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Effects of planting date and seed origin on yield, essential oil and compositions content of *Pimpinella affinis* Ledeb. in Iran

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Abstract
In order to investigation the effects of seed origin and planting date on yield and essential oil of *Pimpinella affinis*, a field experiment was carried out in Alborz Research Center in 2007. This experiment was performed under factorial in the base of (RCBD) with three replications. The treatments were included locations of seeds collection and planting dates. Essential oils were extracted from different parts in all experimental units separately. The results showed that the effects of locality on seed yield, inflorescence oil percentage, seed oil percentage, seed oil yield and limonene percentage had significant difference with other treatments. Effects of planting date on seed yield, Inflorescence oil percentage and seed oil yield was significant different with others. Interaction between locality and planting date on height, stem plus leaf oil percentage, Inflorescence oil percentage, seed oil percentage, seed oil yield, limonene percentage and trans α-bergamotene percentage were significant different. The major compositions of Tehran chemotype were limonene (>83%) and geijerene and the major composition of Mazandaran chemotype was trans α-bergamotene (>91%). As a result, seed origin from Mazandaran is recommended to cultivation due to presence of trans α-bergamotene in essential oils, and Tehran seed origin for its seed oil yield and biomass.

Key words: *Pimpinella affinis*; Planting Date; Seed yield; Essential Oil; Limonene; Trans α-bergamotene

Introduction
The genus *Pimpinella* L. (Umbelliferae) comprises 150 species distributed in Eurasia and Africa, over 16 of which are present in Europe and 22 species present in Iran. *Pimpinella affinis* presents in different regions of Turcoman, Iran, Afghanistan, Iraq, Soiree and Israel. It grows wild in center, west and north of Iran and more distributed in mountainous regions with cold weather **(**Mozaffarian, 2007; Rechinger, 1972; Jodral, 2004).
Between *Pimpinella* species, *Pimpinella anisum* is cultivated abundantly. For cultivating anise, a warm, sunny, and dry climate with long and dry autumns is ideal. Therefore, as a rule, cultivation in the northern regions of the earth does not pay, as the fruits do not usually ripen, and harvests are often poor. The cultivation plain should be protected from wind. The 1000-grain weight of the fruits lies between 1.07 and 1.53 g. The purity of the seeds for drilling should be at least 90% and the germination rate at least 70%. Because the ability to germinate decreases rapidly when using inadequate conditions of storage, the best seeds will come from the previous year's harvest (Jodral, 2004).
Essential oils were obtained from the stems plus the leaves, inflorescences and seeds of *Pimpinella affinis* Ledeb. Individually, those were collected from Khojir and Chaloos. The yields of stem plus the leaf, inflorescence and seed oils for the Khojir sample were 0.04%, 1.98% and 5.33% w/w and for the Chaloos sample were 0.37%, 1.74% and 4.05% w/w, respectively (Askari and Sefidkon, 2006).
Trans-α-bergamotene is an aromatic compound. It has been used for the synthesis of other aromatic compounds. The aerial parts and seeds of *Pimpinella affinis* collected from Noshahr in 2003 and 2004. The yields of stem plus the leaf, inflorescence and seed oils for

the first year were 0.26%, 1/1% and 4.1% w/w and for the second year were 0.26%, 0.86% and 2.45%, respectively. Major constituent of the stem plus the leaf, inflorescence, and seed oils was trans-*α-Bergamotene* (91.1%, 96.2% and 90.2% in 2003 and 94.3% 84.9% and 95.5% in 2004, respectively) (Askari et al, 2006).

The oil yields of stems plus the leaves, inflorescence and seed oils of *Pimpinella tragioides* (Boiss.) Benth. et Hook. were 0.15%, 0.79% and 2.49% w/w, respectively. The major constituent in the stem/leaf oil and inflorescence oil was trans-α-bergamotene (77.1% and 70.3%, respectively), whereas the major constituent of the seed oil was pregeijerene (87.0%). Nonadecane (8.6%) and isoacarone (15.1%) were other major constituents in the stem/leaf and inflorescence oils, respectively (Askari and Sefidkon 2007).

In the oil of *P. affinis*, trans-α-bergamotene (56.2 %) and (E)-g-bisabolene (11.3%) were found to be the major constituents (Rahmani *et al.*, 2008).

Twenty-four components were identified in the essential oil of *Pimpinella affinis* Ledeb., whose major constituents were geijerene (17.68%), limonene (12.86%), pregeijerene (9.92%), germacrene D (8.54%) and trans-β-ocimene (4.94%). The oil showed antimicrobial activity against all the tested microorganisms, excepted *Pseudomonas aeruginosa*. Maximum activity was observed against fungal microorganisms (Verdian-rizi, 2008).

Fruit characterization of *P. affinis* Ledeb.: Mericarps are two, homomorphic, elliptic to round in transverse section. Epidermal surface pubescent or puberulous. Exocarp includes small cells with thin walls; Endocarp consists of only one layer of thin-walled cells (Khajepiri *et al*. 2010).

The anise seeds (*pimpinella anisum* L.) were sowed on three dates (15 Nov., 1 Jan., 15 Feb.) in experimental bloke. The results showed that there were significant differences ($P \leq 0.05$) between sowing dates. The best sowing date was on 15 Feb which reflected in the yield and percentage of essential oil (1115 $kg.hec^{-1}$, 4.09 % respectively), while the best sowing date for anethole percentage was on 15 Nov., which gave (91.83 %). Generally, the best anise plant was in Karahta, in 15 Feb sowing date regarding yield and essential oil production, and on 15 Nov. sowing date for anethole percentage (Hasani, 2010).

Different researches on oil composition of *Pimpinella affinis* from different locality in Iran (Askari and Sefidkon, 2006; Askari *et al*., 2006; Rahmani *et al*., 2008) showed that quality and quantity of *P. affinis* seed oil was important. This species were cultivated for more careful investigation.

To the best of our knowledge, there is no previous work concerning the planting of *P. affinis.* The aim of this study was to investigation on the best planting time on seed yield, quantity and quality of essential oil of *Pimpinella affinis*.

Material and methods

This study was performed under factorial in the base of randomized complete blocks design with 3 replications in 2006-7.

The treatments were included location of collection seeds and cultivation date. Seeds were collected from Khojir and Noshahr (northeast of Tehran province and Mazandaran province respectively). The herbarium specimens (No.93812 and No. 93814 Respectively) have been deposited in the herbarium of Research Institute of Forests and Rangelands (TARI).

Seeds were planted in November 6th, December 6th and April 9th in 2007. The experiment was performed in the Alborz farm research complex located in Karaj. Phenologic data were measured at flowering stage. Plant materials that cultivated in November (N) and December (D) were collected at flowering stage in the last June and at seed stage in middle of August 2006 and those cultivated in April (A) were collected at flowering stage in the first of July and at seed stage in last August 2007. Essential oil yield of different parts, their chemical composition, aerial parts and seed yields were determined at the end of growth period.

The fresh plants were dried at room temperature. The dried parts of the plants were crushed to small particle. Essential oils were extracted by hydro-distillation from stems plus the leaves (S&L), flowering (IF) (during flowering stage) and seeds (S) plants in all experimental units separately. At each time about 80-100g samples of plant materials were used. Three distillations were performed for each oils then mixed for analysis. The

sample essential oils kept in sealed vials at 4° C temperatures before analysis. Oil yield of different parts and seed yields were determined at the end of at growth period.

GC analysis

The essential oils from the (S&L), (IF) and (S) of *P. affinis* were analyzed using Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m x 0.25 mm, film thickness 0.25 μm, J & W scientific corporation). Oven temperature was held at 40°C for 5 min and then programmed to 260°C at a rate of 4°C/min. Injector and detector (FID) temperature were 270°C; helium was used as carrier gas with a linear velocity of 32 cm/s. Percentages were calculated by area normalization method without the use of response factor correction. The retention indices were calculated for all compounds using a homologous series of n-alkanes.

GC-MS analysis

GC/MS analyses were carried out on a Varian 3400 GC/MS system equipped with a DB-5 fused silica column (30 m x 0.25 mm, film thickness 0.25 μm, J & W scientific corporation); oven temperature program was 50°-260 °C at a rate of 4°C/min. Transfer line temperature 270°C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, ionization energy 70 ev, scan time 1 sec, mass range 40-300 amu.

Identification of compounds

The components of the essential oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature (Davies 1990; Shibamoto 1987) Mass spectra from the literature were also compared (Davies 1990; Adams 1995). The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes.

Results

Seed yield

The results showed that the effects of planting date and locality on seed yield were significant different p< 1% (Table 1). Comparison of treatment means showed that seed yield was cultivated samples in November and December had not significant difference while they have significant difference with April Samples. The highest yield of seed which cultivated samples in November and Khojir locality with 404.6 kg/ha which had no significant difference with planted in December (Table 4).

Height

The results of variation analysis (Table 1) were determined that the effects of planting date, locality and their interaction on height were significant on the level 5%, 1% and 1% respectively. Comparison of treatment means (Table 4) showed that the highest of plant (83.17 Cm) was cultivated samples in December and Noshahr locality.

1000 seed weight

Effect of planting date, locality and interaction between them on the 1000 seed weight were not significant. 1000 seed weight was about 0.48 gram.

Stem plus leaf oil yield

The results of variation analysis (Table 1) were determined that the effects of planting date, locality and their interaction on stem plus leaf oil yield were significant on the level 1%. Comparison of treatment means (Table 4) showed that the highest of yield was in sample of Khojir locality and cultivated in November and December (0.50%).

Inflorescence oil yield

The results of variation analysis (Table 1) were determined that the effects of locality on inflorescence oil yield were significant on the level 1%, Whereas the effects of planting date and interaction between it and locality were significant on the level 5%. Comparison of treatment means showed that the highest yield of inflorescence essential oil was cultivated samples in December and Khojir locality with 2.97%.

Seed oil yield

The results of variation analysis (Table 1) were determined that the effects of planting date on seed oil yield were not significant, whereas the effects of locality and it interaction with planting date were significant on the level 1% and 5% respectively. Comparison of treatment means showed that the highest yield of seed essential oil was samples cultivated in December and Khojir locality with 6.11%.

Table 1- Variation analysis of effects of planting date, locality and their interactions on biological functions and essential oil of *Pimpin*

Source of Variation	Degrees of free-dom	M. S.						
		Seed yield	Height (cm)	1000 seed weight	Stem+leaf oil (%)	Inflorescence oil (%)	Seed oil (%)	Seed oi yield
replication	2	479.885	6.073	0.004	0.002	0.103	0.144	0.013
Locality (a)	1	307196.041**	272.611*	0.000ns	0.133**	12.920**	44.400**	888.873
Planting date (b)	2	72689.696**	435.995**	0.000ns	0.161**	0.739*	1.722ns	193.954
Interaction a×b	2	56181.809	41.257*	0.001ns	0.076**	0.077*	4.257*	178.643
error	10	821.648	35.484	0.002	0.008	0.230	0.606	3.643
%CV		18.95	8.30	8.46	27.53	30.14	22.58	25.63

*=significant at 5% **= significant at 1% ns=non significant

Table 2- Means of effects of planting date on biological functions and essential oil of *Pimpinella affinis* L

Treatments	Seed yield (kg/ha)	Height (cm)	1000 seed weight (g)	Stem+leaf oil (%)	Inflorescence oil (%)	Seed oil (%)	Seed oil yield (kg/ha)
November	218.9a	75.15a	0.471a	0.29b	1.46ab	2.86b	8.77b
December	210.6a	78.11a	0.485a	0.50a	1.99a	3.92a	12.36a
April	24.26b	62.09b	0.484a	0.18b	1.32b	3.57ab	1.22 c

Similar words in columns show no significant.

Table 3- Mean of effects of locality on biological functions and essential oil of *Pimpinella affinis* L.by Duncan's test at 5%

Treatments	Seed yield(kg/ha)	Height (cm)	1000 seed weight (g)	Stem+leaf oil (%)	Inflorescence oil (%)	Seed oil (%)	Seed oil y (kg/ha
Noshahr	20.6b	75.67a	0.479a	0.24b	0.74b	1.88b	0.42b
Khojir	281.9a	67.89b	0.480a	0.41a	2.44a	5.20a	14.48

Similar words in columns show no significant.

Table 4- Mean of effects of locality and planting date interactions on biological functions and essential oil of *Pimpinella affinis* L.by D

Treatments	Seed yield(kg/ha)	Height (cm)	1000 seed weight (g)	Stem+leaf oil (%)	Inflorescence oil (%)	Seed oil (%)	Seed oil yield (kg/ha)
November* Noshahr	33.19b	80.87a	0.455a	0.07b	0.67b	2.25bc	0.72 c
December* Noshahr	23.35b	83.17a	0.489a	0.50a	1.01b	1.72 c	0.46 c
April* No-shahr	5.36b	62.98bc	0.493a	0.13b	0.55b	1.67 c	0.09 c
November* Khojir	404.6a	69.42bc	0.487a	0.50a	2.24a	3.48b	16.82b
December* Khojir	397.9a	73.05bc	0.480a	0.50a	2.97a	6.11a	24.26a
April* Khojir	43.17b	61.20 c	0.474a	0.22b	2.10a	5.47a	2.35 c

Similar letters in each columns show no significant

Seed oil function

The results of variation analysis (Table 1) were determined that the effects of planting date, locality and their interaction on seed yield were significant on the level 1%. Comparison of treatment means showed that the highest seed oil function was samples cultivated in December and Khojir locality with 24.26 kg/he.

Limonene and trans α-bergamotene percentages

The results of variation analysis (Table 1) were determined that the effects of planting date on limonene and trans α-bergamotene percentage were not significant, whereas the effects of locality and it interaction with planting date were significant on the level 1% and 5% respectively. Comparison of the treatments means in 2007 showed that the highest limonene percentage was in December cultivation (44.40%) and the highest percentage of trans α-bergamotene was in November cultivation with the 47.92%, while were not significant differences in other planting dates. Effect of locality on limonene percentage determined that seeds collected from Khojir had the highest amount (86.03%), while the percentage of trans α-bergamotene in essential oil had the lowest (0.05%). However the Noshahr locality percentage of trans α-bergamotene (93.87%) was the highest value and Limonene percentage was the lowest value (0.16%) which showed a significant difference. Interaction between locality and planting date on Limonene percentage showed that December planting Khojir seed with 88.47% had the highest amount and November planting Noshahr seed had the highest amount of trans α-bergamotene that differences were significant to the other treatments.

Discussion

Hasani Al-Awak (2010) reported that the best sowing date of *P. anisum* was on 15 Feb regarding yield and essential oil production (1115 kg/ha-1, 4.09% respectively), and on 15 Nov. sowing date for anethole percentage (91.83 %). In present research, the best sowing date for seed yield (about 210 kg/ha-1) were Nov. and Dec., but April sowing date is not sufficient (seed yield was 24 kg/ha-1).

In present research, 1000 seed weight of *P. affinis* was about 0.48 gram while 1000 seed weight of *P. anisum* lies between 1.07 and 1.53 g (Jodral, 2004).

Rahmani et al (2008) was reported the major constituents In the oil of *P. affinis*, trans-α-bergamotene (56.2 %) and (E)-g-bisabolene (11.3%). Verdian-rizi (2008), were identified in the essential oil of *P. affinis* geijerene (17.68%), limonene (12.86%), pregeijerene (9.92%), germacrene D (8.54%). In this research Chemical compositions of seed essential oil were two completely distinct chemotype, according to natural samples. The major compositions of Tehran province chemotype (region Khojir) were limonene and geijerene and composition of Mazandaran province chemotype Noshahr was trans α-bergamotene. As a result, Mazandaran chemotype seed is recommended to cultivation due to presence of trans α-bergamotene in essential oils, despite its seed oil yield and its biomass is less than Khojir chemotype seed.

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