Effect of different fertilizers on peppermint - Essential and non-essential nutrients, essential oils and yield

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Highlights

- *Nitrogen fertilizer applications increased herb yield.*
- *In terms of plant nutrients, mono ammonium phosphate and 15:15:15 fertilizers were more effective than other fertilizer applications.*
- *Heavy metal concentrations of peppermint herb were determined below the limit values.*
- *The main compound in peppermint essential oils was menthol in both years.*

Abstract

Peppermint (*Mentha x piperita* L.) plant was grown in this study using different mineral fertilizers combinations. Effects of fertilizer treatments on green and drug herb yields, herb essential nutrients (N, P, K, Ca, Na, Mg, Fe, Zn, Mn, Cu), essential oil yield, essential oil components (menthol, menthone, 1,8 cineole and menthofuran) and non-essential elements (heavy metal) (Pb, Ni, Co, Cr and Cd) were determined in two successive years (2011-2012). The highest green and drug herb yields were obtained from mono ammonium phosphate (MAP) treatments (24,980 kg ha^{-1} and 3070 kg ha^{-1}) in the first year and from 15:15:15 treatments (16,950 kg ha^{-1} and 3080 kg ha^{-1}) in the second year. Nutrient elements nitrogen 2.70% in MAP application, phosphorus 0.55% in mono potassium phosphate (MKP) application, potassium 3.12% in MAP application, calcium 1.47% in di ammonium phosphate (DAP) application, magnesium 0.36% in 15:15:15 application, iron 106 mg kg^{-1} in 15:15:15 application, copper 11.83 mg kg^{-1} in MAP application, zinc 35 mg kg^{-1} in MKP application and manganese 89 mg kg^{-1} in MAP application the highest value were respectively obtained from treatments. Concentrations of non-essential elements (Pb, Ni, Cr and Cd) in herb were found to be below toxic values. In both years, the highest essential oil yields were obtained from MAP treatments (4.10% in the first year and 2.90% in the second year). The essential oil components of peppermint were menthol, menthone, 1,8 cineole and menthofuran and menthol was the major component in both years.

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Introduction

Peppermint (*Mentha x piperita* L.) is an important medicinal and aromatic plant belonging to the Lamiaceae family (Gupta *et al*., 2017). *Mentha* is a popular herb that has aromotherapeutic and medicinal properties, with the most commonly used mints for cultivation being *M. piperita* L. and *M. spicata* L. (Gholamipourfard *et al*., 2021). The quality of peppermint essential oils mostly relies on the percentages of menthol, menthone, menthofuran and pulegone, and essential oil quality is improved by higher menthol and menthone content and lower menthofuran and pulegone contents (Barros *et al*., 2015). The chemical composition of peppermint essential oils is affected by variety and environmental conditions such as moisture, nutrients in the soil, temperature, and biotic and/or abiotic stress (De Sousa Barros *et al*., 2015). Nutrient availability has a key role in improving biomass productivity and qualitative properties of essential oils in medicinal and aromatic plants (Machiani *et al*., 2019). Fertilization, especially N, P and micro elements (Fe, Mn, Zn), has a positive effect on the quality of peppermint essential oil. (Amooaghaie and Golmohammadi, 2017). The application of chemical fertilizer, especially N and P, could enhance the plant growth characteristics and yield by improving the photosynthetic rate (Iqbal *et al*., 2019). It was stated that the quality of peppermint essential oil was related to the increase in the percentage of menthol and the decrease of mentofuran with the application of 50% chemical fertilizer+nano-chelated fertilizer (Ostadi *et al*., 2020). Moreover, fertilizer compound NPK [nitrogen (N), phosphorus (P), and potassium (K)] increased dry matter yield (Sheykholeslami *et al*., 2015). Nitrogen, phosphorus, and potassium, the so-called three fertility elements, are taken into account because they are often the most used in agricultural practice (Carrubba, 2015). In this study, the effects of different fertilizer combinations of inorganic fertilizers compound NKP (15:15:15, NH_4NO_3, K_2SO_4 and DAP) on green and drug herb yields, essential macro- and micro-nutrients, non-essential elements (*i.e.* some heavy metal contents), essential oil yield and components of peppermint were investigated in *Mentha x piperita* L.

Materials and methods

This study started in the first week of May 2011 in a conventional farming field in Kuşadası town of Aydın province (37° 46' 06'' N, 27° 17' 26'' E). Meteorological data for the experimental area are presented in Figure 1. The Kusadası town of Aydın province, where the research area is located, has a Mediterranean climate, with hot dry summers and cool rainy winters. According to the 2011 meteorological data of the study area, the monthly average minimum temperature was 13.5°C, the average maximum temperature was 21.9°C and the monthly average precipitation was 27.9 mm. *Meteorological data of 2012*: the monthly average minimum temperature was 14.8°C, the average maximum temperature was 22.6°C and the monthly average precipitation was 33.3 mm (Anonymous, 2021). The climate data were obtained from the Turkish State Meteorological Service data base.

The commercial name of the mint cultivar used for this study was mentha/*Mentha x piperita* L. The seedlings were grown for 15 days in an organically certified nursery and the growing media was prepared for every new sowing. Mint seedlings were produced in multi-cell polyester viols. The total porosity of the plant growing medium was 80% (Landis, 1990), slightly acidic pH 5.5-6.5, electrical conductivity (EC) 0.42 dS cm^{-1} (Abad *et al*., 2001), volume weight 0.07 g cm^{-3}, C/N ratio 20 (Klock and Fitzpatrick, 1997), nitrogen 0.92%, phosphorus 0.022% and potassium 1.36%, (Tüzel, 2017) Sphagnum peat with was used. The 15 day old mint plants were transplanted to our experimental field. The plants were planted by hand in the first week of May 2011 and 2012. Mentha seedlings were planted in rows spaced 50 cm apart with 25 cm intra row spacing and were irrigated according to class A pan. Well waters close to the study area were used as irrigation water source. The pH and EC values of the irrigation water were determined as 7.04 and 1.267 dS cm^{-1}. The plants were irrigated with drip irrigation. In the years before the establishment of this experiment, different vegetables were produced in the experimental area. The experiment was conducted in a randomized block design with 3 replications on 20 m^2 in size and 30 cm side effect was considered between the plots. The physical and chemical properties of the soil used for the study are listed in Table 1. The field experiment had five different treatments in the following proportions of fertilizers combinations per plot: i) 15:15:15 (266 kg ha^{-1}) + NH_4NO_3 (188 kg ha^{-1}) + K_2SO_4 (160 kg ha^{-1}) (applications 15:15:15); ii) MAP (65.5 kg ha^{-1}) + NH_4NO_3 (278 kg ha^{-1}) + K_2SO_4 (240 kg ha^{-1}) (applications MAP); iii) DAP (87 kg ha^{-1}) + NH_4NO_3 (255 kg ha^{-1}) + K_2SO_4 (240 kg ha^{-1}) (applications DAP); iv) MKP (77 kg ha^{-1}) + NH_4NO_3 (303 kg ha^{-1}) + K_2SO_4 (188 kg ha^{-1}) (applications MKP); and v) Control (unfertilized).

Before the seedlings were planted, mineral fertilizers were applied to the plots at a depth of 10-15 cm and mixed with the soil by disc harrow. No pests were observed. The 15:15:15, di ammonium phosphate (DAP), mono ammonium phosphate (MAP) and mono potassium phosphate (MKP) fertilizers, commonly used in conventional farming, were used in the fertilizer treatments. Phosphorus and potassium fertilizers 15:15:15, K_2SO_4, MAP, DAP and MKP were only applied during transplanting. In conventional cultivation half of the ammonium nitrate fertilizer is given from the basic fertilizers (1/2), and the remaining half is given after the first harvest as a top dressing fertilizer. Polythene mulching technique has been used to reduce weed prevention and control. Plastic mulch in both black and clear film form was used for this purpose. Polythene mulching was done by spreading plastic layers between or around the plant rows on the soil surface (Steinmetz *et al*., 2016; Chopra and Koul, 2020). No plant protection products were used during cultivation. Deficit quantities of fertilizers for application were completed to a sufficient volume equivalent to complete fertilization with K_2SO_4 and NH_4NO_3 like single fertilizers. Throughout the vegetation period of peppermint plants, two harvests were performed at the 50% of flowering stage of peppermint in both years (Machiani *et al*., 2018) (the whole plants were used for extraction of essential oil). In the first year, the first harvest was made on 7 July 2011 and the second harvest on 28 August 2011. During the second year, the first harvest was made on the 8 July 2012 and second harvest on 29 August 2012. The harvest was carried out mechanically, and the whole plants in each plot were cut 5

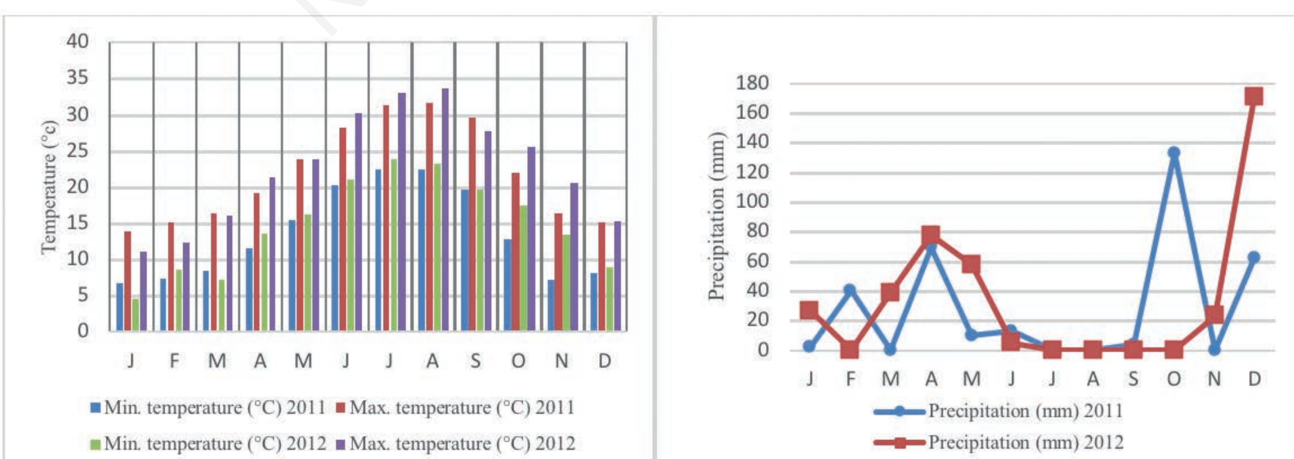

Figure 1. Climate data temperature and precipitation of the experimental area.

cm above the surface of soil and green herb yields were determined. After determining the fresh weight of peppermint at each harvest, the samples were dried in the shade and their dry biomass yield measured. Green herb samples were dried under shade, and drug herb yields were determined. For nutrient analyses, fresh samples taken from each plot were washed through distilled water, then the plant samples were dried at 65-70°C for 48 h and grinded made ready for plant nutrients analysis. Results were expressed on dry matter (105°C) bases. Nitrogen content was determined in accordance with a modified Kjeldahl method (Bremner, 1965). Dry ash-extracts were used to determine phosphorus content colorimetrically with the use of the P vanadomolibdo phosphoric yellow colour method (Lott *et al*., 1956). Potassium, Ca and Na contents were determined by a flame photometer and magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), lead (Pb), nickel (Ni), cobalt (Co), chromium (Cr) and cadmium (Cd) contents by an atomic absorption spectrophotomer (Kacar and İnal., 2008).

Essential oils were obtained through a simple distillation method. Just before flowering, 1/3 of the upper section of the plants including flower, bud and young leaves were cut and then air-dried in shade at ambient temperature (Mrlianova *et al*. 2001). Distillations were performed in accordance with an essential oil distillation method registered in European Pharmacopoeia with the use of a Clevenger-type distillation apparatus. Samples were preserved in dark-colour bottles at +4°C in the dark until analysis (European Pharmacopoeia, 1997).

Qualitative and quantitative essential oil analyses: GS and GC-MS method; the chemical compositions of essential oils were determined with gas chromatography (GC) and mass spectrometry systems (GC/MS). GC-MS analyses were performed using a HP Agilent 6890 combined with HP 5973 selective mass detector and HP-5 capillary column (60 m × 0.25 mm i.d, film thickness of 0.25 µm). In the GC-MS system, electron ionization system with ionization energy of 70 eV was used and helium was used as the carrier gas at 1 mL min^{-1} flow rate. System temperature was gradually increased from 50°C to 300°C with an increment rate of 10°C min^{-1}, injector temperature was set as 150°C and detector temperature was set as 250°C. Essential oil samples were diluted with ethyl acetate in 1 100^{-1} (do you mean: 1 and 100^{-1}) , h h^{-1} ratio and 1.0 µL of the sample was injected with an auto-sampler at unsplit mode (Öztürk *et al*., 2005). Essential oil quantitative analyses were conducted by comparing retention times of sample compounds with retention times of commercial standards of the main compounds, through comparing arithmetic indices of essential oils calculated by retention times of the compounds in alkane mixture of FID detector with the index list of Adams and through comparing mass spectrums obtained from GC-MS analyses with Willey 275 mass database in the computer (Adams, 1995).

Statistical analyses were conducted using SPSS Statistics 20.0 software in accordance with a randomized block design, and significant means were compared. The LSD *post-hoc* test was used to find if differences in the treatments were significant at P<0.05. Since the effect of the years on the applications was not significant, the years were analysed statistically separately.

Results and discussion

Green herb yield

Based on optimum peppermint yield values of previous studies (peppermint fresh herb yields of between 6700-13,500 kg ha^{-1}), plant nutrient requirements were determined to be 280 kg ha^{-1} N, 200 kg ha^{-1} P_2O_5 and 280 kg ha^{-1} K_2O and applied accordingly (Mitchell and Farris, 1996; Scavroni *et al*., 2005). Fertilizer treatments on green herb yields were found to have a significant impact (P<0.01); in the first year the lowest green herb yield (6540 kg ha^{-1}) was obtained from the control treatment and the highest green herb 24,980 kg ha^{-1} from MAP application. The lowest green herb yield in the second year (7690 kg ha^{-1}) was again obtained from the control treatment and the highest (16,950 kg ha^{-1}) from the 15:15:15 treatment. In both years, the lowest green herb yields were found in the control application and the greatest values were seen in MAP and 15:15:15 treatments. The effects of mineral fertilizers on menta yield are presented in Table 2. The present findings on green herb yields were similar to the results given by Yeşil *et al*. (2018) (6816.7-18,337.7 kg ha^{-1}), and different to the values of Sadowska *et al*. (2020) (14.8-18.5 t ha^{-1}). These differences can be explained as follows: many studies indicated that herb yields varied according to growing regions, climate, plant age (Telci and Şahbaz,

Table 1. Physical and chemical characteristics of experimental soils.

Properties	Values	Properties	Values
pH[a]	7.60	Mg[c] (mg kg^{-1})	290
Total salt (%)	0.033	Na[c] (mg kg^{-1})	42.4
Clay (%)	50.56	Fe[c] (mg kg^{-1})	8.65
Silt (%)	30.00	Cu[c] (mg kg^{-1})	2.71
Clay (%)	19.44	Zn[c] (mg kg^{-1})	1.32
Texture	Loam	Mn[c] (mg kg^{-1})	7.61
Organic matter (%)	0.82	Pb[d] (mg kg^{-1})	20.50
$CaCO_3$ (%)	19.94	Ni[d] (mg kg^{-1})	40.66
N[b] (%)	0.090	Co[d] (mg kg^{-1})	126
P[c] (mg kg^{-1})	2.17	Cr[d] (mg kg^{-1})	21.19
K[c] (mg kg^{-1})	104	Cd[d] (mg kg^{-1})	Bdl
Ca[c] (mg kg^{-1})	5600	CEC[f] (meq/100gr)	15.36

[a]w:v, 1:2.5 water; [b]Total; [c]Available P, water extractable by the Bingham - Available K, Ca, Mg and Na:1N NH4OAc pH=7 - Available Fe, Cu, Mn and Zn; DTPA-extractable; Bdl: below detection limit; [d]Total Pb, Co, Cr and Cd extracted with aqua regia; [f]Cation Exchange Capacity (CEC).

2005a), cultivar (Lothe *et al*., 2021) and growing conditions (Singh *et al*., 1995).

Drug herb yield

Fertilizer treatments had significantly different effects on drug herb yields. The effects of treatments on drug herb yields were found to be significant at the $P<0.05$ level in the first year (1690-3070 kg ha^{-1}) and at $P<0.01$ level in the second year (1400-3080 kg ha^{-1}). In both years, the lowest value (1690 kg ha^{-1}) was obtained from the control treatment. The highest drug herb yield was obtained from the MAP treatment (3070 kg ha^{-1}) in the first year and from 15:15:15 treatment (3080 kg ha^{-1}) in the second year. This suggests that the ratio of fresh to dry yields of MAP, DAP and 15:15:15 fertilizer applications containing N in its formulation are higher than the non-nitrogen MKP and control applications due to the effect of nitrogen fertilization (Ardalani *et al*., 2017). The present findings of drug herb yields were different from the results of Sadowska *et al*. (2020) (4.10-5.07 t ha^{-1}). Plant genetics, climate conditions and cultural practices significantly influence drug herb yields. Nitrogenous fertilizer treatments were also reported to increase drug herb yields (Mahantesh *et al*., 2018; Sadowska *et al*., 2020). Fertilizer applications containing nitrogen (15:15:15, MAP and DAP) increased mentha yield compared to those without nitrogen (MKP and control). Shangguan *et al*. (2000) reported that most absorbed N (about 75%) is allocated to the chloroplasts and its availability could increase the photosynthetic rate and improve morphological traits and productivity. Ram *et al*. (2005) and Zheljazkov *et al*. (2010) reported an increase in mint biomass yield with nitrogen doses.

Essential elements of the herb

Results of the research into the effect of fertilizer treatments on the content of macro elements are shown in Table 3.

Nitrogen: Fertilizer treatments on herb N contents were found to be significant at the $P<0.01$ level. Nitrogen content varied between 1.38-1.92% in the first year and between 2.08-2.70% in the second year. The higher herb N content of the second year than the first year could mostly be attributed to soil characteristics and the remaining effects of fertilizers (Sadowska *et al*., 2020). The current findings on N contents were similar to Aziz *et al*. (2011) (1.68-2.12%) and Mahantesh *et al*. (2018) (1.34-2.40%). In both years, the lowest N contents were obtained from the control treatments and the highest from MAP treatments. Since MAP fertilizer had greater solubility than that of 15:15:15 and DAP fertilizers, the effects of MAP were more remarkable.

Phosphorus: The effect of fertilizer sources on herb P contents were found to be significant at the $P<0.01$ level in the first year. The lowest P content (0.41%) was observed in the control treatment and the greatest (0.55%) in the MKP treatment. Phosphorus concentration was determined between 0.37-0.40%, P contents were not significant in the second year. Herb P contents were greater in the first year than in the second year. Our results were greater than the values reported by Ostadi *et al*. (2020) (0.150-0.223%) and different to those of Pytlakowska *et al*. (2012) and Mahantesh *et al*. (2018). Such differences were attributed to growing conditions, genetic factors and geographical position (Kızıl *et al*., 2010).

Potassium: Fertilizer treatments had significant effects on herb K contents at the $P<0.01$ level. Herb K contents varied between 2.40-3.12% in the first year and between 2.30-2.96% in the second year. In both years, the lowest values were found in the control treatments. In the first year, treatment effects resulted in differentiation of herb K contents and the greatest value was obtained from the MAP treatment. In the second year, the greatest herb K content

Table 2. The effect of mineral fertilizers on mentha yield.

	2011 (first year)		2012 (second year)	
Treatments	Green herb yield	Drug herb yield (kg ha^{-1})	Green herb yield	Drug herb yield
15:15:15[a]	18,470[b]	2850[a]	16,950[a]	3080[a]
MAP[b]	24,980[a]	3070[a]	16,450[a]	2990[a]
DAP[c]	17,830[c]	2950[a]	16,200[a]	2940[a]
MKP[d]	14,150[d]	2240[ab]	12,310[ab]	2240[ab]
Control	6540[e]	1690[b]	7690[b]	1400[b]
Fertilizer treatment LSD	(%1) 50.843	(%5) 84.49	(%1) 688.393	(%1) 125.11

[a]Inorganic fertilizers compound NKP; [b]mono ammonium phosphate; [c]di ammonium phosphate; [d]mono potassium phosphate.

Table 3. Contents of macro nutrients of mentha.

	2011 (first year) (%)					2012 (second year) (%)				
Treatments	N	P	K	Ca	Mg	N	P	K	Ca	Mg
15:15:15[a]	1.74[b]	0.46[bc]	2.62[c]	1.34[a]	0.21[a]	2.50[ab]	0.38	2.53[b]	1.25[ab]	0.36[a]
MAP[b]	1.92[a]	0.47[b]	3.12[a]	1.11[b]	0.10[c]	2.70[a]	0.40	2.61[ab]	1.37[a]	0.15[b]
DAP[c]	1.44[d]	0.44[c]	2.52[d]	1.13[b]	0.12[b]	2.37[b]	0.37	2.96[a]	1.47[a]	0.15[b]
MKP[d]	1.64[c]	0.55[a]	3.02[b]	1.12[b]	0.08[d]	2.55[ab]	0.39	2.65[ab]	1.42[a]	0.11[bc]
Control	1.38[e]	0.41[d]	2.40[e]	1.17[b]	0.07[d]	2.08[c]	0.40	2.30[b]	1.01[b]	0.09[c]
Fertilizer treatment LSD	(%1) 0.05	(%1) 0.02	(%1) 0.03	(%1) 0.14	(%1) 0.01	(%1) 0.27	ns	(%1) 0.41	(%5) 0.32	(%1) 0.05

ns, not significant. [a]Inorganic fertilizers compound NKP; [b]mono ammonium phosphate; [c]di ammonium phosphate; [d]mono potassium phosphate.

was obtained from the DAP treatment. Results from both years were quite similar to each other. Herb K contents determined in this study were greater than the findings of Özcan and Akbulut (2008) and Aziz *et al*. (2011), but similar to the reports of Queralt *et al*. (2005).

Calcium: In the first year, herb Ca content ranged from 1.11-1.34% with the lowest value from the MAP treatment and the greatest from the 15:15:15 treatment, and the effects were significant at $P<0.01$ level. In the second year, herb Ca content varied between 1.01-1.47%; the lowest Ca being in the control treatment and the highest in DAP. The effects of fertilizer treatments on Ca contents were found to be significant at the $P<0.05$ level. The present findings on herb Ca contents were similar to those of Kızıl *et al*. (2010) and Rubio *et al*. (2012), but different to the findings of Queralt *et al*. (2005) and Özcan and Akbulut (2008). Some of our results of mineral contents of medicinal and aromatic plants show differences when compared with the literature (Özcan, 2004). These differences might be due to growth conditions, genetic factors, geographical variations and analytical procedures (Guil *et al*., 1998).

Magnesium: Herb Mg contents varied between 0.076-0.217% in the first year, the lowest being in the control treatment and the highest in the 15:15:15 treatment. The effects of fertilizers were found to be significant at the $P<0.01$ level. In the second year, the lowest herb Mg content (0.99%) was obtained from the control treatment, and the greatest (0.36%) from the 15:15:15 treatment. In terms of the effects of fertilizer treatments, the 15:15:15 treatment was found to be prominent in both years. The present findings on herb Mg contents were lower than the values of Queralt *et al*. (2005) and Özcan and Akbulut (2008), but similar to the results of Kızıl *et al*. (2010) and Rubio *et al.* (2012). Arrobas *et al*. (2018) reported nitrogen, phosphorus, potassium, calcium and magnesium contents respectively as between 3.2-4.2, 0.12-0.45, 1.0-3.0, 0.70-2.3 and 0.40-1.0 (%DM), our findings were characterized as being consistent with low N and Mg concentrations.

Sodium: Concentration of Na varied between 284-354 mg kg^{-1} with the lowest value from the DAP treatment. Micro element results are presented in Table 4. In the first year and the greatest concentration from the MKP treatment with effects were significant at $P<0.01$ level. In the second year, herb Na contents varied between 304-377 mg kg^{-1}, and the effects of fertilizer treatments were found to be significant at the $P<0.05$ level. MKP treatments resulted in greater herb Na contents. The present findings on herb Na content were lower than the values of Queralt *et al*. (2005), Özcan and Akbulut (2008), and Rubio *et al*. (2012). This may be due to irrigation water and soil characteristics.

Iron: Effect of fertilizer treatments on herb Fe contents was found to be significant at the $P<0.01$ level in the first year. The concentration of Fe varied between 50-93 mg kg^{-1} with the lowest values in the MKP treatment and greatest values in the 15:15:15 treatment. In the second year Fe concentration varied between 68-106 mg kg^{-1} with lower values in the control and greater values in the 15:15:15 treatment. Fe values were lower than the reference of Rubio *et al*., (2012) (406.0 mg kg^{-1}) and Kızıl *et al*. (2010) (531.5 mg kg^{-1}), but within the range of Schulze *et al*. (2005) who recommended 50-250 mg kg^{-1}. Herb Fe concentration was greater in the second year than in the first year. Fertilizer treatments had similar effects and the greatest Fe content was obtained from the 15:15:15 treatment and micro elements are shown in Table 4.

Copper: Fertilizer treatment had significant effects on herb Cu contents at the $P<0.01$ level in the first year. The lowest concentration Cu 8.09 mg kg^{-1} was obtained from the control application and the greatest Cu concentration was 9.86 mg kg^{-1} from the 15:15:15 treatment. In the second year, the effects of fertilizer treatments on herb Cu contents were found to be significant at the $P<0.05$ level. The lowest value 8.00 mg kg^{-1} was determined in the control treatment and the greatest concentration of Cu was 11.83 mg kg^{-1} in the MAP treatment. The greatest Cu contents were generally obtained from the 15:15:15 and MAP treatments respectively. The plant Cu concentration was greater than the values of Rubio *et al*. (2012) (3.68 mg kg^{-1}), but similar to Kizil *et al.* (2010) (11.52 mg kg^{-1}), Kloke *et al.* (1984) (3-5 mg kg^{-1}) and Kabata-Pendias and Pendias (1992) indicated a sufficient Cu level to be 20 mg kg^{-1}.

Zinc: Fertilizer treatments had significant effects on herb concentration of Zn at the $P<0.01$ level. Herb Zn concentration varied between 21-27 mg kg^{-1} in the first year and between 25-35 mg kg^{-1} in the second year. In both years, the lowest herb Zn contents 21 mg kg^{-1} and 25 mg kg^{-1} were respectively obtained from the control treatments, and the greatest concentration 27 mg kg^{-1} and 35 mg kg^{-1} from MKP treatments. The present findings on herb Zn contents were greater than the values given by Kızıl *et al*. (2010) (12.64 mg kg^{-1}) and Rubio *et al*. (2012) (15.72 mg kg^{-1}) and within the range of Kloke *et al*. (1984) (15-150 mg kg^{-1}).

Manganese: Fertiliser treatments had significant ($P<0.01$) effects on herb Mn concentration in the first year the lowest concentration 42 mg kg^{-1} was measured in the control treatment and the highest 69 mg kg^{-1} in the DAP treatment. In the second year, the effects of fertilizer treatments on herb Mn contents were found to be significant at the $P<0.05$ level. The greatest herb Mn value was obtained from the MAP application. The present findings on herb Mn contents were similar to the results of Kızıl *et al*. (2010) (71.82 mg kg^{-1}), greater than those of Rubio *et al*. (2012) (33.43 mg kg^{-1}) and within the range, Misra and Mani (1991) recom-

Table 4. Contents of micronutrients of mentha.

	2011 (first year) (mg kg^{-1})					2012 (second year) (mg kg^{-1})				
Treatments	Fe	Cu	Zn	Mn	Na	Fe	Cu	Zn	Mn	Na
15:15:15[a]	93[a]	9.86[a]	24[b]	67[b]	312[c]	106[a]	8.69[bc]	26[c]	55[b]	310[bc]
MAP[b]	63[c]	8.65[bc]	22[bc]	43[d]	344[ab]	83[bc]	11.83[a]	30[b]	89[a]	346[ab]
DAP[c]	74[b]	8.38[bc]	22[c]	69[a]	284[d]	88[b]	10.89[ab]	28[bc]	56[b]	304[c]
MKP[d]	50[d]	8.90[b]	27[a]	48[c]	354[a]	71[c]	10.74[ab]	35[a]	72[ab]	377[a]
Control	51[d]	8.09[c]	21[c]	42[e]	332[b]	68[c]	8.00[c]	25[c]	67[ab]	321[bc]
Fertilizer treatment LSD	(%1) 1.97	(%1) 0.62	(%1) 1.57	(%1) 1.64	(%1) 15.97	(%1) 15.715	(%5) 2.287	(%1) 3.842	(%5) 21.668	(%5) 37.30

[a]Inorganic fertilizers compound NKP; [b]mono ammonium phosphate; [c]di ammonium phosphate; [d]mono potassium phosphate.

mended for plants (15-100 mg kg^{-1}). Dried and fresh *M. piperita* leaves contain a considerable amount of macro and micronutrients. In general, peppermint leaves have high values of K, Ca, P, Mg, and Na minerals (Uribe *et al*., 2016). Santos *et al*. (2014) stated that metal uptake by plants can be affected by a variety of factors, including plant species, soil structure, climate, and agricultural practices. Nutrients play a vital role in the growth yields and quality attributes of the plants. For the return of the crops, nitrogen, phosphorous, and potassium (NPK) are the building blocks amongst the other plant nutrients, and integrated management is also necessary. For the maximum oil content and oil yields, cultivators apply excessive amounts of nitrogen (N) fertilizer to maximize oil yield. This stress, like an inadequate amount of nitrogen, reduces yield (Grigoleit and Grigoleit, 2005). Variation in chemical composition is strongly related to environmental factors, such as climate, soil type (Arrobas *et al*., 2018), agronomic conditions and genotype content (Burt, 2004), field management (Amani Machiani *et al*., 2018).

Non-essential elements of the herb

The content of heavy metals in the mentha plants grown under different fertilization regimes are presented in Table 5.

Lead: The effects of treatments on herb Pb contents were found to be significant at the P<0.01 level. Pb contents varied between 0.10-0.27 mg kg^{-1} in the first year and between 0.13-0.32 mg kg^{-1} in the second year. In the first year, the lowest herb Pb contents were determined from MKP and MAP treatments and the greatest from DAP application. A similar case was determined in the second year with the lowest value (0.17 mg kg^{-1}) from the control treatment and the greatest (0.32 mg kg^{-1}) from DAP treatment. In both years, low Pb concentrations were seen in MAP treatments and the highest in DAP application. Pb results in this study were below the limiting value of 10 mg kg^{-1} Pb of the World Health Organization (Rao *et al*., 2011) and the values reported by Kloke *et al*. (1984) (1-5 mg kg^{-1}).

Nickel: Fertilizer treatments had significant influences on nickel in the first year at the P<0.01 level, and varied between 2.64-2.90 mg kg^{-1}, being lowest in control treatment and highest in the 15:15:15 treatment. In the second year, fertiliser treatments significantly (P<0.05 level) affected the herb Ni contents with a variation from 2.76-3.96 mg kg^{-1}, with the lowest value from the control treatment and the greatest value from MAP treatment. The herb Ni values were greater in the second year than in the first year. In two years, the 15:15:15 and MAP treatment peppermint plants had greater Ni contents than the other treatments. The present findings on herb Ni contents were parallel with the findings of Kloke *et al*. (1984) (0.1-5.0 mg kg^{-1}) and Rubio *et al*. (2012) (0.53-4.84 mg kg^{-1}).

Cobalt: In the case of Co, the first year findings showed that treatments induced a significant effect at the P<0.05 level, and the change with respect to treatments was between 0.70-0.76 mg kg^{-1}. In the second year, effects were significant at the P<0.01 level and the values varied between 0.63-0.77 mg kg^{-1}. In both years, the lowest values were obtained from the control treatments (0.70 mg kg^{-1} and 0.63 mg kg^{-1}). Compared to the other treatments, the 15:15:15 treatment yielded higher Co concentrations in both years (0.76 and 0.77 mg kg^{-1}). The present results were higher than Rubio *et al*. (2012) (0.26 mg kg^{-1}) and lower than the values specified in Turkish Food Codex and WHO/FAO for food/vegetable and some foodstuffs (0.02-1.0 mg kg^{-1}) (WHO, 2005; WHO, 2006).

Chrome: Our findings with respect to the effect of fertiliser

Table 5. Contents of heavy metals of mentha.

	2011 (first year) (mg kg^{-1})					2012 (second year) (mg kg^{-1})				
Treatments	Pb	Ni	Co	Cr	Cd	Pb	Ni	Co	Cr	Cd
15:15:15[a]	0.20[ab]	2.90[a]	0.76[a]	0.27[b]	0.053	0.27[a]	3.57[ab]	0.77[a]	0.37[a]	0.12
MAP[b]	0.10[c]	2.76[ab]	0.75[a]	0.17[c]	0.043	0.14[b]	3.96[a]	0.77[a]	0.18[b]	0.10
DAP[c]	0.27[a]	2.81[a]	0.75[a]	0.32[a]	0.053	0.32[a]	3.04[bc]	0.72[a]	0.37[a]	0.11
MKP[d]	0.10[c]	2.77[ab]	0.75[a]	0.21[c]	0.053	0.17[b]	3.26[bc]	0.71[a]	0.31[a]	0.10
Control	0.13[bc]	2.64[b]	0.70[b]	0.17[c]	0.053	0.13[b]	2.76[c]	0.63[b]	0.14[b]	0.09
Fertilizer treatment LSD	(%1) 0.09	(%1) 0.15	(%5) 0.04	(%1) 0.04	ns	(%1) 0.05	(%5) 0.64	(%1) 0.07	(%1) 0.10	ns

ns, not significant. [a]Inorganic fertilizers compound NKP; [b]mono ammonium phosphate; [c]di ammonium phosphate; [d]mono potassium phosphate.

Table 6. Effect of mineral fertilizers on essential oil components of mentha.

	2011 (first year) (%)					2012 (second year) (%)				
Treatments	Essential oil yields	1,8-cineole	Menthone	Menthofuran	Menthol	Essential oil yields	1,8-cineole	Menthone	Menthofuran	Menthol
15:15:15[a]	3.65[c]	6.44[c]	22.65[e]	2.95[d]	39.40[a]	2.10[c]	6.51[a]	18.91[d]	8.13[a]	38.17[b]
MAP[b]	4.10[a]	6.13[d]	26.97[a]	3.72[a]	34.88[d]	2.90[a]	5.72[bc]	25.23[c]	4.57[d]	36.60[d]
DAP[c]	3.85[b]	7.04[b]	26.02[b]	3.05[c]	36.71[c]	2.40[b]	5.61[c]	26.73[b]	5.33[c]	34.92[e]
MKP[d]	3.80[b]	7.11[b]	24.67[d]	3.47[b]	36.81[c]	2.20[c]	5.29[d]	27.69[a]	2.80[e]	37.08[c]
Control	3.55[d]	7.27a	25.58[c]	2.76[e]	37.59[b]	2.10[c]	5.92[b]	14.33[e]	5.78[b]	41.85[a]
Fertilizer treatment LSD	(%1) 0.08	(%1) 0.12	(%1) 0.313	(%1) 0.08	(%1) 0.515	(%1) 0.13	(%1) 0.20	(%1) 0.07	(%1) 0.07	(%1) 0.10

[a]Inorganic fertilizers compound NKP; [b]mono ammonium phosphate; [c]di ammonium phosphate; [d]mono potassium phosphate.

type on peppermint herb Cr contents were found to be significant at the P<0.01 level and measurements indicated a variation between 0.17-0.32 mg kg^{-1} in the first year and between 0.14-0.37 mg kg^{-1} in the second year. In the first year, control treatment plants had the lowest Cr content (0.17 mg kg^{-1}) and DAP the highest (0.32 mg kg^{-1}) In the second year, the lowest value (0.14 mg kg^{-1}) was observed in the control application and the greatest value (0.37 mg kg^{-1}) from the DAP and 15:15:15 treatments. Among the fertilizer treatments, the lowest values (0.17 mg kg^{-1}) in the first year and 0.18 mg kg^{-1} in the second year) were obtained from the MAP treatment. The present findings on herb Cr contents were lower than the findings of World Health Organization, indicating 2 mg kg^{-1} for medicinal plants (WHO, 2005), of Rubio *et al*. (2012) (1.65 mg kg^{-1}) and of Kızıl *et al*. (2010) (5.41 mg kg^{-1}). Greater Cr values were seen in the second year.

Cadmium: Herb Cd contents significantly changed with respect to fertilizer treatments in the first year, being significant at the P<0.05 level and the values varied between 0.043-0.053 mg kg^{-1}. In the second year, the effects were not significant, and results varied between 0.090-0.12 mg kg^{-1}, with the lowest value from the control and the greatest from the 15:15:15 treatment. Cd contents were lower than the findings of Kızıl *et al*. (2010) (0.210-0.220 mg kg^{-1}) of Rubio *et al*. (2012) (0.22 mg kg^{-1}) and the limit indicated by World Health Organization (0.3 mg kg^{-1} Cd) (Rao *et al*., 2011). Conventionally grown and fertilized peppermint plants generally had greater heavy metal contents in the second year of the study. Despite the fertilization, the lowest heavy metal levels were seen in MAP and MKP treatments.

Essential oil yields

The results presented in Table 6 show the effect of fertilizer treatment on essential oil components. Fertiliser treatment effects on essential oil yields were found to be significant at the p<0.01 level. In the first year, the lowest essential oil yield (3.55%) was observed in the control application and the greatest essential oil yield (4.10%) from the MAP treatment. In the second year, essential oil yields varied between 2.10-2.90%, the lowest yield was from the control treatment and the highest from the MAP treatment (Table 6). Greater essential oil yields were determined in the first year of the study. The greatest essential oil yields were obtained from the MAP treatments in both years. Similar to the effects on total N content, MAP fertilizer had significant effects on essential oil yields. The essential oil yields reported in this study were different from the findings of Machiani *et al*. (2018) (2.2-1.1%) and of Ostadia *et al*. (2020) (1.4-2.7%) indicating significant effects of genotype, environment, fertilizer sources and harvest times on essential oil yields.

Essential oil components

The essential oil gas chromatograms in the first and second years are shown in Figures 2 and 3. In both years menthol, menthone, 1,8-cineole and menthofuran were identified as the essential oil components. Essential oils are secondary metabolites and are composed of highly complex compounds (Tabatabaie and Nazari, 2007). Fertiliser treatments on 1,8 cineole contents were found to be significant at the P<0.01 level. In the first year, the lowest 1,8 cineole value (6.13%) was obtained from MAP application and the greatest (7.27%) from the control treatment. The 1,8 cineole contents varied within a narrow range. In the second year, the lowest value (5.29%) was determined from the MKP treatment and the highest (6.51%) from the 15:15:15 treatment. The present findings on 1,8 cineole contents were similar with the results of Telci *et al*. (2011) (5.59-5.76%) and Kraft and Hobbs (2004) (6-8%). Effects of fertilizer treatments on menthone contents were also significant

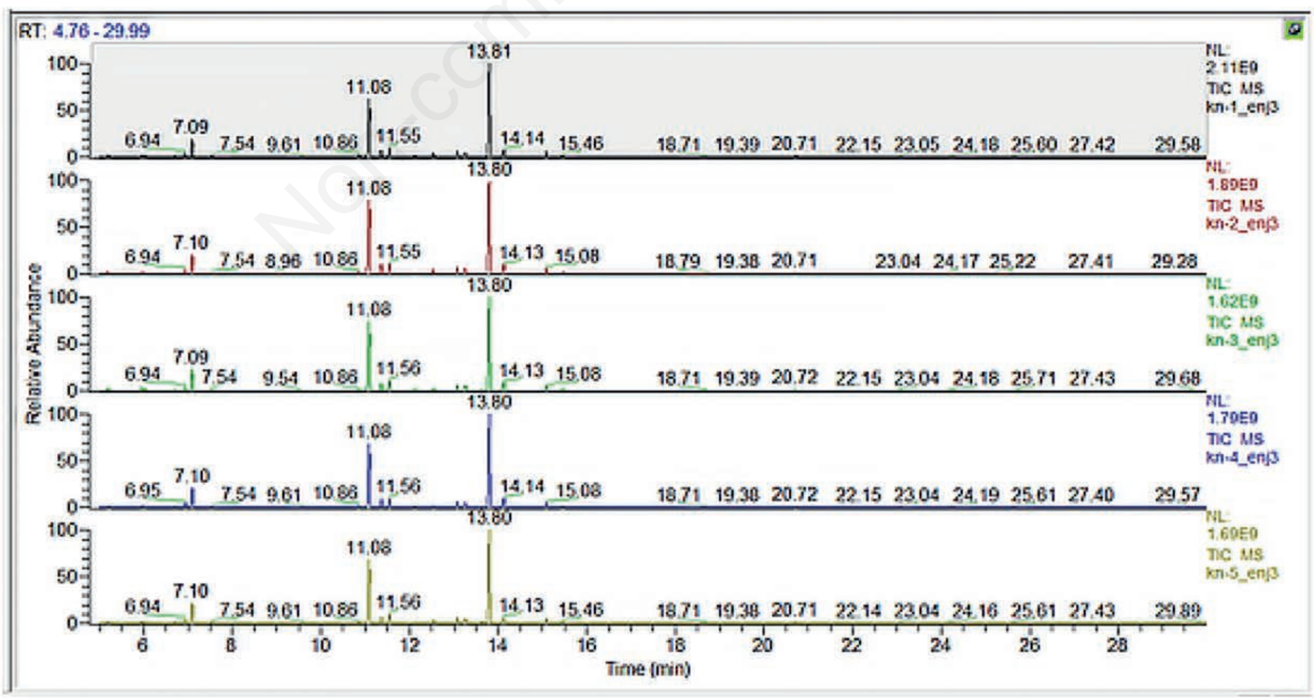

Figure 2. Essential oil gas chromatogram in the mass spectrometry (MS) detector obtained from treatment in the first year. Kn1; 15:15:15, Kn2; MAP, Kn3; DAP, Kn4; MKP and Kn5; control treatment.

at the P<0.01 level. In the first year, the lowest value (22.65%) was obtained from the 15:15:15 treatment and the greatest (26.97%) from the monoammonium phosphate (MAP) treatment. In the second year, the lowest menthone value (14.33%) was obtained from the control treatment and the greatest (27.69%) from MKP. Menthone contents were similar to those of Telci *et al*. (2011) (23.08-25.17%) and Kraft and Hobbs (2004) (15-20%). Similar to the above described oil components, Menthofuran contents also changed with respect to fertiliser treatments, being significant at the P<0.01 level. In the first year, the lowest value (2.76%) was determined from the control treatment and the greatest (3.72%) from MAP. In the second year, the lowest menthofuran (2.80%) was determined from MKP and the highest (8.13%) from the 15:15:15 treatment. Menthofuran contents were generally greater in the second year of the study. Menthofuran contents reported in this study were lower than the findings of Telci *et al*., (2011) (7.80-11.19%). In the fresh samples, the principal component was as follows menthol > menthone > 1,8-cineole > menthofuran. The results obtained were similar to Beigi *et al.* (2018), who identified menthol (44.39%), menthone (15.36%), menthofuran (10.27%), 1,8-cineole (5.81%). However, our findings differed to Pino *et al*. (2002) who characterised the major constituents in peppermint grown in Jalisco (Italy) to be menthol (35.4%), menthofuran (18.2%), menthone (15.4%). Scavroni *et al*. (2005) identified menthyl acetate (35.01-55.68%), menthol (11.28-42.32%), menthofuran (4.56-17.45%), and 1,8-cineole (2.93-5.56%,) as the main compounds in the oil. The differences between the chemical compounds of peppermint and the results reported in the open literature for medicinal and aromatic plants in general can be attributed to the geographical origin, genetics and harvest time of the plant, as well as the extraction method (Scavroni *et al*., 2005). The chemical composition of mint essential oils was shown to be affected by many factors such as cultivar, environmental conditions, humidity, the nutrient in soil, temperature and biotic or abiotic stress (De Sousa Barros *et al*., 2015), the geographical origin of the plants, plant genetics, harvest time, method of essential oils extraction (Beigi *et al*., 2018) and photoperiod (Gholamipourfard *et al*., 2021). The most valuable compound designating the quality of peppermint essential oil is menthol which also significantly changed with respect to fertilizer treatments at the P<0.01 level. In the first year, the lowest value (34.88%) was obtained from the MAP treatment and the greatest value (39.40%) from the 15:15:15 treatment. In the second year, menthol contents varied between 34.92-41.85%. The present findings on menthol contents of this study were found to be similar to those of Kraft and Hobbs (2004) (35-40%), Kumar *et al*. (2011) (30-55% for menthol and 14-32 for menthone) and Telci *et al*. (2011) (33.24-37.02%). In both years, among the essential oil components of peppermint, menthol was the major component. Values reported in the present study were within the range of pharmacopoeia (30-35%). The second major component was menthone, and the present values were again within the range of pharmacopoeia (14-32%) (WHO, 2002). The amount and components of the essential oil vary between regions, depending mostly on climate, soil composition (Telci *et al*., 2011; Benabdallah *et al*., 2016), agronomic conditions, harvesting season (Giray *et al*., 2008), technique of oil extraction and the drying method (Singh, 2020). The difference in essential oil content and essential oil yield may be due to climatic factors such as temperature, rainfall and light (Kassahun *et al*., 2014).

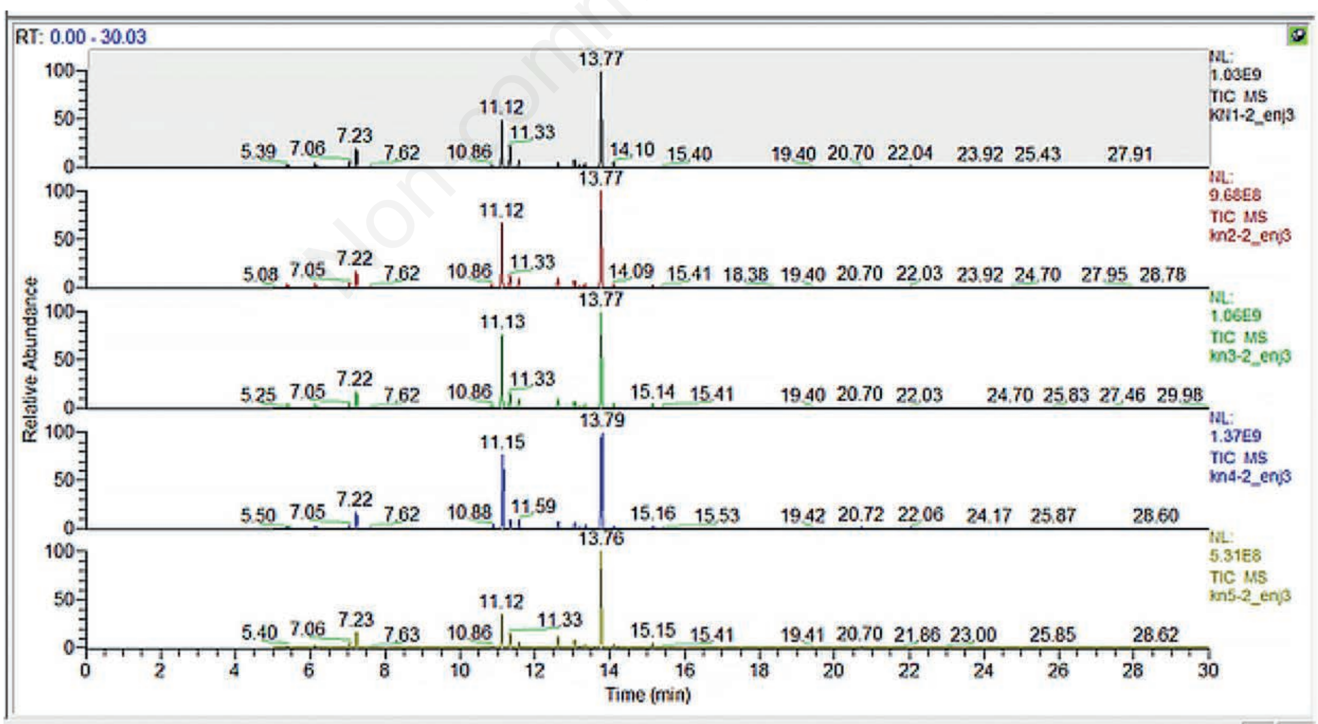

Figure 3. Essential oil gas chromatogram in the mass spectrometry (MS) detector obtained from treatment in the secont year. Kn1; 15:15:15, Kn2; MAP, Kn3; DAP, Kn4; MKP and Kn5; control treatment.

Conclusions

In generally MAP and 15:15:15 treatments were more effective in terms of green herb, drug herb yield and essential plant nutrients. The minimum non-essential element (heavy metal) levels were seen in MAP and MKP treated peppermint plants. In both years, the greatest essential oil yields were obtained from MAP treatments. However, oil yields should not only be expressed as a ratio (%), but also be related to drug herb yield and expressed in unit area yield (kg ha^{-1}). In both years, menthol was identified as the major essential oil component. Non-essential element (Pb, Ni, Co, Cr and Cd) concentrations of peppermint herb were below the allowed limits. Based on present findings of this study, 15:15:15 and MAP fertilizers can be recommended for conventional peppermint farming.

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