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Chemical composition and larvicidal activity of Algerian *Foeniculum vulgare* seed essential oil

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Abstract A laboratory study was conducted to determine the effect of three extraction parameters (soaking time, extraction time and the ratio of solid to liquid) on the yield and chemical composition of *Foeniculum vulgare* seeds essential oils. The bioactivity of the essential oil extracted for the optimum extraction parameters was assessed against *Culex pipiens* mosquito. *F. vulgare* essential oil composition included large amounts of phenylpropanoids. Through an extraction time of 6 h and a ratio solid to liquid of 300 g/L we can get over than 72% of *trans*-anethol without soaking the seeds. With bioassays, essential oils showed different activities on *C. pipiens* larvae and pupae. Results show that a concentration at 40 mg/L was sufficient to register 50% mortality for the second instars larvae and this, after 2 h exposition time. Moreover, concentration at 60 mg/L ensured after 4 h exposition time 90% mortality for the fourth instars larvae. However, pupae needed 24 h exposition time to show promising mortalities when using concentration at 200 mg/L. Even if laboratory bioassays are only the first step towards the use of essential oils in practical applications, these substances represent a potential alternative to chemical insecticides in some markets.

1. Introduction

Fennel is an annual, biennial or perennial plant, depending on the variety, belonging to Apiaceae family and is native to the Mediterranean area (Barros et al., 2010). It has been cultivated and introduced into many regions outside that zone; it is grown commercially in some of them, such as Russia, India, China and Japan (Damjanovic et al., 2005). Mature fennel fruit and essential oil are used as flavoring agents in food products such as liqueurs, bread, pickles, pastries, and cheese. They are also used as a constituent in cosmetic and pharmaceutical products (Telci et al., 2009). Many researches have been carried out on fennel oils composition from various origins

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Table 1 Soaking time, extraction time, and ratio of solid to liquid effect's on *Foeniculum vulgare* seed essential oil composition.

Compound	Composition (%)														
	Soaking time (h)[a]					Extraction time (h)[b]					Ratio of solid to liquid (g/L)[c]				
	0	6	12	18	24	2	3	4	6	8	50	150	200	250	300
α-Pinene	0.59	0.55	0.63	0.53	0.60	0.59	0.78	0.82	0.89	0.93	0.97	1.45	1.27	1.05	1.22
Camphene	0.09	0.09	0.10	0.09	0.10	0.09	0.12	0.13	0.14	0.14	0.16	0.22	0.19	0.16	0.19
β-Phellandrene	0.23	0.22	0.24	0.21	0.24	0.23	0.27	0.27	0.28	0.28	0.25	0.34	0.30	0.26	0.28
β-Myrcene	0.39	0.35	0.41	0.35	0.42	0.39	0.43	0.44	0.46	0.45	0.60	0.78	0.74	0.64	0.69
α-Phellandrene	0.08	0.08	0.08	0.07	0.09	0.08	0.09	0.09	0.10	0.09	0.11	0.16	0.13	0.12	0.13
Benzene,1-methyl-4-(1-methylethyl)-	0.07	0.08	0.08	0.08	0.07	0.07	0.09	0.08	0.08	0.09	0.07	tr	0.12	0.07	0.08
Limonene	5.82	5.15	5.72	5.01	5.81	5.82	6.19	6.12	6.43	6.68	5.79	7.38	6.80	6.05	6.37
1,3,6-Octatriene,3,7-dimethyl-, (E)-	0.37	0.31	0.37	0.31	0.39	0.37	0.38	0.39	0.41	0.41	0.47	0.61	0.58	0.52	0.54
3-Carene	0.10	0.09	0.10	0.09	0.11	0.10	0.11	0.11	0.12	0.12	0.15	0.19	0.17	0.16	0.17
Fenchone	11.13	11.39	12.24	11.77	11.88	11.13	11.35	11.04	10.04	10.29	12.65	14.17	13.26	12.58	12.93
Camphor	0.20	0.21	0.24	0.23	0.23	0.20	0.21	0.21	0.18	0.18	0.20	0.21	0.20	0.20	0.21
Estragol	4.75	5.01	5.16	5.13	5.03	4.75	4.95	4.93	4.58	4.93	3.66	3.49	3.43	3.35	3.41
Fenchyl acetate	0.12	0.09	0.10	0.09	0.12	0.12	0.11	0.11	0.13	0.12	0.16	0.38	0.53	0.08	0.14
trans-Anethol	76.00	76.37	74.50	76.01	73.99	76.00	74.86	75.16	75.97	75.11	74.27	70.13	71.84	74.34	72.86
Apiol	0.03	–	–	–	0.03	0.03	–	–	0.04	0.03	0.04	–	–	–	0.05
n-Hexadecanoic acid	–	–	–	–	0.06	–	0.04	0.06	0.09	0.12	0.08	–	–	–	0.07
Yield (%)	1.003	0.850	0.946	0.922	0.934	0.725	1.050	1.174	1.231	1.240	0.811	1.201	1.072	1.219	1.264

–: Not detected.

[a] The ration solid to liquid was set at 100 g/L and the extraction time was of 2 h.

[b] The ration solid to liquid was of 100 g/L without soaking the seeds.

[c] The extraction time was of 3 h without soaking the seeds.

(Napoli et al., 2010). It has been found that the principal constituents are *trans*-anethol and fenchone (Akgül and Bayrak, 1988).

Herbs and spices are amongst the most important targets to search for natural antimicrobials and antioxidants from the point of view of safety (Singh et al., 2006). They may provide an alternative to currently used pest control agent (Prajapati et al., 2005). In East Asia, work has been done with plant extracts and essential oils to control stored food mites (Lee et al., 2006). Other works have shown that essential oil could be used also as herbicides like pine oil, clove oil (Matran®), lemongrass oil and citronella oil (Dayan et al., 2009).

Insect vectors, especially mosquitoes are responsible for spreading serious human diseases. *Culex pipiens* mosquitoes are well known vectors of several disease causing pathogens. They are vectors of west Nile virus and an important pest to humans, causing allergic responses that include local skin reaction and systemic reactions such as angioedema, and urticaria (Cheng et al., 2008). Most of the mosquito control programmes target the larval stage in their breeding sites with larvicides, because adulticides may only reduce the adult population temporarily.

Essential oils have a broad spectrum of bioactivity because of the presence of several active ingredients that act through several modes (Liu et al., 2006). Their lipophilic nature facilitates them to interfere with basic metabolic, biochemical, physiological and behavioural functions of insects. *Foeniculum vulgare* is an aromatic plant widespread in Algeria. The essential oils, compounds and their antimicrobial, insecticidal as well as repellent activities of this medicinal plant have been reported (Han et al., 2006; Villalobos and Robledo, 1998). They have the potential of being acute ovicidal, fumigant, insect growth regulatory and insecticidal against various insect species (de Mendonca et al., 2005). With increasing problems of toxicity to non-target organisms and mosquitoes resistance to synthetic insecticides, interest in natural or botanical insecticides has been revived (Seyoum et al., 2002) to find alternatives to these synthetic pesticides. Botanical pesticides are effective, environment-friendly, easily biodegradable and also inexpensive (Dharmagadda et al., 2005). Therefore, the objective of the current study was to optimize the extraction procedure (soaking, extraction time and mass/volume ratio) and to discuss the yields and the chemical composition variations. In addition, for the optimum hydrodistillation parameters the larvicidal toxicity of *F. vulgare* seeds essential oil was determined against the second and fourth instars larvae and pupae of *C. pipiens*, the most widespread and abundant mosquito species in Algiers city.

2. Materials and methods

2.1. Plant material

F. vulgare seeds (5 kg) were collected from cultivated plants in Sétif region (Eastern Algeria, 1096 m above sea level) during May 2007. Only full green seeds are harvested.

2.2. Essential oil isolation

Dried *F. vulgare* seed were subjected to hydrodistillation in Clevenger-type apparatus. The resulting essential oil was dried over anhydrous sodium sulfate and stored at +4 °C until use. The effect of three extraction parameters on the yield and chemical composition of the extracted oil was studied. The considered parameters are: soaking time (0, 6, 12, 18 and 12 h), extraction time (2, 3, 4, 6 and 8 h) and the ratio of solid to liquid (50, 100, 150, 200, 250 and 300 g/L). On the basis of single-factor test, the optimum ratio of solid to liquid after the optimum soaking time was placed in a 4 L round bottomed flask. The flask was connected to a hydrodistillation apparatus (Clevenger-type apparatus) and the water was boiled for the optimum extraction time. The yield of essential oil was calculated by three extractions time.

2.3. GC–FID and GC–MS analysis

Analytical gas chromatography was carried out on a Hewlett–Packard 6890 gas chromatograph equipped with a flame ionization detector. An apolar HP-5 column (30 m × 0.32 mm, 0.25 μm film thickness) was used. The flow of the carrier gas (Helium) was 1 mL/min. The split ratio was 50:1. The analysis was performed using the following temperature program: oven isotherm at 40 °C for 8 min, from 40 to 250 °C at the rate of 2 °C/min and isotherm at 250 °C for 5 min. Injector and detector temperatures were held at 250 °C. The injection volume

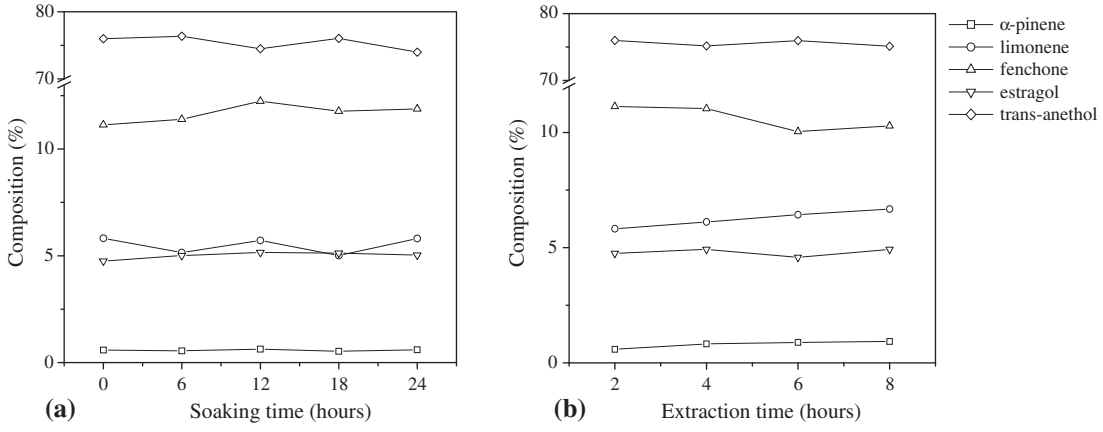

Figure 1 Soaking (a) and extraction (b) times effects on majors *Foeniculum vulgare* seed essential oil components.

was 0.5 µL. GC–MS analysis was performed on a gas chromatograph HP 6890 coupled with an HP 5973 mass spectrometer with electron impact ionization (70 eV). An HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness) was used. GC conditions were the same as described above.

The identification of the essential oil constituents was made based on matching retention time and mass spectrum of unknown components with the identified standards. The retention indices were calculated, for all volatile constituents using a homologous series of *n*-alkanes C_7–C_{27}. Essential oil components are reported as a relative percent of the total oil by peak area.

2.4. Larvicidal investigations

The larvicidal activity assays were developed using a known methodology (WHO, 2005). Concentrations ranging from 1 to 250 mg/L of the dissolved emulsified oil in water were prepared and three replicates were run for each concentration. Control tests were carried out in parallel, using the emulsifier solution without oil. Number of dead larvae was counted after 0.5, 1, 2, 4 and 24 h of exposure and the percentage mortality was reported from the average of three replicates.

3. Results and discussion

3.1. Chemical composition of the essential oils

The qualitative and semi-quantitative essential oil compositions for the three hydrodistillation conditions are presented in Table 1. Compounds are listed in order of their elution on the HP-5MS column. Results have shown that soaking time had no significant influence on *F. vulgare* essential oil yield. We calculated a yield of 0.93 ± 0.07%, this is still true for the considered extraction time and ration solid to liquid, 2 h and 100 g/L, respectively. Through Fig. 1a we notice that soaking time has no influence on the chemical composition. For the studied *F. vulgare* samples we calculate a variation of ±1.6% for *trans*-anethol, ±0.8% for fenchone, ±0.5% for limonene, ±0.3% for estragol, and ±0.05% for α-pinene. For the second studied parameter we perceive that *F. vulgare* essential oil yield increases parallely with the extraction time. We calculate a yield of 0.70% after 2 h versus 1.20% after 6 h extraction time. The four major compound relative chemical variations were presented in Fig. 1b. For the present study, 6 h extraction time was fit to achieve the effective yield. The ration solid to liquid study allowed us to say that there is no

Table 2 Main *F. vulgare* seed essential oil components (in %) as reported in the literature.

Compound (%)	Our study[a]	Akgül and Bayrak (1988)	Telci et al. (2009)	Damjanovic et al. (2005)	Singh et al. (2006)	Fang et al. (2006)
α-Thujene	–	–	–	0.05	tr	–
α-Pinene	1.22	3.18	0.12	2.81	0.2	0.42
Camphene	0.19	0.93	–	0.34	tr	–
Sabinene	–	–	–	0.56	tr	–
β-Pinene	–	1.17	0.05	–	0.2	0.08
β-Phellandrene	0.28	–	0.01	–	–	0.26
β-Myrcene	0.69	1.32	0.18	1.68	0.1	0.01
α-Phellandrene	0.13	1.15	tr	0.73	–	0.33
Benzene,1-methyl-4-(1-methylethyl)-	0.08	–	–	–	–	–
p-Cymene	–	1.78	–	0.28	3.1	–
Limonene	6.37	2.87	2.96	3.15	3.1	6.29
1,8-Cineol	–	–	–	1.20	0.1	0.53
1,3,6-Octatriene, 3,7-dimethyl-, (E)-	0.54	–	–	–	–	–
3-Carene	0.17	–	–	–	–	0.11
β-Ocimene	–	–	0.83	0.22	–	–
γ-Terpinene	–	0.83	–	1.05	2.1	2.35
Fenchone	12.93	13.85	1.19	20.30	8.6	3.28
Linalool	–	–	–	–	1.2	–
Camphor	0.21	–	tr	–	0.3	0.09
Estragol	3.41	4.96	5.16	4.90	4.7	5.95
Fenchyl acetate	0.14	–	0.13	–	0.2	0.11
trans-Anethol	72.86	64.71	87.85	62.00	70.1	73.20
Germacene D	–	–	–	0.18	–	–
Anisketone	–	1.12	–	–	–	–
Apiol	0.05	–	–	–	–	–
4-Methoxy-benzaldehyde	–	–	–	–	–	1.99
n-Hexadecanoic acid	0.07	–	–	–	–	–
Yield (%)	1.284	5.6	–	–	–	–
Density	0.961	–	–	–	–	–
Refraction indice	1.537	–	–	–	–	–

tr: Traces (< 0.05%); –: not detected.

[a] The extraction time was set at 6 h, a ration solid to liquid was equal to 300 g/L and the seeds were not soaked.

significant yield variation for this parameter, and the observed yield fluctuations might be the result of the non-uniform grain shape distribution. Therefore, an extraction time of 6 h and a ration solid to liquid of 300 g/L were the conditions to get 72.86 ± 2.00% of *trans*-anethol toward fenchone and limonene without soaking the seeds.

In addition, we summarize in Table 2 previous investigations of authors on the analysis of the volatile oils from fennel. The

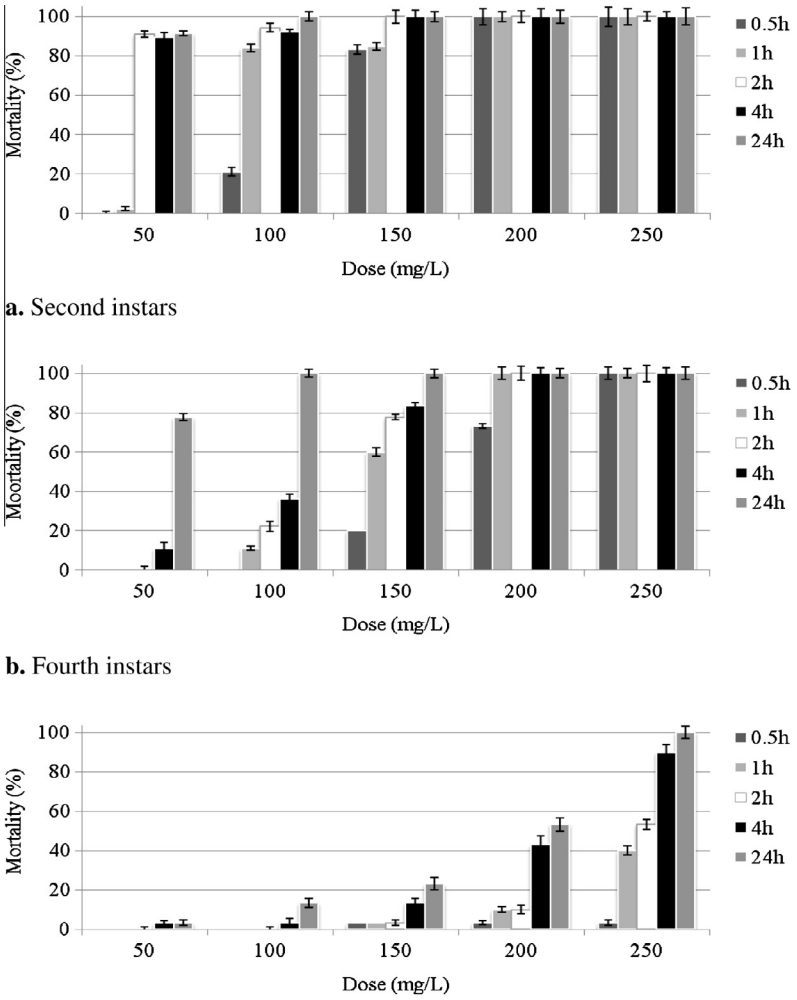

Figure 2 Mortality of *Culex pipiens* resulting from 0.5, 1, 2, 4 and 24 h after treatment with different dosages of *Foeniculum vulgare* seed essential oil. Means (n = 3) using 50 larvae per replicate.

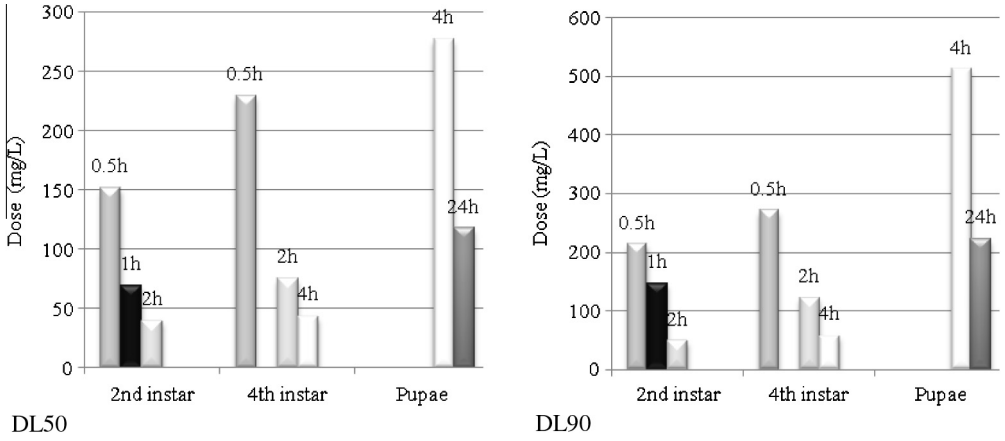

Figure 3 Lethal dosages of *Foeniculum vulgare* seed essential oil for 0.5, 1, 2, 4 and 24 h exposure time to record 50% and 90% mortality of *Culex pipiens* second, four instars and pupae.

chemical composition of the examined Algerian *F. vulgare* seed oil was different from that observed with Turkish (Akgül and Bayrak, 1988; Telci et al., 2009), Serbian (Damjanovic et al., 2005), Indian (Singh et al., 2006), and Chinese (Fang et al., 2006) materials. The similar oil constituents are α-pinene (3.18%, Akgül and Bayrak, 1988), β-myrcene (1.68%, Damjanovic et al., 2005), limonene (6.37%, our work), fenchone (20.30%, Damjanovic et al., 2005), estragol (5.95%, Fang et al., 2006) and *trans*-anethol (87.85%, Telci et al., 2009). However, in the studied Algerian *F. vulgare* seed oil, β-pinene (1.17%, Akgül and Bayrak, 1988), 1,8-cineol (1.20%, Damjanovic et al., 2005), β-ocimene (0.83%, Telci et al., 2009), γ-terpinene (1.05%, Damjanovic et al., 2005), linalool (1.2%, Singh et al., 2006), anisketone (1.12%, Akgül and Bayrak, 1988), and 4-methoxy-benzaldehyde (1.99%, Fang et al., 2006) are not detected.

3.2. Mosquito larvicidal effect of F. vulgare seed essential oil

The essential oil was subjected to laboratory bioassay studies on second, fourth instars and pupae. Results of *C. pipiens* mortalities (Fig. 2) show that dose and time increases amplify the observed mortalities percentages. As a general observation, we perceive that for a dosage of 50 mg/L the mortality value doubles when we pass from 2 to 4 h of exposure for the two larvae considered stages.

Fig. 2a shows that the second instars are very sensitive and the use of the concentration at 250 mg/L ensure 90% mortality after only 30 min treatment time. Moreover, we notice that we reach the 100% mortality for all the studied dosage after 4 h treatment time. The total elimination of the second instars is also insured after 2 h for the concentration at 150 mg/L. The fourth instars are less sensitive (Fig. 2b). To register a 100% mortality using the concentration at 150 mg/L, 4 h treatment time is needed. Besides, to record 100% mortality after 2 h of exposure the use of the concentration at 250 mg/L is required. Pupae are more resistant. Mortalities still less than 23% after 2 h of exposure (Fig. 2c). The use of the concentration at 200 mg/L guarantees 40% mortality after 4 h of exposure. Results became promising after 24 h of exposure. Fig. 3 shows lethal concentrations that insure 50% and 90% mortalities. These results could be useful in search of newer, safer and more effective natural compounds as larvicides.

4. Conclusion

In this study it was observed that *F. vulgare* seed essential oil has showed larvicidal deterrent activity against the mosquito *C. pipiens*. The activity of this plant seed oil may be due to the presence of *trans*-anethol as the main compound. *F. vulgare* seed essential oil can be suggested as natural larvicidal for controlling *C. pipiens* mosquito in Algeria. Nevertheless, field trials with suitable formulations need to be carried out to further assess the efficacies of essential oil as larvicides.

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