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Productivity and essential oil quality of Dracocephalum kotschyi under organic and chemical fertilization conditions

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A B S T R A C T

Organic manures play an important role in the growth and biomass of aromatic and medicinal plants leading to organic and cleaner production. Also, organic manures can improve chemical compositions and the quality of aromatic and medicinal plants. Hence, a field experiment was conducted to study the usage of organic manures and inorganic fertilizers on biomass, essential oil content, essential oil yield and chemical compositions of *Dracocephalum kotschyi* Bioss (*D. kotschyi*) in the first and second cuttings. The treatments consisted of: broiler litter, cow manure, sheep manure, chemical fertilizer and the control (without fertilizer). The results showed that the highest aboveground biomass and essential oil yield of *D. kotschyi* was obtained in plants treated with broiler litter in both cuttings. All treatments in the second cutting showed higher aboveground biomass and essential oil yield as compared with the first cutting. Plants amended with broiler litter and sheep manure had the highest essential oil content in the first and second cuttings, respectively. Neral, geranial, geranyl acetate and α-pinene were major chemical compounds of *D. kotschyi* in both cuttings. At the first cutting, the maximum content of neral (28.24%) and geranial (26.85%) were recorded in plants treated with sheep manure and chemical fertilizer, respectively. In the first cutting, the greatest α-pinene content (15.52%) was achieved in the control treatment that had insignificant difference with the broiler litter. Unlike the first cutting, plants amended with cow manure had maximum content of neral (23.89%) and geranial (29.27%) content upon the second cutting. The present study indicated that the usage of organic manures by improving of photosynthetic conditions and increasing of biomass lead to improve the quality and aroma profile of *D. kotschyi*.

1. Introduction

One main challenge in agriculture is to increase crop production in a sustainable way for the present and the future. Agricultural production and sustainable intensification goals over both short and long-term require a proper plant nutrient management (Pandey et al., 2015). Although chemical fertilizers are used extensively to increase agricultural production (Siddiqui et al., 2011), unfortunately, long-term excessive application of these fertilizers has contributed to soil organic matter decrease, soil quality reduction, and consequently agricultural production decline as well as environmental pollution (Guo et al., 2010; Dinesh et al., 2010; Roelcke et al., 2004). All of these issues have become chief concerns (Chaudhry et al., 2009).

Substituting of chemical fertilizers with organic manures is one of the most important goals of sustainable agriculture (Fallah et al., 2018). Chemical fertilizers are known to be taken up by plants immediately after their application (Prasada et al., 2015; Ahmad et al., 2008), while nutrients derived from organic manures must be mineralized and transformed into forms that can be taken up by plants (Li et al., 2017). Nitrogen from organic manures is slowly released into the soil. Phosphorus recovery is slightly better from organic manures than from chemical fertilizers, as CO_2 released by decomposition improves availability from soil (Gopalakrishnan, 2007). Organic manures can provide micronutrients such as zinc, copper, iron, and manganese at optimum levels and better supplies. Moreover, availability of nutrients (macro-micronutrients) from

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organic manure leads to higher biomass and secondary metabolites production as compared with chemical fertilizer.

Numerous studies on aromatic and medicinal plants have shown that the usage of organic manures enhances biomass and improves the essential oil content and chemical compositions of these plants (Rostaei et al., 2018a,b; Fallah et al., 2018). Bajeli et al. (2016) demonstrated that adding organic manure such as farmyard manure, vermicompost, and poultry manure to the soil, either alone or combined, led to an increase in biomass and menthol content of Japanese mint (*Mentha arvensis* L.). Fallah et al. (2018) reported the addition of organic manure enhanced biomass and piperitone content of dragonhead (*Dracocephalum moldavica*). Rostaei et al. (2018a) similarly observed that the application of organic manure enhanced biomass and α-phellandrene and p-cymene content of dill (*Anethum graveolens*). The biomass and essential oil content of leaves of *Dysphania ambrosioides* L. were enhanced by the application of chicken manure (Bibiano et al., 2019).

Dracocephalum kotschyi Bioss. (Lamiaceae), known as Badernjboei-Dennaei or Zarrin-giah in Persian, is a medicinal plant endemic to Iran (Fattahi et al., 2016). This plant is mainly distributed in central and northern Iran and grows at an altitude of 2000–3000 m in high mountainous regions (Fattahi et al., 2013).

Various pharmacological effects such as antihyperlipidemic (Ebrahim Sajjadi et al., 1998), immunomodulatory (Amirghofran et al., 2000), antinociceptive (Golshani et al., 2004), and cytotoxic effects (Jahaniani et al., 2005) have been reported for *D. kotschyi*. This plant is also considered as a source of essential oil (Fattahi et al., 2013), the major chemical compositions of which include geranial, α-pinene, geranyl acetate (Ashrafi et al., 2017), limonene, carvacrol, γ-terpinene (Golparvar et al., 2016), 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 used mainly by the food and pharmaceutical industries.

Semi-arid agroecosystems are often under cereal cultivation. Yearly tillage operations in these systems cause instability and, consequently, unsustainability in systems. Therefore they are prone to pest outbreaks of which chemical control is an integral part. The addition of annual medicinal herbs to the cropping pattern of these areas can increase stability, but tillage, erosion and soil degradation are still observed. Therefore, the development of perennial medicinal plants can increase the stability of the system and help clean production by preventing chemical control of pests. Additionally, cleaner production of medicinal plants is more crucial than other crops due to their direct role in human health. Consequently, the substitution of chemical fertilizers with organic manure in these plants is a top priority. Although, several reports underline the benefits of applying organic manures compared with chemical fertilizers in most annual medicinal plants, there is no information available about the effect of organic manures on multi-cut medicinal plants such as *D. kotschyi*. The present study is the first report of *Dracocephalum kotschyi* response to fertilization source. It was designed to investigate the application of organic manure on biomass, essential oil content, essential oil yield, and the chemical composition of essential oil in the *D. kotschyi* plant over the first and second cuttings.

2. Materials and methods

2.1. Cultivation, experimental design and details of treatments

The *D. kotschyi* seeds were obtained from Pakan Bazr Company, Isfahan, Iran. Initially, the seeds of *D. kotschyi* were sown in plastic germination trays containing 70% coco peat plus 30% peat moss (v/v basis) and then were germinated. Two months after sowing, seedlings (with uniform size) were selected and then transplanted into experimental plots on November 19, 2017.

The climate of experimental site was classified as a cold semi-arid climate, with average annual precipitation of 496 mm and an average monthly temperature of 10.61 °C. The field experiment was conducted based on a randomized block design with five treatments and three replications at the Research Farm of Fereydoun Shahr (50°18E, 32°93 N; 2393 m above sea level). The treatments comprised of: chemical fertilizers (100-50-50 kg/ha N, P and K, respectively), broiler litter: 1.7 t/ha, cow manure: 2.5 t/ha, sheep manure: 1.7 t/ha and the control (without fertilizer and manure). Micronutrients were not used in any treatment. Seedlings were grown according to organic farming practices and chemical inputs such as pesticides, herbicides and fungicides were not used.

Before seedlings' transplantation, the soil samples were randomly collected from a depth of 0–30 cm at ten points using a soil auger. All soil samples were air-dried in the laboratory during four days and then were crushed and sieved through a 2 mm sieve. Details of the properties of soil and organic manures are indicated in Table 1.

Organic manures and chemical fertilizers (urea, triple superphosphate and potassium sulphate) were added to the soil before transplanting of the seedlings. The plots were irrigated immediately after seedlings' transplantation to proper establishment in the field. Each plot consisted of five rows and its size was 2.40 × 3.60 m. Plots were kept weed free by hand-weeding during the growing season.

2.2. Determination of D. kotschyi biomass

Aerial parts of *D. kotschyi* were cut at 50% of flowering on June 30, 2018 (first cutting was 223 days after the seedlings' transplantation) and August 26, 2018 (second cutting was implemented 57 days after the first cutting). At both cuttings, samples were dried under the shade conditions at room temperature (20–25 °C) over a period of seven days. The moisture content of the shade-dried samples was 7%. After the shade-drying process, the leaf, stem biomass, flower and aboveground biomass were calculated as kg/ha.

2.3. Isolation of essential oil

After the shade-drying, about 50 g of pulverized *D. kotschyi* aerial parts were hydrodistilled for 3.5 h using a Clevenger apparatus according to British Pharmacopoeia (1988). The samples of essential oils were collected and dehydrated over anhydrous sodium sulphate. Then the obtained essential oil was stored in sealed vials at 4 °C until the time of gas chromatography analysis. Essential oil yield was determined as kg/ha.

2.4. 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). FID is sensitive to quantification of compounds; essential oil of various treatments was injected to GC/FID. Helium was used as the carrier gas at a flow rate of 0.5 ml/min. The oven temperature was initiated at 60 °C, then increased to 285 °C at a rate of 40 °C/min and was kept for 3 min at 285 °C. The temperature of injector and FID detector was 280 °C.

2.5. Gas chromatography–mass spectrometry analysis

Gaschromatography–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). Essential oil of various treatments was injected to GC/FID for quantification of compositions but one of them was injected to GC/MS for detection of compounds. 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 up to 240 °C at 20 °C/min and was kept at this temperature for 3 min. The injector and transfer line temperature were 260 °C and 280 °C, respectively. The 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.

2.6. Identification of essential oil chemical compositions

Chemical compositions of essential oil samples were determined through 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 (C8–C24) that were injected after the essential oil under the same conditions. The identities of chemical components were confirmed by the comparison of relative retention indices, either with those of authentic components or the data published in the literature (Adams, 2007; Fattahi et al., 2016).

2.7. Statistical analysis

All measured parameters (biomass and content, yield and chemical compositions of essential oil) in both cuttings were statistically analyzed based on a randomized block design with three replications using statistical analysis system software (SAS, Ver. 9.1). Each data point was the mean of three replications. The means were compared through the least significant difference (LSD) test at 5% probability level. Furthermore, to evaluate the similarities' likelihood among chemical compounds of essential oils of the *D. kotschyi* under different fertilizer sources, hierarchical cluster analysis was performed by SPSS (Ver.16) and according to the Ward method. Principal component analysis (PCA) was used to explore the interrelationship among six major chemical compositions (α-pinene, limonene, neral, geranial, methyl geranate, and geranyl acetate) and the five treatments (broiler litter, cow manure, sheep manure, chemical fertilizer, and control) at each cutting of *D. kotschyi*. Using the software R ver. 3.6.1, the principal components were calculated and two-dimensional biplots were generated.

3. Results and discussion

3.1. Aboveground biomass

As presented in Supplementary Table 1, the aboveground biomass of *D. kotschyi* was considerably affected by different fertilization treatments ($p < 0.001$) in the first cutting, second cutting, and sum of the two consecutive cuttings. At the first cutting, the aboveground biomass of *D. kotschyi* was similar in the treatments of cow manure (on average 1291 kg/ha) and sheep manure (on average 1306 kg/ha) (Fig. 1). No significant difference was observed between the plants treated with chemical fertilizer (on average 1340 kg/ha) and those treated with sheep manure (on average 1306 kg/ha). However, a significant difference was seen between (cow manure, sheep manure and chemical fertilizer) and the broiler litter treatment and the control (without fertilizer). In the first cutting, the highest (on average 1558 kg/ha) and lowest (on average 755 kg/ha) aboveground biomass values of *D. kotschyi* were recorded in the plants treated with broiler litter and the control, respectively (Fig. 1).

In the second cutting, the aboveground biomass of *D. kotschyi* in the sheep manure (on average 1920 kg/ha), chemical fertilizer (on average 1918 kg/ha) and cow manure (on average 1889 kg/ha) treatments was similar. The aboveground biomass in these treatments was significantly less than its amount in the broiler litter (Fig. 1). The results indicated that the lowest aboveground biomass was obtained in unfertilized plants upon the second cutting (Fig. 1).

The results confirmed that no significant difference in total aboveground biomass (in the sum of the two consecutive cuttings) was observed among the plants receiving chemical fertilizer (on average 3259 kg/ha), sheep manure (on average 3226 kg/ha), and cow manure (on average 3181 kg/ha) (Fig. 1). The magnitude of the total aboveground biomass enhancement was exhibited in the following order: broiler litter (on average 3901 kg/ha) > chemical fertilizer (on average 3259 kg/ha) > sheep manure (on average 3226 kg/ha) > cow manure (on average 3118 kg/ha) > control (on average 2041 kg/ha) (Fig. 1).

Aboveground biomass of *D. kotschyi* within the second cutting was enhanced compared with the first cutting (Fig. 1). The lowest difference (43%) between the two cuttings was observed in the chemical fertilizer treatment (Fig. 1). Because *D. kotschyi* is harvested several times during the growing season, it seems that the plants are obliged to consume the assimilation for their proper establishment at early growth stages, while they complete their growth and development (life cycle) and increase biomass production after the first cutting. Also, the plants treated with chemical fertilizer had higher biomass in the first cutting than the second cutting (Fig. 1). In the first cutting, the increase in biomass of the plants fertilized with chemical fertilizer could be attributed to the

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)	0.22	4.73	1.95	2.43
pH	8.25	8.19	8.70	8.33
Nitrogen (g/kg)	0.7	29.1	19.9	28.6
Phosphorus (g/kg)	0.018	5.5	2.6	2.7
Potassium (g/kg)	0.49	16.3	16.7	23.6
Zink (mg/kg)	0.58	401.29	188.23	135.97
Iron (mg/kg)	7.99	998.23	652.47	1102.11
Manganese (mg/kg)	8.53	411.35	164.28	247.08
Copper (mg/kg)	0.81	92.11	36.92	18.43
Organic carbon (%)	0.66	27.81	29.61	28.47

EC: Electrical conductivity.

further release (supply) of nutrients such as nitrogen and phosphorus during the early growth stages. After the first cutting, the nitrogen of chemical fertilizers is simply lost and the phosphorus is easily fixed by soil particles. Under such conditions, the root development will be of greater priority for nutrients acquisition. As a result, photosynthetic assimilation would be allocated more to the roots than to the shoots.

The increase in *D. kotschyi* biomass subsequent to the use of broiler litter might be related to enhancing soil aggregation, increasing water holding capacity, and it offers good environmental conditions for root system. Nutrients of organic manure are released more slowly and stored in the soil for longer periods of time, thus ensuring a long residual effect (Abou El-Magd et al., 2005, 2006; Sharma and Mittra, 1991). Concerning the soil pH (Table 1), the organic matter of organic manures with the modification of rhizosphere pH, can improve the availability of nutrients (especially phosphorus) in the root rhizosphere, while this condition is not created in the chemical fertilizer treatment (Salehi et al., 2018). The decomposition of cow manure's organic matter is slower than that of the broiler litter, because the carbon:nitrogen ratio is higher in cow manure than the broiler litter. Therefore, in the first cutting, the biomass of the plants fertilized with cow manure was less than that of plants in the broiler litter. Consequently, sheep manure can be considered as an intermediate for broiler litter and cow manure (Table 1).

Previous studies have indicated that the application of organic manure has positive effects on the biomass of annual aromatic and medicinal plants. Askary et al. (2018) reported that the application of organic manure enhanced the biomass of *Thymus daene*nsis and *Thymus vulgaris.* Bajeli et al. (2016) observed the usage of organic manures such as farmyard manure, vermicompost and poultry manure either alone, or in a mixed format, increased the biomass of Japanese mint (*Mentha arvensis* L.). Fallah et al. (2018) illustrated that the application of organic manure improved biomass of dragonhead (*Dracocephalum moldavica*) more considerably than synthetic fertilizer.

3.2. Essential oil content

The current results revealed a significant difference in the essential oil content of *D. kotschyi* among different fertilization treatments (see Supplementary Table 1) over the first and second cuttings. At the first cutting, the essential oil content of *D. kotschyi* did not show any significant difference between the plants fertilized with cow manure (on average 0.39%) and the unfertilized plants (on average 0.48%). The essential oil content of *D. kotschyi* was similar in the treatments of broiler litter (on average 0.93%), chemical fertilizer (on average 0.87%) and sheep manure (on average 0.74%) (Fig. 2). The essential oil content in these treatments (broiler litter, chemical fertilizer and sheep manure) was significantly enhanced compared with its content in the cow manure treatment and the control. At the first cutting, the highest essential oil content of *D. kotschyi* (on average 0.93%) was achieved in plants grown under broiler litter usage, whereas, the lowest essential oil content of *D. kotschyi* (on average 0.39%) was recorded in the plants treated with cow manure (Fig. 2).

As presented in Fig. 2, in the second cutting, the essential oil contents of *D. kotschyi* were alike in cow manure (on average 1.19%) and sheep manure (on average 1.23%) treatments. NO significant difference was observed in the essential oil content of *D. kotschyi* between plants treated with cow manure (on average 1.19%) those treated with and broiler litter (on average 1.07%). Essential oil content was significantly higher in these treatments than the chemical fertilizer and unfertilized treatments (Fig. 2). The minimum essential oil content in the second cutting was obtained in the plants treated with chemical fertilizer.

Overall, at the second cutting, the essential oil content was increased in broiler litter, cow manure, sheep manure and unfertilized treatments (Fig. 2). The maximum difference between the cuttings was recorded in the cow manure treatment. Plants amended with chemical fertilizer had lower essential oil content in the second cutting compared with the first cutting (Fig. 2).

The lower essential oil percentage obtained from the cow manure usage at the first cutting, was perhaps due to the slow decomposition of cow manure (Singh et al., 2014) (Fig. 2). Cow manure has a higher carbon:nitrogen ratio compared with other animal manures (Table 1) and as a result, it releases less nutrients in the plants rhizosphere (Alizadeh et al., 2012). However, in the second cutting, the constant nutrients mineralization of cow manure, increases the essential oil more notably compared with

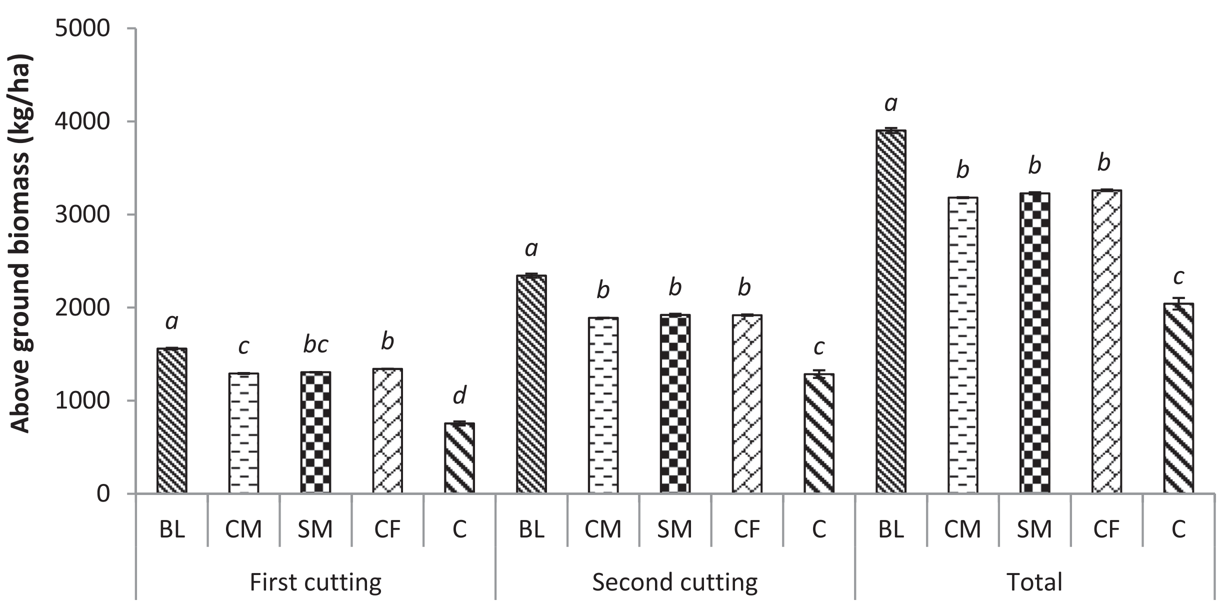

Fig. 1. The effect of different fertilizer sources on above ground biomass of *D. kotschyi*. In each cutting, means with different letters are significantly different, according to LSD test at $P < 0.05$. Error bars represent the mean ± SE. BL, CM, SM, CF and C are broiler litter, cow manure, sheep manure, chemical fertilizer and control, respectively.

the first cutting (Fig. 2). Moreover, the greater presence duration of the cow manure within the soil (from seedlings transplantation to second cutting) affects soil chemical and physical properties and improves nutrients availability and other required favorable conditions for a plant growth and as a consequence, the plant can produce more assimilates. In contrast, a higher supply of nutrients such as nitrogen and phosphorus from chemical fertilizer in the early growth stages (Alizadeh et al., 2012) leads to an increase in essential oil content in the first cutting compared with the second cutting (Fig. 2).

The increased essential oil production created by the application of organic manure (broiler litter and sheep manure) can be attributed to faster decomposition (Alizadeh et al., 2012) and a higher supply of nutrients such as nitrogen, phosphorus and micronutrients (Table 1). Nitrogen generally enhances oil content and yield in aromatic and medicinal plants by increasing the amount of biomass yields per unit area, leaf area development and the photosynthetic rate in these plants (Sangwan et al., 2001; Ram et al., 1995). Phosphorus is an important nutrient in metabolic processes and as a main constituent of energy compounds, phospholipids, nucleic acids and co-enzymes (Hafez and Mahmoud, 2009). Micronutrients such as zinc, iron, copper and manganese facilitate the enhancement of the content of secondary metabolites. Manganese plays an important role in several physiological processes and acts as cofactor for oxidases, dehydrogenases, and sugar transferases (Culotta et al., 2005; Keen et al., 2000; Crowley et al., 2000). Iron is a cofactor for a large number of enzymes that catalyze several biochemical processes within the plants (Marschner, 1995; Brittenham, 1994). It plays a key role in the chlorophyll formation, thylakoid synthesis, and chloroplast development and serves in the transportation of energy in the plants (Nasiri and Najafi, 2015). Zinc and copper are essential for plants and play important roles in metabolism, photosynthesis and respiratory electron transport chain, chlorophyll and protein synthesis, auxin synthesis and cell division, as a metal component of different enzymes and as regulatory cofactor and saccharide metabolism (El-Sawi and Mohamed, 2002).

Bajeli et al. (2016) similarly indicated that the addition of organic manures enhanced the essential oil content of Japanese mint. Another research on French basil plant, reported that the addition of vermicompost at 10 t/ha improved the essential oil content by 15% and 9% in the control and chemical fertilizer treatments, respectively (Anwar et al., 2005).

3.3. Essential oil yield

The essential oil yield of *D. kotschyi* was significantly affected by different fertilization treatments ($p < 0.001$) in the first, second and the sum of two consecutive cuttings (Supplementary Table 2). In the first cutting, no significant difference was recognized in the essential oil yield of *D. kotschyi* between the plants receiving chemical fertilizer (on average 11.66 kg/ha) and those receiving the sheep manure (on average 9.74 kg/ha). The essential oil yield of *D. kotschyi* was similar in the treatments of broiler litter (on average 14.56 kg/ha) and chemical fertilizer (on average 11.66 kg/ha) (Fig. 3). The essential oil yields in broiler litter, sheep manure and chemical fertilizer treatments were significantly enhanced in comparison with the cow manure and unfertilized treatments. In the first cutting, the greatest essential oil yield of *D. kotschyi* was obtained in the broiler litter treatment (Fig. 3).

In the second cutting, the essential oil yield of *D. kotschyi* was analogous in the broiler litter, sheep manure and cow manure treatments (on average 25.08, 23.71 and 22.61 kg/ha, respectively) (Fig. 3). Essential oil yield in these treatments was significantly increased compared with the chemical fertilizer treatment and the control (without fertilizer). The maximum *D. kotschyi* essential oil yield was recorded in plants grown under the application of broiler litter (Fig. 3).

According to the results, total essential oil yield responses in all fertilization options were significantly higher than the responses obtained in the unfertilized treatment (Fig. 3). In sum, the extent of total essential oil yield increased by applying different fertilization sources could be exhibited in the following order:

Broiler litter (on average 39.65 kg/ha) > sheep manure (on average 33.46 kg/ha) > cow manure (on average 27.67 kg/ha) > chemical fertilizer (on average 24.16 kg/ha) > the control treatment (on average 15.35 kg/ha) (Fig. 3).

As presented in Figs. 1 and 2 the application of broiler litter produced the highest aboveground biomass in both first and second cuttings. It also created the maximum essential oil content in

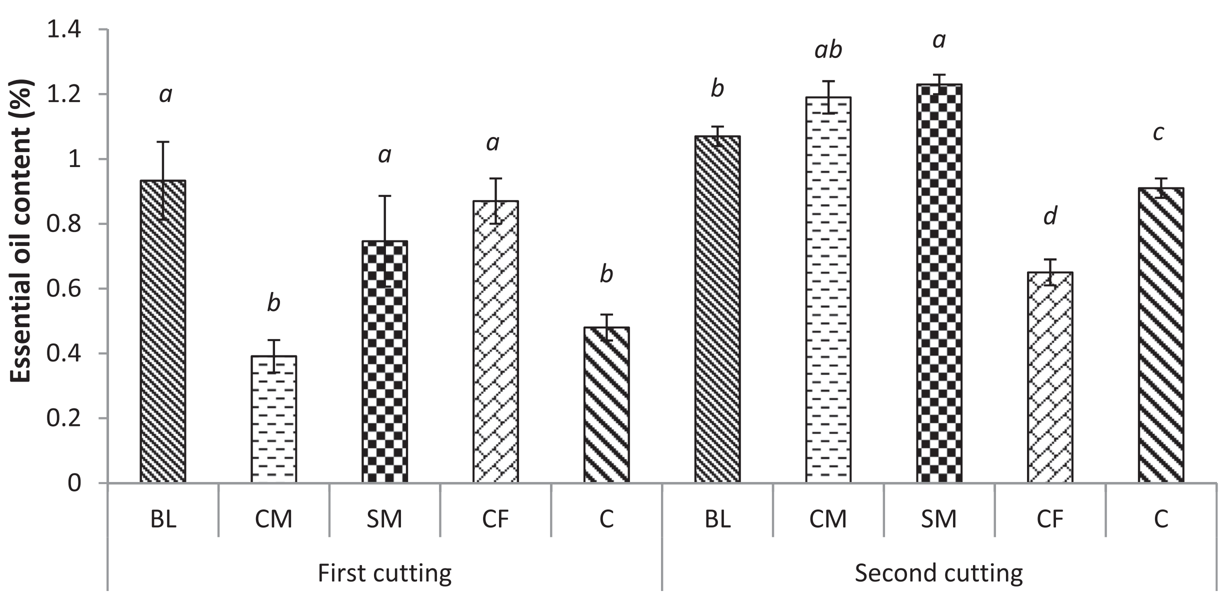

Fig. 2. The effect of different fertilizer sources on essential oil content of *D. kotschyi*. In each cutting, means with different letters are significantly different, according to LSD test at $P < 0.05$. Error bars represent the mean ± SE. BL, CM, SM, CF and C are broiler litter, cow manure, sheep manure, chemical fertilizer and control, respectively.

the first cutting, and ultimately, enhanced essential oil yield in both cuttings. Upon the first cutting, the plants treated with chemical fertilizer had relatively higher aboveground biomass and essential oil content than the second cutting. Thus, these plants had a higher essential oil yield in the first cutting than in the second cutting. The cow and sheep manure treatments had higher aboveground biomass and essential oil content in the second cutting in compared with the first cutting. At the first and second cuttings, the lowest essential oil yield of *D. kotschyi* was obtained in untreated plants.

The increase in the essential oil yield of *D. kotschyi* under the application of organic manures could be related to the higher and longer availability of essential nutrients such as nitrogen, phosphorus and micronutrient (Table 1). This would enhance the uptake of nutrients by the plants, leading to higher biomass accumulation (Fig. 1) and greater production of secondary metabolites (Fig. 2) (Emami Bistgani et al., 2018; Anwar et al., 2005; Patra et al., 2000). Similarly, several studies have indicated that organic manures enhanced the essential oil yield of medicinal plants. Singh et al. (2014) illustrated that the addition of farmyard manure improved essential oil yield of basil (*ocimum basilum* L.) as compared with commercial fertilizer. Rostaei et al. (2018a) reported that the application of organic manure led to the enhancement of essential oil yield in dill plants. Another research revealed that the essential oil yield of dragonhead (*Dracocephalum moldavica*) was increased by applying organic manure instead of synthetic fertilizer (Fallah et al., 2018).

3.4. Chemical compositions of essential oil

The chemical compositions of the essential oil achieved from the aerial parts of *D. kotschyi* are listed in Tables 2 and 3. GC–MS analysis indicated that in the first cutting, 23 chemical compounds were identified and represented between 97.57% and 98.51% of the total essential oils (Table 2). Neral (19.86%–28.24%), geranial (15.89%–26.85%), geranyl acetate (7.16%–20.68%) and α-pinene (11.28%–15.52%) were determined to be the major chemical constituents of *D. kotschyi* in the first cutting.

A number of 22–23 chemical compositions were detected over the second cutting using GC–MS analysis and represented between 97.46% and 98.15% of various treatments (Table 3). At the second cutting, the main chemical components of essential oil were geranial (15.74%–29.27%), neral (19.71%–23.89%), geranyl acetate (11.61%–20.53%) and α-pinene (9.93%–15.37%). Camphene was found in unfertilized plants in both cuttings; however, it was not detected in the fertilizer treatments (Tables 2 and 3). Trans limonen oxide, p-mentha-1,5-dien-8-ol, trans carveol and geraniol were noted to be significantly higher in the unfertilized treatment than in the other treatments in both the first and second cuttings.

Geranial, α-pinene, geranyl acetate (Ashrafi et al., 2017), limonene, carvacrol, γ-terpinene (Golparvar et al., 2016), E-β-ocimene, nerol, 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. kotschyi* in previous studies on the plant at flowering stage. The highest neral (28.24%) content in the first cutting was recorded in the sheep manure treatment. At the first cutting, the maximum geranial content (26.85%) was obtained in the plants treated with chemical fertilizer; no significant difference was found among the chemical fertilizer treatment and the manure treatments. The highest percentage of α-pinene in the first cutting was acquired in the control and the broiler litter treatment (15.52% and 15.09%, respectively). In the first cutting, the maximum content of geranyl acetate (20.68%) was measured in the control treatment. In all fertilization treatments (broiler litter, cow manure, sheep manure and chemical fertilizer) neral and geranial contents were increased compared with the control treatment.

At the first cutting, a cluster analysis of the chemical compositions of *D. kotschyi* essential oils, was implemented and the plants were classified into two groups (Fig. 4). On the basis of the cluster analysis, the plants fertilized with the broiler litter, cow manure, sheep manure and chemical fertilizer that had high amounts of neral (25.83%–28.24%) and geranial (23.93%–26.85%), moderate amount of α-pinene (11.28%–15.09%) and low amount of geranyl acetate (7.16%–9.65%) were classified as the first group. The second group was comprised of unfertilized plants which were extremely rich in geranyl acetate (20.68%) and α-pinene (15.52%).

The results of the principal component analysis based on six chemical compositions (α-pinene, limonene, neral, geranial, methyl

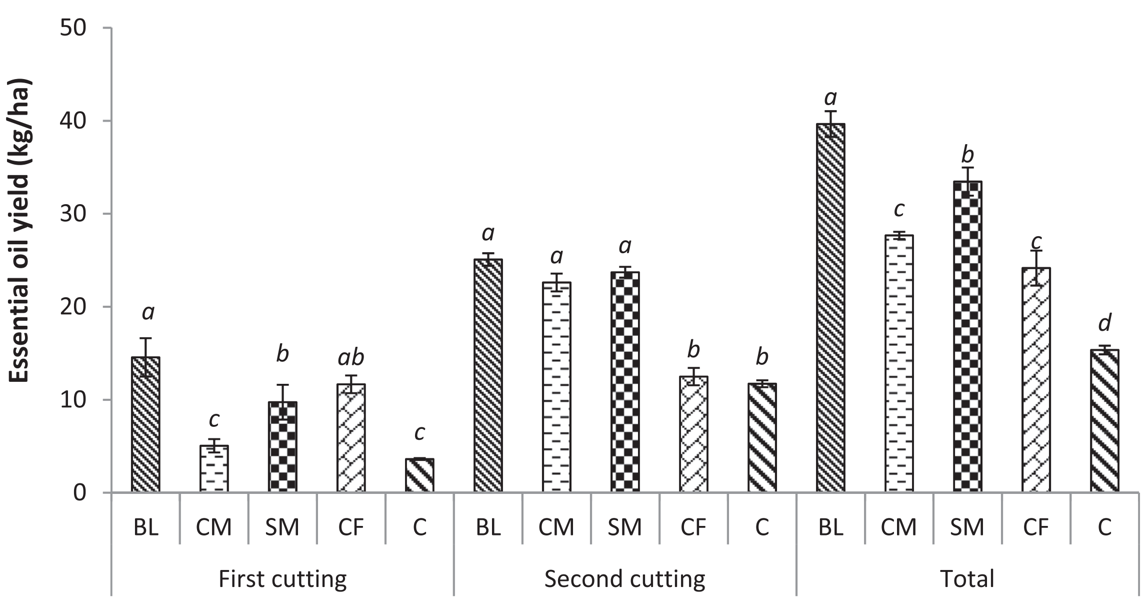

Fig. 3. The effect of different fertilizer sources on essential oil yield of *D. kotschyi*. In each cutting, means with different letters are significantly different, according to LSD test at $P < 0.05$. Error bars represent the mean ± SE. BL, CM, SM, CF and C are broiler litter, cow manure, sheep manure, chemical fertilizer and control, respectively.

Table 2
The chemical compositions *of D. kotschyi* treated with different fertilizer sources in the first cutting.

Compound (%)	KI[a]	KI[b]	Treatments				
			Broiler litter	Cow manure	Sheep manure	Chemical fertilizer	Control
α-Pinene	**943**	**939**	**15.09 ± 4.02**	**14.74 ± 4.79**	**12.36 ± 2.33**	**11.28 ± 1.81**	**15.52 ± 1.46**
Camphene	960	954	–	–	–	–	0.27 ± 0.04
Sabinene	978	975	0.46 ± 0.11	0.46 ± 0.17	0.36 ± 0.09	0.39 ± 0.07	0.19 ± 0.05
β-Pinene	986	979	1.9 ± 0.27	1.99 ± 0.32	2.09 ± 0.23	1.58 ± 0.18	0.95 ± 0.09
Myrcene	998	991	0.26 ± 0.05	0.25 ± 0.07	0.22 ± 0.04	0.21 ± 0.02	0.22 ± 0.01
α-Terpinene	1013	1017	0.44 ± 0.10	0.55 ± 0.20	0.49 ± 0.10	0.45 ± 0.05	0.67 ± 0.10
p-Cymene	1028	1025	0.14 ± 0.01	0.11 ± 0.06	0.13 ± 0.02	0.05 ± 0.06	0.21 ± 0.01
Limonene	1035	1029	6.16 ± 0.92	7.51 ± 2.79	7.76 ± 2	5.5 ± 0.81	4.27 ± 0.62
1,8-Cineole	1038	1031	0.91 ± 0.18	0.97 ± 0.37	0.5 ± 0.25	0.76 ± 0.08	–
(Z) β-Ocimene	1040	1037	0.34 ± 0.04	0.12 ± 0.12	0.52 ± 0.20	0.33 ± 0.04	0.88 ± 0.05
Unknown	–	–	0.19 ± 0.07	0.05 ± 0.05	0.06 ± 0.06	0.32 ± 0.08	0.15 ± 0.03
Linalool	1100	1097	0.22 ± 0.01	0.19 ± 0.04	0.21 ± 0.03	0.19 ± 0.02	0.17 ± 0.04
α-Campholenal	1132	1126	1.2 ± 0.08	1.03 ± 0.18	1.19 ± 0.09	1.22 ± 0.11	1.57 ± 0.07
Trans limonen oxide	1146	1142	1.38 ± 0.11	1.04 ± 0.15	0.98 ± 0.02	1.46 ± 0.20	3.37 ± 0.16
Unknown	–	–	0.81 ± 0.09	0.68 ± 0.09	0.7 ± 0.09	0.79 ± 0.08	0.57 ± 0.04
p-Mentha-1,5-dien-8-ol	1175	1170	1.24 ± 0.22	1.03 ± 0.30	0.93 ± 0.10	1.27 ± 0.17	2.76 ± 0.08
Unknown	–	–	1.17 ± 0.12	0.99 ± 0.08	1.06 ± 0.06	1.11 ± 0.12	0.62 ± 0.05
α-Terpineol	1191	1189	0.37 ± 0.05	0.41 ± 0.13	0.52 ± 0.06	0.53 ± 0.06	0.52 ± 0.01
Myrtenol	1198	1196	0.24 ± 0.14	0.35 ± 0.11	0.62 ± 0.34	0.25 ± 0.02	0.34 ± 0.07
Trans carveol	1210	1217	0.56 ± 0.15	0.55 ± 0.11	0.43 ± 0.07	0.56 ± 0.10	1.52 ± 0.07
Neral	**1242**	**1238**	**26.4 ± 2.16**	**25.83 ± 4.62**	**28.24 ± 2.95**	**27.73 ± 2.88**	**19.86 ± 1.47**
Geraniol	1250	1253	0.13 ± 0.02	0.19 ± 0.06	0.51 ± 0.32	0.12 ± 0.06	0.74 ± 0.02
Geranial	**1265**	**1267**	**26.36 ± 1.69**	**23.93 ± 2.28**	**24.75 ± 1.10**	**26.85 ± 0.9**	**15.89 ± 1.67**
Methyl geranate	1328	1325	5.9 ± 0.81	6.61 ± 0.9	6.32 ± 0.84	6.17 ± 0.54	6.76 ± 0.09
α-Cubebene	1360	1351	0.45 ± 0.06	0.62 ± 0.16	0.53 ± 0.09	0.68 ± 0.11	0.89 ± 0.05
Geranyl acetate	**1376**	**1381**	**7.16 ± 0.79**	**9.30 ± 2.53**	**7.95 ± 1.04**	**9.65 ± 1.28**	**20.68 ± 1.55**
Germacrene D	1480	1485	0.28 ± 0.09	0.26 ± 0.06	0.34 ± 0.08	0.34 ± 0.06	0.26 ± 0.06
Total identified			97.59	98.04	97.95	97.57	98.51

KI[a] Kovats indices on DB-5MS column, experimentally calculated using homologue series of n-alkanes.
Ki[b]: Kovats indices on DB-5MS column taken from Adams. Results are the mean of three replications ±SE.

Table 3
The chemical compositions of *D. kotschyi* treated with different fertilizer sources in the second cutting.

Compound (%)	KI[a]	KI[b]	Treatments				
			Broiler litter	Cow manure	Sheep manure	Chemical fertilizer	Control
α-Pinene	**943**	**939**	**13.86 ± 0.81**	**9.93 ± 1.68**	**13.41 ± 0.41**	**14.39 ± 1.51**	**15.37 ± 2.53**
Camphene	960	954	–	–	–	–	0.12 ± 0.02
Sabinene	978	975	0.51 ± 0.04	0.45 ± 0.07	0.5 ± 0.02	0.57 ± 0.08	0.04 ± 0.01
β-Pinene	986	979	1.68 ± 0.08	1.41 ± 0.14	1.65 ± 0.04	2.25 ± 0.33	0.8 ± 0.12
Myrcene	998	991	0.24 ± 0.01	0.19 ± 0.02	0.26 ± 0.01	0.27 ± 0.03	0.07 ± 0.02
α-Terpinene	1013	1017	0.28 ± 0.04	0.29 ± 0.01	0.26 ± 0.01	0.55 ± 0.09	0.52 ± 0.07
p-Cymene	1028	1025	0.22 ± 0.05	0.2 ± 0.01	0.26 ± 0.03	0.22 ± 0.0	0.06 ± 0.02
Limonene	1035	1029	6.82 ± 1.01	6.59 ± 0.75	6.22 ± 0.66	10.14 ± 1.48	4.12 ± 0.51
1,8-Cineole	1038	1031	0.27 ± 0.27	–	–	–	–
(Z) β-Ocimene	1040	1037	0.71 ± 0.21	0.94 ± 0.08	1.02 ± 0.04	1.13 ± 0.11	1.03 ± 0.12
Unknown	–	–	0.51 ± 0.02	0.54 ± 0.02	0.54 ± 0.03	0.49 ± 0.05	0.3 ± 0.12
Linalool	1100	1097	0.25 ± 0.01	0.23 ± 0.02	0.27 ± 0.01	0.31 ± 0.03	0.32 ± 0.06
α-Campholenal	1132	1126	0.86 ± 0.03	0.97 ± 0.0	0.93 ± 0.04	0.91 ± 0.01	1.72 ± 0.07
Trans limonen oxide	1146	1142	0.98 ± 0.15	0.95 ± 0.06	1.09 ± 0.11	0.71 ± 0.07	3.52 ± 0.17
Unknown	–	–	0.44 ± 0.02	0.45 ± 0.0	0.47 ± 0.02	0.42 ± 0.0	0.72 ± 0.12
p-Mentha-1,5-dien-8-ol	1175	1170	1.38 ± 0.2	1.67 ± 0.06	1.73 ± 0.06	1.23 ± 0.16	2.91 ± 0.12
Unknown	–	–	0.78 ± 0.07	0.74 ± 0.01	0.74 ± 0.01	0.72 ± 0.02	0.77 ± 0.16
α-Terpineol	1191	1189	0.27 ± 0.02	0.3 ± 0.03	0.28 ± 0.02	0.21 ± 0.01	0.67 ± 0.17
Myrtenol	1198	1196	0.32 ± 0.03	0.3 ± 0.04	0.29 ± 0.03	0.29 ± 0.01	0.49 ± 0.17
Trans carveol	1210	1217	0.6 ± 0.07	1.31 ± 0.29	0.77 ± 0.06	1.5 ± 0.23	1.67 ± 0.17
Neral	**1242**	**1238**	**20.95 ± 0.50**	**23.89 ± 0.81**	**21.38 ± 0.49**	**20.41 ± 1.13**	**19.71 ± 1.28**
Geraniol	1250	1253	0.19 ± 0.05	0.19 ± 0.03	0.18 ± 0.01	0.09 ± 0.05	0.59 ± 0.05
Geranial	**1265**	**1267**	**25.75 ± 0.95**	**29.27 ± 1.05**	**26.01 ± 0.27**	**23.07 ± 1.80**	**15.74 ± 1.24**
Methyl geranate	1328	1325	6.86 ± 0.21	6.59 ± 0.56	6.71 ± 0.20	5.53 ± 0.50	6.61 ± 0.12
α-Cubebene	1360	1351	0.69 ± 0.05	0.59 ± 0.10	0.61 ± 0.09	0.58 ± 0.06	0.74 ± 0.14
Geranyl acetate	**1376**	**1381**	**14.2 ± 1.40**	**11.61 ± 0.45**	**13.96 ± 1**	**13.58 ± 0.06**	**20.53 ± 2.04**
Germacrene D	1480	1485	0.16 ± 0.03	0.17 ± 0.01	0.22 ± 0.02	0.21 ± 0.01	0.11 ± 0.02
Total identified		98.05	98.04	98.01	98.15	97.46	

KI[a]: Kovats indices on DB-5MS column, experimentally calculated using homologue series of n-alkanes.
Ki[b]: Kovats indices on DB-5MS column taken from Adams. Results are the mean of three replications ±SE.

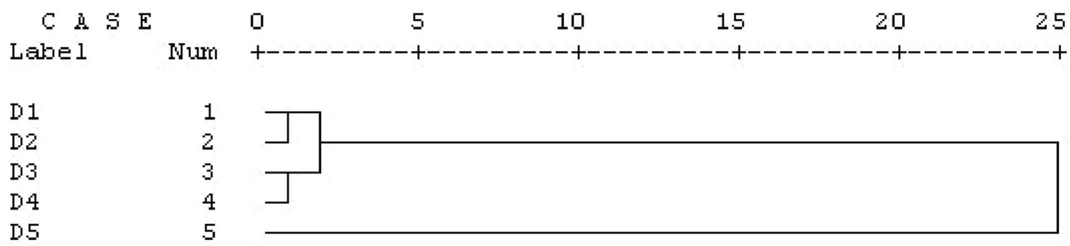

Fig. 4. Classification of five treatments of *D. kotschyi* based on essential oil chemical compounds in the first cutting using Ward clustering method. D1, D2, D3 and D4 are *D. kotschyi* treated with broiler litter, cow manure, sheep manure and chemical fertilizer, respectively. D5 is control treatment (without fertilizer).

geranate and geranyl acetate) in the first cutting indicated that the first and second components explained 72.1% and 14.7% of the total variations, respectively (Fig. 5). Application of organic manures (broiler litter, cow manure and sheep manure) and chemical fertilizer enhanced the content of limonene, neral, geranial compared with the control treatment, but usage of organic manures and chemical fertilizer reduced geranyl acetate and α-pinene contents. A positive correlation was noted among the content of limonene, neral and geranial, but these compounds had a negative correlation with geranyl acetate. A positive correlation was seen between α-pinene and methyl geranate.

At the second cutting, the maximum percentage of geranial (29.27%) and neral (23.89%) were recorded in the plants treated with cow manure. At the second cutting, the maximum content of α-pinene (15.37%) and geranyl acetate (20.53%) were attained in the unfertilized treatment. Geranial and neral together make a mixture called citral (Dudareva and Pichersky, 2008) which is widely used in cosmetics and as a flavor additive in the food industry because of its strong and lemon-like odor (Marcus et al., 2013). Numerous studies have investigated citral's antibacterial (Fisher and Phillips, 2006), antimicrobial (Yang et al., 2013) antifungal (Mesa-Arango et al., 2009; Araújo et al., 2003), and anticancer (Chaouki et al., 2009) bioactivities. Considering the various applications of citral (neral plus geranial), it seems that the cow manure treatment can be an appropriate economical option for different industries.

At the second cutting, the cluster analysis of the chemical compositions of *D. kotschyi* essential oils, was accomplished and the plants were classified into three different groups (Fig. 6). The first group comprised the plants receiving broiler litter, sheep manure and chemical fertilizer. This group was rich in geranial (23.07%–26.01%) and neral (20.41%–21.38%). The plants treated with cow manure which had the highest amounts of geranial (29.27%) and neral (23.89%) were classified as the second group. The third group consisted of unfertilized plants were very rich in geranyl acetate (20.53%) and α-pinene (15.37%).

Results of the principal component analysis in the second cutting revealed that most (93.9%) of the variation could be explained by the first and second components. Similar to the first cutting, the control treatment was extremely rich in geranyl acetate and α-pinene (Fig. 7) but contained very low amounts of limonene, neral, and geranial.

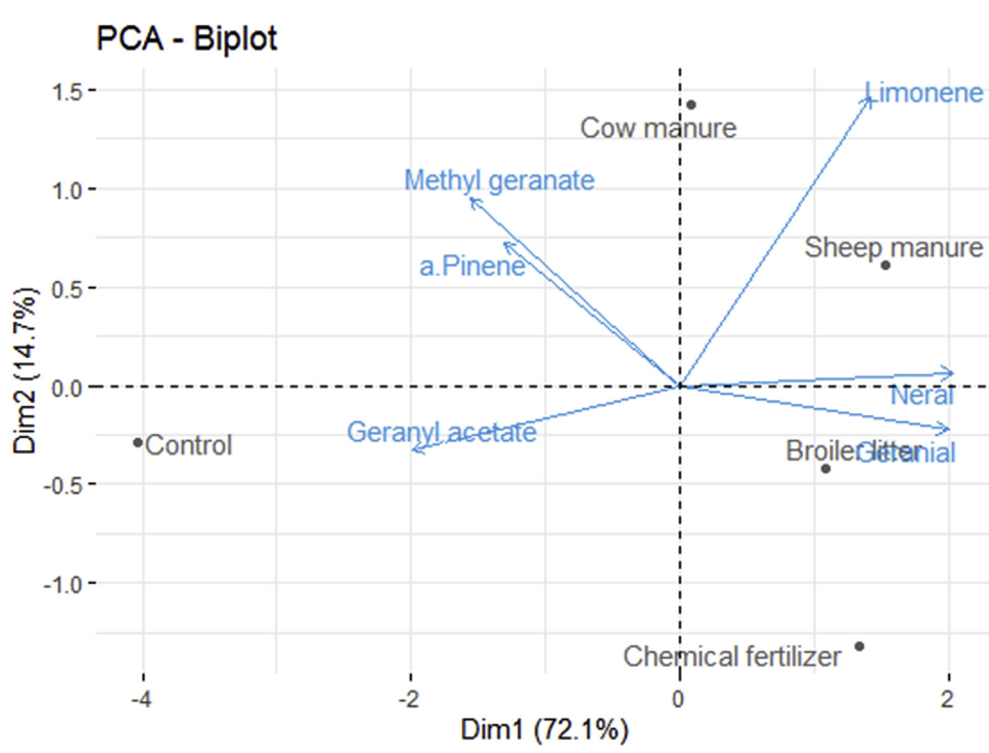

Fig. 5. Biplot of the first two principal component of five treatments (broiler litter, cow manure, sheep manure, chemical fertilizer and control) according to their six major chemical compositions (α-pinene, limonene, neral, geranial, methyl geranate and geranyl acetate) in the first cutting of *D. kotschyi*.

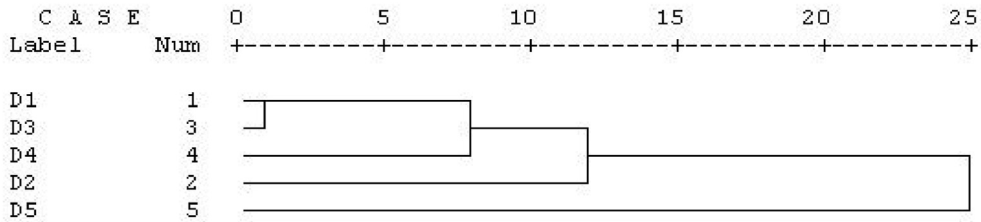

Fig. 6. Classification of five treatments of *D. kotschyi* based on essential oil chemical compounds in the second cutting using Ward clustering method. D1, D2, D3 and D4 are *D. kotschyi* treated with broiler litter, cow manure, sheep manure and chemical fertilizer, respectively. D5 is control treatment (without fertilizer).

The cow manure treatment had the highest amounts of neral and geranial. However, the chemical fertilizer had the greatest and lowest limonene and methyl geranate contents, respectively.

The results could be interpreted to mean that the application of organic manures and chemical fertilizer affects chemical compounds of *D. kotschyi* (Tables 2 and 3). The current results revealed the fact that organic manure has a positive impact on major commercial compositions. It is noteworthy that crop production and, consequently secondary metabolites are very much related to fertilization management (macro-micronutrients); organic manure mainly supplies the available macro-micronutrients for crop growth (Fallah et al., 2013). Nitrogen might enhance the biosynthesis processes of essential oil through its direct or indirect role in plant metabolism resulting in more plant metabolites (Said-Al Ahl et al., 2009). This nutrient is most recognized in plants for its key role in the biosynthesis of amino acids, proteins, enzymes and nucleic acids. Enzymes and amino acids play a pivotal role in the biosynthesis of several compounds that are assumed to be essential oil constituents (Koeduka et al., 2006). According to Rouached et al. (2010) phosphorus plays a significant role in different metabolic processes, being a component of nucleic acid, phospholipids, and coenzymes and activating the amino acid production used in protein synthesis, ATP, DNA and RNA. Furthermore, iron and zinc act as either metal components of different enzymes or regulatory, functional, structural co-factors. Thus, these two nutrients are associated with protein synthesis, saccharide metabolism and photosynthesis (Marschner, 1995). Copper is involved in several physiological processes, acts as a co-factor in different enzymes and facilitates the transportation of photosynthetic electrons (Yruela, 2005). Manganese also has important tasks in plant metabolism especially in chlorophyll synthesis, photosynthesis, amino acids and protein synthesis, nitrate reduction, the activation of various enzymes and phytohormone regulation (Ghannadnia et al., 2014).

Anwar et al. (2005) indicated that the application of organic manure (farmyard manure and vermicompost) better increased methyl chavicol and linalool contents in French basil plant than the chemical fertilizer. Also Pandey et al. (2016) demonstrated that the application of poultry manure more considerably enhanced methyl chavicol content (72.60%) of basil in comparison with chemical fertilizer. Pandey et al. (2015) reported the greater enhancement of marigold E-ocimenone content (*Tagetes minuta* L.) with the use of farmyard manure compared with chemical fertilizer.

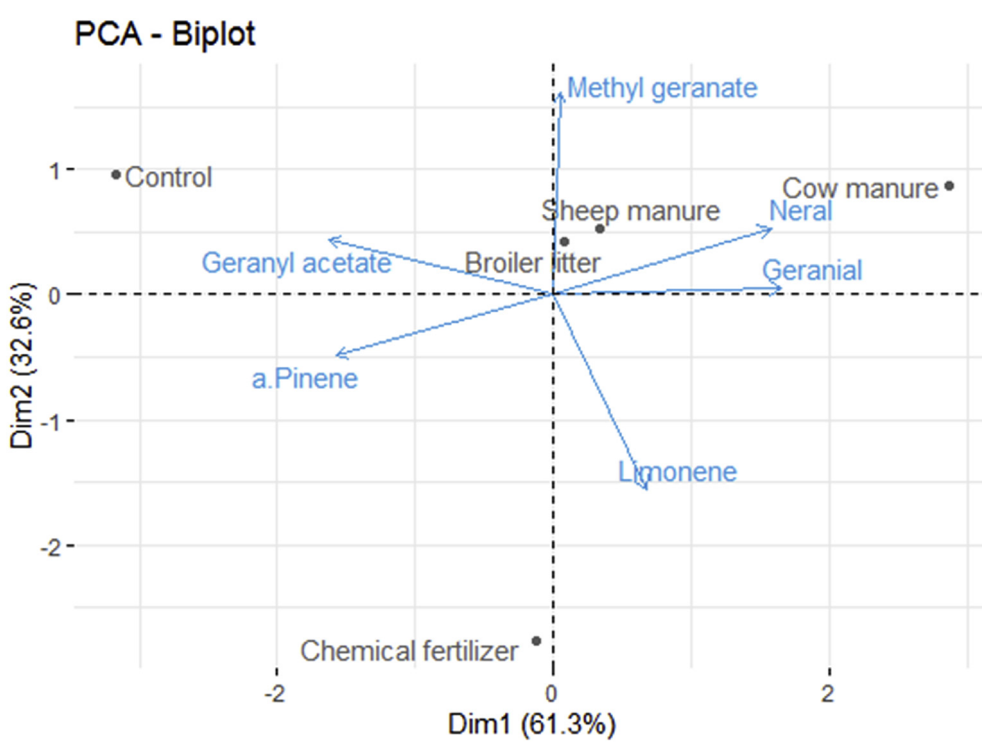

Fig. 7. Biplot of first two principal component for five treatments (broiler litter, cow manure, sheep manure, chemical fertilizer and control) according to their six major chemical compositions (α-pinene, limonene, neral, geranial, methyl geranate and geranyl acetate) in the second cutting of *D. kotschyi*.

4. Conclusions

The results of the present study indicated that the quantity and quality of *D. kotschyi* in both cuttings were more affected by the application of broiler litter and sheep manure. Because of the higher and longer availability of essential nutrients such as nitrogen, phosphorus and micronutrients, organic manures enhanced the quantity and quality of *D. kotschyi.* Neral, geranial, geranyl acetate and α-pinene were determinated to be major chemical compounds of *D. kotschyi* in both cuttings. At the first cutting, the plants amended with organic manures, had maximum neral and limonene contents. At the second cutting, the greatest neral and geranial content was yielded by plants treated with organic manures as well. Plants fertilized with chemical fertilizer had the highest limonene content in the second cutting. Overall, substituting organic manure for synthetic fertilizer increases the quality of the chemical compositions of *D. kotschyi* and eliminates the need for additional chemical fertilizer for the production of multi-cut plants.

Author contributions

Sina Fallah: performed Writing - review & editing; Conceptualization; Supervision. Soroosh Mouguee: performed Investigation. Maryam Rostaei: performed data analysis and Formal analysis, Writing - original draft. Zohrab Adavi: performed Project administration. Zahra Lorigooini: performed the GC data interpretation. Ehsan Shahbazi: performed PCA analysis, Formal analysis,

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2020.120189.

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