

Original Article**Larvicidal Activity of Essential Oils of Apiaceae Plants against Malaria Vector, *Anopheles stephensi***MM Sedaghat¹, A Sanei Dehkordi¹, MR Abai¹, M Khanavi², F Mohtarami¹, Y Salim Abadi¹, F Rafi¹, *H Vatandoost¹¹Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran²Department of Pharmacognosy and Medicinal Plant Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

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Abstract**Background:** Plant extracts and oils may act as alternatives to conventional pesticides for malaria vector control. The aim of this study was to evaluate the larvicidal activity of essential oils of three plants of Apiaceae family against *Anopheles stephensi*, the main malaria vector in Iran.**Methods:** Essential oils from *Heracleum persicum*, *Foeniculum vulgare* and *Coriandrum sativum* seeds were hydro distilled, then their larvicidal activity were evaluated against laboratory-reared larvae of *An. stephensi* according to standard method of WHO. After susceptibility test, results were analysis using Probit program.**Results:** Essential oils were separated from *H. persicum*, *F. vulgare* and *C. sativum* plants and their larvicidal activities were tested. Result of this study showed that *F. vulgare* oil was the most effective against *An. stephensi* with LC₅₀ and LC₉₀ values of 20.10 and 44.51 ppm, respectively.**Conclusion:** All three plants essential oil can serve as a natural larvicide against *An. stephensi*. *F. vulgare* oil exhibited more larvicidal properties.**Keywords:** Malaria, Apiaceae, Vector, *Anopheles stephensi***Introduction**

Mosquitoes play an important role in transmission of some human diseases such as malaria, dengue fever, yellow fever and filariasis, which consider them among the greatest health problems across the globe (James 1992). *Anopheles* species are considered as vectors of human malaria, filariasis and certain arboviruses (Sedaghat and Harbach 2005). Malaria is considered as one of the most important health problem in Iran especially in southern parts. In the south parts of Iran there are six anopheline vectors including *Anopheles culicifacies*, *An. stephensi*, *An. dthali*, *An. fluviatilis*, *An. superpictus*, and *An. pulcherrimus*, (Naddaf et al.

2003, Vatandoost and Moinvaziri 2004, Vatandoost et al. 2004, 2005ab, Hanafi-Bojd et al. 2006, Soltani et al. 2008, Hanafi-Bojd et al. 2010, Vatandoost et al. 2006, 2007, 2009, 2011). *Anopheles sacharovi* and *An. maculipennis* can transmit human malaria in northern part of the country (Sedaghat et al. 2003a, 2003b, Oshaghi et al. 2003, Doosti et al. 2007).

Anopheles stephensi, an oriental malaria vector, is distributed in Indo-Persian area from India, Pakistan and Iran, to countries around the Persian Gulf (Nagpal and Sharma 1995). In Iran, it occurs in the southern areas of the country in Khuzestan, Fars, Kerman,

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Hormozgan, Sistan and Baluchistan and southern Kermanshah Provinces (Manouchehri et al. 1976, Sedaghat and Harbach 2005).

Although there are several methods for control of *Anopheles* mosquitoes however environmental effect and resistance is a main human concern. Synthetic pyrethroids which considered as the most effective insecticides against anophelines, are still expensive and beyond the financial resources of some countries.

Using botanical insecticides is a technique which can apply as an alternative to synthetic chemical formulations. Most botanicals are rapid acting and breakdown quickly in the environment. The extract of whole leaf and essential oil of some certain plants have been investigated against some public health pests (Saxena and Sumithra 1985, Kumar and Dutta 1987, Chariandy et al. 1999, Hadjiakhoondi et al. 2000 ab, Markouk et al. 2000, Hadjiakhoondi et al. 2003, Tare et al. 2004, Vatan-doost et al. 2004, 2008, Hadjiakhoondi et al. 2005–2006, Oshaghi et al. 2008 a).

Apiaceae (Umbelliferae) is one of the best known families of flowering plants, which comprise 300–450 genus and 3000–3700 species (Constance 1971, Pimenov and Leonov 1993). They are aromatic plant and have a distinctive flavor which diverse volatile compounds from the fruits and leaves. This family also encompasses toxic plants that some had used in ancient Athens to execute those sentenced to death (Constance 1971, Pimenov and Leonov 1993). *Heracleum persicum* Desf., known as Persian Hogweed or “Golpar”, is an annual native plant native to Iran with a wide distribution across the country. It is used in traditional medicine because of its antioxidant and anticonvulsant activity (Parsa 1948, Sourì et al. 2000, Sayyah et al. 2005). *Foeniculum vulgare* Mill., known as Fennel or “Razianeh” in Iran and neighboring countries, has been used by humans since antiquity. It is generally considered indigenous to the shores of the Mediterranean, but now can be found in many parts of the world. (Mucken-

sturm et al. 1997). The bioactivities of this plant have been investigated for its antioxidant activity (Oktaya et al. 2003) fumigant activity (Kim et al. 2003), antimicrobial activity (Ruberto et al. 2000), larvicidal activity (Chantraine et al. 1998, Pitasawat et al. 2007), insecticidal activity (Laurent et al. 1997), and acaricidal activity (Lee 2004).

Coriandrum sativum L, known as Coriander, is native to Iran, however it is widely distributed around the world. The seeds contain an essential oil (up to 1%) and the seeds are used in traditional medicine for indigestion, against worms, rheumatism and pain in the joints (Wangensteen 2004). It has also the nematicidal activity (Kim et al. 2008), antibacterial activity (Cantore et al. 2004) and larvicidal activity (Harve and Kamath 2004).

In recent years, much effort has been focused on the exploration of bioactive chemical compounds from indigenous plants for mosquito control in Iran. So, the purpose of this study was to determine bioactivity of three essential oils obtained from the seeds of plants against 4th instar larvae of *An. stephensi*.

Materials and Methods

Mosquito rearing

The fourth-instars larvae of *An. stephensi* used for bioassays. The laboratory-reared *An. stephensi* Bandar-Abbas strain were reared in the Department of Medical Entomology, Tehran University of Medical Sciences, and maintained at 27° C with a photoperiod of 12 hours light and 12 hours e dark in 80±10% relative humidity. A 10% percentage yeast suspension was used as food source.

Plant materials

In this study, the three plants from Apiaceae (Umbellifera) family were collected from three provinces of Iran in 2009 (Table 1). Examined plants were authenticated and identified by the Department of Pharmacognosy, Faculty of Pharmacy, Tehran Univer-

sity of Medical Sciences. The seeds were air-dried at room temperature and kept in an air-tight light-protected container.

Dried seeds of *H. persicum*, *F. vulgare* and *C. sativum* were subjected to hydro dis-

tillation using a modified Clevenger-type apparatus for 3 hours, dried over anhydrous sodium sulphate, and stored in amber-colored vials at 5 °C until required for further work.

Table 1. Collection locations, physical properties and yields of volatile oils derived from seeds of *H. persicum*, *F. vulgare* and *C. sativum*

Plant species	Location	Harvest date (2009)	Color	Appearance	%Yield (w/w)
<i>H. persicum</i>	Mazandaran	August	Pale Yellow	Liquid	1.6
<i>C. sativum</i>	Chaharmahal and Bakhtiary	September	Pale Yellow	Liquid	0.9
<i>F. vulgare</i>	East Azarbaijan	September	Pale yellow	Liquid	0.8

Bioassays and larval mortality

Fourth instar larvae of *An. stephensi* Bandar-Abbas strain was exposed to test concentrations of 5, 10, 20, 40, 80, 160 and 320 ppm of essential oil for 24 hours according to standard method described by WHO (1981). As the oils do not dissolve in water, they were first dissolved in ethanol. The test 400 ml glass beaker were used by adding 1 ml of appropriate dilution of essential oil in ethanol and mixed with 249 ml of water to make up 250 ml of test solution (Dharmagadda et al. 2005). In control beakers, ethanol was applied into the water (1%). A minimum of 20 larvae per each concentrations were used for all the experiments. The dead larvae were counted after 24 hour recovery period, and percentage of mortality was reported from the average for the five replicates. The larvae considered dead were those that did not move when touched with a needle.

Statistical analysis

The lethal concentrations (LC₅₀ and LC₉₀) were calculated using Probit analysis (Finney 1971). For all bioassays, the percentages of mortality were adjusted for the mortality in controls by using Abbott's correction (Abbot

1925). Differences between means were considered significant at $P \leq 0.05$ (SAS Institute 2001).

Results

Hydro distillation of *H. persicum*, *C. sativum* and *F. vulgare* yielded (w/w, dry weight) of volatile oils (Table 1). The highest yield of volatile oil was obtained from *H. persicum*, whereas that of *F. vulgare* was the lowest.

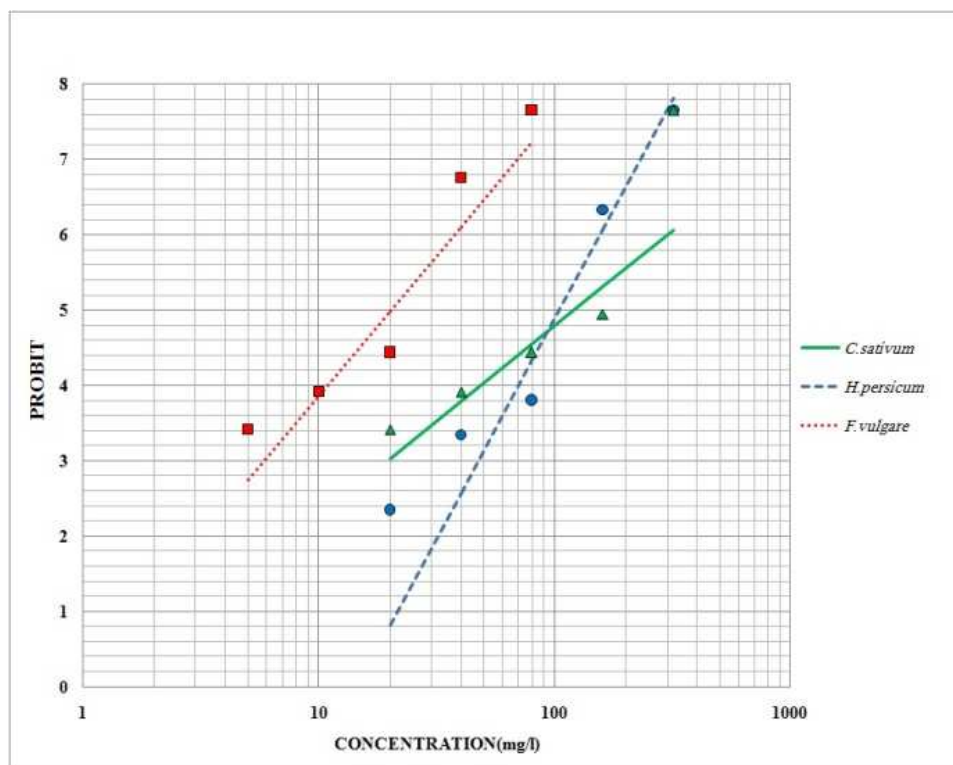
The larvicidal activities of the essential oils against *An. stephensi* larvae under laboratory conditions are given in Table 2. All plants oil exerted significant larvicidal potential against *An. stephensi* after exposure for 24 h (Table 2). The lethal dosage of 50% (LC₅₀) ranged between 20.10 and 120.95 ppm.

Foeniculum vulgare oil was the most effective against *An. stephensi* with LC₅₀ and LC₉₀ values of 20.10 and 44.51 ppm, respectively, while *C. sativum* had the least mortality with LC₅₀ and LC₉₀ values of 120.95 and 389.90 ppm, respectively. This was also 5 times lower than *H. persicum*, which showed an LC₅₀ of 104.80 ppm.

In regression line a positive correlation were observed between the essential oil concentrations and the percent mortality (Fig. 1).

Table 2. Parameters of probit regression line *Anopheles stephensi* to essential oil derived from *H. persicum*, *F. vulgare* and *C. sativum* seeds at different interval concentrations

Specimens	A	B±SE	LC ₅₀ , 95% C.I.	LC ₉₀ , 95% C.I.	X ² (df)	P value
<i>C. sativum</i>	-5.25	2.52±0.652	47.68 120.95 479.9	181.62 389.90 181172.4	43.82 (3)	<0.05
<i>H. persicum</i>	-11.73	5.81±1.725	26.30 104.80 336.90	114.40 174.22 1073788.4	41.85 (3)	<0.05
<i>F. vulgare</i>	-4.83	3.71±1.023	6.05 20.10 67.62	24.30 44.51 15839.5	53.6 (3)	<0.05

**Fig. 1.** Probit regression line of *An. stephensi* exposed to different interval concentrations of *H. persicum*, *F. vulgare* and *C. sativum* seed essential oils

Discussion

The use of plant essential oils in vector control is an alternative method for minimizing the side effects of chemical pesticides on the environment (Fatope et al. 1993). In recent surveys, it has been found that some of secondary plant metabolites act as botanical

insecticides (Watanabe et al. 1993, Vatan-doost et al. 2004, Nathan 2007). According to the biological results of present study, all of three plant oils exerted significant larvicidal potential against *An. stephensi* and the essential oil of *F. vulgare* had an appro-

appropriate effect. A study in Bolivia reported that essential oil of *F. vulgare*, was one of the most toxic oils on *Ae. aegypti* larvae. Also its LC₅₀ and LC₉₀ were 24.3 and 30.8 ppm, respectively (Chantraine et al. 1998). In the other study in Lebanon for assay of repellency and toxicity of aromatic plant extracts against the mosquito *Culex pipiens* form *molestus*, results showed that *F. vulgare* was one of the most significant repellency against this species (52.0 min of protection with concentration of 3%) also the LC₅₀ and LC₉₀ from essential oil of *F. vulgare* was 24.5 and 34 mg/l respectively (Traboulsi et al. 2005). In several study on chemical compositions of plants by using of gas chromatography and mass spectrometry (GC/MS), results showed that different compounds were present in different parts of the plants. For example, in the study on seeds essential oil of *H. persicum* it is found that it contains hexyl butyrate (35.5%), octyl acetate (27%) and hexyl isobutyrate (3.2%) (Sefidkon et al. 2004). In the other study on the leaves of *C. sativum*, Matasyoh et al (2009) reported four main compounds, including: 2E-decenal (15.9%), decanal (14.3%), 2E-decen-1-ol (14.2%) and n-decanol (13.6%). In the two same study on fruit and flower of *F. vulgare*, the trans-anethole (85.63%), estragole (5.27%) and D-limonene (3.8%) were present in the fruit (Dadalioglu and Evrendilek 2004), While other report showed limonene (63.6%), anethole (25.5%), fenchyl acetate (2.6%), α -Pinene (0.97%), myrcene (0.98%) and estragole (1.1%) from flower (Traboulsi et al. 2005). It seems that the larviciding effect of these essential oils of plant is attributed to these main compounds. We propose test different components of the plants against mosquitoes.

Results of the current study showed that the essential oil of these plants exhibited the biological effect on the larvae of *An. stephensi*. The use of botanical pesticide may help in reducing the environmental side effects by the synthetic insecticides. The results ob-

tained suggest that the essential oils of *H. persicus*, *F. vulgare* and *C. sativum* are promising as larvicide against *An. stephensi*. Moreover, these results could be useful in the search for newer, more selective, and biodegradable larvicidal natural compounds. Further investigations are currently underway to isolate these compounds.

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