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Wash resistance and bio-efficacy of Olyset® Plus, a long-lasting insecticide-treated mosquito net with synergist against malaria vector, *Anopheles stephensi*

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ABSTRACT

Objective: To determine the wash resistance of Olyset® Plus using World Health Organization Pesticide Evaluation Scheme standard washing procedure and to assess the value of knock down and mortality rates of *Anopheles stephensi* at different regimens of long lasting insecticide treated nets washings.

Methods: The study was conducted at the Bioassay Laboratory of Culicidae Insectary, School of Public Health, Tehran University of Medical Sciences, Iran. The net was made of polyester impregnated with permethrin and piperonyl butoxide at a ratio of 2:1. The washing resistance was assessed using Le Chat® soap and a shaker incubator set at a speed of 155 r/min, 30 °C for 10 min. The cone bioassay test was carried out according to World Health Organization recommended guideline with tolerant field strain of female *Anopheles stephensi* to pyrethroids.

Results: The knockdown and mortality rates of female mosquitoes exposed to Olyset® Plus from un-washed nets to 2 washings were 79.7% and 88.8% respectively. Mortality was dropped to zero while active ingredient estimated 0.532 μg/100 cm^2 to 0.481 μg/100 cm^2 after 15 washings. A positive correlation was seen between residues of permethrin on nets, knockdown rate and mortality rate of female *Anopheles stephensi* exposed to different regimes of washed Olyset® Plus ($r = 0.954$, $P = 0.001$).

Conclusions: It is recommended that a preliminary survey conducted on resistance level of *Anopheles* vectors before the distribution of Olyset® Plus in malaria endemic communities.

1. Introduction

Diseases transmitted by mosquitoes have negative effects on the productivity of labor, especially in the countries with tropical and subtropical climates where the burden of malaria is the highest and are known to be endemic areas [1]. Currently, due to climate and environmental changes, the risk for the transmission of mosquito borne diseases has been increased the transmission of certain species of pathogens, *e.g.*, malaria, filariasis and arboviruses [2]. On the other hand, mosquitoes can cause annoyance to the residents of both urban and rural areas especially in the evenings, and even if the mosquitoes do not transmit the pathogens, they still act as nuisance to humans [3]. The main combative approach to mosquito related diseases is the application of insecticide-based methods [4]. Environmental concerns and human safety on the application and use of pesticides, their high cost and development of resistance of mosquitoes to more compounds have made it necessary to find safe and environmental friendly components which can replace the old ones [5]. Personal protection against mosquito bites using long lasting insecticide treated nets (LLINs) is cost-effective and specifically reduce the contact between

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target vectors and human hosts [6]. The use of LLINs is one of the main strategies for malaria control in endemic areas [7] as well as the most prominent malaria preventive measures for vector control [8]. It has been reported that Olyset® nets are highly effective against malaria vectors and moderately against other non-target household insects [9]. The use of LLINs is more effective than the application of indoor residual spraying with the possibility of high public participation as well as integrating primary health care which shows reduction of overall morbidity and mortality in children aged 1–4 years by 53% and 70% respectively [10]. Application and content of permethrin is usually taken into consideration for the evaluation of impregnated bed nets (ITNs) [11,12], military uniforms [13,14] as well as the industrial impregnated nets (LLINs) [15,16] for both malaria and cutaneous leishmaniasis control in Iran. This study aimed to determine the wash resistance of Olyset® Plus using the standard washing procedure as well as conducting a bioassay test, assessing the value of knock down and mortality rates of *Anopheles stephensi* (*An. stephensi*) at different regimens of net washing. The high performance thin layer chromatography (HPTLC) was also used to determine the residue variation of permethrin on the washed nettings and the assessment of the correlation of insecticide residue with the bioassay indicators.

2. Materials and methods

2.1. Location of bioassays

In order to maintain a contaminated free environment and procedure, all the bioassay tests were carried out in the Bioassay Laboratory of Culicidae Insectary (BLCI), School of Public Health, Tehran University of Medical Sciences.

2.2. Mosquitoes rearing

Pyrethroid tolerant strain of *An. stephensi* that originated from Chabahr Port, Southeastern Iran was used in this study. The strain was transferred at larvae and pupae stages to BLCI and the emerged adults were fed on 10% sucrose and for the mass production, they were artificially fed on expired human blood taken from the Iranian Blood Transfusion Organization.

Fish flake food produced in Iran (Nirooza®) was used to feed the larvae and after three generations, the adults adopted for blood feeding on guinea pigs. Two to three days old female mosquitoes were used in the experiments. The environmental condition of BLCI was set at temperature of (29 ± 1) °C, (65 ± 5) % relative humidity and the dark to light period (L:D) of 14:10 h.

2.3. Materials tested

The Olyset® Plus is made of polyester incorporating both permethrin and piperonyl butoxide at a ratio of 2:1. This mixture breaks down the mosquito resistance and increase the efficacy of the nets. Olyset® Plus is produced by Sumitomo Chemical Company Ltd and was received from the company following a request. The batches of LLINs was measured 25 cm × 25 cm and were prepared from one net used for both bioassays and analytical tests.

2.4. Washing resistance of LLINS

The LLINs were attributed into two groups: control with untreated polyester netting and treatment with treated polyester. For the treatment group, each LLINs (25 cm × 25 cm) was put into a liter glass bottle containing 500 mL of deionized water and 1.0 g of Le Chat® soap, the bottles were then put into shaker incubator and set at a speed of 155 r/min, 30 °C for 10 min followed by two rinsing stages with a similar procedure, but without any detergent. The total time used for one washing of the net was 30 min. The washed nettings were allowed to dry at room temperature and packed into the sheets of aluminum foil. For the control group, untreated polyester netting was used and underwent the same process of washings described above.

2.5. Bioassay tests

The World Health Organization (WHO) recommended cone test was carried out using 2–3 day old sugar-fed tolerant strain of female *An. stephensi* under controlled conditions in BLCI of School of Public Health Tehran University of Medical Sciences. Four standard WHO cones were fitted into plexiglas sheet [12]. For the assessment of change in the efficacy of both unwashed and washed batches of Olyset® Plus, five females *An. stephensi* were released into one standard cone and into three other cones simultaneously. A total of 20 mosquitoes were considered as one replicate with 3 min of exposure time [17]. The knockdown of exposed mosquitoes was observed between 1 and 64 min after the initial 3 min of exposure. The mortality rate was read after 24 h and all the tests were carried out at (29 ± 1) °C and (65 ± 5) % of relative humidity.

2.6. Chemical analysis of LLINs

HPTLC was used for determining the permethrin residue on the nets. After the bioassay testing, the pieces of netting were inserted into 10 mL glass bottles with caps and sealed with parafilm to prevent drying. The bottles were kept in the refrigerator at 4 °C until residue determination. Acetone was used as a solvent for quantitative analysis of the Olyset® Plus.

2.7. Statistical analysis

The data on mortality and knockdown rates were corrected to control mortality using Abbott's formula if the control mortality was between 5% and 20%. Mortality and knockdown rates from WHO cone bioassays were first transferred using arcsine formula and compared between each regimen of washed LLINs using one-way analysis of variance. Pearson coefficient was calculated to examine the correlation between mortality and knockdown rates as well as the residue of permethrin on the LLINs. The significance was set at 5% level. All analysis were done using PASW® software ver. 18 released by SPSS® Inc.

3. Results

3.1. Bioassay tests

The knockdown and mortality rates of *An. stephensi* exposed to Olyset® Plus after different washing regimes (from unwashed

Table 1
Mortality and knockdown of *An. stephensi* exposed to Olyset® Plus using cone test.

Times of washing	Mortality (%)		Knockdown (%)	
	Treatment (n = 100)	Control (n = 40)	Treatment (n = 100)	Control (n = 40)
0	100.0 ± 0.0	5.0 ± 0.5	100.0 ± 0.0	0.0 ± 0.0
1	96.0 ± 0.4	0.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0
2	84.0 ± 1.0	0.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0
3	79.0 ± 5.3	5.0 ± 0.6	73.0 ± 6.6	4.8 ± 0.5
6	85.0 ± 2.7	2.5 ± 0.3	95.0 ± 1.6	2.4 ± 0.3
8	17.0 ± 2.5	2.5 ± 0.2	33.0 ± 1.0	0.0 ± 0.0
15	6.0 ± 1.9	0.0 ± 0.0	5.0 ± 0.0	0.0 ± 0.0
20	3.0 ± 1.2	2.5 ± 0.2	3.0 ± 0.0	0.0 ± 0.0
25	1.0 ± 1.0	2.5 ± 0.4	1.0 ± 0.0	0.0 ± 0.0

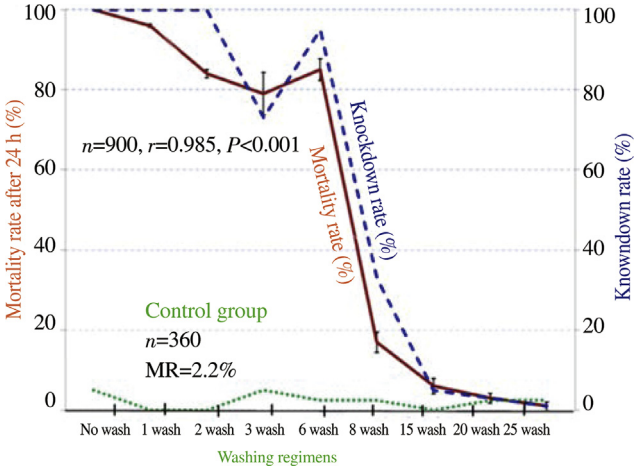

Figure 1. Trend of knockdown and mortality rates of *An. stephensi* to different washing regimens of Olyset® Plus using Le Chat®.

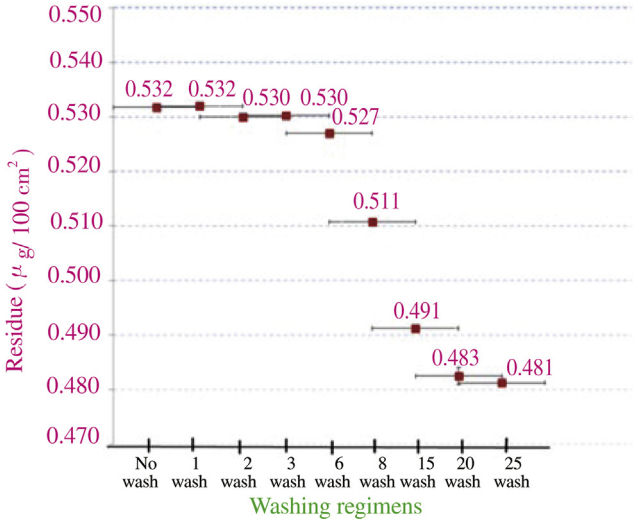

Figure 2. Trend of permethrin residue and confidence intervals followed different washing times of Olyset® Plus with Le Chat®.

to 2 washings) were found to be 79.7% and 88.8% respectively. In the control group, the average knockdown and mortality rates were 2.2% and 0.8%. After 15 washings, the mortality dropped to zero (Table 1). Majority of the knockdown mosquitoes were died after 24 h recovery period (Figure 1). The mean knockdown of tolerant strain *An. stephensi* was correlated with the mean of mortality during different regimens of washing (r = 0.928, P < 0.001).

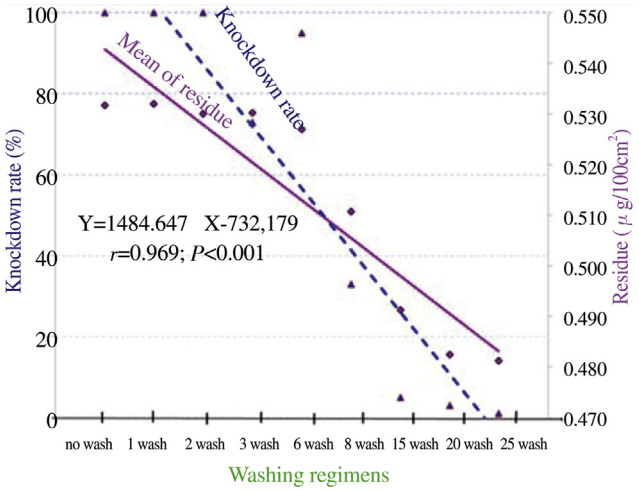

Figure 3. Correlation of permethrin residue with knockdown rate of *An. stephensi* following standard washings of Olyset® Plus batches with Le Chat®.

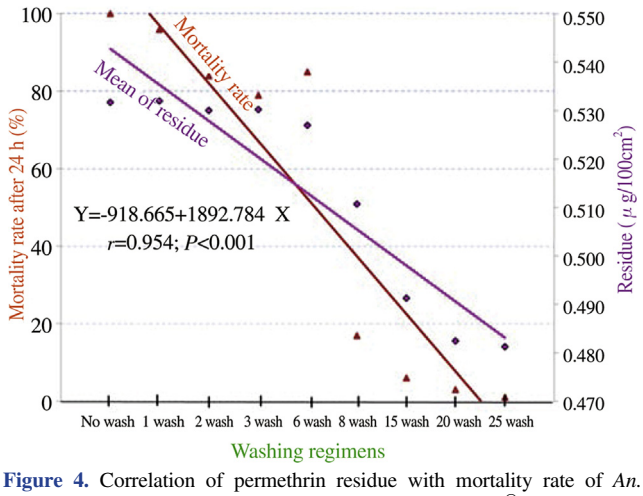

Figure 4. Correlation of permethrin residue with mortality rate of *An. stephensi* followed different washing regimens of Olyset® Plus with Le Chat®.

3.2. Chemical analysis of Olyset® Plus

The active ingredient on the Olyset® Plus was measured using HPTLC before washing and found to be 0.532 μg/100 cm^2, but decreased to 0.481 μg/100 cm^2 after 25 washings (Figure 2). A positive correlation was established between residues of permethrin on the nets, and the knockdown rate and the

mortality rate of female *An. stephensi* were exposed to different regimes of washed Olyset® Plus ($r = 0.969$, $P = 0.001$; Figure 3, $r = 0.954$, $P = 0.001$; Figure 4).

4. Discussion

LLINs are still the main malaria vector control priority based on WHO recommendation [7]. Tolerance to some conventional pyrethroid insecticides has been confirmed from field population of *An. stephensi* in endemic foci of southeastern Iran, as a result of operational issues with malaria vectors and agricultural pest control [18]. The values of LT_{50} and LT_{90} for *An. stephensi* tolerant strain was found to be 5.6 and 21.1 min with a mortality rate of 98% after 1-h exposure to permethrin which indicates susceptibility of this strain [19].

In this study, the observed mortality of tolerant strain *An. stephensi* exposed to different regimes of bi-treated net "Olyset® Plus" never reached 100% even with the unwashed nets and the rate remained low throughout the experiment. Significant correlation was seen between knockdown, mortality rates and the permethrin residues on different regimes of washed bi-treated nets using HPTLC ($r = 0.985$, $P = 0.001$). A comparative study by Allossogbe *et al.* reported a lower efficacy of Olyset® Plus 23%–41% mortality after 24 h against multi-resistance strain of *Anopheles gambiae* s.l. in Benin and also highlighted the mechanisms of resistance (kdr + metabolic resistance) [20]. Another study by Pennetier and colleagues reported mortality rate and knockdown rates of *Anopheles gambiae* s.l. were exposed to unwashed net samples of Olyset® Plus to be 100% but were decreased to 64.0% after 20 washings whereas the storage of the LLINs surprisingly increased the mortality after washing [21]. Evaluation of Olyset® Plus in Odisha state, India against wild strains of *Anopheles fluviatilis* reported mortality rate of 100.0% and 97.2% respectively [22]. Our study showed that the efficacy of mosquito net long-lasting Olyset® Plus could be affected by susceptibility of *An. stephensi* to insecticides especially pyrethroids. We recommend that LLINs be checked for resistance level before introduction into the malaria endemic foci.

Conflict of interest statement

The authors hereby declared that there is no any financial benefit associated with the materials used in this article. The authors declared that they have no competing interests.

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