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## Comparison of different populations of fennel (*Foeniculum vulgare* Miller.) in terms of chemical compounds in the callus induced from tissue culture

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### Abstract

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Fennel (*Foeniculum vulgare* Miller.) herbaceous, perennial aromatic Apiaceae family is valuable secondary metabolites that are widely used in the food industry. This medicinal plant in the treatment of oral flatulence, indigestion and muscle spasms, as well as enhancing milk is used as a topical antimicrobial used. Seed Fennel After sterilization, the Petri dishes containing MS medium without growth regulators on the bridge of the paper medium, and the temperature 24 ° C with 16 hours light and 8 hours dark placed, after 15 days, the plant height of about 10 cm was the nodes first (hypocotyls) are used as explants. Induction of callus and explants cultured on MS medium with hormonal treatments 2,4-D +Kinetin and NAA+ BAP Factorial based on completely randomized design with six treatments in each population were used and callus weight was measured. In order to identify the compounds present in the extracts from the callus were used method GC/MS. Lorestan population with the highest limonene and Khroslari population with the highest alpha-pinene in the MS (1mg l-1 2,4-D +1mg l-1 Kin) with of hormonal treatments showed.

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## Introduction

Biotechnology comprises a set of techniques that allow the use of the ability and efficiency of cells of *living organisms, including plant, animal, or microbial* for scientific, industrial, and economic purposes. Numerous studies in the field of agricultural biotechnology which have been done in recent years given to its importance and value and they also provided this possibility for crops to use tissue culture and plant cells methods to produce plants of better compatibility and efficient performance based on human needs (Afify et al., 2011). 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., 1996). 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 types (Lawless, 1992). 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 (Hunault and Maatar, 1995).

## Materials and methods

Plant material contain 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 percent 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 (Anzidei et al, 1996). Induction of callus from cultured explants on MS medium of 1mg l<sup>-1</sup> 2,4-D + 1mg l<sup>-1</sup> Kin and 1mg l<sup>-1</sup> NAA + 1mg l<sup>-1</sup> BAP hormonal treatment was performed on 16 hours of light, 8 hours of darkness (16L:8D) at 24 ° C (Afify et al., 2011). 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 was set using GC/MS device. EXCEL, MSTATC and SPSS were used for statistical analyses.

**Table 1.** percentage composition of the oils from callus induced from callus of fennel (*Foeniculum vulgare* Miller.).

Population-Hormonal treatment	Ingredients																		
	$\alpha$ -Pinene	Camphene	Sabynene	$\beta$ -Pinene	Myrcene	P-cymene	Limonene	1,8-Cineole	Terpinene	Camphore	Cis-anethole	Trans-anethole	Estragole	Carene	Fenchone	Fenchyl acetate	Codeine	Morphine	Unborn
izmir=1mg l <sup>-1</sup> NAA+1mg l <sup>-1</sup> BAP	4.55	3.4	1.1	28.37	0	0	2.9	0	0	4.6	0	0	30.5	0	0	14	0	0	11
izmir=1mg l <sup>-1</sup> KIN+1mg l <sup>-1</sup> 2,4-D	39	0	0.41	34.7	0	0	3.2	0	0	7.6	0	0	0	0	0	0	0	0	15
madan=1mg l <sup>-1</sup> NAA+1mg l <sup>-1</sup> BAP	4.1	0	0	7.5	0	42.5	0	0	0	0	0	0	19.0	5.1	0	0	0	0	21
madan=1mg l <sup>-1</sup> KIN+1mg l <sup>-1</sup> 2,4-D	0	0	0	0	0	37.9	4.3	18.6	0	0	0	0	0	2.4	17.7	0	5.6	0	14
hb=1mg l <sup>-1</sup> NAA+1mg l <sup>-1</sup> BAP	0	7.32	7.34	0	0	26.4	0	0	0.8	15.3	0	0	0	5.4	18	0	0	0	19
hb=1mg l <sup>-1</sup> KIN+1mg l <sup>-1</sup> 2,4-D	11.8	4.6	6.8	0	0	20.7	0	0	0.9	14.4	0	0	0.5	4.3	15.2	0	0	0	21
lrean=1mg l <sup>-1</sup> NAA+1mg l <sup>-1</sup> BAP	0	0	3.8	0	7.22	0	32.0	0	0.5	0	0	0	0	9.4	31.6	0	0	0	0
lrean=1mg l <sup>-1</sup> KIN+1mg l <sup>-1</sup> 2,4-D	0	7.8	18.85	0	0	0	53.0	0	0	0	0	0	0	11	6.67	2.7	0	0	0
l=1mg l <sup>-1</sup> NAA+1mg l <sup>-1</sup> BAP	0	6.45	1.2	0	0.94	46.2	0	0.27	0	18.1	0	0	0	3.1	19	0	0	0	4.6
l=1mg l <sup>-1</sup> KIN+1mg l <sup>-1</sup> 2,4-D	0	4.3	0	0	0.16	31.3	0	0.5	0	11.5	0	0	0	0	16.3	0	0	0	3.6
l=1mg l <sup>-1</sup> NAA+1mg l <sup>-1</sup> BAP	0	1.15	0	0	0	16.7	8.6	1.2	0	5.2	36.0	0	9.6	0	1.87	4.5	16.16	34	0
l=1mg l <sup>-1</sup> KIN+1mg l <sup>-1</sup> 2,4-D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

## Results and discussion

Compounds obtained from different treatments in this experiment are  $\alpha$ -Pinene, Camphene, Sabynene,  $\beta$ -Pinene, Myrcene, P-cymene, Limonene, 1,8Cineole, Camphore, Cis-anethole, Trans-anethole, Estragole, Carene, Fenchone, Fenchyl acetate, Codeine and Morphine. According to the results of the GC/MS, extract of studied populations callus, Khorouslory population under 1mg l<sup>-1</sup> 2,4-D + 1mg l<sup>-1</sup> Kin hormonal treatment, had the highest amount of  $\alpha$ -Pinene (39 percent) and Sabynene (7.34 percent), and with 1mg l<sup>-1</sup> NAA + 1mg l<sup>-1</sup> BAP hormonal combination, also had the highest amount of  $\beta$ -Pinene (28.37 percent) and the lowest amount of Estragole (30.55 percent). Population of Lorestan, with 1mg l<sup>-1</sup> NAA + 1mg l<sup>-1</sup> BAP hormonal combination had the highest amount of Limonene (53 percent) and Myrcene (7.22 percent) among the mentioned populations. Trans-anethole was not observed in any of these populations and Izmir was the only population which had Cis-anethole (36.0 percent). Production of anethole has not been observed in most studies that have been conducted on Fennel tissue culture (Kirici et al., 2010). But Afifi et al (2011) observed Trans-anethole in the callus tissue

culture. Furthermore, the population of Izmir, with 1mg l<sup>-1</sup> NAA + 1mg l<sup>-1</sup> BAP (6.16 percent) hormonal combination contains Codeine (16.16 percent) and Morphine (34 percent). Population of Tabriz had the highest amount of P-cymene (46.22 percent), Camphore (18.13 percent), and Carene (11 percent) with 1mg l<sup>-1</sup> NAA + 1mg l<sup>-1</sup> BAP hormonal combinations. Population of Bonab produced the maximum amount of Camphene (32.7 percent) with the same hormonal combination and eventually the population of Hamadan, with 1 mg l<sup>-1</sup> 2,4-D + 1 mg l<sup>-1</sup> Kin hormonal combination had the highest amount of 1,8Cineole (62.18 percent). In a study by Kirici et al (2010), the results of the GC/MS in regenerated plants showed that monoterpene materials including Pinene and Limonene and had the highest percentage (Table 1).

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