*International Journal of Horticultural Science and Technology*
*Vol. 7, No. 1; March 2020, pp 13-26*
Print ISSN: 2322-1461 Online ISSN: 2588-3143
DOI: 10.22059/ijhst.2020.287709.317
Web Page: https:// ijhst.ut.ac.ir, Email: ijhst@ut.ac.ir

# Alteration in Essential Oil Yield and Compositions of Multi-Cut *Dracocephalum polychaetum* Grown under Different Fertilization Methods

**Abbas Fallah[1] and S-Zahra-Hosseini Cici[2*]**
*1. Sustainable Agriculture Research, Iran*
*2. Department of Agriculture, Payam Noor University, Tehran, Iran*

(Received: 22 August 2019, Accepted: 15 December 2019)

**Abstract**
Sustainable agriculture can increase the efficiency of resource utilization, provide longer productivity and create a balance in the environment. A field experiment was conducted to study the use of organic and chemical fertilizers on biomass, essential oil content and essential oil yield as well as chemical compositions of *Dracocephalum polychaetum* Burnm in the first and second cutting. The treatments were: broiler litter (1700 kg ha$^{-1}$), cow manure (2500 kg ha$^{-1}$), sheep manure (1700 kg ha$^{-1}$), chemical fertilizer (110 kg ha$^{-1}$) and control (no fertilizer) in three replications. The results showed that *D. polychaetum* was most responsive to application of broiler litter as compared with the other treatments. Gas chromatography–mass spectrometry analysis indicated that neral, geranial, geranyl acetate and α-pinene were the major chemical compounds of *D. polychaetum* in the both cuttings. In the first cutting the plants treated with sheep manure had the highest neral content (28.24%). The highest geranial content (26.85%) in the first cutting was recorded in plants amended with chemical fertilizer, without significant difference with broiler litter (26.36%). In the first cutting the greatest α-pinene (15.52%) content was observed in the control treatment without significant difference with broiler litter (15.09%). Unlike the first cutting, plant amended with cow manure had the maximum neral (23.89%) and geranial (29.27%) contents in the second cutting. The present study demonstrated that the application of organic manure improved essential oil content and quality and aroma profile of *D. polychaetum.*

**Keywords**: Medicinal herb, Essential oil, Geranial, Neral, Organic production.

## Introduction
The main challenge in agriculture is to increase crop production in a manner that is sustainable for the present and the future. The agricultural production and sustainable intensification goals in commercially important crops over both short and long-terms demand depends on plant nutrient and soil management (Pandey et al., 2016). Although chemical fertilizers have been extensively used in agriculture to improve yield (Siddiqui et al., 2011), long-term excessive application of these fertilizers has decreased soil quality and sustainability (Guo et al., 2010; Dinesh et al., 2010; Roelcke et al., 2004), which has become a major concern (Chaudhry et al., 2009).

Manure can be used as an alternative to chemical fertilizer in sustainable agriculture (Dinesh et al., 2010). It improves chemical and physical properties of soil and enhances

* Corresponding Author, Email: sydzaracici@gmail.com

organic matter, cation exchange, water holding capacity and ultimately adjusts the appropriate supply of essential nutrients for crop growth (Schlegel et al., 2015a,b; Govahi et al., 2015; Pandey et al., 2016). Manure may affect the chemical compositions and quality of medicinal plants (Emami Bistgani et al., 2018). Bajeli et al. (2016) showed that addition of manure such as cow manure, vermicompost and poultry manure led to increase in biomass and methanol content of Japanese mint (*Mentha arvensis* L.).

*Dracocephalum polychaetum* Burnm. (Lamiaceae), which is known as “Badernjboei-Dennaei” or “Kermani Zarrin-giah” in Persian, is a medicinal herb endemic to Iran (Mozaffarian, 1998). This plant is mainly distributed in central and northern of Iran and grows at an altitude of 1000-1800 m in high mountainous regions (Boroomand et al.. 2017; Rechinger, 1986).

Various pharmacological effects such as antihyperlipidemic (Taghizadeh et al. 2019), immunomodulatory (Pouraboli et al., 2015), antinociceptive (Yaghoobi et al., 2018) and cytotoxic effect (Boroomand et al., 2017) have been reported for D. *polychaetum*. The plant is also a source of essential oil (Saeidnia et al., 2004). The major chemical compositions of essential oil of this plant includes E-β-ocimene, nerol, α-pinene (Fattahi et al., 2016), α-pinene and caryophyllene oxide (Javidnia et al., 2005), geranial (Saeidnia et al., 2007), α-pinene and methyl geranate (Monsef-Esfahani et al., 2007). These chemical compositions are widely used in food and pharmaceutical industries.

Organic farming of medicinal plants is very important due to their direct role in human health. Hence, the replacement of chemical fertilizers by manure in cropping these plants is a priority. Although there are relatively lots of reports on the benefits of manures in annual medicinal plants compared to chemical fertilizers, there is a lack of information on multi-cut medicinal plants such as *D. polychaetum*. On the other hand, multi-cut *D. polychaetum* only grows in cold and mountainous regions and in this specific condition the effect of organic manures is not studied. Therefore, the present study aimed to investigate the effect of application of manure on biomass, essential oil content, essential oil yield and chemical composition of essential oil in *D. polychaetum* plant in its first and second cuttings.

## Materials and methods

### *Location and experimental design*

The seeds of *D. polychaetum* were obtained from Kerman Herb Company, Kerman, Iran. Initially, seeds of *D. polychaetum* were sown in coco-peat: peat moss mix (70:30 w:w) on plastic germination trays and then germinated. Sixty days after sowing, seedlings (with uniform size) were selected and then were transplanted into the experimental plots in 21 November, 2017.

A field experiment was conducted based on randomized block design with five treatments and three replications in 2018 at the research field of Darab, Fars, Iran (54°55E, 28°75 N; 1180 m above sea level). According to Koppen climate classification, the region is cold semi-arid, with an average annual precipitation of 221 mm and an average monthly temperature of 16.1 °C.

The treatments were included: 110 kg $ha^{-1}$ chemical fertilizer (100:50:50 NPK), 1700 kg $ha^{-1}$ broiler litter, 2500 kg $ha^{-1}$ cow manure, 1700 kg $ha^{-1}$ sheep manure, and control (without fertilizer) (Rostaei et al., 2018a).

Soil samples were randomly collected from a depth of 0-30 cm from 10 different spots using a soil auger. All soil samples were air-dried in the laboratory for 4 days and then crushed and sieved using a 2 mm sieve. The physical and chemical properties of the soil and the organic manures were analyzed and indicated in Table 1.

Seedlings were grown in accordance with organic farming practices with no use of herbicide or pesticide applications. Manures and chemical fertilizer were applied to the soil before transplanting the seedlings. The plots were irrigated immediately after transplanting the seedlings in November. The size of each plot was 2.40 × 3.60 m and consisted of five rows. Weeds were controlled by hand weeding during the growing season.

***D. polychaetum* biomass**

Aerial parts of *D. polychaetum* were cut at 50% of flowering on 30th June 2018 (first cutting; 223 days after transplanting the seedling) and 26th August 2018 (second cutting; 55 days after first cutting). The whole plants were harvested when they were 10 cm above ground level in each plot using manual shears. Leaves, stems and flowers were separated, dried in the shade for 5 days and weighed.

***Isolation of essential oil***

The above ground parts of the *D. polychaetum* were harvested in September, dried in shade and powdered. About 50 g of the powder were hydro-distilled for 3.5 hours using a Clevenger apparatus according to British Pharmacopoeia (1988). The samples of essential oils were dehydrated using anhydrous sodium sulphate. Then the extracted essential oil was stored in sealed vials at 4 °C until gas chromatography analysis. The essential oil yield was determined as kg $ha^{-1}$ and calculated using the following equation (Emami Bistgani et al., 2018):

Essential oil yield (kg $ha^{-1}$) = (biomass kg. $ha^{-1}$×essential oil content g $kg^{-1}$)/1000

***Gas chromatography analysis***

Gas chromatography (GC) was performed using a Thermo-UFM gas chromatography equipped with a flame ionization detector (FID) and DB-5 column (10 m × 0.1 mm i.d., film thickness 0.4 μm). Helium was used as the carrier gas at a flow rate of 0.5 mL per min. The oven temperature was initiated at 60 °C, then increased to 285 °C at a rate of 40 °C/min and kept for 3 min at 285 °C. The temperature of injector and FID detector was 280 °C.

***Gas chromatography–mass spectrometry analysis***

Gas chromatography–mass spectrometry (GC–MS) analysis was carried out using an Agilent 7890A/5975C GC-MS system equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was programmed as follows: The oven temperature was initiated at 60 °C, and then was increased from 60 to 220 °C at a rate of 3 °C/min; subsequently the temperature was enhanced to 240 °C at 20 °C/min and kept at this temperature for 3 min. The injector and transfer line temperature were 260 °C and 280 °C, respectively. Carrier gas was helium with a linear velocity of 30.6 cm/s; split ratio 1:100, ionization energy 70 Ev and scan time 1 s. The mass range was 40–300 m/z (Adams 2007).

***Identification of essential oil chemical compositions***

The chemical compositions of the essential oil samples were determined by comparing their mass spectra with those held in the computer library or achieved using authentic components. Retention indices were calculated using the retention times of *n*-alkanes (C6-C24) injected after the essential oil under the same conditions. The identities of the chemical components were confirmed by comparing with relative retention indices, either with those of authentic components or with data published in the literature (Adams, 2007; Fattahi et al., 2016).

***Statistical analysis***

All the measured traits (biomass, essential oil content, essential oil yield and chemical compositions of essential oil) in both cuttings were statistically analyzed based on randomized block design using software statistical analysis system (SAS, Ver. 9.4). Means were compared using the least significant difference (LSD) test at 5% probability level.

**Table 1. Physical and chemical properties of field soil (depth of 0-30 cm) and organic manures (broiler litter, cow manure and sheep manure).**

| Parameter | Soil | Broiler litter | Cow manure | Sheep manure |
|---|---|---|---|---|
| Texture | Silty clay loam | - | - | - |
| EC (dS $m^{-1}$) | 0.23 | 4.73 | 1.95 | 2.43 |
| pH | 7.55 | 8.19 | 8.70 | 8.33 |
| Nitrogen (g $kg^{-1}$) | 0.06 | 29.1 | 19.9 | 28.6 |
| Phosphorus (g $kg^{-1}$) | 0.028 | 5.5 | 2.6 | 2.7 |
| Potassium (g $kg^{-1}$) | 0.48 | 16.3 | 16.7 | 23.6 |

## Results

### *Aboveground biomass*

The aboveground biomass of *D. polychaetum* was significantly affected by different fertilization treatments ($p<0.001$) in the first cutting, second cutting and sum of two consecutive cuttings (Table 2). In the first cutting, the aboveground biomass of *D. polychaetum* was similar to those of treatments of cow manure (on average 1291 kg $ha^{-1}$) and sheep manure (on average 1306 kg $ha^{-1}$) (Fig. 1A). Also, the aboveground biomass of *D. polychaetum* was not significantly different in plants treated using chemical fertilizer and sheep manure. The aboveground biomass in cow manure, sheep manure and chemical fertilizer application was significantly different from those of broiler litter and control. In the first cutting the aboveground biomass of broiler treatment was twice as much as that of the control (Fig. 1A).

In the second cutting, the aboveground biomass of *D. polychaetum* using sheep manure, cow manure and chemical fertilizer was similar and significantly less than that of the broiler litter (Fig. 1B).

The results demonstrated that the total aboveground biomass (sum of two consecutive cuttings) was not significantly different in plants that were treated with chemical fertilizer, sheep manure and cow manure (Fig. 1C). The highest aboveground biomass of *D. polychaetum* was recorded in broiler litter; it was 49% more than the one in control (Fig 1C).

The aboveground biomass of *D. polychaetum* in the second cutting was significantly enhanced compared with the one in the first cutting (Fig. 1A and 1B). It seems that plants at early growth stage allocate their assimilation to proper establishment, while after the first cutting; the plants complete their growth and development and increase biomass production. Furthermore, plants treated with chemical fertilizer in the first cutting had higher biomass than the second cutting (Fig. 1A and 1B). In the first cutting, increase in biomass in plants treated with chemical fertilizer could be possibly attributed to the further release of nutrients such as nitrogen and phosphorus in the early growth season.

### *Essential oil content*

Our results showed that there was a significant difference in essential oil content of *D. polychaetum* under different fertilization treatments (Table 2) in the first and second cuttings. In the first cutting, essential oil content of *D. polychaetum* was not significantly different in plants fertilized with cow manure and those that were not fertilized. The essential oil content of *D. polychaetum* was similar in treatments using broiler litter (on average by 0.93 %), chemical fertilizer (on average by 0.87%) and sheep manure (on average by 0.74%) (Fig. 2A). The results showed that the highest and lowest essential oil contents of

*D. polychaetum* in the first cutting were respectively obtained from the treatment in which broiler litter (0.93%) and cow manure (0.39%) were used.

As presented in Fig. 2B in the second cutting, the essential oil content of *D. polychaetum* was similar to that of the treatments in them cow manure (on average by 1.19%) and sheep manure (on average by 1.23%) were used. Also essential oil content of *D. polychaetum* was not significantly different in plants treated with cow manure and broiler litter. The essential oil content in these treatments was significantly higher than that of chemical fertilizer and control treatments (Fig. 2B). The minimum essential oil content in the second cutting was obtained from the plants treated with chemical fertilizer.

Overall, at the second cutting essential oil content was increased in broiler litter, cow manure, sheep manure and control (Fig. 2A and 2B). The maximum difference between cuttings was recorded in the cow manure treatment. In the second cutting, plants amended with chemical fertilizer had lower essential oil content as compared with that of the first cutting (Fig. 2A and 2B).

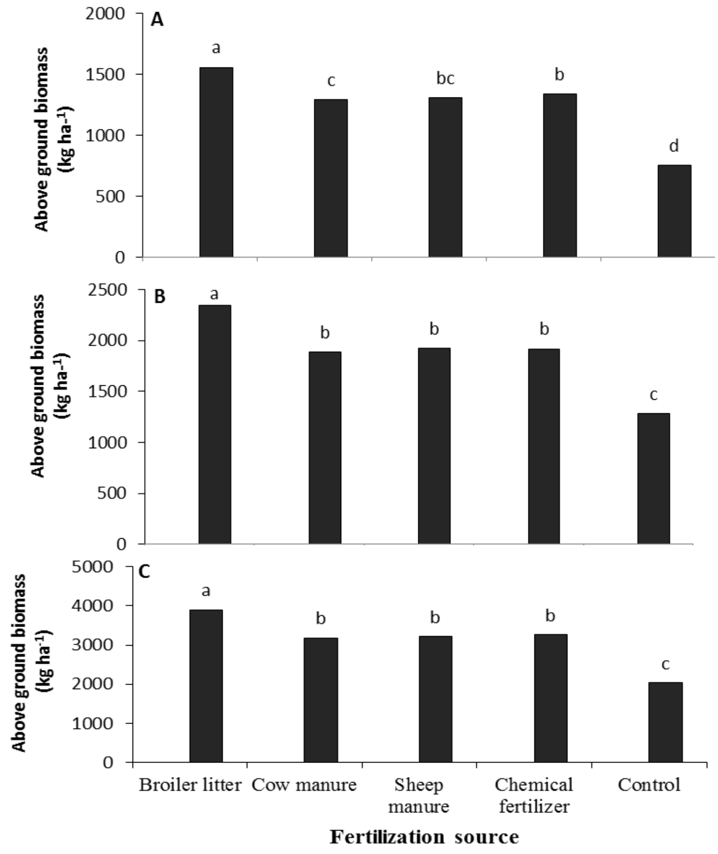



**Fig. 1. Effects of different fertilizer sources on above ground biomass of *D. polychaetum* in the first cutting (A), second cutting (B) and sum of two cuttings (C). Means with different letters are significantly different**

**Table 2. Analysis of variance for above ground biomass and essential oil content of multi-cut *D. polychaetum* as affected by different fertilizer sources**

| Source of variation | df | Above ground biomass | | | Essential oil content | |
|---|---|---|---|---|---|---|
| | | First cutting | Second cutting | Sum of two cuttings | First cutting | Second cutting |
| Block | 2 | 87 ns | 1148 ns | 1832 ns | 0.062 ns | 0.001 ns |
| Treatment | 4 | 264438** | 428111** | 1356998** | 0.17** | 0.17** |

| | | | | | | |
|---|---|---|---|---|---|---|
| Error | 8 | 412 | 1495 | 3210 | 0.017 | 0.005 |
| CV (%) | - | 1.62 | 2.06 | 1.81 | 19.5 | 7.16 |
| LSD | - | 38.22 | 72.82 | 106.69 | 0.25 | 0.13 |

$^{ns}$ and **: non-significant and significant at $P \le 0.001$, respectively

**Table 3. Analysis of variance for essential oil yield of multi-cut *D. polychaetum* as affected by different fertilizer sources**

| Source of variation | df | Essential oil yield | | |
|---|---|---|---|---|
| | | First cutting | Second cutting | Sum of two cuttings |
| Block | 2 | 12 ns | 0.45 ns | 8.03 ns |
| Treatment | 4 | 62.25** | 0.125** | 255** |
| Error | 8 | 3.84 | 1.92 | 4.09 |
| CV (%) | - | 21.93 | 7.26 | 7.21 |
| LSD | - | 3.68 | 2.61 | 3.81 |

$^{nS}$ and **: non -significant and significant at $P \le 0.001$, respectively.

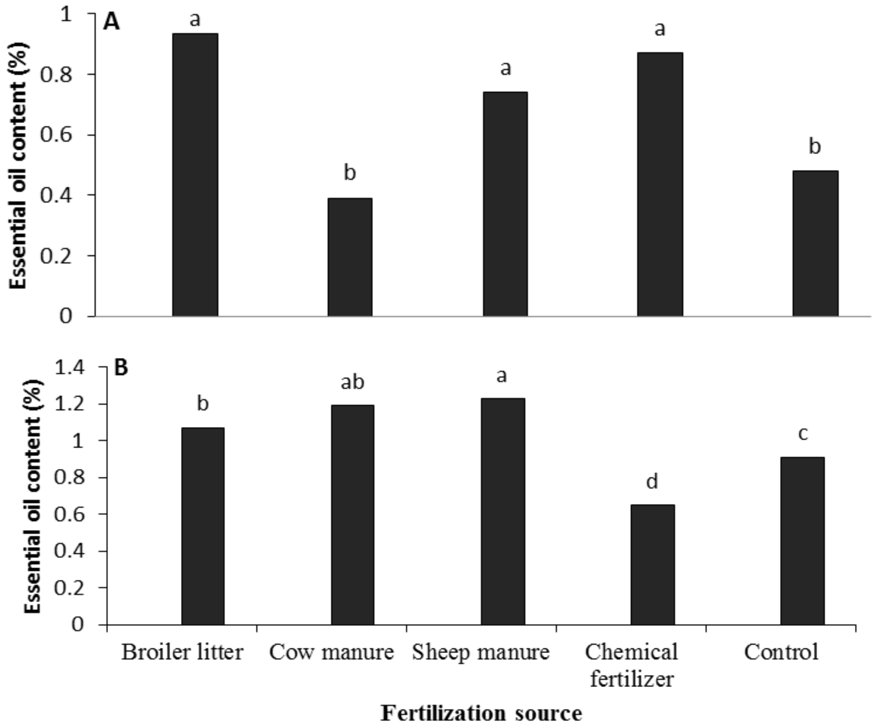



**Fig. 2. Effect of different fertilizer sources on essential oil content of *D. polychaetum* in the first cutting (A) and second cutting (B). Means with different letters are significantly different**

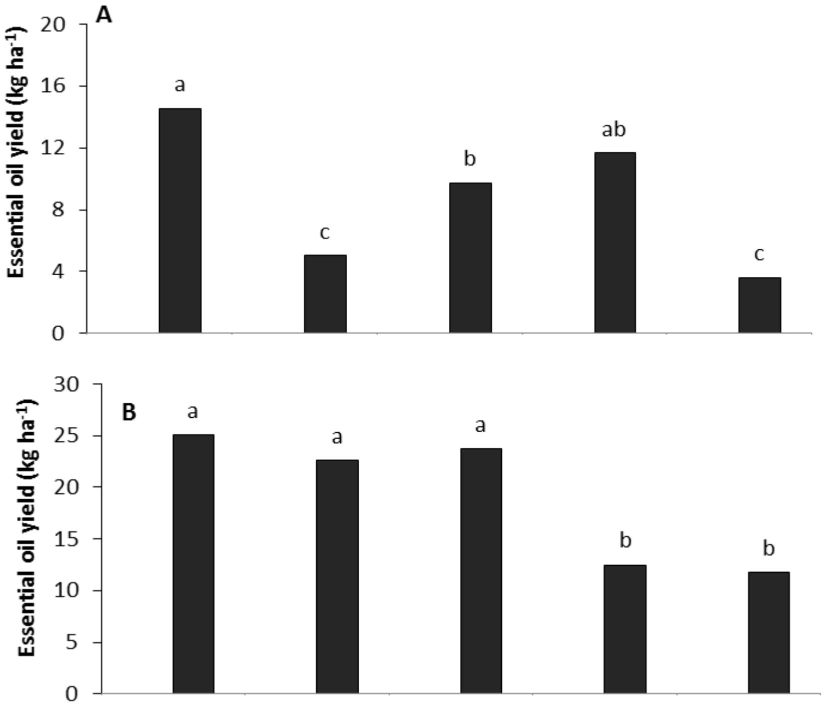
### *Essential oil yield*

Essential oil yield of *D. polychaetum* was significantly affected by different fertilization treatments (p<0.001) in the first and second cuttings (Table 3.). In the first cutting, essential oil yield *D. polychaetum* was not significantly different in plants received chemical fertilizer (on average by 11.66 kg ha$^{-1}$) and sheep manure (on average by 9.74 kg ha$^{-1}$). Also the essential oil yield of *D. polychaetum* was similar in treatments of broiler litter (on average by 14.56 kg ha$^{-1}$) and chemical fertilizer (on average by 11.66 kg ha$^{-1}$) (Fig. 3A). The essential oil yield in treatments of broiler litter, sheep manure and chemical fertilizer significantly enhanced compared with cow manure and unfertilized treatments. The greatest essential oil yield of *D. polychaetum* was achieved in broiler litter treatment (Fig. 3A).

In the second cutting, the essential oil yield of *D. polychaetum* was similar in broiler litter, sheep manure and cow manure treatments (on average by 25.08, 23.71, and 22.61 kg ha$^{-1}$, respectively) (Fig. 3B). The essential oil yield in these treatments significantly increased in comparison with the chemical fertilizer and control. The greatest essential oil yield of *D. polychaetum* was recorded in broiler litter (Fig. 3B).

According to our results, total essential oil yield responses were significantly positive under all fertilization methods relative to those obtained from treatments in them no fertilizers were used (Fig. 3C). The magnitude of total essential oil yield elevation exhibited the following order: broiler litter 39.65 kg ha$^{-1}$) > sheep manure (33.46 kg ha$^{-1}$) > cow manure (27.67 kg ha$^{-1}$) > chemical fertilizer (24.16 kg ha$^{-1}$) > control (15.35 kg ha$^{-1}$) (Fig. 3C).

As presented in Fig. 2A, 2B, 3A and 3B, application of broiler litter produced the highest aboveground in first and second cuttings, and created maximum essential oil content in the first cutting and ultimately enhanced essential oil yield in both cuttings. In the first cutting, plants treated with chemical fertilizer had relatively high amount of aboveground and essential oil content as compared with those in the second cutting. Therefore, these plants had higher essential oil yield in the first cutting than those in the second cutting. Cow manure and sheep manure had higher aboveground and essential oil content in the second cutting over the first cutting. In the first and second cuttings the lowest essential oil yield of *D. polychaetum* was recorded in untreated plants.



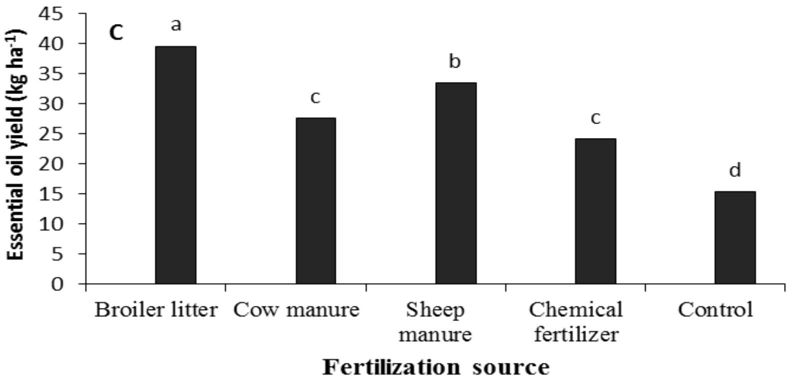



**Fig. 3. Effect of different fertilizer sources on essential oil yield of *D. polychaetum* in the first cutting (A), second cutting (B) and sum of two cuttings (C). Means with different letters are significantly different**

## Chemical composition of essential oil

The essential oil chemical compositions that were identified from the aerial parts of *D. polychaetum* are listed in Table 4 and 5. According to GC–MS analysis, 23 chemical compounds were identified in the first cutting and presented 97.57% - 98.51% of the total essential oils (Table 4). Neral (19.86%±2.55-28.24%±5.10), geranial (15.89%±2.89-26.85%±1.55), geranyl acetate (7.16%±1.37-20.68%±2.68) and α-pinene (11.28%±3.12-15.52%±2.52) were major chemical constituents of *D. polychaetum* in the first cutting.

In the second cutting (Table 5), the main chemical components of the essential oil were geranial (15.74%±2.14-29.27%±1.81), neral (19.71%±2.21-23.89%±1.40), geranyl acetate 11.61%±0.77-20.53%±3.54) and α-pinene (9.93%±2.91-15.37%±4.37). Camphene was found in control in both cuttings, but was not detected in fertilized treatments (Table 4 and 5). Trans limonen oxide, cis-p-mentha-2, 8-dien-1-ol, trans carveol and geraniol were higher in control as compared with other treatments in the first and second cuttings.

**Table 4. The chemical compositions (%) of the first cutting *of D. polychaetum* treated with different fertilizers**

| Compound | KI[a] | Treatments | | | | |
|---|---|---|---|---|---|---|
| | | Broiler litter | Cow manure | Sheep manure | Chemical fertilizer | Control |
| α-Pinene | 943 | 15.09 ±6.96 | 14.74 ±8.28 | 12.36 ±4.03 | 11.28 ±3.12 | 15.52 ±2.52 |
| Camphene | 960 | - | - | - | - | 0.27 ±0.07 |
| Sabinene | 978 | 0.46 ±0.19 | 0.46 ±0.29 | 0.36 ±0.15 | 0.39 ±0.11 | 0.19 ±0.09 |
| β-Pinene | 986 | 1.9 ±0.47 | 1.99 ±0.55 | 2.09 ±0.39 | 1.58 ±0.31 | 0.95 ±0.15 |
| Myrcene | 998 | 0.26 ±0.09 | 0.25 ±0.12 | 0.22 ±0.07 | 0.21 ±0.03 | 0.22 ±0.02 |
| α-Terpinene | 1013 | 0.44 ±0.17 | 0.55 ±0.34 | 0.49 ±0.16 | 0.45 ±0.08 | 0.67 ±0.17 |
| p-Cymene | 1028 | 0.14 ±0.02 | 0.11 ±0.09 | 0.13 ±0.04 | 0.05 ±0.09 | 0.21 ±0.01 |
| Limonene | 1035 | 6.16 ±1.59 | 7.51 ±4.82 | 7.76 ±3.45 | 5.5 ±1.39 | 4.27 ±1.07 |
| 1,8-Cineol | 1038 | 0.91 ±0.31 | 0.97 ±0.63 | 0.5 ±0.44 | 0.76 ±0.14 | - |
| (Z) β-Ocimene | 1040 | 0.34 ±0.06 | 0.12 ±0.21 | 0.52 ±0.34 | 0.33 ±0.07 | 0.88 ±0.08 |
| Unknown | - | 0.19 ±0.12 | 0.05 ±0.08 | 0.06 ±0.1 | 0.32 ±0.13 | 0.15 ±0.05 |
| Linalool | 1100 | 0.22 ±0.02 | 0.19 ±0.07 | 0.21 ±0.04 | 0.19 ±0.02 | 0.17 ±0.07 |
| α-Campholenal | 1132 | 1.2 ±0.13 | 1.03 ±0.30 | 1.19 ±0.15 | 1.22 ±0.18 | 1.57 ±0.12 |
| Trans limonen oxide | 1146 | 1.38 ±0.19 | 1.04 ±0.25 | 0.98 ±0.03 | 1.46 ±0.34 | 3.37 ±0.27 |
| Unknown | - | 0.81 ±0.16 | 0.68 ±0.16 | 0.7 ±0.15 | 0.79 ±0.13 | 0.57 ±0.07 |
| Cis-p-mentha-2,8-dien-1-ol | 1175 | 1.24 ±0.38 | 1.03±0.51 | 0.93 ±0.17 | 1.27 ±30 | 2.76 ±0.14 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Unknown | - | 1.17 ±0.20 | 0.99 ±0.13 | 1.06 ±0.10 | 1.11 ±0.21 | 0.62 ±0.08 |
| α-Terpineol | 1191 | 0.37 ±0.09 | 0.41 ±0.21 | 0.52 ±0.10 | 0.53 ±0.1 | 0.52 ±0.02 |
| Myrtenol | 1198 | 0.24 ±0.25 | 0.35 ±0.19 | 0.62 ±0.59 | 0.25 ±0.03 | 0.34 ±0.12 |
| Trans carveol | 1210 | 0.56 ±0.26 | 0.55±0.19 | 0.43 ±0.12 | 0.56 ±0.16 | 1.52 ±0.12 |
| Neral | 1242 | 26.4 ±3.74 | 25.83 ±7.99 | 28.24 ±5.10 | 27.73 ±4.98 | 19.86 ±2.55 |
| Geraniol | 1250 | 0.13 ±0.03 | 0.19 ±0.10 | 0.51 ±0.55 | 0.12 ±0.1 | 0.74 ±0.04 |
| Geranial | 1265 | 26.36 ±2.92 | 23.93 ±3.95 | 24.75 ±1.89 | 26.85 ±1.55 | 15.89 ±2.89 |
| Methyl geranate | 1328 | 5.9 ±1.41 | 6.61 ±1.55 | 6.32 ±1.46 | 6.17 ±0.92 | 6.76 ±0.16 |
| α-Cubebene | 1360 | 0.45 ±0.1 | 0.62 ±0.28 | 0.53 ±0.16 | 0.68 ±0.18 | 0.89 ±0.09 |
| Geranyl acetate | 1376 | 7.16 ±1.37 | 9.30 ±4.37 | 7.95 ±1.80 | 9.65 ±2.21 | 20.68 ±2.68 |
| Germacrene D | 1480 | 0.28 ±0.15 | 0.26±0.1 | 0.34 ±0.13 | 0.34 ±0.1 | 0.26 ±0.1 |
| Total identified | | 97.59 | 98.04 | 97.95 | 97.57 | 98.51 |

a: Calculated Kovats Index. b: Least significant difference (LSD α= 0.05).

**Table 5. The chemical compositions (%) of the second cutting of *D. polychaetum* treated with different fertilizers**

| Compound | KI[a] | Treatments | | | | |
|---|---|---|---|---|---|---|
| | | Broiler litter | Cow manure | Sheep manure | Chemical fertilizer | Control |
| α-Pinene | 943 | 13.86 ±1.41 | 9.93 ±2.91 | 13.41 ±0.71 | 14.39 ±2.61 | 15.37 ±4.37 |
| Camphene | 960 | - | - | - | - | 0.12 ±0.04 |
| Sabinene | 978 | 0.51 ±0.07 | 0.45 ±0.11 | 0.5 ±0.03 | 0.57 ±0.14 | 0.04 ±0.02 |
| β-Pinene | 986 | 1.68 ±0.13 | 1.41 ±0.24 | 1.65 ±0.07 | 2.25 ±0.56 | 0.8 ±0.2 |
| Myrcene | 998 | 0.24 ±0.02 | 0.19 ±0.04 | 0.26 ±0.01 | 0.27 ±0.05 | 0.07 ±0.03 |
| α-Terpinene | 1013 | 0.28 ±0.07 | 0.29 ±0.02 | 0.26 ±0.01 | 0.55 ±0.16 | 0.52 ±0.12 |
| p-Cymene | 1028 | 0.22 ±0.08 | 0.2 ±0.01 | 0.26 ±0.05 | 0.22 ±0.0 | 0.06 ±0.04 |
| Limonene | 1035 | 6.82 ±1.75 | 6.59 ±1.29 | 6.22 ±1.14 | 10.14 ±2.56 | 4.12 ±0.88 |
| 1,8-Cineol | 1038 | 0.27 ±0.47 | - | - | - | - |
| (Z) β-Ocimene | 1040 | 0.71 ±0.36 | 0.94 ±0.13 | 1.02 ±0.06 | 1.13 ±0.18 | 1.03 ±0.2 |
| Unknown | - | 0.51 ±0.04 | 0.54 ±0.04 | 0.54 ±0.04 | 0.49 ±0.09 | 0.3 ±0.2 |
| Linalool | 1100 | 0.25 ±0.02 | 0.23 ±0.04 | 0.27 ±0.02 | 0.31 ±0.06 | 0.32 ±0.1 |
| α-Campholenal | 1132 | 0.86 ±0.06 | 0.97 ±0.0 | 0.93 ±0.06 | 0.91 ±0.02 | 1.72 ±0.12 |
| Trans limonen oxide | 1146 | 0.98 ±0.26 | 0.95 ±0.1 | 1.09 ±0.19 | 0.71 ±0.11 | 3.52 ±0.3 |
| Unknown | - | 0.44 ±0.04 | 0.45 ±0.005 | 0.47 ±0.02 | 0.42 ±0.005 | 0.72 ±0.2 |
| Cis-p-mentha-2,8-dien-1-ol | 1175 | 1.38 ±0.35 | 1.67 ±0.10 | 1.73 ±0.1 | 1.23 ±0.28 | 2.91 ±0.2 |
| Unknown | - | 0.78 ±0.12 | 0.74 ±0.01 | 0.74 ±0.01 | 0.72 ±0.03 | 0.77 ±0.28 |
| α-Terpineol | 1191 | 0.27 ±0.04 | 0.3 ±0.05 | 0.28 ±0.03 | 0.21 ±0.02 | 0.67 ±0.3 |
| Myrtenol | 1198 | 0.32 ±0.05 | 0.3 ±0.06 | 0.29 ±0.05 | 0.29 ±0.02 | 0.49 ±0.29 |
| Trans carveol | 1210 | 0.6 ±0.12 | 1.31 ±0.49 | 0.77 ±0.1 | 1.5 ±0.4 | 1.67 ±0.3 |
| Neral | 1242 | 20.95 ±0.86 | 23.89 ±1.40 | 21.38 ±0.84 | 20.41 ±1.96 | 19.71 ±2.21 |
| Geraniol | 1250 | 0.19±0.08 | 0.19 ±0.05 | 0.18 ±0.02 | 0.09 ±0.09 | 0.59 ±0.09 |
| Geranial | 1265 | 25.75 ±1.64 | 29.27 ±1.81 | 26.01 ±0.45 | 23.07 ±3.11 | 15.74 ±2.14 |
| Methyl geranate | 1328 | 6.86 ±0.36 | 6.59 ±0.96 | 6.71 ±0.34 | 5.53 ±0.87 | 6.61 ±0.21 |
| α-Cubebene | 1360 | 0.69 ±0.09 | 0.59 ±0.17 | 0.61 ±0.16 | 0.58 ±0. 1 | 0.74 ±0.24 |
| Geranyl acetate | 1376 | 14.2 ±2.43 | 11.61 ±0.77 | 13.96 ±1.73 | 13.58 ±0.1 | 20.53 ±3.54 |
| Germacrene D | 1480 | 0.16 ±0.04 | 0.17 ±0.02 | 0.22 ±0.04 | 0.21±0.01 | 0.11 ±0.04 |
| Total identified | | 98.05 | 98.04 | 98.01 | 98.15 | 97.46 |

a: Calculated Kovats Index. b: Least significant difference (LSD α= 0.05).

## Discussion

The biomass increase of *D. polychaetum* following the use of broiler litter may be linked to the instant availability of both macro- and micro-nutrients for a longer period during plant growth (Pandey et al., 2016; Chand et al., 2012), improvement of soil physical, chemical and biological properties (Schlegel et al. 2015 a,b) and enhancement of water holding and cation exchange capacities (Pandey et al., 2016). Also regarding soil pH (Table 1), organic matter in organic manures with modification of rhizosphere pH can improve the availability of nutrients (especially phosphorus) in the root rhizosphere, and this condition is not created for chemical fertilizers treatment (Salehi et al., 2018). Organic matter of cow manure has lower decomposability compared to that of broiler litter, due to its higher ratio of carbon and nitrogen (Pandey et al., 2016). For this reason, in the first cutting, the biomass of different parts of the plants fertilized with cow manure was lower than those fertilized with broiler litter (Table 1).

Previous studies also proved the positive effects of manure application on the biomass of aromatic plants (Dinesh et al., 2010). For instance in dill (*Anethum graveolens*), in comparison with the commercial fertilizer treatment, its biomass was significantly increased by the application of broiler litter (Rostaei et al., 2018a). Bajeli et al. (2016) also observed that the use of manures such as farmyard manure, vermicompost and poultry manure either alone, or in combination with one another increased the biomass of Japanese mint (*Mentha arvensis* L.). Fallah et al. (2018) illustrated that manure application improves the biomass of dragonhead (*Dracocephalum moldavica*) compare to synthetic fertilizers.

Lower essential oil content that obtained from the use of cow manure in the first cutting could be result of slow decomposition in cow manure (Singh et al., 2014) (Fig. 2A), which had the high carbon: nitrogen ratio compared with the other kinds of manures (Table 1). Cow manure released a little amount of nutrients in the plant rhizosphere (Alizadeh et al., 2012). In the second cutting, constant mineralization of the nutrients from the cow manure, increased the essential oil compared with the first cutting (Fig. 2B). In addition, a greater duration for cow manure in the soil (from transplanting of the seedlings to second cutting) can affect the chemical and physical properties of soil and improve nutrients availability and provide other beneficial conditions for plant growth, thus the plant can produce more secondary metabolite. In contrast, higher supply of nutrients such as nitrogen and phosphorus of chemical fertilizer in early growth season (Alizadeh et al., 2012) can increase essential oil content in the first cutting compared with the second cutting (Fig. 2A and 2B).

Increase in essential oil production by broiler litter and sheep manure application could be attributed to faster decomposition (Alizadeh et al., 2012) and the higher supply of nutrients such as nitrogen, phosphorus and micronutrients (Table 1). Nitrogen nutrition plays a key role in the development and division of cells containing essential oil, essential oil channels, secretory ducts and glandular trichomes (Yousefzadeh et al., 2013). Also, phosphorus and micronutrients play an important role in enhancement of secondary metabolites content (Alizadeh et al., 2010). Bajeli et al. (2016) indicated that the addition of manure enhanced essential oil content of Japanese mint. With regard to dill, compared to control and chemical fertilizer treatments, the addition of vermicompost at 10 t $ha^{-1}$ improved the essential oil content by 15% and 9% (Anwar et al., 2005).

The increased of the essential oil yield of *D. polychaetum* under organic manure application is due to the increase of higher and longer availability of essential nutrients such as nitrogen, phosphorus and micronutrient (Table 1) that enhances the uptake of nutrients by plants, leading to higher biomass accumulation (Fig. 2A and 2B) and secondary metabolites production (Fig. 3A and 3B) (Anwar et al., 2005; Emami Bistgani et al., 2018; Rostaei et al., 2018b). Several studies indicated that organic manure enhanced the essential oil yield of medicinal plants. Singh et al. (2014) illustrated that addition of farmyard manure enhances the essential oil yield of basil (*ocimum basilum* L.) as compared to commercial fertilizers. Rostaei et al. 2018a reported that the application of organic manure led to an increase in essential oil yield of dill. The essential oil yield of dragonhead (*Dracocephalum moldavica*) increased due to the application of organic manure over synthetic fertilizer (Fallah et al., 2018).

*E*-β-ocimene, nerol and α-pinene (Fattahi et al., 2016), geranial (Saeidnia et al., 2007), α-pinene and caryophyllene oxide (Javidnia et al., 2005) have been reported as major chemical compositions of *D. polychaetum* in previous studies of this plant at flowering stage. The highest neral (28.24%±5.10) content in the first cutting was recorded in treatment of sheep manure. In the first cutting, the maximum geranial content (26.85%±1.55) was obtained in the plants treated with chemical fertilizer that was not significantly different from manure treatments. The highest percentage of α-pinene in the first cutting was achieved in control and broiler litter (15.52±2.52% and 15.09%±6.96, respectively). The maximum content of geranyl acetate (20.68%±2.68) in the first cutting was achieved in control treatment. In all fertilized treatments (broiler litter, cow manure, sheep manure and chemical fertilizer) the amount of neral and geranial was increased in comparison with their amounts in control.

In the present study, application of organic manure and chemical fertilizer affected the chemical compounds of *D. polychaetum* (Table 4 and 5). Our results showed positive effects of organic manure on commercial major compositions. It seems that production of secondary metabolites depends on fertilization management (macro- and micro-nutrients) and organic manure mainly supplies available macro- and micro-nutrients for crop growth (Fallah et al., 2013). Nitrogen plays a key role in the biosynthesis of many organic compounds, including amino acids, proteins, enzymes and nucleic acids. Amino acids and enzymes play a pivotal role in the biosynthesis of numerous compounds that are essential oil constituent (Koeduka et al., 2006). According to Rouached et al. (2010) phosphorus plays a significant role in different metabolic processes, being a constitute of nucleic acid, phospholipids and coenzymes activating the amino acid production used in protein synthesis, ATP, DNA and RNA. Additionally, iron and zinc act either as metal components of different enzymes or as regulatory, functional, structural cofactors. Thus these two nutrients are associated with protein synthesis, saccharide metabolism and photosynthesis (Marschner, 1995). Copper is involved in several physiological processes and acts as a cofactor for different enzymes and helps in photosynthetic electron transport (Yruela, 2005). Manganese has important functions in plant metabolism especially in chlorophyll synthesis, photosynthesis, amino acids and protein synthesis, nitrate reduction, activation of various enzymes and phytohormone regulation (Ghannadnia et al., 2014). Rostaei et al. (2018a) indicated that applying of broiler litter will lead to a significant increase in p-cymene content (33.69%) in dill. Also Pandey et al. (2016) demonstrated that in comparison with chemical fertilizers, application of poultry manure enhances methyl chavicol content (72.60%) in basil. Pandey et al. (2015) reported compare to chemical fertilizers, E-ocimenone content of marigold (*Tagetes minuta* L.) increases with the use of farmyard manure.

## Conclusion

The present study indicated that quantity and quality of multi-cut plant of *D. polychaetum* was significantly enhanced by various fertilization sources. The application of different fertilization sources enhanced biomass, essential oil content and essential oil yield in both first and second cuttings. The positive effect of organic manure was higher than the chemical fertilizer. The usage of broiler litter and sheep manure led to enhancement of quantity and quality in the both cuttings, while the advantage of chemical fertilizer and cow manure was considerable in the first and second cuttings, respectively. Neral, geranial, geranyl acetate and α-pinene were detected as the major chemical compounds of *D. polychaetum* in both cuttings. The Control had the highest geranyl acetate content in both cuttings. In the first cutting, plants amended with sheep manure had the maximum neral content (28.24%). The greatest geranial content in the first cutting was obtained in plants treated with chemical fertilizer and broiler litter. At the first cutting the highest α-pinene content was obtained in control and broiler litter treatments. Plants fertilized with cow manure had the highest neral and geranial content in the second cutting. Overall, the advantages of application of organic manure can be summarized as the followings: improvement of the quality and commercial chemical compositions of *D. polychaetum*, removal the need for additional chemical fertilizer and added benefit to a sustainable agriculture.

## Acknowledgments

This research was funded by Payam Noor University and there was no external funding for this research.

## Conflict of interest

The authors declare that they have no conflict of interest.

## Reference

1. Adams RP, 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th ed Allured Publishing Corporation, Carol Stream, Illinois.
2. Alizadeh A, Khoshkhui M, Javidnia K, Firuzi O, Tafazoli E, Khalighi A. 2010. Effects of fertilizer on yield, essential oil composition, total phenolic content and antioxidant activity in *Satureja hortensis* L. (*Lamiaceae*) cultivated in Iran. Journal of Medicinal Plants Research 4: 33-40.
3. Alizadeh P, Fallah S, Raiesi F. 2012. Potential N mineralization and availability to irrigated maize in a calcareous soil amended with organic manures and urea under field conditions. International Journal of Plant Production 6: 493-512.
4. Anwar M, Patra DD, Chand S, Alpesh K, Naqvi AA, Khanuja SPS. 2005. Effect of organic manures and inorganic fertilizer on growth, herb and oil yield, nutrient accumulation, and oil quality of French basil. Communications in Soil Science and Plant Analysis 36: 1737-1746.
5. Bajeli J, Tripathi S, Kumar A, Tripathi A, Upadhyay RK. 2016. Organic manures convincing source for quality production of Japanese mint (*Mentha arvensis* L.). International Journal of Plant Production 83: 603-606.
6. Boroomand N, Moghbeli M, Hosseini Grouh MS, Farajpour M. 2017. Phytochemical components, total phenol and mineral contents and antioxidant activity of six major medicinal plants from Rayen, Iran. Natural Product Research 32, 564-567.
7. British Pharmacopoeia. 1988. HMSO, London, pp. 2: A137-A138.
8. Chand S, Pandey A, Patra DD. 2012. Influence of nickel and lead applied in combination with vermicompost on growth and accumulation of heavy metals by *Mentha arvensis* Linn. cv. 'Kosi'. Indian Journal of Natural Products and Resources 3: 256–261.
9. Chaouki W, Leger DY, Liagre B, Beneytout JL, Hmamouchi M. 2009. Citral inhibits cell proliferation and induces apoptosis and cell cycle arrest in MCF-7 cells. Fundamental and Clinical Pharmacology 23: 549-556.
10. Chaudhry AN, Jilani G, Khan MA, Iqbal T (2009) Improved processing of poultry litter to reduce nitrate leaching and enhance its fertilizer quality. Asian Journal of Chemistry 21: 497-503.
11. De Martino L, D'Arena G, Minervini MM, Deaglio S, Fusco BM, Cascavilla N, De Feo V. 2009. *Verbena officinalis* essential oil and its component citral as apoptotic-inducing agent in chronic lymphocytic leukemia. International Journal Immunopathol Pharmacol. 1097–1104.
12. Dinesh R, Srinivasan V, Hamza S, Manjusha A. 2010. Short term incorporation of organic manures and biofertilizers influences biochemical and microbial characteristics of soils under an annual crop [Turmeric (*Curcuma longa* L.)]. Current opinion in biotechnoly 4697-4702.
13. Dudareva N, Pichersky E. 2008. Metabolic engineering of plant volatiles. Current Opinion in Biotechnology. 19: 181-189.
14. Emami Bistgani Z, Siadat SA, Bakhshandeh A, Pirbalouti AG, Hashemi M. Maggi F, Morshedloo MR. 2018. Application of combined fertilizers improves biomass, essential oil yield, aroma profile, and antioxidant properties of *Thymus daenensis* Celak. Industrial Crops and Products 121: 434-440.
15. Fallah S, Ghalavand A, Raiesi F. 2013. Soil chemical properties and growth and nutrient uptake of maize grown with different combination of broiler litter and chemical fertilizer in a calcareous soil. Communications in Soil Science and Plant Analysis 44: 3120-3136.
16. Fallah S, Rostaei M, Lorigooini Z, Surki AA. 2018. Chemical compositions of essential oil and antioxidant activity of dragonhead (*Dracocephalum moldavica*) in sole crop and dragonhead-soybean (*Glycine max*) intercropping system under organic manure and chemical fertilizers. Industrial Crops and Products 115: 158-165.

17. Fattahi M, Bonfill M, Fattahi B, Torras-Claveria L, Sefidkon F, Cusido RM, Palazon J. 2016. Secondary metabolites profiling of *Dracocephalum kotschyi* Boiss at three phenological stages using uni-and multivariate methods. Journal of Applied Research on Medicinal and Aromatic Plants 3: 177-185.

18. Ghannadnia M, Haddad R, Zarinkamar F, Sharifi M. 2014. Manganese treatment effects on terpene compounds of *Cuminum cyminum* flowers. Industrial Crops and Products 53: 65-70.

19. Govahi M, Ghalavand A, Najafi F, Sorooshzade A. 2015. Comparing different soil fertility systems in Sage (*Salvia officinalis*) under water deficiency. Industrial Crops and Products 74: 20–27.

20. Guo JH, Liu XJ, Zhang Y, Shen JL, Han WX, Zhang WF, Christie P, Goulding KWT, Vitousek PM, Zhang FS. 2010. Significant acidification in major Chinese croplands. Science 327: 1008-1010.

21. Javidnia K, Miri R, Fahham N, Mehregan I. 2005. Composition of the essential oil of *Dracocephalum kotschyi* Boiss. Journal of Essential Oil Research 17: 481-482.

22. Koeduka T, Fridman E, Gang DR,Vassao DG, Jackson BL, Kosh ChM, Orlova L, Spassova SM, Lewis NG, Noel JP, Bagia TJ, Dudareva N, Pichersky E. 2006. Eugenol and isoeugenol, characteristic aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester. Proceedings of the National Academy of Sciences103: 10128-10133.

23. Marcus J, Klossek ML, Touraud D, Kunz W. 2013. Nano-droplet formation in fragrance tinctures. Flavour and Fragrance Journal 28: 294-299.

24. Marschner H. 1995. Mineral Nutrition of Higher Plants. 2ed New York: Academic Press, p. 889.

25. Mozaffarian V. 1998. A dictionary of Iranian plant names. Tehran: Farhang Moaser Publishers.

26. Mesa-Arango AC, Montiel-Ramos J, Zapata B, Durán C, Betancur-Galvis L, Stashenko E. 2009. Citral and carvone chemotypes from the essential oils of *Colombian Lippia alba* (Mill.) N.E. Brown: Composition, cytotoxicity and antifungal activity. Memorias do Instituto Oswaldo Cruz 104: 878-884.

27. Monsef-Esfahani HR, Karamkhani F, Nickavar B, Abdi K, Faramarzi MA. 2007. Thevolatile constituents of *Dracocephalum kotschyii* oils. Chemistry of Natural Compounds 43: 40-43.

28. Pandey V, Patel A, Patra DD. 2015. Amelioration of mineral nutrition, productivity, antioxidant activity and aroma profile in marigold (*Tagetes minuta* L.) with organic and chemical fertilization. Industrial Crops and Products 76: 378-385.

29. Pandey V, Patel A, Patra DD. 2016. Integrated nutrient regimes ameliorate crop productivity, nutritive value, antioxidant activity and volatiles in basil (*Ocimum basilicum* L.). Industrial Crops and Products 87: 124-131.

30. Pouraboli I, Nazari S, Sabet N, Sharififar F. 2015. Antidiabetic, antioxidant, and antilipid peroxidative activities of *Dracocephalum polychaetum* shoot extract in streptozotocin-induced diabetic rats: In vivo and in vitro studies. Pharmaceutical biology 54: 272-278.

31. Rechinger H. 1986. Labiatae in flora iranica. (Vol 150).Graz, Austria: Akademische Druck verlagsantalt, pp. 218-230.

32. Roelcke M, Han Y, Schleef KH, Zhu JG, Liu G, Cai ZC, Richter J. 2004. Recent trends and recommendations for nitrogen fertilization in intensive agriculture in eastern China. Pedosphere 14: 449-460.

33. Rostaei M, Fallah S, Lorigooini Z, Surki AA. 2018a. The effect of organic manure and chemical fertilizer on essential oil, chemical compositions and antioxidant activity of dill (*Anethum graveolens*) in sole and intercropped with soybean (*Glycine max*). Journal of Cleaner Production 199: 18-26.

34. Rostaei M, Fallah S, Lorigooini Z, Surki AA. 2018b. Crop productivity and chemical compositions of black cumin essential oil in sole crop and intercropped with soybean under contrasting fertilization. Industrial Crops and Products 125: 622-629

35. Rouached H, Arpat AB, Poirier Y. 2010. Regulation of phosphate starvation responses in plants: signaling players and cross-talks. Molecular Plant 3: 288-299.

36. Saeidnia S, Gohari AR, Uchiyama N, Ito M, Honda G, Kiuchi F. 2004. Two new monoterpene glycosides and *trypanocidal terpenoids* from *Dracocephalum kotschyi*. Chem Pharm bull 52: 1249-1250.

37. Saeidnia S, Gohari AR, Hadjiakhoondi A, Shafiee A. 2007. Bioactive compounds of the volatile oil of *Dracocephalum kotschyi*. Zeitschrift fur Naturforschung 62: 793-796.

38. Salehi A, Mehdi B, Fallah S, Kaul HP, Neugschwandtner RW. 2018. Productivity and

nutrient use efficiency with integrated fertilization of buckwheat-fenugreek intercrops. Nutrient Cycling in Agroecosystems 110: 407-425.

39. Schlegel AJ, Assefa Y, Bond HD, Wetter SM, Stone LR. 2015b. Corn response to long-term applications of cattle manure, swine effluent, and inorganic nitrogen fertilizer. Agronomy Journal 107: 1701-1710.

40. Schlegel AJ, Assefa Y, Bond HD, Wetter SM, Stone LR. 2015a. Soil physicochemical properties after 10 years of animal waste application. Soil Science Society of America Journal 79: 711-719.

41. Siddiqui Y, Islam TM, Naidu Y, Meon S. 2011. The conjunctive use of compost tea and inorganic fertilizer on the growth, yield and terpenoid content of *Centella asiatica* (L.) urban. Scientia Horticulturae 130, 289-295.

42. Singh K, Chand S, Yaseen M. 2014. Integrated nutrient management in Indian basil (*Ocimum basilicum*). Industrial Crops and Products 55: 225-229.

43. Taghizadeh M, Ghanati F, Nasibi F, Manouchehri Kalantari K. 2019. Evaluation of secondary metabolites and antioxidant activity in *Dracocephalum polychaetum* Bornm. cell suspension culture under magnetite nanoparticles and static magnetic field elicitation. Plant Cell Tissue and Organ Culture 132: 489–498.

44. Yaghoobi MM, Khaleghi M, Rezanejad H, Parsia P. 2018. Antibiofilm Activity of *Dracocephalum polychaetum* Extracton Methicillin-Resistant Staphylococcus aureus. Avicenna Journal of Clinical Microbiology and Infection 5: 72-78.

45. Yang TS, Liou ML, Hu TF, Peng CW, Liu TT. 2013. Antimicrobial activity of the essential oil of *Litsea cubeba* oncariogenic bacteria. Journal of Essential Oil Research 25: 120- 128.

46. Yousefzadeh S, Modarres-Sanavy SAM, Sefidkon F, Asgarzadeh A, Ghalavand A, Sadat-Asilan K. 2013. Effects of Azocompost and urea on the herbage yield and contents and compositions of essential oils from two genotypes of dragonhead (*Dracocephalum moldavica* L.) in two regions of Iran. Food Chemistry 138: 1407–1413.

47. Yruela I. 2005. Copper in plants. Braz. Journal of Plant Physiology 17: 145-156.