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Repellency effects of picaridin and DEET against *Anopheles stephensi* on human volunteers

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Abstract
Background: Reduction of mosquito contact by adopting personal protection measures is recommended as a strategy for the management of vector borne diseases. One of the common ways for malaria prevention is to use the chemical repellents. Researchers are trying to introduce more powerful repellents with less side effects. One of these newly found repellents is picaridin (Trade name: Byreple®or KBR2030®). The aim of this study was to determine the repellency effect of picaridin in Iran.
Methods: The study was carried out on four male volunteers, under laboratory conditions and the results compared to the repellency effect of standard DEET by the use of ASTM 951-94 standard method.
Results: The effective doses (ED^{50} and ED^{90}) of picaridin were determined as 0.0023mg/cm^2 and 0.009 mg/cm^{2}respectively, while the ED^{50} and ED^{90} of DEET were determined as 0.0018mg/cm^2 and 0.0248 mg/cm^2 respectively.
Conclusion: This study showed that there is a significant difference between the effective Doses of DEET and Picaridin ($P<0.05$); Also the t-test showed that the mean protection time of picaridin 20% is significantly higher than DEET 25% ($P<0.05$).

Keywords: *Anopheles Stephensi*, DEET, Picaridin, Protection time, Repellency

1. Introduction
Malaria contributes to the major disease burden and its control is hampered by many operational and technical reasons and among the technical reasons is insecticidal resistance. Development of insecticide resistance in malaria vectors to the commonly used synthetic chemical insecticides in public health and this has made the disease control more difficult [1]. The high price of these kinds of insecticides as well as environmental and economic concerns have led to the search of safer insecticides and finding appropriate substitutes for them is imperative [2]. *Anopheles stephensi* is considered as one of the main malaria vectors in Iran. Resistance of the species to organochlorine, organophosphorus, carbamates and some pyrethroids insecticides have been reported in the country [3]. It is necessary to consider more effective and accessible methods to malaria prevention [4]. Personal protection by the use of insect repellants is an appropriate way for preventing human contact with the vectors of malaria. Some factors are important in the use of repellants, such as appropriate price, accessibility and acceptance of users [5]. Applying the repellants on the skin is one of the common methods for personal protection against the insects. Repellants could be used not only in confined disease communicable places, but also in many places for preventing insect bites during the night. One of the oldest and commonly used repellants is DEET (Di Ethyl Tolo Amid) which has a high repellency effect against a large number of mosquitoes [6] and is usually considered as a Gold Standard that provides more than eight hours of protection. Despite the widespread and increased interest in the use of DEET in Public Health programmes, controversies remain concerning both the identification of its target sites at the olfactory system and its mechanism of toxicity in humans [7]. One of the recently reported repellents is Picaridin. In this investigation, the repellency effect of picaridin was determined and finally compared with DEET.

2. Materials and Methods
Mosquitoes: In this investigation, the Indian and Kazerooni strains of *An.stephensi* of School in the school of Public Health insectarium were used. At the insectarium, the adult female mosquitoes were fed on the blood of Guinea pigs. The larvae were fed on larvae feed named

"Bemax" which contained the powder of wheat sprout. For preventing oxygen reduction and providing better condition for the larvae, water was slowly added to their containers, every two days. Finally, the emerged female adults were used for hematophagy in the volunteers. The standard condition for raising adult *An. stephensi* was ± about 27±3 ºC by the relative humidity of 80±10 percents and light cycle of 16 hours light with 8 hours darkness.

2.1 Chemical Repellents

DEET: CAS number: 134-62-3, A color less fluid with a weak smell, Purity degree: 99% and was provided by Merck company, Germany

Picaridin: CAS number: 119515-38-7, A color less or very light yellow fluid**,** Purity degree: 97.3%, Density: 1.07 G/m^3 and was provided by the Research Institute of Chemical Industry Testing Center, Shanghai, China

2.2 Testing method: Sensitivity test was carried out before testing the repellents on male human volunteers. If there was no sign of dermal sensitivity like burning, itching, inflammation or redness after three days and volunteers were fully normal, they could enter the study by signing the written consents. The adult female mosquitoes which were used for the test, were starved for seven to eight days. During that time, they were just fed on 10% sucrose solution. According to the suggestion of EPA, the sucrose solution was exited from the cages, 10-12 hours before the test. The laboratory temperature was 28-30℃ during the repellant assay. The relative humidity was 50-80%. Before starting the tests and for assuring the appropriate condition for the mosquitoes, their biting pressures were measured.

Biting pressure includes at least 10 times of landing, and or siting inserting or probing without hematophagy, in 30 seconds. If this figure were less than 10 or more than 20, then the mosquitoes would not be appropriate for the test.

Before applying the repellant, the volunteers had to wash their hand with water and non-aromatic soap and also their lower arm with 70% alcohol, and then dry it thoroughly. The area of the lower arm of an adult man is usually about 550-650 cm^2. According to the measured areas, 1.5-2 ml of the repellants were applied on the lower arms of the volunteers, using a 1000 micro liter sampler. Before applying the repellants, the volunteers put their arms into the test cage and if at least 15 landing, probing or biting occurred, the test could start. During the tests, the temperature and relative humidity of the insectarium were 29± ºC and 60±2 percents, respectively.

In this study, the protection and failure time of DEET 25% and Picaridin 20% were tested. The base of preparation for the different concentrations of the repellants was absolute Ethanol. After five minutes of applying the repellants, the volunteers dipped their lower arms into the test cage which contained about 200 mosquitoes, for three minutes. During this time, all of the landing, probing and biting cases were recorded in the special forms. Then, the volunteers rested for 30 minutes. This frequency of three minutes contact and thirty minutes rest continued until two bitings at a three minute test at two following three minute test, with 30 minute intervals, occurred. For determination of the studied repellants failure times, the test continued until the tenth biting. For determination of the effective dose, the standard method of ASTM was used [11]. In this study, the ASTM E951-94 method was used. During the test, the temperature and relative humidity of the insectariums was 29±3 ºC and 60±2 percents, respectively. Using the standard model, five similar circles with 29 mm diameter which were according to the holes of the plastic cages of ASTM model, were drawn on the lower arms of the volunteers. Four out of the five circles were used for the different concentrations of the repellants and the last circle was used as the control (absolute ethanol). Absolute ethanol was applied on the control circle. Also, 25 ml of different concentrations of the repellants were used for three other circles. Five minutes after applying the repellants on the lower arm of the volunteers, the ASTM test cage was closed. The plastic test cage was 5×4×18 centimeters cube with five 29mm holes with similar distances. Under each hole, there was a drawer slide. In each chamber of the cage, five adult female mosquitoes that had not fed aspirated on any blood in the past 7-8 days and had not fed for 12 hours, were entered using an aspirator. By opening the drawers, the mosquitoes concomitantly had access to the skins of the volunteers who had different concentrations of the repellants or absolute ethanol. The test continued for five minutes and the number of biting was recorded for each test. Three and two test cages were used for right and left arms, respectively. A total of five test kits for each volunteer and each test cage were used for five minutes. The whole time of each test didn't exceed 30 minutes.

2.3 Data Analysis: Number of the selected doses for exact determination of the effective doses (ED_{50} and ED_{90}) of the repellants was at least four, and number of repetitions for each concentration and studied method was four. After finding the repelling status of the selected concentrations against each of the strains, ED_{50} and ED_{90} were calculated using probit regression analysis. Difference between the protection time indicator, for each of the tested repellants on the human volunteers, was separately analyzed by t- test.

Difference between the indicator of ED_{50} and ED_{90}, separately for each of the tested repellants on the human volunteers, was also analyzed using t- test. The diagrams were drawn by Excel software.

3. Results

Determination of the effective doses (ED_{50} and ED_{90}) for DEET and Picaridin repellents was carried out. According to the results of probit analysis, we used amounts of 0.0021 mg/cm^2 0.0169 mg/cm^2 for determination of ED_{50} and ED_{90} for DEET respectively. (Table 1). There wasn't any significant statistical difference between amounts of ED_{50} and ED_{90} of the two studied repellents ($p>0.05$) (diagram 2).

Table 1: Determination of the repelling indicators (ED_{50} and ED_{90}) of DEET and Picaridin against two strains of *An. stephensi* on human volunteers

Repellant	Number of mosquitos	ED_{50} mg/cm^2	95% C.L mg/cm^2	ED_{90} mg/cm^2	95% C.L mg/cm^2
DEET	500	0.0021	0.0020-0.0027	0.0169	0.0071-0.0127
Picaridin	500	0.0024	0.0012-0.0023	0.0197	0.0134-0.0829

The equation of DEET regression line was Y=5.7588+2.1903X by the use of probit software. This equation for picaridin was Y=3.0759+1.1173X (Table 2 and diagram 1).

Table 2: Equation of regression line and $x^2 \pm SE$ (df) for effectiveness of DEET and Picaridin against *An.stephensi* on human volunteers

Repellant	Equation of regression line	$x^2 \pm SE$ (df)	P-Value
DEET	Y=5.7588+2.1903X	16.185(2) $\pm$ 0.221	0.05
Picaridin	Y=3.0759+1.1173X	13.107(2) $\pm$ 0.196	0.05

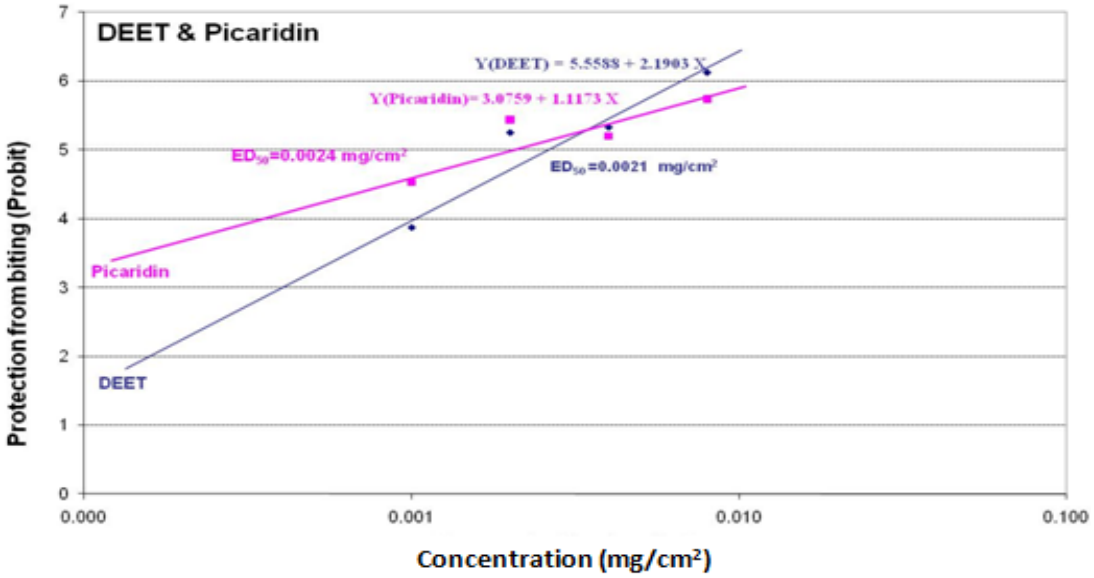

Diagram 1: Regression line and equation of doses comparison; responses of DEET and picaridin against *An.stephensi* on human volunteers by the use of the standard model (ASTM-E951-94)

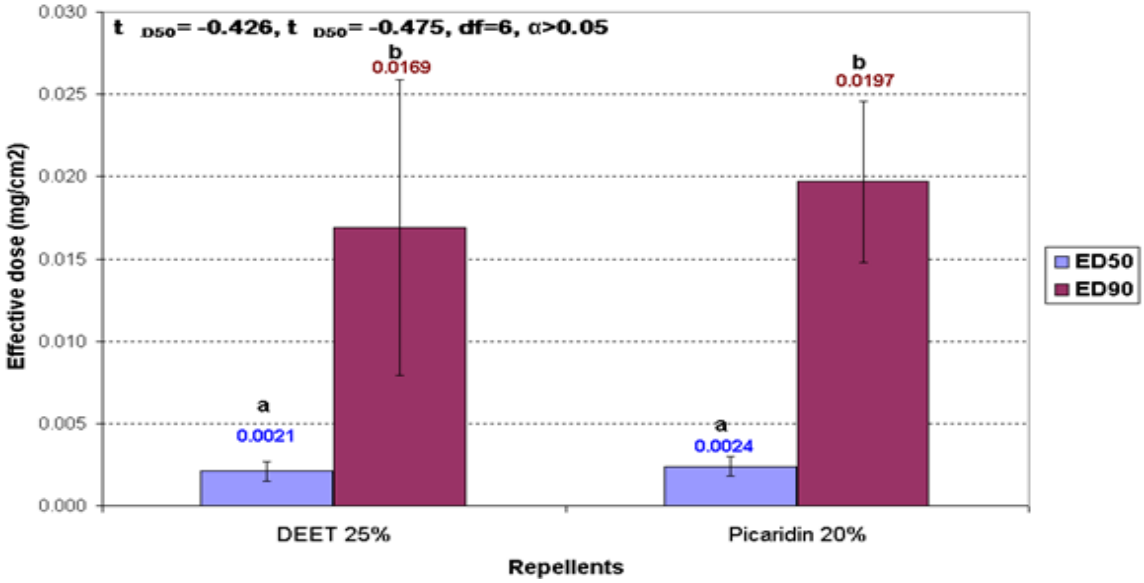

Diagram 2: DEET and Picaridin statistical comparison of $ED_{50}$and ED_{90}amounts (mg/cm^2) against *An.stephensi* on human volunteers by the use of the standard model (ASTM-E951-94).

3.1 Protection and failure times of DEET and Picaridin determination

According to the results, the mean of the protection and failure times of DEET 25% and Picaridin 20% were calculated as 6.23, 7.30, 10.02 and 11.15 hours, respectively (table 3).

Table 3: Protection and failure times of the essence of the case plants, Calendula, DEET and picaridin against *An.stephensi* on human volunteers under laboratory condition

Repellant	Concentration (V/V)	Protection time (hour)		Mean of failure time (hour)
		Mean	Range	
DEET	25%	6.23 $\pm$ 0.16	6.5-7.00	7.30
Picaridin	20%	10.02 $\pm$ 0.59	8.25 $\pm$ 10.30	11.15

According to the results, there was a significant statistical difference between the protection times of DEET 25% and the protection times of DEET 25% and picaridin 20% ($p<0.05$) (diagram 4).

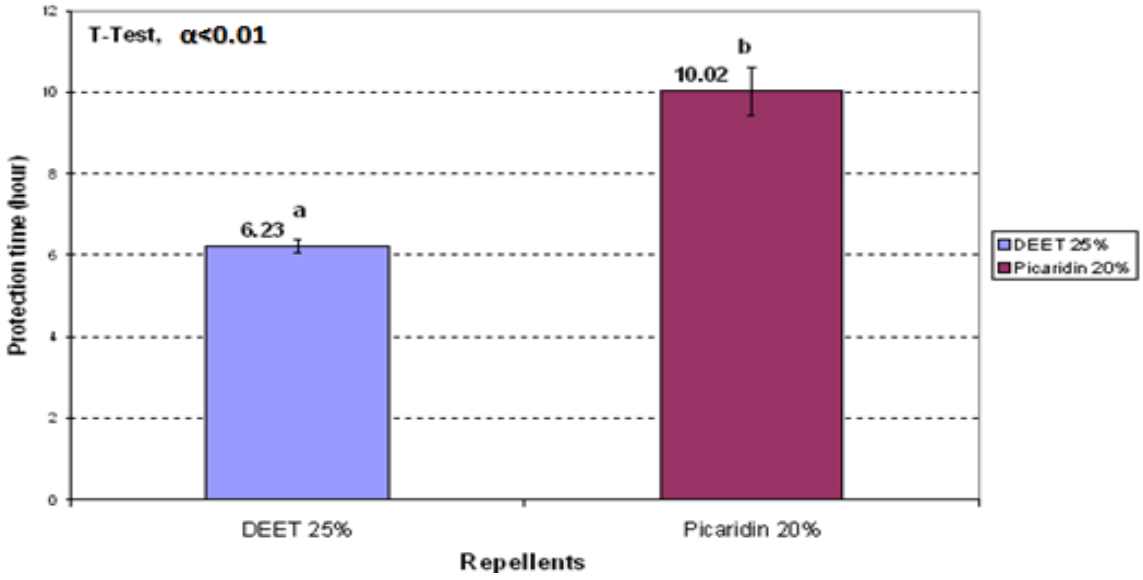

Diagram 3: Statistical comparison of protection times of DEET 25% and picaridin 20% against *An.stephensi* on human volunteers under laboratory conditions

4. Discussion

For more than 50 years, DEET has been known as a highly effective repellant against several species of insects. DEET is considered as a "Gold Standard" repellant with a long protection time. Since 2000 some new repellants have been introduced in the world including Picaridin (Icaridin, KBR3023®) with trade name of Byreple®, which can be an appropriate substitute for the old fashioned DEET [8]. These newly introduced repellants are chemically safer and provide higher protection. In this study, the repelling effect of picaridin against *An.stephensi* which is the main vector of malaria in southern Iran, was tested for the first time under laboratory conditions. The results revealed that picaridin has an appropriate repelling effect against this species of *Anopheles*. Vatandoost *et al.* [9], reported the ED_{50} of DEET against laboratory and field species of *Anopheles Stephensi*, as 0.007 and 0.005 mg/cm^2, respectively. The results of our study are in accordance with the aforementioned investigation, regarding the effective dose of DEET against *An.stephensi*, for both laboratory and field species.

Badolo *et al.* [10], determined the ED_{50} of DEET and picaridin as 0.018 and 0.78 mg/cm^2, against *An. stephensi*, respectively. In their study, DEET had a lower effective dose and a higher failure time against *An.stephensi*, compared to picaridin. Also, DEET and picaridin had a higher effective dose and a lower failure time, compared to the results of the study on *An.gambiae*. These differences, however, can be as a result of the different species studied and or the methods used for the tests. Like DEET, Picaridin prevents hematophagy of *An.stephensi* through the intervention in its smelling system. In agreement with DEET, Picaridin can decrease the hematophagy of *An.stephensi*, *Phlebotomus papatasi* and *Aedes aegypti*, by about 50% [11]. Costantini *et al.* [12], reported the protection time of picaridin against *An.nili*, as about 10 hours which is higher than the protection times of DEET and IR3535. The results of our investigation are also in accordance with the results of the Costantini's one.

Scheinfeld *et al.* [13], estimated the protection time of picaridin 19.2% and DEET 35% as 9 and 7 hours, respectively against *Anopheles* species. They also showed that Picaridin had lower concentrations compared with DEET, which had a higher protection time. Also, the results of our study are in accordance with the aforementioned results. Abdolkarim Amer *et al.* [14], implemented a test on repellency effect of some chemical and botanical compounds and revealed the protection times of DEET 20% and picaridin 20% against *Aedes*, *Anopheles* and *Culex* mosquitoes as minutes respectively. These results are in concordance with the results of our study in our investigation, the repellency effect of picaridin was carried out against one of the most important vectors of malaria (*An.stephensi*) and compared to DEET'S effect, under laboratory conditions, for the first time in Iran. The results revealed that the protection time of picaridin 20% is significantly higher than the protection time of DEET 25% ($p<0.05$); however, we could not find any significant differences between the effective doses (ED_{50} and ED_{90}) of two repellents ($p>0.05$). Our study showed that picaridin has a high repelling power and could be used instead of DEET in many aspects. This is the first study in Iran; so it is suggested that investigations be carried on the repellency aspects and also other aspects of picaridin for achieving more realistic results under laboratory condition as well as field condition.

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