Original Article

Larvicidal Activity of *Bunium persicum* Essential Oil and Extract against Malaria Vector, *Anopheles stephensi*

Hassan Vatandoost [1, 2], Arezoo Rustaie [3], Zeynab Talaeian [3], Mohammad Reza Abai [1], Fatemeh Moradkhani [3], Mahdi Vazirian [3], Abbas Hadjiakhoondi [3], Mohammad Reza Shams-Ardekani [3, 4], *Mahnaz Khanavi [3, 5]

[1]Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Chemical Pollutants and Pesticides, Institute for Environmental Research, Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Pharmacognosy, School of Pharmacy and Persian Medicine and Pharmacy Research Centre, Tehran University of Medical Sciences, Tehran, Iran
[4]Department of Traditional Pharmacy, School of Traditional Medicine, Tehran University of Medical Sciences, Tehran, Iran
[5]Faculty of Land and Food Systems, University of British Columbia, Vancouver, Canada

(Received 21 Sep 2016; accepted 12 Dec 2017)

Abstract

Background: Malaria, a mosquito-transmitted disease, is still a major human health problem all over the world. Larviciding is a component of comprehensive control program to overcome the disease. Negative aspects of synthetic insecticides application, such as environmental safety concerns, have favored use of natural insecticides.

Methods: Larvicidal activity of essential oil, extracts and fractions of a wild grown and a cultivated type of *Bunium persicum* fruits against malaria vector *Anopheles stephensi* was assessed according to the method described by WHO.

Results: *Bunium persicum* showed remarkable potency against *An. stephensi* larvae. LC_{50} values for essential oil, total extract, petroleum ether fraction and methanol fraction were 27.4284, 64.9933, 85.9933 and 255.7486ppm for wild type, and 21.3823, 63.2580, 62.7814 and 152.6357ppm for cultivated one.

Conclusion: The results of this study suggest *B. persicum* as a valuable source of natural insecticides against malaria vector *Anopheles stephensi*.

Keywords: *Anopheles stephensi*, *Bunium persicum*, Larvicidal activity, Extract, Essential oil

Introduction

Despite progresses made over the past decades to decline the mortality rate of malaria all over the world, it is still prevalent in some tropical countries and areas with about 200 million affected cases in 2013. Vector control interventions have had substantial contribution on the recent reduction in global malaria burden. Larviciding, with the aim of adult vector density reduction, as an auxiliary to core interventions, is helpful especially in urban regions, where breeding of vectors take places in permanent or semi-permanent aquatic habitats (1). The mosquito *Anopheles stephensi* is one of the six main vectors of human malaria in southern parts of Iran (2). Larvicidal potentials of some herbal extracts and essential oils on *An. stephensi* larvae have been investigated previously (3-5).

Bunium persicum is a perennial plant belonging to Apiaceae family, growing wild in Iran (6). The fruit of *B. persicum* is used as spice, antiseptic and carminative agent (7). Several studies have analyzed essential oil composition of the fruits and mostly reported γ-ter-

***Corresponding author:** Dr Mahnaz Khanavi, E-mail: khanavim@tums.ac.ir

http://jad.tums.ac.ir
Published Online: March 18, 2018

pinene, cuminaldehyde and ρ-cymene as main components (8-10). Kaempferol, caffeic and p-coumaric acid have been isolated from polar fraction of the fruits as major antioxidant constituents (11) but according to our knowledge no other comprehensive study has been organized to identify other phytochemicals in the extract. Overexploitation and unscientific harvesting of *B. persicum* as well as climate changes, has threatened its existence in wild (12). Cultivation of endangered species could preserve their genetic resources (13). In recent years, *B. persicum* is cultivated in limited areas in Iran especially in Khorasan Razavi Province. As a part of our ongoing studies on larvicidal activity of plants extracts and essential oils against *An. stephensi* (4, 5, 14-20), in the present study, we have studied larvicidal activity of the essential oil, extract and fractions from *B. persicum* fruits against late third instar larvae of *An. stephensi*. Moreover, we have compared the activities of a wild and a cultivated type.

Materials and Methods

Plant material

The fruit of wild *B. persicum* was purchased from Kerman, and cultivated type was supplied from agricultural research fields of Ferdowsi University of Mashhad (2013). The samples were authenticated at the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, where voucher specimens were deposited (PMP-649 and PMP-689).

Essential oil preparation

100g powdered fruits of cultivated and wild *B. persicum* were subjected to hydrodistillation for 3 hours using Clevenger type apparatus. The obtained essential oils were dried over anhydrous sodium sulfate and kept in refrigerator until needed.

Extraction and fractionation

250g dried and powdered fruits from both samples were separately extracted with meth anol (5× 1.5L) to afford total methanol extracts. The solvent was removed under reduced pressure by rotary evaporator at 40 °C, and subsequently lyophilized by freeze dryer at -40 ºC for 24h (Lyotrap Ultra, LTE Scientific Ltd., Oldham, UK). Fractionation of total extracts was performed with sufficient volumes of petroleum ether, ethyl acetate and methanol. The fractions were then concentrated to dryness by rotary evaporation.

Larval mortality bioassay

Anopheles stephensi larvae (Bandar Abbas strain) were supplied by the Department of Medical Entomology, Tehran University of Medical Sciences. The mosquito colony was maintained under a constant insectarium condition at 27 ºC and 75–85% relative humidity with 12:12 light and dark photoperiod. Late third and early fourth instars larvae were used for experiments.

Larvicidal activity of total extracts, fractions and essential oils were evaluated according to the procedure recommended by WHO (21). The larvae were exposed to different concentrations of samples for 24 hours. Tests were carried out in four replicates. One ml of solvents (DMSO for essential oil and petroleum ether fraction, DMSO2: water 3 for total extract and ethanol for methanol fraction) were added separately into control bakers. Mortality was scored 24 hours post exposure.

Analysis method

The mortality percentages were calculated and corrected relative to the associated controls using Abbott's formula (22). The concentration-mortality data were subjected to Probit analysis (23) and lethal concentrations (LC_{50} and LC_{90}) were determined with 95% confidence intervals from the regression lines.

http://jad.tums.ac.ir
Published Online: March 18, 2018

Results

Hydro distillation of wild and cultivated *B. persicum* fruits yielded 2.5% and 2.25% (w/w) essential oil respectively. Both essential oils had a lot of commonalities in composition. γ-Terpinene (30.77% and 27.57%), cuminaldehyde (20.49% and 21.1%), ρ-cymene (20.1% and 18.32%) and γ-terpinen-7-al (8.29% and 7.84%) constituted main components in the wild and cultivated oils respectively (24). The results of larvicidal activity of essential oils, total extracts, petroleum ether and methanol extracts against *An. stephensi* under insectary condition are presented in table 1 and plotted in Figs. 1 to 4. All tested samples showed significant anti-larval effect against the malaria vector *An. stephensi*, of which, the essential oils from cultivated and wild types with LC_{50} values of 21.3823ppm and 27.4284ppm were the strongest samples and methanol fractions with LC_{50} values of 152.6357ppm and 255.7486 ppm exhibited least larvicidal activity among the samples. Comparison of lethal concentration values of efficient tested samples reveals there is no difference in efficacy of them between wild and cultivated types.

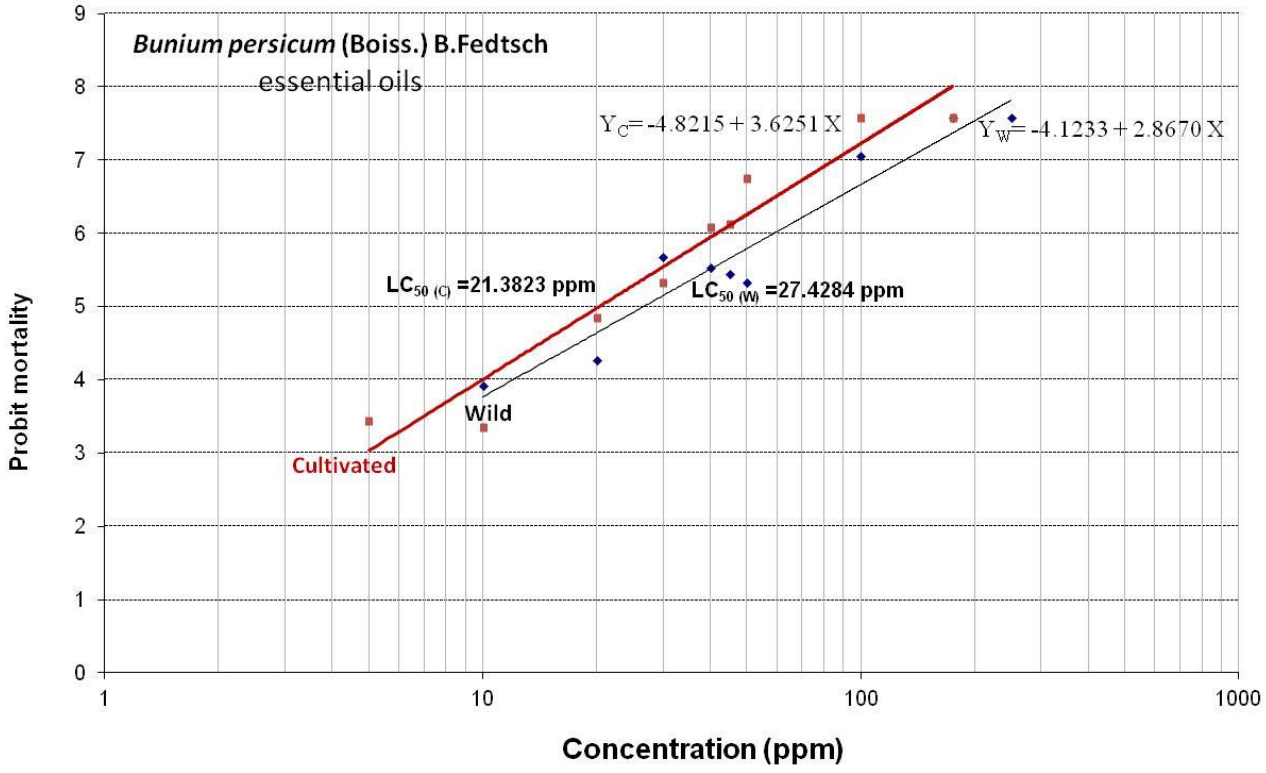

Fig. 1. Comparison of lethal concentrations (LC_{50}) of cultivated and wild types of *Bunium persicum* essential oils against larvae of *Anopheles stephensi*

http://jad.tums.ac.ir
Published Online: March 18, 2018

Table 1. Probit regression line parameters of essential oil, total extract, petroleum ether and methanol fraction of wild and cultivated *Bunium persicum* fruits against *Anopheles stephensi*

Specimen	Intercept	Slope ± SE	LC_{50}	95% CI	LC_{90}	95% CI	χ^2	P
W EO	-4.1233	2.8670 ± 0.471	27.4284	19.7868-35.0421	76.7752	56.3038-139.1275	41.682	<0.05
C EO	-4.8215	3.6251 ± 0.486	21.3823	13.6913-25.9482	48.2608	38.6334-68.5159	33.107	<0.05
W T	-3.9100	2.1568 ± 0.270	64.9933	44.8917-89.5814	255.3195	170.9457-498.5673	16.725	<0.05
C T	-4.6503	2.5819 ± 0.181	63.2580	55.8062-71.3261	198.3795	167.9464-243.6008	10.718	<0.05
W PE	-5.0602	2.6158 ± 0.502	85.9933	47.2631-130.9756	265.7116	166.2611-869.8822	28.442	<0.05
C PE	-4.7365	2.6346 ± 0.186	62.7814	55.4789-70.6893	192.4364	163.2242-235.7975	12.880	<0.05
W M	-6.6009	2.7414 ± 0.365	255.7486	159.3871-405.0692	750.4194	459.8467-2245.3156	26.381	<0.05
C M	-9.6475	4.4181 ± 1.524	152.6357	94.5358-262.7553	297.6718	202.8848-6675.4919	18.475	<0.05

W: wild, C: cultivated, EO: essential oil, T: total extract, PE: petroleum ether fraction, M: methanol fraction, SE: standard error, LC_{50}: lethal concentration to cause 50% mortality in population, LC_{90}: lethal concentration to cause 90% mortality in population, CI: confidence interval, χ^2: heterogeneity about the regression line.

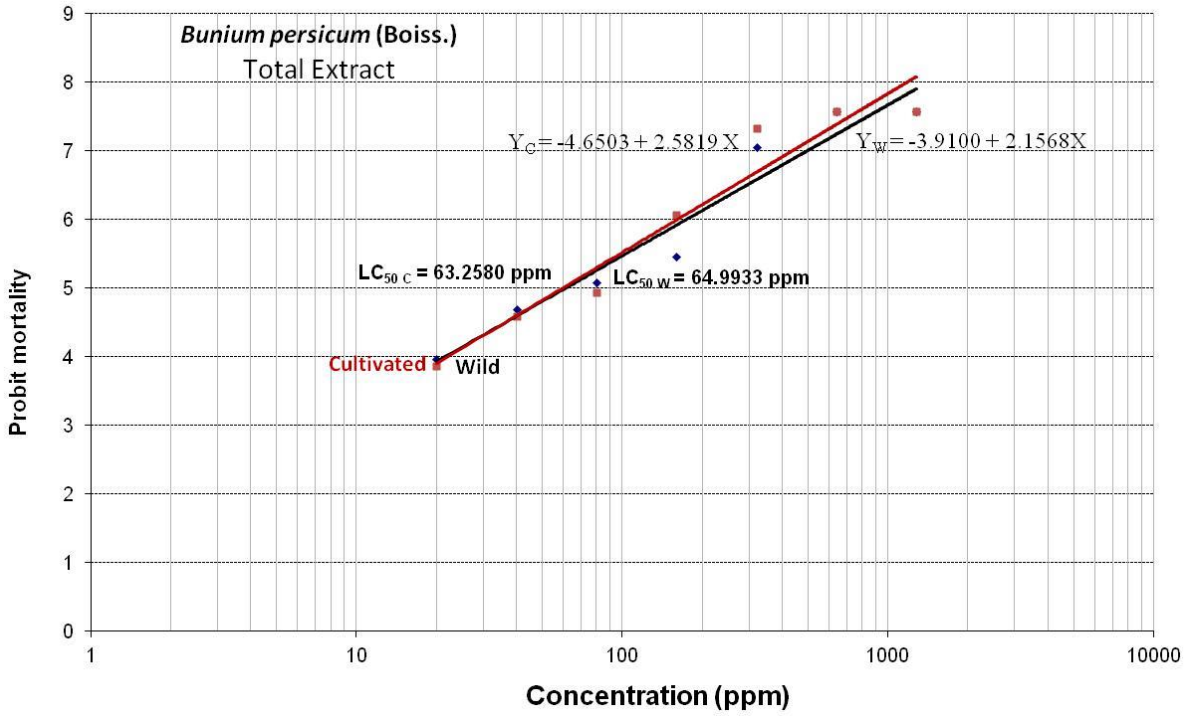

Fig. 2. Comparison of lethal concentrations (LC_{50}) of cultivated and wild types of *Bunium persicum* total extracts against against larvae of *Anopheles stephensi*

http://jad.tums.ac.ir
Published Online: March 18, 2018

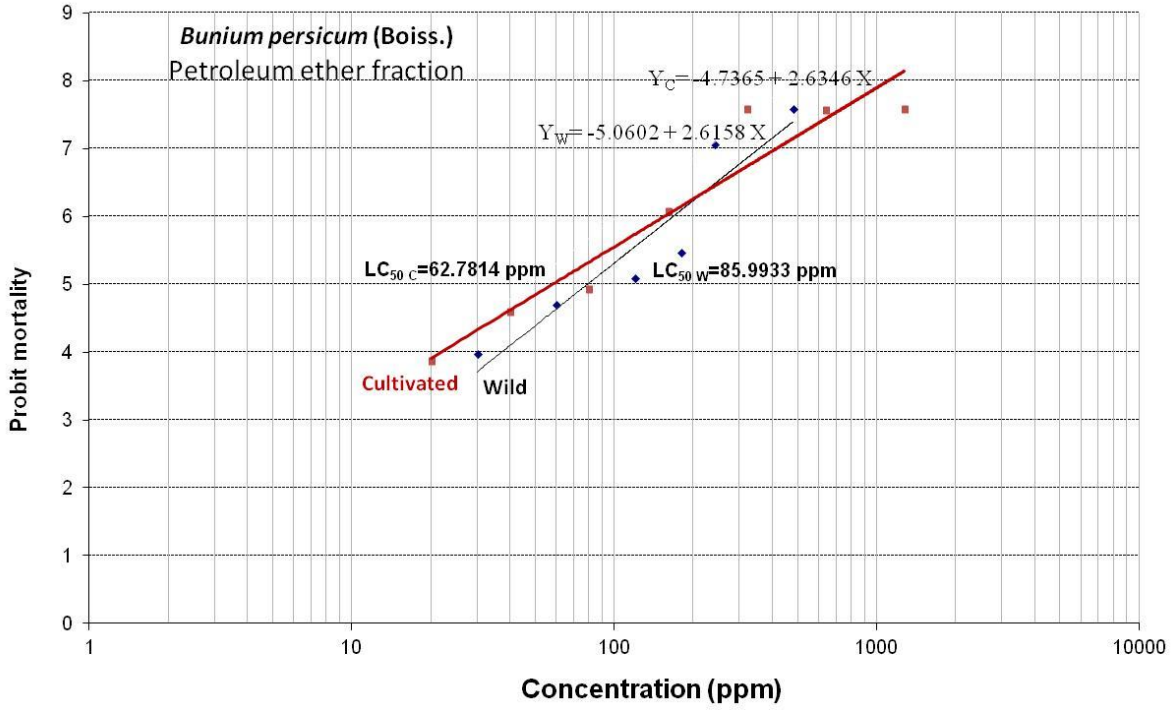

Fig. 3. Comparison of lethal concentrations (LC_{50}) of cultivated and wild types of *Bunium persicum* petroleum ether fraction against against larvae of *Anopheles stephensi*

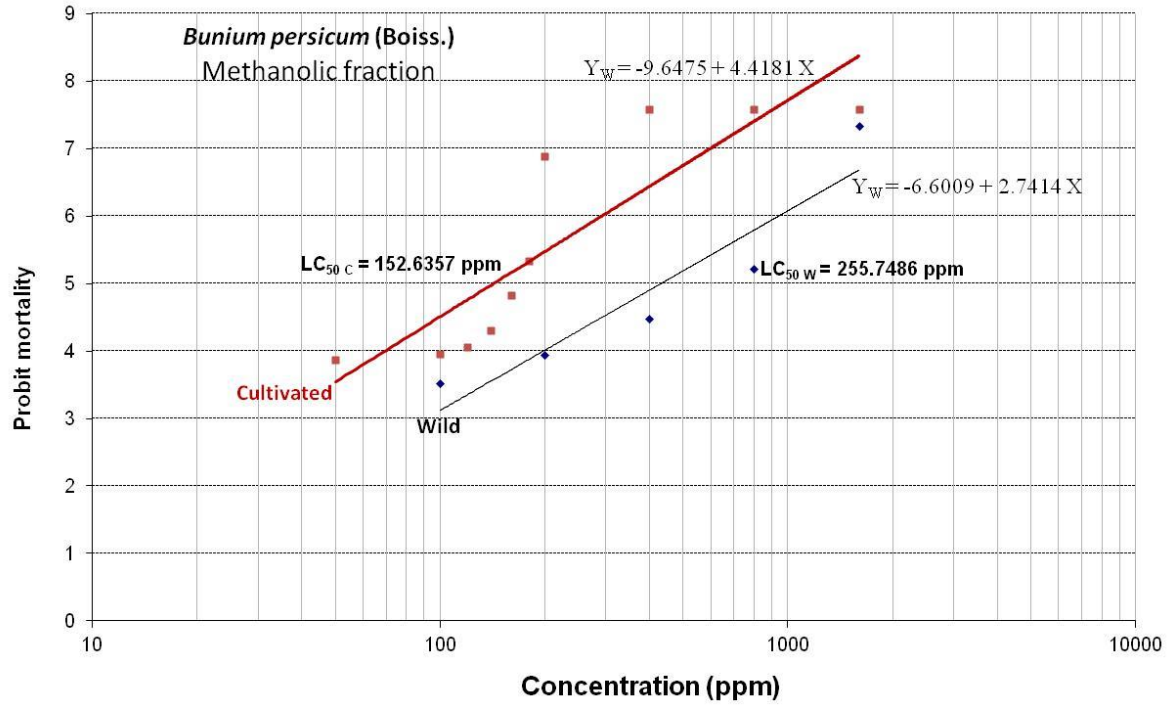

Fig. 4. Comparison of lethal concentrations (LC_{50}) of cultivated and wild types of *Bunium persicum* methanol fraction against larvae of *Anopheles stephensi*

http://jad.tums.ac.ir
Published Online: March 18, 2018

Discussion

Many researchers have already studied larvicidal potentials of plant derived compounds, extracts and essential oils against various insects, with the aim of finding active phytochemicals to replace synthetic insecticides. High cost of various commercial insecticides beside their food and environmental safety concerns, toxicity problems and increasing resistance rates have made their utilization undesirable (25, 26). Anti-larval activity of essential oil from a wild grown *B. persicum* against *An. stephensi* and *Culex pipiens* has been previously reported with LC_{50} values of 27.72 and 20.61ppm respectively (27). According to the results of our study, the essential oil, methanol total extract and petroleum ether fraction of both wild and cultivated samples had significant larvicidal activity against late third and early fourth instar larvae of *An. stephensi*. The larvicidal potential of γ-terpinene, cuminaldehyde and ρ-cymene, main constituents of both wild and cultivated type *B. persicum* fruits, against various insect larvae has been previously proved in several experiments. γ-Terpinene has shown potent larvicidal activity with LC_{50} value of 29.21 ppm against *Anopheles anthropophagus* (28) and 30.7 and 29.8ppm against *Aedes aegypti* and *Aedes albopictus* respectively (29). Zahran and Abdelgaleil (30) documented toxicity of cumin aldehyde on *Culex pipiens* larvae, which was more stronger than other tested monoterpenes in that experiment, with LC_{50} values of 38.9 and 21.4ppm for 24 and 48h exposures respectively. Anti-larval potential of ρ-cymene, the other main constituent, towards *A. aegypti* and *Ae. albopticus* has also been demonstrated (LC_{50}= 19.2 and 46.7ppm) (29). Higher lethal effect of the petroleum ether fraction in comparison to the methanol fraction, suggests higher potency of non-polar components than polar phenolics towards *An. stephensi* larvae. LC_{50} value of 85.9933 and 62.7814ppm for petroleum ether fraction from wild and cultivated types makes it suitable choice for further studies to isolate the active principles. Anti-larval activity of efficient samples from cultivated type was comparable to those from wild grown, so it can be concluded that cultivation of *B. persicum* has not affected chemical constituents' biosynthesis or concentration, which are responsible for larvicidal activity of the fruit.

Conclusion

The extract and fractions from *B. persicum* fruits, ie, petroleum ether fraction and total extract, beside the essential oil, have shown significant larvicidal effects on *An. stephensi*, and can be a great candidate to develop an eco-friendly insecticide to combat malaria vector breeding. More precise investigation will require revealing phytochemical composition of extract. Since cultivated type showed comparable results as wild grown, cultivation of *B. persicum*, as a solution to preserve its wild resources, is highly recommended. There are several studies on larvicidal activities of different plants against malaria vectors in Iran (16, 31-46). We recommend formulation of plant extract which have the lowest LC_{50} for field evaluation.

Acknowledgment

This project was supported by the deputy of research at Tehran University of Medical Sciences (TUMS) (grant number 26183) and was a part of Dr Z Talaeian, Pharmacology department.

References

1. WHO (2013) Larval Source Management: a supplementary measure for malaria vec-

tor control: an operational manual. Geneva: World Health Organization. p. 116.
2. Soleimani-Ahmadi M, Vatandoost H, Hanafi-Bojd AA, Zare M, Safari R, Mojahedi A, Poorahmad-Garbandi F (2013) Environmental characteristics of anopheline mosquito larval habitats in a malaria endemic area in Iran. Asian Pac J Trop Med. 6: 510–515.
3. Saxena R, Harshan V, Saxena A, Sukumaran P, Sharma M, Kumar ML (1993) Larvicidal and chemosterilant activity of *Annona squamosa* alkaloids against *Anopheles stephensi*. J Am Mosq Control Assoc. 9: 84–87.
4. Hadjiakhoondi A, Vatandoost H, Jamshidi A, Amiri EB (2003) Chemical constituents of efficacy of *Cymbopogon olivieri* (Boiss) Bar essential oil against malaria vector, *Anopheles stepensi*. Daru. 11: 125–128.
5. Khanavi M, Vatandoost H, Dehghani NK, Sanei-Dehkordi A, Sedaghat MM, Hadjiakhoondi A, Hadjiakhoondi F (2013) Larvicidal activities of some Iranian native plants against the main malaria vector, *Anopheles stephensi*. Acta Med Iran. 51 (3): 141–147.
6. Mozaffarian VA (2006) A Dictionary of Iranian Plant Names. Tehran: Farhang Moaser. pp. 198–515.
7. Amin G (2005) Popular Medicinal Plants of Iran. Vice chancellorship of Research, Tehran University of Medical Sciences, Tehran, Iran, p. 54.
8. Baser K, Oezek T, Abduganiev B, Abdullaev U, Aripov KN (1997) Composition of the essential oil of *Bunium persicum* (Boiss.) B. Fedtsch. from Tajikistan. J Essent Oil Res. 9: 597–598.
9. Foroumadi A, Asadipour A, Arabpour F, Amanzadeh Y (2002) Composition of the essential oil of *Bunium persicum* (Boiss.) B. Fedtsch. from Iran. J Essent Oil Res. 14: 161–162.
10. Jahansooz F, Sefidkon F, NajafiA, Ebrahimzadeh H, Najafi MS (2012) Comparison of essential oils of *Bunium persicum* (Boiss.) populations grown in Iran, Pakistan and India. J Essent Oil Bear Plants. 15: 761–765.
11. Sharififar F, Yassa N, Mozaffarian V (2010) Bioactivity of major components from the seeds of *Bunium persicum* (Boiss.) Fedtch. Pak J Pharm Sci. 23: 300–304.
12. Saeidnejad AH, Kafi M, Khazaei HR, Pessarakli M (2013) Effects of drought stress on quantitative and qualitative yield and antioxidative activity of *Bunium persicum*. Turk J Botany. 37: 930–939.
13. WHO, IUCN, WWF (1993) Guidelines on the conservation of medicinal plants. p. 120.
14. Hadjiakhoondi A, Vatandoost H, Abousa-ber H, Khanavai M, Abdi L (2008a) Chemical composition of the essential oil of *Tagetes minuta* L and its effect on *Anopheles stephensi* larvae in Iran. J Med Plants. 7(26): 33–39.
15. Vatandoost H, Khazani A, Rafinejad J, Khoobdel M, Kebriai-Zadeh A, Abai MR, Hanafi-Bojd AA, Akhavan AA, Abtahi SM, Rafi F (2008) Comparative efficacy of Neem and dimethyl phthalate (DMP) against malaria vector, *Anopheles stephensi* (Diptera: Culicidae). Asia Pac J Trop Med. 1(3): 1–6.
16. Shahi M, Hanafi-Bojd A, Iranshahi M, Vatandoost H, Hanafi-Bojd M (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: 185–188.
17. Khanavi M, Bagheri-Toulabi P, Abai MR, Sadat N, Hadjiakhoondi F, Hadjiakhoondi A, Vatandoost H (2011b) Larvicidal activity of marine algae, *Sargassums wartzii* and *Chondriada syphylla*, against malariavector, *Anopheles stephensi*. J

Vector Borne Dis. 48: 241–244.
18. Vatandoost H, Sanei-Dehkordi A, Sadeghi SMT, Davari B, Karimian F, Abai MR, Sedaghat MM (2012) Identification of chemical constituents and larvicidal activity of *Kelussia odoratissima* Mozaffarian essential oil against two mosquito vectors *Anopheles stephensi* and *Culex pipiens* (Diptera: Culicidae). Exp Parasitol. 132(4): 470–474.
19. Khanavi M, Fallah A, Vatandoost H, Sedaghat M, Abai MR, Hadjiakhoondi A (2012) Larvicidal activity of essential oil and methanol extract of *Nepeta menthoides* against malaria vector, *Anopheles stephensi*. Asia Pac J Trop Med. 5 (12): 962–965.
20. Torabi Pour H, Shayeghi M, Vatandoost H, Abai MR (2016) Study on larvicidal effects of essential oils of three Iranian native plants against larvae of *Anopheles stephensi* (Liston). Vector Biol J. 1: 2.
21. WHO (1981) Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. Geneva: WHO/VBC. p. 6.
22. Abbott W (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol. 18: 265–267.
23. Finney DJ (1971) Probit Analysis. 3d Ed. Cambridge University Press. Cambridge, p. 333.
24. Rustaie A, Keshvari R, Samadi N, Khalighi-Sigaroodi F, Shams Ardekani MR, Khanavi M (2016) Essential oil composition and antimicrobial activity of the oil and extracts of *Bunium persicum* (Boiss.) B. Fedtsch., wild and cultivated fruits. Pharm. 22: 296–301.
25. Ghosh A, Chowdhury N, Chandra G (2012) Plant extracts as potential mosquito larvicides. Indian J Med Res. 135(5): 581–598.
26. Shaalan EAS, Canyon D, Younes MWF, Abdel-Wahab H, Mansour AH (2005) A review of botanical phytochemicals with mosquitocidal potential. Environ Int. 31: 1149–1166.
27. Sanei-Dehkordi A, Vatandoost H, Abaei MR, Davari B, Sedaghat MM (2016) Chemical composition and larvicidal activity of *Bunium persicum* essential oil against two important mosquitoes vectors. J Essent Oil Bear Pl. 19: 349–357.
28. Zhu L, Tian Y (2011) Chemical composition and larvicidal effects of essential oil of *Blumea martiniana* against *Anopheles anthropophagus:* a malarial vector mosquito. Parasitol Res. 109: 1417–1422.
29. Cheng SS, Huang CG, Chen YJ, Yu JJ, Chen WJ, Chang ST (2009) Chemical compositions and larvicidal activities of leaf essential oils from two eucalyptus species. Bioresour Technol. 100: 452–456.
30. Zahran HEDM, Abdelgaleil SAM (2011) Insecticidal and developmental inhibitory properties of monoterpenes on *Culex pipiens* L. (Diptera: Culicidae). J Asi Pac Entomol. 14: 46–51.
31. Hajiakjoondi A, Aghel N, Xamanizadehnadgar N, Vatandoost H (2000) Chemical and biological study of *Mentha spicata* L. essential oil from Iran. Daru. 8(1 and 2): 19–21.
32. Hadjiakhoondi A, Vatandoost H, Khanavai M, Abai MR, Karami M (2005) Biochemical investigation of different extracts and larvicidal activity of *Tagetes minuta* L. on *Anopheles stephensi* larvae. Iran J Pharmaceutical Sci. 1: 81–84.
33. Hadjiakhoondi A, Sadeghipour-Roodsari HR, Vatandoost H, Khanavi M, Abaee M, Vosoughi M, Kazemi M (2006a) Fatty acid composition and toxicity of *Melia azedarach* L. fruits against malaria vector *Anopheles stephensi*. Iran J Pharmaceutical Sci. 2: 97–102.
34. Hadjiakhoondi A, Vatandoost H, Khanavi M, Sadeghipour-Roodsari HR, Vosoughi M, KazemiM,Abai MR (2006b) Chemical composition and toxicity of *Melia*

azedarach L. against malaria vector *Anophels stephensi*. Iran J Pharmaceutical Sci. 2: 97–102.
35. Hadjiakhoondi A, Vatandoost H, Khanavi M, Abousaber M, Abdi M (2008b) Chemical components and efficacy of fresh and dry *Tagetes minuta* L. against *Anopheles stephensi*. J Med Plants. 26 (7): 33–39.
36. Oshaghi MA, Ghalandari R, Vatandoost H, *Anopheles stephensi* on human volunteers. J Entomol Zool Stud. 3(2): 343–347.
37. Mozaffari E, AbaiMR, Khanavi M, Vatandoost H, SedaghatMM,Sanei-Dehkordi A, Moridnia A, Saber-Navaei M, Rafi F (2014) Chemical composition, larvicidal and repellent properties of *Cionuraerecta* (L.) Griseb. Against malaria vector, *Anopheles stephensi* Liston (Diptera: Culicidae) under laboratory conditions. J Arthropod Borne Dis. 8(2): 147–155.
38. Hosseini SA, Bazrafkan S, Vatandoost H, Abaei MR, Ahmadi MS, Tavassoli M, Shayeghi M (2014) The insecticidal effect of diatomaceous earth against adults and nymphs of *Blattella germanica*. Asian Pac J Trop Biomed. 4(Suppl 1): S228–S232.
39. Pirmohammadi M, Shayeghi M, Vatandoost H, Abaei MR, Mohammadi A, Bagheri A, Khoobdel M, Hasan Bakh-shi H, Pirmohammadi M, Tavassoli M (2016) Chemical composition and repellent activity of *Achillea vermiculata* and *Satureja hortensis* against *Anopheles stephensi.* J Arthropod Borne Dis. 10 (2): 201–210.
40. Shayeghi M, Kamalinejad M, Tourabi-Khaledi H, Abolhassani M, Hashemzadeh M (2003) Repellent effect of extracts and essential oil of *Citrus limon* (Rutaceae) and *Melissa officinalis* (Labiatae) against main malaria vector, *Anopheles stephensi* (Diptera: Culicidae) in Iran. Iran J Public Health. 32(4): 47–52.
41. Vatandoost H, Moinvaziri M (2004) Larvicidal activity of a neem tree extract (Neemarin) against mosquito larvae in the Islamic Republic of Iran. East Mediterr Health J. 10(4–5): 573–581.
42. Sedaghat M, Sanei-Dehkhordi AR, Khanavi M, Abai MR, Hadjiiakhondi A, Mohtarami F, Vatandoost H (2010) Phytochemistry and larvicidal activity of *Eucalyptus camaldulensis* against malaria vector, *Anopheles stephensi*. Asia Pac J Trop Med. 3(11): 841–845.
43. Sedaghat MM, SaneiDehkordi AR, Khanavi M, Abai, MR, Mohtarami F, Vatandoost H (2011) Chemical composition and larvicidal activity of essential oil of *Cupressus Arizona* E.L. Greene against malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). Pharmacognosy Res. 3(2): 135–139.
44. Khanavi M, Rajabi A, Behzad M, Hadjiakhoondi A, Vatandoost H, Abai MR (2011a) Larvicidal activity of *Centaurea bruguierana* ssp. *Belangerana* against *Anopheles stephensi* Larvae. Iran J Pharm Res. 10(4): 829–833.
45. Tavassoli M, ShayeghiM, Abai MR, Vatandoost H, Khoobdel M, Salari M, Ghaderi M, Rafi F (2011) Repellency effects of essential oils of Myrtle (*Myrtus communis* L.), Marigold (*Calendula officinalis* L.) compared with DEET against *Anopheles stephensi* on human volunteers. Iran J Arthropod Borne Dis. 5(2): 10–22.
46. Tavassoli M, Shayeghi M, Abai MR, Vatandoost H, Khoobdel M, Salari M, Ghaderi A, Rafi F (2015) Repellency effects of picaridin and DEET against *Anopheles stephensi* on human volunteers. J Entomol Zool Stud. 3(2): 343–347.