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## An investigation on permethrin-treated military uniforms against diurnal mosquitoes under field conditions

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## Abstract

**B**ackground: Military forces are exposed to mosquito bites because of their duties. In addition to the nuisance caused by mosquito bites, they are the vector of some vector-borne diseases in many countries of the world. Impregnation of military uniforms with permethrin is one of the most effective methods of protecting military personnel. The purpose of this study was to evaluate the effect of permethrin-impregnated uniforms against diurnal mosquitoes under field conditions.

**Methods:** In this study, one of the most common uniforms used in the Iranian military was treated by permethrin (0.125 mg. permethrin ai/cm<sup>2</sup>) and evaluated against the natural population of day-biting mosquitoes, in the rural area with Eight participants, three of them put on the treated uniforms and the other one of them wore the untreated uniforms and Four officers were also appointed as collectors.

**Results:** The average number of bites in those who wore untreated uniforms (control) was 1.8/min/person (107.8/hr), while it was 0.16 (9.83/hr) for treated uniforms. The protection of treated uniforms was about 91%. The knockdown rate for *Culex* and *Aedes* mosquitoes was 95.92% and 94.44%, respectively. The mortality rate in *Culex* was 98.59% and 98.61% in *Aedes* mosquitoes when exposed to treated uniforms.

**Conclusion:** In this study, military uniforms impregnated with permethrin showed significant protection against the bites of *Culex* and *Aedes* mosquitoes. This intervention can be used to protect military personnel from the bites of *Culex* and *Aedes* these mosquitoes and to reduce the diseases transmitted by these mosquitoes.



## Introduction

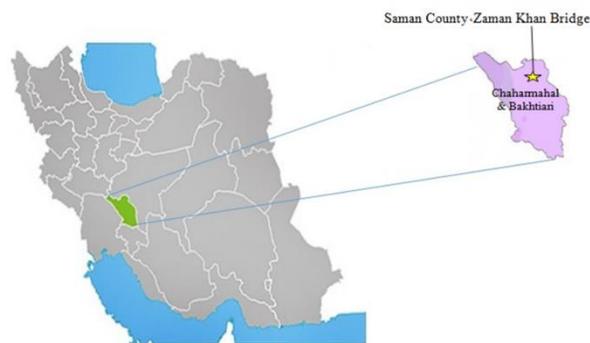
Mosquitoes are the most important group of insects medically and due to the transmission of various diseases as well as the nuisance caused by bites can pose a threat to human health in different areas from residents to travelers and military personnel deployed or dispatched to the area. Military personnel have close contact with the environment and are more susceptible to insect bites than other people and are exposed to related diseases. Mosquitoes vectors are known to transmit many dreadful diseases like malaria, dengue fever, chikungunya, other diseases of arbovirus, and filariasis. Research statistics reveal that casualties of soldiers from military wars and direct combat conflicts are less in number as compared to losses due to vector-borne diseases like malaria [1, 2]. One of these conditions is the high density in the workplace. Others include guarding or conducting military operations in open areas, with a high risk of transmitting the disease and the inability to use control and protection facilities such as the insecticide-treated net. Also, people exposed to the risk of dangerous diseases such as malaria and other diseases, including arboviruses, maybe returning to their areas and as a reservoir, unintentionally transmit the disease to non-endemic areas and increase the prevalence of the disease [3-6]. There are various ways to combat and reduce the risk of mosquito bites. The personal protection method, due to the displacement characteristics of the military forces, is suitable for the protection of insect bites. Permethrin-impregnated clothing can reduce the level of contact between humans and vectors, as reducing the risk of disease [7]. In arthropod-borne diseases, self-protection measures are important as the first line of defense against vectors and to reduce the problems caused by bites. Therefore, to protect endangered persons (eg soldiers, travelers, and outdoor workers), the use of repellent agents and Impregnation clothing and tents with insecticides can be very helpful in reducing the risk of insect bites [8, 9]. Personal protection first started with repellent agents [10]. In World War II, during the high prevalence of typhus in 1944, the use of personal protective equipment became increasingly important, and soldiers uniformly sprayed dimethyl phthalate (DMP) on clothing and tents to avoid insect bites [11]. Pyrethroids are generally used to impregnate clothing, but not all pyrethroids are suitable for clothing impregnation. Caution should be exercised when impregnating clothing because the toxins in the impregnated clothing come in close contact with the skin of the human body. Until now, only permethrin has been permitted at a specified dose for clothing impregnation [12, 13]. Today, the use of permethrin is common in military clothing and is used by many armies around the world, including the United States and some European countries. Initial evaluations of permethrin-impregnated clothes that were not rinsed, with the use of diethyltoluamide on the skin, provided excellent protection against insect bites [14]. This study was conducted to evaluate the effect of permethrin-

impregnated uniforms on protection against diurnal mosquitoes in field conditions.

## Methods

### Study sites and volunteers

The study site was located in Chaharmahal and Bakhtiari province on the edge of Zayandehrood and around Zaman Khan Bridge in Saman County (32°19' N, 50°51' E) (Figure 1). The volunteers were selected from the local community. Four volunteers were selected, three of them put on the treated uniforms and the other one of them wore the untreated uniforms. Their ages ranged from 20 to 29 years and their mean age was  $24 \pm 2.91$  years. All subjects were stable throughout the study. The volunteers were justified in how they performed the test and the time it took, and the collectors were justified in how they recorded the data. Candidates received written consent to participate in the study and to wear permethrin-impregnated clothing. At the end of the study, none of the volunteers showed any skin or respiratory sensitivity or any other complication.



**Figure 1:** The geographical location of Zaman Khan Bridge, Saman County, Chaharmahal Bakhtiari province.

### Treating uniforms

For this study, a sample of one of the most common uniforms in Iran was selected. Then, the physical Features of uniforms such as weight and water absorption coefficient of each uniform were determined. The uniforms were treated at a rate of 0.125 mg. active ingredient (AI)/cm<sup>2</sup> of cloth with aqueous suspensions of permethrin from 10% emulsifiable concentration (EC) formulation (cis:trans = 25:75). The control uniforms were treated just by the water. The uniforms were kept in laboratory conditions (temperature: 23-26°C, relative humidity:30-40%). The dipping method was used for impregnation. Uniforms were put together in a tray or pan. The prepared insecticide solution (at a specified concentration) was poured onto a uniform. The tray was placed in a non-porous plastic bag for 15-20 minutes and the lid of the bag was tightened to allow the insecticide solution to penetrate the clothing fibers well. The tray was removed from the plastic bag and the uniform was immediately removed from the insecticide solution. For about 2 to 3 minutes, the uniform was kept on top of the tray to pour the excess solution. The uniform was spread horizontally on a non-absorbent surface such as aluminum foil and rotated every 10 to 15 minutes to

release the toxin solution into the uniform layer of the coating and its thickness uniformly. The control uniforms were impregnated simultaneously.

**Field test**

This study was conducted in early July and the average temperature during the study was 24.6 degrees and the average relative humidity was 24%. After primary investigation, the participants were selected and trained about the test. They were told not to use insect attractive or repellent materials like alcohol, spearmint, perfumes, garlic, smoke and other aromatic materials. participants received the uniforms three hours before sunset. three of the participants wore the treated uniforms and the other one wore the untreated uniforms (control) and Four officers were also appointed as collectors. participants sat at a suitable distance from each other (10 meters). The control participant sat in the same way at a distance of 40-50 meters from the case participants. Field tests were conducted for 3 hours during the evening and until sunset. One collector was considered for each subject. A collector was considered for each participant. Collectors inserted biting data into the sheets. Mosquitoes are collected with mouth aspirator and flashlight and placed in separate cups for further identification. The collector and officer determined the species of mosquitoes (Anophelinae or Culicinae). The mosquito monitored for 64 minutes at different times (1, 2,4, 8, 16, 32, 64) and then transferred to the laboratory. And sugar-coated cotton was placed on each glass and after 24 hours mosquito mortality was recorded in each glass. The subjects didn't have any activity during the test and ate their dinner before starting the test. After the test, the uniforms were collected and given to the participants the next day.

**Species identification**

The identification of mosquitoes was performed using valid and identification keys [15].

**Data analysis**

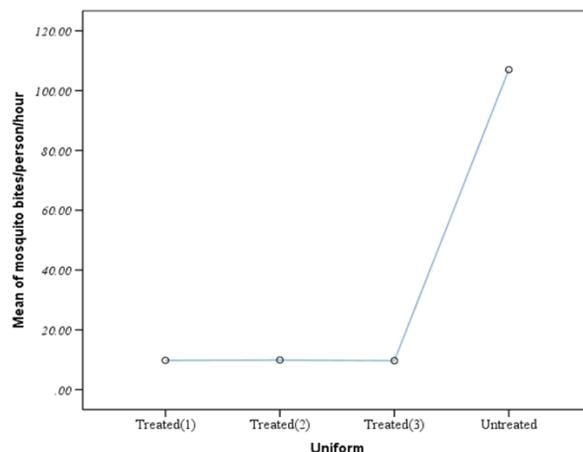
Data for each participant were set and standardized based on the number of biting per minute. Because the statistical analysis of this study was based on quantity, the data were first transferred with the equation  $0.5 + y$  (y: number of bitings per minute) and then Poisson distribution for statistical analysis of the bitings number in Case and control groups are used. Analysis of variances (ANOVA) was used for comparing the biting means (on the skin and through uniforms) in participants who had worn military uniforms. For comparing the means of biting and landing (on the skin and through uniforms) in Case and control groups, the T-test was used. Protection percent of treated uniforms was calculated using the following equation:

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

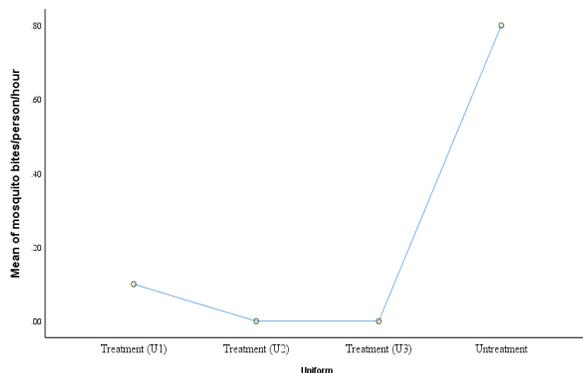
**Results**

In this study, field operations were carried out over five days. At each stage, three insecticide-treated uniforms

(treated by 0.125 mg/cm<sup>2</sup> permethrin) and one control were compared together. The mean number of bites in different uniforms (treated and untreated) was calculated and recorded on the clothes and on the skin (Figures 2 and 3). The average number of mosquito bites on the skin of individuals per hour was 9.8 in treated uniforms and 107 in untreated uniforms, indicating 91% protection. Also, the average number of mosquito bites per hour was 0.03 in treated uniforms and 0.8 in untreated uniforms, indicating 96% protection and overall protection against permethrin-impregnated uniforms in contrast, non-impregnated uniforms were 91% (Table 1). The knockdown and mortality rate of mosquitoes exposed to the permethrin-treated uniform were reported within five days of testing for mosquito species separately (Figure 4).



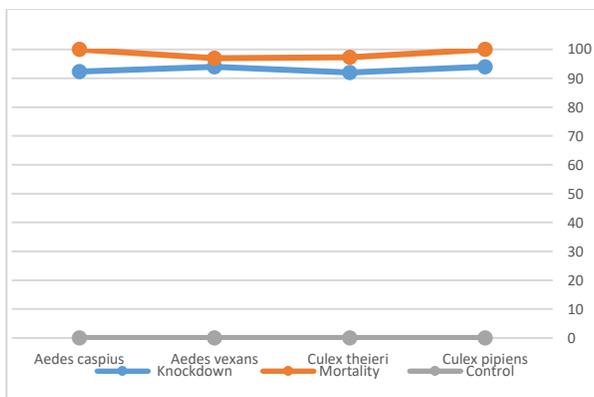
**Figure 2:** Mean of mosquito bites received per person per hour on exposed skin.



**Figure 3:** Mean of mosquito bites received per person per hour through the clothing.

Uniforms	Through clothing	On skin	Overall
Treated	0.03	9.8	9.83
Untreated	0.8	107	107.8
Percent Protection (%)	96	91	91

**Table 1:** Percent protection and Mean of mosquito bites per persons per hour of permethrin-impregnated military uniform.



**Figure 4:** knockdown and mortality rates of species exposed to treated and control uniforms.

## Discussion

Impregnation of military uniforms with permethrin is considered as one of the most effective and safe methods for the military [16]. Today, the use of permethrin-treated military uniforms is among the hygienic programs of many armies around the world. This method widely used by the US Army in the 1991 Gulf War [17]. There are, several oral reports of this technique being used in the 2003 US-Iraq war. In recent years, in some countries, including France, the use of permethrin-treated military uniforms embedded as one part of programs and strategies for controlling vectors and protecting troops against insect-bite has been established [18, 19]. The benefits of this study are field experiments, and the results showed that the mosquitoes exposed were only *Culex* and *Aedes*, probably due to environmental conditions and time of the study. In this study, the protection rate of insecticides-impregnated uniforms was 91%, indicating high protection against diurnal mosquitoes bites. In a study by Khoobdel *et al* (2005) on the protection of permethrin-impregnated uniforms on the bite of three *Culex* species, the difference was significant between the impregnated and control uniforms and the impregnated uniforms had a much greater protective effect. But there was no significant difference in the degree of protection between the impregnated uniforms. And after two weeks of testing, thin-layer chromatography results indicated that the permethrin in the uniforms was not significantly different from the beginning of the test [20]. In another study, the percentage protection of permethrin-impregnated uniforms on *Culex pipiens* was reported to be 87% [21]. In another study, the protection of permethrin-impregnated uniforms on *Culex pipiens* was reported to be 87%. In a study by Schreck *et al* on *Aedes taeniorhynchus* and *Culex sitiens*, the protection of permethrin-impregnated uniforms was reported to be 99.99% and 43%, respectively [22]. In another study, the effects of permethrin-impregnated uniforms on field conditions against *Anopheles stephensi* and 4 *Culex* species were evaluated and it was found that there was a significant difference in bite protection between control and test subjects [23]. Until today, many studies have been conducted on permethrin-impregnated uniforms

around the world, which in most cases have been satisfactory, but unfortunately, this technique has not been widely used by the Iranian military [24, 25]. Field studies have reported very different statistics. Even within the same geographical range, sometimes the percentage of Protection of different species are different, which may be due to variations in behavior and feeding (Skin or clothing) of different species. In the present study, the Knockdown rate in *Aedes* mosquitoes was 94.44% and in *Culex* mosquitoes was 92/95. In 2003 Faulde *et al* working on military clothing in Australia, the results showed that the Knockdown rate in *Aedes aegypti* was between 84.8% and 100%. when exposed to treated uniforms with permethrin and bifenthrin [6]. In another study in 2014, fabrics were impregnated with permethrin by factories in the US and Europe that were 100% Knockdown to *Anopheles farauti* [25]. In a 2014 study by Sukumaran on military clothing with permethrin, showed 93.33% Knockdown to *Aedes aegypti* mosquitoes in vitro [26]. Permethrin can be a good choice because of its two important repellency and insecticidal properties as well as the high knockdown properties [27, 28]. Although most laboratory studies have reported 100% mortality from different species, different statistics have been reported in field studies. Environmental conditions and working methods can have an impact on the results [21, 29]. In the present study, the uniforms were impregnated without washing and the mortality rate of mosquitoes was high and satisfactory. In this study mortality rates in *Culex* and *Aedes* were 98/59 and 98/61, respectively. In the study of Khoobdel *et al.*, although mortality was high in the non-washed mosquitoes (between 82 to 100%), the mortality, knockdown and the toxin residues in the uniforms decreased sharply undergone five washing regimes. Much research has been conducted in different parts of the world to evaluate permethrin-impregnated uniforms, and the effectiveness of these methods against insects and arthropods has been proven [12, 30]. Finally, based on the results obtained here, military uniforms impregnated with permethrin showed significant protection against diurnal mosquito bites. This method can be used to protect people from the bites of these mosquitoes and to reduce diseases transmitted by *Aedes* and *Culex* mosquitoes.

## Authors' Contribution

MK conducted the study, MA, SHMK and HY helped in field and lab work, MRA and KD did data analysis, MM helped in drafting, AAA reviewed and approved the draft.

## Competing Interest

The authors declare that they have no competing interests.

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