

Effect of different fertilizer sources and harvesting time on the growth characteristics, nutrient uptakes, essential oil productivity and composition of *Mentha x piperita* L.

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ABSTRACT

The excessive use of chemical fertilizers in conventional agricultural systems decreased the nutrient use efficiency and caused serious environmental problems such as waterway pollution, mineral depletion, soil acidification and other issues. In order to achieve the desirable essential oil productivity and reduction consumption of chemical inputs in peppermint (*Mentha x piperita* L.), a 2-year field experiment was carried out using a split-plot approach based on a randomized complete block design (RCBD), with 7 treatments and three replications at two harvesting times. The main factor was given by different fertilizer treatments including no fertilizer (control), chemical fertilizer, arbuscular mycorrhiza fungus, 50 % chemical fertilizer + arbuscular mycorrhiza fungus, nano chelated fertilizer, 50 % chemical fertilizer + nano chelated fertilizer, nano chelated fertilizer + arbuscular mycorrhiza fungus, and the sub-factor included two harvesting times (first harvest and second harvest). The results demonstrated that the highest and lowest growth parameters including plant height, number of lateral branches per plant and leaf greenness (SPAD index) were achieved with integrative application of 50 % chemical fertilizer + nano chelated fertilizer (in the first harvest) and control conditions (in the second harvest), respectively. Also, the maximum concentration of N, P, K and Fe was reached in the first harvest with application of 50 % chemical fertilizer + nano chelated fertilizer. Furthermore, the highest peppermint dry matter yield (354.8 g/m²), essential oil content (2.7 %) and essential oil yield (6.6 g/m²) was achieved at the first harvest with application of 50 % chemical fertilizer + nano chelated fertilizer. GC-MS analysis of peppermint essential oil showed that the major components at first harvest were menthol (31.82–37.87 %), menthone (23.85–30.90 %), 1,8-cineole (6.39–6.82 %), δ -terpineol (3.61–4.11 %) and *neo*-menthol (2.67–3.33 %), whereas at second harvest menthol (44–47.31 %), *p*-menth-1-en-9-ol (11.66–14.96 %), menthofuran (3.44–5.14 %), menthone (3.82–10.62 %), 1,8-cineole (5.51–5.99 %) and *neo*-menthol (5.03–5.90 %). Notably, menthol reached the highest amount with application of 50 % chemical fertilizer + nano chelated fertilizer. Overall, an integrative application of chemical fertilizers with nano fertilizers can be suggested to farmers as an alternative and environmentally friendly strategy to improve the quali-quantitative characteristics of peppermint essential oil.

1. Introduction

Nowadays, the rapid thrive of global population increased the requirements for food and energy, leading to the enhancement of agricultural productivity in regions with limited cultivation area (Liu and

Lal, 2015; Chen and Yada, 2011). In the conventional agricultural systems, the excessive use of chemical inputs allowed to increase the agricultural productivity. Detrimental implications on the environment and human health from intensive agricultural practices and long-term use of chemical fertilizers have been well evidenced (Bansal, 2017).

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Table 1
Physico-chemical properties of field soil.

Soil texture	Sand (%)	Silt (%)	Clay (%)	Organic matter (%)	EC (ds/m)	pH	Amount of exchangeable potassium (mg/kg)	Amount of cation exchange capacity (Cmol/kg)	Available phosphorus (mg/kg)	Total nitrogen (%)
sandy clay loam	56	16.5	27.5	0.83	1.18	8.16	570.85	27	11.05	0.089

Intensive application of chemical fertilizers has different negative impacts on environment including the aggregation of pesticides and fertilizers, soil erosion, soil and water pollution, genetic erosion, leaching of nutrients and reduction of agrobiodiversity (Daneshmandi and Seyyedi, 2019).

The soil health management is crucial for ensuring ecological and agricultural productions and maintaining plant diversity. The negative impacts of chemical fertilizers forced the agrochemical companies to replace them with biofertilizers in sustainable agricultural systems in order to achieve a desirable crop productivity (Sharma et al., 2013). Over 80 % of plant species like those belonging to Fabaceae (Weisany et al., 2016), Asteraceae (Kapoor et al., 2007), Apiaceae (Kapoor et al., 2002) and Lamiaceae (Tarraf et al., 2017) establish mutualistic associations with arbuscular mycorrhiza (AM) fungi. The coexistence between AM fungi and host plants not only allows the exploration of bulk of the soil, but also improves water relations and concentration of nutrients such as phosphorus (Read and Perez-Moreno, 2003; Prasad et al., 2012) and nitrogen (Varma et al., 2018; Cavagnaro et al., 2015), and finally increases plant resistance to abiotic tension (Lenoir et al., 2016), pathogens and soil-borne diseases (Abo-Elyousr et al., 2014). These conditions allow to improve the growth characteristics and productivity of crops with reduction of the application of chemical fertilizer (Pirzad and Mohammadzadeh, 2018). In fact, previous studies reported that the inoculation of medicinal and aromatic plants (MAPs) with AM fungi could enhance the quantity and quality of secondary metabolites such as essential oils (Rydlová et al., 2016; Weisany et al., 2016; Amiri et al., 2015; Mahfouz and Sharaf-Eldin, 2007).

In recent years, the use of MAPs and their derivatives for the treatment of several diseases has increased because of the negative side effects produced frequently by synthetic drugs. Peppermint (*Mentha x piperita* L.), belonging to the Lamiaceae family, is one of the most important aromatic and medicinal herbs. The essential oil obtained from its leaves is a valuable source of pharmaceutically and cosmetically important compounds such as menthol and menthone (Gupta et al., 2017). On a global scale, the peppermint oil is the second most important essential oil after citrus oil, with about 14,000 tons produced annually and 300 million \$ of income (Tiwari, 2016). Lubbe and Verpoorte (2011) reported that the commercial value of peppermint essential oil varies between 7 and 45 €/kg depending on the quality and origin of the sample. The main essential oil constituents are menthol, menthone, 1,8-cineole, germacrene D and (*E*)-caryophyllene (Amani Machiani et al., 2018b).

The nutrient availability has a key role in improving the biomass productivity and the quali-quantitative characteristics of the essential oil in MAPs (Amani Machiani et al., 2019). In recent years, the application of nanotechnology for the production of smart and slow releasing fertilizers, known as nano fertilizers, represents one the best solutions for improving the nutrient use efficiency (NUE) ratio in plants. Nanoparticles are ascertained as particles with dimension ranging from 1 to 100 nm. They are used in different fields such as agriculture, biotechnology and pharmaceutical industry (Chakravarty et al., 2015; Siddiqui et al., 2015). Because of the small size and large surface, the chemical, physical and biological properties of nanoparticles are enhanced when compared with the conventional materials (Mishra et al., 2018; Liu and Lal, 2015). The application of nano fertilizers in agricultural systems has several benefits such as the controlling release of nutrients in order to match the crop absorption pattern, the improvement of solubility and dispersion of insoluble nutrients, and the enhancement of NUE, with an extended duration of nutrient supply and a decreased rate of fertilizer loss (Calabi-Floody et al., 2018; Naderi and Danesh-Shahraki, 2013; Baruah and Dutta, 2009).

Given the negative consequences from the use of chemical fertilizers on the human health and environment, alternative methods for decreasing their application in agriculture are urgently needed. The current study was aimed to compare the effects of different fertilizers sources including sole chemical fertilizer, bio-fertilizer, nano chelated

Table 2
Monthly average temperature and total Monthly Precipitation in 2017 and 2018 growing seasons and long-term averages in the experimental area.

Year	April	May	June	July	August	September	October
Monthly average temperature (°C)							
2017	13	19.4	24.7	29.2	29.01	24.7	15.1
2018	12.6	16.6	24.1	30.2	27.7	23.6	15.9
2-year mean	12.8	18	24.4	29.7	28.4	24.2	15.5
10-year mean	12.9	17.9	23.8	27.9	27.4	22.0	14.9
Total monthly precipitation (mm)							
2017	33.6	4.4	1.2	0.5	0.01	0	0.03
2018	44.9	54.5	1.7	0.1	0	0.2	19.5
2-year mean	39.3	29.4	1.5	0.3	0.0	0.1	9.7
10-year mean	38.3	22.5	5.6	0.4	0.3	8.5	31.4

fertilizer and their combinations on the growth characteristics, macro and micro-nutrients uptake, essential oil productivity and constituents of peppermint at two different harvest times.

2. Materials and methods

2.1. Site description

The experiment was conducted during 2017 and 2018 growing seasons in the research farm of Maragheh University, East Azerbaijan Province, Maragheh, Iran (E 46°16'E; N 37°23', 1485 m a.s.l.). The soil physico-chemical characteristics of the experimental site are listed in Table 1. The soil was composed of sandy clay loam with pH 8.16, 1.23 % organic carbon, 0.09 % total N, 11.05 and 570.85 mg/kg of available P and K, respectively (depth of 0–30 cm). The climatic data in the research area are presented in Table 2.

2.2. Treatments details

The experimental study was performed using a split-plot approach based on a randomized complete block design (RCBD) with seven treatments, three replications and two harvesting times. The main factor was given by different fertilizer treatments containing no fertilizer (control), chemical fertilizer, arbuscular mycorrhiza fungus, 50 % chemical fertilizer + arbuscular mycorrhiza fungus, nano chelated fertilizers, 50 % chemical fertilizer + nano chelated fertilizer, nano chelated fertilizer + arbuscular mycorrhiza fungus, and the sub factor included the two harvest times (first harvest and second harvest). For chemical fertilizer, 135 kg/ha of triple superphosphate (before planting) and 200 kg/ha of nitrogen fertilizer in the form of urea (in three times including planting, and before and after flowering) were added to the soil. In arbuscular mycorrhiza fungus (inoculation with *Glomus mosseae*) treatments, 100 g of the soil including mycorrhizal fungal hyphae and the remains of the root and spores (1000 g spore/10 g soil) were added to the soil at planting. Also, the nano chelated fertilizers of N (total N 20 %), P (P₂O₅ 25 %), K (K₂O 23 %), Fe (FeO 10 %), Zn (ZnO 20 %) and Mn (MnO 25 %) were obtained from Sepehr Parmis Company and used at concentration of 2 mL/L, 1 g/L, 1 g/L, 1 g/L, 1 g/L and 0.5 g/L, respectively. The foliar application of nano chelated fertilizers was performed in two stages (one month after the planting and one after the first cutting). The size of each plot was 2 × 3 m and consisted of 5 rows. Seedlings of peppermint were planted on 15 May 2017 and 2018 with a row distance of 40 cm and density of 10 plant per row. Seedlings were irrigated immediately after planting and during the growth stage every 7–10 days using a drip irrigation system. Weeds were regulated early in the growing season by hand weeding.

2.3. Harvesting and measurements

The aerial parts of peppermint were harvested at 50 % of flowering

stage on the first harvest (92 days after sowing) and the second harvest (62 days after first harvesting). Before harvesting, the growth characteristics of peppermint including plant height, number of nodes per plant, number of leaves per plant, leaf/stem ratio and number of lateral branches per plant were measured among 10 randomly selected plants of each treatment.

2.3.1. Root colonization

At the end of the growing season, the fresh root samples of peppermint were taken randomly from the soil and washed with water to remove the residual soil particles. The root samples were cut into small pieces (1 cm) and cleared in hot 10 % KOH for 10 min. The samples were rinsed with tap water and then acidified with 2% HCl at room temperature for 15 min and stained with trypan blue (0.05 %) in 80 % lactic acid for 12 h (Phillips and Hayman, 1970; Koske and Gemma, 1989). Finally, the samples rinsed with water and saved in a solution including water, glycerol and lactic acid in proportions of 1:1:1 (v/v/v) until investigation. The root mycorrhizal colonization was assessed using grid-line intersection method suggested by Giovannetti and Mosse (1980).

2.3.2. SPAD chlorophyll meter

Chlorophyll Meter reading (SPAD) used for measuring the relative leaf greenness (level of chlorophyll) in peppermint leaves (SPAD 502, Minolta Ltd., Osaka, Japan). The SPAD values of peppermint were measured on the middle part of the leaf blade.

2.3.3. Dry matter yield

The peppermint seedlings were randomly taken from the ground level and the middle row of each plot. To calculate the dry yield (as g/m²), the harvested samples were transferred in a ventilated room and dried under shade for one week.

2.3.4. Essential oil extraction and analysis

Forty g of the shade-dried peppermint leaves were grounded and hydrodistilled for 3 h using a British Pharmacopoeia model Clevenger-type apparatus. Anhydrous sodium sulfate was added to each extracted essential oil for removing possible water drops, and then kept at 4°C before analysis. The essential oil content and yield, expressed on a dry weight basis, were calculated according to the following equations (Amani Machiani et al., 2018b):

$$\text{Essential oil content of peppermint} = (\text{distilled essential oil (g)} / 40\text{g}) \times 100$$

$$\text{Essential oil yield of peppermint} = \text{dry yield of peppermint (g/m}^2\text{)} \times \text{essential oil content}$$

The peppermint essential oils were analyzed using GC-FID and GC-MS following previously reported methods by Amani Machiani et al. (2018a, b). Briefly, the analysis was conducted using an Agilent 7990 B gas chromatograph equipped with a 5988A mass spectrometer and a HP-5MS (0.25 mm i.d., 30 m l., 0.25 μm f.t., 5% phenyl methylpolysiloxane). The following oven temperature was used: 5 min at 60 °C, then up to 240 °C with the rate of 3 °C/min, held for 10 min. Helium (carrier gas) flow rate was 1 mL/min; the injector split ratio was 1:30; the mass range and electron impact (EI) was 400 m/z and 70 eV, respectively. The identification of constituents was performed using the procedure explained by Morshedloo et al. (2017), which is based on the interactive combination of linear retention indices (RIs), calculated respect to a homologue series of n-alkanes (Supelco, Bellefonte, CA), and the mass spectrum (MS) matching with commercial libraries (ADAMS, WILEY 275 and NIST 17). GC-FID analysis was performed using an Agilent 7990 B gas chromatograph equipped with a flame ionization detector (FID), capillary column VF-5MS (30 m l., 0.25 mm i.d., 0.50 μm f.t., 5% phenyl methylpolysiloxane). The same oven temperature reported for GC-MS was used. The injection volume of the essential oil

was 1 μ l of a solution in *n*-hexane (1:100). Quantification of the constituents was performed by peak area normalization without using correction factors (Morshedloo et al., 2018).

2.3.5. Nutrients concentration

The extracts of peppermint samples were used to measure K concentration via a flame photometer. The N content was calculated via Kjeldahl and P concentration using the yellow method, in which vanadate-molybdate was used as an indicator. The P content was measured at 470 nm using a spectrophotometer (Tandon et al., 1968). Also, the Mn, Fe and Zn contents were determined using an atomic absorption spectrometer (AA-6300 F; Shimadzu, Kyoto, Japan) (Jones, 1972).

2.4. Statistical analysis

All data were subjected to normality test via Anderson-Darling method and homoscedasticity of data were checked through Levene test. Then data were subjected to combined ANOVA using SAS software (version 9.1). Also, the significant differences among means were compared with the LSD (last significant difference) method at $P < 0.05$. PCC (Pearson's correlation coefficient) was calculated between dry weight, essential oil content, essential oil yield and major components of peppermint essential oil.

3. Results

3.1. Root colonization

The results showed that the root colonization percentage was significantly impacted by treatments. The highest percentage of root colonization (77.7 %) was achieved with application of arbuscular mycorrhiza fungus followed by application of nano chelated fertilizer + arbuscular mycorrhiza fungus (72.5 %). Also, the lowest percentage of root colonization (56.9 %) was recorded in the 50 % chemical fertilizer + arbuscular mycorrhiza fungus (Fig. 1).

3.2. Plant height

The results demonstrated that the plant height of peppermint was significantly affected by fertilization, harvesting date and interaction of fertilization \times harvesting date. The highest (59.6 cm) and lowest (33.1 cm) plant height was achieved with combination of 50 % chemical + nano chelated fertilizer (in the first harvest) and control (in the second harvest), respectively. Also, the plant height of peppermint in

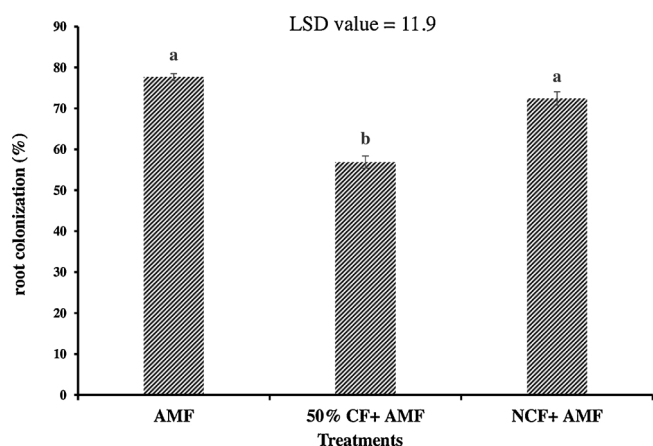


Fig. 1. Mean of the root colonization in different treatments. Dissimilar letters indicate significant differences at the 5% level according to LSD's test. AMF: arbuscular mycorrhiza fungus; CF: chemical fertilizer; NCF: nano chelated fertilizer

the second harvest was 28 % lower than first harvest (Table 3).

3.3. Number of nodes per plant

Based on the obtained results, the number of nodes per plant was significantly impacted by fertilization, harvesting date and interaction of fertilization \times harvesting date. The highest (21.3) and lowest (6.4) number of nodes per plant was recorded with the application of 50 % chemical fertilizer + arbuscular mycorrhiza fungus (in the first harvest) and control (in the second harvest), respectively. In addition, the number of nodes per plant in the second harvest decreased 62 % compared with first harvest (Table 3).

3.4. Number of leaves per plant

The number of leaves per plant was significantly affected by fertilization, harvesting date and interaction of fertilization \times harvesting time. The highest number of leaves per plant was achieved in the first harvest treatment with chemical fertilizer that did not significantly differ from treatment with 50 % chemical fertilizer + nano chelated fertilizer. In addition, the lowest number of leaves per plant was recorded in the control at second harvest. The results demonstrated that the application of chemical fertilizer, 50 % chemical fertilizer + arbuscular mycorrhiza fungus and 50 % chemical fertilizer + nano chelated fertilizer enhanced the number of leaves per plant by 98, 87 and 96.1 %, respectively, when compared with control. In addition, the number of leaves per plant in the second harvest decreased 48 % compared with first harvest (Table 3).

3.5. Leaf /stem ratio

The leaf /stem ratio per plant was significantly affected by harvesting time and interaction of fertilization \times harvesting date. The highest leaf /stem ratio (2.6) was observed at the first harvest with application of 50 % chemical fertilizer + nano chelated fertilizer, while the lowest ratio (1.6) was recorded at the second harvest with nano chelated fertilizer application. Also, the leaf /stem ratio in the second harvest reduced 22 % compared with first one (Table 3).

3.6. Number of lateral branches per plant

Different fertilization sources, harvesting times and interaction of fertilization \times harvesting time had a significant impact on the number of lateral branches per plant. The highest number of lateral branches (30.2) was achieved in the first harvest with application of 50 % chemical fertilizer + nano chelated fertilizer followed by application of 50 % chemical fertilizer + arbuscular mycorrhiza fungus, chemical fertilizer and nano chelated fertilizer + arbuscular mycorrhiza fungus. Also, the lowest number of lateral branches (10.1) was observed in the control at second harvest. The application of chemical fertilizer, arbuscular mycorrhiza fungus, 50 % chemical fertilizer + arbuscular mycorrhiza fungus, nano chelated fertilizer, 50 % chemical fertilizer + nano chelated fertilizer and nano chelated fertilizer + arbuscular mycorrhiza fungus increased the number of lateral branches by 32, 22, 32, 16, 34 and 23 %, respectively, when compared with control. Furthermore, the number of lateral branches in the second harvest decreased 52 % in comparison to first harvest (Table 3).

3.7. Leaf greenness (SPAD index)

The results demonstrated that the leaf greenness (SPAD index) content of peppermint was significantly impacted by fertilization, harvesting date and interaction of fertilization \times harvesting time. Based on the interaction of fertilization \times harvesting time, the maximum and minimum SPAD content was obtained in the first harvest with application of 50 % chemical fertilizer + nano chelated fertilizer and control

Table 3
Means of morphological traits in different fertilizer sources and harvesting time (an average over the two years).

Treatments	Plant height (cm)	Number of nodes per plant	Number of leaves per plant	Leaf /stem ratio per plant	Number of lateral branches per plant	leaf greenness (SPAD index)	Dry matter yield (g/m ²)	Essential oil content (%)	Essential oil yield (g/m ²)
Fertilization									
Control	40.3 d	12.4 b	440.5 d	2 a	16.7 c	43.4 e	169.1 b	1.79 d	2.9 b
CF	50.9 ab	14.5 a	871.5 a	1.9 a	22.1 a	65.3 b	268.8 a	2.16 ab	5.8 a
AMF	49.3 abc	14.4 a	704.3 b	2.1 a	20.4 ab	48.4 d	199.5 b	1.96 c	3.8 b
50 % CF + AMF	51.3 a	14.9 a	821.7 a	2 a	22.1 a	62.7 b	285.3 a	2.10 b	5.5 a
NCF	46.4 c	14.3 a	588.6 c	1.9 a	19.3 b	54.5 c	196.5 b	1.85 d	3.5 b
50 % CF + NCF	51.9 a	14.8 a	863.7 a	2.3 a	22.3 a	72.1 a	306.9 a	2.22 a	6.3 a
NCF + AMF	47.2 bc	14.5 a	603.1 c	2.1 a	20.5 ab	53.4 c	201.9 b	1.86 d	3.7 b
LSD	3.99	0.86	85.6	0.37	2.44	2.8	40.9	0.09	0.91
Harvesting date									
First harvest (H1)	56 a	20.7 a	918.1 a	2.27 a	27.7 a	62.7 a	270 a	2.40 a	4.69 a
Second harvest (H2)	40.4 b	7.9 b	480.1 b	1.78 b	13.3 b	51.5 b	195.1 b	1.58 b	4.32 b
LSD	1.19	0.33	31.01	0.12	1.08	1.79	13.68	0.09	0.36
Fertilization × Harvesting date									
(Control)*H1	47.4 d	18.5 b	599.5 e	2.1 cde	23.4 d	49.8 e	182.5 e	2.1 e	3.3 cd
(CF) *H1	57.8 ab	21.2 a	1190.9 a	2 def	29.5 ab	70.6 b	318.1 b	2.6 ab	5.8 ab
(AMF) *H1	58.8 a	20.7 a	910.5 c	2.5 ab	26.8 bc	55.9 d	222.6 d	2.4 bcd	4.2 c
(50 % CF + AMF) *H1	58.5 a	21.3 a	1050.8 b	2.2 bcd	29.7 a	62.7 c	347.7ab	2.5 abc	5.4 b
(NCF) *H1	54.6 c	21.1 a	763.8 d	2.2 bcd	26.1 cd	62.6 c	237.4 cd	2.3 cde	3.5 cd
(50 % CF + NCF) *H1	59.6 a	21.1 a	1145.3 a	2.6 a	30.2 a	75.8 a	354.8a	2.7 a	6.6 a
(NCF + AMF) * H1	55.3 bc	21.1 a	765.4 d	2.4 abc	28.1 abc	61.7 c	227.0 cd	2.2 de	3.9 c
(Control)*H2	33.1 g	6.4 e	281.5 i	1.9 defg	10.1 f	36.9 g	155.6 e	1.4 h	2.6 d
(CF) *H2	44.1 e	7.8 cd	552.2 ef	1.8 efg	14.7 e	60.1 cd	219.5 d	1.7 fg	5.9 ab
(AMF) *H2	39.8 f	8.2 cd	498.1 fg	1.7 fg	13.9 e	40.9 fg	176.4 e	1.5 gh	3.3 cd
(50 % CF + AMF) *H2	44.1 e	8.6 c	592.6 e	1.8 efg	14.5 e	62.8 c	222.9 cd	1.7 fg	5.5 b
(NCF) *H2	38.3 f	7.5 d	413.4 h	1.6 g	12.6 ef	46.3 e	158.6 e	1.5 h	3.4 cd
(50 % CF + NCF) *H2	44.3 de	8.6 c	582.1 e	1.9 defg	14.4 e	68.4 b	258.9 c	1.7 f	6.0 ab
(NCF + AMF) * H2	39 f	7.9 cd	440.8 gh	1.7 fg	12.9 e	45.2 ef	176.7 e	1.5 fgh	3.4 cd
LSD	3.15	0.87	82.04	0.32	2.9	4.7	36.2	0.24	0.95
significance									
Fertilization	**	**	**	ns	**	**	**	**	**
Harvesting date	**	**	**	**	**	**	**	**	*
Fertilization × Harvesting date	**	**	**	*	**	**	**	**	**

Control (No fertilizer), CF (Chemical fertilizer), AMF (arbuscular mycorrhiza fungus), 50 % CF + AMF (50 % Chemical fertilizer + arbuscular mycorrhiza fungus), NCF (Nano chelated fertilizer), 50 % CF + NCF (50 % Chemical fertilizer + nano chelated fertilizer), NCF + AMF (Nano chelated fertilizer + arbuscular mycorrhiza fungus). ns, * and ** indicated no significant difference, significant at 5% probability level and significant at 1% probability level, respectively. Different letters at each column indicate significant difference at $p \leq 0.05$.

(in the second harvest), respectively. The SPAD content following application of chemical fertilizer, arbuscular mycorrhiza fungus, nano chelated fertilizer, 50 % chemical fertilizer + arbuscular mycorrhiza fungus, 50 % chemical fertilizer + nano chelated fertilizer and nano chelated fertilizer + arbuscular mycorrhiza fungus was 50, 12, 44, 26, 66 and 23 % higher than that of control, respectively. In addition, the SPAD index in the second harvest decreased 18 % compared with first one (Table 3).

3.8. Dry matter productivity

Dry matter yield of peppermint was significantly affected by fertilization, harvesting date and interaction of fertilization × harvesting time. The highest (354.8 g/m²) and lowest (155.6 g/m²) dry matter yield was recorded in the first harvest with the integrated application of 50 % chemical fertilizer + nano chelated fertilizer, and in the control at second harvest, respectively. The application of chemical fertilizer, arbuscular mycorrhiza fungus, 50 % chemical fertilizer + arbuscular mycorrhiza fungus, nano chelated fertilizer, 50 % chemical fertilizer + nano chelated fertilizer and nano chelated fertilizer + arbuscular mycorrhiza fungus increased the total dry yield by 59, 18, 69, 16, 81 and 19 %, respectively, when compared with control. Furthermore, the dry matter yield of peppermint in the second harvest reduced 28 % in comparison to first one (Table 3).

3.9. Nutrients concentrations

Based on the obtained results, the concentration of macro- and micronutrients was significantly impacted by different fertilizer sources and interaction of fertilization × harvesting time. Harvesting times did not significantly impact the macro- and micronutrients concentrations. The highest content of N (4.37 %), P (0.223 %), K (2.68 %) and Fe (2.95 mg/g dry matter) was observed in the first harvest with application of 50 % chemical fertilizer + nano chelated fertilizer. The highest concentrations of Zn (0.86 mg/g dry matter) and Mn (0.48 mg/g dry matter) were observed in the first harvest with application of nano chelated fertilizer followed by 50 % chemical fertilizer + nano chelated fertilizer. The lowest concentration of macro- and micronutrients was detected in the control at both harvests (Table 4).

3.10. Essential oil content

Different fertilization sources, harvesting times and interaction of fertilization × harvesting time significantly impacted the essential oil content of peppermint. The highest (2.7 %) and lowest (1.4 %) essential oil content of peppermint was obtained in the first harvest with application of 50 % chemical fertilizer + nano chelated fertilizer and in the second harvest with no application of fertilizer (control), respectively. The essential oil content following application of chemical fertilizer, arbuscular mycorrhiza fungus, 50 % chemical fertilizer + arbuscular mycorrhiza fungus, nano chelated fertilizer, 50 % chemical fertilizer + nano chelated fertilizer and nano chelated fertilizer + arbuscular mycorrhiza fungus increased the total dry yield by 59, 18, 69, 16, 81 and 19 %, respectively, when compared with control. Furthermore, the dry matter yield of peppermint in the second harvest reduced 28 % in comparison to first one (Table 3).

Table 4
The macro and micro-nutrients concentration of peppermint in different fertilizer sources and harvesting time (an average over the two years).

Treatments	N content (%)	P content (%)	K content (%)	Fe content (mg/g dry matter)	Zn content (mg/g dry matter)	Mn content (mg/g dry matter)
Fertilization						
Control	2.13 d	0.155 d	2.11 b	0.82 e	0.06 c	0.14 d
CF	3.49 b	0.168 cd	2.18 b	2.33 b	0.74 a	0.26 c
AMF	2.33 d	0.160 cd	2.13 b	1.59 d	0.07 c	0.13 d
50 % CF + AMF	3.37 b	0.200 ab	2.35 ab	1.78 cd	0.47 b	0.33 b
NCF	2.94 c	0.182 bc	2.18 b	2.17 bc	0.83 a	0.46 a
50 % CF + NCF	4.24 a	0.217 a	2.60 a	2.90 a	0.75 a	0.36 b
NCF + AMF	3.61 b	0.203 ab	2.30 ab	2.84 a	0.70 a	0.27 c
LSD	0.40	0.03	0.30	0.49	0.14	0.06
Harvesting date						
First harvest (H1)	3.18 a	0.185 a	2.27 a	2.08 a	0.536 a	0.285 a
Second harvest (H2)	3.13 a	0.182 a	2.26 a	2.04 a	0.499 a	0.275 a
LSD	0.053	0.004	0.05	0.098	0.04	0.02
Fertilization × Harvesting date						
(Control)*H1	2.03 i	0.160 fg	2.10 f	0.84 f	0.07 f	0.14 f
(CF) *H1	3.44 ed	0.167 def	2.17 ef	2.35 c	0.81 ab	0.27 e
(AMF) *H1	2.32 h	0.163 efg	2.10 f	1.64 de	0.08 f	0.12 f
(50 % CF + AMF) *H1	3.40 ed	0.207 b	2.32 cd	1.81 d	0.48 e	0.35 bc
(NCF) * H1	3.05 f	0.173 d	2.17 ef	2.25 bc	0.86 a	0.48 a
(50 % CF + NCF) * H1	4.37 a	0.223 a	2.68 a	2.95 a	0.77 abc	0.39 b
(NCF + AMF)* H1	3.68 c	0.210 b	2.28 cde	2.84 a	0.73 bcd	0.29 de
(Control) * H2	2.23 h	0.150 h	2.13 f	0.80 f	0.05 f	0.13 f
(CF) * H2	3.53 d	0.170 de	2.18 ef	2.32 bc	0.66 d	0.26 e
(AMF) * H2	2.33 h	0.157 gh	2.16 ef	1.55 e	0.07 f	0.13 f
(50 % CF + AMF) * H2	3.34 e	0.193 c	2.38 c	1.75 de	0.46 e	0.32 cd
(NCF) * H2	2.83 g	0.190 c	2.19 def	2.08 c	0.80 ab	0.45 a
(50 % CF + NCF) * H2	4.11 b	0.210 b	2.52 b	2.84 a	0.73 bcd	0.34 c
(NCF + AMF) * H2	3.53 d	0.197 c	2.32 c	2.83 a	0.68 cd	0.25 e
LSD	0.14	0.009	0.13	0.26	0.11	0.04
significance						
Fertilization	**	**	*	**	**	**
Harvesting date	ns	ns	ns	ns	ns	ns
Fertilization × Harvesting date	**	**	*	**	**	**

Control (No fertilizer), CF (Chemical fertilizer), AMF (arbuscular mycorrhiza fungus), 50 % CF + AMF (50 % Chemical fertilizer + arbuscular mycorrhiza fungus), NCF (Nano chelated fertilizer), 50 % CF + NCF (50 % Chemical fertilizer + nano chelated fertilizer), NCF + AMF (Nano chelated fertilizer + arbuscular mycorrhiza fungus). ns, * and ** indicated no significant difference, significant at 5% probability level and significant at 1% probability level, respectively. Different letters at each column indicate significant difference at $p \leq 0.05$.

fertilizer + arbuscular mycorrhiza fungus was about 21, 10, 17, 3, 24 and 4% higher than that of control, respectively. In addition, the essential oil content in the second harvest was 34 % lower than that in the first harvest (Table 3).

3.11. Essential oil yield

The peppermint essential oil yield was significantly impacted by fertilization sources, harvesting time and interaction of fertilization × harvesting time. The highest essential oil yield (6.6 g/m²) was recorded in the first harvest with application of 50 % chemical fertilizer + nano chelated fertilizer, whereas the lowest value was measured in the control at second harvest. The essential oil yield following application of chemical fertilizer, arbuscular mycorrhiza fungus, 50 % chemical fertilizer + arbuscular mycorrhiza fungus, nano chelated fertilizer, 50 % chemical fertilizer + nano chelated fertilizer and nano chelated fertilizer + arbuscular mycorrhiza fungus was about 100, 31, 90, 20, 117, and 28 % higher than control, respectively. In addition, the essential oil yield in the second harvest was 8% lower than that in the second harvest (Table 3).

3.12. Essential oil constituents

In total, 23 constituents were identified in the essential oils of peppermint (based on GC–MS analyses), accounting for 96.5–97.6 % and 97.1 %–98.1 % of the total compositions at the first and second harvest, respectively (Fig. 2). The main constituents of peppermint essential oil at the first harvest were menthol (31.82–37.87 %), menthone

(23.85–30.90 %), 1,8-cineole (6.39–6.82 %), δ -terpineol (3.61–4.11 %) and *neo*-menthol (2.67–3.33 %). The highest menthol content (37.87 %) was achieved with application of 50 % chemical fertilizer + nano chelated fertilizer, while the lowest content (31.82 %) was observed with application of chemical fertilizer. In particular, the application of 50 % chemical fertilizer + nano chelated fertilizer increased the menthol content at the first harvest by 5 and 19 % when compared with treatments with chemical fertilizer and control, respectively (Table 5). Alike, the highest content of oxygenated monoterpenes (86.94 %) was recorded with application of 50 % chemical fertilizer + nano chelated fertilizer.

At the second harvest, the main essential oil constituents were menthol (44–47.31 %), *p*-menth-1-en-9-ol (11.66–14.96 %), menthofuran (3.44–5.14 %), menthone (3.82–10.62 %), 1,8-cineole (5.51–5.99 %) and *neo*-menthol (5.03–5.90 %). The highest menthol content (47.31 %) was achieved with application of 50 % chemical fertilizer + nano chelated fertilizer. This treatment increased the menthol content by 9% when compared with application of chemical fertilizer. The highest content of menthone (10.62 %) was observed with application of 50 % chemical fertilizer + arbuscular mycorrhiza fungus, followed by treatments with 50 % chemical fertilizer + nano chelated fertilizer (8.50 %) and chemical fertilizer (8.22 %). The menthone content in these treatments was enhanced by 178, 123 and 115 %, when compared with control, respectively. The highest (5.14 %) and lowest (3.44 %) content of menthofuran at the second harvest was achieved in the control and with application of 50 % chemical fertilizer + nano chelated fertilizer, respectively. Similar to first harvest, the highest amount of oxygenated monoterpenes (88.86 %) was recorded

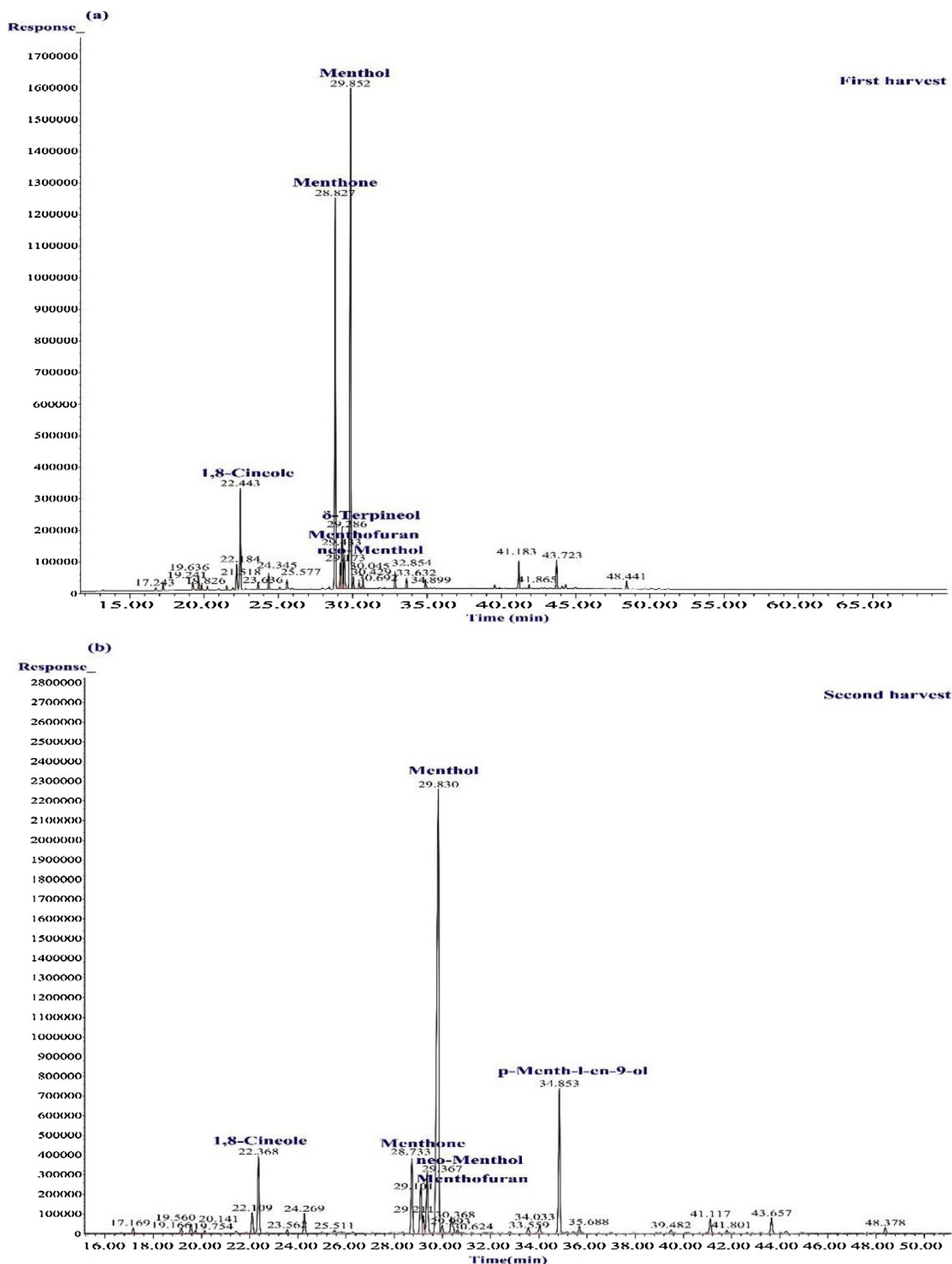


Fig. 2. *M. piperita* essential oil gas chromatogram (GC-FID) that obtained from 50 % chemical fertilizer + Nano chelated fertilizers treatment in the first (a) and second harvest (b).

with application of 50 % chemical fertilizer + nano chelated fertilizer (Table 6).

3.13. Correlation

The results demonstrated a significant and positive correlation between peppermint essential oil yield, dry matter yield and essential oil content ($r = 0.98$ and 0.99 , respectively). Also, the content of

menthone showed a significant and positive correlation with the dry matter yield, essential oil content and essential oil yield ($r = 0.86$ and 0.80 and 0.85 , respectively). In contrast, the content of *p*-menth-1-en-9-ol showed a significant and negative correlation with the dry matter yield, essential oil content and essential oil yield ($r = -0.87$ and -0.88 and -0.89 , respectively). The content of 1,8-cineole, *neo*-menthol, menthofuran and germacrene D were positively correlated with the ones of *p*-menth-1-en-9-ol. The content of the mentioned constituents

Table 5
Chemical compositions of peppermint essential oil at the first harvest under several fertilizer treatments (average over the two years).

No	Components	RI	LIT. RI	Treatments							ID
				Control	CF	AMF	50 % CF + AMF	NCF	50 % CF + NCF	NCF + AMF	
1	α -Pinene	931	932	0.56 ± 0.03	0.56 ± 0.02	0.56 ± 0.02	0.56 ± 0.02	0.53 ± 0.01	0.56 ± 0.02	0.56 ± 0.01	Std
2	Sabinene	970	969	0.56 ± 0.02	0.56 ± 0.01	0.57 ± 0.01	0.56 ± 0.01	0.55 ± 0.02	0.57 ± 0.02	0.55 ± 0.01	Std
3	β -Pinene	975	974	0.94 ± 0.04	0.93 ± 0.03	0.95 ± 0.02	0.93 ± 0.02	0.91 ± 0.02	0.93 ± 0.03	0.94 ± 0.02	Std
4	Myrcene	988	988	0.48 ± 0.07	0.52 ± 0.08	0.53 ± 0.08	0.52 ± 0.08	0.52 ± 0.08	0.50 ± 0.07	0.52 ± 0.07	Std
5	3-Octanol	1000	998	0.28 ± 0.03	0.36 ± 0.03	0.36 ± 0.03	0.36 ± 0.04	0.36 ± 0.03	0.34 ± 0.03	0.35 ± 0.03	RI-MS
6	α -Terpinene	1017	1014	0.17 ± 0.06	0.15 ± 0.06	0.16 ± 0.07	0.17 ± 0.07	0.17 ± 0.08	0.16 ± 0.07	0.17 ± 0.08	RI-MS
7	Limonene	1026	1024	1.91 ± 0.18	1.79 ± 0.18	1.88 ± 0.15	1.75 ± 0.13	1.81 ± 0.15	1.76 ± 0.18	1.91 ± 0.18	RI-MS
8	1,8-Cineole	1029	1026	6.82 ± 0.22	6.55 ± 0.25	6.70 ± 0.36	6.53 ± 0.30	6.75 ± 0.21	6.39 ± 0.38	6.72 ± 0.19	Std
9	γ -Terpinene	1058	1054	0.37 ± 0.05	0.29 ± 0.06	0.38 ± 0.05	0.31 ± 0.07	0.40 ± 0.06	0.37 ± 0.07	0.40 ± 0.06	Std
10	cis-Sabinene hydrate	1066	1065	1.49 ± 0.25	1.81 ± 0.23	1.41 ± 0.20	1.62 ± 0.28	1.36 ± 0.23	1.67 ± 0.27	1.37 ± 0.25	RI-MS
11	Linalool	1103	1095	0.58 ± 0.04	0.60 ± 0.07	0.57 ± 0.05	0.58 ± 0.05	0.57 ± 0.05	0.56 ± 0.06	0.57 ± 0.04	Std
12	Menthone	1152	1148	24.29 ± 0.79	30.90 ± 1.83	23.85 ± 0.75	28.35 ± 1.38	24.95 ± 0.30	25.60 ± 0.28	24.84 ± 0.74	Std
13	Menthofuran	1161	1159	1.82 ± 0.11	2.08 ± 0.09	1.96 ± 0.12	1.91 ± 0.12	1.77 ± 0.12	1.81 ± 0.07	1.82 ± 0.07	RI-MS
14	δ-Terpineol	1162	1162	3.71 ± 0.15	4.10 ± 0.03	3.61 ± 0.15	3.99 ± 0.09	3.77 ± 0.15	4.11 ± 0.05	3.74 ± 0.13	RI-MS
15	neo-Menthol	1163	1161	3.31 ± 0.33	2.67 ± 0.06	3.33 ± 0.30	2.91 ± 0.11	3.21 ± 0.23	2.69 ± 0.03	3.21 ± 0.22	RI-MS
16	Menthol	1175	1167	36.22 ± 1.13	31.82 ± 2.32	36.74 ± 1.26	34.52 ± 1.99	36.81 ± 1.00	37.87 ± 0.33	36.39 ± 1.01	Std
17	Terpinene-4-ol	1177	1177	0.82 ± 0.05	0.76 ± 0.04	0.87 ± 0.04	0.78 ± 0.04	0.87 ± 0.03	0.77 ± 0.03	0.87 ± 0.02	RI-MS
18	neo-iso-Menthol	1184	1184	1.69 ± 0.67	1.99 ± 0.66	2.28 ± 0.76	2.19 ± 0.74	2.33 ± 0.79	2.06 ± 0.67	2.16 ± 0.71	RI-MS
19	Pulegone	1236	1233	1.16 ± 0.10	1.26 ± 0.11	1.38 ± 0.13	1.22 ± 0.07	1.19 ± 0.11	1.22 ± 0.07	1.24 ± 0.12	Std
20	Piperitone	1252	1252	0.66 ± 0.01	0.72 ± 0.02	0.66 ± 0.0	0.74 ± 0.01	0.68 ± 0.02	0.75 ± 0.01	0.67 ± 0.01	RI-MS
21	neo-Menthyl acetate	1273	1271	0.15 ± 0.05	0.05 ± 0.04	0.14 ± 0.04	0.06 ± 0.03	0.09 ± 0.04	0.04 ± 0.02	0.22 ± 0.05	RI-MS
22	p-Menth-1-en-9-ol	1294	1294	1.51 ± 0.37	1.41 ± 0.26	1.58 ± 0.39	1.38 ± 0.28	1.46 ± 0.31	1.41 ± 0.22	1.47 ± 0.33	RI-MS
23	iso-Menthyl acetate	1307	1304	tr.	–	tr.	–	–	tr.	tr.	RI-MS
24	β -Bourbonene	1382	1387	0.60 ± 0.12	0.61 ± 0.13	0.69 ± 0.14	0.63 ± 0.15	0.67 ± 0.15	0.60 ± 0.14	0.67 ± 0.14	RI-MS
25	(E)-Caryophyllene	1416	1417	2.11 ± 0.32	1.77 ± 0.31	1.96 ± 0.17	1.70 ± 0.16	1.84 ± 0.13	1.66 ± 0.16	2.10 ± 0.17	Std
26	(E)- β -Farnesene	1457	1454	0.30 ± 0.05	0.21 ± 0.04	0.28 ± 0.02	0.21 ± 0.01	0.27 ± 0.01	0.25 ± 0.02	0.31 ± 0.02	RI-MS
27	Germacrene D	1479	1484	2.18 ± 0.25	1.79 ± 0.27	2.06 ± 0.14	1.76 ± 0.10	1.88 ± 0.06	1.72 ± 0.12	2.14 ± 0.17	Std
28	Elixene	1494	1492	0.38 ± 0.05	0.24 ± 0.05	0.35 ± 0.03	0.26 ± 0.02	0.32 ± 0.01	0.22 ± 0.01	0.37 ± 0.03	RI-MS
29	Viridiflorol	1589	1592	0.69 ± 0.04	0.48 ± 0.03	0.66 ± 0.04	0.53 ± 0.02	0.59 ± 0.02	0.50 ± 0.03	0.65 ± 0.04	RI-MS
	Total identified (%)			96.79	96.98	96.51	97.01	96.62	97.57	96.90	
	Essential oil yield (g/m ⁻²)			3.34	5.76	4.23	5.42	3.51	6.65	3.9	
	Grouped compounds (%)										
	Monoterpene hydrocarbons			5.01	4.81	5.04	4.79	4.90	4.84	5.04	
	Oxygenated monoterpenes			85.26	86.71	85.14	86.78	85.79	86.94	85.29	
	Sesquiterpene hydrocarbons			5.57	4.62	5.34	4.56	4.97	4.45	5.58	
	Oxygenated sesquiterpenes			0.69	0.48	0.66	0.53	0.59	0.50	0.65	
	Others			0.28	0.36	0.36	0.35	0.36	0.34	0.35	

Control (No fertilizer), CF (Chemical fertilizer), AMF (arbuscular mycorrhiza fungus), 50 % CF + AMF (50 % Chemical fertilizer + arbuscular mycorrhiza fungus), NCF (Nano chelated fertilizer), 50 % CF + NCF (50 % Chemical fertilizer + nano chelated fertilizer), NCF + AMF (Nano chelated fertilizer + arbuscular mycorrhiza fungus).

showed a significant and negative correlation with the dry matter yield, essential oil content and essential oil yield. The correlation between δ -terpineol and dry matter yield, essential oil content, essential oil yield and menthone was positive and significant, while the correlation between δ -terpineol and *p*-menth-1-en-9-ol, 1,8-cineole, *neo*-menthole, menthofuran and germacrene D was significant and negative (Table 7).

4. Discussion

The aim of our work was to evaluate the impact of different fertilizer sources including chemical fertilizer, arbuscular mycorrhiza fungus, nano chelated fertilizer and their combinations on the growth characteristics, macro and micro-nutrients uptake, essential oil content and compositions of peppermint at two different harvest times. Our results revealed that the percentage of peppermint root colonization increased significantly with application of arbuscular mycorrhiza fungus. On the other hand, the colonization percentage decreased after application of chemical fertilizer. The excessive application of chemical fertilizer, especially the higher level of nitrogen and phosphor may change the plant community structure, reducing the species diversity

and suppressing the arbuscular mycorrhiza fungus colonization (Albizua et al., 2015; Lin et al., 2012). Alike, Liu et al. (2016) and Bakhshandeh et al. (2017) noted that the application of N and P fertilizers suppressed significantly the arbuscular mycorrhiza fungus colonization in crops.

In this study, the highest morphological traits including plant height, number of lateral branches and leaf/stem ratio were observed with application of 50 % chemical fertilizer + nano chelated fertilizer. Nitrogen deficiency in semi-arid and arid regions plays a key role in the plant growth and productivity. 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). Shangguan et al. (2000) reported that most of absorbed N (about 75 %) is allocated into the chloroplasts and its availability could increase the photosynthetic rate and improve the morphological traits and productivity. Similarly, Janmohammadi et al. (2018) noted that application of nano-Fe and Zn, together with chemical fertilizer, enhanced significantly the plant height and the number of secondary branches in safflower (*Carthamus tinctorius* L.). In addition, the application of nano fertilizer could enhance the morphological traits by increasing the

Table 6
Chemical compositions of peppermint essential oil at the second harvest time under several fertilizer treatments (an average over the two years).

No	Components	RI	LIT. RI	Treatments							ID
				Control	CF	AMF	50 % CF + AMF	NCF	50 % CF + NCF	NCF + AMF	
1	α -Pinene	931	932	0.49 ± 0.03	0.46 ± 0.03	0.50 ± 0.02	0.47 ± 0.02	0.45 ± 0.03	0.45 ± 0.02	0.48 ± 0.02	Std
2	Sabinene	970	969	0.44 ± 0.03	0.42 ± 0.02	0.46 ± 0.02	0.43 ± 0.01	0.43 ± 0.02	0.43 ± 0.01	0.44 ± 0.02	Std
3	β -Pinene	975	974	0.72 ± 0.11	0.76 ± 0.05	0.83 ± 0.04	0.77 ± 0.04	0.77 ± 0.04	0.76 ± 0.04	0.81 ± 0.04	Std
4	Myrcene	988	988	0.24 ± 0.02	0.25 ± 0.02	0.26 ± 0.02	0.25 ± 0.02	0.22 ± 0.01	0.24 ± 0.02	0.24 ± 0.02	Std
5	3-Octanol	1000	998	0.19 ± 0.03	0.18 ± 0.01	0.18 ± 0.03	0.15 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.02	RI-MS
6	α -Terpinene	1017	1014	0.18 ± 0.03	0.18 ± 0.04	0.19 ± 0.04	0.18 ± 0.04	0.17 ± 0.03	0.17 ± 0.03	0.18 ± 0.03	RI-MS
7	Limonene	1026	1024	1.60 ± 0.11	1.57 ± 0.08	1.57 ± 0.11	1.61 ± 0.02	1.50 ± 0.09	1.62 ± 0.06	1.58 ± 0.07	RI-MS
8	1,8-Cineole	1029	1026	5.89 ± 0.34	5.65 ± 0.44	5.99 ± 0.49	5.47 ± 0.45	5.78 ± 0.36	5.51 ± 0.46	5.76 ± 0.33	Std
9	γ -Terpinene	1058	1054	0.30 ± 0.05	0.28 ± 0.05	0.28 ± 0.04	0.27 ± 0.05	0.27 ± 0.04	0.26 ± 0.04	0.28 ± 0.04	Std
10	cis-Sabinene hydrate	1066	1065	1.43 ± 0.08	1.64 ± 0.13	1.49 ± 0.08	1.69 ± 0.12	1.45 ± 0.11	1.63 ± 0.12	1.48 ± 0.06	RI-MS
11	Linalool	1103	1095	0.26 ± 0.02	0.29 ± 0.01	0.27 ± 0.01	0.29 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.26 ± 0.02	Std
12	Menthone	1152	1148	3.82 ± 0.59	8.22 ± 1.24	3.94 ± 0.60	10.62 ± 2.04	4.32 ± 0.80	8.50 ± 1.41	5.88 ± 0.90	Std
13	Menthofuran	1161	1159	5.14 ± 0.49	3.67 ± 0.62	4.75 ± 0.65	3.52 ± 0.43	4.49 ± 0.59	3.44 ± 0.46	4.01 ± 0.43	RI-MS
14	δ -Terpineol	1162	1162	0.93 ± 0.04	1.36 ± 0.12	0.80 ± 0.04	1.41 ± 0.14	0.84 ± 0.11	1.43 ± 0.16	1.02 ± 0.09	RI-MS
15	neo-Menthol	1163	1161	5.55 ± 0.20	5.33 ± 0.18	5.90 ± 0.18	5.37 ± 0.26	5.65 ± 0.16	5.03 ± 0.23	5.57 ± 0.07	RI-MS
16	Menthol	1175	1167	46.08 ± 0.91	43.6 ± 0.51	46.25 ± 0.73	44 ± 0.78	45.73 ± 0.76	47.31 ± 0.67	45.99 ± 0.52	Std
17	Terpinen-4-ol	1177	1177	0.67 ± 0.05	0.62 ± 0.06	0.65 ± 0.05	0.60 ± 0.05	0.65 ± 0.07	0.63 ± 0.07	0.67 ± 0.06	RI-MS
18	neo-iso-Menthol	1184	1184	1.91 ± 0.09	1.80 ± 0.07	1.93 ± 0.10	1.30 ± 0.18	1.79 ± 0.12	1.51 ± 0.11	1.76 ± 0.13	RI-MS
19	Pulegone	1236	1233	0.07 ± 0.01	0.09 ± 0.01	0.05 ± 0.02	0.10 ± 0.01	0.06 ± 0.02	0.11 ± 0.01	0.06 ± 0.02	Std
20	Piperitone	1252	1252	0.37 ± 0.01	0.51 ± 0.02	0.39 ± 0.01	0.49 ± 0.02	0.40 ± 0.02	0.49 ± 0.03	0.42 ± 0.02	RI-MS
21	neo-Menthyl acetate	1273	1271	0.85 ± 0.07	0.74 ± 0.03	0.89 ± 0.07	0.75 ± 0.04	0.91 ± 0.05	0.72 ± 0.06	0.83 ± 0.04	RI-MS
22	p-Menth-1-en-9-ol	1294	1294	13.45 ± 0.74	13.05 ± 0.76	13.55 ± 0.68	12.50 ± 0.71	14.96 ± 0.72	11.66 ± 0.53	13.47 ± 0.39	RI-MS
23	iso-Menthyl acetate	1307	1304	0.65 ± 0.04	0.63 ± 0.03	0.67 ± 0.04	0.61 ± 0.03	0.73 ± 0.03	0.62 ± 0.05	0.68 ± 0.02	RI-MS
24	β -Bourbonene	1382	1387	0.51 ± 0.04	0.42 ± 0.05	0.47 ± 0.06	0.41 ± 0.05	0.47 ± 0.06	0.40 ± 0.05	0.48 ± 0.05	RI-MS
25	(E)-Caryophyllene	1416	1417	1.93 ± 0.12	1.71 ± 0.19	1.77 ± 0.18	1.60 ± 0.17	1.82 ± 0.18	1.64 ± 0.16	1.85 ± 0.16	Std
26	(E)- β -Farnesene	1457	1454	0.42 ± 0.03	0.36 ± 0.05	0.38 ± 0.05	0.34 ± 0.05	0.40 ± 0.05	0.36 ± 0.05	0.40 ± 0.04	RI-MS
27	Germacrene D	1479	1484	2.06 ± 0.15	1.92 ± 0.26	1.91 ± 0.23	1.82 ± 0.24	1.93 ± 0.23	1.86 ± 0.23	1.96 ± 0.21	Std
28	Elixene	1494	1492	0.31 ± 0.02	0.29 ± 0.03	0.29 ± 0.03	0.27 ± 0.03	0.30 ± 0.03	0.27 ± 0.03	0.30 ± 0.03	RI-MS
29	Viridiflorol	1589	1592	0.66 ± 0.03	0.56 ± 0.06	0.63 ± 0.06	0.56 ± 0.05	0.65 ± 0.05	0.55 ± 0.04	0.64 ± 0.04	RI-MS
	Total identified (%)			97.13	97.05	97.23	97.86	97.63	98.05	97.69	
	Essential oil yield (g/m ⁻²)			2.63	5.92	3.33	5.49	3.44	6.02	3.45	
	Grouped compounds (%)										
	Monoterpene hydrocarbons			3.97	3.92	4.08	3.98	3.82	3.94	4.02	
	Oxygenated monoterpenes			87.07	88.68	87.51	88.71	88.05	88.86	87.85	
	Sesquiterpene hydrocarbons			5.24	4.71	4.82	4.45	4.93	4.53	5.00	
	Oxygenated sesquiterpenes			0.66	0.56	0.63	0.56	0.65	0.55	0.64	
	Others			0.19	0.18	0.18	0.15	0.17	0.17	0.17	

Control (No fertilizer), CF (Chemical fertilizer), AMF (arbuscular mycorrhiza fungus), 50 % CF + AMF (50 % Chemical fertilizer + arbuscular mycorrhiza fungus), NCF (Nano chelated fertilizer), 50 % CF + NCF (50 % Chemical fertilizer + nano chelated fertilizer), NCF + AMF (Nano chelated fertilizer + arbuscular mycorrhiza fungus).

Table 7
Pearson's correlation factors (coefficients) between total dry weight, essential oil content, essential oil yield and chemical compositions of peppermint essential oil.

	dry matter yield	Essential oil content	Essential oil yield	Menthol	Menthone	p-menth-1-en-9-ol	1,8-Cineole	neo-Menthol	Menthofuran	δ -Terpineol	Germacrene D
dry matter yield	1.00										
Essential oil content	0.98**	1.00									
Essential oil yield	0.99**	0.99**	1.00								
Menthol	-0.31	-0.26	-0.29	1.00							
Menthone	0.86*	0.80*	0.85*	-0.72	1.00						
p-menth-1-en-9-ol	-0.87*	-0.88**	-0.89**	0.07	-0.68	1.00					
1,8-Cineole	-0.94**	-0.88**	-0.94**	0.23	-0.84*	0.87*	1.00				
neo-menthol	-0.93**	-0.86*	-0.93**	0.23	-0.80*	0.80*	0.93**	1.00			
Menthofuran	-0.86*	-0.78*	-0.84*	0.25	-0.80*	0.80*	0.94**	0.83*	1.00		
δ -Terpineol	0.97**	0.91**	0.96**	-0.37	0.91**	-0.86*	-0.98**	-0.95**	-0.92**	1.00	
Germacrene D	-0.88**	-0.87*	-0.87*	0.29	-0.77*	0.67	0.87*	0.74	0.80*	-0.85*	1.00

*, **, Significant at 5 and 1% levels of probability, respectively.

availability of nutrients because of the small size and large surface of nanoparticles (Naderi and Danesh-Shahraki, 2013).

In our experiments, the integrative application of 50 % chemical fertilizer + arbuscular mycorrhiza fungus enhanced significantly the number of nodes in peppermint. The higher number of nodes with combination of chemical fertilizer with mycorrhiza could be explained by the improvement of soil microbial activities and balanced nutrient supply that increase the NUE in plants (Abdel Latef and Chaoxing, 2011). In addition, the symbiosis of mycorrhizal fungi improves the water relations and biochemical properties of the soil (Liu et al., 2019; Amiri et al., 2015). Being in close accord with this result, Weisany et al. (2016) noted that the arbuscular mycorrhiza fungus colonization increased the mineral (P, K, Fe, Zn and Mn) uptake of dill (*Anethum graveolens* L.) leading to higher biomass dry weight.

According to the obtained results, the highest SPAD index was achieved with application of 50 % chemical fertilizer + nano chelated fertilizer and chemical fertilizer. The availability of micro- and macronutrients, especially N, Mn and Zn, could significantly affect the chlorophyll content of plants (White and Brown, 2010). Therefore, the higher chlorophyll content (SPAD index) under integrative application of 50% chemical fertilizer + nano chelated fertilizer and chemical fertilizer could be explained by the enhancement of nutrients availability that increases the RUBISCO activity, photosynthetic rate and plant growth parameters (Vishekaii et al., 2019). Rop et al. (2019) reported that no significant differences were observed in the growth parameters of maize (*Zea mays* L.), capsicum (*Capsicum annuum* L.) and kale (*Brassica oleracea* L.) after application of chemical fertilizer and nano-NPK. Yousefzadeh et al. (2013) showed that the chlorophyll content of dragonhead (*Dracocephalum moldavica* L.) improved under nutrients availability (especially nitrogen) after application of different fertilizer sources. Similarly, Vishekaii et al. (2019) reported that after application of nano-boron the number of leaves per shoot in olive trees enhanced by 84 % when compared with control. Also, these authors concluded that the highest chlorophyll and carbohydrate contents were achieved with application of nano-boron. Babaei et al. (2017) showed that the maximum values of chlorophyll *a*, chlorophyll *b* and total chlorophyll of wheat (*Triticum aestivum* L.) was recorded after application of nano-Zn + Fe oxide.

Our results indicated that the maximum concentrations of macronutrients (N, P and K) were reached with application of 50 % chemical fertilizer + nano chelated fertilizer. In addition, the highest content of micro-nutrients (Fe, Zn and Mn) was achieved using treatment with nano chelated fertilizer and 50 % chemical fertilizer + nano chelated fertilizer. The improvement of macro- and micronutrient concentrations with application of nano fertilizer could be explained by the higher surface area to volume ratio and slower release of nanoparticles that promote NUE in plants (Elemike et al., 2019). Similarly, Marzouk et al. (2019) concluded that the concentration of P, K, Zn, Mn and Cu increased significantly after foliar application of nano-micronutrient fertilizers. In addition, Al-Juthery et al. (2018) noted that the concentration of N, P and K in wheat (*Triticum aestivum* L.) after application of nano fertilizer increased by 3.17, 0.66 and 2.88 % when compared with control, respectively.

The maximum dry matter yield of peppermint was recorded with application of 50 % chemical fertilizer + nano chelated fertilizer. It seems that application of micro and macro elements, especially under a nanoparticle formula, could improve the plant's NUE (Hänsch and Mendel, 2009). In addition, they enhance the plant growth characteristics such as plant height, chlorophyll content and number of leaves. Marzouk et al. (2019) reported that the enhancement of vegetative growth and dry matter yield of snap bean (*Phaseolus vulgaris* L.) cultivars may be due to the stimulatory effects of nano micronutrients fertilizer on the chlorophyll productivity, hormone biosynthesis (gibberellic acid, jasmonic acid and ethylene) and improvement of the mitochondrial respiration and photosynthesis. Similarly, Rop et al. (2019) concluded that the dry matter yield of capsicum, maize and kale

was higher after application of nano-NPK fertilizer compared with chemical fertilizer.

The maximum essential oil content and essential oil yield of peppermint were observed after application of 50 % chemical fertilizer + nano chelated fertilizer. In fact, a higher availability of nutrients after application of fertilizers could increase the essential oil synthesis in MAPs by improving the plant growth characteristics such as the chlorophyll content and the essential oil glands (Fallah et al., 2018). In addition, the higher essential oil productivity in peppermint under integrative application of fertilizer could be explained by the increase of the aerial parts dry weight and number of branches. Amooaghaie and Golmohammadi (2017) noted that the higher supply of micro (Zn, Fe, Mn and Cu) and macronutrients (N and P) after application of organic fertilizer increased significantly the essential oil productivity in *Thymus vulgaris* L. Based on the correlation and principal components analysis, there was a positive correlation between the dry matter yield and essential oil content and yield. Therefore, the higher essential oil yield under integrative application of chemical fertilizer and nano chelated fertilizer was related to the higher dry matter yield and essential oil content of peppermint (Amani Machiani et al., 2019). Being in close accord with this result, Mahmoodi et al. (2018) reported that application of nano-urea enhanced the essential oil content and yield in *Borago officinalis* L. compared with chemical and nano fertilizers. Also, Gholinezhad (2017) reported that the best morphological parameters, essential oil content and yield of dill were achieved with application nano-iron fertilizer.

Ecological factors, genetic differences, geographic origins and also techniques and methods used in the cultivation of MAPs are responsible for creating quantitative and qualitative differences in essential oils. In this study, the highest content of menthol and oxygenated monoterpenes at both harvests were achieved with application of 50 % chemical fertilizer + nano chelated fertilizer. Essential oils are made up of terpenoid components so that the availability of needed Acetyl-CoA, NADPH and ATP and mineral nutrients like N and P is essential for the biosynthesis of these compounds (Ormeno and Fernandez, 2012). It seems that any factor inducing the uptake of these nutrients will ultimately enhance the percentage of these components in essential oils. Thus, a better uptake of nutrients, particularly N, P and microelements (Fe, Mn, Zn) by integrative application of chemical fertilizer and nano fertilizer, improves the quality of peppermint essential oil (Amooaghaie and Golmohammadi, 2017). In addition, the lowest content of menthol (as the most important constituent of peppermint essential oil) at both harvests was observed after application of chemical fertilizer. Indeed, the excessive application of chemical fertilizer can reduce the content of essential oil and some main constituents by enhancing the gland size, which dilutes the concentration of essential oil in plant organs (Yousefzadeh et al., 2013). In addition, the quality of peppermint essential oil improved with decreasing menthofuran percentage (Amani Machiani et al., 2018a). In the second harvest, the content of menthofuran with application of 50 % chemical fertilizer + nano chelated fertilizer decreased by 33 and 6% when compared with control and chemical fertilizer, respectively. Overall, the quality of peppermint essential oil with application of 50 % chemical fertilizer + nano chelated fertilizer was high as assured by the percentage increase of menthol and decrease of menthofuran.

The results showed that the growth characteristics, dry matter yield, essential oil content and essential oil yield of peppermint at the first harvest were higher than those at the second harvest. This could be explained by the better climatic conditions, such as temperature and period of lighting, observed at the first harvest (Sangwan et al., 2001). Hassiotis et al. (2014) reported that reduced temperatures have a negative impact on the essential oil productivity of *Lavandula angustifolia* Mill. due to breaking of essential oil gland cells or alteration of the terpene biosynthesis. Same results were reported by Amani Machiani et al. (2018a). These authors noted that the essential oil content and essential oil yield of peppermint at the first harvest (July) was 20 and

52 % higher than that at the second harvest (October), respectively.

5. Conclusions

This study focused on the impacts of different fertilizers sources on the nutrient uptake, growth parameters and essential oil qualitative properties of peppermint. The results of study demonstrated that the highest values of growth parameters, dry matter yield, essential oil content and essential oil yield were recorded in the first harvest after application of 50 % chemical fertilizer + nano chelated fertilizer. In addition, the quality of peppermint essential oil, in terms of menthol content, improved with application of 50 % chemical fertilizer + nano chelated fertilizer. Overall, the integrative application of chemical fertilizer with nano chelated fertilizer, not only optimizes the application of the former allowing a lower impact on the environment, but also improves the qualitative characteristics of peppermint supporting its recommendation to farmers as an efficient and eco-friendly strategy for cultivation of peppermint.

Author statement

Ali Ostadi: Study conception and design, Acquisition of data
 Abdollah Javanmard: Study conception and design, Acquisition of data
 Mostafa Amani Machiani: Drafting of manuscript
 Mohammad Reza Morshedloo: Drafting of manuscript
 Mojtaba Nouraein: Analysis and interpretation of data
 Farzad Rasouli: Analysis and interpretation of data
 Filippo Maggi: Drafting of manuscript, Critical revision

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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