



Research Article

Wash Resistance and Bioefficacy of PermaNet® 2.0, PowerNet and K-O-Tab 123 Treated Bed Nets against Malaria Vectors of Myanmar

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Abstract: Eradication of vector mosquito is necessary for the prevention of malaria. Insecticide treated bed nets (ITNs) may provide necessary safe guard against malaria but not evaluated in Myanmar for bio-efficacy. Evaluation of insecticidal bio-efficacy of Insecticide-treated nets (ITNs) after washing under laboratory conditions against vector *Anopheles dirus* and *Anopheles minimus* mosquitoes. *Anopheles minimus* and *An. dirus* mosquitoes were used for insecticidal tests and PermaNet 2.0, Power net, K-O-Tab:123® net and K-O-Tab were used for monitoring regeneration, knockdown and mortality of vector mosquitoes periodically for 0 to 20 hand washing (Laboratory hand washed) of bed nets for 20 months. *An. dirus* mosquitoes were used to evaluate the vulnerability to selected insecticides. Microsoft excel was used for determining the knockdown and mortality rates of mosquitoes. Tested insecticide nets showed 86-100% knockdown and mortality efficacy against malaria vectors *An. dirus* and *An. minimus* in 20 washing regimen. However, K-O-Tab net exhibited wash resistance up to 15 washing against *An. minimus* only. ITNs bednets can be used for malaria vector control programme in Myanmar.

INTRODUCTION

Mosquitoes are main vector responsible for the spread of malaria and a major public health problem in Myanmar. Its outbreak leads to extensive morbidity and mortality in Myanmar due inaccessible terrains and inhospitable weather, also economic limitations prevents the local population for affording costly treatment [1]. Therapeutic drugs resistance to the medication are limiting the malaria control programme [2, 3]. Several methodologies have been developed for the controlling and elimination of vector mosquitoes is found to be most pragmatic [4-8]. World Health Organization (WHO) introduced and recommended a simple and cost effective technique for the prevention of malaria vector mosquito's i.e. introduction of insecticide-treated mosquito nets (ITNs) and long lasting insecticidal nets (LLINs) and now forms a part of operational strategies adopted by the National Vector Borne Diseases Control Programme around the world [9-11]. It has been reported that ITNs and LLINs provide safe protection against malaria by preventing human-vector contact and act as a physical barrier [12-14]. World Health Organization (WHO) has recommended several insecticide impregnated bed nets in different dosage e.g. alphacypermethrin,

cyfluthrin, deltamethrin, permethrin, etofenprox and bifenthrin for the use in LLINs and ITNs bed nets e.g. PermaNet 2.0, Olyset net and Power nets etc. and are widely used in the malaria infested endemic areas [15, 16]. Recently an advanced LLIN K-O-Tab 1-2-3 insecticide impregnated bed nets were introduced against existing simple K-O-Tab only plus binder and other bed nets, with a claim that it is economical and more effective against vector mosquitoes [17, 18]. Present study was conducted to evaluate bio-efficacy of LLINs and ITNs by multiple washing during 20 months period by knockdown/mortality method on malaria vector mosquitoes. It is also our endeavour to evaluate the manufacturer recommended period of efficacy of insecticide impregnated bed nets against vector mosquitoes, because it not well understood that repeated washings of LLINs and ITNs does not alter the insecticidal efficacy against malaria vector mosquitoes. Bio-efficacy of PermaNet 2.0, Power net, K-O-Tab:1-2-3 (plus binder to make LLIN net) and K-O-Tab only treated bed nets after washing i.e. on 0 day and after end of every month up to 20 months by 20 hand washing (Laboratory hand washed) of bed nets (including vital transmission phase: Total Testing:21). The knockdown and mortality rate of malaria vectors *An. dirus* and *An. minimus* were monitored, as mosquitoes are the main transmission vectors of malaria in Myanmar and around the world [19, 20].

MATERIALS AND METHODS

Study design, study areas and study period

Laboratory based cross sectional expressive design study was done to assess the suitability of ITNs for malaria control. Wild blood fed *An. minimus* and larvae were collected from Laikkyi village, Taikkyi Township, Yangon Region. Blood fed *An. dirus* and larvae were collected from Mudon Township Mon State, from June 2012 to January 2014. Insecticidal bio-efficacy of repeated laboratory hand washed PermaNet 2.0, Power net, K-O-Tab:1-2-3@ plus binder net and K-O-Tab only treated net against malaria vectors (*An. dirus* and *An. minimus*) were studied in the laboratory of Medical Entomology Research Division DMR (Lower Myanmar) during the main malaria transmission period for 20 months.

Mosquitoes collection

An. minimus and *An. dirus* mosquitoes were collected for 3-5 days from the two above mentioned townships respectively by animal bait big net (K-net=330x330x220cm) and indoor and outdoor human bait from 18:00 to 02:00 hour with WHO Sucking tube in 20 times field surveys. All collected mosquitoes were put in paper cups containing glucose and moisture with covering the mouth of cup with water soaked damp towel.

Larval collection

Larvae of *An. minimus* and *An. dirus* were collected from beyond 3 km area of study vicinity. Collected larvae were put in labeled plastic bags containing water and all larvae in plastic bags were brought to laboratory. Larvae of *An. minimus* and *An. dirus* were reared separately to till adult in conducive environment (26-28°C, 70-80% RH).

Species Identification

Species identification of collected *Anopheles* mosquitoes and adult mosquitoes which were emerged from pupae, collected during larval survey were done by intra-specific variations suggested by different authors [21-23]

Mosquitoes rearing in Laboratory

Field collected blood fed *An. minimus* and *An. dirus* were ovi-positive and hatched larvae from eggs during rearing and produced F1 generation of mosquitoes for our LLINs and ITNs bio-efficacy study.

Details and preparation of Insecticide Treated mosquito bed nets samples

1. PermaNet 2.0: PermaNets are long lasting insecticide-treated mosquito nets and manufactured by M/S Vestergaard Frandsen, Denmark. It is impregnated with Deltamethrin insecticide (55 mg/m²) inside or around the netting fibers. It is made of 100% polyester material with multiple wash-resistant properties. The size of bed net

(160x180x150cm) consisting 100 denier polyester yarn providing a mesh of 156/inch².

- 2. Power net:** China made long lasting insecticide-treated mosquito net and impregnated with Deltamethrin insecticide (55 mg/m²). Netting material used for fabrication of net is polyester and insecticide deltamethrin incorporated in and around the fibers. This bed net is also multiple wash-resistant. The size of bed net measures 160x180x150cm and has a mesh pore of 312/inch².
- 3. K-O-Tab:123@ net:** It is a Deltamethrin insecticide plus binder treated wash resistant bed net {using conventional K-O-Tab (Byer Environmental Science) and mixing it with tap water having a special binder (Byer Environmental Science) to make long lasting insecticide treated net}. This mosquito net is further treated by WHO Dipping Method [14]. The developed K-O-Tab:123 bed net is dried in shade (25°C-27°C). It is made of 100% polyester netting material and measures 160x180x150cm in size, with a mesh size of 312/inch².
- 4. K-O-Tab only net:** It is a Deltamethrin insecticide treated bed net and produced by using conventional K-O-Tab (Byer Environmental Science) only and mixing it with tap water. Later on this mosquito net is treated with WHO Dipping Method [14]. The K-O-Tab only treated bed net is dried in shade (25°C-27°C). It is made of 100% polyester netting material and measuring 160x180x150cm in size, having a mesh of 312/inch².

Insecticide Bed Nets Samples

Six pieces each of PermaNet 2.0, Power net, K-O-Tab:123@ net and K-O-Tab treated bed nets were used for mosquitoes knockdown and mortality after serial hand washing for insecticidal bio-efficacy experiment. In other set of insecticide experiment 30cmx30cm mesh pieces were cut from each insecticide net and used for bio-efficacy testing with malaria vector.

Washing Procedure

One ITNs bed net piece (no washing) of each net (bed nets of four types were unwashed for a 20 months) was kept as a control for base line evaluation of bio-efficacy for positive control. Remaining ITNs pieces were washed by hand for 20 months after the end of every month i.e. total 20 washing. Two gram of Fuji Detergent Cream (Shwe Tha Zin Super Soap Co. Ltd, Yangon, Myanmar) was dissolved in 2 litres of normal tap water in 10 litres plastic bucket. Individual bed net pieces was soaked and kept in detergent water for 10 minutes. The soaked net was rubbed thoroughly between the hand palms for 5 minutes and after washing, was rinsed in plain tap water for 3 times to remove the detergent. After washing the nets pieces were shade dried for 5-7 hours at (25-30°C) depending on the month.

Mosquito susceptibility test

Before evaluation of insecticidal properties of malaria vector *An. minimus* and *An. dirus* for ITNs, laboratory reared three to five days old *An. dirus* mosquitoes (5 each) were used for the

evaluation of insecticide susceptibility properties to different insecticides (DDT 4%, Deltamethrin 0.05%, Permethrin 0.75% and Cyfluthrin 0.15%) in order to confirm that vector mosquitoes are susceptible strain to above mentioned insecticides for control. Two replicates testing's of vector mosquitoes against different insecticides were done (4tests+1control x 5 mosquitoes= 25 *An. dirus* mosquitoes in one testing followed by replicate testing = 25x2=50 mosquitoes after two testings). Susceptibility test of vector mosquitoes of malaria was performed by following WHO Treated Papers and Kits Test [24].

Assessment of insecticidal bio-efficacy of nets after washing

Insecticidal efficacy of PermaNet 2, Power net, K-O-Tab:123@net and K-O-Tab only bed nets after laboratory hand washing and drying them at 27-30°C were determined by contact bioassays using a WHO prescribed standard procedure for testing insecticide resistance of net surfaces for vectors (WHO 1998). Persistence of insecticide on bed nets were conducted on one time washed and dried nets and three times consecutively washed and dried nets were carried out on net samples as described in the WHO biological assays [25, 26]. 3-5 days old unfed females *An. dirus* were used in the biological bioassay testing. Five mosquitoes were released in each cone with the help of a suction tube and exposed to each type of bed net for 3 min by using WHO contact cone test technique [25, 26]. The percentage of mosquitoes knocked down after the 60 minutes exposure period and effective mortality after 24 h were recorded. After 3 minute bioassay, mosquitoes were removed from the cone and placed in paper cup containing 10% glucose solution in cotton soaked swab and moisture in cup was maintained by covering the opening by damped towel during the recovery period. The untreated nets served as negative controls. Schedule of bioassay for washing of bed nets were conducted after one day of washing the nets and continued until initial and complete biological activity was restored i.e. (3 days continuous bioassay test = 5mosquitoes x 8 nets pieces (4test+4control) x 3 days = 120 mosquitoes). Insecticide efficacy curves of nets vs. mosquitoes were plotted and compared for nets washed once and three time consecutively. The time required (in days) to reach the plateau was the period required for persistence (regeneration) of the net.

Determination of bio-efficacy of wash resistance of washed nets pieces:

Cut pieces (30x30cm) of bed nets were assessed by 3 min. cone bio-assay test before and after washing by hand (e.g. 0x, 1x, 5x,10x,15x.....x20washing) i.e. total twenty hand washing. ITNs net cut pieces were attached to WHO specified cones horizontally. Five F1 generation (3-5 days old) unfed *An. dirus* mosquitoes were introduced in cones by sucking tube and the experiment was done as above WHO contact cone test method [25, 26]. Also, cone bio assays were carried out on day 3 after each wash by exposing females of *An. dirus* and *An. minimus* mosquitoes. Two replicate testing's were carried out for each category of washed net pieces (5mosquitoes x 5 net

pieces (4test+1control) x 2 replicates =50 mosquitoes). The mosquitoes were then removed from the cones and placed in paper cups with glucose, the experiment was done as described above. Percentage of knockdown was measured after 60 min exposure and effective mortality was accessed 24 hr after exposure. The procedure as described above was followed for *An. minimus* on the same washed nets cut pieces for the determination of persistence of insecticidal properties after repeated hand washing.

Data analysis by Statistical method

Knockdown and mortality data were analyzed by Microsoft excel software. The percentage of corrected mortality was calculated and where control mortality was between 5 and 20%, the percentage mortality was corrected using Abbott's formula [27].

RESULTS

Results of the bioassay tests on insecticide impregnated PermaNet 2.0, Power net and K-O-Tab:123 bed nets showed 100% mortality of females mosquitoes of *An. dirus* and *An. minimus* within 3-min exposure. *An. dirus* was used as control and used for persistence of insecticidal time testing for one to three times washed nets efficacy.

Mosquito susceptibility test

Laboratory reared *An. dirus* mosquitoes (5each x 2 replicates) were used for the evaluation of insecticide susceptibility properties to different insecticides (DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Cyfluthrin 0.15% and control) in order to confirm that vector mosquitoes are susceptible strain to above mentioned insecticides for control and found that F1 strain of *An. dirus* was susceptible to above insecticides (Fig. 1).

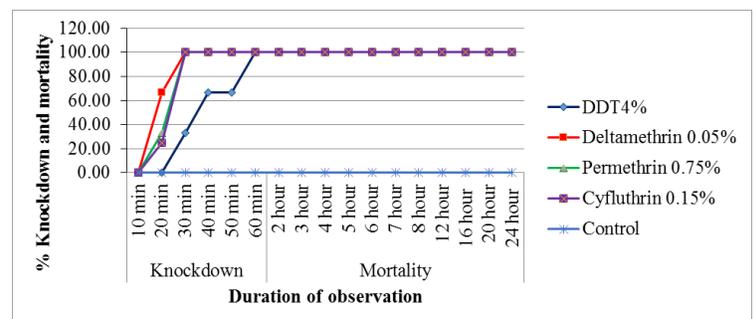


Fig. 1 Susceptibility status of laboratory colony *An. dirus* to different insecticides used in malaria control

Persistency test after one to three washing of nets

The persistence effect of insecticide nets study of continuous three days bioassay (Five F1 generation of 3-5 days old unfed *An. dirus* mosquitoes were introduced in cones by sucking tube of each pieces of nets. Two replicates were done.) showed that the efficacy of insecticide fully recovered i.e. 100% knockdown and mortality by 24 hours after one washing of all tested nets. Also, 3 times washed ITNs were

found to produce 98 -100% knock down and mortality effect within one day. After three days it was found 100% mortality against *An. dirus*. (Fig. 2 & 3).

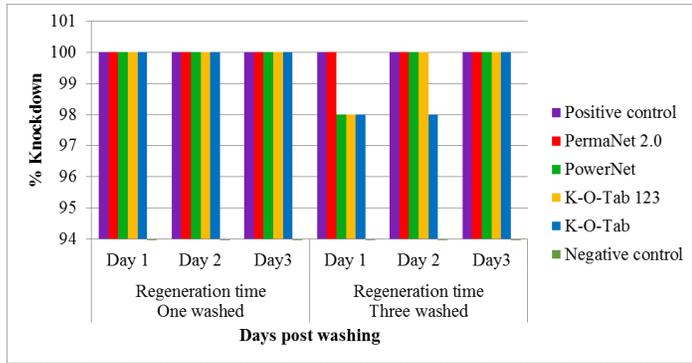


Fig. 2 Percentage knockdown of insecticide susceptible strain of *An. dirus* for 60 minutes post exposure in a 3 min WHO cone test with one to three washed different insecticide nets

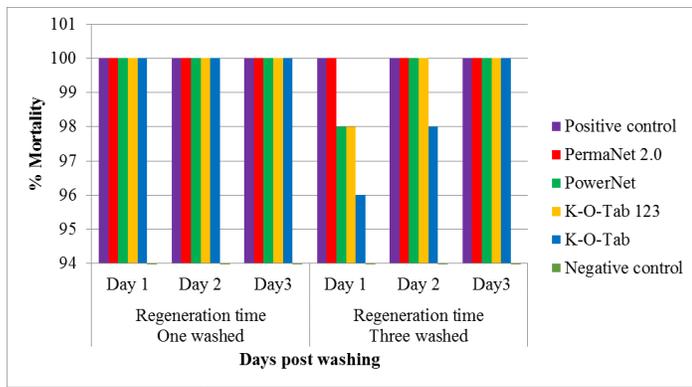


Fig. 3 Percentage mortality of insecticide susceptible strain of *An. dirus* for 24-hour post exposure in a 3 min WHO cone test with one to three washed different insecticide nets

Bio-efficacy of washed LLINs and ITNs nets

The bio-efficacy of 20 time washed PermaNet2.0, Power net and K-O-Tab:123@net exhibited 100% knockdown and mortality effect against F₁ generation of *An. dirus* and F₁ generation of *An. minimus* (Fig. 4 & 5). Bio-efficacy of K-O-Tab123 treated net showed 100% mortality of *An. dirus* till 20 washing. However, *An. minimus* showed 100% mortality of mosquitoes till 15 washing and after which 86% mortality with K-O-Tab only net (Fig. 5) was recorded. Wash resistance of insecticide PermaNet2.0 and Power net nets effect in terms of 100% knockdown and 100% mortality persisted in 20 washing for F₁ generation of *An. dirus* and *An. minimus*. ITN K-O-Tab only treated net was found to be wash resistant and persisted for 15 washes against *An. minimus*.

DISCUSSION

Susceptibility and bio-efficacy of nets

Eradication of vector mosquitoes is necessary for the prevention of malaria; hence there is a need to develop effective and long lasting preventive measures. It has been

found that insecticide treated mosquito nets can provide better alternative for community-based action-oriented schemes. Mosquito net mesh fabric impregnated with insecticide such as pyrethroids provide effective protection against vector of malaria parasite [28-33]. *An. dirus* and *An. minimus* were highly susceptible to all the insecticides as DDT 4%, Deltamethrin 0.05%, Permethrin 0.75% and Cyfluthrin 0.15%. Bio-efficacy of PermaNet2.0 Power net and K-OTab 123@ nets effect in terms of 100% knockdown and mortality persisted in 20 washing for F₁ generation of *An. dirus* and *An. minimus*. Bio-efficacy of insecticide treated bed nets against malaria vectors have been studied in several countries and it was found that LLINs and ITNs bed nets can be used as an effective long lasting and low cost intervention tool for the eradication of malaria disease vector. Sreehari et al.[33] studied vector controlling by LLINs and ITNs and found that PermaNet 2.0 produced >80% mortality to *An. culicifacies* and *An. stephensi* mosquitoes after 20 hand washings and 10 machine washings [34]. Washing of K-O-Tab:123 treated nets, PermaNet and K-O-Tab only treated net reported that mortality rate of mosquitoes steadily decreased after 5th washing for K-O-Tab only treated net, but K-O-Tab123 and PermaNet showed 100% mortality even after 30 wash cycles [35,36].

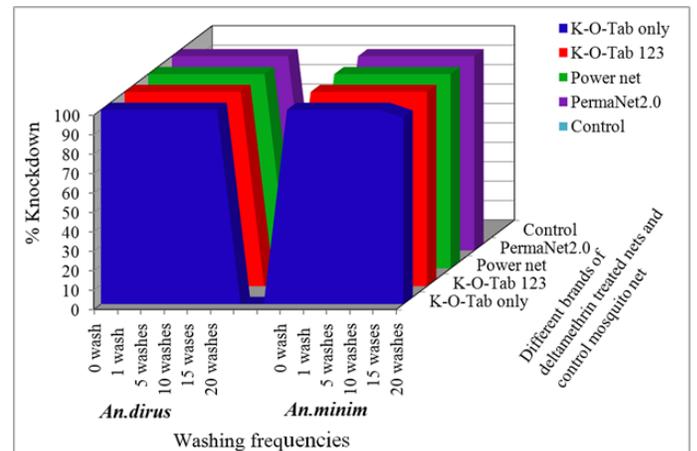


Fig. 4 Percentage knockdown of main vector *An. dirus* and *An. minimus* after 60 minutes, exposure, after 3 minute WHO cone bioassay

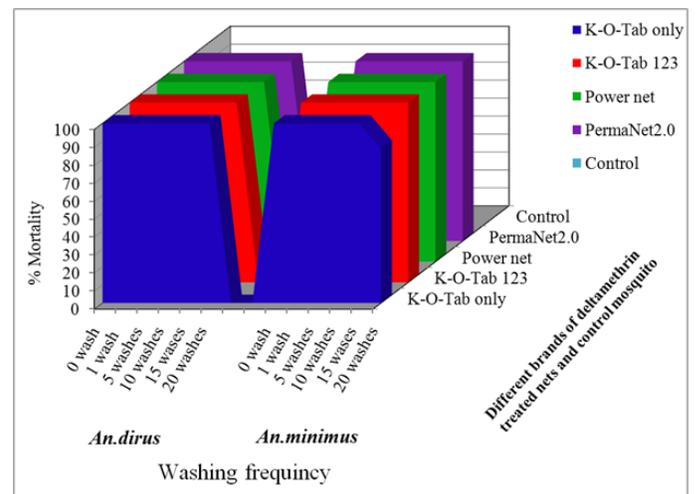


Fig. 5 Percentage mortality of main vector *An. dirus* and *An. minimus* after 24 hours, exposure, after 3 minute WHO cone bioassay

Wash resistance and persistence of nets

In Myanmar LLINs and ITNs bed nets are commonly used for the prevention and control of malaria. Our laboratory study assessed the bio-efficacy and wash-resistance properties of four types of insecticide bed nets i.e. PermaNet 2.0, Power net, K-O-Tab:123@net and K-O-Tab only nets. These bed nets were impregnated with Deltamethrin insecticides at different dilutions. Our study investigated two main malaria causing breeds of Lower Myanmar i.e. *An. dirus* and *An. minimus* for effectiveness of insecticidal properties after repeatedly washed LLINs and ITNs bed nets. The regeneration evaluation of PermaNet 2.0, PowerNet, K-O-Tab 123@ and K-O-Tab only treated net found that total efficacy was recovered by 24 hour after one and three washes for *An. dirus*. Although fully recovery 100% regeneration was found in day three test after one and three washes of tested nets. The study help to evaluate wash resistance in all LLINs and ITNs, and found regeneration time persisted for 20 washed without losing efficacy. This study not difference than results from other studies, in which the efficacy of PermaNet 2.0 was maintained even 20 washes under laboratory and field conditions against some of the most important malaria vectors around the World [28, 29, 30, 34, 36].

We also report that *An. minimus* has high tolerance to Deltamethrin insecticide compared to *An. dirus*, however both the vectors are susceptible to 0.05% Deltamethrin and result in 100% mortality with 20 hand washing of PermaNet 2.0, Power Net and K-O-Tab 123@ net. Results of earlier insecticidal studies of LLINs and ITNs bed nets shows that Deltamethrin impregnated nets cause 100% mortality on unwashed nets as examined by WHO cone and tube test [25,26]. Our study found that washing does not alter the insecticidal effectiveness of PermaNet2.0, Power net and K-O-Tab123@ net and insecticidal potency recovers with in within 24 hours. It is also reported that insecticidal effect in ITNs against malaria vectors remains there even after 20 months hand washing including transmission period [17,18]. Both types of insecticidal bed nets caused 100% knockdown and mortality effect in *An. dirus* mosquitoes. Jaramillo et al. also reported that PermaNet 2.0 insecticidal bed net effectiveness recovers with in 24 hour after one and three washing and produces effective mortality of mosquitoes [36]. In our study K-O-Tab only treated bed net showed small decrease in wash resistance against malaria vector *An. minimus* after 20 hand washing and produced 86% insecticidal mortality effect but bio-efficacy remains more than cut off value of WHO (80% mortality). ITNs satisfy WHO Pesticide Evaluation Scheme phase 1 criteria of a knock down effect above 95% after 20 washes. Mortality rate does not decrease below 80% (26). The bio-efficacy study found that repeated laboratory hand washings of the LLINs and ITNs bed nets retains insecticidal efficacy against malaria vector mosquitoes.

CONCLUSIONS

Malaria is a major infectious disease in Myanmar causing immense sufferings to mankind. Its prevention and control is tackled by several methodologies. This study evaluated the wash resistance and efficacy of LLINs and ITNs bed nets and

found that insecticidal persistence in mosquito nets remained even after 20 months washing (20 times of the laboratory hand washing). Present laboratory study found that PermaNet 2.0, Power net and K-O-Tab 123@ net can result in 100% mortality of malaria vector *An. dirus* and *An. minimus* in terms of efficacy and wash resistance. Maximum bioavailability was obtained 98-100% knock down and 96-100% mortality against *An. dirus* within 24 hours after one and three washes of all tested nets and three days continuous study observed that 100% mortality in terms of regeneration time of insecticidal efficacy appeared within 3 day. Bio-efficacy of 3 insecticide impregnated nets suggests that LLINs and ITNs can be effective alternative tools for malaria vector control in support of National Malaria Control Programme. Deltamethrin treated nets during night time can help prevent malaria effectively in malaria infested and anti-malarial drug resistance areas. Hence, it is proposed that Deltamethrin nets may be used for the prevention of malaria in remote forested areas and malaria endemic regions in Myanmar. Further bio-efficacy study of LLINs and ITNs in fields are needed to know the real situation from the villages or field, as people wash their nets more than 20 times and also other factors affecting the bio-efficacy of treated nets, like physical damage, use of bleach, washing method, and drying in the sun.

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