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The effects of different nitrogen and phosphorus doses on essential oil components of some *Mentha* genotypes

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Abstract: The present study was conducted in Erzurum during the years 2010 and 2011 to determine the effects of different nitrogen and phosphorus doses on essential oil components of spearmint genotypes (*Mentha spicata* L. and *Mentha villoso-nervata* Opiz.). Two genotypes of *Mentha spicata* (genotypes 2 and 6) and one genotype of *Mentha villoso-nervata* (genotype 4) were tested under three nitrogen and phosphorus doses (0, 50, and 100 kg/ha) in a randomized complete block design with three replications. Essential oil components of the genotypes were determined and subjected to variance analysis. Effects of experimental year and nitrogen and phosphorus doses on investigated traits were found to be significant. Considering the experimental years of 2010 and 2011 and the nitrogen-phosphorus fertilizer doses, 1.8-cineole contents varied respectively between 2.12% and 3.98% (for nitrogen treatments) and between 2.42% and 3.77% (for phosphorus treatments); 4-terpineol contents varied between 1.34% and 2.74% and between 1.30% and 2.73%; pulegone contents between 3.41% and 9.64% and between 4.11% and 8.93%; carvone contents between 21.05% and 33.64% and between 21.77% and 33.54%; piperitone contents between 2.41% and 17.75% and between 2.04% and 17.60%; and finally caryophyllene between 3.98% and 4.93% and between 3.62% and 4.49%, respectively. The greatest 1.8-cineole content was observed in genotype 4 in the first year and in genotype 2 in the second year. Genotype 6 was prominent for 4-terpineol, pulegone, and caryophyllene contents in both years and genotype 4 was prominent for carvone content. The greatest piperitone content was observed in genotype 6 in the first year and in genotype 2 in the second year.

Key words: Carvone, essential oil components, fertilizer, *Mentha spicata*, *Mentha villoso-nervata*, spearmint

1. Introduction

Medicinal and aromatic plants are effective with their active ingredients (Kamel, 2000). Several essential oils of such plants have antimicrobial, carminative, chlorotic, sedative, diuretic, and antispasmodic impacts in various diseases (Maksimovic et al., 2005). Peppermint cultivars contain menthone, isomenthone, carvone, and pulegone and all these peppermint essential oils have antimicrobial characteristics. They are also used as active ingredients in toothpastes, food stuffs, soaps, creams, lotions, and perfumes (Sivropoulou et al., 1995; Baydar, 2013).

Nitrogen treatments are commonly used worldwide in plant culture to improve the yields (Ladha et al., 2005). Nitrogenous and phosphorus fertilizers may also improve essential oil yield and quality of medicinal and aromatic plants (Kotheri et al., 1987; Ram et al., 1995; Meneghini et al., 1998; Rajeswara Rao, 2001; Sangwan et al., 2001; Singh et al., 2002; Kiran and Patra, 2003; Kapoor et al., 2004; Yeşil and Kara, 2014). However, it is worth mentioning that fertilizer treatments should be performed at proper times and in sufficient amounts (Fageria et al., 2006; Abbasi et al., 2012; Bindraban et al., 2015).

The present study was conducted to investigate the effects of different nitrogen and phosphorus doses on quality attributes of three spearmint genotypes of *Mentha spicata* (genotypes 2 and 6) and *Mentha villoso-nervata* (genotype 4) under the ecological conditions of Erzurum Province of Turkey.

2. Materials and methods

2.1. Materials

In the present experiments, two genotypes of *M. spicata* and one genotype of *M. villoso-nervata* were used for field studies. The genotypes were supplied from the Gaziosmanpaşa University Agricultural Faculty Field Crops Department (Tokat, Turkey). Three nitrogen and three phosphorus doses (0, 50, and 100 kg/ha) were studied. Ammonium sulfate with 21% N and triple super phosphate (45%) were used as nitrogenous and phosphorus fertilizers.

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2.2. Experimental site

Experiments were conducted in experimental field #4 of the Atatürk University Agricultural Faculty, Erzurum, Turkey, in 2010 and 2011. Erzurum Province, at an altitude of 1853 m and with a dominant terrestrial climate, is located in the northeast of Turkey at 39°55′N, 41°61′E. Because of the terrestrial climate and high altitude, the temperature differences both between the seasons and between day and night are quite high. Soil samples taken from the 0–20 cm profile of the experimental site revealed that experimental soils had a clay-loam texture with a pH of 7.27, organic matter content of 0.11%, available P_2O_5 of 142 kg/ha, and K_2O of 1636 kg/ha. According to these data, soils were slightly alkaline, medium in available phosphorus, rich in potassium, and poor in organic matter (Sezen, 1991).

2.3. Methods

2.3.1. Soil preparation and propagation of spearmint cuttings

The experimental site was leveled, tilled at a depth of 15–20 cm, and left for wintering. Then the field was shallow-tilled in spring and experimental plots were harrowed with a disk-harrow.

Spearmint was propagated with stem cuttings in this study. During the first year of experiments, cutting propagations were performed in a greenhouse at the Atatürk University Agricultural Faculty. To obtain a sufficient number of plants from experimental cuttings, they were planted into pots (21 cm in diameter and 20 cm high) containing a growth mixture (1/3 sand, 1/3 soil, 1/3 manure). Stem cuttings of the plants that developed from these cuttings were used to get a sufficient amount of material. Experiments were set up with these resultant plants, planting them into the field by leaving axillary shoots and terminal leaves aboveground. Fertilization was performed right after transplantation of the plants into the field in the setup year and in May of both years through spreading over the field.

2.3.2. Experimental design

Experimental plots were treated with three different nitrogen and phosphorus doses (0, 50, and 100 kg/ha). Plots had 3.6 × 1.6 m dimensions (5.76 m^2 in size). Each plot had 4 rows with row spacing of 40 cm and in-row plant spacing of 30 cm. There were 81 experimental plots and thus the total experimental area was 1532.16 m^2. A single harvest was performed in both years and harvest was performed at the beginning of flowering since plants have the highest essential oil contents in this period. In the first year, side rows and 30-cm strips from the ends of the plots were omitted so as to consider side effects and the rest of the plot was harvested. Since harvested plants of the first year covered the plot surface and spoiled the row and in-row plant spacing arrangements, a frame of 200 × 80 cm was used and the plants within this frame were harvested in the second year of the experiments.

The essential oil components obtained through distillation method were analyzed in a gas chromatography/mass spectrometry (GC-MS) device. HP1 (30 m × 0.25 mm, film thickness: 0.25 µm) was used as the column and helium was used as the carrier gas. GC-MS parameters were as follows: MS source temperature, 230 °C; MS quadrupole temperature, 150 °C; ionization energy, 70 eV; ionization current, 60 µA; screening area, 35–350 amu; screening/s, 4.51 (Martins Maldao et al., 2004). Electronic libraries of Flavor2.L, Wiley7n.1, and NIST98.L (Martins Maldao et al., 2004; Arslan et al., 2010) and standard substances were used for essential oil characterization. Based on fertilizer doses, the Flavor2.L, Wiley7n.1, and NIST98.L electronic libraries yielded peaks between 98 (N_0P_0 fertilizer dose of genotype 2 of the first year) and 205 (N_5P_5 fertilizer dose of genotype 2 of the second year) and components were denoted based on these peaks. The components with reliability standard of 70% or above were used in this study and variance analysis was performed on significant components influencing quality in peppermint.

3. Results and discussion

The effects of nitrogen and phosphorus doses on all traits of spearmint genotypes were found to be significant ($P < 0.01$). Current findings comply with the findings of earlier studies indicating the effects of years, cultivars, ecology, fertilization, irrigation, harvest period, and genetic factors on spearmint essential oil composition (Misra et al., 1989; Özgüven and Kırıcı, 1999; Kirakosyan et al., 2004; Zobayed et al., 2005; Gopichand et al., 2013). The 1.8-cineole, 4-terpineol, and pulegone contents were higher in the second year than in the first year and such findings comply with the results of Kirakosyan et al. (2004), proposing that cold weather or water stress might have increased polyphenolic compound contents. The temperature and precipitation of the second year (average temperature: 4.6 °C; average precipitation: 39.4 mm) were lower than the first year (average temperature: 7.9 °C; average precipitation: 39.7 mm).

3.1. 1.8-Cineole content

The 1.8-cineole contents of experimental factors are provided in Table 1 and variance analysis results are provided in Table 2.

Average 1.8-cineole contents of the genotypes at 0, 50, and 100 kg/ha nitrogen doses were respectively observed as 2.14%, 3.13%, and 2.12% in the first year and 3.98%, 3.00%, and 3.36% in the second year. The highest 1.8-cineole content was obtained from the 50 kg nitrogen treatment (3.13%) in the first year and from the genotypes without nitrogen treatment (3.13%) in the second year. These findings do not comply with the findings of Baranauskiene et al. (2003), indicating insignificant effects of nitrogenous fertilization on essential oil components of

Table 1. The 1.8-cineole contents (%), by nitrogen and phosphorus doses, of spearmint genotypes.

Years	Nitrogen doses (kg/ha)	Phosphorus doses (kg/ha)	Spearmint genotypes			Average of nitrogen doses
			2	4	6	
2010	0	0	1.10	2.78	1.50	2.14B
		50	1.50	3.26	1.83	
		100	2.95	3.03	1.33	
	50	0	3.67	4.27	2.30	3.13A
		50	3.42	3.73	3.21	
		100	2.13	3.02	2.47	
	100	0	2.43	2.00	2.11	2.12C
		50	1.50	3.39	0.73	
		100	2.23	2.38	2.28	
Average of genotypes			2.32B	3.09A	1.97C	2.46B
Average of phosphorus doses			0: 2.46B	50: 2.51A	100: 2.42C	
2011	0	0	6.85	2.90	5.30	3.98A
		50	3.23	3.50	2.32	
		100	3.77	3.03	4.97	
	50	0	3.44	3.97	2.37	3.00C
		50	2.84	3.31	2.97	
		100	4.09	4.05	0.00	
	100	0	1.73	3.15	4.26	3.36B
		50	2.72	3.80	3.04	
		100	3.30	3.98	4.30	
Average of genotypes			3.55A	3.52B	3.28C	3.45A
Average of phosphorus doses			0: 3.77A	50: 3.08C	100: 3.50B	

Differences among means indicated with capital letters are significant at $P < 0.01$; differences among means indicated with lowercase letters are significant at $P < 0.05$.

Table 2. Variance analysis results for 1.8-cineole contents of nitrogen and phosphorus doses.

Sources of variation	SD	1.8-Cineole F-values	
		2010	2011
Nitrogen doses (N)	2	4705.973**	2896.321**
Phosphorus doses (P)	2	24.476**	1432.877**
Genotypes (G)	2	4585.854**	258.813**
N × P	4	963.489**	1767.735**
N × G	4	171.360**	4174.481**
P × G	4	310.645**	711.252**
N × P × G	8	850.466**	1974.735**
Error	54		

**: F-values are significant at $P < 0.01$.

thyme, or the findings of Singh (2013), indicating again insignificant effects of different nitrogen doses on the 1.8-cineole content of rosemary.

With regard to phosphorus treatments, the greatest 1.8-cineole content was observed with 5 kg/ha phosphorus treatment (2.51%) in the first year and in treatments without phosphorus (3.77%) in the second year. The lowest value was obtained from 100 kg/ha phosphorus treatment (2.42%) in the first year and from 50 kg/ha phosphorus treatment (3.08%) in the second year.

With regard to genotypes, the greatest 1.8-cineole content was observed in genotype 4 (3.09%) in the first year and in genotype 2 (3.55%) in the second year, and the lowest value was seen in genotype 6 (1.97% and 3.28%) in both years

3.2. 4-Terpineol content

Average 4-terpineol contents of the genotypes at 0, 50, and 100 kg/ha nitrogen doses were respectively observed as 1.43%, 1.34%, and 1.60% in the first year and as 2.74%, 2.34%, and 2.60% in the second year (Table 3). These findings complied with the results of Kandeel et al. (2002).

Considering the phosphorus doses, the lowest 4-terpineol content was obtained from 100 kg/ha phosphorus treatment (1.30%) in the first year and from 50 kg/ha phosphorus treatment (2.25%) in the second year. The greatest 4-terpineol contents were observed in treatments without phosphorus (1.59% and 2.73%) in both years (Table 3).

In both years, the greatest 4-terpineol contents were observed in genotype 6 (1.74% and 3.37%), followed by

Table 3. The 4-terpineol contents (%), by nitrogen and phosphorus doses, of spearmint genotypes.

Years	Nitrogen doses (kg/ha)	Phosphorus doses (kg/ha)	Spearmint genotypes			Average of nitrogen doses
			2	4	6	
2010	0	0	1.18	1.13	2.84	1.43B
		50	1.50	1.16	1.77	
		100	0.62	1.34	1.36	
	50	0	1.58	1.37	1.60	1.34C
		50	1.13	1.88	1.45	
		100	1.25	1.05	0.72	
	100	0	1.60	1.25	1.78	1.60A
		50	2.15	0.88	1.42	
		100	1.26	1.33	2.76	
Average of genotypes			1.36B	1.27C	1.74A	1.46B
Average of phosphorus doses			0: 1.59A	50: 1.48B	100: 1.30C	
2011	0	0	1.62	1.67	7.65	2.74A
		50	2.00	0.00	2.47	
		100	2.42	2.76	4.07	
	50	0	2.43	2.11	1.73	2.34C
		50	2.25	2.83	2.94	
		100	2.33	2.12	2.94	
	100	0	2.59	1.16	3.65	2.60B
		50	3.22	1.24	3.30	
		100	4.11	1.96	2.22	
Average of genotypes			2.55B	1.76C	3.37A	2.56A
Average of phosphorus doses			0: 2.73A	50: 2.25B	100: 2.70A	

Differences among means indicated with capital letters are significant at P < 0.01; differences among means indicated with lowercase letters are significant at P < 0.05.

genotype 2 (1.36% and 2.55%) and genotype 4 (1.27% and 1.76%) (Table 3).

Results of variance analysis of 4-terpineol contents are provided in Table 4.

3.3. Pulegone content

As an average of experimental factors, pulegone content was observed as 4.94% in the first year and as 7.71% in the second year (Table 5). These findings comply with

Table 4. Variance analysis results for 4-terpineol contents of nitrogen and phosphorus doses.

Sources of variation	SD	4-Terpineol	
		F-values	
		2010	2011
Nitrogen doses (N)	2	595.816**	29.138**
Phosphorus doses (P)	2	717.861*	52.338**
Genotypes (G)	2	2092.964**	463.172**
N × P	4	739.110**	135.500**
N × G	4	1243.978**	249.122**
P × G	4	541.312**	100.462**
N × P × G	8	984.330**	86.556**
Error	54		

*: F-values are significant at P < 0.05, **: F-values are significant at P < 0.01.

Table 5. The pulegone contents (%), by nitrogen and phosphorus doses, of spearmint genotypes.

Years	Nitrogen doses (kg/ha)	Phosphorus doses (kg/ha)	Spearmint genotypes			Average of nitrogen doses
			2	4	6	
2010	0	0	0.00	1.01	0.00	3.41C
		50	0.00	0.32	13.94	
		100	1.23	0.79	13.45	
	50	0	0.60	1.19	17.38	6.86A
		50	0.53	0.83	20.02	
		100	0.47	0.86	19.89	
	100	0	0.00	0.00	16.82	4.54B
		50	0.00	1.96	21.25	
		100	0.30	0.00	0.54	
Average of genotypes			0.35C	0.77B	13.70A	4.94B
Average of phosphorus doses			0: 4.11C	50: 6.54A	100: 4.17B	
2011	0	0	0.00	0.00	25.01	7.18B
		50	0.00	0.00	18.35	
		100	0.00	0.00	21.30	
	50	0	0.00	0.00	21.60	6.31C
		50	19.91	0.00	20.63	
		100	0.00	0.00	14.56	
	100	0	0.00	4.77	20.37	9.64A
		50	0.00	0.00	21.46	
		100	0.00	0.00	20.25	
Average of genotypes			2.21B	0.53C	20.39A	7.71A
Average of phosphorus doses			0: 7.97B	50: 8.93A	100: 6.23C	

Differences among means indicated with capital letters are significant at P < 0.01; differences among means indicated with lowercase letters are significant at P < 0.05.

the results of studies indicating changing essential oil compositions for spearmint with years (Guenther, 1961; Smith and Levi, 1961; Thomas et al., 1961).

With regard to nitrogen doses of 0, 50, and 100 kg/ha, the greatest pulegone content was obtained from 5 kg/ha nitrogen treatment (6.86%) in the first year and from 100 kg/ha nitrogen treatment (9.64%) in the second year. The lowest value was observed in samples without nitrogen treatment (3.41%) in the first year and in 50 kg/ha nitrogen treatment (6.31%) in the second year (Table 5). These findings were similar to results of studies indicating varying essential oil compositions with fertilization (Ahlgrimm, 1956; Gopichand et al., 2013) and were different from the results of studies reporting decreased pulegone contents with increasing nitrogen doses (Court et al., 1993) and indicating insignificant effects of different nitrogen doses on essential oil components (Baranauskiene, 2003).

Effects of phosphorus doses on pulegone contents were found to be significant in both experimental years ($P < 0.01$) (Table 6). The greatest pulegone contents were observed in 50 kg/ha phosphorus treatment (6.54% and 8.93%) in both years. The values were respectively observed as 4.11% and 7.97% in samples without phosphorus treatment and as 4.17% and 6.23% in 100 kg/ha phosphorus treatments. Pulegone contents increased up to a certain point with increasing phosphorus doses and such findings comply with the results of El-Habbasha (2005) for groundnut and Tuncturk and Tuncturk (2006) for *Cuminum cyminum* L., indicating positive impacts of increasing phosphorus doses on essential oil components.

Significant differences were observed in the pulegone contents of the genotypes ($P < 0.01$) (Table 6). The greatest pulegone contents were obtained from genotype 6 in both years (13.70% and 20.39%), followed by genotype 2 (0.35% and 2.21%) and genotype 4 (0.77% and 0.53%). These results are lower than the pulegone contents of *M. spicata* reported by Telci (2010).

3.4. Carvone content

As an average of experimental factors, carvone content was 31.03% in the first year and 23.93% in the second year (Table 7). The higher carvone content of the first year was due to lower total precipitation (178.6 mm) and relative humidity (57.6%) and higher total temperature (66.1 °C) in the first year than the second year (208.9 mm, 58.6%, 63.2 °C) (Yeşil, 2012). Current carvone contents were lower than the values reported by Tyler et al. (1988) and Özgüven and Kırıcı (1998) and the values reported in a pharmacopeia (42%–67%) (Wagner et al., 1984).

The greatest carvone contents were observed in samples without nitrogen treatment (33.64% and 26.54%) in both years and the lowest value was observed with the 50 kg/ha nitrogen dose (29.47%) in the first year and 100 kg/ha nitrogen (21.05%) in the second year (Table 7). These findings are parallel to the results of Wander and Bouwmeester (1998), indicating increasing carvone contents with increasing nitrogen doses.

With regard to phosphorus doses, the greatest carvone contents were observed with the 100 kg/ha phosphorus dose (33.54% and 25.38%) in both years, followed by the samples without phosphorus treatments (30.68% and 24.65%), and the lowest value was seen in 50 kg/ha phosphorus treatment (28.86% and 21.77%). These findings comply with the results of previous studies indicating positive impacts of fertilization on secondary metabolites of medicinal and aromatic plants (Rajeswara Rao, 2001; Kapoor et al., 2004; Ram et al., 2006; Sifola and Barbieri, 2006).

Table 6. Variance analysis results for pulegone contents of nitrogen and phosphorus doses.

Sources of variation	SD	Pulegone	
		F-values	
		2010	2011
Nitrogen doses (N)	2	11,166.659**	104,298.981**
Phosphorus doses (P)	2	6929.478**	65,489.380**
Genotypes (G)	2	208,021.036**	4,250,785.774**
N × P	4	9823.861**	63,297.512**
N × G	4	9724.946**	48,950.690**
P × G	4	6613.203**	77,344.673**
N × P × G	8	8683.838**	74,780.841**
Error	54		

**: F-values are significant at $P < 0.01$.

Table 7. The carvone contents (%), by nitrogen and phosphorus doses, of spearmint genotypes.

Years	Nitrogen doses (kg/ha)	Phosphorus doses (kg/ha)	Spearmint genotypes			Average of nitrogen doses
			2	4	6	
2010	0	0	46.13	45.44	23.58	33.64A
		50	39.86	41.83	9.34	
		100	43.36	43.72	9.54	
	50	0	36.73	43.31	7.53	29.47C
		50	42.42	42.53	3.46	
		100	36.13	43.06	10.08	
	100	0	33.29	35.98	4.17	29.97B
		50	26.83	44.94	8.51	
		100	36.81	42.73	36.48	
Average of genotypes			37.95B	42.61A	12.52C	31.03A
Average of phosphorus doses			0: 30.68B	50: 28.86C	100: 33.54A	
2011	0	0	44.06	37.73	0.00	26.54A
		50	34.14	43.38	2.47	
		100	34.50	40.73	1.82	
	50	0	34.95	35.83	0.00	24.21B
		50	30.07	37.78	0.00	
		100	37.25	37.61	4.44	
	100	0	26.09	39.42	3.74	21.05C
		50	0.86	44.17	3.06	
		100	33.27	38.84	0.00	
Average of genotypes			30.58B	39.50A	1.72C	23.93B
Average of phosphorus doses			0: 24.65B	50: 21.77C	100: 25.38A	

Differences among means indicated with capital letters are significant at $P < 0.01$; differences among means indicated with lowercase letters are significant at $P < 0.05$.

Among the spearmint genotypes, the greatest carvone content was obtained from genotype 4 (42.61% and 39.50%), followed by genotype 2 (37.95% and 30.58%) and genotype 6 (12.52% and 1.72%) (Table 7). The differences in carvone contents of the genotypes were due to the genetics of the genotypes (Telci, 2001). Previous researchers indicated that essential oil content and composition of spearmint mostly depend on ecology, cultivars, years, fertilization, irrigation, harvest period, and genetics (Misra et al., 1989; Özgüven and Kırıcı, 1999).

Results of variance analysis of carvone contents are provided in Table 8.

3.5. Piperitone content

Across the nitrogen doses, the average piperitone content of the first year (16.05%) was higher than the piperitone content of the second year (4.91%) (Table 9).

The greatest piperitone content was obtained from the 100 kg/ha phosphorus dose (17.60%) in the first year and from 50 kg/ha phosphorus treatment (6.64%) in the second year (Table 9). Such a finding complies with the results of Prasad et al. (2012), indicating positive effects of phosphorus fertilization on essential oil concentrations of *Pelargonium* species.

With regard to genotypes, the greatest piperitone content was obtained from genotype 6 (19.30%) in the first year and from genotype 2 (8.14%) in the second year. The lowest piperitone content was observed in genotype 2 (13.38%) in the first year and in genotype 4 (2.12%) in the second year (Table 9).

Effects of nitrogen and phosphorus doses on piperitone contents of the genotypes were found to be significant ($P < 0.01$) (Table 10).

Table 8. Variance analysis results for carvone contents of nitrogen and phosphorus doses.

Sources of variation	SD	Carvone	
		F-values	
		2010	2011
Nitrogen doses (N)	2	11,605.761**	82,776.305**
Phosphorus doses (P)	2	12,479.135**	39,803.501**
Genotypes (G)	2	586,439.872**	4,253,986.689**
N × P	4	22,038.066**	23,195.672**
N × G	4	14,630.762**	123,044.629**
P × G	4	7448.508**	96,137.695**
N × P × G	8	10,585.938**	40,950.523**
Error	54		

**: F-values are significant at P < 0.01.

Table 9. The piperitone contents (%), by nitrogen and phosphorus doses, of spearmint genotypes.

Years	Nitrogen doses (kg/ha)	Phosphorus doses (kg/ha)	Spearmint genotypes			Average of nitrogen doses
			2	4	6	
2010	0	0	0.36	12.81	19.14	14.34C
		50	10.65	14.43	22.44	
		100	11.34	14.77	23.11	
	50	0	11.98	14.94	23.27	16.07B
		50	10.68	13.11	19.51	
		100	11.99	15.92	23.26	
	100	0	25.21	20.06	22.71	17.75A
		50	19.05	14.67	0.00	
		100	19.14	18.58	20.32	
Average of genotypes			13.38C	15.48B	19.30A	16.05A
Average of phosphorus doses			0: 16.72B	50: 13.84C	100: 17.60A	
2011	0	0	1.37	8.93	21.33	6.41A
		50	11.26	0.00	14.83	
		100	0.00	0.00	0.00	
	50	0	0.00	10.17	0.00	2.41C
		50	7.54	0.00	4.02	
		100	0.00	0.00	0.00	
	100	0	12.63	0.00	0.00	5.90B
		50	22.11	0.00	0.00	
		100	18.39	0.00	0.00	
Average of genotypes			8.14A	2.12C	4.46B	4.91B
Average of phosphorus doses			0: 6.05B	50: 6.64A	100: 2.04C	

Differences among means indicated with capital letters are significant at P < 0.01; differences among means indicated with lowercase letters are significant at P < 0.05.

Table 10. Variance analysis results for piperitone contents of nitrogen and phosphorus doses.

Sources of variation	SD	Piperitone	
		F-values	
		2010	2011
Nitrogen doses (N)	2	815,166.115**	3,139,511.182**
Phosphorus doses (P)	2	1,087,279.731**	4,143,773.727**
Genotypes (G)	2	2,534,020.731**	6,107,646.545**
N × P	4	1,615,118.481**	2,198,444.591**
N × G	4	2,951,978.827**	11,287,978.227**
P × G	4	556,523.596**	3,648,497.591**
N × P × G	8	407,875.529**	927,838.295**
Error	54		

**: F-values are significant at $P < 0.01$.

3.6. Caryophyllene content

In the first year, caryophyllene content was observed to be 4.10% with 0 and 50 kg/ha nitrogen doses and 4.93% with the 100 kg/ha nitrogen dose. In the second year, caryophyllene contents increased with increasing nitrogen doses and the values for 0, 50, and 100 kg/ha doses were respectively observed as 3.98%, 4.31%, and 4.41% (Table 11).

Decreasing caryophyllene contents were observed in both years with increasing phosphorus doses. Thus, caryophyllene contents in 0, 50, and 100 kg/ha phosphorus treatments were respectively observed as 4.51%, 4.49%, and 4.13% in the first year and as 4.56%, 4.52%, and 3.62% in the second year (Table 11).

With regard to spearmint genotypes, the greatest caryophyllene content was obtained from genotype 6 in both years (5.87% and 5.42%), followed by genotype 2 (3.82% and 4.07%) and genotype 4 (3.45% and 3.21%) (Table 11).

Effects of phosphorus doses on spearmint caryophyllene contents were also found to be significant ($P < 0.01$) (Table 12).

3.7. Conclusion

The current findings revealed that experimental factors had significant effects on the investigated traits in both years. While carvone, piperitone, and caryophyllene contents were higher in the first year, 1.8-cineole, 4-terpineol, and pulegone were higher in the second year.

Genotypes 2 and 4 were prominent in 1.8-cineole content; genotype 6 was prominent in 4-terpineol, pulegone, and caryophyllene content; genotype 4 was prominent in carvone content; and, finally, genotypes 2 and 6 were prominent in piperitone.

Considering 0, 50, and 100 kg/ha nitrogen treatments, 100 kg/ha N treatment increased 4-terpineol, piperitone, and caryophyllene contents of the first year and pulegone and caryophyllene contents of the second year more than 50 kg/ha N treatment did. On the other hand, 50 kg/ha N treatment increased 1.8-cineole and pulegone contents of the first year but did not yield significant increases in the second year compared to other fertilizer doses. In samples without nitrogen treatment, only the carvone contents increased in the first year, but the greatest 1.8-cineole, 4-terpineol, carvone, and piperitone contents were observed in these samples in the second year.

With 100 kg/ha phosphorus treatments, piperitone and carvone contents increased in the first year, but only the carvone content increased in the second year. While greater 1.8-cineole and pulegone contents were obtained from 50 kg/ha phosphorus treatments in the first year, increase was observed only in piperitone content in the second year. On the other hand, in samples without phosphorus treatments, 4-terpineol and caryophyllene contents were higher in both years than with the other phosphorus doses and an increase was observed only in 1.8-cineole contents in the second year.

Considering the investigated genotypes, the greatest 1.8-cineole content was observed in genotype 4 in the first year and in genotype 2 in the second year. Genotype 6 was prominent in 4-terpineol, pulegone, and caryophyllene contents in both years and genotype 4 was prominent in carvone content in both years. The greatest piperitone content was observed in genotype 6 in the first year and in genotype 2 in the second year.

To conclude, an increase was observed in the studied components at almost every nitrogen and phosphorus

Table 11. The caryophyllene contents (%), by nitrogen and phosphorus doses, of spearmint genotypes.

Years	Nitrogen doses (kg/ha)	Phosphorus doses (kg/ha)	Spearmint genotypes			Average of nitrogen doses
			2	4	6	
2010	0	0	3.33	2.77	4.56	4.10B
		50	3.70	3.49	6.73	
		100	2.47	3.21	6.67	
	50	0	2.71	3.28	5.73	4.10B
		50	3.08	2.48	6.97	
		100	3.90	3.97	4.81	
	100	0	6.80	5.22	6.22	4.93A
		50	5i19	2.62	6.17	
		100	3i25	3.98	4.96	
Average of genotypes			3.82B	3.45C	5.87A	4.38A
Average of phosphorus doses			0: 4.51A	50: 4.49B	100: 4.13C	
2011	0	0	2.94	3.55	5.97	3.98C
		50	4.03	2.38	6.04	
		100	3.63	2.69	4.59	
	50	0	3.37	4.07	6.17	4.31B
		50	4.18	2.80	8.35	
		100	3.46	2.82	3.59	
	100	0	4.76	5.01	5.17	4.41A
		50	5.61	2.11	5.23	
		100	4.63	3.46	3.71	
Average of genotypes			4.07B	3.21C	5.42A	4.23B
Average of phosphorus doses			0: 4.56A	50: 4.52A	100: 3.62B	

Differences among means indicated with capital letters are significant at $P < 0.01$; differences among means indicated with lowercase letters are significant at $P < 0.05$.

Table 12. Variance analysis results for caryophyllene contents of nitrogen and phosphorus doses.

Sources of variation	SD	Caryophyllene F-values	
		2010	2011
Nitrogen doses (N)	2	5180.593**	55.971**
Phosphorus doses (P)	2	1013.898**	312.878**
Genotypes (G)	2	38,197.231**	1378.617**
N × P	4	4736.315**	71.077**
N × G	4	2572.523**	196.218**
P × G	4	3208.787**	360.959**
N × P × G	8	1723.475**	39.803**
Error	54		

**: F-values are significant at $P < 0.01$.

dose. Considering fertilizer cost as a significant input in agricultural practices, genotypes 2 and 4 can be recommended for 50 kg/ha nitrogen and phosphorus doses and 1.8-cineole content; genotype 6 for 4-terpineol, pulegone, and caryophyllene contents; genotype 4 for carvone content; and, finally, genotypes 2 and 6 for piperitone content.

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