

Excito-Repellency Effects of *Salvia sclarea* L. (Lamiaceae) Extracts on Adult House Flies, *Musca domestica* L. (Diptera: Muscidae)

Mohammad Reza Fakoorziba¹,
 Mohammad Djaefar Moemenbellah-Fard¹,
 Kourosh Azizi¹,
 Heshmatollah Shekarpoor²,
 Hamzeh Alipoor²

¹Department of Medical Entomology, Research Centre for Health Sciences, School of Health, Shiraz University of Medical Sciences, P.O. Box: 71645-111, Shiraz, Iran;

²Department of Medical Entomology, School of Health, Shiraz University of Medical Sciences, P.O. Box: 71645-111, Shiraz, Iran

Correspondence:

Mohammad Djaefar Moemenbellah-Fard, Ph.D.,
 Department of Medical Entomology, Research Centre for Health Sciences, School of Health, Shiraz University of Medical Sciences, P.O. Box: 71645-111, Shiraz, Iran

Tel: +98-711-7251001

Fax: +98-711-7260225

Email: momenbf@yahoo.com
 momenbf@sums.ac.ir

Received: 1 October 2013

Revised: 15 November 2013

Accepted: 12 December 2013

Abstract

Background: Medicinal plant extracts such as those obtained from *Salvia* species have a wide variety of chemical compounds in their essential oils. The repellency of a number of essential oils including those from the labiates like *Salvia* against several insect species including the common house flies is reported.

Objective: The aim of this investigation was to find out the excito-repellency effects of *Salvia sclarea* L. (Lamiaceae) extracts against adult house flies, *Musca domestica* L. (Diptera: Muscidae).

Methods: Air-dried plant material from the aerial parts of *S. sclarea* was subjected to hydro-distillation in a Clevenger type glass apparatus model Soxhlet with acetone, benzene, petroleum ether, chloroform, and aqueous solvents. Only adult house flies were inserted into an exposure chamber and their behavior was monitored for feeding tendency, repellency rate and deterrence rate. Statistical analyses were carried out by one-way analysis of variance (ANOVA) with computation of the significance of differences in the outcome of various treatments.

Results: There were significant differences among most of the various extracts with their controls in the sequential effects of feeding ($P=0.04$), deterrent ($P=0.023$) and repellency ($P=0.01$) rates of house flies. The order of potency for various extracts with a concentration of 100 g/l was as follows: petroleum ether > benzene > water > acetone > chloroform.

Conclusion: It is thus conceivable to search for native means of combating house flies by fractionating the active ingredients in the Clary sage in the light of its excito-repellency effects.

Please cite this article as: Fakoorziba MR, Moemenbellah-Fard MD, Azizi K, Shekarpoor H, Alipoor H. Excito-Repellency Effects of *Salvia sclarea* L. (Lamiaceae) Extracts on Adult House Flies, *Musca domestica* L. (Diptera: Muscidae). J Health Sci Surveillance Sys. 2014;2(1):2-7.

Keywords: Medicinal plant; House fly; *Musca*; *Salvia* extracts; Repellency

Introduction

Plants and animals, particularly insects, exhibit an intimate integrated relationship on an evolutionary time scale. Medicinal plants are rich sources of pesticides discovery, especially insecticides, partly due to the long association between the coexistence of insects, humans and species of plants. Herbal extracts have long been

used for centuries as remedies in ailments ranging from headaches to microbial infections; yet, only in the last 2-3 decades have researchers begun to evaluate whether traditional plant-based remedies are effective and, if so, discover their mode of action. Of ~250 000 world's flowering plant species, <10% have so far been tested for their pharmaceutical properties but almost 25% of active medical compounds currently prescribed in some

western countries were isolated from higher plants.¹

Insect infestation and microbial contamination naturally account for a wide variety of diseases in human and his domesticated animals.²⁻⁶ They cause significant economic losses as well as health problems. The use of synthetic insecticides against pest insects has been a major part of an integrated pest management strategy.⁷ Insect resistance, environmental pollution, ozone depletion, mammalian toxicity and other unfavorable effects have been reported.⁸ Alternative pest control methods should thus be looked for. Of the known alternative control strategies, the use of plant-derived essential oil products instead of the conventional chemical insecticides has attracted much attention.^{9,10}

The flowering plant family of Lamiaceae or Labiatae (Order: Tubiflorae) is very important since it is a highly diverse and rich source of secondary metabolites including essential oils which are volatile natural compounds with a strong odor. The medicinal plant genus, *Salvia* (sage), has about 800 species worldwide.¹¹ This genus is represented by 58 species of sage plants, of which 17 are native in Iran.¹² Many *Salvia* species have a wide variety of chemical compounds in their essential oils comprising mono- and sesquiterpenes, carbonylic and phenolic compounds, acids/esters, and hydrocarbons.¹³ The increasingly acceptable use of essential oils is due to their low toxicity to warm-blooded animals, high volatility and also toxicity to insect pests.¹⁴ These botanical compounds are effective, easily-biodegradable, environmentally-friendly, and often cheaper than the synthetic ones. The aerial parts of the herbaceous plant, *Salvia sclarea* (also known as Clary sage), have been used for different purposes in rural areas in many parts of Iran.¹⁵

The common house fly, *Musca domestica* L., is a famous cosmopolitan pest and an important mechanical vector of various pathogens. It is a major synanthropic pest near human dwellings. Over 100 different pathogens are associated with the house fly and they could cause disease in humans and animals, including anthrax, typhoid, trachoma, cholera, leprosy, tuberculosis, Q-fever, bacillary dysentery and infantile diarrhea.¹⁶ These pathogens are picked up by flies from garbage, sewage and other sources of filth and carried on their mouthparts, through vomits, feces and contaminated external body surfaces to human and animal food.¹⁷

The repellency of a number of essential oils, including those from the labiates like *Salvia*, against several insect species is known. These have previously been reported in mosquitoes such as *Aedes albopictus*,^{9,18} *Ae. aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus*,^{19,20} and *C. pipiens*,^{21,22} as well as the moth *Spodoptera littoralis*,²³ the aphid *Lipaphis pseudobrassicae*,²⁴ and the house fly *M. domestica*.^{25,26} The main aim of this investigation was to find out the excito-repellency effects of *Salvia sclarea* extracts against adult house flies, *M. domestica*, in Iran.

Materials and Methods

Plant identification and Preparation

In May 2012, Clary sage, *S. sclarea*, during blossoming stage were collected from the foothills of Cheram district (County of Dehdasht), 160 km to the west of the capital city of Yasuj in the southwest mountainous Iranian province of Kohgiluyeh and Boyer-Ahmad (30°45'N, 50°45'E, at an altitude of about 780 m above the sea level) (Figure 1).

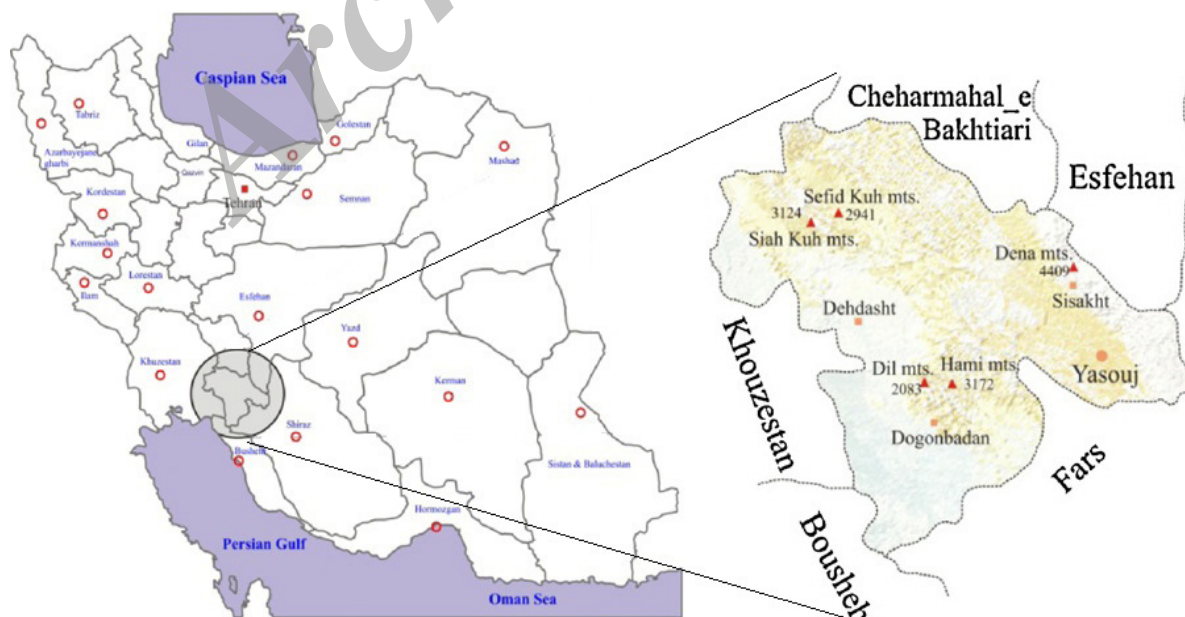


Figure 1: Map of the study area in Kohgiluyeh and Boyer-Ahmad province, Iran, shows the location of Dehdasht County in the mid-western part of the province (with slight modifications by courtesy of Mosadegh¹⁵).

This province is covered mostly with oak forests. A voucher specimen was deposited in the herbarium of Eram botanical garden of Shiraz University, Shiraz, Iran. Aerial parts (leaves and flowers) of the plants were dried in the shade at ambient temperature. Morphological features of *S. sclarea* were determined using Flora Iranica key²⁷ and a Stereomicroscope. Its broad mint-like triangularly-shaped mutual leaves on the cross-sectional squared stem are representative (Figure 2). The leaf margin is serrated. The height of this plant amounts to 60-100 cm with bi-lobed lilac flowers on the extremities of the shoot.

Isolation of the Extracts

The plants were subjected to automated grinding in an electric stainless steel grinder. Air-dried plant material (50g) from the aerial parts of *S. sclarea* was subjected to hydro-distillation for 3 h in a Clevenger type glass apparatus model Soxhlet with acetone, benzene, petroleum ether, chloroform and aqueous solvents. After extraction, the resultant samples were dried over anhydrous magnesium sulfate to delete water and kept in amber vial at 4 degree centigrade prior to the biological

assays. The sample yielded 4% of solvent extracts and 18% of aqueous extract on a dry weight basis of 100 g.

House flies Rearing

Since only adult flies were to be used in this investigation, a representative sample of puparia of *Musca domestica* together with some cow pats were collected from animal pens and dens around Cheram district. These were brought to the lab and kept under optimal conditions with a temperature of $30\pm 2^{\circ}\text{C}$ and relative humidity of $70\pm 5\%$. The emerging adults from puparia were transferred to predesigned cages with water and sugar cubes *ad libitum*.

Exposure Chamber for House Flies

Following earlier tests on malaria vector mosquitoes,²⁸ an exposure chamber was prepared with dimensions of $70\times 70\times 70$ cm and a small (radius=5cm) entry hole equipped with a short (15 cm long) netting sleeve on the outside for leading the house flies into the system and a window trap cage on the opposite side measuring $30\times 30\times 30$ cm, all made up of fiberglass (Figure 3).



Figure 2: The Clary sage, *Salvia sclarea*, grows naturally in its native habitat of Cheram district, Iran.

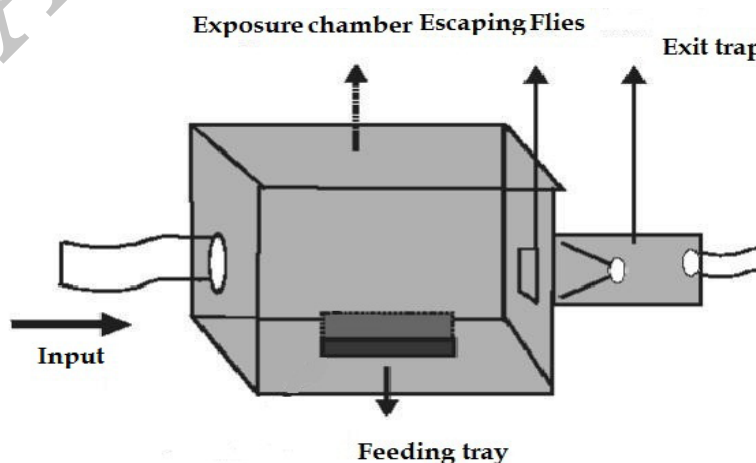


Figure 3: Exposure chamber shows the arrangement of different parts in the excito-repellency of house flies under various conditions.

To prevent the loss of extract odor from the exposure chamber, its sides were equipped with glass walls to make the interior part visible. After preparation of each extract, a small piece of white polyester net (mesh size=2 mm, area=0.1 m²) was impregnated by standard dipping with the herbal extract (the control was left untreated) and placed over the Petri dish containing sugar cubes with water inside it on the centre stage of the exposure chamber. The house flies (25 in each trial) were starved 48 h prior to the start of each test. They were then released into the chamber following our earlier method.²⁸ Each trial was followed up for one hour and the behavior of the house flies was investigated. The data were recorded in specific log books.

Repellent Activity Bioassay

This study was done with two distinct doses (0.5 and 1.0 g/10 ml solvent) each replicated four times. Five extracts (aqueous, acetone, petroleum ether, benzene, chloroform) were separately tested. To fulfill the aims of this research, the tests were performed to compare the different extracts at different doses and repellency versus deterrent rates among house flies.

Data analysis

Statistical analyses were carried out using one-way analysis of variance (ANOVA) with computation of the significance of differences in the outcome of various treatments. These were determined by using arcsine-transformed data ($Y = \text{Arc Sine } \sqrt{P}$) in SPSS. A *P*-value of <0.05 was considered statistically significant.

Results

The present results indicated that there were significant differences among most of the various extracts with their controls in the sequential effects of feeding ($P=0.04$), deterrent ($P=0.023$) and repellency ($P=0.01$) rates of house flies. It was shown that there was a significant difference between the repellency rate (entry into the exit trap) of house flies using aqueous extract in relation to the other extracts ($P=0.01$). There was also a significant difference between the two concentrations used ($P=0.05$), so that the entry index of the house flies at 100 g/l herbal extract was more than at 50 g/l, indicating that the higher concentration gave a larger value of repellency (Table 1).

The excito-repellency indices of house flies in the top entry cage under both concentrations indicated that the petroleum ether and benzene extracts were more effective in the deterrence of house flies than the other extracts (Table 2). The order of potency for various extracts with a concentration of 100 g/l was as follows: petroleum > benzene > water > acetone > chloroform (Table 2).

Discussion

There was an overall excito-repellency effect of *S. sclarea* extracts against adult house flies, *M. domestica*, in the southern parts of Iran. Although in comparison to the other extracts, the aqueous extract clearly revealed a better repellent activity against house flies, the petroleum and benzene extracts exhibited a marked deterrent activity against these insects. Even though the number of reports on aromatic plants used in different countries for their

Table 1: The excito-repellency index (shown as %) of the common housefly, *Musca domestica*, on exposure to different concentrations in various extracts of Clary sage, *Salvia sclarea*

Extract type	100 g/l H ₂ O			50 g/l H ₂ O		
	Tendency to feed	Entry to exit trap	Deterrence in top entry cage	Tendency to feed	Entry to exit trap	Deterrence in top entry cage
Aqueous	12	48	40	25	37	38
Acetone	27	30	43	41	23	36
Petroleum	16	32	52	31	19	51
Benzene	19	32	49	36	20	44
Chloroform	34	34	32	46	24	30
Control	82	4	14	86	3	11

Table 2: The average rates of houseflies' tendency to feed, repellency rate and deterrent rate in relation to the extract types and tested concentrations (Concn.)

Rates (Concn.)/ Extracts	Aqueous	Acetone	Petroleum	Benzene	Chloroform	Control
Feeding tendency (100 g)	3	6.5	4	4.7	8.2	19.3
(50 g)	6.2	10.2	7.7	7.7	11.5	18.9
Repellency rate (100 g)	12	7.5	8	8	8.2	1.2
(50 g)	9.2	5	4.7	5	6	1.3
Deterrent rate (100 g)	10.7	10.5	13	12.2	8	4.4
(50 g)	10.5	9.7	12.2	12.2	7.5	4.2

house fly-repellent activities continues to increase, to the best of our knowledge, no studies have been published on the repellent activity of *Salvia sclarea* against the common house fly, *Musca domestica* adults in Iran.

This research focused solely on excito-repellent activity with instant effectiveness after the exposure, of which the highest was within one hour of exposure to petroleum ether extract. Effectiveness is generally likely to be the most real factor in the search for noble herbal compounds. Apart from the direct impact of these compounds on repellency of house flies, secondary impacts may culminate in reduced fecundity and eggs delivery.²⁶ The essential oils (like β -pinene in balsamic sage) of some *Salvia* species have revealed significant insecticidal activities which vary in different insects depending on the developmental stage and the species of the insect and the plant origin of the essential oil.¹⁰ The Clary sage is native to Iran. This plant is not only traditionally used in folk remedies but also it was found to have remarkable excito-repellent activity against house flies.

Conclusions

As a result of the strong excito-repellency effects of various extracts, particularly the petroleum and benzene extracts, of *S. sclarea* on adult house flies, it is thus conceivable to search for native means of combating these insects by fractionating the active ingredients in the Clary sage.

Acknowledgements

The authors deeply appreciate the valuable help provided by criticism of anonymous referees on preprint drafts. This article is a part of MSc thesis in Medical Entomology by the fourth author (H Shekarpoor) contract number 90-01-42-3286 dated 18th. September 2012 carried out in the School of Health (SUMS). The authors wish to thank the deputy chancellor for research and technology at SUMS due to financial support and authentication of this research plan. We would also like to extend our gratitude to Dr F. Naghibi and colleagues for their permission to reproduce the area map in Figure 1 with minor modifications. Thanks are also due to Ms. T. Dabaghmanesh for assistance with figures.

Conflict of Interest: None declared

References

- 1 Anthony JP, Fyfe L, Smith H. Plant active components-a resource for antiparasitic agents? *Trends Parasitol* 2005; 21(10): 462-468.
- 2 Azizi K, Abedi F, Moemenbellah-Fard MD. Identification and frequency distribution of *Leishmania*

(*L.*) *major* infections in sand flies from a new endemic ZCL focus in southeast Iran. *Parasitol Res* 2012; 111: 1821-1826.

- 3 Fakoorziba MR, Eghbal F, Hassanzadeh J, Moemenbellah-Fard MD. Cockroaches (*Periplaneta americana* and *Blattella germanica*) as potential vectors of the pathogenic bacteria found in nosocomial infections. *Ann Trop Med Parasitol* 2010; 104(6): 521-528.
- 4 Fakoorziba MR, Golmohammadi P, Moradzadeh R, Moemenbellah-Fard MD, Azizi K et al. Reverse transcription PCR-based detection of Crimean-Congo hemorrhagic fever virus isolated from ticks of domestic ruminants in Kurdistan province of Iran. *Vector-Borne Zoo Dis* 2012; 12(9): 794-799.
- 5 Moemenbellah-Fard MD, Benafshi O, Rafinejad J, Ashraf H. Tick-borne relapsing fever in a new highland endemic focus of western Iran. *Ann Trop Med Parasitol* 2009; 103(6): 529-537.
- 6 Moemenbellah-Fard MD, Saleh V, Banafshi O, Dabaghmanesh T. Malaria elimination trend from a hypo-endemic unstable active focus in southern Iran: predisposing climatic factors. *Pathog Glob Health* 2012; 106: 358-365.
- 7 Moemenbellah-Fard MD, Fakoorziba MR, Azizi K, Mohebbi-Nodezh M. Carbamate insecticide resistance monitoring of adult male German cockroaches, *Blattella germanica* (L.), in southern Iran. *J Health Sci Surveillance Syst* 2013; 1: 41-47.
- 8 Skinjar MM, Nemet NT. Antimicrobial effects of species and herbs essential oils. *APTEFF* 2009; 40(1): 195-209.
- 9 Conti B, Benelli G, Leonardi M, Afifi FU, Cervelli C, Profeti R, et al. Repellent effect of *Salvia dorisiana*, *S. longifolia*, and *S. sclarea* (Lamiaceae) essential oils against the mosquito *Aedes albopictus* Skuse (Diptera: Culicidae). *Parasitol Res* 2012; 111: 291-299.
- 10 Ulukanli Z, Karaborklu S, Cenet M, Sagdic O, Ozturk I, Balcilar M. Essential oil composition, insecticidal and antibacterial activities of *Salvia tomentosa* Miller. *Med Chem Res* 2013; 22: 832-840.
- 11 Jassbi AR, Asadollahi M, Masroor M, Schuman MC, Mehdizadeh Z, Soleimani M, et al. Chemical classification of the essential oils of the Iranian *Salvia* species in comparison with their botanical taxonomy. *Chemist Biodiversit* 2012; 9: 1254-1271.
- 12 Ebrahimabadi AH, Mazoochi A, Jookar Kashi F, Djafari-Bidgoli Z, Batooli H. Essential oil composition and antioxidant and antimicrobial properties of the aerial parts of *Salvia eremophila* Boiss. From Iran. *Food Chem Toxicol* 2010; 48: 1371-1376.
- 13 Tenore GC, Ciampaglia R, Arnold NA, Piozzi F, Napolitano F, Rigano D, et al. Antimicrobial and antioxidant properties of the essential oil of *Salvia lanigera* from Cyprus. *Food Chem Toxicol* 2011; 49: 238-243.
- 14 Taarit MB, Msaada K, Hosni K, Chahed T, Marzouk B. Essential oil composition of *Salvia verbenaca* L.

- growing wild in Tunisia. *J Food Biochem* 2010; 34: 142-151.
- 15 Mosaddegh M, Naghibi F, Moazzeni H, Pirani A, Esmacilli S. Ethnobotanical survey of herbal remedies traditionally used in Kohghiluyeh va Boyer Ahmad province of Iran. *J Ethnopharmacol* 2012; 141: 80-95.
- 16 Service M. *Medical Entomology for Students*. Fifth Edit. Cambridge University Press, Cambridge, UK, 2012.
- 17 Zurek L, Denning SS, Schal C, Watson DW. Vector competence of *Musca domestica* (Diptera: Muscidae) for *Yersinia pseudotuberculosis*. *J Med Entomol* 2001; 38: 333-335.
- 18 Mathew J, Thoppil JE. Chemical composition and mosquito larvicidal activities of *Salvia* essential oils. *Pharm Biol* 2011; 49(5): 456-463.
- 19 Amer A, Mehlhorn H. Larvicidal effects of various essential oils against *Aedes*, *Anopheles*, and *Culex* larvae (Diptera, Culicidae). *Parasitol Res* 2006; 99: 466-472.
- 20 Amer A, Mehlhorn H. Repellency effect of forty-one essential oils against *Aedes*, *Anopheles* and *Culex* mosquitoes. *Parasitol Res* 2006; 99: 478-490.
- 21 Cetin H, Cinbilgel I, Yanikoglu A, Gokceoglu M. Larvicidal activity of some Labiatae (Lamiaceae) plant extracts from Turkey. *Phytother Res* 2006; 20: 1088-1090.
- 22 Koliopoulos G, Danae Pitarokili D, Kioulos E, Michaelakis A, Tzakou O. Chemical composition and larvicidal evaluation of *Mentha*, *Salvia*, and *Melissa* essential oils against the West Nile virus mosquito *Culex pipiens*. *Parasitol Res* 2010; 107: 327-335.
- 23 Pavela R. Insecticidal activity of some essential oils against larvae of *Spodoptera littoralis*. *Fitoterapia* 2005; 76: 691-696.
- 24 Sampson BJ, Tabanca N, Kirimer N, Demirci B, Baser KHC, Khan IA, et al. Insecticidal activity of 23 essential oils and their major compounds against adult *Lipaphis pseudobrassicae* (Davis) (Aphididae: Homoptera). *Pest Manage Sci* 2005; 61: 1122-1128.
- 25 Maganga ME, Gries G, Gries R. Repellency of various oils and pine oil constituents to house flies (Diptera: Muscidae). *Environ Entomol* 1996; 25: 1182-1187.
- 26 Pavela R. Insecticidal properties of several essential oils on the house fly (*Musca domestica* L.). *Phytother Res* 2008; 22: 274-278.
- 27 Rechinger KH (Ed.), *Flora Iranica*, No. 1-164. Akademische Druck-u, Verlagsanstalt, Graz-Austria, 1965-2008.
- 28 Alipour H, Ladonni H, Abai MR, Moemenbellah-Fard MD, Fakoorziba MR. Laboratory efficacy tests of pyrethroid-treated bed nets on the malaria vector mosquito, *Anopheles stephensi*, in a baited excito-repellency chamber. *Pakistan J Biol Sciences* 2006; 9(10): 1877-1883.