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

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ABSTRACT

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Keywords

Mosquitoes PermaNet 2.0 Icon LLIN Deltamethrin Icon Cotton Nylon Net Washes. 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 5min and 15min 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 bate 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 24:00hours. 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 10washes with PermaNet 2.0 and Icon LLINs in both season. Knockdown efficacy was significantly declined between 5 and 20washes, 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 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]. Pyrethroides (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 weather 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: $156/inch^2=25/cm^2$) coated with a deltamethrin-containing ($55mg/m^2$), (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/m^2) according to WHO dipping method.

Icon LLIN (160x180x150cm) net (mesh: $156/inch^2=25/cm^2$) is a 100% polyethylene net in which Icon is coated ($50mg/m^2$), made by Thailand.

Icon treated cotton net and Nylon net were treated with $Icon (50 \text{mg/m}^2)$ 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 pecies 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. minumus* 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.





Figure-1. Knock down effect of *Anopheles* mosquitoes against different washing frequencies of LLINs and Deltamethrm insecticide treated nets under 5 min exposure time in raining 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 effecs were found with unwashed deltamethrin treated cotton and nylon nets. After 5 washed 100% knockdown were found in *An. minimus, An. culicifacies, An. aconatus, 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 10washes 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.culicfacies* 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. culicifacies, An. annularis, An aconitus* and *An. tersellatus* and reaming were found 46-53%.





Figure-2. Knock down effect of *Anopheles* mosquitoes against different washing frequencies of Icon LLIN and Icon treated nets under 5 min exposure time in raining season. Source: Myaypyinthar village, Myothit Township.

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. minumus* 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.



Deltamethrin treated different mosquito nets

Figure-3. Knock down effect of *Anopheles* mosquitoes against different washing frequencies of PermaNet 2.0 and Deltamethrin treated nets under 5 min exposure time in cold season. Source: Myaypyinthar village, Myothit Township.

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. minumus* and secondary vectors of *An. annularis, An. culicifacies, An. tersellatus* and *An. maculatus* and 10washes 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. Knockdow 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 affect was decreased to 13 -20% in all mosquitoes.

After 20 washes of Icon LLIN found 40 to 46% 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. min*umus 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

Figure-5. Knockdown effect of Anopheles mosquitoes against different washing frequencies of PermaNet 2.0, deltamethrin treated Cotton and Nylon nets under 15 min exposure time in raining season. Source: Myaypyinthar village, Myothit Township.

Table 5 and Figure 5 shows that PermaNet 2.0 LLIN, Deltamethrin treated cotton net and Deltamethrin treated nylonnet : After testing 15 min exposure, Knockdown effect of all anopheles 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

Figure-6. Knockdown effect of Anopheles mosquitoes against different washing frequencies of Icon LLIN, Icon treated Cotton and Icon treated Nylon nets under 15 min exposure times in raining season. Source: Myaypyinthar village, Myothit Township.

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. minumus* 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

Figure-7. Knock down effect of Anopheles mosquitoes against different washing frequencies of PermaNet 2.0, Deltamethrin treated cotton and Deltamethrin treated Nylon nets under 15 min exposure time in cold season. Source: Myaypyinthar village, Myothit Township. 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.

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% 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 An. culicifacies was caught followed by An vagus in cattle bate k net. The main vector of 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. An.annularis, An.culicifacies, An. minimus, An.tersellatus, An. mimus. The main vector of An minimus were caught in Second quarter of night at 9:00-12:00 pm. Other researcher also found that An minimus is a midnight blood sucker in Thabwewa village Bago Division [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 An.minimus and 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 5 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 agreed with a susceptibility study of An. mininus in Assam India [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 An. albimanus that regeneration time persisted for 15 washes without losing efficacy [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 20washes (60-80% knockdown). These results confirm the long lasting insecticidal action of these nets have higher wash resistance. Graham, et al. [25] reported an approximately 82% knockdown after 20 washing of the PermaNet in Pakistan against An. stephensi and also 237 revealed that Knockdown and mortality rate declined to 72% after 15 washes then 47% after 20 washes. These results are agreed 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 [25-29]. This could be due to difference in insecticide susceptibility or landing behavior of the 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. Sreehari, et al. [28] observed that increased efficacy of PermaNet in producing more than 80% mortality in An. culicifacies and 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 [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 5wshes 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 [10].

PermaNet 2.0 net had reasonably good wash resistance for up to 15 washes against *An.minimus* the major vector of malaria and *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.

Species	No. of tested mosq		P Nun	ermaNet nber of wa	2.0 ashes		Deltam	ethrin tre Number o	ated cot of washes	ton ITN s	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	15 100	14 93.33	$\begin{array}{c} 13\\ 86.67\end{array}$	$\frac{11}{73.33}$	$\begin{array}{c} 10 \\ 66.67 \end{array}$	15 100	$\begin{array}{c} 13\\ 86.67\end{array}$	$\begin{array}{c} 11\\73.33\end{array}$	9 60.00	2 13.33	15 100	$\begin{array}{c} 13\\ 86.67\end{array}$	7 46.67	3 20.00	$\begin{array}{c}1\\6.67\end{array}$		
An. barbirostris	15	15 100	14 93.33	12 80	$\begin{array}{c} 10 \\ 66.67 \end{array}$	8 53.33	15 100	12 80.00	9 60.00	7 46.67	2 13.33	15 100	12 80.00	6 40.00	$\frac{2}{13.33}$	0 0.00		
An. culicifacies	15	15 100	15 100	14 93.33	12 80.00	10 66.67	15 100	12 80.00	9 60.00	8 53.33	2 13.33	15 100	12 80.00	6 40.00	3 20.00	$1 \\ 6.67$		
An. minimus	15	15 100	15 100	14 93.33	12 80.00	10 66.67	15 100	13 86.67	12 80.00	$\begin{array}{c} 10\\ 66.67\end{array}$	3 20.00	15 100	$\begin{array}{c} 13\\ 86.67\end{array}$	7 46.67	3 20.00	$1 \\ 6.67$		
An. aconitus	15	15 100	15 100	$\begin{array}{c} 14\\93.33\end{array}$	11 73.33	10 66.67	15 100	13 86.67	11 73.33	9 60.00	2 13.33	1510 0	$\begin{array}{c} 13\\ 86.67\end{array}$	7 46.67	3 20.00	0 0.00		
An. vagus	15	15 100	14 93.33	12 80.00	11 73.33	9 60.00	15 100	12 80.00	10 66.67	9 60.00	2 13.33	15 100	12 80.00	6 40.00	2 13.33	0 0.00		
An. tersellatus	15	15 100	15 100	$\frac{13}{86.67}$	11 73.33	$\begin{array}{c} 10 \\ 66.67 \end{array}$	15 100	$\begin{array}{c} 13\\ 86.67\end{array}$	10 66.67	8 53.33	3 20.00	15 100	$\frac{13}{86.67}$	6 40.00	2 13.33	0 0.00		
An. maculatus	15	15 100	15 100	14 93.33	11 73.33	10 66.67	15 100	13 86.67	10 66.67	8 53.33	2 13.33	15 100	$\begin{array}{c} 13\\ 86.67\end{array}$	6 40.00	3 20.00	$1 \\ 6.67$		
Average	15	15 100	$14.63 \\ 97.50$	13.25. 88.33	$11.13 \\ 74.17$	$9.63 \\ 64.17$	15.00 100	$12.63 \\ 84.17$	$10.25 \\ 68.33$	$8.50 \\ 56.67$	$2.25 \\ 15.00$	15.00 100	$12.63 \\ 84.17$	$6.38 \\ 42.50$	$2.63 \\ 17.50$	0.50 3.33		
Mean SD	15	15 0	14 0.51	12 0.88	11 0.64	8 0.74	15	12 0.51	9 1.03	7 0.92	2 0.46	15	12 0.517	6 0.517	2 0.517	0 0.534		

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.

	No. of tested			Icon LLI	N			Icon trea	ated cot	ton ITN		Icon treated nylon ITN					
Species	mosq.		Nun	nber of w	ashes			Num	ber of wa	ashes			Num	ber of w	ashes		
	_	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	
		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	
An. barbirostris	15	15	13	11	7	4	15	13	11	5	1	13	7	5	3	0	
		100	86.67	73.33	46.67	26.67	100	86.67	73.33	33.33	6.67	86.67	46.67	33.33	20.00	0.00	
An. culicifacies	15	15	14	12	9	5	15	14	12	6	3	15	9	6	4	1	
		100	93.33	80.00	60.0	33.33	100	93.33	80.00	40.00	20.00	100	60.00	40.00	26.67	6.67	
An. minimus	15	15	14	12	10	6	15	14	12	6	2	15	9	6	4	2	
		100	93.33	80.00	66.67	40.00	100	93.33	80.00	40.00	13.33	100	60.00	40.00	26.67	13.33	
An. aconitus	15	15	14	11	10	6	15	14	11	6	1	14	9	6	4	0	
		100	93.33	73.33	66.67	40.00	100	93.33	73.33	40.00	6.67	93.33	60.00	40.00	26.67	0.00	
An. vagus	15	15	14	12	10	5	15	14	12	6	1	14	8	5	3	1	
		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	15	14	11	10	6	15	14	10	6	1	14	9	6	3	1	
		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	15	14	12	9	6	15	14	12	7	2	14	8	7	2	1	
		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	
	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	
Average		100	92.50	78.33	61.67	35.83	100	92.50	76.67	40.00	11.67	95.00	56.67	40.00	22.50	6.67	
Mean	15	15	13	11	7	4	15	13	10	5	1	14	7	6	2	0	
SD		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	
		5534	553	098	24	24	553	929	522	9252	405	107	929	929	24	929	

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		Pe Nun	ermaNet iber of w	2.0 vashes		Delt	amethrii Num	n treated ber of wa	l cotton ashes	ITN	Deltamethrin treated nylon ITN Number of washes						
•	Ĩ	0 %	5 %	10 %	15 %	20 %	0 %	5 %	10 %	15 %	20 %	0 %	5 %	10 %	15 %	20 %		
An. annularis	15	15 100	14 93.33	$\begin{array}{c} 12\\ 80.00 \end{array}$	11 73.33	9 60.00	15 100	$\begin{array}{c} 13\\ 86.67\end{array}$	12 80.00	7 46.67	3 20.00	15 100	$\begin{array}{c} 13\\ 86.67\end{array}$	$7 \\ 46.67$	$\frac{4}{26.67}$	$1 \\ 6.67$		
An. barbirostris	15	15 100	$\begin{array}{c} 13\\ 86.67\end{array}$	12 80.00	10 66.67	8 53.33	15 100	12 80.00	10 66.67	5 33.33	$\begin{array}{c}1\\6.67\end{array}$	15 100	12 80.00	6 40.00	2 13.33	0 0.00		
An. culicifacies	15	15 100	14 93.33	12 80.00	11 73.33	9 60.00	15 100	$\begin{array}{c} 13\\ 86.67\end{array}$	$\begin{array}{c} 12\\ 66.67\end{array}$	7 46.67	3 20.00	15 100	$\begin{array}{c} 13\\ 86.67\end{array}$	8 53.33	3 20.00	$1 \\ 6.67$		
An. minimus	15	15 100	14 93.33	12 80.00	$\frac{11}{73.33}$	9 60.00	15 100	$\frac{13}{86.67}$	$\begin{array}{c} 12\\ 66.67\end{array}$	$7 \\ 46.67$	3 20.00	15 100	$\begin{array}{c} 13\\ 86.67\end{array}$	7 46.67	2 13.33	$1 \\ 6.67$		
An. tersellatus	15	15 100	13 86.67	12 80.00	$\frac{11}{73.33}$	9 60.00	15 100	$\frac{13}{86.67}$	$\begin{array}{c} 12\\ 66.67\end{array}$	8 53.33	2 13.33	15 100	$\begin{array}{c} 13\\ 86.67\end{array}$	6 40.00	3 20.00	$1 \\ 6.67$		
An. maculatus	15	15 100	14 93.33	12 80.00	11 73.33	9 60.00	15 100	$\begin{array}{c} 13\\ 86.67\end{array}$	$\begin{array}{c} 12\\ 66.67\end{array}$	9 60.00	3 20.00	15 100	13 86.67	8 53.33	$\frac{4}{26.67}$	$1 \\ 6.67$		
Average		15.00 100	$13.67 \\ 91.11$	12.00 80.00	$10.83 \\ 72.22$	8.83 58.89	15.00 100	$12.83 \\ 85.56$	$11.67 \\ 77.78$	$7.17 \\ 47.78$	$2.50 \\ 16.67$	15.0 100	$12.83 \\ 85.56$	$7.00 \\ 46.67$	3.00 20.00	$0.83 \\ 5.56$		
SD		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		
Mean	15	15	13	12	10	8	15	12	10	5	1	15	12	6	2	0		

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.

	No. of tested		Р	ermaNet	2.0		Delt	amethri	n treate	d cotton	ITN	Deltamethrin treated nylon ITN					
	mosq		Nur	nber of w	ashes			Num	ber of w	ashes			Num	ber of wa	ashes		
Species		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	
		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	
Mean		14	13	12	8	5	14	11	9	5	1	13	11	5	2	0	
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	

Table-4. Knockdown effect of different Anopheles mosquitoes against different washes of Icon LLIN and Icon treated Cotton and Nylon nets for 5min exposure in cold season.

Species	No. of tested mosq		P Nu	PermaNe mber of v	t 2.0 washes		Del	tamethri Num	n treated ber of wa	l cotton I ashes	TN	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	15 100	15 100	$\begin{array}{c} 14\\ 93.33\end{array}$	12 80.00	10 66.67	15 100	15 100	$\begin{array}{c} 13\\ 86.67\end{array}$	9 60.00	5 33.33	15 100	15 100	12 80.00	$7 \\ 46.67$	3 20.00	
An. barbirostris	15	15 100	15 100	$\begin{array}{c} 14\\93.33\end{array}$	11 73.33	9 60.00	15 100	13 86.67	12 80.00	7 46.67	4 26.67	15 100	13 86.67	9 60.00	5 33.33	2 13.33	
An. culicifacies	15	15 100	15 100	15 100	12 80.00	11 73.33	15 100	15 100	13 86.67	8 53.33	6 40.00	15 100	15 100	10 66.67	7 46.67	3 20.00	
An. minimus	15	15 100	15 100	15 100	$\frac{13}{86.67}$	11 73.33	15 100	15 100	$\begin{array}{c} 13\\ 86.67\end{array}$	9 60.00	6 40.00	15 100	15 100	$\begin{array}{c} 10\\ 66.67 \end{array}$	7 46.67	$\frac{4}{26.67}$	
An. aconitus	15	15 100	15 100	14 93.33	12 80.00	10 66.67	15 100	14 93.33	$\begin{array}{c} 13\\ 86.67\end{array}$	8 53.33	6 40.00	15 100	14 93.33	10 66.67	6 40.00	$\frac{3}{20.00}$	
An. vagus	15	15 100	15 100	14 93.33	11 73.33	10 66.67	15 100	14 93.33	$12 \\ 80.00$	8 53.33	5 33.33	15 100	$\begin{array}{c} 13\\ 86.68\end{array}$	$10 \\ 66.67$	6 40.00	$3 \\ 20.00$	
An. tersellatus	15	15 100	15 100	$\frac{14}{93.33}$	$\begin{array}{c} 13\\ 86.67\end{array}$	10 66.67	15 100	14 93.33	$\begin{array}{c} 13\\ 86.67\end{array}$	9 60.00	7 46.67	15 100	$\begin{array}{c} 14\\93.33\end{array}$	11 77.33	7 46.67	$\frac{4}{26.67}$	
An. maculatus	15	15 100	15 100	15 100	$\begin{array}{c} 13\\ 86.67\end{array}$	$\begin{array}{c} 10 \\ 66.67 \end{array}$	15 100	14 93.33	$\begin{array}{c} 13\\ 86.67\end{array}$	9 60.00	7 46.67	15 100	$\begin{array}{c} 14\\93.33\end{array}$	11 73.33	7 46.67	$\frac{4}{26.67}$	
Average	15	15 100	15 100	$14.37 \\ 95.83$	12.12 80.83	$10.12 \\ 67.50$	15 100	$14.12 \\ 95.00$	$12.75 \\ 85.00$	$8.37 \\ 55.83$	5.75 38.33	15 100	$13.87 \\ 94.17$	$10.37 \\ 69.17$	$6.5 \\ 43.33$	$3.25 \\ 21.67$	
Mean		15	15	14	11	9	15	13	12	7	4	15	13	9	5	2	
SD		0.00	0.00	0.52	0.83	0.64	0.00	0.64	0.46	0.74	1.04	0.00	0.83	0.92	0.76	0.71	

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.		Nu	Icon LLI mber of v	IN vashes			Icon tre Num	ated cot ber of w	ton ITN ashes		Icon treated nylon ITN Number of washes					
		0	5 %	10 %	15 %	20 %	0	5 %	10 %	15 %	20 %	0	5 %	10 %	15 %	20 %	
4 7 1		/0	/0	/0	/0	/0	/0	/0	/0	/0	/0	/0	/0	/0	/0	/0	
An. annularıs	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	13	12	8	4	15	12	10	4	2	
		100	100	86.67	66.67	53.33	100	86.67	80.00	53.33	26.67	100	80.00	66.67	26.67	13.33	
An. tersellatus	15	15	15	13	11	8	15	13	12	8	5	15	12	8	4	2	
		100	100	86.67	73.33	53.33	100	86.67	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	
	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	
Average		100	98.33	85.83	69.17	50.83	100	90.00	79.17	49.17	29.17	100	87.50	58.33	32.50	15.83	
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	0.74	

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.

	No. of tested		Р	ermaNet	2.0		Del	tamethrir	ı treated	cotton I'	ГN	Deltamethrin treated nylon ITN					
Species	mosq		Nun	nber of w	ashes			Numb	ber of wa	shes			Num	ber of w	ashes		
		0	5	10	15	20	0	5	10	15	20	0	5	10	15	20	
		%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	
An. annularis	15	15	15	14	13	12	15	15	13	12	9	15	15	12	10	5	
		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. barbirostris	15	15	14	13	12	10	15	14	12	9	8	15	14	10	7	4	
		100	93.33	86.67	80.00	66.67	100	93.33	80.00	60.00	53.33	100	93.33	66.67	46.67	26.67	
An. culicifacies	15	15	15	14	13	12	15	15	13	11	9	15	15	12	7	6	
		100	100	93.33	86.67	80.00	100	100	86.67	73.33	60.00	100	100	80.00	46.67	40.00	
An. minimus	15	15	15	14	14	13	15	15	13	12	9	15	15	12	8	6	
		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	8	15	15	12	8	5	
		100	100	93.33	86.67	80.00	100	100	86.67	80.00	53.33	100	100	80.00	53.33	33.33	
An. maculatus	15	15	15	14	13	12	15	15	13	12	9	15	15	11	8	5	
		100	100	93.33	86.67	80.00	100	100	86.67	80.00	60.00	100	100	73.33	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	
		100	98.89	92.22	86.67	78.89	100	98.89	85.56	75.56	57.78	100	98.89	76.67	53.33	33.44	
SD		0.00	14	13	12	10	0.00	14	12	9	8	0.00	14	10	7	4	
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	

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

		1	•									Loss treated unlaw ITN						
	No. of tested		I	con LLI	N			Icon tre	ated cot	ton ITN			Icon tr	eated ny	lon ITN			
Species	mosq.		Num	ber of w	ashes			Num	ber of w	ashes			Nun	ber of w	ashes			
-	-	0	5	10	15	20	0	5	10	15	20	0	5	10	15	20		
		%	%	%	%	%	%	%	%	%	%	%	%	%	%	%		
An. annularis	15	15	14	12	10	7	15	14	12	7	5	15	13	8	4	3		
		100	93.33	80.00	66.67	46.67	100	93.33	80.00	46.67	33.33	100	86.67	53.33	26.67	20.00		
An. barbirostris	15	15	12	12	9	6	15	12	10	6	4	15	12	6	2	1		
		100	80.00	80.00	60.00	40.00	100	80.00	66.67	40.00	26.67	100	80.00	40.00	13.33	6.67		
An. culicifacies	15	15	15	12	10	8	15	15	12	8	6	15	14	8	4	2		
		100	100	80.00	66.67	53.33	100	100	80.00	53.33	40.00	100	93.33	53.33	26.67	13.33		
An. minimus	15	15	15	12	10	8	15	15	12	8	6	15	15	8	4	3		
		100	100	80.00	66.67	53.33	100	100	80.00	53.33	40.00	100	100	53.33	26.67	20.00		
An. tersellatus	15	15	14	12	10	8	15	13	11	7	5	15	13	8	4	3		
		100	93.33	80.00	66.67	53.33	100	86.67	73.33	46.67	33.33	100	86.67	53.33	26.67	20.00		
An. maculatus	15	15	15	12	10	7	15	15	11	6	5	15	13	7	5	3		
		100	100	80.00	66.67	46.67	100	100	73.33	40.00	33.33	100	86.67	46.67	33.33	20.00		
Average	15	15	14.17	12.00	9.83	7.33	15.00	14.00	11.33	7.00	5.17	15.00	13.33	7.50	3.83	2.50		
_		100	94.44	80.00	65.56	48.89	100	93.33	75.56	46.67	34.44	100	88.89	50.00	25.56	16.67		
Mean	15	15	12	12	9	6	15	12	10	6	4	15	12	6	2	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		

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.

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