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**Field Evaluation of Permethrin-treated Military Uniforms Against
Anopheles stephensi and 4 species of *Culex* (Diptera: Culicidae) in Iran**

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Abstract: In this study, six types of current military uniforms of Iran were treated by permethrin (0.125 mg permethrin [AI] cm⁻²) and examined against the biting of natural population mosquitoes of rural areas of kazeroon, Fars Province, south of Iran, namely *Culex* and *Anopheles*. Eight volunteers were selected for this study. Six of them put on the treated uniforms and the other two ones wore the untreated uniforms. All the subjects participated in the night biting test for eight active nights of July and August 2004. There was no significant difference in the protection of different treated uniforms against mosquitoes biting. In this study, the average number of biting among who wore untreated uniforms (controls) was 3.21 mosquito biting/min/person (192.8 h⁻¹). This amount was 0.26 (15.6 h⁻¹) for who wore treated uniforms (cases). The relative protection level of treated uniforms, in comparison with untreated ones, was about 91.9%. The protection percent of treated uniforms for different species of mosquitoes, in comparison with untreated ones, was calculated through determining the species of captured mosquitoes, separately from case and control subjects. The results showed that the relative protection percent of treated uniforms against species of *Culex bitaeniorhynchus*, *Cx. tritaeniorhynchus*, *Cx. perexiguus*, *Cx. theileri* and *An. stephensi* was 72.7, 87, 89.8, 84.3 and 78.7, respectively. The results of chemical analysis with High Performance Thin Layer Chromatography (HPTLC) method, before and after field test, showed that in two weeks of study, there hasn't been any significant decrease in the amount of uniforms' permethrin.

Key words: Permethrin, mosquitoes, fabrics, impregnation, military uniform, personal protection, *Culex bitaeniorhynchus*, *Cx. tritaeniorhynchus*, *Cx. perexiguus*, *Cx. theileri*, *Anopheles stephensi*, HPTLC, TLC, Iran

Introduction

Insects and other arthropods, with nuisance and transmission different diseases to human, is one of the most important health threatening factors for mankind.

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Personnel of military forces have close contact with the environment and can be bitten by insects and exposed to the related diseases, more than other people (Schreck, 1977; Debboun *et al.*, 2001).

In recent years, great progresses have been occurred in protecting people, especially military personnel, against arthropod bites and vector-borne disease, using repellents and treating tents, mosquito nets and clothes with insecticides.

Treating mosquito nets, tents, curtains, blankets and clothes with Pyrethroid, has become current since many years ago. Allowed doses of permethrin, is a safe repellent and insecticide. So, it has been widely used for treating military uniforms and has protected them against hematophagous arthropods like mosquitoes, ticks, mites, fleas and many other insects (Fryauff *et al.*, 1996). Using permethrin-treated military uniforms in its allowed and safe doses (0.125 mg cm^{-2}) has been known as an effective method for protecting military personnel against hematophagous insects (Schreck *et al.*, 1978a, b; 1980; 1982; 1988; 1989). Several laboratory and field researches have been done in many armies of the world to evaluate the effectiveness of permethrin treated uniforms; the results have been satisfactory in most cases.

Today, using permethrin-treated military uniforms is among the hygienic programs of many armies of the world and the U.S army widely used these clothes in Persian Gulf war (Magill *et al.*, 1993).

Using permethrin-treated uniforms has also been introduced in some countries like France, as a vector control strategy and protection method for military personnel against insects (Deparis *et al.*, 2001).

In this study which has been performed for the first time in Iran (Khoobdel *et al.*, 2003), the protection of permethrin-treated military uniforms against anophelinae and culicinae mosquitoes in a rural field has been examined.

Permethrin-treated military uniforms retain their insecticide quality even after several washing, thus they don't need retreating after every washing. Meanwhile, by using specific treating techniques, the insecticide quality of permethrin can be retained for a very long time (Schreck *et al.*, 1978; Gonzalez *et al.*, 2002).

Materials and Methods

Study Sites and Volunteers

According to the previous studies and the primary estimations, Islamabad village (Kazeroon, Fars Province) was selected for this study. The reasons of this selection include facilities of Kazeroon's health research site, experiences of the researchers of the site in performing night biting tests and presence of local volunteers for attending the test. Moreover, mosquito-borne diseases were rarely reported in this area. On the other hand, species of culicinae and anophelinae were abundant in this area due to the numerous breeding place and plentiful flowing and stagnant water supplies in Islamabad village. So, the selected places had the required technical condition, regarding to the amount of biting/ person (biting pressure). According to the EPA standard, at least 5 biting per 5 min acceptable for mosquitoes test.

Eight local male subjects were selected for wearing permethrin-treated and untreated uniforms (six as cases and two as controls). In order to decrease subject caused errors, they wore the uniforms in a revolving manner (subjects in eight nights wore all treated and untreated uniforms).

Subjects were 18-28 years old and their age average was 19.5 years. Eight officers were also appointed as collectors. The subjects were trained about the test time and the needed activities. The

officers were also trained about the method of data registration in special sheets. The field study was performed in July and August, 2004.

Fabric Treatment

The uniforms were treated at rate of 0.125 mg active ingredient (AI) cm⁻² of cloth with aqueous suspensions of permethrin from 10% Emulsifiable Concentration (EC) formulation (cis/trans isomers with 25/75% ration, respectively). The concentrations of treating solutions of permethrin were determined based on the quality and absorption coefficient of six uniforms' textile and also the area of uniforms. Then, treating was done by dipping method. Sufficient liquid (450-500 ML) was used to saturate each article of clothing without runoff. Saturated fabrics were placed in plastic bags for 24 h to enhance liquid penetration. The uniforms 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 (Schreck *et al.*, 1980). The control uniforms were treated just by water. The uniforms were kept in laboratory condition (temperature: 23-26°C, humidity: 30-40%).

For assuring to achieve a deposition rate of 0.125 mg (AI) cm⁻² of fabrics, three to four 2 cm² pieces of uniforms' textile from pockets and collars were cut and separately put in small vials. After sealing the vials by parafilm, they were kept in 8°C temperature. Before starting field test, their permethrin were extracted and measured by HPTLC technique. In cases of inappropriate concentrations, the uniforms were retreated.

Field Test

After primary reviews, the subjects were selected among local people of study villages and trained about the test. They were asked not to use insect repellent or attractive materials like perfumes, alcohol, smoke, garlic, mint and other odorous materials. Subjects received the uniforms, half an hour before sunset. Six of subjects wore the treated uniforms and the other two ones, wore the control untreated uniforms. Subjects sat in appropriate distance of each other (5-10 m). The control subjects sat in the same manner, in 50-100 m distance of case subjects. Field test was started at sun set. One collector was considered for each subject. The collectors entered the night biting data in the sheets. They did the task by direct watching of the biting point. Then they captured the mosquitoes and transferred them in to the cups. The mosquitoes that only landed were just counted and not captured. The related collector and officer determined the species of mosquitoes (Anophelinae, or Culicinae) if possible. The mosquitoes that aspirators and then their species captured bited subjects were determined. The cups were changed every half an hour, in order to calculation of the activity peak of anophelinae and culicinae mosquitoes. Meanwhile, subjects changed there place every half an hour to face the untried mosquitoes and prevent a fatigue or knockdown effect on mosquitoes because of repeated contacts with treated uniforms in the same areas (Schreck *et al.*, 1989).

Night biting test was started at 20:00 and ended at 1:00 every night, when number of bitings decreased. The subjects ate their dinner before starting the test and didn't have any activity during the test, except a 15 min rest at 11:00, when the bitings had a decrease. At the start and end of every hour, the temperature and humidity of air were recorded. After ending the test every night, the uniforms were collected and given back to the subjects, the next night.

Chemical Test

As explained earlier, some 2 cm² pieces of uniforms were cut before and after the field test and kept at 8°C for assuring of the treating confidency and preventing errors and also determining the remained permethrin in the uniforms.

One milliliter pure acetone was added to the vials containing 2 cm² pieces of uniforms. The vials were sealed and the permethrin of uniform pieces was extracted by 10 min shaking with medium round and then allowed to stand 1 h just before analysis to ensure extraction of a representative quantity of permethrin (Schreck *et al.*, 1982). The spotting on a silica gel containing aluminum plate (Silica gel 60 F₂₅₄), was performed by an applicator and capillary (5 µL). Volume of each spot was 10 µL and the distance between spots was 1 cm.

The standard permethrin (10 mg) was bought from Accustandard Company. For spotting the samples, the multiple level method was used. In this method, some different concentrations or different volumes of a standard concentration are used for spotting the standard sample. After spotting and drying the spots, the ready plate is put inside the chamber tank. The mobile phase solvent used for permethrin, was n-hexane-ethyl acetate, with 95:5% ratio, respectively (Sherma, 1997; Gupta *et al.*, 1998). This was poured into the tank and the ready plate was put in it after saturation of tank (about 30 min).

Then, plate was exited of the tank. After drying, spots were observed in UV cabinet by fluorescence light with 254 nm wavelength. Finally, the spots were scanned (207 nm) by TLC Scanner 3 (CAMAG), using CATS4 software, (the measurement mode was Absorption/ Reflection) (Chen and Wang, 1996).

Species Identification

The mounted mosquitoes were identified in laboratory, using valid identification keys (Zaim and Cranston, 1986; Harbach, 1988; Colick, 1992).

Data Analysis

Data was set and standardized based on the number of bitings per minute, for each subject. Since the statistical analysis of this study was done bases on the quantity, data was firstly transferred by $\sqrt{y+0.5}$ formula (y: number of bitings per minute) and then Poisson distribution was used for statistical analysis of bitings number in case and control groups. For comparing the biting means (on skin and through uniforms) in subjects who had worn 6 types of military uniforms, Analysis of Variances (ANOVA) was used. Also Tukey method was used for Multiple Comparison. For comparing the means of biting and landing (on skin and through uniforms) in treated and untreated groups, t-test was used. For comparing protection percent of treated uniforms in different species of *culex* and *Anopheles* mosquitoes, Crucial and Alice test was used. After statistical analysis, the means retransformed to original units and converted to mosquito bites per minute. Protection percent of treated uniforms was calculated using the following formula:

$$\text{Protection percent} = \frac{\text{Untreated biting} - \text{treated biting}}{\text{Untreated biting}} \times 100$$

Results

The results showed that in spite of the differences in fiber type, water absorption coefficient, thickness, light stability and some other physical factors of uniforms, there was not a significant differences in protection percent of 6 types of uniforms ($p > 0.05$). The mean of biting per person for all treated uniforms was 15.6 h⁻¹ (SE±1.57). Comparison of treated and untreated uniforms showed

Table 1: Mean of mosquito bites per person, in 6 kinds of Iranian military uniforms (treated and untreated)

	Uniforms	Mean	SD	SE
Treated	U ₁	8.8	1.75	0.44
	U ₂	22.3	11.27	2.82
	U ₃	18.3	8.87	2.22
	U ₄	14.3	3.50	0.88
	U ₅	17.7	7.71	1.93
	U ₆	12.3	4.59	1.15
Untreated	U ₀ *	192.8	30.7	7.68

Statistical analysis was performed on the squar-root transformation of number of mosquito bites per minute
* is significantly different $p < 0.01$, U: Uniforms

Table 2: Mean of mosquito bites per person per hour , through skin and clothing

	Mosquito bites per hour				Mosquito Landing per hour	
	On skin±SE	Through clothing	Overall	Percent protection	On clothing±SE	Percent repellency on clothing
Treated	15.6±1.57	0.00	15.6	91.9	1.6±0.13	97
Untreated	192.8±7.68	0.61	193.4	0	52.3±8.9	0

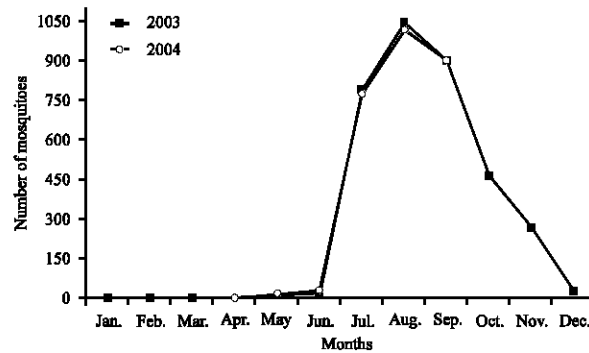


Fig. 1: Monthly activity of *An. stephensi* and *Culex* spp. in Islamabad village, Kazeroon, Fars province; 2003, 2004 (with Total catch method)

that the protection percent of treated uniforms against mosquito biting was totally 91.9%, which was significantly higher than untreated uniforms ($p < 0.01$). The mean of biting per percent for untreated uniforms was 192.8 h^{-1} ($SE \pm 7.68$) (Table 1).

Natural population of mosquitoes in this study was mainly of two genus, namely *Culex* and *Anopheles*. These species usually bite at nights and through skin. Since they rarely bite through clothes, 95% of bitings in this study occurred through skin. Comparison of the mean of bitings through skin in treated and untreated groups showed that there is significant difference between them ($p < 0.01$) (Table 2).

Comparison of landing on the uniforms of case and control groups showed that there was a significant difference between them ($p < 0.01$). Landing on the uniforms was 1.6 ($SE \pm 0.13$) and 52.3 ($SE \pm 8.9$) per hour per person for case and control groups, respectively. This shows a 97% of repellency for treated uniforms, in comparison with untreated ones (Table 2). Since the peak of seasonal activity of mosquitoes, especially *An. stephensi* (the main vector of malaria in Iran), in study region is July and August, (Fig. 1) this study was performed at the end of July and beginning

Table 3: Protection percent of permethrin-treated uniforms against *Culex* (4 species) and *Anopheles stephensi*

Species	Captured on volunteer per night		
	Untreatment	Treatment	Percent protection (%)
<i>Cx. bitaeniorhynchus</i>	100.3	27.3	72.7
<i>Cx. tritaeniorhynchus</i>	96.0	12.5	87.0
<i>Cx. perexiguus</i>	126.0	12.8	89.8
<i>Cx. theileri</i>	35.0	5.5	84.3
<i>An. stephensi</i>	124.5	26.5	78.7
<i>An. fluviatilis</i>	4.0	1.0	-
<i>An. turkhudi</i>	1.0	0.4	-
<i>An. superpictus</i>	2.0	2.0	-

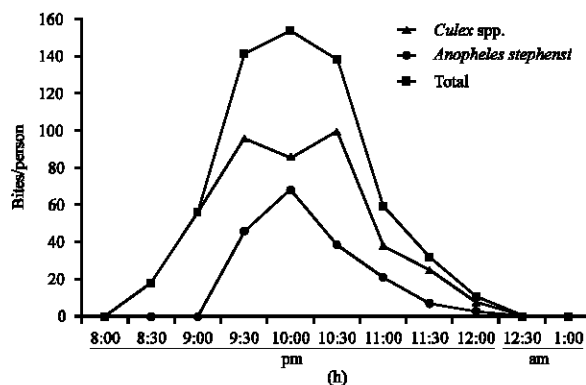


Fig. 2: Night biting (bites/person) activity of *An. stephensi* and *Culex* spp. in Islamabad village, Kazeroon, Fars province; 24 July-1 August, 2004

of August. In this study, night biting of *An. stephensi* and *Culex* mosquitoes of Islamabad village was also determined (Fig. 2).

As it can be seen at Fig. 2, the peak of night biting report of *Culex* and *Anopheles* mosquitoes is different in study region, so that *Culex* mosquitoes start their activity at 20:00 which reaches the peak at 21:30, which *Anopheles* start their night biting at 21:00 and have the peak of their activity at 22:00. *Culex* have also one other peak of activity at 22:30 (Fig. 2).

The mean of air temperature during study and at different hours (20-01) was 27.4°C (20-33°C) and the mean of relative humidity was 60.1% (42-75%).

As it mentioned before, all of bitings on subjects, were recorded in especial sheets by collectors. Besides, the biting mosquitoes were captured by aspirator.

Totally, 70-80% of mosquitoes bited case and control subjects were captured. So, the protection percent of each mosquitoes species was calculated using the number of captured mosquitoes from case and control subjects. The results of this study showed that the protection of treated uniforms, in comparison with untreated ones, was different for various species, so that the protection percent of these uniforms against *Cx. bitaeniorhynchus*, *Cx. tritaeniorhynchus*, *Cx. perexiguus* and *Anopheles stephensi*, was 72.7, 87, 89.8, 84.3 and 78.7, respectively (Table 3). In addition to the abovementioned species, three other species of *Anopheles* namely *An. fluviatilis*, *An. turkhudi* and *An. superpictus* were also captured during the study, but since their number were too low and their biting pressure were less than 5 biting per 5 min, calculation of protection percent was impossible for them, even at the peak hour of their night biting activity.

The results of chemical analysis by HPTLC method showed that the concentration of permethrin in different uniforms was 0.124 mg cm^{-2} (0.121-0.130) at the start of field test and 0.120 mg cm^{-2} (0.118-0.131) at the end of it (two weeks later), which was not a significant decrease ($p > 0.05$). In this study that *n-hexane-ethyl acetate* solvent system was used, two separate spots of trans-permethrin and cis-permethrin were observed. R_f value was also calculated for the two isomers:

$$R_f(\text{trans}) = 0.41$$

$$R_f(\text{cis}) = 0.48$$

Discussion

Different fibers and textiles such as cotton, jute, wool, nylon and polyester, may show different insecticidal qualities after treating (Curtis *et al.*, 1996). For example, some studies show that pure fibers of wool or cotton, retain their insecticidal quality up to one year after treating, while the pure fibers of polyester or polyamide lose this quality only one week after treating (Wood *et al.*, 1998).

Since every kind of fiber has its own physical specifications, insecticide molecules may act differently in penetration and making physical bonds with them. This difference may appear immediately after treating. It has been shown in some studies that LD_{50} of permethrin-treated cotton fibers is three times more than Permethrin-treated nylon fibers (Hossain and Curtis, 1989). Differences of various fibers in retaining insecticidal quality after treating, may appear with time and under influence of some physical and environmental factors like washing, aging, rinsing, wearing and weathering. On the other hand, efficacy of fibers may be different by various insecticides. For example, efficacy of deltamethrin for treating cotton fibers is more than other pyrethroids, while efficacy of cyfluthrin on jute fibers is more than other insecticides. Efficacy of Lambda-cyhalothrin, however, is similar on all fibers including cotton, Jute, nylon and polyester (Ansari *et al.*, 1998).

Although the study uniforms contained different percents of cotton and polyester fibers and a small percent of nylon and other fibers, there was not a significant difference in their insecticidal quality ($p > 0.05$). Meanwhile, there was not a significant difference in the remained amount of permethrin of different uniforms, three weeks after treating ($p > 0.05$). But regarding to the variety of fibers of the six uniforms, environmental and physical factors may have different effects on them. In a similar study, the quality of fibers of two various treated textile (100% cotton and 50% cotton-50% nylon) has been ineffective in their protection against mosquito biting, but environmental factors like weathering have been effective in this regard (Gupta *et al.*, 1998).

For example, in Olyset Net, a strong bond is made between permethrin molecule and polyethylene fibers by a polymer that isn't broken even by frequent washing and sun light and heat (Gonzalez *et al.*, 2002). Also in Prema Net, deltamethrin insecticidal has been mixed by resin and made a bond around polyester fibers (WHO, 2000). So, insecticidal molecules gradually are released from polymer and resin and come to the surface. In this way, lasting of insecticides on fibers increases (Guessan *et al.*, 2001). According to the studies, protection through permethrin-treating of clothing against arthropods that are external parasites of human, like pediculus and mite and or insects that remain in the body and clothing for a long time, is very effective, about 90-100% (Frances and Sweeney, 1996; Sholdt *et al.*, 1989; Breeden and Schreck, 1982). Besides, this kind of protection have been reported very effective against insects and arthropods that bite through clothing (Schreck *et al.*, 1988; Romi *et al.*, 1997; Harlan *et al.*, 1983).

Although the studied species of this survey don't usually bite through clothing, but even their landing on treated uniforms decreased dramatically, so that the treated uniforms had a 97% repellency in comparison with untreated ones (Table 2). Also according to several studies, permethrin-treated clothing have more than 90% efficacy in preventing biting of different species of ticks (Schreck *et al.*, 1978; Chen and Wang, 1996; Mount and Snoddy, 1983; Lane and Anderson, 1984; Evans *et al.*, 1990; Faulde *et al.*, 2003; Fryauff *et al.*, 1998).

Although most of laboratory studies have been reported 100% mortality of different species of mosquitoes in bioassays on permethrin-treated clothing (Schreck and Kline, 1989; Romi *et al.*, 1997), but the results of field test and evaluation of permethrin-treated clothing is some different; so that in field studies, protection of permethrin-treated clothing against *Culiseta impatiens* (Lillie *et al.*, 1988) and *Aedes taeniorhynchus* and *Culex sitiens* (Schreck and Kline, 1989), have been reported 93, 99 and 43%, respectively. In one other study in Pakistan, the mean Protection percent of permethrin-treated uniforms against different species of mosquitoes has been estimated 57% (Schreck *et al.*, 1988).

Also our study showed that even in one geographical region (one village), protection percent of permethrin-treated military uniforms is different against different species of mosquitoes, so that the protection of permethrin-treated military uniforms against *Cx. bitaeniorhynchus* and *Cx. tritaeniorhynchus*, was 72.7 and 87%, respectively (Table 3). It can totally be said that protection of permethrin-treated clothing against different species of mosquitoes is different, regarding to the variety of biological behaviors in biting (through clothing or on skin) and also searching was and host selection of various species of mosquitoes.

In some other studies, the efficacies of permethrin-treated clothing in preventing arthropod-borne diseases have been directly studied. For example, a study has shown that using permethrin-treated military uniforms has decreased malaria and leishmaniosis among Colombian soldiers (Soto *et al.*, 1995). Of course, one other study in Thailand has shown that using permethrin-treated uniforms without using repellents on skin, is ineffective on reducing malaria among Tai soldiers (Emsila *et al.*, 1994).

It should be noted that although using permethrin-treated uniforms has decreased the biting of *An. stephensi* and made a 78.7% protection in our study, but this technique may be ineffective in protection of people against malaria in hyperendemic areas like Sistan and Baloochestan and Hormozgan Provinces (in southeast of Iran), because although treating clothing decreases biting, but one can be affected just by one biting. So, this method should be accompanied by using repellents like deet, on exposed skin, in hyperendemic area (Evans *et al.*, 1990; Faulde *et al.*, 2003).

In alaska, where the biting density is 1188 per person per hour, concomitant use of permethrin-treated military uniforms and application of repellent deet 35% on exposed skin, against *Culiseta impatiens*, made 99.9% protection (Lillie *et al.*, 1988). Of course a study that has recently been done among French soldiers, showed that concomitant use of permethrin-treated military uniforms and application of repellent deet, has not decreased the incidence of malaria among them in an endemic region (DeParis *et al.*, 2004).

There is no doubt that using permethrin-treated clothing is a useful technique which makes a considerable protection against insects biting and if it be accompanied with application of repellents on skin, makes the maximum protection (Barnard, 2000), but it is not enough for preventing arthropod borne diseases in hyperendemic areas.

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