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CHEMICAL CHARACTERIZATION of *Foeniculum vulgare* MILL ESSENTIAL OIL COMPOSITION AND ITS TOXICOLOGICAL EFFECTS AGAINST MOSQUITO: *Aedes caspius* (PALLAS, 1771) SPECIES

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

In this study, the essential oil (EO) of the aerial parts of *Foeniculum vulgare* Mill (Umbelliferae: Apiaceae) was extracted by hydro-distillation and subsequently, its chemical composition was analyzed using gas chromatography (GC) and the profile was identified by gas chromatography-mass spectrometry (MS). Then, its larvicidal potential was evaluated against the larvae of *Aedes caspius* (Pallas, 1771). Using the CPG-MS analyses fourteen volatile compounds representing 99.22% of the essential oil were identified; namely are: Camphor (38.2%), Fenchone (28.24%) and o-Cymene (11.44%) which were predominant in the EO of *F.vulgare.* The toxicity of the EO was evaluated, after 24h of exposure time, against the fourth instar larvae of *Aedes caspius* and the sublethal and lethal concentrations, $LC_{25,}$ LC_{50} and LC_{90} values were estimated with their confidence limits and their values are 27.65, 37.76 and 70.40 µl respectively. The obtained bioassay results showed that *F. vulgare* EO exhibited a toxic effect against *Ae. caspius* larvae with a dose-response relationship. Based on the results of the toxicity of *F. vulgare* EO, it can be concluded that, the present aromatic plant species showed a significant toxicological effect and could be used as a promising alternative for the mosquito control. This result opens interesting perspectives for its application in the production of a new source of various larvicidal active compounds for controlling mosquito vectors.

Keywords: Essential oil; Aedes caspius; toxicity; mosquito control; insecticide.

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1. INTRODUCTION

Mosquitoes are one of the major causative agents of devastating diseases like dengue, chikungunya, malaria, yellow fever, filariasis, Japanese encephalitis, Zika and lyme which result in millions of deaths each year [1,2]. These arthropod disease vectors are generally controlled by conventional neurotoxic insecticides, that their misuse can cause serious problems such as insecticide resistance [3,4], environmental pollution and toxicity to human and non-target organisms [5,]. Such problems have accentuated the need for newer strategies, using new alternatives, for mosquito control. The proposition of a new potential insecticides from plant extracts such as essential oils (EOs), are relatively cost-effective, without environmental secondary effects and has therapeutic benefits [7]. Consequently, the scientific community was highly interested to valorize the natural bioactive chemicals, as potential substitutes for manufactured compounds for insect vector control. Since they were found a variety of bioactive chemicals that are highly toxic to mosquitoes but safe to non-target organisms and the environment [8, 9, 1]. subsequently, the utilization of EOs from aromatic plants could be particularly significant, when used against mosquito control, because they are more potent, secure, and environmentally friendly. In addition, observed physiological changes in neuroendocrine system function, influencing insect behavior, growth, molting, histological aberrations, and metamorphosis, EOs have been studied to explain their mode of action [10-14]. In this regard it has been reported that Fennel EO is used as a cure for pediatric colic and some respiratory disorders [15] analgesic, anti-inflammatory [16,17], hepathoprotective [18] and neuroprotective [19]. *F. vulgare* Mill, from the Apiaceae family, is an aromatic plant widespread in Algeria and its seeds are widely utilized in herbal medicine [20, 21]. The plant has several traditional uses, and they interestingly have an impact against insects [22, 23, 20]. The EO profile of different sources of fennel might be different, so it is necessary to characterize its chemical composition, by GC-MS/MS analyses. Following go-green conceptions, this study proceeded to the extraction of EO from *F. vulgare* grown in Setif, investigating its chemical analysis and its potential larvicidal effects against fourth instar larvae of *Aedes caspius*.

2. MATERIALS AND METHODS

2.1 Plant Materials and Oil Extraction

Foeniculum vulgare Mill, known as fennel, is one of the Apiaceae family members. This plant is native but not limited to Mediterranean areas. The plant is widely used by local people as a source of spice and medicinal uses [24, 25]. The aerial parts of *F. vulgare* were collected in the month of October from Hammam Guergour, high-plains region (Setif, Northeast Algeria; 36°34’77’’ N, 5°06’26’’E). The collected plant has been identified by the botany department, Ferhat Abbes University, Algeria. The Completely dried samples were subjected to grinding with a commercial blender. A sample of 100g of plant powder was hydrodistilled for 3h using a Clevenger-type apparatus. The obtained EO was stored in dark vials at 4°C until the insecticidal bioassays and chemical analyses. The EO yield was estimated according to dry mass, which is estimated by the ratio between the weight of extracted oil and that of the treated plant. It is expressed as a percentage; using the following equation: Oil yield=oil content (g) ⁄ dried weight of the sample ×100.

2.2 Essential Oil Analysis using GC-MS

The plant EO of *F. vulgare* was analyzed by gas chromatography-mass spectrophotometry GC / FID and GC-MS/MS. The volume of the solution was adjusted to 1 ml with hexane before being transferred into a GC vial for analysis. All used solvents (Fisher Scientific, Loughborough, United Kingdom) are highly performance liquid chromatography grade. The quantification of the components of EO was carried out by chromatogram gas type Agilent 7890A Gas Chromatograph, equipped with a FID detector. An auto-sampler and air-cooling multimode inlet were implemented through the used GC device. The vaporizer temperature is set at 280 °C. The injection volume is 1 µl in split mode and the split ratio was set at 10:1. The concentration of the injected oil is 1% in hexane. A HP-5MS capillary column (30 m length; 0.25 mm inner diameter; 0.25 µm film thickness) is used. Helium of high purity (N60) was used as a carrier gas at a 1 mL/min flow rate. The temperature of the oven was kept at 50 °C for 1 min, increased by 9 °C/min up to 280 °C. The final temperature was kept for 5 min. Under the same chromatographic conditions as GC-FID, the identification of the EO components are determined using Gas Chromatography coupled to Tandem Mass Spectrometry (GC/MS-MS) using an Agilent 7000 Triple Quadrupole instrument, which combines a GC 7890A for the separation and a triple quadrupole mass spectrometer (QqQ) that operated at 70V to perform tandem mass spectrometry. Constituent identification was found with MassHunter (MH) Workstation Software Qualitative Analysis Workflows (version B.10.00, Agilent Technologies Inc., Santa Clara, CA, USA). It was operated with the following parameters: compounds were discovered by chromatogram deconvolution with the default settings; substances

were identified using an MS library (NIST17) search.

2.3 Toxicological Assays

Aedes caspius eggs were obtained from the untreated breeding sites and there were maintained in controlled laboratory conditions; at a temperature of 26± 3 °C and 12/12 h (light: dark) photoperiods. Each larval stage was kept separately in storage jars containing 500 ml of stored tap water, they were daily fed with fish food and the water was changed every two days. The 4th instar larvae of *Ae. caspius* were used for the bioassay using *F. vulgare* EO. The larvicidal bioassays were carried out following the World Health Organization (OMS) standard protocol recommendations [26]. 1ml of EO was dissolved in ethanol to obtain 1% and 10% and stored as a stock solution. From the stock solution, 0,1-1 ml were added to 100 ml of tap water. Through this process, the following concentrations were obtained: 10, 20, 40, 50, 60, 80, 100, 200 and 400µl. The positive controls were exposed to 1ml ethanol, while the negative controls were exposed to water only. The toxicological tests were performed with three repetitions of 25 larvae for each used concentration. The toxicity effect was estimated by recording the larval mortality during the period of 24, 48 and 72 h, after the treatment period. Larvae that showed no movement were recorded as being dead.

2.4 Statistical Analysis

Statistical analysis of the experimental data was performed with R 3.6.3 (packages drc) to determine LC25, LC50 and LC 90 values and their 95% FL. Comparison between the different series was presented as mean & SD, and made using one-way analysis of variance (ANOVA) followed by Tukey's test, using (packages ggplot2).

3. RESULTS

3.1 Yield and Chemical Composition of *F. vulgare* Essential Oil

Hydrodistillation of the fennel areal part provided a pale yellow-colored EO with a 1.40 ± 0.13% (w/w) yield. Phytochemical screening of EO of *F. vulgare* by GC–MS/MS showed the presence of fourteen phyto-constituents representing 99.22% of the total identified compounds (Tab.1, Fig. 1). The percentage of the compounds in the EO was calculated according to the area of the chromatographic peaks (Fig. 1) and the retention index relative to n-alkanes of the components, using the chromatograms resulting from the analysis by GC-FID among which Camphor (38.2%), Fench one (28.24%) and o-Cymene (11.44%), were the predominant components in the oil of *F. vulgare*, while the other components exhibit lower percentages like Limonene (3.87%), alpha-Phellandrene (2.18%) and Estragole (1.80%), in addition to others that exist in traces with less than 1%.

3.2 Larvicidal Activity of *F. vulgare* EO against *Ae. caspius*

The results of the larvicidal activity, of *F. vulgare* against *Ae. caspius* larvae are shown in (Fig. 2). After treatment, the observed mortality, which is recorded at different periods during the treated developmental stage increased accordingly with the concentrations in function of time (Fig. 2). Concentration-response relation-ship was determined against the 4th instar larvae of *Ae. caspius.* The recorded mortality of the treated 4th instar larvae varies between 1.33% and 100%, where *F. vulgare* EO in a concentration of 80µl was the most effective and with the highest toxicity, while a concentration of 10µl showed the lowest toxicity. The data shows that 100% mortality rate was observed at 100, 200 and 400 µl of concentrations. Yet no significant effect against *Ae. caspius* larvae was noted for the positive controls.

The statistical analyses reveal the existence of very highly significant differences between the concentrations used for the same period (P= 0.000). As far as the time factor is concerned, there are no significant differences (P= 0.693). Statistical analyses using Fisher's LSD test of obtained results indicated that different *F. vulgare* EO concentrations demonstrated meaningful statistical differences in relation to the larvicidal efficacy as a dependent variable (P= 0.000 for $P \leq 0.001$). However, the time intervals were not statistically significant (P= 0.693 for $P > 0.05$) (Table 2).

The regression equation of the mosquito mortality rate for the EO of the present study is illustrated in the graph and the table as below (Fig.4) and (Table 3) indicating the lethal estimated concentrations with their fiducial limits (95 %) from the linear regression curves, expressed by the mortality probits and the logarithm of *F. vulgare* oil doses.

The results of regression analysis of used oil showed that *F. vulgare* EO possessed a high larvicidal efficiency against *Ae. caspius* larvae with LC_{25} value of 27.65, 22.87 and 19.28 µl and LC_{50} value of 37.76, 33.14 and 29.43 µl and LC_{90} value of 70.40, 69.56 and 68.60µl for the fourth instar larvae after 24.48 and 72 h of exposure respectively.

Table 1. Chemical composition of *F. vulgare* essential oil

N°	RT (mn)	Compound	Formule	RI	Area %
1	7.100	α-Pinene	C10 H16	876.2628977	4.06
2	7.394	2(10)-Pinene	C10 H16	882.9306595	0.37
3	7.825	Nopinene	C10 H16	891.9950901	0.3
4	7.921	Melilotal	C9 H10 O	893.9112696	0.45
5	8.086	.alpha.-Phellandrene	C10 H16	897.1247334	2.18
6	8.429	o-Cymene	C10 H14	1006.619323	11.44
7	8.793	Limonene	C10 H16	1018.650488	3.87
8	8.886	Tricyclo[5.2.1.0(2,5)]dec-5(6)ene	C10 H14	1021.610225	6.65
9	9.428	gamma.-Terpinene	C10 H16	1038.028249	0.96
10	10.102	Fenchone	C10 H16 O	1056.705717	28.24
11	11.136	Fenchone	C10 H16 O	1082.309243	0.55
12	12.115	Camphor	C10 H16 O	1402.951373	38.2
13	13.568	Estragole	C10 H12 O	1450.809246	1.8
14	16.876	Anise camphor	C10 H12 O	1539.515138	0.15
Total identified					99.22%

RT: Retention time; RI: Retention index: Kovats retention index relative to n-alkanes on column; Area %: Value expressed as relative area percentages to total identified compounds.

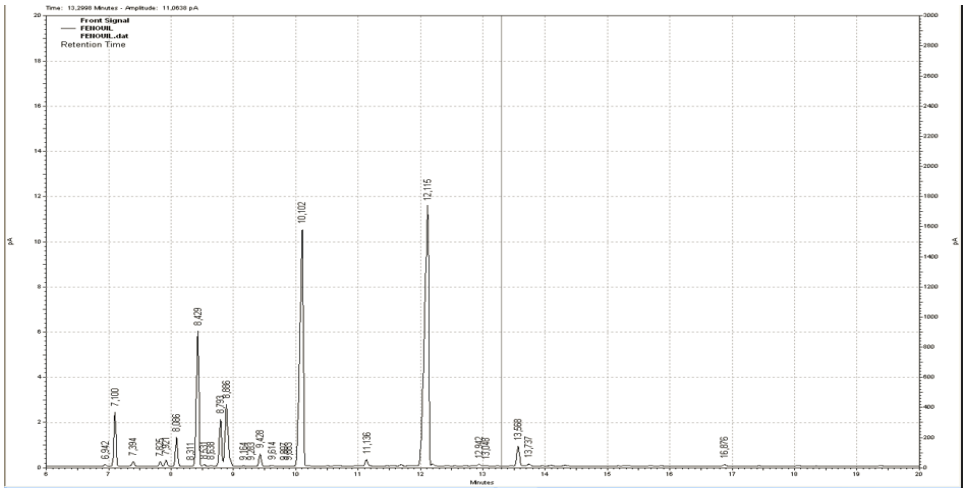

Fig. 1. GC-FID chromatogram for essential oil of *F.vulgare*

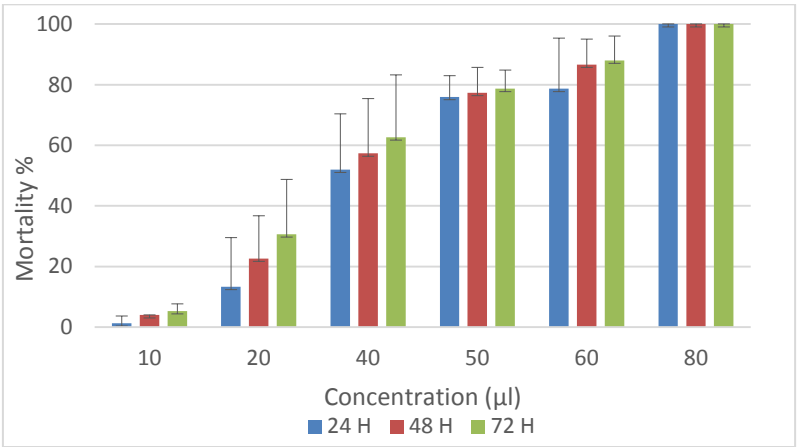

Fig. 2. Concentration-response relationship of the treatment of EO of *F. vulgare* applied to the newly exuviated fourth instar larvae of *Ae. caspius*

Table 2. The results of the Fisher's LSD post-hoc test (concentrations and exposure times: 24. 48 and 72 hours)

Time		Concentration	
(df=2)		(df=9)	
***P* value**	**Observation**	**P value**	**Observation**
0.693	ns	0.000	***

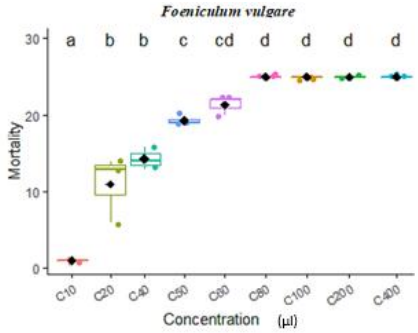

Fig. 3. Larvicidal efficacy of EO of *F. vulgare* applied on fourth instar of larvae of *Ae. caspius* depending on different concentrations (a, b and c) indicate that the variation is significant at p <0.05, using Tukey's test. Boxplots labeled with the same letter are not significantly different at p > 0.05. The boundaries of the central box show the interquartile range (IQR) with the first quartile (lower bound) and the third quartile (upper bound). Outliers are indicated by small circles

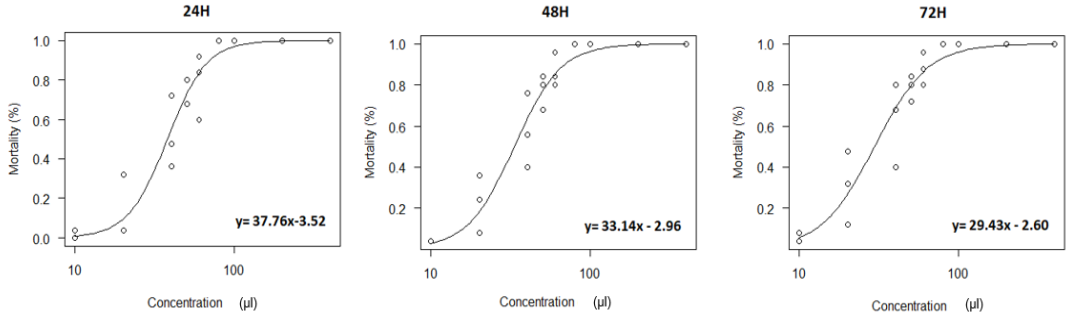

Fig. 4. Probit transformed responses with equation regression for *F. vulgare* EO tested on 4th instars larvae of *Ae. caspius* for 24, 48 and 72 h

Table 3.The sublethal and lethal concentrations, LC_{25}, LC_{50} and LC_{90} values of *F. vulgare* EO against the late 4th instar larvae of *Ae. caspius*, after 24, 48 and 72 hours of exposure time; with regression equations

Time	Equation regression	LCL>LC_{25}>UCL(µl) Confidence limit (95%)	LCL>LC_{50}>UCL(µl) Confidence limit (95%)	LCL>LC_{90}>UCL (µl) Confidence limit (95%)
24 h	37.76x-3.52	22.46<27.65<32.85	33.66<37.76<41.87	58.86<70.40<81.93
48 h	33.14x-2.96	18.87<22.87<26.87	29.48<33.14<36.80	59.24<69.56<79.87
72 h	29.43x-2.60	15.56<19.28<22.99	25.65< 29.43< 29.44	56.14<68.60<81.07

4. DISCUSSION

4.1 Yield and Chemical Composition of Essential Oil

The yield of EO extractionof *F. vulgare* was 1.40 % ± 0.13. The EO yield of fennel herbage was between 0.69 % and 4.60 % [27, 28]. The obtained yield in this experiment is higher than the EOs extracted from the same species; collected in Pithoragarh (0.6 %) and Didihat (0.9 %) of North India [29]. However, EO yield in the seeds of *F. vulgare* cultivated in the same region (Setif) collected during May was lower than studied yield (0.93 ± 0.07%) [20]. The EO yield varies, whether the extraction method is different or

the same. Its variety could be affected by the quality of the used plant material in addition to other factors, such as the growth stage, soil quality, climate conditions, time of harvest and drying period [30]. For the present study, it was noted that the main constituents of the EO from the dry aerial parts of *F. vulgare* are Camphor (38.2%), Fenchone (28.24%) and o-Cymene (11.44%). While, Belabdelli [21] found that major components were Estragole (84.8%), limonene (7.8%), Fenchone (3.1%) and α-pinene (1.3%) in the *F. vulgare* EO of Algerian seeds. Chemical analysis of *F. vulgare* by other authors [31, 32, 25] also showed that the main constituents of this oil are trans-anethole, estragole, fenchone and limonene. Variation in chemical composition of EOs may be caused by several factors such as the method of extraction, period of plant collection. It is also influenced by both internal and external factors affecting the plant, such as genetic structures and environmental conditions [33, 34].

4.2 Insecticidal Activity

In this study, the results indicate that the *F. vulgare* EO exhibited a larvicidal activity against *Ae. caspius* larvae; while Zoubiri [20] showed the insecticidal activity of *F. vulgare* EO against larvae of *Cx. pipiens.* In the other study [35] the larvicidal activity, of the same species of plant EO, was the most effective against *Aedes stephensi* with LC_{50} and LC_{90}.Chantawee and Soonwera [36] found 100% mortality in the larvae of *Aedes aegypti* treated with *F. vulgare* EO at a concentration of 10% with LT_{50} achieved at a concentration of 5%. Studies reported by Zoubiri [20] show clearly that the activity of this plant seed oil may be due to the presence of *trans*-anethol as the main compound. *F. vulgare* seed EO can be suggested as natural larvicidal for controlling *Cx. pipiens* mosquito. Thus, *F. vulgare* can serve as a natural larvicidal agent along with other previous studies which reported significant toxicity of the fennel EO against different arthropod species [37, 38, 39, 40].

5. CONCLUSION

Plant-based pesticides are promising alternatives to synthetic insecticides. *F.vulgare* essential oil exhibited larvicidal activity and the identified components support the future development of novel pesticides from EOs as potential natural sources for the pest control programs.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rathy M C, Sajith U, Harilal C C. Larvicidal efficacy of medicinal plant extracts against the vector mosquito *Aedes albopictus*. International Journal of Mosquito Research. 2015; 2(2):80-82.
2. Nwabor O F, Nnamonu E I, Martins P E, Odiachi O. Synthetic insecticides, phytochemicals and mosquito resistance. Academia Journal of Biotechnology. 2019;5: 118-25.
3. Pavlidi N, Vontas J, Van Leeuwen T. The role of glutathione S-transferases (GSTs) in insecticide resistance in crop pests and disease vectors. Current Opinion in Insect Science. 2018; 27:97-102.
4. Richard S L, Byrd B D, Reiskind MH, White A V. Assessing Insecticide Resistance in Adult Mosquitoes: Perspectives on Current Methods. Environmental Health Insights. 2020; 14:1-7.
5. Sarwar M, Ahmad N, Toufiq M. Host plant resistance relation-shiphs in chickpea (*Cicer arietinum* L.) against gram pod borer (Helicoverpa armigera Hubner. Pakistan Journal of Botany. 2009;41(6):3047-3052.
6. Smith C J, Perfetti, T A. A comparison of the persistence, toxicity, and exposure to high-volume natural plant-derived and synthetic pesticides. Toxicology Research and Application. 2020; 4:1-15.
7. Dadalioğlu I, Evrendilek G A. Chemical compositions and antibacterial effects of essential oils of Turkish oregano (Origanum minutiflorum), bay laurel (*Laurus nobilis*), Spanish lavender (Lavandula stoechas L.), and fennel (Foeniculum vulgare) on common foodborne pathogens. Journal of agricultural and food chemistry. 2004;52:8255-8260.
8. Aissaoui L, Bouaziz A, Boudjelida H. Toxic effect of a certain medicinal plant extracts against larvae of mosquito species *Culiseta longiareolata* (Maquart, 1838). Uttar Pradesh Journal of Zoology. 2022;43(6):19 -25.
9. Djeddar H, Boudjelida H, Arroussi, D E R. New alternative for culicidian fauna control using *Borago officinalis* and *Drimia maritima* plant extracts. Biodiversitas Journal of Biological Diversity. 2021;22:5688-5694.
10. 10.Pavela R, Maggi F, Ngahang Kamte S L, Rakotosaona R, Rasoanaivo P, Nicoletti M, Canale A, Benelli, G. Chemical composition of

Cinnamosma madagascariensis (Cannelaceae) essential oil and its larvicidal potential against the filariasis vector *Culex quinquefasciatus* Say. South African Journal of Botany. 2017; 108:359-363.

11. 11.Djeghader N, Aïssaoui L, Amira K, Boudjelida H, Toxicity evaluation and effects on the development of a plant extract, the Saponin, on the domestic mosquito, *Culex pipiens*. International Journal of Mosquito Research. 2018;5(1):01-05.
12. Shoba V. Biochemical status and histological changes of Catharanthus roseus ethanolic leaves extract against mosquito larvae *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquifasciantus* (Diptera: Culicidae). International Journal of Zoology and Applied Biosciences. 2018;3(2):352-358.
13. Corzo F L, Traverso L, Sterkel M, Benavente A, Ajmat M T, Ons S. *Plodia interpunctella* (Lepidoptera: Pyralidae): Intoxication with essential oils isolated from *Lippia turbinata* (Griseb.) and analysis of neuropeptides and neuropeptide receptors, putative targets for pest control. Archives of insect biochemistry and physiology. 2020; 104, 21684-21697.
14. Dris D, Bouabida H. Larvicidal activity of an Algerian *Ruta graveolens* Essential oil on Mosquito Species *Culex pipens*. Transylvanian Review. 2020; 47:14717-14728.
15. Liu J, Luo JG, Ye H, Sun Y, Lu ZX, Zeng X. Medium optimization and structural characterization of exopolysaccharides from endophytic bacterium *Paenibacillus polymysa* EJS-3. Carbohydrate Polymer. 2010;79:206-213.
16. Choi E M, Hwang J K. Anti-infammatory, analgesic and antioxidant activities of the fruit of *Foeniculum vulgare*. Fitoterapia. 2004;75:557-565.
17. Lee H S, Kang P, Kim K Y, Seol G H. *Foeniculum vulgare* Mill. protects against lipopolysaccharide-induced acute lung injury in mice through ERK-dependent NF-KB activation. Korean Journal of Physiology and Pharmacology. 2015;19:183-189.
18. Ozbek H, Ugras S, Dulger H, Bayram I, Tuncer I, Ozturk G, Ozturk A. Hepatoprotective effect of *Foeniculum vulgare* essential oil. Fitoterapia. 2003;74:317-319.
19. Sheharbano B, Syed A A S, Touqeer A, Saadia Z. Neuroprotective efects of *Foeniculum vulgare* seeds extract on lead induced neurotoxity in mice brain. Drug Chemical Toxicology. 2018;41(4):399- 407.
20. Zoubiri S, Baaliouamer A, Seba N, Chamouni N. Chemical composition and larvicidal activity of Algerian *Foeniculum vulgare* seed essential oil, Arabian Journal of Chemistry. 2014;7(4):480-485.
21. Belabdelli F, Piras A, Bekhti N, Falconieri D, Belmokhtar Z, Merad Y. Chemical Composition and Antifungal Activity of *Foeniculum vulgare* Mill. Chemistry Africa. 2020;3:323-328.
22. Mendonca F A C, da Silva K F S, dos Santos K K, Junior K A L R, Ana A E G S. Activities of some Brazilian plants against larvae of the mosquito *Aedes aegypti*. Fitoterapia. 2005;76:629-636.
23. Conti B, Canale A, Bertoli A, Gozzini F, Pistelli L. Essential oil composition and larvicidal activity of six Mediterranean aromatic plants against the mosquito *Aedes albopictus* (Diptera: Culicidae). Parasitology Research. 2010;107:1455-1461.
24. Hendawy S F, Ezz El-Din A A. Growth and yield of *Foeniculum vulgare* var.azoricum as influenced by some vitamins and amino acids. Ozean Journal of Applied Sciences. 2010; 3(1).
25. Bahmani K, Darbandi A I, Ramshini H A, Moradi N, Akbari A. Agro-morphological and phytochemical diversity of various Iranian fennel landraces. Industrial Crops and Products. 2015; 77, 282-294.
26. WHO. Guidelines for laboratory and field-testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP. 2005; 13.
27. Marotti M, Piccaglia R, Giovannelli E, Effects of variety and ontogenetic stage on the essential oil composition and biological activity of fennel (Foeniculum vulgare Miller). Journal of Essential Oil Research.1994;6:57-62.
28. Garcia-Jimenez N, Perez-Alonso M J, Velasco-Negueruela A. Chemical composition of fennel oil, *Foeniculum vulgare* Miller, from Spain. Journal of Essential Oil Research. 2000;12:159-162.
29. Punetha D, Tewari G, Pande C, Bhatt S. Effect of Climatic Conditions on the Volatile Compounds of the Aerial Parts of *Foeniculum vulgare* Mill. Journal of Essential Oil Bearing Plants. 2019;22(4).
30. Mohammedi H, Mecherara-Idjeri S, Hassani A. Variability in essential oil composition, antioxidant and antimicrobial activities of *Ruta montana* L. collected from different geographical regions in Algeria. Journal of Essential Oil Research. 2020;32:88-101.
31. Ahmed A F, Shi M, Liub C, Kang W. Comparative analysis of antioxidant activities of essential oils and extracts of fennel (*Foeniculum vulgare* Mill.) seeds from Egypt

and China. Food Science and Human Wellness. 2019; 8, 67-72.
32. Sabzi Nojadeh M, Pouresmaeil M, Younessi-Hamzekhanlu M, Venditti A. Phytochemical profile of fennel essential oils and possible applications for natural antioxidant and controlling *Convolvulus arvensis* L, Natural Product Research. 2020; 35, 4164-4168.
33. Saxena S N, Agarwal D, John S, Dubey P N, Lal G. Analysis of fennel (*Foeniculum vulgare*) essential oil extracted from green leaves, seeds and dry straw. International Journal of Seed Spices. 2018; 8(1), 60-64.
34. Venditti A, Frezza C, Rossi G, Di Cecco M, Ciaschetti G, Serafini M, Bianco A. Secondary metabolites with ecologic and medicinal implications in *Anthemis cretica* subsp. petraea from Majella National Park. Aims Molecular Science. 2016; 3(4), 648-660.
35. Sedaghat M M, Sanei Dehkordi A, Abai M R, Khanavi M, Mohtarami F, Salim Abadi Y, Rafi F, Vatandoost H. Larvicidal Activity of Essential Oils of Apiaceae Plants against Malaria Vector, *Anopheles stephensi*. Iran Journal of Arthropod-Borne Diseases. 2011; 5(2), 51-59.
36. Chantawee A, Soonwera M. Efficacies of four plant essential oils as larvicide, pupicide and oviposition deterrent agents against dengue fever mosquito, *Aedes aegypti* Linn. (Diptera: Culicidae). Asian Pacific Journal of Tropical Biomedicine. 2018;8:217-225.
37. Amizadeh M, Hejazi M J, Saryazdi G A. Fumigant toxicity of some essential oils on *Tetranychus urticae* (Acari:Tetranychidae). International Journal of Acarology. 2013; 39, 285-289.
38. Gemeda A, Sendi J, Aliakbar A, Razmjou J. Chemical Composition and acaricidal effects of essential oils of *Foeniculum vulgare* Mill. (Apiales: Apiaceae) and *Lavandula angustifolia* Miller (Lamiales: Lamiaceae) against *Tetranychus urticae* Koch (Acari: Tetranychidae). Psyche. 2014;42:67-78.
39. Pavela R, Zabka M, Bednar J, Triska J, Vrchotova N. New knowledge for yield, composition and insecticidal activity of essential oils obtained from the aerial parts or seeds of fennel (*Foeniculum vulgare* Mill). Industrial Crops and Products. 2016;83:275-282.
40. Aboelhadid S M, Arafa W M, Abdel-Baki A A, Sokmen A, Al-Quraishy S, Hassan A O. Acaricidal activity of *Foeniculum vulgare* against *Rhipicephalus annulatus* is mainly dependent on its constituent from trans-anethone. PLOS ONE. 2021;16(12): 1-15.