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Original Article

Repellency Effects of Essential Oils of Myrtle (*Myrtus communis*), Marigold (*Calendula officinalis*) Compared with DEET against *Anopheles stephensi* on Human Volunteers

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Abstract

**Background:** Malaria and leishmaniasis are two most significant parasitic diseases which are endemic in Iran. Over the past decades, interest in botanical repellents has increased as a result of safety to human. The comparative efficacy of essential oils of two native plants, myrtle (*Myrtus communis*) and marigold (*Calendula officinalis*) collected from natural habitats at southern Iran was compared with DEET as synthetic repellent against *Anopheles stephensi* on human subjects under laboratory condition.

**Methods:** Essential oils from two species of native plants were obtained by Clevenger-type water distillation. The protection time of 50% essential oils of myrtle and marigold was assessed on human subject using screened cage method against *An. stephensi*. The effective dose of 50% essential oils of two latter species and DEET were determined by modified ASTM method. ED50 and ED90 values and related statistical parameters were calculated by probit analysis.

**Results:** The protection time of 50% essential oils of marigold and myrtle were respectively 2.15 and 4.36 hours compared to 6.23 hours for DEET 25%. The median effective dose (ED50) of 50% essential oils was 0.1105 and 0.6034 mg/cm2 respectively in myrtle and marigold. The figure for DEET was 0.0023 mg/cm2.

**Conclusion:** This study exhibited that the repellency of both botanical repellents was generally lower than DEET as a synthetic repellent. However the 50% essential oil of myrtle showed a moderate repellency effects compared to marigold against *An. stephensi*.

**Keywords:** *Calendula officinalis, Myrtus communis, DEET, Anopheles stephensi, Repellent, Essential oils, Iran*

Introduction

Malaria is still a major endemic disease in foci located in south and southeast of Iran. The annual malaria cases have been reported from 66075 to 6211 during 1995–2009, indicating the sharp decline of disease. It is unstable with two seasonal peaks mainly in spring and autumn. These areas include the provinces of Sistan and Baluchistan, Hormozgan and Kerman. In this part of the country six anopheline mosquitoes including *Anopheles culicifacies* s.l., *An. stephensi* Lindsey, *An. dhalii* Patton, *An. fluviatilis* s.l., *An. superpictus* Grassi, and *An. pulcherrimus* Theobald are known to be the malaria vectors and *An. sacharovi* (Favre, 1903) and *An. maculipennis* s.l. are considered as malaria vector in northern part of the country (Manouchehri et al. 1992, Zahirnia et al. 1998, 2001, Enayati et al. 2003, Naddaf et al. 2003, Oshaghi et al. 2003ab, Salari Lak et al. 2003, Vatandoost et al. 2004ab, 2005ab, 2006ab, 2008ab, 2009a, 2010, 2011, Sedaghat et al. 2005, 2011).

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2003ab, Doosti et al. 2006, 2007, Davari et al. 2007, Abai et al. 2008).

One of the widely used and effective insect repellents is the synthetic compound, N, N-diethyl-m-toluam ide (DEET) which is generally considered the “gold standard” repellent, providing long-lasting protection of up to 8 hours from time of application. “There are some rare reports of severe reactions in people, additionally DEET melts plastics causing spoilage of equipment, such as glasses and mobile phones, and many consumers find the odor and sensation on the skin unpleasant” (Logan et al. 2010). Therefore researchers are trying to improve the efficacy with reducing the side effects of new generation of repellents. In the past few years, a plant derived repellent, para-methane 3–8, diol (PMD) has been proven to be suitably efficacious and safe to compete with DEET in the field of disease prevention, and repellents have been recognized by WHO as a useful disease prevention tool to complement insecticide-based means of vector control. Many plants have substances that are toxic, antifeedant properties or repellency for insects. Repellent of plant material refers to a plant origin have an inherent and naturally defensive and repellency effects on insects (Maia and Moore 2010). This material includes extracts and essential oils. Their effect is significantly differs from different parts of plants, including flowers, tubers, leaves, fruit, branches and roots.

Myrtle (*Myrtus communis* L.) is a native plant distributed in south, north and central parts of Iran (Rechinger 1996). A study in Iran showed that the myrtle essential oil (*M. communis*) is very active against *Streptococcus pneumoniae*, *Moraxella catarrhalis* and *Haemophilus influenzae* in vitro. This confirms the application of herbal medicines for treating a range of infectious diseases in ancient times (Pourmand et al. 2008). Study of the conditions in vitro effect of myrtle essential oil, the extracts form soluble in water, and soluble dichloromethane corn oil was studied on a variety of microbes. It was determined that the oil could prevent the growth of bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Its effect of antiviral ointment containing about 10% of myrtle essential oil on patients with herpes simplex virus was tested (Zolfaghari et al. 1997). The essential oil of myrtle is effective in protection of biting insects. In addition insecticidal action was also observed (Yaghoobi-Ershadi et al. 2006). Marigold also exhibits great effects in treating some skin disorders such as leaving the skin, acne and dermatitis. The *Calendula* ointment are presented and is believed that the spring flowers of this plant had been used for reducing swelling, treat injuries, and a disinfectant material. For domestic use, this plant has been used for soothing effects of mucosal ulcers, swelling of the stomach. The repellency property makes an impact effect on the insects away from humans.

For the first time in the country, the repellency of essentials oils myrtle and marigold which are native in southern Iran, were assessed using *An. stephensi* and compared with DEET on human volunteers under laboratory condition.

**Materials and Methods**

**Mosquitoes**

The tested mosquitoes were the established colony of *An. stephensi* obtained from the Insectary of School of Public Health, Tehran University of Medical Sciences, Iran. Mosquitoes were reared and maintained at 27±3 °C and 80±10% relative humidity (RH) under a 12: 12 (L: D) photoperiod. Larvae were fed on a diet of enriched wheat germ. The adults were maintained in screen cages and fed with 10% aqueous sucrose solution as a source of energy and guinea pigs as blood-feeding female mosquitoes for maturing the eggs. Starved 7 to 10 days old females were used for the repellency tests. The sucrose solution was
picking up from the cage, 12 hour before starting the experiments.

**Repellents**

The chemical repellent, DEET (N, N-diethyl-meta-toluamid), CAS NUMBER: 134-162 and assay 98.8%, Density: 0.998 g/cm\(^3\) were purchased from Merck Co, Germany. The myrtle (*M. communis*) is evergreen shrubs or small tree which was collected from southern extension of natural habitat in Noorabad district at coordinate 30º 7’E 51º 31’N, 920 meters above sea level, southern Iran. The marigold (*Calendula officinalis* L.) was also collected in suburb of Shiraz City, at coordinates 29°37’N 52°32’E at elevation of 1481 meter above sea level, Fars Province, southern Iran. The leaves and flowers of myrtle and the flowers of marigold were dried at room temperature under good ventilation and chopped into small pieces using a knife mill. The essential oil was extracted from the plants using a Clevenger-type water steam distillation apparatus. The distilled essential oils were stored in a refrigerator at 4°C until being used in the experiments which were diluted by absolute ethanol at 50% concentration. The composition of the volatile constituents was established by gas chromatography-mass spectrometry.

**Test method**

All series of the experiments were carried out in laboratory condition. In the first stage, in order to reveal the probable allergic reaction of chemical and natural repellents to human volunteers, the scratch test was done on skin of the upper arm. The treated skin of arm was observed up to 72 hours for allergic reaction.

The 25% solution of DEET was prepared using absolute ethanol as well as 50% essential oils myrtle and marigold was obtained in same manner and tested against *An. ste-phantesi* on four male volunteers. Observation was based on the variable dose-response of the mosquito to the serial dilutions of the repellents. The procedures for determination of effective dosages of the repellents were adopted by the standard method of American Society for Testing and Material (ASTM E951-94). The testing kit was made of Plexiglas cube at dimension of 4 x 5 x 18 cm having five circles in 29 mm diameters. Before selecting the mosquitoes, the willingness of mosquitoes for introducing in repellency tests were monitored based on determination of biting pressure on untreated, alcohol washed arm which should be at least 10 landings/probes per 30 seconds. Each of 5 adjacent cells in the ASTM modules was provided with 5 female 7–10 days mosquitoes that randomly selected from a cage containing 200 starved mosquitoes. The effective dose tests were conducted by applying each repellent directly to the human skin. Five circles (29 mm in diameter) were drawn on the volunteer's forearm using a felt tipped pen and a plastic pattern. The drawn circles on the human subjects were treated with 25 µl of the diluents. The serial dilutions were applied on 4 holes as well as the absolute ethanol was applied in control circle. The treated circles were allowed to dry, and then test apparatus containing starved mosquitoes were fixed on the treated skin of the volunteers and opened the slide for exposure with treated skin. The counts of probing and biting were recorded at 1 minute intervals up to 5 minutes. The experiments were done at 2 replicates on right forearm and 2 replicates on left forearm of the volunteers. After each test, the mosquitoes were removed from the test apparatus using aspirator and then transferred into a screened cup. Mortality of mosquitoes was read after 24 hours. The protection and failure times were also determined on human subjects. Before starting the experiments, 1 ml DEET 25%, as well as 1 ml of 50% essential oils both myrtle and marigold were dissolved in
absolute ethanol and spread evenly between the elbow and wrist of a volunteer’s arm. The other arm, acting as a control and was treated only with 1 ml of absolute ethanol. After drying the test arm, a rubber glove was put by volunteers and inserted the arms into 50×50×50 cm cage containing 150–170 starved mosquitoes for 3 minutes. The mosquitoes which landed and attempted to bite were recorded. The procedure was repeated at 30 minutes intervals and was used consistently throughout the experiment. If more than 1 mosquito bite was recorded during an observation, the test of repellency was terminated, and the period of repellent protection was calculated as the time between the repellent application and multiple mosquito bite. If only 1 mosquito attempted to bite during an observation period, any addition mosquito bites during that next observation period (30 min later) confirmed that the initial bite represented the time of repellent failure. The period was repeated up to 10th bites and took into account as failure time. The successive expose of the control arm were made prior to inserting the treated arm in order to provide a standard for comparing mosquito biting activity during the experiments. The similar tests were also repeated on 4 human volunteers.

Statistical analysis
The data were subjected to statistical analyses using SPSS software ver. 11.5. In order to estimate the ED50 and ED90 values, the cumulative results were subjected to the probit analysis which had been repeated in different days with four volunteers (Finney 1971, 1978). The regression lines were plotted and the ED50 and ED90 values with confidence limits and regression parameters were calculated. Data were transformed using arc-sine √x transformation to meet the normality. Significant differences between three repellents were assessed by ANOVA. The latter test was also used for assessment of the significant differences between protection times of the tested repellents. Means of protection times and ED were compared by the Tukey’s honest significance test or games-Howell test depending on significance of Levene’s test. The 1% level was employed in tests of significance.

Ethical approval
This study received formal ethical approval from the Medical Ethics and History of Medicine of Research Center, Tehran University of Medical Sciences. Informed consent was taken from each volunteer.

Results
Protection time
The protection time of 50% essential oils myrtle and marigold compared with DEET 25% against An. stephensi on human subject is shown in Table 1. The protection time (PT) of botanical repellents was ranged between 4.25–4.40 and 1.00–3.30 hours with mean of PT 4.36 and 2.15 hours respectively with myrtle and marigold essential oils (Table 1). The mean of protection time of DEET was ranged between 6.05–7.00 hours with mean of 6.23 hours. The difference between botanical repellents and DEET was significant compared to both botanical repellents (P< 0.01). On the other hand, the difference of protection times of myrtle compared with marigold essential oil were significant (P< 0.01) (Fig. 1).

Effective doses
The ED50 values (with 95% confidence limits) of the myrtle (M. communis) and the marigold (C. officinalis) essential oils were 0.1105 (0.0772–0.1399) and 0.6034 mg/cm2 (0.4464–0.7476), respectively on 4 human subjects. The ED90 values with 95% confidence limits were respectively 0.5404 (0.4281–0.7683) and 3.4905 mg/cm2 (2.6203–5.4534) for myrtle and marigold plants (Table 2). The ED50 and ED90 values for DEET as a golden repellent were 0.0023 (0.002–0.0027)
and 0.009 mg/cm² (0.0071–0.0127). Statistical comparison of the data was revealed that the ED values for essential oils marigold and myrtle is significantly higher than ED values of DEET, showing less repellency of botanical repellents compared with DEET ($P<0.01$). The ED$_{50}$ of myrtle was close to ED$_{50}$ of marigold and was significant different (Fig. 2). Comparing the ED$_{50}$ values, it can be concluded that the ED$_{50}$ for DEET is significantly higher than those of ED$_{50}$ for essential oils marigold and myrtle, showing higher repellency effect ($P<0.01$). The related dose-response lines, ED values and regression equations for DEET, marigold and myrtle are shown in Fig. 3.

### GC-mass analysis

The result of GC-mass analysis showed that the number of chemical contents were 65 and 33 constituents respectively in essential oils marigold and myrtle. The main compounds of marigold were Alpha-cadinol (18.3%), beta-eudesmol (14.5%) and tau-muurolol (13.0%) compared to alpha-pinene (47.8%), 1, 8-cineole (25.9%), linalool (8.4%) and linalyl acetate (4.3%) in myrtle.

Table 1. Protection and failure times values of essential oils both marigold and myrtle compared to DEET against An. stephensi on human subjects

| Repellents   | Protection time (hour) | Failure time (hour) |
|--------------|------------------------|---------------------|
|              | Range                  | Mean ± SE           |                      |
| DEET 25%     | 6.05–7.00              | 6.23 ± 0.16         | 7.30                 |
| Marigold 50% | 1.00–3.30              | 2.15 ± 0.66         | 3.30                 |
| Myrtle 50%   | 4.25–4.40              | 4.36 ± 2.18         | 4.40                 |

Table 2. Parameters of probit analysis on chemical and botanical repellents against An. stephensi using standard method (ASTM- E951-94)

| Repellents   | No. mosquitoes | ED$_{50}$ (mg/cm²) | 95% C.L. (mg/cm²) | ED$_{90}$ (mg/cm²) | 95% C.L. (mg/cm²) | $X^2$ (df, SE) | $X^2$ (table) | $P$-value | Equation of regression lines |
|--------------|----------------|--------------------|-------------------|--------------------|-------------------|----------------|---------------|----------|-----------------------------|
| DEET 5%      | 100            | 0.0023             | 0.0020–0.0027     | 0.009              | 0.0071–0.0127     | 16.185(2)±0.221 | 5.99          | <0.05    | Y = 5.7588 +2.1903X         |
| Marigold -   | 100            | 0.6034             | 0.4464–0.7476     | 3.4905             | 2.6203–5.4530     | 8.110(2)±0.196 | 5.99          | <0.05    | Y = 0.3688+1.6813 X          |
| Myrtle 50%   | 100            | 0.1105             | 0.0772–0.1399     | 0.5404             | 0.4281–0.7683     | 12.043(2)±0.618 | 5.99          | <0.05    | Y = 1.7785+1.8589 X          |
Fig. 1. Statistical comparison of protection time of essential oils of marigold and myrtle as well as DEET on human subjects using *An. stephensi*.

Fig. 2. Statistical comparison of effective doses of essential oils marigold and myrtle as well as DEET on human subjects using *An. stephensi*.
Discussion

Insect repellents are used to prevent nuisance bites from mosquitoes as well as other blood-feeding arthropods and may aid in lowering disease transmission e.g. malaria, leishmaniasis, filariasis and West Nile virus.

Anopheles stephensi is the main malaria vector in the country and is rearing easily and used for different biological assays such as irritability tests, olfaction studies, bioassay tests for bednets and indoor residual spraying, biological tests for plant extraction and repellents (Hadjiahoondi et al. 2000ab, 2003, 2005, 2006, Sadat Ebrahimi et al. 2005, Rafinejad et al. 2006, Vatandoost et al. 2006a, 2008b, 2009b, 2011, Davari et al. 2007, Omrani et al. 2010, Shahi et al. 2010, Hanafi-Bojd et al. 2011, Sedaghat et al. 2011). In this study, the effect of essential oils myrtle and marigold as the botanical repellents were compared with DEET. The N,N-diethyl-m-toluamide (DEET) as a broad-spectrum repellent is provide longer-lasting protection against many species of biting arthropods including mosquitoes which has been used worldwide since 1957 (USEPA 1998). It is commonly assumed that plant-based repellents are safer than DEET because they have natural origin (Maia and Moore 2010). The botanical repellents were developed from definite species of plants are environment-friendly, with pleasant natural aroma and less harmful than synthetic repellents which have been reported to cause many undesirable side effects to human.

In our study, 25% DEET provided an average of 6.23 hours of complete protection against An. stephensi bites. DEET-based repellents have been shown in other studies to provide complete protection against arthropod bites for as long as 12 hours under laboratory conditions (Fradin and Day 2002) which depend on the concentration, formulation and mosquito species tested (Klun et al. 2006).
Our ED₅₀ estimates for DEET is 0.0023 mg/cm² on 4 human subjects. On the animal model, the ED₅₀ value of DEET was calculated 0.005 mg/cm² against An. stephensi (Vatandoost 2008b). In the laboratory condition, the mean of relative effectiveness of 50% DEET was showed 97.0% protection against An. stephensi on guinea pig and 80.5% protection on human hand (Oshaghi et al. 2003b).

A cream formulation of DEET was evaluated at 10 mg/cm² with 96.2% protection against An. stephensi and provided protection up to 6.75±0.2 hours up to 4 hours observation (Mittal et al. 2011).

There are no published data describing the repellency of essential oil of marigold. The mean of protection time of 50% myrtle essential oil showed considerable repellency on human subjects and provided 4.36 hours protection against An. stephensi, the main malaria vector at laboratory condition. In other study which conducted on 41 natural repellents, the protection time of myrtle essential oil was reported 6.5 hours against Anopheles species (Abdelkrim et al. 2006). The past studies revealed that the most natural product-based repellents provided 3 hours protection which is comparable with protection provided by 7 or 15% DEET (Barnard and Xue 2004). The mean of protection time of 50% essential oil marigold provided only 2.15 hours protection against An. stephensi bites. In other laboratory study, the values ED₅₀ and ED₉₀ for myrtle essential oil were respectively calculated as 0.1140 and 0.6711 mg/cm² on animal model (rabbit) using K and D apparatus against lab-bred Phlebotomus papatasi Scopoli (Yaghoobi-Ershadi et al. 2006). Surprisingly, in our study, the ED₅₀ and ED₉₀ values for myrtle essential oil against An. stephensi was very close to latter studies on animal model with P. papatasi (respectively 0.1105 and 0.5404 mg/cm²) which assessed on 4 male human subjects.

The repellent effects of the essential oils indicated that they contained active constituents which responsible for the repellency activity. The major components of these two essential oils are monoterpenes, primarily 1, 8-cineole and linalyl acetate which detected in moderate percentages (13.0–18.3%) in the essential oil of studied myrtle (M.communis) compared to lower percentage (<0.5%) in marigold (C. officinalis). The higher repellency and insecticidal effects of the myrtle could be attributed to the major aforementioned constituents. The insecticidal effect of myrtle at 1.6 mg/cm² was reported 62.2% against lab-bred P. papatasi on animal model (Yaghoobi-Ershadi et al. 2006). In marigold (C. officinalis), carvacrol and thymol were extracted in low percentage (0.18–0.28%) had been shown to have insecticidal properties. On the other hands, 1, 8-cineole and linalyl acetate could be responsible for the high repellency activity of the essential oil of myrtle (Klocke et al. 1987, Abdurrahman et al. 2006, Günter et al. 2009,).

The protection time provided by 25% DEET (PT=6.23 hours) is contrastable with the results of 50% myrtle essential oil (PT= 4.36 hours) which shows that the myrtle essential oil can be useful and safe in preventing mosquito bites and have potential use as a botanical repellent.

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References

Abai MR, Mehravaran A, Vatandoost H, Oshaghi MA, Javadian E, Mashayekhi M, Mosleminia A, Piyazak N, Edallat H, Mohtarami F, Jabbarie H, Rafi F (2008) Comparative performance of imagicides on Anopheles stephensi, main malaria vector in a malarious area, southern, Iran. J Vector Borne Dis. 45: 307–312.

Abdelkrim A, Mehlhorn H (2006) Larvicidal effects of various essential oils against Aedes, Anopheles, and Culex larvae (Diptera, Culicidae). Parasitol Res. 99: 466–472.

Abdurrahman A, Osman S, Salih K, Ismet O (2010) Insecticidal activity of the essential oils from different plants against three stored-product insects. J Insect Sci. 10: 21.

Barnard DR, Xue RD (2004) Laboratory Evaluation of Mosquito Repellents Against Aedes albopictus, Culex nigripalpus, and Ochlerotatus triseriatus (Diptera: Culicidae) J Med Entomol. 41(4): 726–730.

Davari B, Vatandoost H, Oshaghi MA, Ladonni H, Enayati AA, Shaeghi M, Basseri HR, Rassi Y, Hanafi-Bojd AA (2007) Selection of Anopheles stephensi with DDT and dieldrin and cross-resistance spectrum to pyrethroids and fipronil. Pest Bioch Physio. 89: 97–103.

Doosti S, Azari-Hamidian S, Vatandoost H, Oshaghi MA, Hosseini M (2006) Taxonomic differentiation of Anopheles sacharovi and An.maculipennis s.l. (Diptera: Culicidae) larvae by seta 2 (ante-palmate hair). Acta Med Iran. 44: 41–43.

Doosti S, Vatandoost H, Oshaghi MA, Hosseini M, Sedaghat MM (2007) Applying Morphometric variation of seta 2 (ante-palmate hair) among the larvae of the members of the Maculipennis subgroup (Diptera: Culicidae) in Iran. Iran J Arthropod-Borne Dis. 1: 28–37.

Enayati AA, Vatandoost H, Ladonni H, Townson H, Hemingway J (2003) Molecular evidence for a Kdr-like pyrethroid resistance mechanism in the malaria vector mosquito Anopheles stephensi. Med Vet Entomol. 17: 138–144.

Finney DJ (1971) Probit analysis, 3rd ed. Cambridge Univ Press, Cambridge, England.

Finney DJ (1978) Statistical method in biological assay, 3rd ed. Charles Griffin and Co, London.

Fradin MS, Day GF (2002) Comparative efficacy of insect repellents against mosquito bites. The New England J Med. 47: 13–18.

Günter C, Müller AJ, Jerry B, Vassiliy DK, Edita ER, Robert WW, Schlein Y (2009) Efficacy of the botanical repellents geraniol, linalool, and Citronella against mosquitoes. J Vector Ecol. 34(1): 2–8.

Hadjiahkoondi A, Aghel N, Zamanizadeh N, Vatandoost H (2000a) Chemical and Biological study of Mentha spicata L. essential oil from Iran. Daru. 8: 19–21.
Hadjiakhoondi A, Vatandoost H, Abousaber M, Khanavi M, Abdi L (2000b) Chemical composition of the essential oil of *Tagetes minuta* L. and its effects on *Anopheles stephensi* larvae in Iran. J Med Plants. 7: 33–100.

Hadjiakhoondi A, Vatandoost H, Jamshidi A, Amiri EB (2003) Chemical constituents and efficacy of *Cymbopogon olivieri* (Boiss.) bar essential oil against malaria vector, *Anopheles stephensi*. Daru. 11: 125–128.

Hadjiakhoondi A, Vatandoost H, Khanavi M, Abaee MR (2005) Biochemical investigation of different extracts and larvicidal activity of *Tagetes minuta* L. on *Anopheles stephensi* larvae. Iran J Pharm Sci. 1: 81–84.

Hadjiakhoondi A, Sadeghipour-Roodsari HR, Vatandoost H, Khanavi M, Vosoughi M, Kazemi, M Abai, MR (2006) Fatty acid composition and toxicity of Melia azedarach L. fruits against malaria vector *Anopheles stephensi*. Iran J Pharm Sci. 2: 97–102.

Hanafi-Bojd AA, Vatandoost H, Jafari R (2006) Susceptibility status of *An. dthali* and *An. fluviatilis* to commonly used larvicides in an endemic focus of malaria, southern Iran. J Vector Borne Dis. 43: 34–38.

Hanafi-Bojd AA, Azari-Hamidian S, Vatandoost H, Charrahy Z (2011) Spatio-temporal distribution of malaria vectors (Diptera: Culicidae) across different climatic zones of Iran. Asian Pac J Trop Med. 6: 498–504.

Hanafi-Bojd AA, Vatandoost H, Oshaghi MA, Haghdooost AA, Shahi M,Se- aghat MM, Abedi F, Yeryan M, Pakari A (2011) Entomological and epidemiological attributes for malaria transmission and implementation of vector control in southern Iran. Acta Trop. Acta Trop. [Epub ahead of print]

Klocke JA, Darlington MV, Balandrin MF (1987) 8-Cineole (Eucalyptol), a mosquito feeding and ovipositional repellent from volatile oil of *Hemizonia fitchii* (Asteraceae)”. J Chem Ecol. 13(12): 23–27.

Klut JA, Khrimian A, Debboun M (2006) Repellent and deterrent effects of SS220, Picardin, and DEET suppress human-blood feeding by *Aedes aegypti*, *Anopheles stephensi*, and *Phlebotomus papatasi*. J Med Entomol. 43: 34–39.

Logan JG, Stanczyk NM, Hassanali A, Kemei J, Santana AES, Ribeiro KAL, Pickett JA, Mordue (Luntz) JA (2010) Arm-in-cage testing of natural human-derived mosquito repellents. Malar J. 9: 239.

Maia FM, Moore SJ (2010) Plant-based insect repellents: a review of their efficacy, development and testing. Malar J. 10 (Suppl 1): S11.

Manouchehri AV, Zaim M, Emadi AM (1992) A review of malaria in Iran, 1957–1990. J Am Mosq Control Assoc. 8: 381–385.

Mittal PK, Sreehari U, Razdan RK, Dash AP, Ansari MA (2011) Efficacy of Advanced Odomos® repellent cream (N, N-diethyl-benzamidine) against mosquito vectors. Indian J Med Res. 133: 426–430.

Naddaf SR, Oshaghi MA, Vatandoost H, Asmar M (2003) Molecular characterization of the *Anopheles fluviatilis* species complex in Iran. East Mediterr Health J. 9: 257–265.

Omrami SM, Vatandoost H, Oshaghi MA, Shokri F, Guerin PM, Yaghoob-Erishadi, MR, Rassi Y, Tirgari S (2010) Fabrication of an olfactometer for mosquito behavioural studies. J Vector Borne Dis. 47: 17–25.

Oshaghi MA, Ghalandari R, Vatandoost H, Shayeghi M, Kmaili-nejad M, Tourabi-Khaledi H Abolhassani M, Hashemzadeh M (2003a) Repellent effect of extracts and essential oil of Citrus limon
and *Melissa officinalis* (Labiatae) against main malaria vector, *Anopheles stephensi* (Diptera: Culicidae) in Iran. Iranian J Public Health. 32: 47–52.

Oshaghi MA, Sedaghat MM, Vatandoost H (2003b) Molecular characterization of the *Anopheles maculipennis* complex in the Islamic Republic of Iran. East Mediterr Health J. 9: 59–66.

Oshaghi MA, Yaaghoobi F, Abai MR (2006a) Pattern of mitochondrial DNA variation between and within *Anopheles stephensi* (Diptera: Culicidae) biological forms suggests extensive gene flow. Acta Trop. 99: 226–233.

Oshaghi MA, Yaghoobi F, Vatandoost H, Abai MR, Akbarzadeh K (2006b) *Anopheles stephensi* biological forms, geographical distribution, and malaria transmission in malarious regions in Iran. Pak J Biol Sci. 9: 294–298.

Oshaghi MA, Chavshin AR, Vatandoost H (2006c) Analysis of mosquito bloodmeals using RFLP markers. Exp Parasitol. 114: 259–264.

Pourmand MR, Yazdi MH, Bayat M (2008) In vitro antimicrobial effects of *Zataria multiflora* Boiss., *Myrtus communis* L. L. and *Eucalyptus officinalis* against *Streptococcus pneumoniae*, *Moraxella catarrhalis* and *Haemophilus influenzae*. Iran J Medicinal and Aromatic Plants. 23: 14–25.

Rafinejad J, Vatandoost H, Nikpoor F, Abai MR, Shaeghi M, Duchen, S, Rafi F (2006) Effect of washing on the bioefficacy of insecticide-treated nets (ITNs) and long-lasting insecticidal nets (LLINs) against main malaria vector *Anopheles stephensi* by three bioassay methods. J Vector Borne Dis. 45: 143–150.

Rechinger KH (1996) Myrtacea in Flora Iranica (No. 34.1). Kademische Druck-U. Verlagsanstalt, Graz.

Sadat Ebrahimi SE, Hadjiakhoondi A, Reza-zadeh Sh, Fereidunian N, Vatandoost H, Abai MR (2005) The components of *Tagetes minuta* L. and its biological activities against malaria vector, *Anopheles stephensi* in Iran. J Med Plants. 4: 43–47.

Salari Lah SH, Vatandoost H, Entezarmahd MR, Ashraf H, Abai MR, Nazari M (2002) Monitoring of insecticide resistance in *Anopheles sacharovi* (Favre, 1903) in borderline of Iran, Armenia, Naxcivian and Turkey.. Iran J Public Health. 31: 96–99.

Sedaghat MM, Linton YM, Nicolescu G, Smith L, Koliopoulos G, Zounos AK, Athanassios K. Zounos, Oshaghi MA, Vatandoost H, Harbach RE (2003a) Morphological and molecular characterization of *Anopheles* (*Anopheles*) *sacharovi* Favre, a primary vector of malaria in the Middle East. Systematic Entomol. 28: 241–256.

Sedaghat MM, Linton YM, Oshaghi MA, Vatandoost H, Harbach RE (2003b) The *Anopheles maculipennis* complex (Diptera: Culicidae) in Iran: molecular characterization and recognition of a new species. Bull Entomol Res. 93: 527–535.

Sedaghat MM, Harbach RE (2005) An annotated checklist of the *Anopheles* mosquitoes (Diptera: Culicidae) in Iran. J Vector Ecol. 30: 272–276.

Sedaghat MM, Dehkordi AS, Khanavi M, Abai MR, Mohtarami F, Vatandoost H (2011) Chemical composition and larvicidal activity of essential oil of *Cupressus arizonica* E.L. Greene against malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). Pharmacognosy Res. 3: 135–139.

Shahi M, Hanafi-Bojd AA, Iranshahi M, Vatandoost H, Hanafi-Bojd MY (2010) Larvicidal efficacy of latex and extract of *Calotropis procera* (Gentianales: Asclepiadaceae) against *Culex quinquefasciatus* and *Anopheles stephensi* (Diptera: Culicidae). J Vector Borne Dis. 47: 185–88.
USEPA (US Environmental Protection Agency) (1998) DEET: Reregistration eligibility decision. Washington, DC: USEPA, Office of Pesticide Programs, Special Review and Registration Division.

Vatandoost H, Shahi M, Abai MR, Hanafi-Bojd AA, Oshaghi MA, Zamani G. (2004a) Larval habitats of main malaria vectors in Hormozgan province and their susceptibility to different larvicides. Southeast Asian J Trop Med Public Health. 35: 22–25.

Vatandoost H, Borhani N (2004b) Susceptibility level and irritability of synthetic pyrethroids against main malaria vectors in the endemic areas of Iran. Acta Med Iran. 42: 247–255.

Vatandoost H, Moinvaziri VM (2004c) Larvicidal activity of neem tree extract (Neemarin) against mosquito larvae in the Islamic Republic of Iran. East Mediterr Health J. 10: 573–578.

Vatandoost H, Mashayekhi M, Abai MR, Aflatoonian MR, Hanafi-Bojd AA, Sharifi I (2005a) Monitoring of insecticides resistance in main malaria vectors in a malarious area of Kahnooj district, Kerman province, southeastern Iran. J Vector Borne Dis. 42: 100–108.

Vatandoost H, Hanafi-Bojd AA (2005b) Current resistant status of Anopheles stephensi Liston to different larvicides in Hormozgan province, southeastern Iran. Pak J Biol Sci. 8: 1568–1570.

Vatandoost H, Dehakia M, Djavadian E, Abai MR, Duchson S (2006a) Comparative study on the efficacy of lambdacyhalothrin and bifenthrin on torn nets against the malaria vector, Anopheles stephensi as assessed by tunnel test method. J Vector Borne Dis. 43: 133–135.

Vatandoost H, Gholizadeh MR, Abai MR, Djavadian E (2006b) Laboratory efficacy of protection rate of torn nets treated with pyrethroids, cyfluthrin, deltamethrin and permethrin against Anopheles ste-

phensi (Diptera: Culicidae). J Biol Sci. 6: 331–336.

Vatandoost H, Oshaghi MA, Aflai MR, Shahi M, Yaaghoobi F, Baghaii M, Hanafi-Bojd AA, Zamani G, Townson H (2006c) Bionomics of Anopheles stephensi Liston in the malarious area of Hormozgan province, southern Iran. Acta Trop. 97: 196–205.

Vatandoost H, Khazani A, Rafinejad J, Khoobdel M, Kebria-Zadeh A, Abai MR, Hanafi-Bojd AA, Akhavan AA, Abtahi SM, Rafe F (2008a) Comparative efficacy of Neem and dimethyl phthalate (DMP) against malaria vector, Anopheles stephensi (Diptera: Culicidae). Asian Pacific J Trop Med. 1: 1–6.

Vatandoost H, Hanafi-Bojd AA (2008b) Laboratory evaluation of 3 repellents against Anopheles stephensi in the Islamic Republic of Iran. East Mediterr Health J. 14: 260–267.

Vatandoost H, Ramin E, Rassi Y, Abai MR (2009a) Stability and wash resistance of local made mosquito bednets and detergents treated with pyrethroids against Anopheles stephensi. Iran J Arthropod-Borne Dis. 3: 19–28.

Vatandoost H, Ramin E, Rassi Y, Abai MR (2009b) Stability and wash resistance of local made mosquito bednets and detergents treated with pyrethroids against Anopheles stephensi. Iran J Arthropod-Borne Dis. 3: 19–28.

Vatandoost H, Abai MR, Abbasi M, Shaeghi M, Abtahi M, Rafe F (2009c) Designing of a laboratory model for evaluation of the residual effects of deltamethrin (K-othrine WP 5%) on different surfaces against malaria vector, Anopheles stephensi (Diptera: Culicidae). J Vector Borne Dis. 46: 261–267.

Vatandoost H, Zahirnia AH (2010) Responsiveness of Anopheles maculipennis to different imagicides during resurgent malaria. Asian Pacific J Trop Med. 3:360-363.
Vatandoost H, Rashidian A, Jafari M, Raeisi A, Hanafi-Bojd A, Yousofzai AW, Daryanavard A, Mojahedi A, Pakari A (2011) Demonstration of malaria situation analysis, stratification and planning in Minab District, southern Iran. Asian Pac J Trop Med. 4(1): 67–71.

Yaghoobi-Ershadi MR, Akhavan AA, Jahaniard E (2006) Repellency effect of myrtle essential oil and DEET against Phlebotomus papatasi under laboratory conditions. Iran J Public Health 35: 7–13.

Zahirnia AH, Vatandoost H, Nateghpour M, Javadian E (1998) Insecticide resistance/susceptibility monitoring in Anopheles pulcherrimus (Dipter: Culicidae) in Ghasreghand district, Sistan and Baluchistan province. Hakim. 1: 97–106.

Zahirnia AH, Taherkhani H, Vatandoost H (2001) Observation of malaria sporozoite in Anopheles culicifacies (Diptera: Culicidae) in Ghasreghand district, Sistan and Baluchistan province. Hakim. 4: 149–153.

Zolfaghari ME, Salamian P, Riazi A, Khaksa G (1997) Clinical trial of Efficacy of myrtle in the treatment of herpes simplex. Iran J Med Sci. 22(3 and 4):137–140.
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کارگاه آنلاین
کاربرد نرم افزار SPSS در پژوهش

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