies show that the knockdown effect is caused by poisoning of the peripheral nerves [20, 21]. In raining 8 species of Anopheles
mosquitoes were caught in Maybintha village, maximum number of \textit{An. culicifacies} was caught followed by \textit{An. vagus} in cattle bate k n. The main vector of \textit{An. minimus} was caught in cattle and human bait collection between 9:00 to 12:00 pm periods and in cold seasons 6 species of Anopheles mosquitoes were caught i.e. \textit{An. annularis, An. culicifacies, An. minimus, An. tersellatus, An. minuscus}. The main vector of \textit{An minimus} were caught in Second quarter of night at 9:00-12:00 pm. Other researcher also found that \textit{An minimus} is a midnight blood sucker in Thabwewa village Bago Division \cite{22}. The efficacy evaluation of 0 wash PermaNet 2.0 and Icon LLIN were found 100% knockdown with all tested Anopheles mosquitoes in raining and cold season and similar results were found unwashed deltamethrin and Icon treated cotton and Nylon nets in both raining and cold season with 5min and 15min exposed in WHO cone after 60 min exposure. After 5 washes, the percentage of knockdown was 100% for \textit{An.minimus} and \textit{An.culicifacies}, remaining mosquitoes were 90-100% knockdown with PermaNet 2.0, Icon LLIN and deltamethrin treated nets and also Icon treated cotton and nylon nets were found 70-85% knockdown of all mosquitoes with cone test method after 5 washes for 5min and 80-100% knockdown after washes for 15min exposure in both season. At 10 washes percentage of knockdown were slightly decrease to 80-90% for PermaNet 2.0 and Icon LLIN. The results are agree with a susceptibility study of \textit{An. minuscus} in Assam India \cite{23}. Deltamethrin and Icon treated cotton net found higher knockdown effect (65-80%) than nylon net (35-65%) after 10 washes. Jaranillo and his associate revealed that 1 to 3 washes Permanet 2.0 showed higher mortality and knockdown of \textit{An. albimanus} that regeneration time persisted for 15 washes without losing efficacy \cite{24}. Present study found that Knockdown efficacy of PermaNet 2.0 and Icon LLIN net decline slowly from 5 washes to 20 washes. The knockdown effect was 1.2-1.5 folds higher than 20 washes (60-80% knockdown). These results confirm the long lasting insecticidal action of these nets have higher wash resistance. \textit{Graham, et al.} \cite{25} reported an approximately 82% knockdown after 20 washing of the PermaNet in Pakistan against \textit{An. stephensi} and also \cite{23} revealed that Knockdown and mortality rate declined to 72% after 15 washes then 47% after 20 washes. These results are agree with the present study but slightly different than results from several other studies, in which the efficacy of PermaNet 2.0 was maintained even after 20 washes under laboratory and field conditions against some of the most important disease vectors around the worlds \cite{25-29}. This could be due to difference in insecticide susceptibility or landing behavior of the \textit{Anopheles} mosquitoes. Deltamethrin and Icon treated cotton nets found gradually declined after 10 washes to 20 washes and also highly decline of knockdown was found with Nylon ITN after 5 washes to 20 washes i.e. 8-25 folds in raining, 10-15 fold in cold season. Wash resistant of PermaNet 2.0 and Icon LLIN against 5, 10, 15 and 20 washes were higher than deltamethrin and Icon treated cotton and Nylon nets. \textit{Sreehari, et al.} \cite{28} observed that increased efficacy of PermaNet in producing more than 80% mortality in \textit{An.culicifacies} and \textit{An. stephensi} mosquitoes after up to 20 hand washes and up to 10 machine washes. PermaNet 2.0 retained its efficacy longer with successive washes using different washing method as machine, hand and washing on rocks \cite{29}. When compare with 0, 5,10,15 and 20 washes deltamethrin and Icon treated cotton ITN net with same insecticide treated Nylon nets, 0 and 5washes were not significantly difference with other but after 10, 15 and 20 washes cotton ITN net were highly effective then Nylon net it mean that higher washes resistant than Nylon ITN net in both season according to 5 and 15 min exposed in WHO cone \cite{10}. PermaNet 2.0 net had reasonably good wash resistance for up to 15 washes against \textit{An.minimus} the major vector of malaria and \textit{An.culicifacies} secondary vector of malaria in Myanmar under the environmental condition of Myebinthar village Myothit Township Magwe Regional Division. PermaNet 2.0 net, Icon LLIN, deltamethrin and Icon treated nets reduced the indoor biting of mosquitoes. They were accepted by community. Large scale studies are needed to study the bio-efficacy of both LLIN nets and ITN net for after long term use in field condition using local washing practices to confirm its insecticidal efficacy against malaria vector species and to retreat the nets in malaria endemic area of Myothit Township, Magwe Regional Division.
Table 1. Knockdown effect of different *Anopheles* mosquitoes against different washes of PermaNet 2.0, Deltamethrin treated Cotton and Nylon nets for 5min exposure in raining season.

| Species       | No. of tested mosq | PermaNet 2.0 Number of washes | Deltamethrin treated cotton ITN Number of washes | Deltamethrin treated nylon ITN Number of washes |
|---------------|---------------------|-----------------------------|-----------------------------------------------|-----------------------------------------------|
|               |                     | 0% | 5%   | 10%  | 15%  | 20% | 0%   | 5%   | 10%  | 15%  | 20% | 0%   | 5%   | 10%  | 15%  | 20% |
| *An. annularis* | 15                  | 15 | 14    | 13   | 11   | 10  | 15   | 13   | 11   | 9    | 2    | 15   | 13   | 7    | 5    | 1   |
|               |                     | 100 | 93.33 | 86.67 | 73.33 | 66.67 | 100 | 86.67 | 73.33 | 60.00 | 13.33 | 100 | 86.67 | 46.67 | 20.00 | 6.67 |
| *An. barbirostris* | 15              | 15 | 14    | 12   | 10   | 8   | 15   | 12   | 9    | 7    | 2    | 15   | 12   | 6    | 2    | 0   |
|               |                     | 100 | 93.33 | 80   | 66.67 | 53.33 | 100 | 80.00 | 60.00 | 46.67 | 13.33 | 100 | 80.00 | 40.00 | 13.33 | 0.00 |
| *An. culicifacies* | 15               | 15 | 15    | 14   | 12   | 10  | 15   | 12   | 9    | 8    | 2    | 15   | 12   | 6    | 3    | 1   |
|               |                     | 100 | 100 | 93.33 | 80.00 | 66.67 | 100 | 80.00 | 60.00 | 53.33 | 13.33 | 100 | 80.00 | 40.00 | 20.00 | 6.67 |
| *An. minimus* | 15                  | 15 | 15    | 14   | 12   | 10  | 15   | 13   | 12   | 10   | 3    | 15   | 13   | 7    | 3    | 1   |
|               |                     | 100 | 100 | 93.33 | 80.00 | 66.67 | 100 | 86.67 | 80.00 | 66.67 | 20.00 | 100 | 86.67 | 46.67 | 20.00 | 6.67 |
| *An. acontius* | 15                  | 15 | 15    | 14   | 11   | 10  | 15   | 13   | 11   | 9    | 2    | 15   | 13   | 7    | 5    | 0   |
|               |                     | 100 | 100 | 93.33 | 86.67 | 66.67 | 100 | 86.67 | 73.33 | 60.00 | 13.33 | 100 | 86.67 | 46.67 | 20.00 | 0.00 |
| *An. vagus* | 15                  | 15 | 14    | 12   | 11   | 9   | 15   | 12   | 10   | 9    | 2    | 15   | 12   | 6    | 2    | 0   |
|               |                     | 100 | 93.33 | 80.00 | 73.33 | 60.00 | 100 | 80.00 | 66.67 | 60.00 | 13.33 | 100 | 80.00 | 40.00 | 13.33 | 0.00 |
| *An. tersellatus* | 15               | 15 | 15    | 13   | 11   | 10  | 15   | 13   | 10   | 8    | 3    | 15   | 13   | 6    | 2    | 0   |
|               |                     | 100 | 100 | 86.67 | 73.33 | 66.67 | 100 | 86.67 | 66.67 | 53.33 | 20.00 | 100 | 86.67 | 40.00 | 13.33 | 0.00 |
| *An. maculatus* | 15                  | 15 | 15    | 14   | 11   | 10  | 15   | 13   | 10   | 8    | 2    | 15   | 13   | 6    | 3    | 1   |
|               |                     | 100 | 100 | 93.33 | 73.33 | 66.67 | 100 | 86.67 | 66.67 | 53.33 | 13.33 | 100 | 86.67 | 40.00 | 20.00 | 6.67 |
| Average       | 15                  | 15 | 14.63 | 13.25 | 11.13 | 9.63 | 15.00 | 12.63 | 10.25 | 8.50 | 2.25 | 15.00 | 12.63 | 6.38 | 2.63 | 0.50 |
|               |                     | 100 | 97.50 | 88.33 | 74.17 | 64.17 | 100 | 84.17 | 68.33 | 56.67 | 15.00 | 100 | 84.17 | 42.50 | 17.50 | 3.33 |
| Mean          | 15                  | 15 | 14    | 12    | 11   | 8   | 15   | 12    | 9    | 7    | 2    | 15   | 12   | 6    | 2    | 0   |
| SD            | 0                   | 0.51 | 0.88 | 0.64 | 0.74 | 0.51 | 0.51 | 0.92 | 0.46 | 0.517 | 0.517 | 0.517 | 0.517 | 0.534 |

Source: Myaypyinthar village, Myothit Township.
Table 2. Knockdown effect of different Anopheles mosquitoes against different washes of Icon LLIN, Icon treated Cotton and Nylon nets for 5min exposure in raining season.

| Species          | No. of tested mosq. | Icon LLIN Number of washes | Icon treated cotton ITN Number of washes | Icon treated nylon ITN Number of washes |
|------------------|---------------------|-----------------------------|-----------------------------------------|----------------------------------------|
|                  |                     | 0%  | 5%  | 10% | 15% | 20% | 0%  | 5%  | 10% | 15% | 20% | 0%  | 5%  | 10% | 15% | 20% |
| An. annularis    | 15                  | 15  | 14  | 13  | 9   | 5   | 15  | 14  | 12  | 6   | 3   | 15  | 9   | 7   | 4   | 2   |
| An. barbirostris | 15                  | 100 | 93.33 | 86.67 | 60.00 | 33.33 | 100 | 93.33 | 80.00 | 40.00 | 20.00 | 100 | 60.00 | 46.67 | 26.67 | 13.33 | 0 |
| An. culicifacies | 15                  | 100 | 93.33 | 86.67 | 73.33 | 66.67 | 100 | 86.67 | 73.33 | 33.33 | 6.67 | 86.67 | 46.67 | 33.33 | 20.00 | 0.00 |
| An. minimus      | 15                  | 100 | 93.33 | 80.00 | 66.67 | 40.00 | 100 | 93.33 | 80.00 | 40.00 | 20.00 | 100 | 60.00 | 40.00 | 26.67 | 13.33 | 0 |
| An. aconitus     | 15                  | 100 | 93.33 | 73.33 | 73.33 | 40.00 | 100 | 93.33 | 73.33 | 40.00 | 6.67 | 93.33 | 60.00 | 40.00 | 26.67 | 0.00 |
| An. vagus        | 15                  | 100 | 93.33 | 80.00 | 66.67 | 33.33 | 100 | 93.33 | 80.00 | 40.00 | 6.67 | 93.33 | 53.33 | 33.33 | 20.00 | 6.67 |
| An. tersellatus  | 15                  | 100 | 93.33 | 73.33 | 66.67 | 40.00 | 100 | 93.33 | 66.67 | 40.00 | 6.67 | 93.33 | 60.00 | 40.00 | 20.00 | 6.67 |
| An. maculatus    | 15                  | 100 | 93.33 | 80.00 | 60.00 | 40.00 | 100 | 93.33 | 80.00 | 46.67 | 13.33 | 93.33 | 53.33 | 46.67 | 13.33 | 6.67 |
| Average          | 15                  | 15.00 | 13.88 | 11.75 | 9.25 | 5.38 | 15.00 | 13.88 | 11.50 | 6.00 | 1.75 | 14.25 | 8.50 | 6.00 | 3.38 | 1.00 |
| Mean             | 15                  | 15.00 | 13.88 | 11.75 | 9.25 | 5.38 | 15.00 | 13.88 | 11.50 | 6.00 | 1.75 | 14.25 | 8.50 | 6.00 | 3.38 | 1.00 |
| SD               | 15                  | 0.353 | 0.353 | 1.035 | 0.7440 | 0.7440 | 0.353 | 0.755 | 0.534 | 0.600 | 0.886 | 0.707 | 0.755 | 0.755 | 0.7440 | 0.755 |

Source: Myaypyinthar village, Myothit Township.
Table-3. Knockdown effect of different *Anopheles* mosquitoes against different washes of PermaNet 2.0, Deltamethrin treated Cotton and Nylon nets for 5min exposure in cold season.

| Species          | No. of tested Mosq | PermaNet 2.0 Number of washes | Deltamethrin treated cotton ITN Number of washes | Deltamethrin treated nylon ITN Number of washes |
|------------------|---------------------|-------------------------------|-----------------------------------------------|-----------------------------------------------|
|                  |                     | 0 % | 5 % | 10 % | 15 % | 20 % | 0 % | 5 % | 10 % | 15 % | 20 % | 0 % | 5 % | 10 % | 15 % | 20 % |
| *An. annularis*  | 15                  | 15  | 14  | 12   | 11   | 9    | 15  | 13  | 12   | 7    | 3    | 15  | 13  | 7    | 4    | 1    |
|                  |                     | 100 | 93.33 | 80.00 | 73.33 | 60.00 | 100 | 86.67 | 80.00 | 46.67 | 20.00 | 100 | 86.67 | 46.67 | 26.67 | 6.67 |
| *An. barbirostris* | 15               | 15  | 13  | 12   | 10   | 8    | 15  | 12  | 10   | 5    | 1    | 15  | 12  | 6    | 2    | 0    |
|                  |                     | 100 | 86.67 | 80.00 | 66.67 | 53.33 | 100 | 80.00 | 66.67 | 33.33 | 6.67  | 100 | 80.00 | 40.00 | 13.33 | 0.00 |
| *An. culicifacies* | 15              | 15  | 14  | 12   | 11   | 9    | 15  | 13  | 12   | 7    | 3    | 15  | 13  | 8    | 3    | 1    |
|                  |                     | 100 | 93.33 | 80.00 | 73.33 | 60.00 | 100 | 86.67 | 66.67 | 46.67 | 20.00 | 100 | 86.67 | 53.33 | 20.00 | 6.67 |
| *An. minimus*    | 15                  | 15  | 14  | 12   | 11   | 9    | 15  | 13  | 12   | 7    | 3    | 15  | 13  | 7    | 2    | 1    |
|                  |                     | 100 | 93.33 | 80.00 | 73.33 | 60.00 | 100 | 86.67 | 66.67 | 46.67 | 20.00 | 100 | 86.67 | 46.67 | 13.33 | 6.67 |
| *An. tersellatus* | 15                 | 15  | 13  | 12   | 11   | 9    | 15  | 13  | 12   | 8    | 2    | 15  | 13  | 6    | 3    | 1    |
|                  |                     | 100 | 86.67 | 80.00 | 73.33 | 60.00 | 100 | 86.67 | 66.67 | 53.33 | 13.33 | 100 | 86.67 | 40.00 | 20.00 | 6.67 |
| *An. maculatus*  | 15                  | 15  | 14  | 12   | 11   | 9    | 15  | 13  | 12   | 9    | 3    | 15  | 13  | 8    | 4    | 1    |
|                  |                     | 100 | 93.33 | 80.00 | 73.33 | 60.00 | 100 | 86.67 | 66.67 | 53.33 | 13.33 | 100 | 86.67 | 40.00 | 20.00 | 6.67 |
| Average          |                     | 15.00 | 13.67 | 12.00 | 10.83 | 8.83 | 15.00 | 12.83 | 11.67 | 7.17 | 2.50 | 15.00 | 12.83 | 7.00 | 3.00 | 0.83 |
| SD               |                     | 100.0 | 91.18 | 80.00 | 72.22 | 58.89 | 100.0 | 85.56 | 77.78 | 47.78 | 16.67 | 100.0 | 85.56 | 46.67 | 20.00 | 5.56 |
| Mean             |                     | 0.00 | 0.52 | 0.00 | 0.41 | 0.41 | 0.00 | 0.41 | 0.82 | 1.33 | 0.84 | 0.00 | 0.41 | 0.89 | 0.89 | 0.41 |

Source: Myaypyinthar village, Myothit Township.
| Species            | No. of tested mosq | PermaNet 2.0 Number of washes | Deltamethrin treated cotton ITN Number of washes | Deltamethrin treated nylon ITN Number of washes |
|--------------------|--------------------|-------------------------------|-------------------------------------------------|-------------------------------------------------|
|                    |                    | 0 % | 5 % | 10 % | 15 % | 20 % | 0 % | 5 % | 10 % | 15 % | 20 % | 0 % | 5 % | 10 % | 15 % | 20 % |
| An. annularis      | 15                 | 15  | 14  | 13  | 9   | 7   | 15  | 12  | 10  | 7   | 2   | 15  | 12  | 6   | 3   | 1   |
|                    | 100                | 93.33 | 86.67 | 60.00 | 46.67 | 100 | 80.00 | 66.67 | 46.67 | 13.33 | 100 | 80.00 | 40.00 | 20.00 | 6.67 |
| An. barbirostris   | 15                 | 14  | 13  | 12  | 8   | 5   | 14  | 11  | 9   | 5   | 2   | 14  | 11  | 5   | 2   | 0   |
|                    | 93.33              | 86.67 | 80.00 | 53.33 | 33.33 | 93.33 | 73.33 | 60.00 | 33.33 | 13.33 | 93.33 | 73.33 | 33.33 | 13.33 | 0.00 |
| An. culicifacies   | 15                 | 15  | 13  | 12  | 10  | 7   | 15  | 13  | 10  | 7   | 3   | 15  | 13  | 7   | 3   | 2   |
|                    | 100                | 86.67 | 80.00 | 66.67 | 46.67 | 100 | 86.67 | 66.67 | 46.67 | 20.00 | 100 | 86.67 | 46.67 | 20.00 | 13.33 |
| An. minimus        | 15                 | 15  | 14  | 13  | 10  | 7   | 15  | 13  | 11  | 9   | 1   | 15  | 11  | 7   | 3   | 1   |
|                    | 100                | 93.33 | 86.67 | 66.67 | 46.67 | 100 | 86.67 | 73.33 | 60.00 | 6.67  | 100 | 73.33 | 46.67 | 20.00 | 6.67 |
| An. tersellatus    | 15                 | 14  | 14  | 13  | 10  | 6   | 14  | 13  | 10  | 7   | 2   | 13  | 11  | 6   | 2   | 1   |
|                    | 93.33              | 93.33 | 86.67 | 66.67 | 40.00 | 93.33 | 86.67 | 66.67 | 46.67 | 13.33 | 86.67 | 73.33 | 40.00 | 13.33 | 6.67 |
| An. maculatus      | 15                 | 14  | 14  | 13  | 10  | 6   | 14  | 13  | 11  | 8   | 1   | 14  | 11  | 7   | 3   | 1   |
|                    | 93.33              | 93.33 | 86.67 | 66.67 | 40.00 | 93.33 | 86.67 | 73.33 | 53.33 | 6.67  | 93.33 | 73.33 | 46.67 | 20.00 | 6.67 |
| Average            |                    | 14.50 | 13.67 | 12.67 | 9.50 | 6.33 | 14.50 | 12.50 | 10.17 | 7.17 | 1.83 | 14.33 | 11.50 | 6.33 | 2.67 | 1.00 |
| Mean               |                    | 96.67 | 91.11 | 84.44 | 63.33 | 42.22 | 96.67 | 83.33 | 67.78 | 47.78 | 12.22 | 95.56 | 76.56 | 42.22 | 17.78 | 6.67 |
| SD                 | 15                 | 0.55  | 0.52  | 0.52  | 0.84  | 0.82  | 0.55  | 0.84  | 0.75  | 1.33  | 0.75  | 0.82  | 0.84  | 0.82  | 0.52  | 0.63 |

**Source:** Myaypyinthar village, Myothit Township.
Table 5. Knockdown effect of different *Anopheles* mosquitoes against different washes of PermaNet 2.0, Deltamethrin treated Cotton and Nylon nets for 15min exposure in raining season.

| Species       | No. of tested mosq | PermaNet 2.0 Number of washes | Deltamethrin treated cotton ITN Number of washes | Deltamethrin treated nylon ITN Number of washes |
|---------------|---------------------|-------------------------------|-----------------------------------------------|-----------------------------------------------|
|               | 0%  | 5%   | 10%  | 15%  | 20%  | 0%  | 5%   | 10%  | 15%  | 20%  | 0%  | 5%   | 10%  | 15%  | 20%  |
| *An. annularis* | 15  | 15   | 14   | 12   | 10   | 15  | 15   | 13   | 9    | 5    | 15  | 15   | 12   | 7    | 3    |
| *An. barbirostris* | 15  | 100  | 100  | 93.33| 80.00| 66.67| 100  | 100  | 86.67| 60.00| 33.33| 100  | 100  | 80.00| 46.67| 20.00|
| *An. culicifacies* | 15  | 100  | 100  | 93.33| 73.33| 60.00| 100  | 100  | 86.67| 60.00| 46.67| 26.67| 100  | 100  | 80.00| 46.67| 20.00|
| *An. minimus* | 15  | 100  | 100  | 100  | 80.00| 73.33| 100  | 100  | 86.67| 53.33| 40.00| 100  | 100  | 66.67| 46.67| 20.00|
| *An. aconitus* | 15  | 100  | 100  | 100  | 86.67| 73.33| 100  | 100  | 86.67| 60.00| 40.00| 100  | 100  | 66.67| 46.67| 26.67|
| *An. vagus* | 15  | 100  | 100  | 100  | 93.33| 73.33| 100  | 100  | 93.33| 80.00| 53.33| 100  | 100  | 88.67| 66.67| 40.00|
| *An. tersellatus* | 15  | 100  | 100  | 100  | 86.67| 73.33| 100  | 100  | 93.33| 60.00| 46.67| 100  | 100  | 77.33| 67.50| 30.00|
| *An. maculatus* | 15  | 100  | 100  | 100  | 93.33| 66.67| 100  | 100  | 93.33| 60.00| 46.67| 100  | 100  | 77.33| 67.50| 30.00|
| Average       | 15  | 100  | 100  | 100  | 14.37| 12.50| 100  | 100  | 93.33| 66.67| 46.67| 100  | 100  | 77.33| 67.50| 30.00|
| Mean          | 15  | 100  | 100  | 100  | 5.00 | 0.52 | 100  | 100  | 95.83| 67.50| 38.33| 100  | 94.17| 69.17| 43.33| 21.67|
| SD            | 0.00| 0.00 | 0.52 | 0.83 | 0.64 | 0.00| 0.04 | 0.46 | 0.74 | 1.04 | 0.00| 0.83 | 0.92 | 0.76 | 0.71 |

Source: Myaypyinthar village, Myothit Township.
Table 6. Knockdown effect of different *Anopheles* mosquitoes against different washes of Icon LLIN, Icon treated Cotton and Icon treated Nylon nets for 15min exposure in raining season.

| Species         | No. of tested mosq. | Icon LLIN Number of washes | Icon treated cotton ITN Number of washes | Icon treated nylon ITN Number of washes |
|-----------------|---------------------|----------------------------|------------------------------------------|------------------------------------------|
|                 |                     | 0 % | 5 % | 10 % | 15 % | 20 % | 0 % | 5 % | 10 % | 15 % | 20 % | 0 % | 5 % | 10 % | 15 % | 20 % |
| *An. annularis* | 15                  | 15  | 15  | 13   | 10   | 7    | 15  | 14  | 12   | 7    | 4    | 15  | 14  | 9    | 6    | 3    |
|                 | 100                 | 100 | 86.67 | 66.67 | 46.67 | 100 | 93.33 | 80.00 | 46.67 | 26.67 | 100 | 93.33 | 60.00 | 40.00 | 20.00 |
| *An. barbirostris* | 15              | 15  | 14  | 12   | 10   | 6    | 15  | 12  | 10   | 6    | 4    | 15  | 12  | 7    | 5    | 1    |
|                 | 100                 | 93.33 | 80.00 | 66.67 | 40.00 | 100 | 80.00 | 66.67 | 40.00 | 26.67 | 100 | 80.00 | 46.67 | 33.33 | 6.67  |
| *An. culicifacies* | 15             | 15  | 15  | 13   | 10   | 8    | 15  | 15  | 12   | 7    | 5    | 15  | 14  | 9    | 5    | 2    |
|                 | 100                 | 100 | 86.67 | 66.67 | 53.33 | 100 | 100 | 80.00 | 46.67 | 33.33 | 100 | 93.33 | 60.00 | 33.33 | 13.33 |
| *An. minimus*   | 15                  | 15  | 15  | 13   | 10   | 8    | 15  | 15  | 12   | 8    | 5    | 15  | 15  | 10   | 6    | 3    |
|                 | 100                 | 100 | 86.67 | 66.67 | 53.33 | 100 | 100 | 80.00 | 53.33 | 33.33 | 100 | 100 | 66.67 | 40.00 | 20.00 |
| *An. aconitus*  | 15                  | 15  | 14  | 13   | 11   | 9    | 15  | 13  | 13   | 7    | 4    | 15  | 13  | 8    | 4    | 3    |
|                 | 100                 | 93.33 | 86.67 | 73.33 | 60.00 | 100 | 86.67 | 86.67 | 46.67 | 26.67 | 100 | 86.67 | 53.33 | 26.67 | 20.00 |
| *An. vagus*     | 15                  | 15  | 15  | 13   | 10   | 8    | 15  | 15  | 12   | 8    | 4    | 15  | 12  | 10   | 4    | 2    |
|                 | 100                 | 100 | 86.67 | 66.67 | 53.33 | 100 | 100 | 80.00 | 53.33 | 26.67 | 100 | 80.00 | 66.67 | 26.67 | 13.33 |
| *An. tersselatus* | 15               | 15  | 15  | 13   | 11   | 8    | 15  | 15  | 12   | 8    | 5    | 15  | 12  | 8    | 4    | 2    |
|                 | 100                 | 100 | 86.67 | 73.33 | 53.33 | 100 | 100 | 80.00 | 53.33 | 33.33 | 100 | 80.00 | 53.33 | 26.67 | 13.33 |
| *An. maculatus* | 15                  | 15  | 15  | 13   | 11   | 7    | 15  | 13  | 12   | 8    | 4    | 15  | 13  | 9    | 5    | 3    |
|                 | 100                 | 100 | 86.67 | 73.33 | 46.67 | 100 | 86.67 | 80.00 | 53.33 | 26.67 | 100 | 86.67 | 60.00 | 33.33 | 20.00 |
| Average         | 15                  | 15  | 14.75 | 12.88 | 10.38 | 7.63 | 15.00 | 13.50 | 11.88 | 7.38 | 4.38 | 15.00 | 13.13 | 8.75 | 4.88 | 2.38 |
| Mean            | 15                  | 15  | 14   | 12   | 10   | 6    | 15  | 12  | 10   | 6    | 4    | 15  | 12  | 7    | 4    | 1    |
| SD              | 0.35                | 0.46 | 0.52 | 0.92 | 0.64 | 1.07 | 0.83 | 0.74 | 0.52 | 0.53 | 1.13 | 1.04 | 0.83 | 0.74 | 1.74 |

Source: Myaypyinthar village, Myothit Township.
Table 7. Knockdown effect of different *Anopheles* mosquitoes against different washes of PermaNet 2.0, Deltamethrin treated Cotton and Deltamethrin treated Nylon nets for 15min exposure in cold season

| Species          | No. of tested mosq | PermaNet 2.0 Number of washes | 0 | 5 | 10 | 15 | 20 | Deltamethrin treated cotton ITN Number of washes | 0 | 5 | 10 | 15 | 20 | Deltamethrin treated nylon ITN Number of washes | 0 | 5 | 10 | 15 | 20 |
|------------------|--------------------|-------------------------------|---|---|----|----|----|-----------------------------------------------|---|---|----|----|----|-----------------------------------------------|---|---|----|----|----|
| *An. annularis*  | 15                 |                               | 15 | 15 | 14  | 13  | 12 | 15 | 15 | 13  | 12  | 9 | 15 | 15 | 12  | 10  | 5 |
| *An. barbirostris* | 15                 |                               | 100 | 100 | 93.33 | 86.67 | 80.00 | 100 | 100 | 86.67 | 80.00 | 60.00 | 100 | 100 | 80.00 | 66.67 | 33.33 |
| *An. culicifacies* | 15                 |                               | 100 | 100 | 93.33 | 86.67 | 80.00 | 100 | 100 | 86.67 | 73.33 | 60.00 | 100 | 100 | 80.00 | 46.67 | 26.67 |
| *An. minimus*    | 15                 |                               | 100 | 100 | 93.33 | 93.33 | 86.67 | 100 | 100 | 86.67 | 80.00 | 60.00 | 100 | 100 | 80.00 | 53.33 | 40.00 |
| *An. tersellatus* | 15                 |                               | 15 | 15 | 14  | 13  | 12 | 15 | 15 | 13  | 12  | 9 | 15 | 15 | 12  | 8   | 5 |
| *An. maculatus*  | 15                 |                               | 100 | 100 | 93.33 | 86.67 | 80.00 | 100 | 100 | 86.67 | 80.00 | 53.33 | 100 | 100 | 80.00 | 53.33 | 33.33 |
| Average          |                    |                               | 15.00 | 14.83 | 13.83 | 13.00 | 11.83 | 15.00 | 14.83 | 12.83 | 11.33 | 8.67 | 15.00 | 14.83 | 11.50 | 8.00 | 5.17 |
| SD               |                    |                               | 0.00 | 0.41 | 0.41 | 0.63 | 0.98 | 0.00 | 0.41 | 0.41 | 1.21 | 0.52 | 0.00 | 0.41 | 0.84 | 1.10 | 0.75 |
| Mean             | 15                 |                               | 15 | 0.41 | 0.41 | 0.63 | 0.98 | 15 | 0.41 | 0.41 | 1.21 | 0.52 | 15 | 0.41 | 0.84 | 1.10 | 0.75 |

Source: Myaypyinthar village, Myothit Township.
### Table 8. Knockdown effect of different *Anopheles* mosquitoes against different washes of Icon LLIN, Icon treated Cotton and Icon treated Nylon nets for 15min exposure in Cold season.

| Species         | No. of tested mosq. | Icon LLIN Number of washes | Icon treated cotton ITN Number of washes | Icon treated nylon ITN Number of washes |
|-----------------|---------------------|-----------------------------|-----------------------------------------|-----------------------------------------|
|                 | 0% | 5% | 10% | 15% | 20% | 0% | 5% | 10% | 15% | 20% | 0% | 5% | 10% | 15% | 20% |
| *An. annularis* | 15 |    | 12  | 10  | 7   |    | 12 | 10  | 8   | 7   |    | 12 | 10  | 7   | 5   |    |
|                 | 100| 15 | 14  | 12  | 10  | 7  | 14 | 12  | 10  | 7   | 5  | 12 | 10  | 7   | 5   | 3  |
| *An. barbirostris* | 15 |    | 12  | 9   | 6   |    | 12 | 10  | 8   | 6   |    | 12 | 10  | 8   | 6   | 2  |
|                 | 100| 15 | 14 | 12  | 9   | 6 | 14 | 12  | 10  | 8   | 6  | 14 | 12  | 10  | 8   | 2  |
| *An. culicifacies* | 15 |    | 12  | 10  | 8   |    | 12 | 10  | 8   | 6   |    | 12 | 10  | 8   | 6   | 1  |
|                 | 100| 15 | 14 | 12  | 10  | 8 | 14 | 12  | 10  | 8   | 6  | 14 | 12  | 10  | 8   | 2  |
| *An. minimus* | 15 |    | 12  | 10  | 8   |    | 12 | 10  | 8   | 6   |    | 12 | 10  | 8   | 6   | 3  |
|                 | 100| 15 | 14 | 12  | 10  | 8 | 14 | 12  | 10  | 8   | 6  | 14 | 12  | 10  | 8   | 3  |
| *An. tersellatus* | 15 |    | 12  | 10  | 8   |    | 12 | 10  | 8   | 6   |    | 12 | 10  | 8   | 6   | 1  |
|                 | 100| 15 | 14 | 12  | 10  | 8 | 14 | 12  | 10  | 8   | 6  | 14 | 12  | 10  | 8   | 3  |
| *An. maculatus* | 15 |    | 12  | 10  | 8   |    | 12 | 10  | 8   | 6   |    | 12 | 10  | 8   | 6   | 3  |
|                 | 100| 15 | 14 | 12  | 10  | 8 | 14 | 12  | 10  | 8   | 6  | 14 | 12  | 10  | 8   | 3  |
| Average         | 15 |    | 12  | 10  | 8   |    | 12 | 10  | 8   | 6   |    | 12 | 10  | 8   | 6   | 1  |
|                 | 100| 15 | 14 | 12  | 10  | 8 | 14 | 12  | 10  | 8   | 6  | 14 | 12  | 10  | 8   | 3  |
| Mean            | 15 |    | 12  | 10  | 8   |    | 12 | 10  | 8   | 6   |    | 12 | 10  | 8   | 6   | 1  |
| SD              | 0.35 | 1.17 | 0.00 | 0.41 | 0.82 | 1.07 | 1.26 | 0.82 | 0.89 | 0.75 | 1.13 | 1.03 | 0.84 | 0.98 | 0.84 |

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