The cytotoxicity of malathion and essential oil of *Nepeta crispa* (lamiales: lamiaceae) against vertebrate and invertebrate cell lines

Amirhossein Zahirnia¹, Mitra Boroomand¹, Hassan Nasirian¹,², Sara Soleimani-Asl¹, Aref Salehzadeh¹, Dara Dastan⁴

¹Department of Medical Entomology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran, ²Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, ³Department of Anatomy, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran, ⁴Medicinal Plants and Natural Products Research Center, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran

*Corresponding author: Hassan Nasirian, Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

Key words: *Nepeta crispa*, cytotoxicity, essential oil, invertebrate cell line, malathion, vertebrate cell line

Received: 07/04/2019 - Accepted: 19/07/2019 - Published: 06/08/2019

Abstract

**Introduction:** Pesticides are used as essential tools to control vector-borne diseases and agricultural pests and maintain quality and quantity crop production. Scientists attempt to use derived plant natural products due to environmental safety and low mammalian toxicity. Therefore, the cytotoxicity of malathion and *Nepeta crispa* essential oil against vertebrate L929 and invertebrate Sf9 cell lines were investigated. **Methods:** About \(2 \times 10^3\) cells were placed into the wells of a 96-well plate experiments. Then appropriate concentrations of malathion and *N. crispa* essential oil added to the wells. The cells were allowed to grow for 3-5 days and estimated the cell numbers. Control cell wells contained only cells with DMSO. All treatments and controls repeated at least four replicates. **Results:** About \(2 \times 10^3\) cells were placed into the wells of a 96-well plate experiments. Then appropriate concentrations of malathion and *N. crispa* essential oil added to the wells. The cells were allowed to grow for 3-5 days and estimated the cell numbers. Control cell wells contained only cells with DMSO. All treatments and controls repeated at least four replicates. **Conclusion:** Plant essential oil not only had no negative effects but also had boosting effects on the L929 cell viability. *Nepeta crispa* essential oil had negative effects on the Sf9 cell viability with the differences that derived plant natural products containing environmentally friendly and readily biodegradable derivatives, hydrolyzing rapidly in nature and nearly having no destructive effects on mammals and environment.

Pan African Medical Journal. 2019;33:285. doi:10.11604/pamj.2019.33.285.18776

This article is available online at: http://www.panafrican-med-journal.com/content/article/33/285/full/

© Amirhossein Zahirnia et al. The Pan African Medical Journal - ISSN 1937-8688. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Introduction

At present, pesticides with increasingly global marketing are used as an essential tool to control of vector-borne diseases and agricultural pests and maintain quality and quantity crop production. They also play a key role in the prevention and control of infectious diseases such as malaria, dengue, and filariasis [1]. Despite their importance for public health, there is concern about pesticides potential side effects. Exposure to insecticides has severe effects on reproductive performance in vertebrates. It may cause increasing rates of cryptorchidism and hypospadias male genital congenital anomalies in human populations. Various types of insecticide exposure may be a risk factor for cancers such as leukemia or lymphoma. The other undesirable effects of pesticides are may be direct toxicity to users, environmental pollution, ozone depletion, pesticide residues, and toxicity to non-target organisms. There is the link between humans who are occupationally in contact with insecticides and muscle fatigue, neurological diseases and psychotic disorders [2-6]. Insecticide resistance in arthropods of vectors of diseases and agricultural pests to synthetic insecticides has been considered as a substantial problem of the vector and pest management programs [7-14]. With great concern about environmental problems and human health of synthetic pesticides, scientists have attempted to use natural products derived from plants that are considered as an appropriate option for vector and pest management due to containing environmentally friendly and readily biodegradable derivatives [6, 15, 16]. Essential oils are fugitive oil compounds that are secondary metabolites of plants. Essential oils hydrolyze rapidly in nature and have less destructive effects on the environment because of their environmental safety and low mammalian toxicity [6, 17]. Many studies have been done on insecticides effects of essential oils. Botanical insecticide compounds inhibit the activity of enzymes that are required to protect insects from oxidative stress, resistance to insecticides and other damage to insects [16, 18-20].

Many Iranian wild flowers have medicinal and insecticidal properties. Several studies have been conducted on Lamiaceae family due to their toxic effects on various insect species. The family of Lamiaceae have high diversity and distribution in flora of Iran containing 46 genera and 420 species and sub-species [21]. Nepeta is a genus of Lamiaceae family that has been spread in many parts of the world, including Asia, Europe and North Africa. About of 280 annual or perennial worldwide species of Nepeta genus, there are 79 species present in Iran with of 38 species of native to the country [22, 23]. In traditional medicine, Nepeta species are widely used as antispasmodic, anti-asthma, sedative, treatment of various digestive, neurological and respiratory diseases [24-26]. It's anti-viral, anti-inflammatory and antioxidant properties also have been reported [27-29]. Many studies have shown the insecticide properties of α-pinene, 1,8-cineole and α-terpineol as the main components of Nepeta genus species [30-32]. Nepeta crispa Willd. (Lamiales: Lamiaceae) is one of the most aromatic plants in Iran which is popular in Iranian traditional medicine, especially people of Hamadan province. Nepeta crispa is autochthonous of Hamadan climate having insecticidal activity, and antimicrobial and antifungal properties [33, 34]. Many studies have been conducted on the cytotoxic and insecticidal properties of N. crispa, but a simultaneous comparative study about the cytotoxicity of N. crispa against the cell lines of invertebrates and vertebrates would be of particular importance. Therefore in this study, we compared the cytotoxicity of N. crispa essential oil with malathion against vertebrate and invertebrate cell lines.

Methods

Providing of insecticide and plant materials: liquid technical grade of malathion (95%) were purchased from India's Haramba Company. The aerial parts (foliage) of Nepeta crispa during their flowering stage were collected from Avicenna Medicinal Herbs Research Center, Hamadan Province of Iran in June 2017. The plant was confirmed by a voucher specimen (no. 72) in the Department of Pharmacognosy, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran.

Plant essential oil isolation: a total of 1000 g powder of shade-dried aerial parts of N. crispa were subjected to hydrodistillation using a clevenger-type apparatus for 4h. The essential oil was dehydrated over anhydrous sodium sulphate and transferred into amber-colored vials to store in a refrigerator at 4ºC for further work.

Providing and maintaining cell lines

Invertebrate cell line: Sf9 cell line which was derived from the ovary of Spodoptera frugiperda (Smith) (Lepidoptera: Noctuidae) was provided from National Cell Bank of Pasture Institute of Iran. Sf9 cell line is routinely cultured and maintained at 27ºC in 5ml of Grace's insect cell culture medium in 25cm² culture flasks, enriched with 10%
fetal bovine serum at Pasture Institute of Iran. The cell doubling time for this cell was found to be 18-24h under optimum conditions. Cells were sub-cultured every 3 days [35].

Vertebrate cell line: L929 vertebrate cell line which was derived from mouse fibroblast cells used for this study. It was provided from National Cell Bank of Pasture Institute of Iran. It was maintained at 37°C in 3ml of DMEM Media (Gibco®) in 25cm² culture flasks, enriched with 10% fetal bovine serum and buffered with 4% sodium bicarbonate in an atmosphere of 5% carbon dioxide. The doubling time for cultures was approximately 24h and the cell was sub-cultured every 6 day [35].

Cell bioassay: technical grade of malathion (95%) was dissolved with ethanol 96% to prepare the concentrations of 10⁻¹⁰, 10⁻⁹, 10⁻⁸, 10⁻⁷, 10⁻⁶, 10⁻⁵, 10⁻⁴ and 10⁻³ μg·μL⁻¹ containing 0.000095, 0.00095, 0.0095, 0.095, 0.95, 9.5 and 95 μg·μL⁻¹, respectively. Herbal essential oil (0.1 mg) of Nepeta crispa was dissolved with 1ml of DMSO (dimethyl sulfoxide) due to hydrophobic properties and then diluted with sterilized distilled water to prepare the concentrations of 10⁻¹⁰, 10⁻⁹, 10⁻⁸, 10⁻⁷, 10⁻⁶, 10⁻⁵, 10⁻⁴ and 10⁻³ ng·μL⁻¹ containing 0.00001, 0.0001, 0.001, 0.01, 0.1, 1, 10 and 100 ng·μL⁻¹, respectively. To consider the cytotoxicity of malathion and N. crispa essential oil against L929 and SF9 cell lines, about 2×10³ cells per 100 μl of culture medium were placed into the wells of a 96-well plate experiments of treatments and then appropriate concentrations of N. crispa essential oil and malathion added to the wells. We allowed the cells to grow for 3-5 days, and estimated the number of cells as described. Control cell wells contained only cells with 1 μl·mL⁻¹ of DMSO. All treatment and control experiments repeated at least four replicates.

Estimation of cell number: the base method for cell estimation is the Mossman method, which uses 3-(4,5-dimethylthiazol-2-yl)-2,5-difenyltetrazolium bromide (MTT, tetrazolium, compound). MTT is a quantitative coloring for living cells and cell proliferation, and it's a known method for invitro cytotoxicity which measures the active metabolism of the cells. In this coloring solution, dehydrogenase enzyme reduced the MTT and produced blue formazan. The wells of 96-well plates containing L929 and SF9 cell lines were incubated with 10 μ MTT for 3h at 36°C and 27°C, respectively. After the blue formazan and cells settled out and the supernatant was removed, 100 μ of DMSO was added to any well of 96-well plate, shaked for 15 minutes and then the absorbance of the solution read at 492 nm using ELISA reader [36].

Statistical analysis: IBM SPSS statistics data editor version 24 was used for any statistical analyses. Wilcoxon signed ranks-test was used for comparing cytotoxicity of malathion and essential oil of Nepeta crispa between control and treatments, and treatments against L929 and SF9 cell lines. P < 0.05 was considered significant. The trends of malathion and N. crispa essential oil cytotoxicity against L929 and SF9 cell lines was estimated by Microsoft Office Excel 2013. The trends were drawn by clicking on graph line distribution and selecting "add trendline" option using Nasirian and Salehzadeh (2017a, b; 2019a, b) style [11, 37-40].

Results

Malathion cytotoxicity: Table 1 and (Figure 1 A,B) show cytotoxicity of malathion and essential oil of Nepeta crispa (Figure 1 C,D) against L929 and SF9 cell lines. Figure 2 also show the cytotoxicity trends of malathion (μg·μL⁻¹) and essential oil of Nepeta crispa (ng·μL⁻¹) against L929 and SF9 cell lines. The cytotoxicity of malathion against L929 and SF9 cell lines were gradually increased with a relatively low decreasing slope in accordance with malathion concentrations from 10⁻¹⁰ to 10⁻³ μg·μL⁻¹ (Figure 1 A,B, Figure 2). Table 2 also shows the results of descriptive analysis and Wilcoxon signed-ranks test between control and treatments and between treatments of cytotoxicity of malathion and essential oil of N. crispa against L929 and SF9 cell lines. There was a significant difference between treatments of 10⁻⁵ to 10⁻³ malathion concentrations against L929 cell lines with control (P < 0.05) (Figure 1 A and Table 2). There was also a significant difference between treatments of 10⁻⁸ to 10⁻³ malathion concentrations against SF9 cell lines with control (P < 0.05) (Figure 1 B and Table 2). Although Wilcoxon signed-ranks test did not show a significant difference between treatments of 10⁻⁸ and 10⁻⁷ malathion concentrations against SF9 cell lines with control (P > 0.05) (Table 2). While there was a significant difference at P < 0.001 level between treatments of 10⁻⁸ and 10⁻⁷ malathion concentrations against SF9 cell lines with control (Figure 1 B).

Nepeta crispa essential oil cytotoxicity: the cytotoxicity of Nepeta crispa essential oil against L929 cell lines were gradually decreased with a moderately increasing slope in accordance with N. crispa essential oil concentrations from 10⁻¹⁰ to 10⁻³ μg·μL⁻¹ (Figure 1 C, Figure 2). While the cytotoxicity of N. crispa essential oil against SF9 cell lines were strongly increased with an intensive increasing slope in accordance with N. crispa essential oil...
concentrations from $10^{-10}$ to $10^{-3} \mu g$\text{L}$^{-1}$ (Figure 1 D, Figure 2). Wilcoxon signed-ranks test revealed a significant difference between treatments of $10^{-10}$ to $10^{-4}$ \textit{N. crispa} essential oil concentrations against L929 cell lines with control ($P < 0.05$) (Figure 1 C and Table 2).

Although Wilcoxon signed-ranks test did not show a significant difference between treatments of $10^{-3}$ \textit{N. crispa} essential oil concentration against L929 cell lines with control ($P = 0.068$) (Table 2). While there was a significant difference at $P < 0.001$ level between treatments of $10^{-3}$ \textit{N. crispa} essential oil concentration against SF9 cell lines with control (Figure 1 C). Wilcoxon signed-ranks test did not show a significant difference between treatments of $10^{-10}$ to $10^{-3}$ \textit{N. crispa} essential oil concentrations against SF9 cell lines with control ($P > 0.05$) (Figure 1 B and Table 2), even though there was a significant difference at $P < 0.001$ level between treatments of $10^{-7}$ and $10^{-3}$ of \textit{N. crispa} essential oil concentrations against SF9 cell lines (Figure 1 D).

**Comparison between cytotoxicity of malathion and \textit{N. crispa} essential oil:** The cytotoxicity of malathion against L929 and SF9 cell lines were gradually increased with a relatively low decreasing slope in accordance with malathion concentrations. The cytotoxicity of \textit{N. crispa} essential oil against L929 cell lines were gradually decreased with a moderately increasing slope in accordance with \textit{N. crispa} essential oil concentrations. While the cytotoxicity of \textit{N. crispa} essential oil against SF9 cell lines were strongly increased with an intensive increasing slope in accordance with \textit{N. crispa} essential oil concentrations (Figure 2). Wilcoxon signed-ranks test revealed a significant difference between treatments of $10^{-10}$ to $10^{-4}$ malathion concentrations with \textit{N. crispa} essential oil, respectively against L929 cell lines ($P < 0.05$) (Table 2). Even though there was a significant difference between treatments of $10^{-3}$ malathion concentration with \textit{N. crispa} essential oil at $P < 0.001$ level. Although Wilcoxon signed-ranks test did not show a significant difference between treatments of $10^{-10}$ to $10^{-3}$ malathion concentrations with \textit{N. crispa} essential oil against SF9 cell lines ($P > 0.05$). While there was a significant difference at $P < 0.001$ level between treatments of $10^{-10}$ to $10^{-7}$ malathion concentrations with \textit{N. crispa} essential oil against SF9 cell lines (Table 2).

**Discussion**

In recent years, insect insecticide resistance, ecosystem and food chain pesticide contamination, extinction of non-target organisms, mutation, and soil and water pollution has been critical problems. The scientists attempt to use natural products derived from plants. Based on the results of the study, malathion had negative effects on the viability of both L929 and SF9 cell lines. This results confirm the reports of the previous studies that concluded insecticides decreased growth of the vertebrate and invertebrate cell lines [41, 42]. Compared with the experiments of malathion treatments, the highest rate of cell viability was observed in the control group which did not receive any toxic agent. While, the viability of cell lines which were exposed to the different concentrations of malathion was lower than control group and decreased with increasing malathion concentrations (Figure 1 A,B, Figure 2). The negative effects of malathion on the viability of both L929 and SF9 cell lines were also confirmed by Wilcoxon signed-ranks test by observing a significant difference between treatments of $10^{-5}$ to $10^{-3}$ and $10^{-8}$ to $10^{-3}$ malathion concentrations with control group against the L929 and SF9 cell lines, respectively at $P < 0.05$ or $P < 0.001$ levels (Figure 1 A,B) and Table 2.

Unlike malathion, essential oil of \textit{Nepeta crispa} did not have negative effects on the viability of L929 cell lines. Compared with the control group, the highest rate of cell viability was observed in the experiment treatments which were treated with \textit{N. crispa} essential oil. The viability of L929 cell lines which were exposed to different concentrations of \textit{N. crispa} essential oil was higher than control group and increased with increasing \textit{N. crispa} essential oil concentrations (Figure 1 C, Figure 2). The boosting effects of \textit{N. crispa} essential oil on the viability of L929 cell lines were also confirmed by Wilcoxon signed-ranks test by observing a significant difference between treatments of $10^{-10}$ to $10^{-3}$ \textit{N. crispa} essential oil concentrations with treatments of malathion $10^{-10}$ to $10^{-3}$ concentrations and control group against the L929 cell lines at $P < 0.05$ or $P < 0.001$ levels (Figure 1 C) and Table 2. In addition to some previous benefits of the natural products deriving from plants [32, 43], this boosting effects of \textit{N. crispa} essential oil on the viability of vertebrate cell lines may be considered as the new benefits of the natural products deriving from plants.

Like malathion, essential oil of \textit{N. crispa} had negative effects on the viability of SF9 cell lines with the differences that the natural products deriving from plants containing environmentally friendly and readily biodegradable derivatives, hydrolyzing rapidly in nature and nearly having no destructive effects on mammals, humans or the environment [6, 15]. In addition, the application of \textit{N. crispa} essential oil concentrations (ng\text{L}$^{-1}$) was 1,000 folds lower than malathion concentrations (\text{ng}\text{L}$^{-1}$). The viability of SF9 cell lines which were
exposed to concentrations of 10⁻⁵ and 10⁻⁷ to 10⁻³ *N. crispa* essential oil was lower than control group and decreased with increasing *N. crispa* essential oil concentrations (Figure 1 D, Figure 2). The negative effects of *N. crispa* essential oil on the viability of SF9 cell lines were also confirmed by Wilcoxon signed-ranks test by observing a significant difference between treatments of 10⁻⁷ to 10⁻³ *N. crispa* essential oil concentrations with control group against the SF9 cell lines at P < 0.001 level (Figure 1 D). But with application of the natural products deriving from plants, there is no concern about their potential side effects like direct toxicity to users, environmental pollution, ozone depletion, pesticide residues and toxicity to non-target organisms. We will not face up to severe insecticide effects on vertebrate reproductive performance, and encounter probably some type of cancers or muscle fatigue, neurological diseases and psychotic disorders [2-6]. Maybe no longer encounter to a substantial problem of the pest and vector management programs due to insecticide resistance in arthropods of the agricultural pests and vectors of diseases [7-14].

**Conclusion**

Pesticides are used as an essential tool to vector-borne diseases and agricultural pests, and maintain quality and quantity crop production. With great concern about environmental problems and human health of synthetic pesticides, scientists have attempted to use natural products derived from plants. *Nepeta crispa* (Lamiales: Lamiaceae) is one of the most aromatic plants in Iran. *N. crispa* is autochthonous of Hamadan climate. A simultaneous comparative study about the cytotoxicity of *N. crispa* against the cell lines of invertebrates and vertebrates would be a particular of importance. Therefore, the cytotoxicity of *N. crispa* essential oil and malathion against L929 cell line of vertebrates and SF9 cell line of invertebrates were investigated. Based on the results of the study, malathion had negative effects on the viability of both L929 and SF9 cell lines. Unlike malathion, essential oil of *N. crispa* not only did not have negative effects on the viability of L929 cell lines, but also have boosting effects on the viability of L929 cell lines. Significant differences are also observed between treatments of 10⁻¹⁰ to 10⁻³ *N. crispa* essential oil concentrations with treatments of malathion 10⁻¹⁰ to 10⁻³ concentrations and control group against the L929 cell lines by Wilcoxon signed-ranks test confirming this fact. Like malathion, essential oil of *N. crispa* had negative effects on the viability of SF9 cell lines with the differences that the natural products deriving from plants containing environmentally friendly and readily biodegradable derivatives, hydrolyzing rapidly in nature and nearly having no destructive effects on mammals, humans or the environment. In addition, the application of *N. crispa* essential oil concentrations was extremely lower than malathion concentrations. On the other hand there is no concern about plant essential oil potential side effects like direct toxicity to users, environmental pollution, ozone depletion, pesticide residues, and toxicity to non-target organisms with application of the natural products deriving from plants. We will not also encounter probably some type of cancers or muscle fatigue, neurological diseases, and psychotic disorders by applying the derived natural products of plants. As well as maybe no longer encounter to a substantial problem of the vector and pest management programs due to insecticide resistance in arthropods of the vectors of diseases and agricultural pests.

**What is known about this topic**

- Scientists attempt to use derived plant natural products due to environmental safety and low mammalian toxicity;
- *Nepeta crispa* (lamiales: lamiaceae) is one of the most aromatic plants in Iran which is popular in Iranian traditional medicine.

**What this study adds**

- Plant essential oil not only had no negative effects but also had boosting effects on the L929 cell viability.
- *Nepeta crispa* essential oil had negative effects on the SF9 cell viability with the differences that derived plant natural products containing environmentally friendly and readily biodegradable derivatives, hydrolyzing rapidly in nature and nearly having no destructive effects on mammals and environment.

**Competing interests**

The authors declare no competing interests.

**Authors’ contributions**

All the authors have read and agreed to the final manuscript.
Acknowledgments

We are thankful to Moshkfam Fars Company, Shiraz, Iran, for supplying malathion insecticide. We also acknowledge the help of Mr. Kheiri for providing plant and for his assistance during the research. This study forms part of Mitra Boroomand’s MSc thesis in Medical Entomology and Vector Control in the Department of Medical Entomology, School of Medicine, which was supported financially by the Research Deputy of Hamadan University of Medical Sciences (Grant no. 9605033004).

Tables and figures

Table 1: cytotoxicity of malathion (μg·μL⁻¹) and essential oil of Nepeta crispa (ng·μL⁻¹) against L929 and Sf9 cell lines

Table 2: results of Wilcoxon signed-ranks test between cytotoxicity of malathion and essential oil of Nepeta crispa against L929 and Sf9 cell lines

Figure 1: cytotoxicity of malathion and essential oil of Nepeta crispa against L929 and Sf9 cell lines. A) Malathion against L929 cell lines; B) Malathion against Sf9 cell lines; C) Essential oil of Nepeta crispa against L929; D) Essential oil of Nepeta crispa against Sf9 cell lines. Sf9 cell line derived from the ovary of Spodoptera frugiperda (Smith) (lepidoptera: noctuidae). L929 vertebrate cell line derived from mouse fibroblast cells

Figure 2: cytotoxicity trends of malathion (μg·μL⁻¹) and essential oil of Nepeta crispa (ng·μL⁻¹) against L929 and Sf9 cell lines. A) Normal data and B) Percent. The trends were drawn by clicking on graph line distribution and selecting “add trendline” option. Sf9 cell line derived from the ovary of Spodoptera frugiperda (Smith) (lepidoptera: noctuidae). L929 vertebrate cell line derived from mouse fibroblast cells

References

1. Matthews G. Pesticides: health, safety and the environment. John Wiley & Sons. 2015. Google Scholar

2. Bhatia R, Shiu A, Petreas M, Weintraub JM, Farhang L, Eskenazi B. Organochlorine pesticides and male genital anomalies in the child health and development studies. Environ Health Perspect. 2004; 113(2): 220-224. PubMed | Google Scholar

3. Milton M, Ambrose K, Abraham C, Charles N, Kiriamiti K. Dichlorodiphenyl trichloroethane (DDT) and its observed effects on body functions in vertebrates. East Afr J Public Health. 2011; 8(4): 277-279. PubMed | Google Scholar

4. Alavanja MC, Bonner MR. Occupational pesticide exposures and cancer risk: a review. J Toxicol Environ Health, Part B. 2012; 15(4): 238-263. PubMed | Google Scholar

5. Menegaux F, Baruchel A, Lescoeur B, Nelken B, Clavel J, Leverger G et al. Household exposure to pesticides and risk of childhood acute leukaemia. Occup Environ Med. 2006; 63(2): 131-134. PubMed | Google Scholar

6. Khani A, Asghari J. Insecticide activity of essential oils of Mentha longifolia, Plicaria gnaphalodes and Achillea wilhelmsii against two stored product pests, the flour beetle, Tribolium castaneum, and the cowpea weevil, Callosobruchus maculatus. J Insect Sci 2012; 12: 73. PubMed | Google Scholar

7. Nasirian H. An overview of German cockroach, Blattella germanica, studies conducted in Iran. Pak J Biol Sci. 2010; 13(22): 1077-1084. PubMed | Google Scholar

8. Nasirian H. New aspects about Supella longipalpa (Blattaria: Blattellidae). Asian Pac J Trop Biomed. 2016; 6(12): 1065-1075. Google Scholar

9. Nazari M, Alipourian Motlagh B, Nasirian H. Toxicity of cypermethrin and chlorpyrifos against German cockroach [Blattella germanica (Blattaria: Blattellidae)] strains from Hamadan, Iran. Pak J Biol Sci. 2016; 19(6): 259-264. PubMed | Google Scholar

10. Davari B, Hassanvand A, Nasirian H, Ghiasian S, Salehzadeh A, Nazari M. Comparison of cockroach fungal contamination in the clinical and non-clinical environments from Iran. J Entomol Acarol Res. 2017; 49(2): 109-115. Google Scholar
11. Nasirian H. Contamination of cockroaches (Insecta: Blattaria) to medically fungi: a systematic review and meta-analysis. J Med Mycol. 2017; 27(4): 427-448. PubMed | Google Scholar

12. Veni T, Pushpanathan T, Mohanraj J. Larvicidal and ovicidal activity of Terminalia chebula Retz. Family: Combretaceae) medicinal plant extracts against Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus. J Parasit Dis. 2017; 41(3): 693-702. PubMed | Google Scholar

13. Davari B, Kashani S, Nasirian H, Nazari M, Salehzadeh A. The efficacy of MaxForce and Avion gel baits containing fipronil, clothianidin and indoxacarb against the German cockroach (Blattella germanica). Entomol Res. 2018; 48(6):459-465. Google Scholar

14. Nasirian H, Salehzadeh A. Control of cockroaches (Blattaria) in sewers: a practical approach systematic review. J Med Entomol. 2019; 56(1): 181-191. PubMed | Google Scholar

15. Prabhu K, Murugan K, Nareshkumar A, Ramasubramanian N, Bragadeeswaran S. Larvicidal and repellent potential of Moringa oleifera against malarial vector, Anopheles stephensi Liston (Insecta: Diptera: Culicidae). Asian Pac J Trop Biomed. 2011; 1(2): 124-129. PubMed | Google Scholar

16. Dunphy BM, Norris EJ, Coats JR, Gross AD, Bartholomay L, Bessette S. Comparison of the insecticidal characteristics of commercially available plant essential oils against Aedes aegypti and Anopheles gambiae(Diptera: Culicidae). J Med Entomol. 2015; 52(5): 993-1002. PubMed | Google Scholar

17. Tong F, Bloomquist JR. Plant essential oils affect the toxicities of carbaryl and permethrin against Aedes aegypti (Diptera: Culicidae). J Med Entomol. 2013; 50(4): 826-832. PubMed | Google Scholar

18. Baeck S-J, Ahn Y-J, Kwon HW, Yoon J-S, Kim S-I, Lee S-H. Toxicity and synergic repellency of plant essential oil mixtures with vanillin against Aedes aegypti (Diptera: Culicidae). J Med Entomol. 2012; 49(4): 876-885. PubMed | Google Scholar

19. Waliwitiya R, Lowenberger CA, Kennedy CJ, Nicholson RA. The synergistic effects of insecticidal essential oils and piperonyl butoxide on biotransformational enzyme activities in Aedes aegypti (Diptera: Culicidae). J Med Entomol. 2012; 49(3): 614-623. PubMed | Google Scholar

20. Thanigaivel A, Senthil-Nathan S, Vasantha-Srinivasan P, Edwin ES, Ponsankan A, Selin-Rani S et al. Chemicals isolated from Justicia adhatoda Linn reduce fitness of the mosquito, Aedes aegypti L. Arch Insect Bioch Physiol. 2017; 94(4): e21384. PubMed | Google Scholar

21. Jamzad Z, Ingrouille M, Simmonds MS. Three new species of Nepeta (Lamiaceae) from Iran. Taxon. 2003; 93-98. Google Scholar

22. Mojab F, Nickavar B, Hooshdar Tehrani H. Essential Oil Analysis of Nepeta crispa and N. menthoides from Iran. Iran J Pharm Sci. 2009; 5(1): 43-46. Google Scholar

23. Akaberi M, Emami SA, Vatani M, Tayarani-Najaran Z. Evaluation of antioxidant and anti-melanogenic activity of different extracts of aerial parts of N. sinesis in murine melanoma B16F10 Cells. Iran J Pharm Res. 2018; 17(1): 225-235. PubMed | Google Scholar

24. Tzakou O, Harvala C, Galati E, Sanogo R. Essential oil composition of Nepeta argolica Bory et Chab. subsp. argolica. Flavour Fragrance J. 2000; 15(2): 115-118. Google Scholar

25. Ghannadi A, Aghazari F, Mehrabani M, Mohagheghzadeh A, Mehregan I. Quantity and composition of the SDE prepared essential oil of Nepeta macrosiphon Boiss. Iran J Pharm Res. 2010; 2(2): 103-105. Google Scholar

26. Rapisarda A, Galati EM, Tzakou O, Flores M, Miceli N. Nepeta sibthorpii Bentham (Lamiaceae): micromorphological analysis of leaves and flowers. Il Farmaco. 2001; 56(5-7): 413-415. PubMed | Google Scholar

27. Bourrel C, Perineau F, Michel G, Bessiere J. Catnip (Nepeta cataria L.) essential oil: analysis of chemical constituents, bacteriostatic and fungistic properties. J Essent Oil Res. 1993; 5(2): 159-167. Google Scholar
28. Cigremis Y, Ulukanli Z, Ilcim A, Akgoz M. In vitro antioxidant and antimicrobial assays of acetone extracts from *Nepeta meyeri* Bentham. Eur Rev Med Pharmacol Sci. 2010; 14(8): 661-668. PubMed | Google Scholar

29. Miceli N, Taviano M, Giuffrida D, Trovato A, Tzakou O, Galati E. Anti-inflammatory activity of extract and fractions from *Nepeta sibthorpii* Bentham. J Ethnopharmacol. 2005; 97(2): 261-266. PubMed | Google Scholar

30. Topcu G, Ulubelen A. Structure elucidation of organic compounds from natural sources using 1D and 2D NMR techniques. J Mol Struct. 2007; 834: 57-73. Google Scholar

31. Mahnaz K, Alireza F, Hassan V, Mahdi S, Reza AM, Abbas H. Larvicidal activity of essential oil and methanol extract of *Nepeta menthoides* against malaria vector *Anopheles stephensi*. Asian Pac J Trop Med. 2012; 5(12): 962-965. PubMed | Google Scholar

32. Gharbani P, Javazi H. The antioxidant, general toxicity and insecticidal activities of Nepeta scrophularioides Rech. f. extracts in different developmental stages. Pak J Pharm Sci. 2015; 28(5 Suppl): 1905-9. PubMed | Google Scholar

33. Asgarpanah J, Sarabian S, Ziarati P. Essential oil of *Nepeta genus* (Lamiaceae) from Iran: a review. J Essent Oil Res. 2014; 26(1): 1-12. Google Scholar

34. Sonboli A, Salehi P, Yousefzadi M. Antimicrobial activity and chemical composition of the essential oil of *Nepeta crispa* Willd. from Iran. Z Naturforsch. 2004; 59(9-10): 653-656. PubMed | Google Scholar

35. Salehzadeh A, Jabbar A, Jennens L, Ley SV, Annadurai RS, Adams R *et al*. The effects of phytochemical pesticides on the growth of cultured invertebrate and vertebrate cells. Pest Manage Sci. 2002; 58(3): 268-276. PubMed | Google Scholar

36. Berridge MV, Tan AS, McCoy KD, Wang R. The biochemical and cellular basis of cell proliferation assays that use tetrazolium salts. Biochem. 1996; 4(1): 14-19.

37. Nasirian H. Infestation of cockroaches (Insecta: Blattaria) in the human dwelling environments: a systematic review and meta-analysis. Acta Trop. 2017; 167: 86-98. PubMed | Google Scholar

38. Nasirian H. Recent cockroach bacterial contamination trend in the human dwelling environments: a systematic review and meta-analysis. Bangladesh J Med Sci. 2019; 8(3): 540-545. Google Scholar

39. Nasirian H. Crimean-Congo hemorrhagic fever (CCHF) seroprevalence: a systematic review and meta-analysis. Acta Trop. 2019; 196: 102-120. Google Scholar

40. Nasirian H, Salehzadeh A. Effect of seasonality on the population density of wetland aquatic insects: a case study of the Hawr Al Azim and Shadegan wetlands, Iran. Vet World. 2019; 12(4): 584-592. Google Scholar

41. Masoud L, Vijayasarathy C, Fernandez-Cabezudo M, Petroianu G, Saleh A. Effect of malathion on apoptosis of murine L929 fibroblasts: a possible mechanism for toxicity in low dose exposure. Toxicol. 2003; 185(1-2): 89-102. PubMed | Google Scholar

42. Saleh M, Hajjar J, Rahmo A. Effect of selected insecticides on Sf9 insect cell line. Leban Sci J. 2013; 14(2): 115. PubMed | Google Scholar

43. Tak JH, Jovel E, Isman MB. Comparative and synergistic activity of *Rosmarinus officinalis* L. essential oil constituents against the larvae and an ovarian cell line of the cabbage looper, *Trichoplusia ni* (Lepidoptera: Noctuidae). Pest Manage Sci. 2016; 72(3): 474-480. PubMed | Google Scholar
| Treatment | C  | R  | Malathion (μg) | EONC (ng) |
|-----------|----|----|----------------|-----------|
| L929      | SF9| L929| SF9            | C  | R  | Malathion (μg) | EONC (ng) |
| Control   |    |    |                |    |    |                |            |
| -         | R₄ | 0.263| 0.177          |    |    |                |            |
| -         | R₃ | 0.245| 0.379          |    |    |                |            |
| -         | R₂ | 0.296| 0.384          |    |    |                |            |
| -         | R₁ | 0.345| 0.424          |    |    |                |            |
| 10⁻¹⁰     | R₄ | 0.268| 0.362          |    |    |                |            |
| 10⁻⁹      | R₄ | 0.240| 0.319          |    |    |                |            |
| 10⁻⁸      | R₄ | 0.244| 0.250          |    |    |                |            |
| 10⁻⁷      | R₄ | 0.253| 0.330          |    |    |                |            |
| 10⁻⁶      | R₄ | 0.249| 0.327          |    |    |                |            |
| 10⁻⁵      | R₄ | 0.238| 0.293          |    |    |                |            |
| 10⁻⁴      | R₄ | 0.219| 0.233          |    |    |                |            |
| 10⁻³      | R₄ | 0.224| 0.285          |    |    |                |            |

C=C= Concentration, EONC=Essential oil of Nepeta crispa and R=Replicate. SF9 cell line derived from the ovary of Spodoptera frugiperda (Smith) (Lepidoptera: Noctuidae). L929 vertebrate cell line derived from mouse fibroblast cells.
### Table 2: results of Wilcoxon signed-ranks test between cytotoxicity of malathion and essential oil of *Nepeta crispa* against L929 and Sf9 cell lines

#### Descriptive statistics

|               | Malathion |                                     | Essential oil of *Nepeta crispa* |                                     |
|---------------|-----------|-------------------------------------|-----------------------------------|-------------------------------------|
|               | Mean      | Std. deviation                      | Mean                              | Std. deviation                      |
| L929          | 0.27388   | 0.03676                             | Control                           | 0.20063                             |
| Sf9           | 0.38788   |                                     | 0.070888                          |                                     |
| Control       | 0.105627  |                                     | 0.65550                           |                                     |
| Treatment     | 0.27600   | 0.034690                            | 0.69175                           |                                     |
| 10^{-10}      | 0.38300   | 0.094820                            | 0.227017                          |                                     |
| 10^{-9}       | 0.045071  |                                     | 0.174798                          |                                     |
| 10^{-8}       | 0.037852  |                                     | 0.151501                          |                                     |
| 10^{-7}       | 0.219546  |                                     | 0.69175                           |                                     |
| 10^{-6}       | 0.249546  |                                     | 0.151629                          |                                     |
| 10^{-5}       | 0.276494  |                                     | 0.29275                           |                                     |
| 10^{-4}       | 0.281898  |                                     | 0.23641                           |                                     |
| 10^{-3}       | 0.300000  |                                     | 0.19243                           |                                     |
| 10^{-2}       | 0.000000  |                                     | 0.000000                          |                                     |
| 10^{-1}       | 0.000000  |                                     | 0.000000                          |                                     |

#### Wilcoxon signed-ranks test

|               | Malathion |                                     | Essential oil of *Nepeta crispa* |                                     |
|---------------|-----------|-------------------------------------|-----------------------------------|-------------------------------------|
|               | Mean ranks| Z                                  | p-value (2-tailed)                | Mean ranks                          | Z                                  | p-value (2-tailed)                |
| L929          | Negative  | Positive                            |                                   | Negative                            | Positive                           |                                   |
| 10^{-10}      | 6.0       | 3.6                                 | 0.00015                            | 1.00                                | 0.001                              | 4.5                               | -2.5215                           | 0.012                             |
| 10^{-9}       | 4.8       | 3.0                                 | -0.8457                            | 0.398                               | 0.001                              | 4.0                               | -2.3665                           | 0.018                             |
| 10^{-8}       | 6.0       | 3.0                                 | -0.8405                            | 0.401                               | 0.001                              | 4.5                               | -2.5215                           | 0.012                             |
| 10^{-7}       | 6.3       | 2.8                                 | -0.9805                            | 0.327                               | 0.001                              | 4.5                               | -2.5215                           | 0.012                             |
| 10^{-6}       | 4.9       | 3.3                                 | -1.6121                            | 0.107                               | 0.001                              | 4.0                               | -2.3665                           | 0.018                             |
| 10^{-5}       | 4.9       | 2.0                                 | -2.2405                            | 0.025                               | 0.001                              | 4.5                               | -2.5215                           | 0.012                             |
| 10^{-4}       | 4.0       | 0.001                              | -2.3665                            | 0.018                               | 1.0                                 | 4.5                               | -2.1955                           | 0.028                             |
| 10^{-3}       | 4.0       | 0.001                              | -2.3665                            | 0.018                               | 0.001                              | 2.5                               | -1.8265                           | 0.068                             |
| Sf9           | Negative  | Positive                            |                                   |                                     |                                    |                                   |                                    |
| 10^{-10}      | 4.0       | 5.3                                 | -0.2805                            | 0.779                               | 2.0                                | 3.0                               | -0.3655                           | 0.715                             |
| 10^{-9}       | 4.0       | 4.0                                 | -1.0155                            | 0.310                               | 3.5                                | 1.5                               | -0.7305                           | 0.465                             |
| 10^{-8}       | 4.6       | 4.0                                 | -1.9605                            | 0.050                               | 2.0                                | 3.0                               | -0.3655                           | 0.715                             |
| 10^{-7}       | 4.4       | 5.0                                 | -1.8205                            | 0.069                               | 2.5                                | 0.001                             | -1.8265                           | 0.068                             |
| 10^{-6}       | 4.9       | 2.0                                 | -2.2405                            | 0.025                               | 2.5                                | 0.001                             | -1.8265                           | 0.068                             |
| 10^{-5}       | 5.0       | 1.0                                 | -3.8095                            | 0.017                               | 2.5                                | 0.001                             | -1.8265                           | 0.068                             |
| 10^{-4}       | 4.5       | 1.0                                 | -2.1975                            | 0.028                               | 2.5                                | 0.001                             | -1.8265                           | 0.068                             |
| 10^{-3}       | 4.0       | 0.001                              | -2.3665                            | 0.018                               | 2.5                                | 0.001                             | -1.8265                           | 0.068                             |

#### Between treatments of malathion and essential oil of *Nepeta crispa*

|               | L929      |                                     | Sf9                                |                                     |
|---------------|-----------|-------------------------------------|-----------------------------------|-------------------------------------|
| 10^{-10}      | 10^{-10}  | 5.0                                 | -2.3805                            | 0.017                               | 2.5                                | 0.001                             | -1.8265                           | 0.068                             |
| 10^{-9}       | 10^{-9}   | 4.0                                 | -2.3665                            | 0.018                               | 2.5                                | 0.001                             | -1.8265                           | 0.068                             |
| 10^{-8}       | 10^{-8}   | 4.5                                 | -2.5215                            | 0.012                               | 2.5                                | 0.001                             | -1.8265                           | 0.068                             |
| 10^{-7}       | 10^{-7}   | 4.5                                 | -2.5215                            | 0.012                               | 2.5                                | 0.001                             | -1.8265                           | 0.068                             |
| 10^{-6}       | 10^{-6}   | 4.0                                 | -3.7175                            | 0.018                               | 2.5                                | 0.001                             | -1.8265                           | 0.068                             |
| 10^{-5}       | 10^{-5}   | 4.5                                 | -2.5215                            | 0.012                               | 2.5                                | 0.001                             | -1.8265                           | 0.068                             |
| 10^{-4}       | 10^{-4}   | 4.5                                 | -1.9705                            | 0.028                               | 3.0                                | 0.001                             | -0.7305                           | 0.465                             |
| 10^{-3}       | 10^{-3}   | 2.5                                 | 0.001                              | -2.3665                            | 0.068                               | 3.0                                | 0.001                             | -0.3655                           | 0.715                             |

*The sum of negative ranks equals the sum of positive ranks, *b*ased on negative ranks and *b*ased on positive ranks. The *p*-value of significant (*P < 0.05*) are shown in bold font style. Sf9 cell line derived from the ovary of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae). L929 vertebrate cell line derived from mouse fibroblast cells.
Figure 1: Cytotoxicity of malathion and essential oil of *Nepeta crispa* against L929 and SF9 cell lines. A) Malathion against L929 cell lines; B) Malathion against SF9 cell lines; C) Essential oil of *Nepeta crispa* against L929; D) Essential oil of *Nepeta crispa* against SF9 cell lines. SF9 cell line derived from the ovary of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae). L929 vertebrate cell line derived from mouse fibroblast cells.
Figure 2: cytotoxicity trends of malathion (μg mL⁻¹) and essential oil of Nepeta crispa (ng mL⁻¹) against L929 and Sf9 cell lines. A) Normal data and B) Percent. The trends were drawn by clicking on graph line distribution and selecting "add trendline" option. Sf9 cell line derived from the ovary of Spodoptera frugiperda (Smith) (Lepidoptera: Noctuidae). L929 vertebrate cell line derived from mouse fibroblast cells.