



EFFECT OF WASHING ON DELTAMETHRIN CONCENTRATION AND BIO-EFFICACY OF INSECTICIDE-TREATED MOSQUITO NETS

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Abstract

The deltamethrin impregnated mosquito nets not only prevent but also control malaria, dengue fever and other vector-borne diseases. However, the deltamethrin concentration and bio-efficacy of mosquito nets can reduce due to washing. The purpose of this study is to investigate the effect of washing on deltamethrin concentration and bio-efficacy of military insecticide-treated mosquito nets. High Performance Liquid Chromatography method was used for the determination of deltamethrin in mosquito-nets. Then, the WHO recommended cone and excito-chamber methods were used to determine the bio-efficacy of mosquito nets. The deltamethrin concentrations in the mosquito-net were after no wash (4590±58.54 ppm), 1× wash (4020±47.39 ppm), 2× wash (3225±45.22 ppm), 3× wash (2771±107.5 ppm), 4× wash (2657±58.59 ppm), 5× wash (1835±47.55 ppm), 10× wash (957±34.85 ppm), 15× wash (197±22.54 ppm) and 20× wash (87±23.75 ppm). The mosquito knockdown effects were reduced by washing of net (no wash to 20× wash) after 60 minutes and 6 hours exposure range from 90-40% and 100-70%. The mosquito mortality rate was reduced by washing of net (no wash to 20× wash) after 24 hours exposure were reduced range from 100-80%. The mosquito repellent action was reduced by washing of net (no wash to 20× wash) after 10 minutes and 24 hours exposure, range from 90-30% and 100-60%. The effective bio-efficacy level was found in 5×wash (1835±47.55 ppm of deltamethrin). Therefore, mosquito net should be impregnated again after the 5× wash to obtain the best protection on the public from mosquito bite.

Keywords: Bio-efficacy, Deltamethrin, Malaria, Military, Mosquito nets

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1. Introduction

Malaria is considered as a main vector-borne disease in the worldwide. Therefore, the different innovative methods for mosquito control have been studied by the researchers¹. The use of deltamethrin impregnated mosquito-nets is one of the main malaria vector control strategies^{1,2}. Pyrethroids insecticides, including deltamethrin are recommended for the impregnation of mosquito nets because they have not only rapid knock-down effects but also high insecticidal potency with low dosages. Moreover, the pyrethroids insecticides have good safety for domestic handling, human contact and low mammalian toxicity¹. Therefore, the pyrethroid insecticide such as deltamethrin is recommended by WHO Pesticide Scheme for indoor spraying and treatment of mosquito nets impregnation^{1,2}. The deltamethrin-impregnated nets have the positive effects on reducing morbidity and mortality due to malaria^{3,4}.

Malaria is one of the major causes of morbidity and mortality in Myanmar. According to National Malaria Control Program (2019), the 44 million people live in malaria endemic areas and 75,159 malaria cases were reported in 2008 (50% caused by *Plasmodium falciparum*)⁵. Malaria control activities in the country have been concentrated on early, mostly clinical, diagnosis and treatment. Limited availability of curative services in remote areas, the difficulties in accessing malarious areas and relatively high costs of effective treatment of multi-drug resistant malaria, has compromised malaria control efforts. The insecticide-treated mosquito nets (ITNs) are appealing approach to the control of malaria in Myanmar^{5,6}.

In several endemic areas of the world, the laboratory and field trial studies with treated and untreated mosquito nets have shown that treated mosquito nets have better protection as compared to untreated nets.^{7,8} The use of insecticide treated nets (ITNs) can reduce the number of bites from malaria vector by 94%. However, the nets have risk of the losing the mosquito repellent action due to washing. Therefore, these nets have to be retreated with insecticide after a few washes and if not, the nets do not serve as a long-term effective solution to the malaria problem.^{2,9}

In Myanmar, the soldiers and their families are living in the different areas of the country. Therefore, the prevention of malaria and other vector bone diseases is very important. The use of deltamethrin impregnated mosquito nets can prevent not only insects and mosquito bite but also malaria, dengue fever and other vector-bone diseases. Moreover, the deltamethrin concentration in ITNs is also important to prevent insect and mosquito bite. However, the deltamethrin concentration can reduce due to frequency of washing. The actual useful lives of ITNs can be estimated based on extrapolations from controlled laboratory washing experiments and field trial. Therefore, this study evaluated the concentration and bio-efficacy of deltamethrin in military insecticide-treated mosquito nets by High Performance Liquid Chromatography (HPLC) and different bioassay methods.

2. Objectives

2.1. General objective

To study the effect of washing on the deltamethrin concentration and bio-efficacy of military insecticide-treated mosquito nets.

2.2. Specific objectives

1. To determine the effect of washing on the losses of deltamethrin concentration in military insecticide-treated mosquito nets by HPLC.
2. To determine the bio-efficacy of military insecticide-treated mosquito nets by bioassay methods.

3. To evaluate the bio-efficacy level of deltamethrin concentration in military insecticide-treated mosquito nets.

3. Research Methodology

This study was laboratory based phase I and II field trial study. And, five deltamethrin treated mosquito repellent nets were collected from Disease Prevention and Control Unit, Naypyitaw. The study site was at Department of Pharmacology Research, DSMRC, Naypyitaw. The study period was from February 2021 to July 2021 and the convenience sampling method was used.

3.1. Materials and Methods

3.1.1. Chemicals and apparatus

The chemicals used in this study were acetonitrile, methanol, acetone, deionized water, deltamethrin standard and apparatus were shaker, autoclave, beakers, filter paper, syringe filter (0.22 μm), oven, rotatory evaporator, deionized machine, solid phase extraction (SPE) manifold and HPLC (LC 20AD, Shimadzu).

3.1.2. Determination of deltamethrin in samples by HPLC

3.1.2.1. Standard solutions preparation

The 1000 ppm deltamethrin stock standard solution was prepared by accurately weighing 100 mg of deltamethrin standard powder and quantitatively transferred into 100 mL volumetric flask and making it to the mark with the acetone. The working standards of 500, 250, 125, 62.5, 31.25 ppm were prepared by serial dilution from the stock solution with the acetone.

3.1.2.2. Calibration curve preparation

The calibration curve was constructed by peak areas versus concentration of the standards. The deltamethrin concentrations of the mosquito-net samples were calculated using the regression equation of the calibration curve.

3.1.2.3. Sample collection

The mosquito-nets were cut and the fifteen (15) pieces (25 \times 25 cm) of the net were used for HPLC analysis. The twenty (20) pieces from the same net (40 \times 40 cm) were used for cone and repellency bioassay tests.

3.1.2.4. Washing procedure

Net samples were added into 1 L beakers containing 0.5 L deionized water with 2 g/L soap (Win detergent powder) added and fully dissolved. The beakers are placed on water bath at 30 $^{\circ}\text{C}$ and shaken for 10 min at 150 rpm. Then, the samples were rinsed twice for 10 min with distilled water under same conditions as above and dried at room temperature¹⁰.

3.1.2.5. Sample extraction and analyze determination

Each 2 g of mosquito net added into 100 mL beakers. Then, the 30 mL of acetone was added into sample adding beaker and shaken for 5 min on vortex mixer. Then, this sample mixture was placed at 80 $^{\circ}\text{C}$ on digital water bath for 15 min and then, shaken for 30 min on orbital shaker (150 rpm). Then, the sample mixture was filtered with Whatman filter paper and concentrated in a Rotary evaporator until residues remained. This residue was reconstituted with 3 mL of acetone and it was

filtered by syringe filter (0.22 μm). Finally, the 20 μL of filtrate solution was injected into the optimized HPLC system.

3.1.3. Bioassay method for determination of deltamethrin impregnated mosquito-net

3.1.3.1. Cone test

The plastic cone was placed on top of the treated surface of the sample and secured using a masking tape. The five mosquitoes were blown into the cone using aspirator and mosquitoes were exposed to the treated surface. The numbers of mosquitoes resting on the deltamethrin treated nets were counted within 3 min of exposure. After 3 min, the mosquitoes were transferred to the plastic cones for further observation. The plastic cup was kept in an insecticide-free air and supplied with 10 % glucose solution. The number of immobilized mosquitoes was determined 1 h, 6 h after the exposure and the mortality rate was determined after 24 h.

3.1.3.2. Excito-chamber test

The excito-chamber box is made with one front and exit panel occupied with single escape portal. It builds up with glass holding frame and door cover. The mosquito was starved 4 h before the test. The behavior of mosquito was observed viz. the number of escaped mosquitoes to another space and remained mosquitoes inside the chamber which filled with treated product. Observation was recorded after 10 min and 24 h of exposure.

4. Results

The chromatogram of deltamethrin standard was shown in figure 1 and the retention time of deltamethrin was found at 9.5 min.

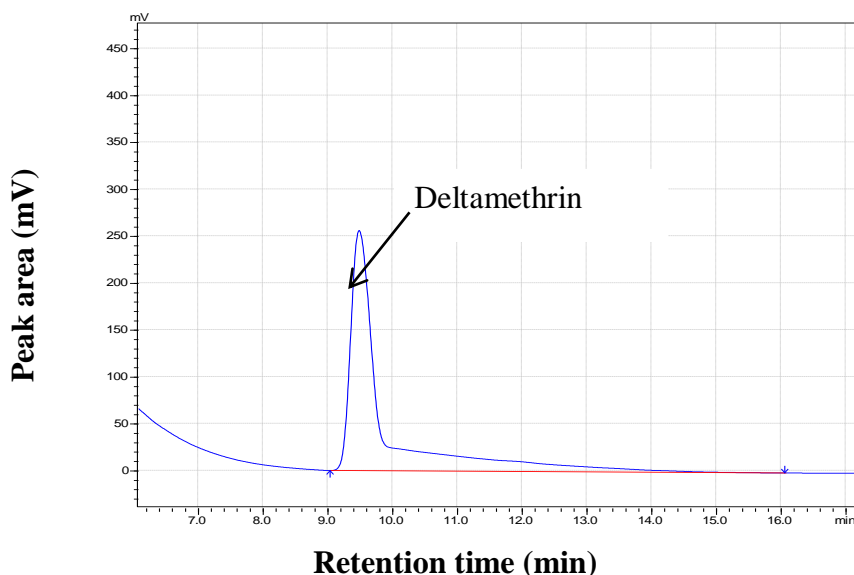


Fig. 1. The HPLC chromatogram of deltamethrin standard (500 ppm)

The regression value R^2 value was 0.998 that is suitable for quantitative determination of deltamethrin in nets.

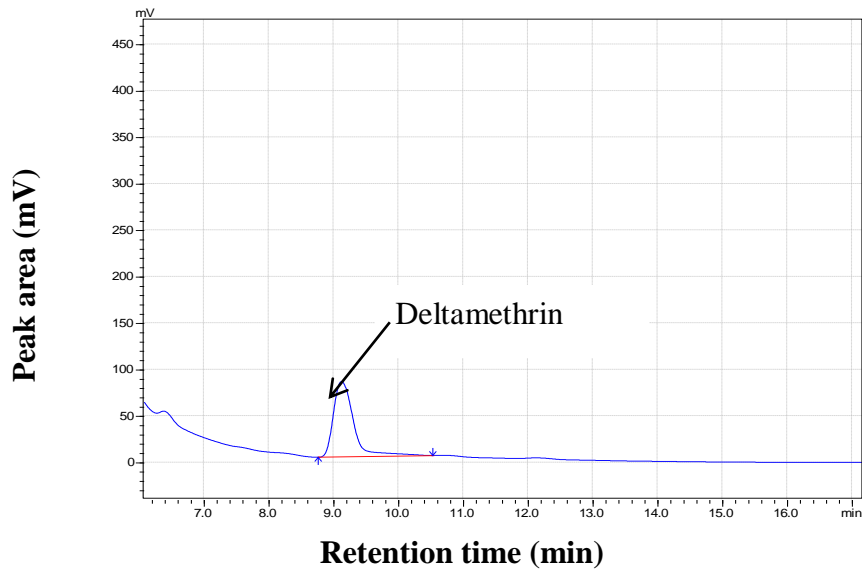


Fig. 2. The HPLC chromatogram of deltamethrin in mosquito-net

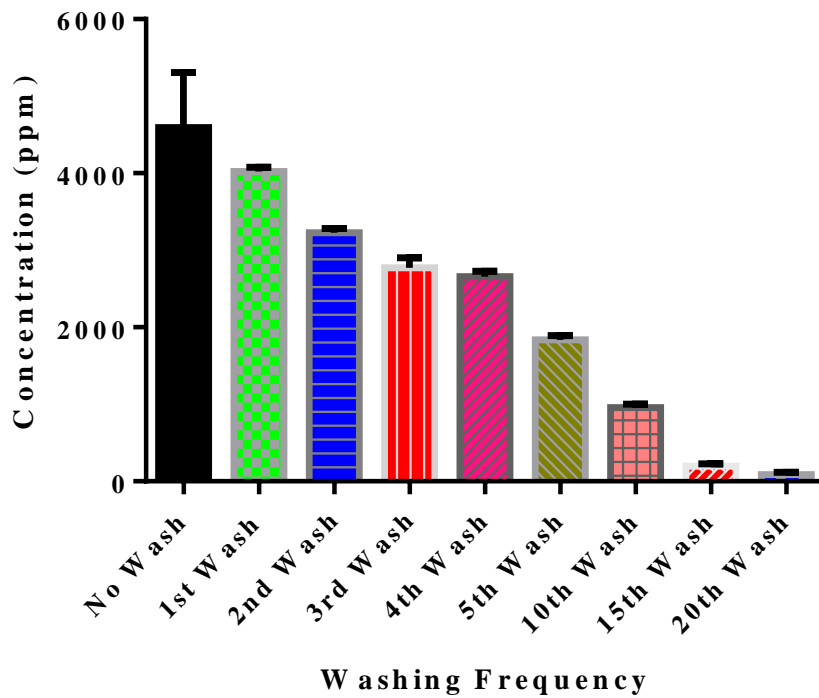


Fig. 3. Washing frequency and reducing of deltamethrin concentration in mosquito-net

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The following figure shows the effect of washing frequency and 60 min and 6 h knockdown of mosquito-nets. Moreover, the washing frequencies also influence the 24 h mortality rate of the mosquito-net.

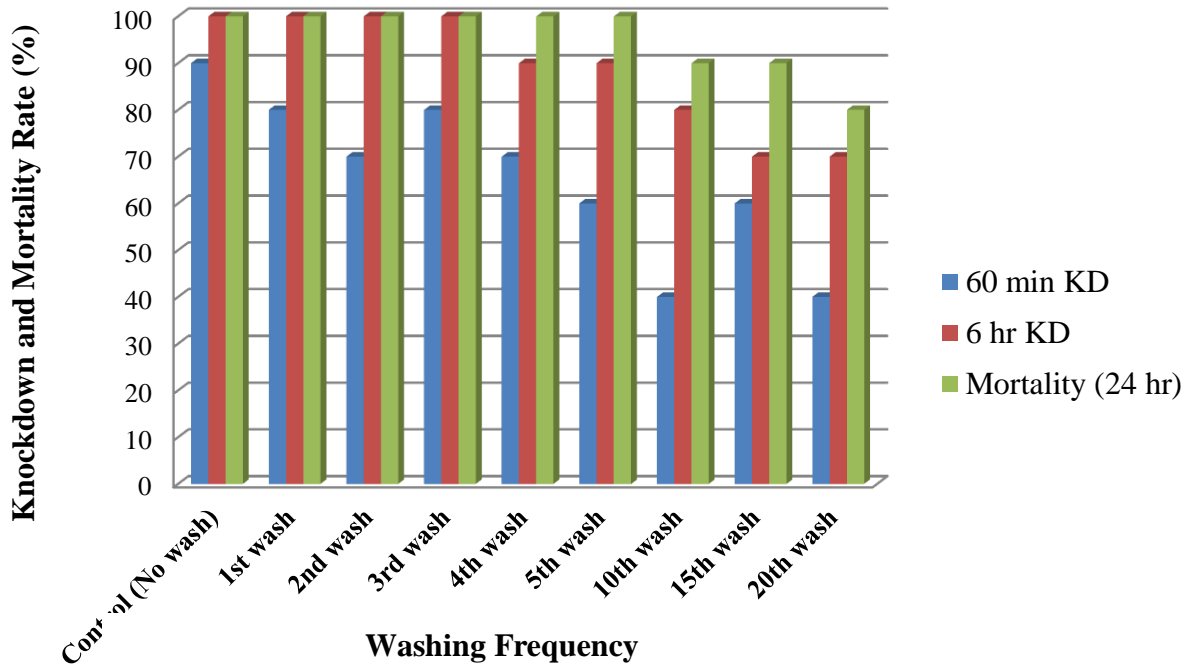


Fig. 4. Effect of washing frequency, knockdown (KD) and mortality rate of military mosquito-net

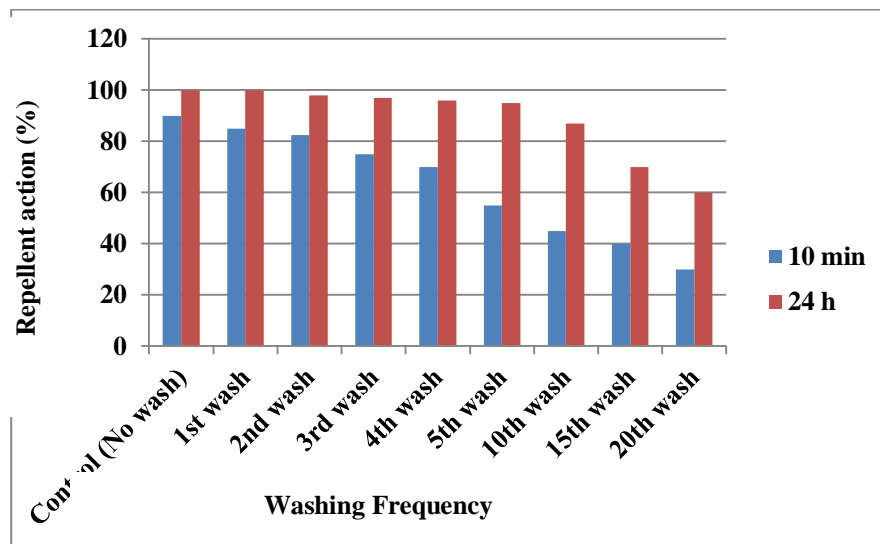


Fig. 5. Washing frequency and mosquito repellent action of mosquito-net

5. Discussion

According to the 2012 world malaria report, the 99 countries in the world still suffer from malaria transmission. As a developing country, especially in Myanmar, the malaria is one of the major public health problems in the malaria endemic areas. Nowadays, the insecticide treated mosquito nets (ITNs) are mostly used in the world to prevent people from malaria vectors and other mosquito-borne diseases. However, the frequencies of washing in the field are important factors which affect the efficacy and durability of mosquito-net^{11,12,13,14}.

In this study, the efficacy of the mosquito-net was determined by using two different bioassay methods. The concentrations of deltamethrin in military mosquito-nets were determined by the optimized and precise HPLC method. The HPLC conditions accepted were wavelength at 245 nm, the flow rate of 1 mL/min, oven temperature of 35 °C, and acetonitrile: water (85:15, v/v). The retention time of deltamethrin in standard and mosquito-nets were found at 9.5 min. The recovery percent of deltamethrin in sample were found to be 80-100 % and therefore, can be accepted according to the international standard.

The deltamethrin concentration in no wash mosquito-net sample was found to be 4590±58.54 ppm. Moreover, the deltamethrin concentration in mosquito-nets were reduced after 1× wash, 2× wash, 3× wash, 4× wash and 5× wash were found that 4020±47.39 ppm, 3225±45.22 ppm, 2771±107.5 ppm, 2657±58.59 ppm, 1835±47.55 ppm, respectively. Therefore, the reducing of deltamethrin concentration from 1× wash to 5× wash was lower than approximately 2000 ppm. However, the deltamethrin concentration in mosquito-nets were markedly reduced after 10× wash, 15× wash and 20× wash were found that 957±34.85 ppm, 197±22.54 ppm and 87±23.75 ppm, respectively. Therefore, the deltamethrin concentrations in military mosquito-nets were very markedly reduce between the 5× wash and 20× wash.

In this study, the effect of washing on the bio-efficacy of nets were studied by the WHO recommended cone method. The knockdown effect of no-wash, 1× wash, and 2× wash mosquito-nets in 60 minutes were 90%, 80%, and 70% respectively. However, after the 3× wash and 15× wash on mosquito-nets were not regular reducing of bio-efficacy level. This may be the unequal distribution of deltamethrin in mosquito-net during impregnating and difference size of mosquitoes. Moreover, after the 5× wash on mosquito-net the knockdown effects were lower than 60 %. Furthermore, the 100 % knockdown effects have on mosquito-net has been studied after no wash, 1× wash, 2× wash, and 3 × wash on 6 hour observation. Moreover, the knockdown actions were decreased from 90% to 70% among 4× wash and 20× wash. The mosquito mortality rate found 100 % from no wash to 5× wash after 24 h observation. However, after 10× wash of mosquito-net, the mortality percentage was lower than from 100 % to 80 %. Therefore, the determination of cone bioassay test on military mosquito-nets and its efficacy was markedly lower after the 5× wash.

In this study, the mosquito repellent action of the military mosquito-net was studied by the excito-chamber method. The repellent action of the military mosquito-net among no wash to 5× wash after 10 minute observation was found to decrease from 90% to 50%. However, the washing frequency after 5× wash such as 10× wash, 15× wash and 20× wash were found to be gradually lower than 50%. Moreover, the mosquito repellent action of military mosquito-net was after 20× wash indicated that only 60 % of repellent action remained after 24 hour observation.

The deltamethrin concentration in mosquito-net is may be depend on made up of various materials, including the cotton, nylon, polyester etc. The efficacy of ITNs depends on the frequency of washing time, impregnated chemicals quality, method of impregnated type and quality of fabrics.

Rafinejada J et al. studied the effect of washing on the bio-efficacy of insecticide-treated nets (ITNs) and long-lasting insecticidal nets (LLINs) against main malaria vector *Anopheles*

stephensi by three bioassay methods. They found that the results of three methods of bioassay tests showed that between two LLINs, PermaNet® was more efficient than OlysetNet®. The results of ITNs exhibited that deltamethrin and permethrin were more effective than etofenprox and bifenthrin as impregnants¹⁵.

Ordonez GJ et al., study revealed that in a trial in Colombia and Bolivia using cone test for evaluation of PermaNet, lambda-cyhalothrin, deltamethrin and alpha-cypermethrin impregnated polyester nets against *Anopheles spp* reported 100% mortality after three washes¹⁶.

The WHO 2002 highlighted that the insecticide treated nets (ITNs) must be impregnated after 3× wash and at least one time per year, if it is not washed¹⁶. In this study, the military insecticide treated-net was effective until the 5× wash and therefore the washing resistance was high. Moreover, the insecticide treated mosquito-net was stored at cool and dark place with room temperature for 6 months. And then, the deltamethrin content was determined by HPLC and it remained that 4350±27.45 ppm in nets.

6. Conclusion

According to the HPLC analysis and bioassay methods, the bio-efficacy level was found at 5× wash with the deltamethrin concentration of 1835±47.55 ppm. After the 5× wash of military insecticide-treated mosquito net, the deltamethrin concentration was lower than about 50% of initial concentration and both the bio-efficacy level of 60 min knockdown and 10 min repellent actions were markedly reduced. Therefore, every washing of 5 times (5× wash) on military insecticide-treated mosquito net that are needed to be impregnated to obtain the best protection on the soldier and their family member from mosquito and insect bite.

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