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Effect of sowing date and plant density on some of the essential oil compounds of Dragon head (*Dracocephalum moldavica* L.) under different harvest times in Erzurum, Turkey

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Dragonhead (*Dracocephalum moldavica* L.) is an annual herbaceous, sometimes biennial, which is aromatic, and belongs to the mint family (*Lamiaceae*). In order to investigate the effect of sowing date, plant density and harvest time on essential oil compounds of dragon head, a split-split plot experiment was done as base of randomized complete blocks design with three replications in turkey at spring and summer of 2018. Sowing date in three levels (5 May, 15 May, 25 May) as main plot, distance on row in three levels (15, 30, 45 cm) as sub-plot and harvest time in three levels (50% flowering, 100% flowering, seeding tame) as sub-sub plot were considered. Results of the analysis of variance revealed that Z-Citral (Neral), Geraniol, E-Citral (Geranial) and Geraniol, belonged to the oxygenated monoterpenes group, were the major compounds of dragon head essential oil. The data mean comparison indicated that Geraniol, as the main compound of essential oil, had the highest amount in first sowing date (5 may), 45 cm distance on row and seeding harvest time with mean of 40.21%.

Keywords: Dragon head, Essential oil, Geraniol, harvest time

INTRODUCTION

The Moldavian dragon's head (*Dracocephalum moldavica* L.) is an annual, herbaceous, essential oil-producing, spicy aromatic medicinal plant of the deadnettle family (*Lamiaceae*), which reaches 25 - 75 cm in height (Nikitina et al. 2008). There are 45 species of herbaceous and shrub's dragonhead in the world and there are 8 species of annual and perennial herbaceous and fragrant in Iran of which three are endemic (Mozafarian, 1996). The origin of this plant is reported from southern Siberia and the Himalaya (Galambosi and Holmes, 1989; Omidbeigi, 2005) and naturally grows in temperate Zones of Europe and Asia (Domokos et al. 1994).

There are two common forms of dragonhead, with white and blue flowers, differing mainly in their flowering period. It flowers mainly in July and sets fruit in August, and contains flavones, terpenes, proteins, polypeptides and 16 amino acids, of which 8 are essential (Sultan et al. 2008). It is frequently consumed as food and drug additives. Dragonhead seed yield is about 1600 and 1900 kg ha-1 for the white and blue form, respectively (Hanczakowski et al. 2009).

Seeds are rich in fatty oil, whose content ranges from 18 to 29%. This oil is rich in unsaturated fatty acids (about 90%), principally the linolenic and linoleic acids (about 60 and 20%, respectively) which belong to essential fatty acids (Domokos et al. 1994). Apart from this, dragonhead seeds (DHS) contain about 21% of protein with beneficial amino acid composition and

high biological value (Hanczakowski et al. 2009).

The aim of present experiment was to investigate the effect of sowing date, plant density and harvest time on essential oil compounds of dragon head in Erzurum region.

MATERIALS AND METHODS

This experiment was conducted in field of agricultural faculty, Ataturk University, Erzurum during 2018. Sowing date in three levels (5 May, 15 May, 25 May) as main plot, distance on row: in three levels (15, 30, 45 cm) as sub-plot and harvest time in three levels (50% flowering, 100% flowering, seeding tame) as sub-sub plot were considered. The extracted essential oil constituents consisted of: 1-Octen-3-ol, 6-Methyl-5-hepten-2-one, *ortho*-Cymene, Linalool, Nonanal (except D, D × H) and 2-Cyclohexen-1-one, 4,5-dimethyl content, Estragole, Nerol, *Z*-Citral (Neral), Piperitone, Geraniol, *E*-Citral (Geranial), Geranyl formate, Carvacrol, *E*-Methyl geranate, Neryl acetate, *α*-Copaene, Geranylacetate, Geranic acid, *Z*-Caryophyllene and β-Ionone. The main constituents of essential oil (Z-Citral (Neral), Geraniol, E-Citral (Geranial) and their mean were compared.

Chromatographic analysis

Samples containing essential oils of the plant were analyzed on an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a HP-5MS 5% phenyl methyl siloxane capillary column (30m × 0.25mm, 0.25µm film thickness; Restek, Bellefonte, PA). The column outlet was simultaneously connected to an Agilent HP-5973 mass selective detector (MSD) in the electron impact mode (ionization energy: 70 eV) and a flame ionization detector (FID) using a Y type 1:10 post column splitter (Agilent part No: 0101-0595). Oven temperature was kept at 60 ˚C for 3 min, then programmed to 246 ˚C at a rate of 3 ˚C /min. Injector temperature was set at 220 ˚C and both of detectors (MSD and FID) temperatures were set at 240 ˚C. Ultra-high pure helium (flow rate: 1.2 ml/min), hydrogen (flow rate: 40 ml/min) and nitrogen (flow rate: 50 ml/min) were used as carrier, fuel and make up gases, respectively. Compressed air (flow rate: 450 ml/min) was used for combustion. Diluted samples (1/1000 in n-pentane, v/v) of 2.0 µl were injected manually in the split mode (split ratio: 1/10). Retention indices (RI) were calculated for all components using a homologous series of n-alkanes injected in conditions identical to the samples injections. Identification of samples components was made based on their retention indices (RI) relative to n-alkanes, computer matching of their mass spectra with Wiley275.L and Wiley7n.L libraries and comparison of the fragmentation pattern of the mass spectra with the data published in the literature. Peak area percent of each compound relative to the area percent of the entire FID spectrum (100%) was used for obtaining its quantitative data.

Statistical analysis

The data set was first tested for skewness and kurtosis. Appropriate transformation was applied for specific characters that showed non-normal distributions. Analysis of variance (ANOVA) for treatment main effect was conducted using the MSTAT-C statistical software. Multiple comparisons of means were calculated by running the Duncan's multiple range test. All statistical significances were determined at $p<0.05$ significance level.

RESULTS

The GC–MS chromatogram yielded for the extracted volatile compounds demonstrated in figure 1. The main components in *Dracocephalum Moldavica* essential oil were identified as Z-Citral (16.17%), Geraniol (26.55%), E-Citral (Geranial) (21.29%) and Geranyl acetate (14.59%).

Results of ANOVA showed the effect of sowing date, distance on row, harvest time, sowing date × distance on row, sowing date × harvest time, distance on row × harvest time and sowing date × distance on row × harvest time were significant on Z-Citral (Neral), Geraniol, E-Citral (Geranial) and Geraniol (Table 1). The data mean comparison indicated that Neral and Geraniol were highest value in S1D1H2 and S1D3H, respectively (Table 6). Results showed that the highest *E*-Citral (Geranial) was observed in S2D2H3 with mean of 28.960%. Results also indicated the most Geranyl acetate was existed in S2D1H1 (Table 6). Based on our findings, the most Geraniol as main constituent of essential oil was gained in first sowing date (5 May) with 45 cm at 50% flowering. Also, the essential oil of *Dracocephalum moldavica* L. was characterized by a high containing oxygenated acyclic monoterpenes, having a keton, aldehyde, and alcoholic function groups, which in dependent on plant origin.

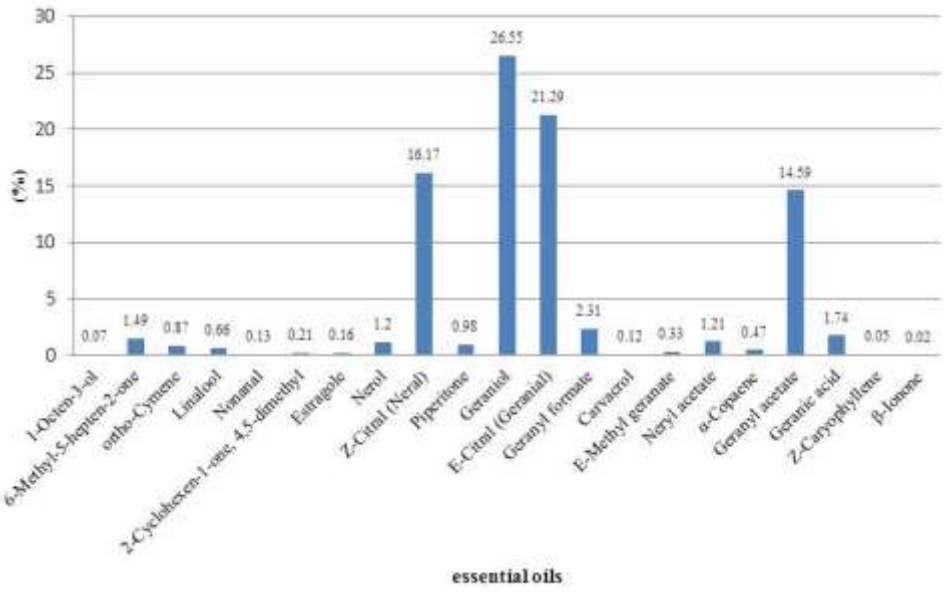

Figure 1: Volatile organic compounds identified in the essential oil of *Dracocephalum Moldavica*

Table 1: The variance analysis of *Z*-Citral (Neral), Geraniol, E-Citral (Geranial) and Geraniol

S.O.V	D.F	*Z*-Citral (Neral)	Geraniol	*E*-Citral (Geranial)	Geranyl acetate
R	2	0.027**	0.053**	0.012**	0.077**
S	2	18.312**	90.385**	58.473**	2.636**
E	4	0.0001	0.0001	0.0001	0.0001
D	2	5.977**	9.014**	1.662**	6.441**
S × D	4	2.162**	28.171**	20.400**	5.338**
E	12	0.0001	0.0001	0.0001	0.0001
H	2	88.668**	1277.070**	544.118**	412.295**
S × H	4	102.366**	39.078**	43.051**	29.765**
D × H	4	9.755**	7.484**	4.133**	0.765**
S × D × H	8	2.989**	31.020**	24.002**	4.923**
E	36	0.0001	0.0001	0.0001	0.000
CV%	-	2.09	2.03	2.05	1.06

**and * significant at 1 and 5 %, respectively; ns non-significant. R: Replication, S: Sowing date, D: Distance on row, H: Harvest time, E: Error

Table 2: The mean comparison of simple effects of sowing date, distance on row and harvest time on *Z*-Citral (Neral), Geraniol, E-Citral (Geranial) and Geraniol

Sowing date	Z-Citral (Neral)	Geraniol	*E*-Citral (Geranial)	Geranyl acetate
S1	16.911	27.357	21.651	14.589
S2	16.330	24.462	22.551	14.908
S3	15.286	27.848	19.674	14.283
SE	±0.004	±0.001	±0.002	±0.002
Distance on row				
D1	16.285	25.890	21.019	14.274
D2	15.660	26.930	21.504	15.156
D3	16.582	26.847	21.353	14.351
SE	±0.003	±0.001	±0.002	±0.002
Harvest time				
H1	14.607	34.458	21.651	18.937
H2	18.160	21.921	22.551	13.478
H3	15.760	23.289	19.674	11.364
SE	±0.003	±0.002	±0.002	±0.002

Sowing date (S): S_1: 5 May, S_2: 15 May, S_3: 25 May, Distance on row (D): D_1: 15, D_2: 30, D_3: 45, Harvest time (H): H_1: 50% flowering, H_2: 100% flowering, H_3: seeding time, SE: Standard Error.

Table 3: the mean comparison of sowing date × distance row interaction on *Z*-Citral (Neral), Geraniol, E-Citral (Geranial) and Geraniol

Sowing date	Distance on row	*Z*-Citral (Neral)	Geraniol	*E*-Citral (Geranial)	Geranyl acetate
S1	D1	17.233	25.770	22.093	14.168
	D2	16.437	27.248	20.632	15.623
	D3	17.063	29.053	22.229	13.974
S2	D1	16.003	22.760	22.714	14.470
	D2	16.301	25.279	23.948	14.661
	D3	16.687	25.348	20.990	15.593
S3	D1	15.618	29.141	18.250	14.183
	D2	14.243	28.264	19.932	15.182
	D3	15.997	26.139	20.840	13.484
SE		±0.004	±0.002	±0.003	±0.003

Sowing date (S): S_1: 5 May, S_2: 15 May, S_3: 25 May, Distance on row (D): D_1: 15, D_2: 30, D_3: 45, Harvest time (H): H_1: 50% flowering, H_2: 100% flowering, H_3: seeding time, SE: Standard Error.

Table 4: the mean comparison of sowing date × harvest time interaction on *Z*-Citral (Neral), Geraniol, E-Citral (Geranial) and Geraniol

Sowing date	Harvest time	*Z*-Citral (Neral)	Geraniol	*E*-Citral (Geranial)	Geranyl acetate
S1	H1	13.530	37.713	14.816	19.257
	H2	20.220	22.060	22.801	12.360
	H3	16.983	22.298	27.338	12.149
S2	H1	13.862	30.163	18.991	20.480
	H2	15.919	20.590	22.920	12.623
	H3	19.210	22.633	25.741	11.621
S3	H1	16.430	35.497	14.847	17.076
	H2	18.340	23.112	23.016	15.451
	H3	11.088	24.936	21.160	10.323
SE		±0.005	±0.003	±0.004	±0.003

Sowing date (S): S_1: 5 May, S_2: 15 May, S_3: 25 May, Distance on row (D): D_1: 15, D_2: 30, D_3: 45, Harvest time (H): H_1: 50% flowering, H_2: 100% flowering, H_3: seeding time, SE: Standard Error.

Table 5: The mean comparison of distance row × harvest time on *Z*-Citral (Neral), Geraniol, E-Citral (Geranial) and Geraniol

Distance on row	Harvest time	*Z*-Citral (Neral)	Geraniol	*E*-Citral (Geranial)	Geranyl acetate
D1	H1	14.281	34.197	15.677	18.664
	H2	19.419	21.919	23.437	13.137
	H3	15.154	21.556	23.944	11.020
D2	H1	14.911	34.712	16.687	19.172
	H2	16.537	22.338	22.386	14.323
	H3	15.533	23.741	25.440	11.971
D3	H1	14.630	34.464	16.290	18.976
	H2	18.523	21.506	22.914	12.974
	H3	16.593	24.570	24.854	11.102
SE		±0.005	±0.004	±0.004	±0.003

Sowing date (S): S_1: 5 May, S_2: 15 May, S_3: 25 May, Distance on row (D): D_1: 15, D_2: 30, D_3: 45, Harvest time (H): H_1: 50% flowering, H_2: 100% flowering, H_3: seeding time, SE: Standard Error.

Table 6: The mean comparison of sowing date × Distance on row × harvest time on *Z*-Citral (Neral), Geraniol, E-Citral (Geranial) and Geraniol

Sowing date	Distance on row	Harvest time	*Z*-Citral (Neral)	Geraniol	*E*-Citral (Geranial)	Geranyl acetate
S1	D1	H1	12.827	35.087	15.990	17.857
		H2	21.317	22.877	22.340	13.013
		H3	17.557	19.347	27.950	11.633
	D2	H1	14.387	37.837	12.847	20.353
		H2	18.567	20.427	23.720	12.673
		H3	16.357	23.480	25.330	13.843
	D3	H1	13.377	40.217	15.610	19.560
		H2	20.777	22.877	22.343	11.393
		H3	17.037	24.067	28.733	10.970
S2	D1	H1	12.920	29.237	18.210	20.693
		H2	17.573	15.667	27.100	11.363
		H3	17.517	23.377	22.833	11.353
	D2	H1	15.250	30.033	22.453	19.043
		H2	14.057	24.077	20.430	14.283
		H3	19.597	21.727	28.960	10.657
	D3	H1	13.417	31.220	16.310	21.703
		H2	16.127	22.027	21.230	12.223
		H3	20.517	22.797	25.430	12.853
S3	D1	H1	17.097	38.267	12.830	17.443
		H2	19.367	27.213	20.870	15.033
		H3	10.390	21.943	21.050	10.073
	D2	H1	15.097	36.267	14.760	18.120
		H2	16.987	22.510	23.007	16.013
		H3	10.647	26.017	22.030	11.413
	D3	H1	17.097	31.957	16.950	15.663
		H2	18.667	19.613	25.170	15.307
		H3	12.227	26.847	20.400	9.483
SE			±0.008	±0.005	±0.007	±0.005

Sowing date (S): S_1: 5 May, S_2: 15 May, S_3: 25 May, Distance on row (D): D_1: 15, D_2: 30, D_3: 45, Harvest time (H): H_1: 50% flowering, H_2: 100% flowering, H_3: seeding time, SE: Standard Error.

DISCUSSION

The results about changes of compositions of essential oil upon employed treatments are in agreement with Argyropoulou et al. (2007) and Santos-Gomes et al. (2005) reports. They believed that the different observations in quality and quantity of essential oil compositions in aromatic and medicinal plants occurred with many factors such as origin, ecological elements, genetic variation, different methods applied in cultivation and etc.

The oil containing a large quantity of Geranyl acetate, Z- Citral (Neral) and Geraniol as oxygenated compounds was demonstrated in bio-autography assay to be a patents antimicrobial activity than other constitutes in the oil (Kim et al. 1995). The present compounds exert antimicrobial activity by (1) impairing a variety of enzyme system, especially of those involved in the production of cellular energy and synthesis of structural components; (2) interfering with the phospholipids bilayer of the cell membranes causing increased permeability and loss of cellular constituents and/or (3) inactivation or destroying genetic material (Kim et al., 1995).

Mundt et al. (2003) reported that essential compounds had a high antifungal properties, which could denature the enzymes responsible for spore germination or interfere with the amino acid involved in germination; interactions with membrane enzymes and proteins would cause on apposite flow of protons, affecting cellular activity or disturb genetic and interact with membrane proteins, causing a deformation in there structure and functionality (Abd El-Baky and El-Baroty, 2007). Some researchers indicated 90% of essential oil of *Dracocephalum moldavica* L. includes Geranial, Geraniol, Neral and Geranial acetate (Holm and Hiltunen, 1988). Hussein et al. (2006) reported that the highest essential oil belonged to Linalool. Yousefzadeh et al. (2013)

and Kakasy et al. (2006) also reported that Geranyl acetate, Geraniol and Geranial are the main essential oil constituents of dragonhead. Based on Helm et al. (1998) findings, main essential oil constituents include Geranyl acetate, Geraniol, Geranial and Neral. Sonboli et al. (2008) stated the main essential oil constituents was formed by Neral, Geranial, Geranyl acetate, Geraniol and Geranial with 32.1, 21.6, 19.9 and17.6%, respectively

CONCLUSION

This study's findings indicate interplay between sowing date, plant density and harvest times in terms of essential oil compounds of dragon head. Oxygenated acyclic monoterpenes had a significant contribution in dragon head essential oils with a keton, aldehyde, and alcoholic function groups. Also, Geraniol, E-Citral (Geranial), Z-Citral and Geranyl acetate were the main compounds of dragon head essential oils, respectively, which were changed in value by applying the different treatments.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest. Also this manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

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AUTHOR CONTRIBUTIONS

R. Rahmatollahi performleed the experiments and also wrote the manuscript. T.Polat supervised the project and reviewed the manuscript. All authors read and approved the final version.

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