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Variation in bioactive compounds and anatomical characteristics of different fennel (*Foeniculum vulgare* Mill.) populations as affected by self-pollination

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Summary

The production of self pollinated plant genotypes could be critical for improving medicinal plants. Pollination in Apiaceae family can also affect secondary metabolites. In this study, 23 fennel populations were used to assess the effect of self pollination on essential oil yield, antioxidant activity (based on three model system), total phenolic (TPC) and flavonoid content (TFC). First, some plant inflorescences were divided in two parts. Then a half was bagged and a second half was permitted for out crossing. The self and outcross pollinated seeds were sown in a randomized complete block design (RCBD) in three replicates. Results revealed that inbreeding led to the increase in the secretory ducts number (9.36%) as well as essential oil yield (25.61%) in all fennel populations. Essential oil yield ranged from 2.4% to 6.4% in seeds produced via out crossing, while it varied from 3.5% to 6.5% in self pollinated ones. Furthermore, self pollination increased TPC (21.66%), TFC (49.40%) and antioxidant activity (6.23%). Among the populations derived from self-pollinated seeds, cv. Tabriz showed the highest TFC (8.4 mg $QUEg^{-1}$ DW) and antioxidant activity (IC_{50} = 83.1 µg/ml), whereas cv. Semirom possessed the highest TPC (150 mg $TAEg^{-1}$ DW). In overall, self-pollination can lead to the populations with higher amount of secondary metabolites.

Introduction

Fennel (*Foeniculum vulgare* Mill) is a species belonging to the Apiaceae family, known and used by human since antiquity. Because of its flavor, it was cultivated in every country surrounding the Mediterranean Sea. It is now grown in the temperate and subtropical areas of Pakistan up to an altitude of 2000 m and is cultivated as an annual crop. Fennel is used in folk medicine for its balsamic, cardiotonic, digestive, lactogogue and tonic properties (Garcia-Jamenz et al., 2000; Patra et al., 2002; Saleha, 2011; Saravanaperumal and Terza, 2012). The essential oil is used in cosmetics and pharmaceutical products. Fennel contains volatile oils, phenolic glycosides, flavonoids, phytosterols, triterpenes and saponins (Ebeed et al., 2010). Fennel is an estrogenic (Annusuya et al., 1988), diuretic, antioxidant and immune booster useful in dyspepsia. It has also bronchodilator effects (Boskabady et al., 2004).

Medicinal plants possess different kinds of valuable metabolites such as essential oil, antioxidants and flavonoids. As known, the biosynthesis of bioactive compounds is highly influenced by various environmental factors such as soil condition (Taarit et al., 2009), light intensity, climate conditions (Mathe et al., 1992), anatomical structure and pollination system (Mediavilla and Steinemann, 1997). Moreover, the production of essential oils in plants is generally associated with the presence of specialized secretory structures such as glandular trichomes and secretory cavities or ducts (Mediavilla and Steinemann, 1997).

Seeds of Apiaceae plants are the most important part in respect to their medicinal properties which can highly affect the biosynthesis of bioactive compounds. Amomg many factors, pollination can highly influence the seed production. Gross et al. (2008) assessed the flowering dynamics and crossability of different populations of fennel (*Foeniclum vulgare* L.). Moreover, previous reports revealed that pollination can influence the essential oil yield in some medicinal plants such as hemp (Mediavilla and Steinemann, 1997).

The study of pollination and production of self pollinated plants could be critical for improving medicinal plants (Faehnrich et al., 2013). Pollination in Apiaceae family can affect the phytochemical as well as anatomical characteristics of plant species (Chu and Liu, 2007). Assessment of pollination system and the effect of out crossing on plant characteristics were reported in some Apiaceae plants such as *Eryngium alpinum* (Gaudeul et al., 2004), *Trachymene incisa* (Yvonney et al., 2007) and *Heracleum mantegazzianum* (Pergl et al., 2006).

One of the most important goals for medicinal plant breeders is to increase the amount of secondary metabolites as well as to achieve a plant with high yield and purity. Among the secondary metabolites, phenolic substances and antioxidants are considered as valuable components in most of the medicinal plants. Previous studies have assessed secondary metabolites of fennel genotypes maintained as outcrossing populations (Aprotosoaie et al., 2010; Semiz et al., 2012; Rahimmalek et al., 2014), but the effect of self-pollination on secondary metabolites has not been evaluated. The present study was aimed to evaluate the effect of self- and outcross pollination on secondary metabolites of fennel populations. Furthermore, the results of this study can provide new insights for breeding fennel regarding higher metabolite content in the next generations. In this context the objectives were (1) to determine the effect of inbreeding on essential oil yield, total flavonoid and phenolic content and antioxidant activity based on three model systems in 23 fennel populations, and (2) to assess variation in some anatomical traits related to secondary metabolites in outcrossed and self-pollinated fennel populations.

Materials and methods

Plant Material

Fennel populations were collected from different geographical regions of Iran. There were nineteen fennel populations and the four others which were from Spain, England, Albania and Poland (Tab. 1). The seeds of the collected samples were sown in a Randomized Block Design (RCBD) in three replicates. For each population, eight individuals and ten umbels per plant were investigated. Each plant inflorescence was divided in two parts. A half (five umbels) was bagged and the second half was permitted for out crossing. The seeds were harvested at full maturity stage when the seeds were completely dried. The self-fertilized seeds were sown in a new RCBD design with three replicates.

The index of self-pollination (%) was measured as the ratio of the number of self-fertilized seeds to cross-fertilized ones. Inbreeding depression (ID) for each trait was also evaluated as follows:

ID (%) = [(Mop – Ms) / Mop] × 100

Mop: Mean value of cross-pollinated population

Ms: Mean value of self-pollinated population

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Tab. 1: Collection site and geographical characteristics of different fennel population

No.	Accession name	Collection site	Altitude [m a.s.l.]	Latitude	Longitude	The minimum temperature (°C)	The maximum temperature (°C)
1	Al1	Karaj, Alborz, Iran	1300	35° 48´ N	51° 00´ E	-20.2	42.1
2	Ya	Yazd, Yazd, Iran	1230	31° 41´ N	53° 49´ E	-11.1	45.2
3	Is1	Isfahan, Isfahan, Iran	1570	32° 39´ N	51° 43´ E	-20.5	42.5
4	Ha1	Nahavand, Hamadan , Iran	1644	34° 52´ N	50° 10´ E	-19.2	36.5
5	Te1	Tehran, Tehran, Iran	1190	36° 52´ N	53° 10´ E	-5.3	12.3
6	Ha2	Hamedan, Hamedan, Iran	1900	34° 52´ N	48° 32´ E	-23.6	20.7
7	Is2	Kashan, Isfahan, Iran	982	51° 35´ N	33° 59´ E	-12.2	44.7
8	Ke1	Pave, Kermanshah, Iran	1530	46° 22´ N	35° 03´ E	-13.4	41.3
9	Az1	Tabriz, Azarbayjan Sharghi, Iran	1561	51° 17´ N	38° 04´ E	-12.4	19.5
10	Te2	Varamin, Tehran, Iran	918	51° 12´ N	34° 12´ E	-11.3	31.6
11	Ke2	Kerman, Kerman, Iran	800	59° 00´ N	30° 16´ E	-7.5	45.7
12	Fa	Shiraz, Fars, Iran	1486	52° 33´ N	29° 36´ E	-10.6	42.5
13	Kh1	Shirvan, Khorasan Shomali, Iran	1160	56° 03´ N	36° 42´ E	-13.8	20.9
14	Kh2	Yasuj, Khozestan, Iran	1870	51° 35´ N	30° 39´ E	-19.2	39.6
15	Is3	Semirom, Isfahan, Iran	2500	51° 34´ N	31° 25´ E	-16.5	42.1
16	Bu	Bushehr, Bushehr, Iran	5	50° 08´ N	27° 17´ E	-6.5	38.1
17	Az2	Ardebil, Azarbayjan Gharbi, Iran	1354	48° 55´ N	37° 45´ E	-33.8	39.2
18	Kh3	Gonabad, Khorasan Razavi, Iran	1150	58° 45´ N	34° 15´ E	-17.3	47.5
19	Kh4	Mashhad, Khorasan Razavi, Iran	979	59° 34´ N	36° 16´ E	-7.7	29.8
20	Sp	Madrid, Spain, Europe	654	40° 40´ N	3° 68´ W	4.5	38.2
21	Al2	Tirana, Albani, Europe	316	41° 32´ N	19° 81´ E	-9.3	29.2
22	Po	Wroclaw, Poland, Europe,	110	51° 11´ N	17° 03´ E	-2.3	32.4
23	En	London, England, United Kingdom	360	51° 30´ N	00° 10´ W	2.1	23.1

Essential oil extraction

Samples were dried at room temperature (25 ± 5 °C) and ground to fine powder using a Moulinex food processor. The essential oil content was estimated based on oil yield (ml / 100 g dry matter) as recommended by the European Pharmacopeia (ver. 8.2, monograph 2.8.12) by collecting the hydro-distilled essential oil with 0.5 ml hexane into the graduated tube (0.01 ml) on the basis of dry matter. All measurements were carried out in triplicates.

For each hydro-distillation run, 50 g of powdered seeds were placed in a round-bottom flask. An aliquot of 400 ml distilled water was added and boiled for 5 hours. Then, the essential oil was collected in a container.

Methanolic extract and evaluation of total phenolics content

The fennel seeds were subjected to air drying. For methanolic extraction, 10 g of seed powder was extracted with 200 ml of 80% methanol. The extraction was carried out by using an orbital shaker (150 rpm) at 25 °C for 24 h. The extracts were filtered through four layers of cheesecloth to remove the fibber debris, and were successively extracted four times. Extraction yield was calculated according to the following formula:

% Extraction yield = m_1-m_2 / m_1 × 100

Where:

m_1= mass in gram of the sample before extraction

m_2= mass in gram of the sample after extraction

The total phenol content was determined colorimetrically using Folin-Ciocalteu reagent as described by Pinelo et al. (2004). In this regard, ten-fold diluted Folin-Ciocalteu reagent (2.5 ml), 7.5% sodium carbonate (2 ml), and methanolic extract (0.5 ml) were mixed; then, after heating at 45 °C for 15 min, the absorbance was measured at 765 nm against a blank. The phenolic content was expressed as tannic acid equivalent per 1g dry weight of sample.

Antioxidant activity

DPPH scavenging activity

The antioxidant activity of the plant seed extracts and standard antioxidant was assessed on the basis of radical scavenging effect on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Braca et al., 2002) purchased from Sigma Chemical Co. (Sigma-Aldrich, Germany). Different concentrations of fennel seed extracts (equivalent to 50, 100, 300 and 500 ppm) were prepared in methanol. The BHT was used as the standard antioxidant in 1-100 µg/ml solutions. Five milliliters of a 0.1 mM methanolic solution of DPPH was mixed with 0.1 ml of sample and standard solutions separately. These solution mixtures were kept in dark for 30 min and after that, optical density was measured at 517 nm. Radical scavenging activity of the extracts was calculated by the following formula:

% Radical scavenging activity = (control OD – sample OD/control OD) × 100.

Methanol (80%) and DPPH solution (0.1 mM, 5 ml) were used separately as blank and control samples, respectively.

Antioxidant activity using the β-carotene-linoleic acid model system

This assay was conducted according to a method developed by GURSOY et al. (2009) with minor modifications. For this evaluation, 0.5 mg β-carotene was dissolved in 1 ml of chloroform and 25 µl linoleic acid and 200 mg Tween 80 were added to prepare the stock solution. The solvent was evaporated by a vacuum evaporator and 100 ml of oxygenated distilled water was added with vigorous shaking. Then, 2.5 ml of reaction mixture was dispersed in test tubes and 0.5 ml of various concentrations (0.5-5 mg per 1 ml) of the extracts and BHT was added and the mixture was incubated at 50 °C. All solvents and chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany). The absorbance was measured at zero time (t = 0) at 490 nm. Absorbance reading was continued at an interval of 25 min until the color of β-carotene disappeared in the control tubes (t = 125 min). Antioxidant activity was expressed as the percentage of inhibition in relation to control according to an equation proposed by KULISIC et al. (2004):

$\%\ \text{Inhibition} = [(A_{A(125)} - A_{C(125)}) / (A_{C(0)} - A_{C(125)})] \times 100.$

$A_{C(0)}$ = Absorbance of the control at the moment of solution preparation.

$A_{C(125)}$ = Absorbance of the control after incubation for 125 minutes.

$A_{A(125)}$ = Absorbance of the sample after incubation for 125 minutes.

Reducing power

The extracts (2.5 ml) and BHT were mixed with 2.5 ml of 1% potassium ferricyanide and 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and incubated at 50 °C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid was added and the mixture was centrifuged at 200 g for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. The absorbance at 700 nm was measured against a blank. The increased absorbance of the reaction mixture has been found to correlate with greater reducing power (ARDESTANI and YAZDANPARAST, 2007).

Total flavonoid content of the extracts

Total flavonoid content was determined spectrophotometrically using a method based on the formation of a Al-flavonoid complex, with some modifications. An aliquot (0.5 ml) of the extract solution was mixed with distilled water (2 ml) and subsequently, with $NaNO_2$ solution (5%, 0.15 ml). After 6 min, $AlCl_3$ solution (10%, 0.15 ml) was added and allowed to stand for further 6 min; thereafter, NaOH solution (4%, 2 ml) was added to the mixture. Immediately, distilled water was added to bring the final volume to 5 ml. Then, the mixture was properly mixed and allowed to stand for 15 min. The intensity of pink color was measured at 510 nm.

Anatomical and seed yield measurements

Some anatomical traits were evaluated in self and outcross fertilized populations including the number of seed coat layers, the number of secretory duct, and thickness of epidermal periclinal wall and the thickness of epidermal anticlinal wall. For preparation of the samples, the schizocarps were cut transversally with a razor blade and stained with aceto-carmine dye solution (EL-OQLAH and KARIM, 1990). Sections were observed under a light microscope coupled to a computer. Secretory ducts were counted at magnification of ×400 in the area of $1.3 \cdot 10^{-6}$ m^2. In fennel, seeds are considered as the most important part for medicinal purposes. So, the seed yield of populations was evaluated in three replicates.

Statistical analysis

ANOVA for all the studied traits was done using SAS ver. 8. Cluster analysis and calculation of correlations among the traits were performed using SPSS ver. 11.

Results and discussion

Anatomical and seed yield variation

As the seeds of fennel possess the highest amount of essential oil yield compared with other parts, some seed-related anatomical traits were evaluated in this study. Analysis of variance showed significant differences among all anatomical characteristics (Tab. 2). Self-pollination lead to the increase in the number of secretory ducts in self-pollination derived populations (Fig. 1), while the other anatomical traits were less influenced. The lowest number of secretory ducts in outcross (16.33) and self (17.33) pollination derived populations belonged to Yazd, while the highest number of secretory duct in outcross (44.33) and self pollination derived populations (46) were observed in the genotype England. Number of seed coat layers was also indicated with high variation among populations. The lowest number of seed coat layers in outcross and self pollination derived population belonged to cv. Shiravan (2.33), cv. Semirom and cv. Albani (1.33), respectively, while the highest number of seed coat layers in outcross (5.66) and self- pollination derived population (5.33) were obtained in cv. Isfahan. Cultivars Nahavand and Semirom had the highest thickness of epidermal anticlinal walls in outcross (5.33 µm) and self- pollination derived populations (4.66 µm), respectively. The highest thickness of epidermal periclinal walls in out-crossed populations was observed in cultivars Kashan, Kerman, Pave, Varamin and Mashhad (4.66 µm), while in self-pollinated ones, the highest values was obtained in cultivars Spain, Poland and Yazd (3.33 µm) (Tab. 2).

Among populations, cv. Gonabad possessed the highest seed yield in outcross pollinated populations, while cultivars Bushehr and Pave showed higher seed yield in self-pollinated populations (Tab. 2). In general, inbreeding decreased seed yield in all of the populations (Tab. 2).

Essential oil variation

Essential oil yield increased as a result of self pollination in populations. The lowest and the highest essential oil yield in out-crossed populations belonged to cultivars Ardebil and Bushehr (2.4%) and cv. Isfahan (6.4%), respectively, while in self-pollinated ones, cv. Shiravan (3.5%) and cv. Hamedan (6.5%) had the lowest and the highest essential oil yield, respectively (Fig. 2). In overall, self-pollination led to an increase of essential oil yield in all populations (Fig. 2). Among populations, cultivars Tehran, Albania and Pave possessed the lowest changes in their essential oil yield during self-pollination, while cultivars Bushehr and Shiravan had the highest and the lowest variations, respectively. In the present study, essential oil yield obtained from Iranian fennel populations showed a range from 2.4% to 6.4%, while the European ones revealed lower essential oil yield compared with Iranian ones ranging from 3.2% in cv. Albania to 6.2% in cv. Poland (Fig. 2).

SALAMI et al. (2015) assessed the seed essential oil composition of different fennel ecotypes (similar ecotypes used in the present study) collected from different regions of Iran and some European countries in outcrossing condition. In their studies, *trans*-anethole was the major compound and ranged from 85.07% in cv. Hamadan to 90.38% in cv. Isfahan. Fenchone and estragol were the major components of the oil, but their amount was much lower than *trans*-anethole. However, evaluation of essential oil composition of self-pollinated fennel populations in the next generations can be suggested for further breeding programs in fennel.

Tab. 2: LSD test for mean comparisons of anatomical characteristics and yield of seed among different outcross and self-pollinated populations and the effect of inbreeding depression (ID) on anatomical characteristics and seed yield of fennel

Ecotypes name	Number of secretory duct		ID of number of secretory duct (%)	Number of periclinal wall layers		ID of number of periclinal wall layers (%)	Thickness of anticlinal wall (µm)		ID of thickness of anticlinal wall (%)	Number of seed coat layers		ID of number of seed coat layers (%)	Seed yield (%)		ID of seed yield (%)
	self	outcross		self	outcross		self	outcross		outcross	self		outcrossed	selfed	
Az1	34.33 ±1.7^{b}	33±1.6^{b}	-4.03	2.33±0.2^{b}	4.33±0.4^{ab}	46.18	2.33±0.2^{b}	3.33±0.3^{b}	30.03	2.33±0.2^{d}	3.33±0.3^{b}	30.03	23.76±1.4^{b}	19.51±1.2^{b}	17.88
Kh2	24±0.8^{c}	21.33 ±0.7^{c}	-12.51	2.66±0.3^{b}	3.33±0.3^{b}	20.12	2.66±0.2^{b}	3.33±0.2$^{3\,b}$	20.12	3±0.3^{c}	4.33±0.4^{ab}	30.71	16.12±1.2^{d}	13.26±0.8^{e}	17.74
Is2	22±0.5^{c}	21±0.8^{c}	-4.76	3.33±0.3^{a}	4.66±0.4^{a}	28.54	3.33±0.3^{ab}	4.33±0.4^{ab}	23.09	3.33±0.3^{c}	3.66±0.3^{b}	9.01	22.21±1.3^{b}	18.15±0.9^{c}	18.28
Is3	28.66±0.8^{bc}	26.66±0.6^{c}	-7.50	1.33±0.2^{cd}	3.33±0.3^{b}	60.06	4.66 ±0.4^{a}	4.66 ±0.4^{ab}	0.00	1.33±0.1^{e}	2.66±0.2^{cd}	50	19.14±1.3^{c}	15.31±0.8^{d}	20.01
Ke2	28 ±0.7^{bc}	26.33±0.7^{c}	-6.34	3.33±0.3^{a}	4.66±0.4^{a}	28.54	1.33±0.1^{c}	2.33±0.2^{c}	42.91	2.66±0.2^{d}	3.33±0.3^{b}	20.12	9.89±0.8^{h}	6.98±0.3^{h}	29.42
Ha2	18.33±0.4^{cd}	17 ±0.4^{cd}	-7.82	1.33±0.2^{cd}	2.33±0.2^{c}	42.91	2.33±0.2^{b}	3.66±0.3^{b}	36.33	1.66±0.1^{e}	2.66±0.2^{cd}	37.59	28.48±1.4^{b}	22.50±1.1^{b}	20.99
Te1	30.33±1.6^{bc}	29.33±0.8^{c}	-3.40	1.66±0.3^{c}	2.33 ±0.2^{c}	28.75	2.33±0.2^{b}	3.66±0.3^{b}	36.33	3.33±0.3^{c}	4.33±0.4^{ab}	23.09	18.70±1.2^{c}	14.39±0.9^{d}	23.04
Bu	22±0.7^{c}	21±0.5^{c}	-4.76	2.66±0.2^{b}	3.66±0.3^{b}	27.32	2.33±0.2^{b}	4.33±0.4^{ab}	46.18	4.33±0.3^{b}	4.66±0.4^{ab}	7.08	32.88±1.6^{ab}	29.43±1.5^{a}	10.49
Ha1	31±1.4^{bc}	29.66±0.7^{c}	-4.51	2.33±0.2^{b}	2.33±0.2^{c}	0.00	4.33 ±0.4^{a}	5.33±0.4^{a}	18.6	3±0.3^{c}	3.33±0.3^{b}	9.90	18.20±0.9^{c}	15.97±0.8^{d}	12.25
Fa	30.33±1.5^{bc}	30±1.4^{b}	-1.1	2.33±0.2^{b}	3.66±0.3^{b}	30.03	2.33±0.2^{b}	2.66±0.2^{c}	12.40	3.33±0.3^{c}	4.66±0.4^{ab}	28.54	11.64±0.5^{g}	9.51±0.4^{g}	18.29
Az2	20.33±0.6^{c}	19.33±0.6^{cd}	-5.17	1.66±0.3^{c}	2.33±0.2^{c}	64.37	1.33±0.1^{c}	2.33±0.2^{c}	42.91	2.33±0.2^{d}	3.33±0.3^{b}	30.03	31.60±1.5^{ab}	27.62±1.3^{ab}	12.59
Al1	21.33 ±0.7^{c}	20.33 ±0.8^{c}	-4.91	2.33±0.2^{b}	3.33±0.3^{b}	50	2.33±0.2^{b}	3.33±0.3^{b}	30.03	1.66±0.1^{e}	2.66±0.2^{cd}	37.59	16.91±0.8^{d}	15.27±0.7^{d}	9.69
Ke1	29.33±0.8^{bc}	27±0.7^{c}	-8.62	3.33±0.3^{a}	4.66±0.4^{a}	-42.91	3.66 ±0.3^{ab}	4.66±0.4^{ab}	21.45	4.33±0.4^{b}	5.33±0.5^{a}	18.76	31.49±1.2^{ab}	29.04±1.2^{a}	7.78
Te2	29.66±0.6^{bc}	27±0.5^{c}	-9.85	3.33±0.2^{a}	4.66±0.4^{a}	23.09	3.33 ±0.3^{ab}	3.66±0.3^{b}	9.01	3.33±0.3^{c}	4.66±0.4^{ab}	28.54	20.96±1.3^{b}	17.92±0.7^{c}	14.50
Is1	27.33±0.7^{c}	19.33±0.6^{cd}	-41.38	3.33±0.2^{a}	2.33±0.2^{c}	30.03	3.66±0.3^{ab}	4.33±0.4^{ab}	15.47	5.33±0.5^{a}	5.66±0.5^{a}	5.83	24.50±1.2^{b}	21.15±0.9^{b}	13.67
Sp	21.33 ±0.5^{c}	20.33±0.6^{c}	-4.91	3.33±0.3^{a}	4.33±0.4^{ab}	23.09	1.33±0.1^{c}	2.33±0.2^{c}	42.91	3.66±0.3^{c}	4.66 ±0.4^{ab}	21.45	14.38±0.7^{e}	12.73±0.5^{e}	11.47
Kh1	29.66±0.6^{bc}	26±0.7^{c}	-14.07	2.33±0.2^{b}	3.33±0.3^{b}	30.03	2.33±0.2^{b}	3.66±0.3^{b}	36.33	2 ±0.2^{d}	2.33±0.2^{cd}	14.16	18.20±0.8^{c}	17.03±0.5^{c}	6.42
Ya	17.33±0.8^{d}	16.33±0.4^{d}	-6.12	3.33±0.3^{a}	4.33±0.4^{ab}	9.01	3.66±0.3^{ab}	4.33±0.4^{ab}	15.47	2.33±0.2^{c}	3.66±0.3^{b}	9.01	20.89±1.2^{b}	19.33±0.6^{b}	7.46
Po	35.66±1.8^{b}	33.33±1.5^{b}	-6.99	3.33±0.2^{a}	3.33±0.3^{b}	0.00	3.33±0.3^{ab}	3.66±0.3^{b}	9.01	3.33±0.3^{c}	4.33±0.4^{ab}	23.09	11.93±0.8^{g}	10.77±0.5^{f}	9.72
En	46±1.9^{a}	44.33±1.8^{a}	-3.76	2.33±0.2^{b}	3.66±0.3^{b}	50	3.33±0.3^{ab}	4.33±0.4^{ab}	23.09	2±0.2^{d}	3.33±0.3^{b}	39.93	9.95±0.5^{h}	6.95±0.2^{h}	30.15
Kh3	23.66±0.7^{c}	29±0.8^{c}	18.41	2.33±0.2^{b}	3.33±0.3^{b}	30.3	2.33±0.2^{b}	3.66±0.3^{b}	36.33	2.33±0.2^{d}	3.33±0.3^{b}	30.03	40.75±1.9^{a}	27.86±1.4^{ab}	31.63
Kh4	29.66±0.8^{bc}	18.33±0.5^{cd}	-61.81	3.33±0.3^{a}	4.66±0.4^{a}	-45.71	1.33±0.1^{c}	2.33±0.2^{c}	42.91	3.33±0.3^{c}	4.66±0.4^{ab}	28.54	18.14±0.9^{c}	15.72±0.7^{d}	13.34
Al2	23.33±0.7^{c}	21.33±0.4^{c}	-9.37	2.66±0.3^{b}	3.33±0.3^{b}	-87.79	1.33 ±0.1^{c}	1.33±0.1^{d}	0.00	1.33±0.1^{e}	3.33±0.3^{b}	60.06	12.36±0.5^{f}	10.01±0.5^{f}	19.01
Mean	27.11	25.09	-9.36	2.61	3.57	6.18	2.66	3.54	25.70	2.85	3.83	25.78	20.56	17.23	16.34

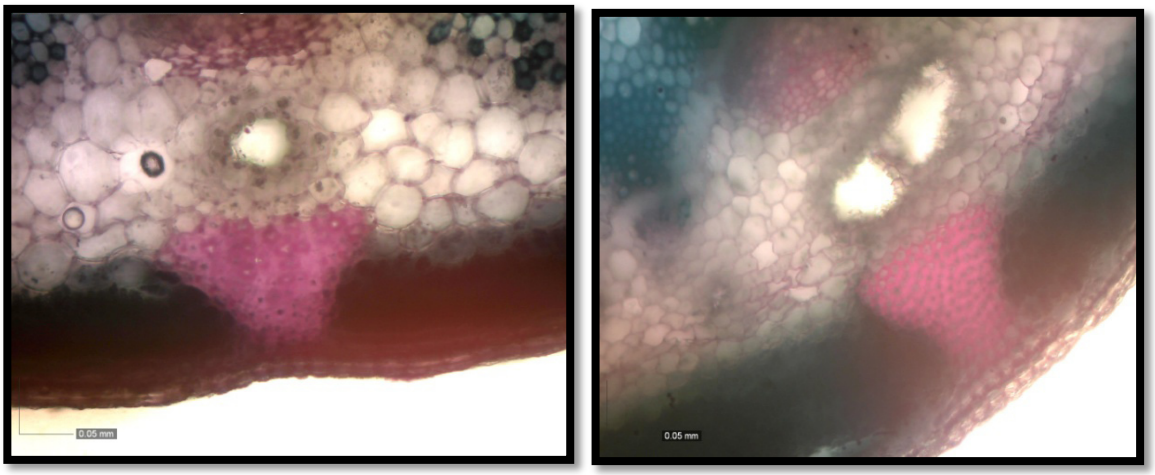


Fig. 1: Number of secretory duct in outcross (A) and self-pollinated (B) fennels.

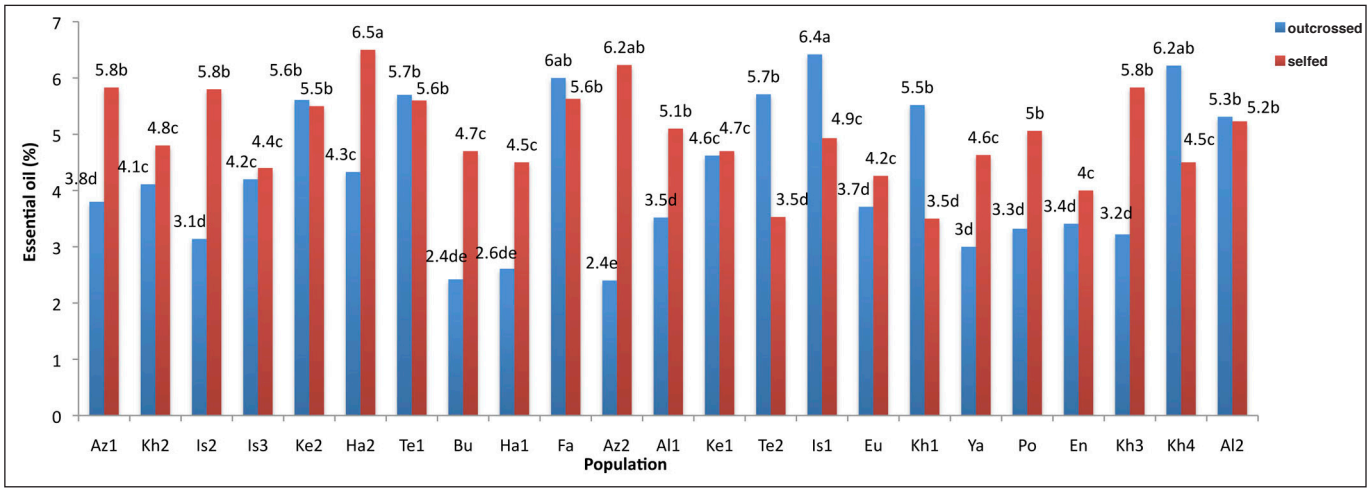


Fig. 2: Essential oil yield in outcross and self-pollinated populations of fennel used in this study.

Total phenolic (TPC) and total flavonoid content (TFC)

Extraction yield, TPC and TFC in selfed and out-crossed populations of fennel are presented in Tab. 3. Most of self-pollinated populations revealed higher extraction yield and TPC compared with out-crossed ones ($P < 0.05$). In self-pollination derived populations, cv. Tabriz (18.73 g/100 g) had the highest extraction yield, while in out-crossed ones, cv. Shiravan (17.73 g/100 g) possessed the highest amount. Cultivar Semirom showed the lowest extraction yield in self-pollinated (5.20 g/100 g) and out-crossed populations (9.99 g/100 g).

TPC in seed extracts of outcross pollination derived populations of fennel ranged from 14.8 to 262.4 mg tannic acid per 1 g dry weight of the samples, while TPC of self-pollinated ones varied from 24.33 to 150 mg TAEg^{-1} DW of the sample. Cultivar Shiravan had the highest TPC in out-crossed populations (262.4 mg TAEg^{-1} DW), while the lowest TPC (14.8 mg TAE g^{-1} DW) was obtained in cv. Tehran. In self-pollinated ones, cultivars Semirom and Pave had the highest (150 mg TAE g^{-1} DW) and the lowest TPC (24.33 mg TAE g^{-1} DW). Similar to TPC, TFC was higher in self-pollinated populations than that found in out-crossed ones (Tab. 3). TFC of outcross pollinated ones ranged from 1.42 to 12.24 mg quercetin per 1 g dry weight of the samples. The lowest and the highest amounts were obtained in cv. Tehran (1.42 mg QUE g^{-1} DW) and cv. Shiravan (mg QUE g^{-1} DW), respectively, while TFC of self-pollinated ones varied from 4.09 to 8.49 mg QUE g^{-1} DW. The lowest and the highest amounts belonged to cv. Tehran (4.09 mg QUE g^{-1} DW) and cv. Tabriz (mg QUE g^{-1} DW), respectively.

DPPH scavenging assay

The ability of different extracts of fennel populations to quench DPPH free radical was measured. The extracts and BHT demonstrated a dose-dependent scavenging activity by reducing DPPH radical (Fig. 3). By plotting the graph of extract concentrations against the scavenging activity, a specific concentration of the sample that needed to provide 50% inhibition (IC_{50}) was calculated. The highest antioxidant activity of seeds was obtained in the self-pollinated populations compared to the out-crossed counterparts. As shown in Tab. 3, the scavenging effect of most of self-pollinated seed extracts was higher than that of out-crossed ones ($P < 0.05$). In overall, self-pollination led to a decrease in IC_{50} in all populations suggesting higher radical scavenging activity than out-cross ones. IC_{50} values ranged from 76.29 to 3062.5 µg/ml for cultivars Shiravan and Tehran, respectively (Fig. 3). The lowest and the highest IC_{50} values belonged to cultivars Tabriz and Mashhad, respectively, while in out-crossed populations, the lowest and the highest IC_{50} values belonged to cv. Shiravan (76.29 µg/ml) and cv. Tehran (3062.5 µg/ml), respectively.

Tab. 3: LSD test for mean comparisons of different secondary metabolite among different outcross and self-pollinated populations and the effect of inbreeding depression (ID) on secondary metabolites of fennel

ID of Essential oil yield	Essential oil yield (%)		ID of Extraction yield (%)	Extraction yield (g/100 g)		ID of Total Phenolics (%)	Total Phenolics (mg/g)		ID of Total Flavonoids (%)	Total Flavonoids (mgQUEg^{-1})		ID of IC_{50}* (%)	IC_{50}* (µg/ml)		Population name	No.
	outcross	self		outcross	self		outcross	self		outcross	self		outcross	self		
-53.42	3.8±0.2^{d}	5.83±0.2^{b}	-31.80	14.2±0.4^{bc}	18.7±0.5^{a}	-26.65	64±1.9^{de}	81±2.1^{b}	-67.45	5±0.5^{c}	8.4±0.8^{a}	61.85	217.9±2.3^{ef}	83.1±2.1^{i}	**Az1**	**1**
-16.78	4.1±0.2^{c}	4.8±0.2^{c}	-0.667	10.8±0.2^{cd}	10.5±0.2^{h}	-82.5	16±0.3^{fg}	29.2±0.5^{h}	-76.66	3.3±0.3^{e}	5.8±0.5^{d}	96.93	2910±5.2^{b}	89.1±2.2^{i}	**Kh2**	**2**
-84.71	3.1±0.1^{d}	5.8±0.3^{b}	-67.22	8.3±0.3^{de}	13.8±0.2^{e}	-70.51	31.2±0.9^{ef}	53.2±1.5^{e}	-83.51	3.7±0.3^{e}	6.7±0.6^{c}	78.20	551.2±3.4^{d}	120.1±2.4^{g}	**Is2**	**3**
-4.76	4.2±0.2^{c}	4.4±0.3^{c}	-92.11	5.2±0.3^{e}	9.9±0.3^{i}	-82.92	82±2.1^{cd}	150±3.1^{a}	-41.00	5.3±0.5^{c}	7.6±0.6^{b}	42.59	209.2±2.3^{fg}	120.1±2.2^{g}	**Is3**	**4**
1.96	5.6±0.3^{b}	5.5±0.2^{b}	-9.41	12.7±0.5^{bc}	13.9±0.2^{e}	-36.75	46.8±1.2^{e}	64±2.1^{d}	-66.20	4.3±0.4^{d}	7.1±0.6^{b}	50.17	300.9±2.9^{e}	149.9±2.1^{g}	**Ke2**	**5**
-50.11	4.3±0.2^{c}	6.5±0.2^{a}	-38.94	9.1±0.2^{d}	12.7±0.2^{f}	-96.02	15.1±0.3^{g}	29.6±0.5^{h}	-76.57	2.8±0.2^{ef}	5±0.5^{d}	93.80	2910.2±5.3^{b}	180.2±2.3^{g}	**Ha2**	**6**
1.75	5.7±0.4^{b}	5.6±0.2^{b}	-55.81	8.4±0.2^{de}	13.1±0.2^{e}	-78.26	13.8±0.2^{gh}	24.6±0.5^{hi}	-80.26	2.2±0.2^{ef}	4±0.4^{e}	93.78	3062.5±5.8^{a}	190.2±2.4^{g}	**Te1**	**7**
-94.21	2.4±0.1^{de}	4.7±0.3^{c}	-20.35	11.2±0.5^{c}	13.5±0.2^{e}	-89.58	28.8±0.5f	54.6±2.1^{e}	-81.98	3.3±0.3^{e}	6±0.6^{c}	61.85	586.2±3.1^{d}	223.6±2.5^{f}	**Bu**	**8**
-72.41	2.6±0.1^{de}	4.5±0.1^{c}	-77.31	8.3±0.3^{de}	14.7±0.3^{d}	-89.02	32.8±0.6^{ef}	62±2.3^{d}	-64.00	4.1±0.4^{d}	6.7±0.6^{c}	40.58	391.9±2.9^{e}	232.8±2.5^{f}	**Ha1**	**9**
6.16	6±0.4^{ab}	5.6±0.2^{b}	22.55	15.9±0.5^{b}	12.3±0.2^{f}	45.48	131.2±2.1^{b}	71.5±2.6^{c}	-36.09	5.6±0.5^{c}	7.7±0.6^{b}	-22.28	114.3±1.9^{g}	139.7±2.1^{g}	**Fa**	**10**
-83.23	2.4±0.1^{e}	6.2±0.3^{ab}	-29.26	10.7±0.4^{cd}	13.8±0.3^{e}	48.17	128±1.3^{bc}	66.3±2.5^{d}	-24.06	5.6±0.5^{c}	6.9±0.6^{c}	-81.70	172.9±1.9^{g}	314.2±2.7^{e}	**Az2**	**11**
-44.88	3.5±0.1^{d}	5.1±0.2^{b}	-34.23	11.8±0.4^{c}	15.8±0.5^{c}	-6.57	60.8±1.5^{de}	64.8±2.3^{d}	-52.88	4.5±0.4^{d}	6.8±0.6^{c}	-36.32	231.6±2.7^{ef}	315.7±2.7^{e}	**Al1**	**12**
-1.73	4.6±0.2^{c}	4.7±0.2^{c}	3.00	12.3±0.5^{bc}	11.9±0.4^{g}	23.96	32±0.6^{ef}	24.3±0.5^{hi}	-59.65	4±0.4^{d}	6.4±0.6^{c}	38.40	534.7±3.5^{d}	329.3±2.7^{e}	**Ke1**	**13**
38.17	5.7±0.3^{b}	3.5±0.1^{d}	-11.45	11.2±0.4^{c}	12.5±0.3^{f}	28.05	72±1.6^{d}	51.8±2.1^{e}	-18.84	5.3±0.5^{c}	6.3±0.5^{c}	-95.35	211.8±2.5^{f}	413.9±2.8^{d}	**Te2**	**14**
23.20	6.4±0.4^{a}	4.9±0.2^{c}	-49.38	9.8±0.3^{d}	14.6±0.4^{d}	-6.93	48±0.7^{e}	51.3±2.3^{e}	-58.95	4.4±0.4^{d}	7±0.6^{b}	-60.57	292.7±2.6^{ef}	470±2.9^{d}	**Is1**	**15**
-14.82	3.7±0.1^{d}	4.2±0.2^{c}	-92.02	9.6±0.3^{d}	18.5±0.5^{ab}	-84.56	16±0.2^{fg}	29.5±0.5^{h}	-89.25	3±0.3^{e}	5.8±0.5^{d}	62.75	1485±4.3^{bc}	553.2±3^{c}	**Sp**	**16**
36.59	5.5±0.3^{b}	3.5±0.1^{d}	29.54	17.3±0.5^{a}	12.2±0.2^{f}	81.78	262.4±2.8^{a}	47.8±1.8^{f}	57.83	14.4±1.2^{a}	6.1±0.6^{c}	-97.52	76.2±0.9^{i}	150.6±2.3^{g}	**Kh1**	**17**
-54.33	3±0.2^{d}	4.6±0.2^{c}	-15.06	12.1±0.5^{bc}	13.9±0.3^{e}	36.67	62.4±1.5^{de}	39.5±1.2^{g}	-35.36	4.7±0.4^{d}	6.4±0.6^{c}	-76.39	218.5±2.8^{ef}	385.4±2.8^{e}	**Ya**	**18**
-52.40	3.3±0.2^{d}	5±0.2^{b}	5.30	12.6±0.5^{bc}	11.9±0.3^{g}	69.94	112±2.1^{c}	33.6±1.2^{g}	-16.12	5.5±0.5^{c}	6.4±0.6^{c}	-79.83	206±2.9^{fg}	370.4±2.6^{e}	**Po**	**19**
-17.30	3.4±0.1^{d}	4±0.1^{c}	-70.64	10.7±0.3^{cd}	18.3±0.6^{ab}	77.30	133.2±2.3^{b}	30.2±1.3^{g}	8.30	6.3±0.6^{b}	5.8±0.5^{d}	-11.50	85.3±0.8^{h}	95.1±2.1^{h}	**En**	**20**
-81.05	3.2±0.1^{d}	5.8±0.3^{b}	-56.16	10.6±0.3^{cd}	16.6±0.5^{c}	9.27	36±1.2^{ef}	32.6±1.3^{g}	-37.44	4.2±0.4^{d}	5.8±0.5^{d}	34.24	317.3±2.9^{e}	208.6±2.2^{f}	**Kh3**	**21**
27.65	6.2±0.4^{ab}	4.5±0.1^{c}	-80.53	9.4±0.3^{d}	16.9±0.5^{b}	-89.54	15.2±0.4^{g}	28.8±0.7^{h}	-75.82	3±0.3^{e}	5.3±0.5^{d}	-25.21	2252.2±4.9^{b}	2820±5.1^{a}	**Kh4**	**22**
1.50	5.3±0.3^{b}	5.2±0.2^{b}	-21.95	12.8±0.5^{bc}	15.7±0.5^{c}	-79.16	18.5±0.5^{fg}	33.2±1.5^{g}	-60.24	3.3±0.3^{e}	5.3±0.5^{d}	-25.01	884.3±4.1^{cd}	1105.6±4.3^{b}	**Al2**	**23**
-25.61	4.3	4.9	-52.34	11.4	14.3	-21.66	63.4	50.1	-49.40	4.7	6.3	6.23	793.3	393.9	**Mean**	

*a specific concentration of the sample that needed to provide 50% inhibition

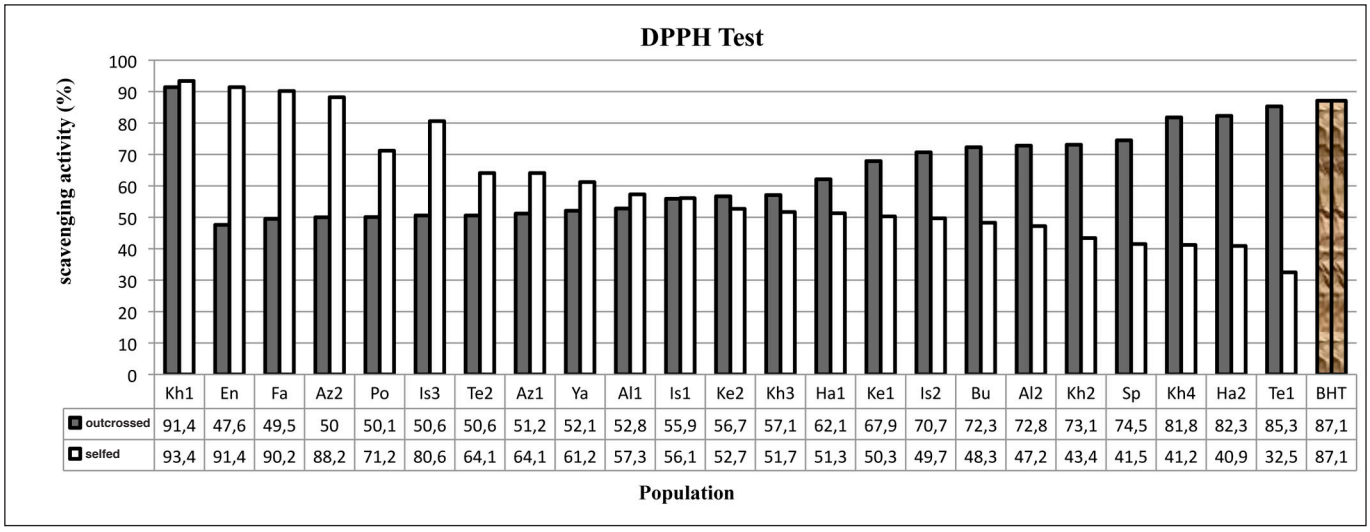


Fig. 3: DPPH radical scavenging activity of outcross and self-pollinated population of fennel extracts compared to BHT.

Reducing power

Reducing capacities of methanolic seed extracts of out-crossed and self-pollinated fennel populations are shown in Tab. 3. Similar to DPPH assay, self-pollinated populations showed higher reducing capacities than out-crossed ones (Fig. 4). The strongest power in out-crossed populations seed extract was found for cv. Shiravan (Fig. 4).

Inhibition of β-carotene bleaching

The antioxidant effect of seed extracts obtained from out-crossed and self-pollinated populations as well as BHT in the model system of β-carotene/linoleic acid are presented in Fig. 5. Oxidation of the linoleic acid was effectively inhibited by the extract from cv. Shiravan followed by cultivars England and Shiraz, while in self-pollinated ones, cultivars Tehran and Shiravan showed the lowest and the highest values, respectively.

Cluster analysis of secondary metabolites

Cluster analysis was carried out to distinguish possible groups among the accessions. Fig. 6 presents the corresponding dendrogram using the Ward's algorithm. Cluster analysis allows dividing 23 out-crossed (A) and self-pollinated (B) populations in six major groups. Group 1 included the highest TFC, while group 2 consisted of the populations with moderate secondary metabolites. Group 3 included low TFC and essential oil yield, while group 4 consisted of populations with high extract yield. Group 5 included moderate essential oil and extract yield, while group 6 consisted of the highest extraction yield.

Correlation analyses

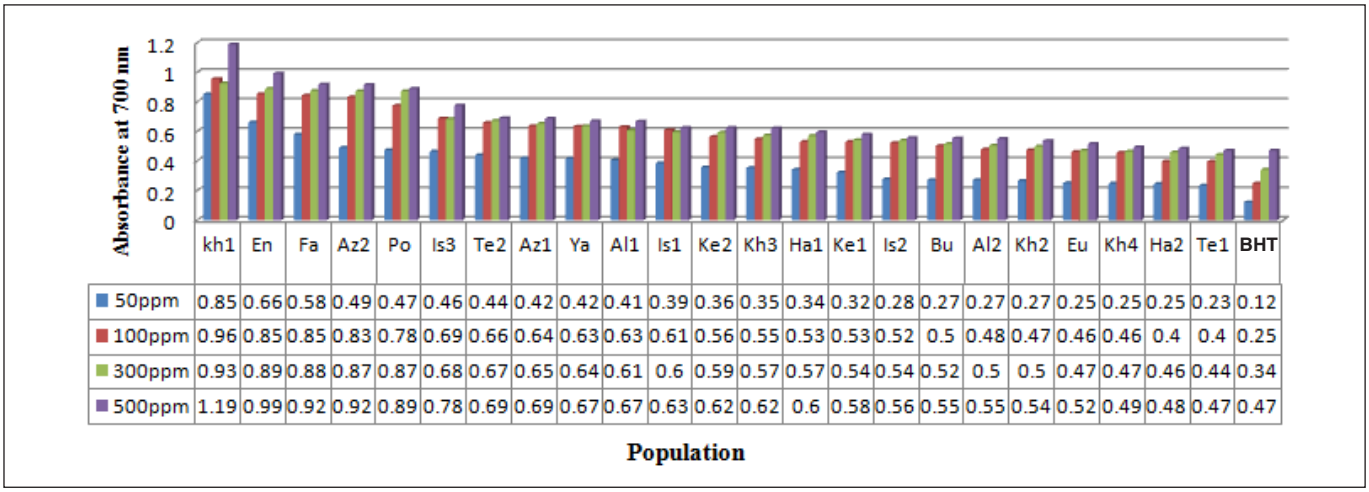
In self-pollinated populations, correlation analysis revealed that the highest correlation coefficient was between the number of seed coat layers and number of periclinal wall layers (+0.568), TPC and TFC (+0.612), extraction yield and TPC (+0.611) and extraction yield and TPC (+0.941) (Tab. 4). The highest negative correlations were between IC_{50} and the number of secretory duct (-0.542) and essential oil yield and TPC (-0.537). In out-crossed populations, high positive correlation was obtained between extraction yield and TPC (+0.711) (Tab. 4).

Apiaceae family exhibits a variety of sex expression including hermaphroditism, andromonoecy, gynodioecy and dioecy. Fennel is categorized in hermapherodite type that exhibits protandrus dichogamy which promotes cross pollination (Koul et al., 1996). Flowering phases of the umbellules within the same umbel are not simultaneous (Nemeth et al., 1999). Thus, flowering process of fennel assures fertilization among the flowers of each single umbel. The previous reports showed that seed setting by isolation of a single umbel can reach to 56% (Nemeth et al., 1999). In fennel and many Apiaceae plants, geitonogamous mode was reported and selfing can be promoted by geitonogamous mode that highly depends to insects and wind pollination (Koul et al., 1996).

However, one of the breeding objectives in medicinal plants is to produce high pure lines with high amount of metabolites. In the present study, in spite of non synchrony between male and female flowers in fennel, the self pollinated seeds were produced and showed high variation in respect to their secondary metabolites. Changes in secondary metabolites might be originated from different factors.

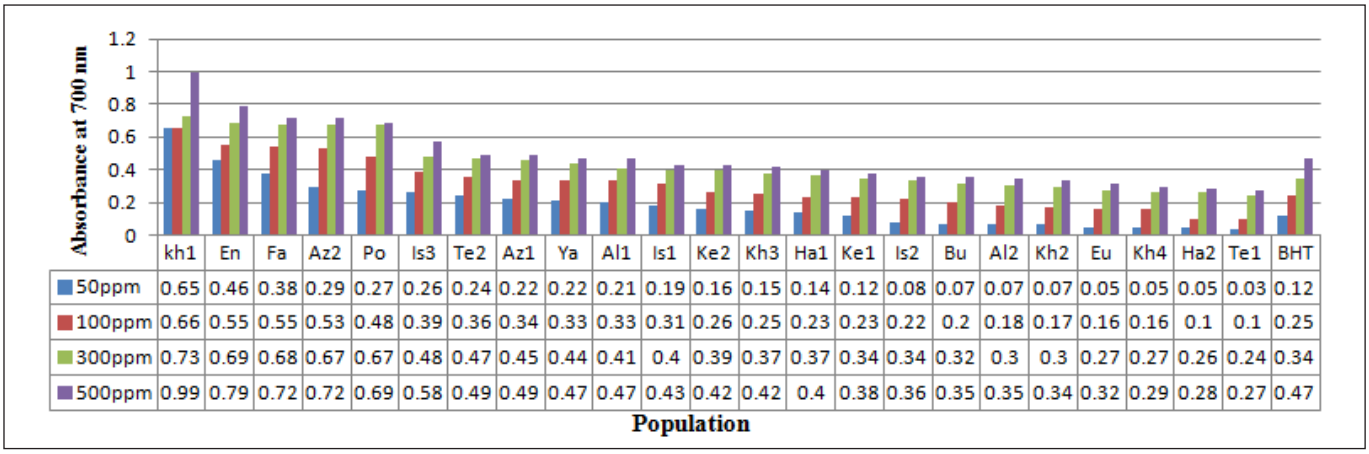
Anatomical characteristics can affect the essential oil yield of plants (Anackov et al., 2009; Hulley et al., 2010; Bombo et al., 2012). Previous researches revealed that some anatomical traits such as number of glandular trichoms, secretory ducts and cavity were more effective to increase or decrease the essential oil yield (Bombo et al., 2012). In different plant families, the frequency of these tissues are different. For example, in Lamiaceae family, the number of glandular trichoms was higher (Baher-Nik et al., 2004; Celep et al., 2014; Venditti et al., 2014), while in Apiaceae, the number of secretory ducts was more crucial in comparison with other traits (Sousa et al., 2005; Chu and Liu, 2007; Mu et al., 2009). In fennel, the frequencies of secretory ducts are more important than the other types of secretory tissues. Sousa et al. (2005) assessed the effect of seasonal variation on secretory duct frequencies and its relationships with essential oil yield. They also suggested that the accumulation of the essential oil in secretory ducts was highly related to environmental factors and phenological stage. In fennel, the seed essential oil is more valuable, because of its higher valuable compounds in comparison with the other parts (Rahimmalek et al., 2014). Moreover, seed formation is one of the most important phenological stages in fennel. Seed set decreases by the lack of synchrony between pollen production and stigma receptivity (Nemeth et al., 1999). So, the type of pollination can lead to more or less seed formation. In this research, self-pollination derived populations possessed more secretory ducts compared with out-crossed ones. It might be due to more differentiation of parenchyma cells to secretory ducts due to self-pollination.

The essential oil yield in many medicinal plants may be influenced by the ploidy level (Nemeth, 2005), plant phenological stage



	kh1	En	Fa	Az2	Po	Is3	Te2	Az1	Ya	Al1	Is1	Ke2	Kh3	Ha1	Ke1	Is2	Bu	Al2	Kh2	Eu	Kh4	Ha2	Te1	BHT
50ppm	0.85	0.66	0.58	0.49	0.47	0.46	0.44	0.42	0.42	0.41	0.39	0.36	0.35	0.34	0.32	0.28	0.27	0.27	0.27	0.25	0.25	0.25	0.23	0.12
100ppm	0.96	0.85	0.85	0.83	0.78	0.69	0.66	0.64	0.63	0.63	0.61	0.56	0.55	0.53	0.53	0.52	0.5	0.48	0.47	0.46	0.46	0.4	0.4	0.25
300ppm	0.93	0.89	0.88	0.87	0.87	0.68	0.67	0.65	0.64	0.61	0.6	0.59	0.57	0.57	0.54	0.54	0.52	0.5	0.5	0.47	0.47	0.46	0.44	0.34
500ppm	1.19	0.99	0.92	0.92	0.89	0.78	0.69	0.69	0.67	0.67	0.63	0.62	0.62	0.6	0.58	0.56	0.55	0.55	0.54	0.52	0.49	0.48	0.47	0.47

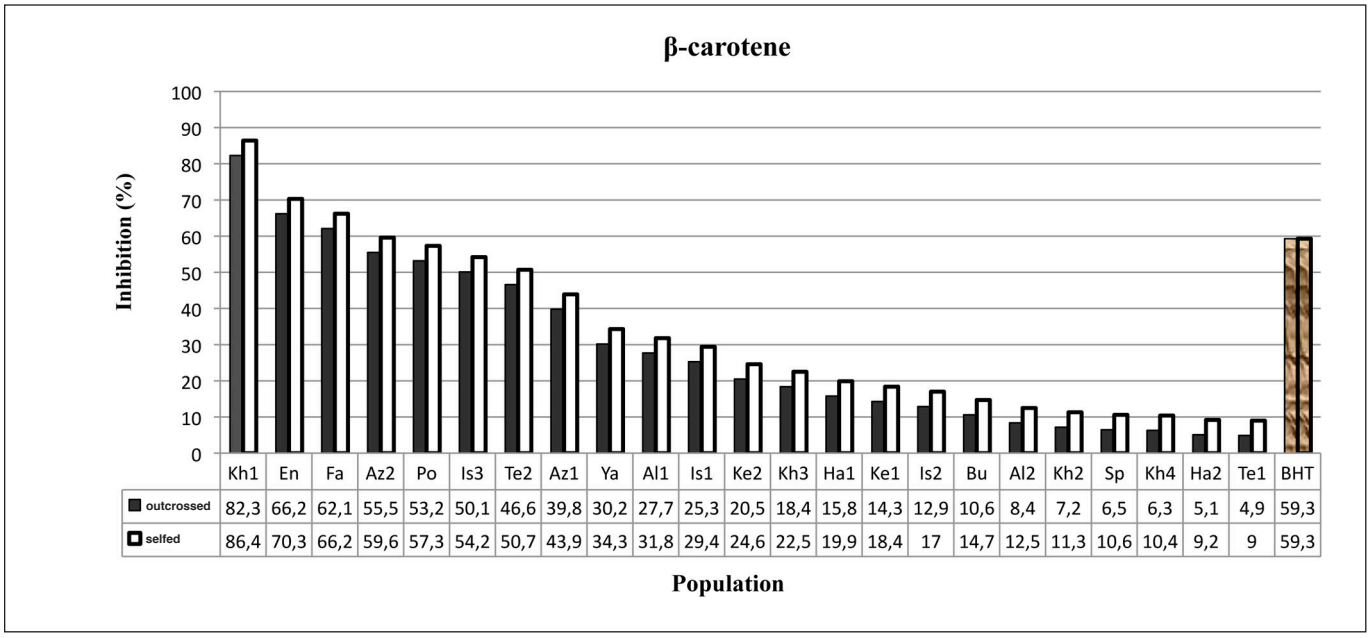
A



	kh1	En	Fa	Az2	Po	Is3	Te2	Az1	Ya	Al1	Is1	Ke2	Kh3	Ha1	Ke1	Is2	Bu	Al2	Kh2	Eu	Kh4	Ha2	Te1	BHT
50ppm	0.65	0.46	0.38	0.29	0.27	0.26	0.24	0.22	0.22	0.21	0.19	0.16	0.15	0.14	0.12	0.08	0.07	0.07	0.07	0.05	0.05	0.05	0.03	0.12
100ppm	0.66	0.55	0.55	0.53	0.48	0.39	0.36	0.34	0.33	0.33	0.31	0.26	0.25	0.23	0.23	0.22	0.2	0.18	0.17	0.16	0.16	0.1	0.1	0.25
300ppm	0.73	0.69	0.68	0.67	0.67	0.48	0.47	0.45	0.44	0.41	0.4	0.39	0.37	0.37	0.34	0.34	0.32	0.3	0.3	0.27	0.27	0.26	0.24	0.34
500ppm	0.99	0.79	0.72	0.72	0.69	0.58	0.49	0.49	0.47	0.47	0.43	0.42	0.42	0.4	0.38	0.36	0.35	0.35	0.34	0.32	0.29	0.28	0.27	0.47

B

Fig. 4: Reducing power of outcross (A) and self-pollinated (B) population fennel extracts compared to BHT.



	Kh1	En	Fa	Az2	Po	Is3	Te2	Az1	Ya	Al1	Is1	Ke2	Kh3	Ha1	Ke1	Is2	Bu	Al2	Kh2	Sp	Kh4	Ha2	Te1	BHT
outcrossed	82,3	66,2	62,1	55,5	53,2	50,1	46,6	39,8	30,2	27,7	25,3	20,5	18,4	15,8	14,3	12,9	10,6	8,4	7,2	6,5	6,3	5,1	4,9	59,3
selfed	86,4	70,3	66,2	59,6	57,3	54,2	50,7	43,9	34,3	31,8	29,4	24,6	22,5	19,9	18,4	17	14,7	12,5	11,3	10,6	10,4	9,2	9	59,3

Fig. 5: Inhibitory effect of outcross and self-pollinated population fennel extracts on β-Carotene bleaching system.

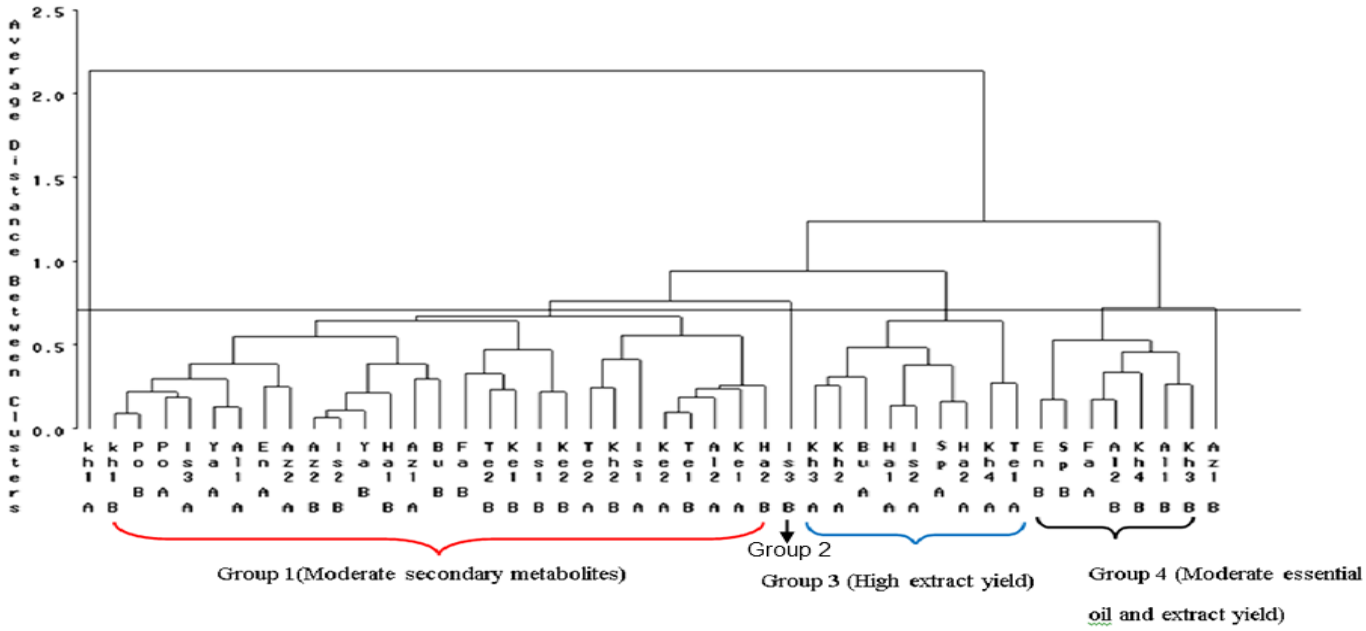


Fig. 6: Grouping of outcross (A) and self-pollinated (B) fennel populations according to their secondary metabolites using Ward's minimum variance. *The codes used in dendrogram were explained in Tab. 1.

Tab. 4: Correlation of some anatomical characters and secondary metabolites in outcross and self-pollinated populations of fennel used in this study

outcrossed populations										
-0.057 ns	-0.068 ns	-0.013 ns	-0.073 ns	-0.042 ns	-0.048 ns	0.266ns	-0.018 ns	1	Number of secretary ducts	self-pollinated populations
-0.048 ns	0.012ns	0.062ns	-0.097 ns	-0.169 ns	0.247ns	-0.135 ns	1	0.043ns	Number of periclinal wall layers	
-0.488 ns	-0.212 ns	-0.229 ns	-0.215 ns	-0.08 ns	0.024 ns	1	-0.013 ns	0.301 ns	Thickness of anticlinal wall	
-0.084 ns	-0.103 ns	-0.111 ns	-0.049 ns	-0.109 ns	1	0.124 ns	0.568 *	0.084 ns	Number of seed coat layers	
0.186 ns	0.07 ns	0.012 ns	0.033 ns	1	0.135 ns	-0.379 ns	0.092 ns	-0.542 **	IC_{50}	
0.280 ns	0.006 ns	-0.237 ns	1	0.2 ns	-0.066 ns	0.226 ns	0.018 ns	-0.203 ns	Total Flavonoids	
-0.236 ns	0.711**	1	0.612**	0.085 ns	0.218 ns	0.069 ns	0.105 ns	-0.236 ns	Total Phenolics	
-0.264ns	1	0.941**	0.611**	0.133 ns	-0.145 ns	0.152 ns	0.234 ns	-0.270 ns	Extraction yield	
1	-0.492**	-0.537**	-0.360 ns	0.228 ns	0.107 ns	0.099 ns	-0.086	-0.109 ns	Essential oil yield	

(Rahimmalek et al., 2009) and bio-regulators (Abd El-Wahed et al., 2004) as well as pollination (Mendoza-Poudereux et al., 2014). This suggests that self-pollination can lead to an increase of essential oil yield among fennel populations in the next generations. In outcross pollinating crops, exploitation of heterosis for higher productivity is advocated (Chopra, 1996). Preliminary studies conducted by Dashora et al. (2003) indicated the presence of heterosis for seed yield and yield contributing characters through varietal diallel analysis in fennel. The cost of hybrid seed production was generally high because of the controlled pollination that was required. Introducing new variation into medicinal plant germplasm is very helpful in the development of new cultivars characterized with higher content of important metabolites. Generally, inbreeding led to an increase of the essential oil content in most populations. One probable reason for that could be the number of secretory ducts. In this study, interestingly the number of secretory ducts elevated as a result of inbreeding. Furthermore, previous researches revealed that in out-crossed plants, the genes encoding the essential oil production in secretory tissues, co-suppressed, while in self-pollinated ones, the co-suppression of these genes was reduced (Mendoza-Poudereux et al., 2014). So, in respect to essential oil yield of fennel, selecting of self pollination derived populations can produce more insightful results.

Inbreeding elevated TPC, TFC and antioxidant activity of populations. Self-pollination decreased cell wall thickness and more lignifications in fennel seeds. Lignin is considered as the precursor of many phenolic compounds (Babar-Ali, 2006; Gross, 2008). So, increasing in pericelinal wall thickness might lead to the elevated TPC. Furthermore, in most of the previous researches, a positive correlation was obtained between TPC and antioxidant activity (Hinneburg et al., 2006; Mohammadi-Motamed et al., 2010; Ramkissoon et al., 2012). So, the higher antioxidant activity in self-pollinated populations might be due to higher TPC. Moreover, many phenolic compounds can biosynthesized through shikimic or phenyl propanoid pathways (Janas et al., 2002). Therefore, it might be concluded that inbreeding can affect the expression of some genes underlying the mentioned metabolic pathways.

Conclusion

The results of the current study provide, for the first time, the data on the effect of inbreeding on secondary metabolites of different fennel populations. The reasons for the observed variation of metabolites

are also discussed. In this research, self-pollination led to an increase in the secretory ducts in self-pollination derived populations and increased the essential oil yield. Furthermore, extract yield, TPC, TFC and antioxidant activity of populations was also elevated as a result of inbreeding. This increase was attributed to higher lignification in self-pollinated fennel seeds. In the present study, TFC showed higher elevation as a result of inbreeding compared to other secondary metabolites. Self-pollination decreased the cell wall number and intensified lignification in fennel seeds. In overall, cv. Tabriz was introduced as the best population with respect to the secondary metabolites content. Finally, self-pollination of these populations in the next generations can help the breeders to introduce new populations with high amount of metabolites as well as high purity.

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