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Increase the residual efficacy of Permethrin-impregnated cloths against mosquitoes by the use of controlled-release formulations

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Abstract
Permethrin-impregnated cloths and uniforms are one of the most effective personal protection measures against a variety of vector arthropods. Today, the dipping method with commercially available permethrin emulsions is a common method to impregnation of fabrics. But these formulations suffered from low durability on fabrics. In this regard, technical permethrin was formulated to permethrin solution (Emulsion), polymeric and microencapsulated formulations and their residual persistency was measured chromatographically on military uniform fabrics. Additionally, impregnated-fabrics efficacy on the knockdown and mortality were evaluated against *Anopheles stephensi* L. (Diptera: Culicidae). The obtained results showed markedly higher residual permethrin quantities with the polymeric and microencapsulated formulation, resulting in 237 and 124 mg/m^2 still present after 50 defined launderings, respectively. The knockdown and mortality rates of *A. stephensi* after 10 and 20 launderings, respectively, were significantly higher ($P<0.05$) for mosquitoes exposed to the polymeric and microcapsule formulations. The evaluation of the laundering-dependent time frame for obtaining 100% knockdown of mosquitoes constantly exposed to permethrin-impregnated fabrics indicated that 100% knockdown time of *A. stephensi* ranging from 3.5 min, 4.2 min and 4.1 min, prior to laundering, respectively. After 50 launderings, 100% knockdown time was > 120 min, 28.6 min and 45.8 min, for permethrin solution, polymeric and microcapsule formulations, respectively. These findings confirm the incremental impact of polymeric and microcapsule formulations on permethrin persistency and residual activity on fabrics against *A. stephensi*.

Keywords: Permethrin; vector control; personal protection; polymer formulation; microencapsulation; *Anopheles stephensi*

1. Introduction

Control and treatment of vector-borne diseases by vaccines and drugs faced numerous restrictions. So, over the past few decades, personal protection measures against mosquitoes and other medically important arthropods have played a significant role in integrated vector management (IVM) programs [1-6, 7]. Personal protection technologies such as insecticide-treated clothes and nets are one of the most effective tools against mosquitoes. Additionally, researches have shown that they have provided protection against ticks, bed bugs, fleas, sandflies and silverfishs [8–11]. Today impregnation of fabrics and clothes with pyrethroid insecticides especially permethrin, constitute the first line of defense against arthropod-borne diseases [12, 13]. Permethrin, as a synthetic pyrethroid insecticide, with features such as having a knockdown, repellency, hot-feet and residual activity, also low human and mammalian toxicity, widely used for control of mosquitoes, especially for impregnation of fabrics and clothes [12–14].

Many methods have developed to impregnation of fabrics and clothes with permethrin or similar compounds, which are categorized into four groups generally. Methods based on absorption (dipping and spraying), incorporation or Eulanisierung, polymer coating and microencapsulation [13]. Dipping fabrics in permethrin solutions and spray permethrin to clothes are basic methods and other three methods have developed from them to one reason generally: control the release rate of permethrin from fabric fibers and consequently increase the residual activity of permethrin-impregnated cloths under environmental conditions such as wearing, laundering and ironing [15, 16]. In the controlled-release formulations, polymer compounds play an essential role in the creation of new features: binding of permethrin molecules to fabrics and controlled-release of them under environmental conditions [12, 13, 15, 17]. It should be considered that permethrin is a persistent ingredient intrinsically [18] but, detergents used in washing reduce the residual activity of impregnated fabrics to almost zero after one–five washing cycles [17,19]. To cope with the problem, permethrin efficacy and bioavailability has increased by polymer coating techniques and microencapsulated formulations.

Permethrin-treated clothes and uniforms are now widely used by military personnel because of their at-risk environments, especially in tropical areas [20]. Infected troops have reduced maneuverability, moreover importing the diseases to their homelands potentially [11]. Using permethrin-impregnated clothing and uniforms in developed countries has become an inevitable issue. But, it isn't well accepted in developing countries such as Iran, which suffers from the vector-borne disease [19]. In this research, we have evaluated the persistence of three different permethrin formulations on the fabrics of military uniforms under different laundering cycles. In addition, residual efficacy on the knockdown and mortality evaluated against *Anopheles stephensi* (Diptera: Culicidae) after different laundering cycles.

2. Materials and Methods

2.1 Test insects

The established colony of a susceptible strain of *A. stephensi* from a continuously (≥20 years) reared colony were obtained from the Insectary of School of Public Health, Tehran University of Medical Sciences. Mosquitoes were kept under a regimen of 28±2 °C with a photoperiod of 16:8 (light: dark cycle) and 65±5% relative humidity (RH). Larvae were fed with fish food and water lettuce until adult emergence. The adults were fed with 10% sucrose solution as a source of energy and guinea pigs as blood-feeding female mosquitoes for maturing the eggs. Starved two to three days old females were used for the tests.

2.2 Preparation of formulations

2.2.1 Permethrin solution.

Technical grade permethrin (92% purity) consisted of a 25:75 blend of cis/trans isomers dissolved in a distinct volume of acetone containing non-ionic (Span 20) and hydrophilic (Tween 80) surfactants. In order to have fabrics with 1200 mg/m^2 permethrin concentration, water was added to the freshly prepared solution. The quantity of water calculated as per the absorption coefficient and dimensions of the fabrics.

2.2.2 Polymeric formulation

Polymeric permethrin formulation prepared according to Hebeish *et al*. [16] with modifications. Briefly distinct volume of polyvinyl acetate as a binder and Arkofix® NG (aqueous solution of Dimethylol dihydroxy ethylene urea (DMDHEU)) as a cross linker was added to permethrin solution. Before impregnation, water (calculated as per the absorption coefficient and dimensions of the fabrics) was added to the solution.

2.2.3 Microencapsulated formulation

Polymethyl methacrylate (PMMA) polymer was used for preparation of microencapsulated permethrin using the solvent evaporation method in Oil/Water emulsion system described by Teeka *et al*. [22] Briefly, PMMA was dissolved in toluene and mixed with permethrin and poured into Polyvinyl acetate (PVA) aqueous solution. After evaporation of toluene encapsulated permethrin was obtained. The rate of encapsulation was analyzed with UV-visible spectroscopy. Finally, formulation diluted in water and applied at 1200 mg/m^2 on fabric pieces by the conventional dipping method using a fixing agent (Arkofix).

2.3 Impregnation of fabrics

Pieces of military uniform fabrics (dimensions 10 cm × 10 cm) were used for permethrin impregnation and the evaluation of insect knockdown and mortality efficacy. Firstly the water absorption coefficient of the fabrics was determined. Fabrics were treated at rate of 1200 mg active ingredient (AI)/m^2 with prepared formulations by the conventional dipping method. Sufficient liquid was used to saturate each piece of clothing without runoff. Saturated fabrics were placed in plastic bags for 24 h to enhance liquid penetration. The fabrics were then removed from the bags, placed horizontally on aluminum foil and turned periodically to air dry without loss of permethrin from dripping. Before testing, the clothing was labeled and stored in fresh plastic bags. The control uniforms were treated just by water. The uniforms were kept in laboratory condition (temperature: 23-26 ºC, relative humidity: 30-40%).

2.4. Laundering procedures

Washing of permethrin-impregnated fabrics was carried out according to WHO protocol [23]. For this, fabric pieces were introduced individually into separate glass beakers (one l capacity) containing 850 ml of deionized water with detergent soap powder at 2 g/l added before washing and fully dissolved. The fabrics were shaken in the beaker manually using a glass rod with 155 movements per minute for 10 min. Later, the soap solution from the beaker was removed, and fresh deionized water was added to the beaker, and sleeves were again shaken for 10 min at the same agitation speed. In a similar way, once again, fresh deionized water was added, and further washing of sleeves was carried out. After washing, samples were taken after 1, 5, 10, 20, and 50 launderings and air-dried.

2.5 Permethrin quantification

The permethrin residue was detected after different laundering cycles and reduced percent was calculated for each formulation according to Frances *et al*. [17] with modifications. Samples were taken from three locations on the pieces and cut into pieces 1 × 1 cm. Pieces of each treatment placed in 2 ml glass vials and permethrin extraction were carried out by adding 1 ml acetonitrile to each vial. The samples were then sonicated for 10 min and aliquots analyzed by gas chromatography (GC). GC analysis according to Torabi and Talebi [24] was performed with an Agilent 7890A gas chromatograph equipped with a nitrogen-phosphorus detector (NPD) and a split/splitless injector. The column was a HP-5 capillary (30 m × 0.32 mm ID × 0.25 µm film). Career and makeup gases were helium and nitrogen at 3 mL/min and five mL/min respectively. Injection volume was 1 µL and splitless. Injector and detector temperatures were set at 240 °C and 250 °C, respectively. The initial oven temperature was kept at 100 °C for one min, increased to 155 °C at 30 °C/min for 1 min, and then raised to 175 °C at 10 °C/min for 2 min. The temperature was finally increased to 240 °C at 30 °C/min for 3 min. the experiment was performed in three replication and the standard curves were plotted using permethrin analytical standard.

2.6 Bioassay for knockdown and mortality

Cone bioassay for the knockdown was evaluated adopting WHO protocol [23]. The inner surface of bioassay tubes was covered by a fabric piece and then 10 numbers of nonblood-fed 2- to 5-day-old female of *A. stephensi* mosquitoes were introduced to the tubes for 3 min (Ten adult female mosquitoes per textile fabric sample).

The sample of fabrics after 0, 1, 5, 10, 20 and 50 washes for each permethrin formulation was exposed in each test (three replications). After 3 min mosquitoes transferred to the holding cylinder which was attached to the test tubes. The percentage knockdowns at 60 min post-exposure and the mortality 24 h post-exposure were determined.

A mosquito was scored as knocked down if it was lying on its back or side and was unable to maintain fight following a gentle tap on the cylinder [17]. In another experiment, the times of exposure necessary to obtain 99 % knockdown were measured continually for up to 120 min. Indeed, instead of 3 min, mosquitoes were exposed to fabrics for 120 min constantly.

Knockdown and mortality rates were corrected using Abbott's formula.

$$\text{Correlated mortality} = \frac{\text{Percent observed mortality} - \text{Percent untreated mortality}}{100 - \text{Percent untreated mortality}} \times 100$$

2.7 Data analysis

Values were reported as the mean ± standard error (SE). Knockdown percentages, times and mortality for *A. stephensi* were analyzed by a two-way analysis of variance (ANOVA) for repeated measures method. The differences between the least squares means and the *p* values associated with these differences were computed and compared by Tukey's test at the fifth percentile of significance using the IBM® SPSS 24 program (SPSS Inc., Chicago, Ill., USA). Graphs were plotted by SIGMAPLOT v.12.3 software (Systat Software, San Jose, CA, USA).

3. Results

3.1 Quantification of permethrin residue in fabrics

Prior to laundering, initial permethrin concentrations was 1200 mg/m^2 for all fabrics. The corresponding residual permethrin content determined after 50 defined launderings and associated percentage losses of permethrin are shown in detail in Table 1. After five launderings cycles, permethrin loss from the fabrics treated by permethrin emulsion up to five laundering was more than 80%, whereas in the fabrics which had treated by polymer and microcapsule formulations, remaining residue was more than 71% and 54% respectively (Fig. 1). The quantitative loss of permethrin after laundering is almost similar for the polymeric and microcapsule formulations. Markedly higher residual permethrin quantities were determined with the polymer-coating method, resulting in 237 mg/m^2 still present after 50 defined launderings (Table 1 and Fig. 1). This amount is equivalent to the quantity of permethrin remaining after five launderings using permethrin solution and after 20 launderings using the microcapsule formulation.

Table 1: Summary of initial and residual permethrin concentrations in the fabrics prior to laundering and after 50 defined launderings

Formulation	Initial Permethrin concentration (mg/m^2)	Permethrin concentration after 50 launderings (mg/m^2)	% Residual permethrin after 50 launderings	% Permethrin loss after 50 launderings
Permethrin solution	1200	0	0	100
Polymeric permethrin	1200	237	19.75	80.25
Microencapsulated permethrin	1200	124	10.33	89.67

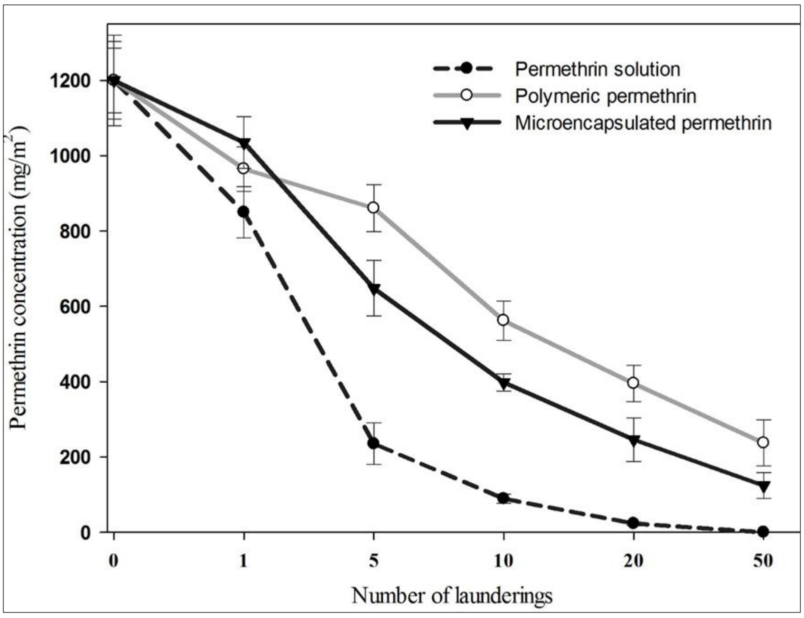

Fig 1: Residual amount of permethrin (mean ± SE) in fabric measured before and after up to 50 launderings

3.2 Knockdown and mortality patterns

The results of cone bioassay, the percent knockdown of *A. stephensi* mosquitoes exposed to permethrin-impregnated fabrics after different cycles of washings at different periods are given in Fig. 2. The knockdown of *A. stephensi* was significantly higher for mosquitoes exposed to the polymeric and microencapsulated formulations of permethrin compared with the permethrin solution (Fig. 2; $F = 39.1$; df = 2, 6; $P < 0.0001$). No significant differences were observed in the amounts of the knockdown percent in *A. stephensi* exposed to fabrics which, were impregnated with polymeric and microcapsule formulations of permethrin. After 50 launderings, no knockdown was observed in permethrin solution treatment (Fig. 2). The mortality pattern of insects exposed to the permethrin-impregnated army uniform cloths is given in Fig. 3. The results showed significantly higher mortality of *A. stephensi* exposed to the polymeric formulation compared with both microcapsule formulation and permethrin solution (Fig. 3; $F = 22.4$; df = 2, 6; $P < 0.002$).

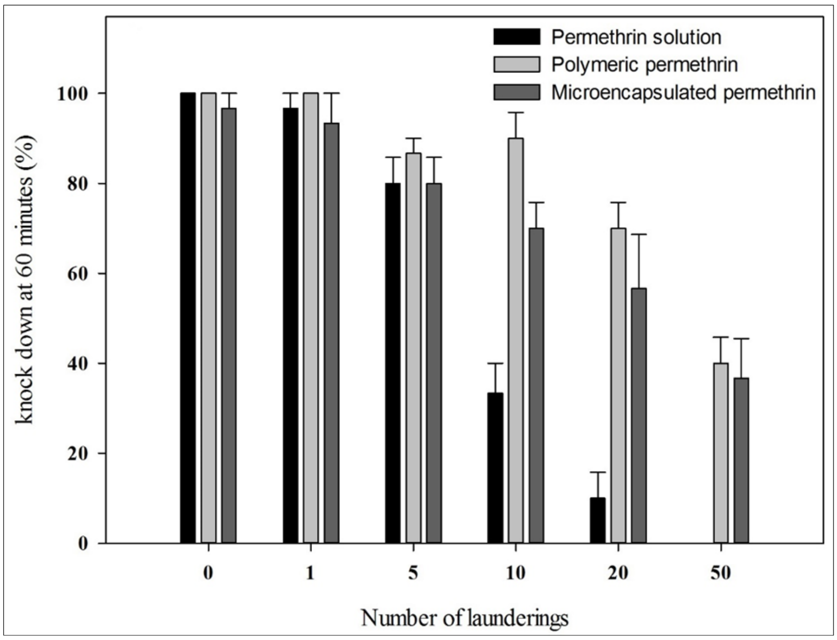

Fig 2: Knockdown of *Anopheles stephensi* at 60 min after 3-min exposure to treated fabric before and after up to 50 launderings

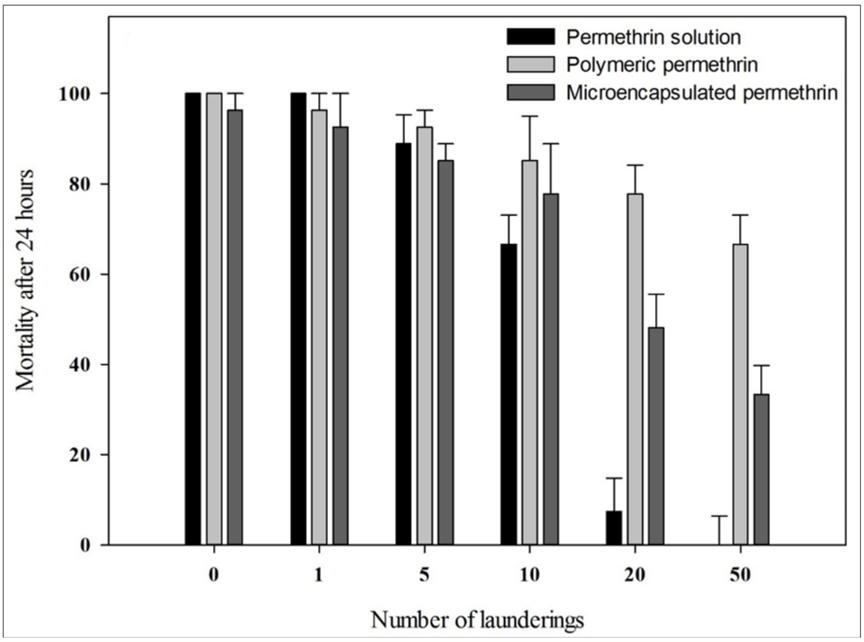

Fig 3: Mortality of *Anopheles stephensi* at 24 h after 3-min exposure to treated fabric before and after up to 50 launderings

The laundering-dependent time frame for obtaining 100% knockdown of *A. stephensi* constantly exposed to permethrin-impregnated fabrics is indicated in Fig. 4. By considering all launderings cycles, the knockdown activity of the polymeric formulation was significantly higher when compared with both microcapsule formulation and permethrin solution (Fig. 4; $F = 95.4$; df = 2, 6; $P < 0.0001$). As can be seen in Fig. 4, the knockdown time for permethrin solution, polymeric and microcapsule formulations on *A. stephensi* ranging from 3.5 min, 4.2 min and 4.1 min prior to laundering to > 120 min, 28.6 min and 45.8 min after 50 launderings respectively.

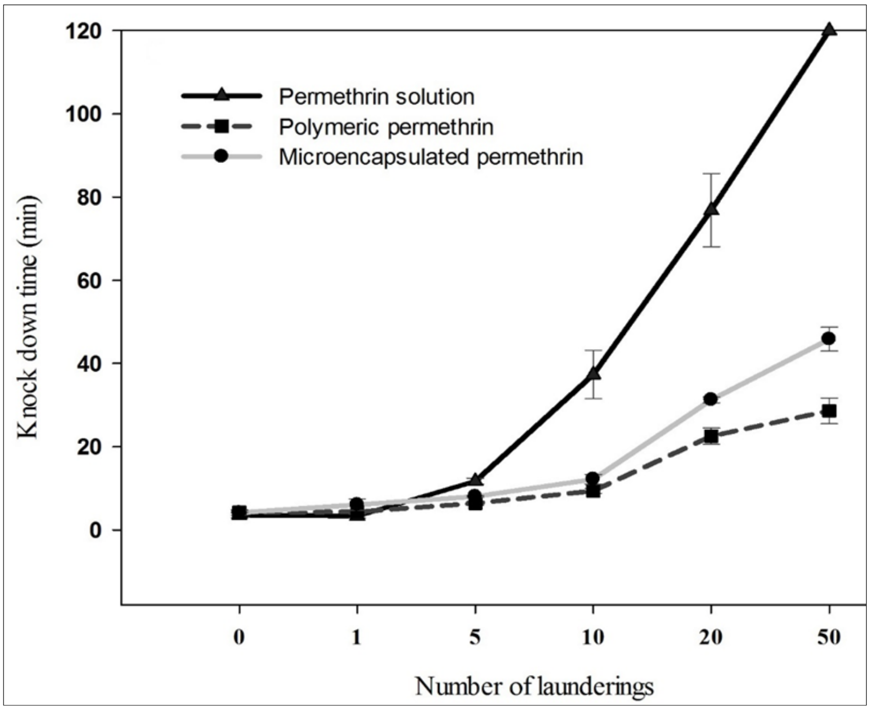

Fig 4: Time necessary to achieve 100% knockdown in A. stephensi before and after up to 50 launderings

4. Discussion

Many studies have performed about the persistence of permethrin in impregnated-fabrics (different methods) by up to 100 laundering and then exposing mosquitoes (or other arthropods) to treated fabrics for the amount of time needed to knockdown or kill them which, some of them reviewed by Banks *et al.* [8]. Laundering is the main effective factor in the elimination of insecticides from clothes, so it has attracted special attention almost in all researches that have done in this field. Our results show that impregnation of cloths by different permethrin formulations has specific features and affected permethrin persistence in fabrics. The results indicate a steep decline in the permethrin residue in permethrin solution treatment after five launderings. This result confirms previous studies that, most permethrin residue disappears up to five launderings in dipping method by usual permethrin solutions [17, 20, 25]. In another study, initial Permethrin residue (1600 mg/m^2) in the fabrics treated by dipping methods by commercial permethrin emulsions (Peripel 10® and IARFT®) after ten launderings reduced to less than 200 mg/m^2 [9]. However in factory-based fabrics treatment (UTXBEL®) after 100 launderings permethrin concentrations were more than 200 mg/m^2 [9,26]. The factory-based fabrics and clothes are produced by polymer coating technique, which polymer molecules create cross-link bounds between permethrin molecules and fabric fibers. In the other hand, factories use pressure and heat to better results. So, permethrin persistence in the conventional dipping method with polymeric emulsion and microencapsulation maybe cannot be as effective as commercial products such as UTXBEL. However, based on our results these formulations compared to permethrin emulsions are very efficient on permethrin durability on fabrics. In accordance with the present results, Ardanuy *et al*. [27] demonstrated that adding polymer compound (tetraethyl orthosilicate) to permethrin emulsion increase persistence and residual activity of permethrin in clothes.

Knockdown and mortality percentages in all three formulations on *A. stephensi* before any laundering were almost equal, while after five launderings significant differences have emerged. It has shown that the effectiveness of treated fabrics with permethrin emulsion (dipping method) against *Aedes albopictus* (S.) dropped under 30% after five launderings [20]. Similar results have reported by dipping method on *Amblyomma Americanum* (L.) [28]. Also, Frances *et al*. [17] showed that impregnation of fabrics by dipping method with permethrin emulsion (Perigen®) shows knockdown and mortality efficacy up to five launderings against *Anopheles farauti* (L.) and *Aedes aegypti* (L.). However, commercial factory treatments (polymer coating method) were effective even after 50 launderings [17]. In another study, Faulde and Undelhoven [9] have compared different impregnation methods and indicated high persistency of permethrin effects on *A. aegypti* and *Ixodes ricinus* (L.) after numerous launderings in polymer coated technique. In other hands, microencapsulation of pyrethroids has increased their effectiveness on *A. albopictus* even after 20 launderings, whereas effects of dipping method were observed only up to 10 launderings [15].

The time required for the knockdown has increased with increasing laundering cycles. There is a correlation between the percentage loss of permethrin during launderings and knockdown time. Washing decrease availability of active ingredients (AI e.g. permethrin). Therefore, based on toxicokinetic models, the time required to absorption and reach AI concentration to toxic levels will increase. Subsequently, along with absorption, detoxifying procedures reduce the bio-availability of AI and fabrics loss their toxic effects after given laundering cycles finally. It should be borne in mind that, in the case of medically important arthropods the increased knockdown time challenged the effectiveness of fabrics against arthropod vectors [13]. However, based on our results in the case of polymer and microcapsule formulations, increased time was significantly lower than permethrin solution. These findings correspond with the results reported by Faulde *et al*. [13, 26], and Faulde and Undelhoven [9] which demonstrated that knockdown required time in polymer-coated technique after different launderings increased much less than dipping method. This fact indicating polymer effects on controlled release and persistence of AI on or in cloths fibers which, affected users of permethrin-treated clothes less than permethrin solutions [13, 14].

5. Conclusion

Today, permethrin-impregnated fabrics are available in many countries around the world commercially. In other hands, permethrin-impregnated military uniforms, e.g., PTBDUs (permethrin treated battle dress uniforms of German armed forces), PTBFUs (permethrin treated battlefield uniform of French armed forces), widely used for protection of troops. Most of these products are factory-based permethrin-impregnated fabrics, which have treated with permethrin by dipping or polymer coating methods. However, it doesn't mean that there is no need for commercial permethrin formulations for impregnation of fabrics and uniforms. Permethrin formulations either liquid solutions or sprays are available and are considered as useful and applicable tools for the enhancement of personal protection in at-risk environments. But, as it mentioned, treatment of fabrics with these formulations will not have long-lasting efficacy and more should be replaced with new formulations such as polymer based ones.

6. Conflict of Interest

The authors have declared that no conflict of interest exists.

7. Acknowledgment

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