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Chemical composition of essential oil compounds from the callus of fennel (*Foeniculum vulgare* Miller.)

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Abstract

Fennel (Foeniculum vulgare Miller.), a herbaceous, perennial and aromatic from Apiaceae family, which is used for pharmaceutical, food, health and cosmatic are cultivated in different parts of Iran and much of the world. In this study, the amount of trans-anethole in callus gained from tissue culture of fennel six belonging to different regions of Iran and Turkey have been compared. Tissue culture is a randomized trial. Factor of evaluating in the first node (hypocotyl) has been as explants and hormonal composition of 2,4-D+Kinetin and NAA+BAP was used for callus induction. Callus extracts were extracted by using organic solvent and finally the effective compound was determined, using GC/MS. The results show that the highest percentage of essential oil compounds in callus of E,E 2,4-Decadienal of 46.22% and cineole were 1,8 of the 35.17 percent, respectively. Our results indicate that the derived callus from fennel plants in the MS environment and with herbal hormone has been able to produce volatile compounds.

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Introduction

In vitro culture of cells, tissues or organs provides the accessibility to important secondary metabolites. Production of these metabolites by plant cell and tissue culture has many advantages such as Standardization and improvement of products quality (Zobayed *et al.*, 2004) In addition, Plant cell culture is considered as an effective system for the study of the biological importance of bioactive metabolites in vitro (Yanpaisan *et al.*, 1999). The focus of this study is the amount of active ingredient in the callus. Despite the cost needed to improve biotechnology techniques for producing aromatic compounds, there are several factors that support the idea. Nature-oriented consumers are concerned about the possible side effects of artificial food additives. Thus, natural products are increasingly preferred. In addition to that aromatic compounds of plant tissue culture systems, microbial fermentation or biological transformations should be more natural than their synthetic type. Consumers are also concerned about pesticide and herbicides residues which are commonly found in agricultural food. Consumer acceptance is not the only crucial factor in pushing the industries to find biotechnology ways for producing aromatic compounds. Other stimuli, such as providing raw materials due to erratic weather conditions, seasonal changes, natural disasters or political instability in plant growth areas are also involved (Zhang, 2000).

Materials and Methods

Plant material contains different populations of fennel which are compared in different combinations of growth regulators in a factorial randomized complete block design with six replications. The seeds are first rinsed with distilled water for disinfection. After they are placed in 70% ethanol for a minute and washed with distilled water, they are placed in the sodium hypochlorite solution with 2.5 percent active ingredient for 20 minutes. Then, they are thoroughly rinsed in double-distilled water for three to five times. Seeds are placed in petri dish containing MS medium without growth regulators on the paper bridge for germination and seedling production. It should be noted that all the above procedures are performed under laminar hood in sterile conditions. The above petri dishes are kept in the darkness at 24°C for the germination of fennel seeds. When seedlings grow, the first node (hypocotyl) is used as the explant. Induction of callus from cultured explants on MS medium of 1 mg l -1 2,4-D 1 mg l-1 Kin 1 mg l -1 NAA 1 mg l -1 BAP hormonal treatment was performed on 16 hours of light, 8 hours of darkness (16L:8D) at 24°C. Each one is done several times for four weeks to get the required amount of callus from hypocotyls callus. Extraction with n-hexane was used to extract oil from the callus tissue culture. The amount of trans-anethole and other active compounds of the extract were set using GC/MS device. EXCEL, MSTATC and SPSS were used for statistical analyses.

All calluses were formed of 2,4 D or αNAA regulators and Kinetin (Anzidei *et al.*,1996) For the present study, eugenol, benzyl alcohol, phenyl ethanol and *anisaldehyde* were isolated from the environment purposefully. But anethole was found in none of them. Even after adding instruments such as phenylalanine or after hydrolysis of the extract, there was no anatole (Hunault and Maatar,1995).

Results and discussion

Fennel (*Foeniculum vulgare* Miller.) is one of the most important and most frequently used herbs which is mainly cultivated and extracted to be used in the oil industry, pharmaceutical, food, and cosmetics. This plant is native to the Mediterranean region and is cultivated in many parts of the world (Afify *et al.*, 2011). Fennel belongs to the aromatic perennial umbellifereae family and it is from 200 to 70 cm in height. It has feathery dark green leaves and more or less deep cuts (Dumanoir *et al.*, 1985). Fennel essential oil is composed of more than 30 types of terpinene compounds. Most important of these compounds are trans-anethole, Fenchone, estragole, and Methyl (Yanpaisan *et al.*, 1999). Other compounds found in the essential oil of fennel can be alpha - pinene, camphene, α-Phellandrene, trans-anethole, Fenchone, estragole, anis aldehyde, and some alkaline compounds (Lawless, 1992).

Kirici and Inan (2010) studied the percentage and composition of the essential oils of four populations of Turkish wild fennel and they observed high genetic diversity in terms of amount of the essential oil among population. Percentage of essential oil in these populations was in the range of 1.5% to 4.6%. GC/MS analysis was performed in the study to explore the components of the essential oil and the six original compositions including pinene, myrcene, limonene, Fenchone, estragole and trans-anethole. The amount of these materials differed in different populations and Yumurtal K population among them was proposed to be used in breeding programs due to its high amount of essential oil and trans-anethole.

Complex structure of many secondary metabolites has prevented them to be synthesized. Thus, plant cells are their only source. If these cells can also be cultured like microorganisms, their production can be increased significantly (Hunault and Dumanoir, 1992).

Numerous studies have been conducted on in vitro culture of fennel in different areas. These studies include micropropagation of fennel (Dumanoir *et al*, 1985). Callus formation (Anzidei *et al.* 1996), suspension culture (Hunault *et al.*, 1989) and fennel regeneration especially via somatic embryogenesis (Hunault and Maatar, 1995). Some of these studies indicate an increased percentage of some important compounds of fennel essential oil, especially trans-anethole, in vitro culture using a mixture of plant growth regulators (Afify *et al.*, 2011) In these studies, one or a limited number of genotypes were studied.

Theiler and Kägi (1991) studied two populations of fennel on MS medium containing 2,4-D and 0.3 mg/l kinetin and the result was successful callus induction from Fennel (Scragg, 1989). studied several fennel populations from Germany, Turkey, France, Italy, Turkey, and France and they announced that only populations from Turkey and France produce callus under MS medium containing 88.0 mM 2,4-D and 3.2 mM kinetin. In this study, Hypocotyl explant was recognized as the effective explant on callus induction. Using hypocotyl explants, the researchers examined the fennel organogenesis, and investigated different levels of NAA growth regulator plus kinetin and BA on Francia pernod population. They reported organogenesis in KIN+NAA medium. Most regeneration was produced from 1:1 ratio of these two materials.

Table 1. Constituents of volatile oils obtained from callus tissue culture Fennel.

Name Of Gompound	Retention time	F150	Callus
Pinene	902	2.63	2.35
2 - ß- Pinene	940	0.84	4.71
1,8 Cinneole	1001	-	17.35
Terpinene	1024	-	7.07
Camphor	1107	0.5	5.57
4 Terpineol	1141	0.67	5.78
Terpineol	1153	0.13	6.13
(E,Z) 2,4-Decadienal	1281	-	10.15
(E,E)2,4-Decadienal	1307	-	22.64

Table 2. Percent of the compounds identified in the essential oil of Fennel callus.

Name of compound	F150	Callus
Monoterpene hydrocarbons	15.6	14.13
Oxygenated monoterpene	13.72	34.83
Total monoterpenes	31.08	48.96
Total hydrocarbons	16.67	14.13
Total oxygenated compounds	74.7	67.62
Etc	-	32.79
Of the identified compounds	91.37	91.59

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