Original Article

Pediculicidal Activity of *Foeniculum vulgare* Essential Oil in Treatment of *Pediculus capitis* as a Public Health Problem

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Abstract

Background: Pediculosis, caused by *Pediculus* spp is an important public health problem in urban and rural areas around the world. Natural compounds such as plant essential oils (EOs) have been suggested as a potential alternative for insect pest control recently. The purpose of this study was to investigate the toxicity of *Foeniculum vulgare* essential oil against the head louse, *Pediculus capitis* under laboratory conditions.

Methods: Fennel essential oil components were analyzed using GC-mass apparatus. Immersion and contact filter paper bioassays were used to evaluate fennel essential oil toxicity at the two-fold concentrations of 2.5, 5, 10, 20, and 40% against nit and nymph/adult stages of the head louse.

Results: Trans-anethole, α-Thujone, and limonene, which consisted of 76.08%, 10.37%, and 5.34% were the most components of fennel oil respectively. The LC_{50} values for the adult /nymphs were 11.5, 6.4, 3.9, 3.1 and 2.5% and LC_{99} values were 29.5, 15.2, 12.8, 10.8, and 7.4% at 10, 20, 30, 45 and 60 minutes after exposure respectively. The lethal times (LT_{50}) for adults/nymphs were 5.2, 8.1, 9.5, 20.5, and 45.8 minutes and LT_{99} were 138.6, 91.3, 23.8, 21.7, and 13.9 minutes in the concentrations of 2.5, 5, 10, 20 and 40%, respectively. LC_{50} and LC_{99} values were 2.32% and 7.36% after 5 days for the eggs.

Conclusion: Fennel essential oil at the concentration of 15% after 20min is suggested to develop as an appropriate formulation to evaluate in clinical trials.

Keywords: Head lice; *Pediculus capitis*; Pediculusis; *Foeniculum vulgare*

Introduction

Pediculus capitis, a blood-sucking insect, belonging to the order Anoplura, the family Pediculidae, completes its life cycle on the human head as obligatory ectoparasite (1, 2). The insect feeds several times during the day every two-three hour. Today, head louse is one of the most important health problems around the world from a village to urban areas (1–3). The main mode of transmission of this infestation is close personal contact and sharing of personal stuff (1–3). Head louse infestation is a global health problem that often infests children in school ages (4 to 13 years), teachers and family members, and other people who

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will be in contact with the infected children (1–5). It is higher in girls and women (2, 3).

According to WHO reports approximately 6–12 million people are annually infected by head louse in different areas of the world. This public health problem is prevalent in many developing countries where the primary healthcare program of WHO is inefficient and haphazard. The prevalence of this infestation worldwide is variable from 0.7–50% and between less than 5% to over 40% among school children, respectively (1–3).

The highest prevalence rate of this infestation was seen in Central and South America (33%), followed by Africa (31%), Australia (19%), Asia (18%), North America (8%), and Europe (5%) (6).

Epidemiological studies in schools in various countries have shown the different frequencies of pediculosis; 13.6% in Mexico, 26.6% in Jordan, 15.3% in South Africa, 23.32% in Thailand, 26.4% in Nigeria, and 28.3% in England (1–3).

Head louse infestation in different regions of Iran has been reported as less than 6% to 30% (4). Varied prevalence rates have been observed in different provinces of Iran such as 4% in Urmia, 13.5% in Hamedan, 1.8% in Kerman, 4.7% in Sanandaj, 7.6% in Qom Province, 27% in Sistan-Balochistan Province, and 0.47% in Isfahan (1–4).

Pediculus capitis infestation is increasing in some areas in Iran along with other communicable diseases (5). Growth of population, people's immigration from villages to cities, marginalization, and the establishment of satellite settlements with minimal health facilities and welfare services can be the reasons for this increase (5). Although head louse does not transmit any disease to humans, it causes problems such as itching, skin lesions, lymph nodes, and secondary fungal and bacterial infections, including yellow ulcers in severe infestation (6–8). It can be caused anemia, particularly in children, headaches, insomnia, bad morals, restlessness, and decentralization, especially in children, social embarrassment, isolation, and mental stress (1–8).

Physical methods such as combing, separating hair lice from the hair, and scrubbing hair have been used to remove head louse infestation in the past (9, 10). Accurate differentiation of nits and hair casts plays important role in treating *P. capitis* (11). Nowadays, anti-pediculosis compounds are recommended to treat the infestation (9, 10). Chemical control, as the main treatment for head lice, involves the use of a wide range of synthetic neurotoxic insecticides such as Permethrine %1, Malathion 0.5%, Lindan 1%, Permethrin 5%, Crotamiton 10%, Ivermectin 0.5% , Spinodad 0.9%, Pyrethrins plus piperonal butoxide and Benzyl alcohol 5% (9, 10). Most of these compounds may have harmful effects on the patients (10). The high prices of anti-lice products and lice resistance concerns in several countries, including Iran (5, 7). Therefore, today, the development of new anti-lice combinations with higher safety and performance is considered a serious necessity.

Alternative control compounds with novel mode of action, low mammalian toxicity and harmless environmental impact are needed to be developed in order to prevent and control vector- borne diseases. It seems the plant based on products and their main components, such as meutropoinids are good alternatives to chemical insecticides because of easily their extracted, biodegradability and little toxicity against mammals (12). Also, they are effective against a wide range of insect pests, including head lice, and unlike chemical pesticides, the problem of resistance development is occurring slowly (12, 13).

Many of these compounds are found in the markets in unusual products offered as pediculicides without proper evaluation (14). The anti-lice properties of the plant compounds may be enhanced by their lipophilic performance and cause better penetration and greater bioavailability in the insect body (15, 16). Several factors which include the lipophilicity of the prod-

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ucts, the rate of diffusion through the cuticle and some physicochemical variables such as the density and the molecular structure of the EO components may affect the penetration rate and finally, contact toxicity of the EOs (18-19). So far, numerous plant essential oils have been studied to determine their pediculicidal properties against head lice around the world (8, 13–26).

Fennel (*Foeniculum vulgare*) is an aromatic herb of the family Apiaceae. This plant is known as a native plant in the Mediterranean and Southern Europe, and warm weather is favorable for its growth. It is distributed in different parts of Iran, including Gorgan, northern Manjil, Baluchistan and Azerbaijan (27). Fennel is one of the medicinal plants that have been introduced in Iran's herbal pharmacopoeia (27), which is known for its medicinal properties, including anti-nausea, digestibility, and diuretic properties. Different parts of the fennel plant are considered for essential oil extraction. There are more than 30 components in fennel essential oils, the most important of which are trans-anethole, funchon, limonene, α-pinene and estragole (28–30). The main fennel oils have acaricidal, anti-fungal, antibacterial properties, and have recently been shown to have a repellent effect against insects (28–30). Its efficiency has been proven against insect-borne diseases such as the mosquitoes *Culex pipiens* and *Aedes aegypti* (29, 30). The aim of this study was to determine the toxicity of *F. vulgare* essential oil against head lice under laboratory conditions.

Materials and Methods

Essential oil extraction

The fennel plant was harvested from Razan area of Hamedan Province during the harvesting season in late summer and early autumn. The dried seed was used for essential oil extraction (28–30). The seed was crushed and powdered with an electric mill and was extracted by the Clevenger apparatus by water distillation. For this purpose, every 100 to 150g of crushed fennel seed was extracted for 4 hours using a Clevenger apparatus. Extracted essential oils were stored in the refrigerator at 4 °C and in the dark glass until the test was performed.

Determination of Essential Oil Components

Gas-chromatography-mass spectrometer (GC-MS) was used for the analysis and identification of fennel essential oil components (GC Agilent 7890, MS Agilent 5975). It was equipped with HP–5MS column (30m× 0.25 mm× 0.25μm). For this purpose, the essential oil samples were first rehydrated with sodium sulfate (Na So4) and diluted with dichloromethane, which was especially spectrophotometric. Then 0.2μl of diluted oil was taken by the micro sampler and injected into the GC-MS apparatus. The essential oil constituents were identified by comparing their retention indices, and mass spectra fragmentation with those in a stored Wiley 7n.1 mass computer library and those of the National Institute of Standards and Technology (NIST).

Bioassay tests

Adults, nymphs, and eggs of *P. capitis* were collected from the head of children 6–13 years old, who attended primary schools in Karoon County, Khuzestan Province from three schools over a 2-month period. The children were not previously treated with anti-lice products for at least 1 month and the head lice were collected using a fine- toothed anti-louse metal comb and transported to the Medical Entomology Laboratory of Ahwaz Jundishapur University of Medical Sciences in glass jars with screw caps.

Adults and nymphs test

Fennel oil was dissolved in ethanol as solvent to obtain the following two-fold doses: 2.5, 5, 10, 20 and 40% (= 0.39, 0.77, 1.5, 3.1, 6.2mg/cm^2). For evaluating pediculicidal activity, the contact bioassay method was used. Petri dishes lined with Wathman no.1 filter pa-

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pers (9cm in diameter) were treated with 1ml of different concentrations of EOs and control filter paper received 1ml of ethanol. After drying of filter papers, batches of 10 adults and 4–5th instar nymphs of head lice were placed on each petri dish, containing a few strands of human hair, and the dishes were covered with lids. Treated and control groups were left in Petri dishes for 15min at 65±5% humidity in the dark chamber and incubated at 35±2 °C and then placed in Petri dishes with untreated filter papers and incubated under mentioned conditions (18, 31). Lice, exposed to the essential oil within 2h after collection. A control test was performed with lice placed on solvent ethanol-impregnated filter paper dried for 5 min. The plates with adults and nymphs of lice were observed by stereomicroscope at 10, 20, 30, 40, and 60min after exposure.

Head louse death was defined as the absence of movement of limbs and gut, with or without stimulation using forceps. Experiments were repeated at least three times (18).

Ovicidal test

Hair-containing eggs were cut from the student's heads with scissors and placed in a container and transported to the laboratory of Medical Entomology for bioassay testing. Ten viable louse eggs were dipped in 1ml of EOs solutions of 2.5, 5, 10, 20 and 40 % (= 6.2, 3.1, 1.5, 0.77 and 0.39mg/cm^2) for 1 minute and then distributed in Petri dishes lined with damped Wathman no.1 filter paper. The control group was treated with ethanol as a solvent. Treated and control groups were incubated at 35±2 °C and 65±5% humidity chamber in darkness. The louse egg hatching was monitored daily under microscopic inspection until 7 days after hatching of the control group. Louse eggs with closed operculum and nymphs inside were the criterion for embryo mortality. Experiments were repeated, at least, three times (14–16, 18).

Data Analysis

The probit regression model was used for determining lethal doses **(**LD_{50} and LD_{99}) and lethal times (LT_{50} and LT_{99}). The P-value and χ^2-tests were used to assess the significance and goodness of fit to the probit regression models, respectively. The mortality means with fennel oil were corrected with Abbott`s formula using natural mortality data.

Abbot`s formula (Abbot):

$$\frac{\text{Treated Mortality \%} - \text{Control Mortality \%}}{100 - \text{Control Mortality \%}} \times 100$$

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software (version 16). A p-value of less than 0.05 was considered statistically significant.

Results

Essential oil extracted

An average of 1.8ml of essential oil was extracted from 150 grams of fennel seed. The density of 1ml of fennel essential oil was calculated to be 0.97g/ml. Approximately 19 main compounds were identified in the fennel essential oil, the highest amount was related to trans-anethole (76.08%), α-thujoneα (10.37%), d1-limonene (5.34%) respectively and then the combination of methyl chavicol (3.55%) (Estragol) (Table 1).

Bioassay test Results

The contact toxicity of fennel essential oil against head lice resulted in significant differences in mortality means over the times of 10, 20, 30, 45 and 60 minutes in a constant dose ($F= 154.528$, $df= 4$, $P< 0.001$). In other words, the effect of time in the means' mortality was significant, so the mortality increases with time increasing. Significant differences were observed between fennel essential oil doses, i.e., the effect of dose was significantly on the head lice mortality means ($F= 265.658$, $df= 5$, $P< 0.001$) and the mortality increased with essential oil increasing dose (Table 2).

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Probit analysis of head lice mortality rates (adult/nymph) at different times is presented in Table 3. As shown, the P values of the model were < 0.05 at different times which indicates that the data fit the Probit model. Also, heterogeneity factors are < 1 which confirms the data accuracy (Table 3). When the heterogeneity factor (which equals the chi-square divided by degrees of freedom) is > 1, a plot of the data should be examined because the data do not fit the model (32).

The concentrations need to eliminate 50% and 99% of adult/nymph stages at different times are presented in Table 3. With increasing time from 10min to 60min, LC_{50} values decreased from 16.05 to 7.38% and LC_{99} changed from 39.5 to 2.46%. A comparison of the lethal doses of 50% and 99% of head lice mortality at different times showed no overlap between the confidence limits (CL) of LC_{50} values at 10min with the other times (20, 30, 45 and 60min). So, it can be concluded that the effect of lethal doses on head lice mortality was significantly different. However, there was an overlap between the confidence limits of LC_{50} at 20, 30, 45 and 60 minutes, indicating no significant differences between the 50% lethal doses at these times. Comparison of LC_{99} confidence limits after different time periods showed CL overlaps at 20, 30 and 45 minutes and so there were no significant differences in LC_{99} values at these time periods, but the highest overlap can be seen in the confidence limits of LC_{99} between 20 and 30min and also between 30 and 45min. LC_{99} values at 10 minutes were significantly different from these values at other times due to a lack of overlap in confidence limits. The lethal doses of 50% and 99% of fennel essential oil against head lice nit were 2.3% and 7.4% after 5 days, respectively. Considering the model's P values and the heterogeneity factor calculation which is < 1 also show that the observed data fit the Probit model appropriately.

Head Lice Lethal Time

The times required killing 50% and 99% of head lice in adult and nymph stages exposed to different concentrations of fennel essential oil are shown in Table 4. It would take 13.9, 21.7, 23.8, 91.3 and 138 minutes to kill 99% of head lice at concentrations of 40, 20, 10, 5 and 2.5%, respectively, which indicate an inverse relationship between lethal times and essential oil concentrations. Also, the negative slope values in Table 4 indicate a decrease in lethal times with the concentration increasing of fennel essential oil. A comparison of the confidence limits of LT_{99} at different concentrations also indicates a significantly different between the time of lethality at concentrations of 2.5% and 5% with other lethal times. The 99 % lethal time at a concentration of 10%, 20% and 40% had no significant difference due to their overlap of confidence limit.

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Table 1. Storage time and percentage of essential oil components of fennel using gas chromatography

Fennel essential oil components	Retention time	(%)
α-Pinene	7.896	0.97
Camphene	8.314	0.13
Sabinene	9.041	0.28
β-pinene	9.121	0.06
β-Myrcene	9.562	0.32
1-Phellandrene	9.939	0.11
Cymene	10.546	0.16
d1-Limonene(Limonene)	**10.672**	**5.34**
1,8-Cineole	10.735	0.35
Terpinene	11.564	0.11
α-Thujone	**12.463**	**10.37**
Camphor	14.088	0.21
Methyl chavicol (Estragol)	15.655	3.55
α-Fenchyl acetate	16.657	0.17
Carvone	16.937	0.05
Anisaldehyde	17.218	0.68
Trans-Anethole	18.247	**76.08**
delta-Cadinene	24.187	0.01

Table 2. Mean mortality percentages of head louse at adult/nymph stages exposure to different concentrations of fennel essential oil by contact bioassay

Essential oil concentration (%)	Time after exposure (min)	Mortality mean (%) ± SE
40	10	85±4.0
	20	90±0.0
	30	90±0.0
	45	90±0.0
	60	90±0.0
20	10	67.5±4.8
	20	77.5±4.8
	30	90±0.0
	45	90±0.0
	60	90±0.0
10	10	42.5±4.8
	20	75±2.9
	30	80±5.8
	45	85±2.9
	60	87.5±4.8
5	10	25±4.1
	20	45±2.9
	30	57.5±6.3
	45	65±2.9
	60	80±4.1
2.5	10	6±2.4
	20	20±3.2
	30	34±4
	45	42±3.7
	60	48±3.7

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Table 3. Lethal concentrations (%) of fennel essential oil against adult and nymphs and egg of head lice using filter paper contact

Head lice	Time (min)	LC_{50} (95%CL)	LC_{99} (95%CL)	Slope (±SE)	$\aleph(df)$	P
Adult/ nymph	10	11.1 (9.5-21.6)	29.5 (23.2-36.9)	0.07 (±0.01)	21.4 (22)	<0.001
	20	6.4 (2.8-7.2)	15.2 (13.6-20.3)	0.22 (±0.04)	7.9 (22)	<0.001
	30	3.9 (1.8-5.6)	12.6 (10.6-16.4)	0.26 (±0.02)	18.9 (22)	<0.001
	45	3.1 (1.2-4.6)	10.8 (8.9-13.9)	0.30 (±0.05)	14.4 (22)	<0.001
	60	2.5 (2.1-3.7)	7.4 (5.9-11.7)	0.90 (±0.18)	3.9 (22)	<0.001
Egg	5 days	2.3 (1.5-4.3)	7.4 (6.1-11.9)	0.50 (±0.09)	14 (21)	<0.001

95% CL: 95% confidence Limits of lethal concentrations of 50% and 99% head lice, $\aleph(df)$: chi-square (degree of freedom), P: significance level of Probit model

Table 4. Lethal times (LT_{50} and LT_{99}) of head louse (adult / nymph) at different concentrations of fennel essential oil in vitro (min)

Concentration (%)	LT_{50} (95%CL)	LT_{99} (95%CL)	Slope (±SE)	$\aleph(df)^a$	P
2.5	45.8 (38.9-58.1)	138 (107.3-214.4)	-1.5±0.21	10.1 (18)	<0.001
5	20.5 (12.1-26.2)	91.3 (74.6-110.3)	-0.68±0.2	8.2 (18)	<0.001
10	9.5 (8.8-14.6)	23.8 (18.8-30.2)	-0.52±0.25	10.9 (18)	<0.001
20	8.1 (6.5-17.8)	21.7 (15.5-35)	-0.3±0.19	6.2 (18)	<0.001
40	5.2 (3.6-8.4)	13.9 (9.8-32.5)	-0.37±0.27	12.2 (18)	<0.001

95% CL: 95% confidence Limits of lethal times of 50% and 99% head lice, a: chi-square (degree of freedom), P: significance level of Probit model

Discussion

In this study, the highest percentages of fennel EO components were belonged to trans-anethole, α -thujone, and limonene which constituted of 76.08%, 10.37%, and 5.34%, respectively. This result is consistent with the other findings. Trans-anethole (32% and 30%, respectively), limonene (28% and 18%, respectively) and fenchone (10% in both cases) were the main compounds identified in the fennel EOs from Cape Verde and Portugal, respectively (30). Trans-anethole constituted 72% of the fennel oil composition in the study by Zoubiri et al. 2014 (29). The results of Lee (2004) on the properties of fennel essential oil against two *Dermatophagoides* dust mites and the essential oil constituents showed that the highest percentage of essential oil components were belonged to trans-anethole, fenchone and estragole with 53.2, 14.2, and 12.7%, and fenchone had high lethal activity against dust mites (28).

Fennel essential oil showed appropriate potential for treating the head louse infestation in nit and nymph/adult stages in our study. At the concentration of 10%, it kills 50% and 99% of the adult/ nymph after 9.5 and 24 minutes, respectively. The lethal times of 50% and 99% mortality decreased to 8 and 21.7 minutes at the concentration of 20%. The LC_{50} and LC_{99} values decreased with an increase in exposure times. After 20 minutes, these values were calculated as 6.4 and 15.4%.

Fennel EOs from Cape Verde and Portugal resulted in 99% mortality of *Ae. aegypti* larva at 37.1 and 52.4µl l^{-1}, respectively (30).

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Application of 40 and 60mg.l^{-1} fennel essential oil eliminated 50% and 90% of the second instar larval population of *Culex pipiens* after 2 hours and 4 hours, respectively (29).

Repellency of antitol and estragole components of fennel essential oil has been reported against stored grain pests, *Rhyzopertha dominica*, *Sitophilus zeamais* and *Tribolium confusum*. The estragole type of fennel essential oil showed more repellency than the anethole type against the three studied pests (26). The mentioned studies indicate that the fennel essential oil has toxicity or repel activity against insect pests.

The results of these studies are consistent with our study in terms of the major constituent of fennel essential oil which is trans-anethole and the potential of this essential oil for repelling or killing of insect pests. Differences in the obtained results can be attributed to the pest species, plant phenology affected by the geographical condition and climate as well as the bioassay test.

Adulticidal and ovicidal activity against *P. capitis* has been reported for some essential oils. Many essential oils that are recommended for the treatment of head lice including eucalyptus, rosemary, geranium, tea tree, lemon, and their components were studied for possible adulticide and repellent effects on head and body lice (13–27).

Tea tree (*Melaleuca alternifolia*) and nerolidol essential oils alone and in combination showed that tea oil with 1% concentration caused mortality of 100% of adult/nymph in 30min and had a better effect than nerolidol oil. The toxicity of nerolidol against head louse egg was better than tea essential oil (50% egg lethality at 1% concentration for 5 days). Combining these two substances together killed the entire lice population within 30 minutes (18). The lethal toxicity of wild bergamot, clove, lavender, tea tree, and verbena essential oils was evaluated against adult head lice using impregnated filter paper bioassay method. Clove oil, diluted either in coconut oil or sunflower oil, demonstrated the best adulticidal activity of > 90% mortality within 2h in lice exposure to 30min contact toxicity (14). The LT_{50} values were calculated as 2.5, 8.1, 9.5, 20.5, and 45.8 minutes in the concentration of 40, 20, 10, 5 and 2.5% (equal to 6.2, 3.1, 1.5, 0.77 and 0.39mg/cm^2) respectively in our study.

Thymus vulgaris, *Aloysia polystachya* and *Aoysia citriodora* EOs showed that Thyme essential oil has significant toxicity for adult and egg stages as well as knock down against the eggs and adults of *P. capitis* by fumigant and contact toxicity bioassays. The calculated KT_{50} values for adults at doses of 0.84, 0.63, 0.42 and 0.21mg/cm^2 were 3.93, 6.30, 6.49 and 9.90 minutes in contact bioassay, respectively (22).

Three *Origanum* species essential oils have been shown to decrease the rate of limb, bowel, and abdomen movements of head louse significantly at the concentration of 1%, producing more than 90% mortality after 12h using the adult immersion test for 5min (27).

Nonconformity in the results can be related to differences in the components of the plant essential oil, the head lice susceptibility, and the bioassay method. Variation in insect responses to different essential oils has been studied previously. The qualitative and/or quantitative chemical composition among plant species may be different and it definitely affects the obtained results (25).

Conclusion

Fennel oil is a potent and useful compound for human head louse treatment. Concentrations of 12.6% to 15.2% of this essential oil killed 99% of adult/nymph at 30 and 20 minutes. It also kills 99% head louse nit in 5 days. But further research is necessary to evaluate the safety of this plant EO on human health and to develop appropriate formulation for improving the pediculicidal activity in clinical trials.

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Ethical considerations

This article was approved by AJUMS (Ethical Code IR.AJUMS.REC.1396.418).

Conflict of interest statement

Authors declare that there is no conflict of interest.

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