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Chemical compositions and antioxidant activity of essential oil of wild and cultivated *Dracocephalum kotschyi* grown in different ecosystems: A comparative study

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ABSTRACT

Dracocephalum kotschyi Boiss. (*D. kotachyi*), is an aromatic and perennial plant endemic to Iran. This plant is commonly used for the treatment of headache, congestion, stomach and also liver disorders. Furthermore, antihyperlipidemic, immunomodulatory, antinociceptive, cytotoxic and antispasmodic effects have already been reported for *D. kotachyi*. However, no information is available about environmental factors' impact on the quantity and quality of this plant. The objectives of this study were to compare the biomass, essential oil content, chemical compositions and antioxidant activity of *D. kotachyi* in three different (natural, agricultural and controlled) ecosystems. In order to do that, the plant samples were selected from each ecosystem in which they consisted of: two natural ecosystems (Nat. 1: altitude of 3040 m and Nat. 2: altitude of 3646 m), two agricultural ecosystems (Agr. 1: altitude of 2539 m and Agr. 2: altitude of 2589 m), and two controlled (Con. 1: altitude of 2393 m and Con. 2: altitude of 2393 m). The results indicated that the maximum aboveground biomass (29.21 g/plant) of *D. kotschyi* belonged to the sample of the Agr. 2. Also, the samples of Nat. 1 and Con. 1, possessed higher oil essential content as compared with other ecosystems. The highest antioxidant activity of *D. kotschyi* was obtained in the sample of Con. 1 under the application of broiler litter. According to the findings, Neral (20.29%-28.24%), geranial (17.32%–26.36%), geranyl acetate (7.16%–20.82%) and α-pinene (11%–15.09%) were identified as major chemical compounds of *D. kotschyi* in all samples except in the Nat. 2. The major chemical compositions in the Nat. 2 ecosystem were *trans*-carveol (52.65%), limonene (20.13%), geranyl acetate (9.52%) and geranial (4.13%). In general, the cultivation of *D. kotschyi* treated with broiler litter (Con. 1) leaded to high essential oil content, main chemical compositions and the highest antioxidant activity. Hence, it could be applied as a valuable medicinal plant in various industries and can be considered as an alternative to protect this plant in the natural ecosystems.

1. Introduction

Gathering wild plants is an ancient tradition that has been persisted in many aboriginal communities (Ladio and Lozada, 2000). Wild plants were collected for a range of various purposes. They were used to meet our ancestors' material needs including food, fodder, firewood and construction materials (Tardío and Pardo-de-Santayana, 2008). Many of these harvested plants are medicinal and may contain desired chemical compositions which are usable in various industries.

Some of previous studies have uncovered that wild plants have higher phenolic content and antioxidant activity comparing cultivated plants. Jung et al (2005) reported that wild ginseng leaves have higher phenolic content and antioxidant activity than the cultivated ginseng leaves. Another research focused on damiana plant (*Turnera diffusa*), represented that wild samples (collected at El Carrizal site) contain higher antioxidant activity than the cultivated samples (Soriano-Melgar et al., 2012). The study of wild plants grown in the natural populations can provide new insights that may lead to introducing new populations with higher essential oil content and greater quantities of desired major chemical compositions which are beneficial for various industrial applications such as food and pharmaceutical purposes (Tohidi et al., 2017). On the other hand, transplanting a plant from its wild ecosystem to a manipulated cropping system can affect its growth and development, and also the content and chemical compounds of secondary

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metabolites (Fennell et al., 2004). Thus, causing any change in the plant growth environment could result in the quantity and quality variation of the plant products.

Dracocephalum kotschyi Bioss. (Lamiaceae), which is known as Badernjboei-Dennaei or Zarrin-giah in Persian, is an endangered herbaceous plant endemic to Iran (Fattahi et al., 2016; Mozaffarian, 1998). This plant is mainly distributed in Northern and Central area of Iran and grows at an altitude of 2000–3000 in high mountainous regions (Fattahi et al., 2013; Rechinger, 1986).

In previous studies, geranial, α-pinene, geranyl acetate, limonene, carvacrol, γ-terpinene, *cis*-β-ocimene, nerol, methyl geranate, and perilla aldehyde were identified as the major chemical compositions of aerial parts of this plant at flowering stage (Ashrafi et al., 2017; Golparvar et al., 2016; Fattahi et al., 2016; Monsef-Esfahani et al., 2007; Moridi Farimani et al., 2017). These compounds are used in different industries such as cosmetic, food and pharmaceutical industries. On the other hand, this plant has been used for the treatment of a variety of diseases such as headache, congestion, stomach and liver disorders (Dorosti and Jamshidi, 2016; Rechinger, 1986). In general, different pharmacological properties of *D. kotachyi* such as antihyperlipidemic (Sajjadi et al., 1998), immunomodulatory (Amirghofran et al., 2000), antinociceptive (Golshani et al., 2004) and cytotoxic effects (Jahaniani et al., 2005) have been reported by researchers.

Excessive harvesting of the wild plants as well as this plant, its limited distribution areas and the lack of its cultivation and domestication are presumed as the main reasons that *D. kotachyi* is now placed in the list of endangered plants (Fattahi et al., 2013). Another crucial occurrence is the fact that this plant is mainly harvested during the flowering period and before the seed set. This obviously results in lowering regeneration and gradual degradation of its wild population (Abbad et al., 2011).

The quantity and quality gap evaluation between wild and cultivated samples of *D. kotachyi* plant has not been reported yet. Therefore, the main objectives of the this study were: (i) to evaluate the above ground biomass of *D. kotachyi* in the wild and cultivated samples, (ii) to compare the chemical compositions and antioxidant activity of *D. kotachyi* essential oil in various ecosystems. The findings of present study can provide further guidance for the cultivation and domestication of *D. kotachyi* as a valuable medicinal plant with desired chemical compositions.

2. Material and methods

2.1. Collection of plant samples and determination of biomass

The samples' aerial parts of the wild (natural ecosystem) and cultivated (agricultural and controlled ecosystems) of *D. kotachyi* were collected from Fereydoun Shahre in Esfahan province of Iran. Then the samples were dried in the shade conditions. After shade-drying, the biomass was measured as g/plant. The collection site, climate, ecosystem type plus its code, geographical attributes, applied fertilizer, harvesting time and water supply of *D. kotschyi* samples in different ecosystems are presented in Table 1. The aerial parts of cultivated samples were harvested at the flowering stage. While, aerial parts of wild samples were harvested shortly before the flowering stage due to their early and excessive harvesting by the local people and grazing possibility by the livestock of animal keepers.

Plant identities were confirmed by Dr. H. Shirmardi and a representative voucher specimen (No. 2225) was placed in the Herbarium of the Research Center of Natural Resources of Chaharmahal va Bakhtiari province of Iran. In controlled samples, organic manures were added to the soil before seedlings' transplantation. All details of the soil properties of different ecosystems and organic manures are illustrated in Tables 2 and 3 , respectively. Thereafter, *D. kotschyi* were planted at 5, 5, 6 and 7 seedling/m^2 in Con. 1, Con. 2, Agr. 1, and Agr. 2 ecosystems, respectively.

2.2. Extraction of essential oil

The samples of *D. kotschyi* collected from different ecosystems were shade-dried and then powderd. About fifty g of *D. kotschyi* pulverized samples were subjected to hydrodistillation for 3.5 h using a Clevenger apparatus. Hydrodistillation was performed according to the method described in the British Pharmacopoeia (1988). Obtained essential oils were dried over anhydrous sodium sulphate and were kept in sealed vials at 4 °C for further analysis. Essential oil yield was determined as g/plant and calculated using the following equation:

$$\text{Essential oil yield (g/plant)} = (\text{D. kotschyi biomass g/plant} \times \text{essential oil content \%})/100 \quad (1)$$

2.3. Analysis of essential oil' chemical compositions

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) using helium as the carrier gas at a flow rate of 0.5 ml per min during the analysis. The oven temperature was programmed as follows: The oven temperature was started at 60 °C, then was raised to 285 °C at a rate of 40 °C/min and was maintained for three min at 285 °C. The injector and FID detector temperature was kept at 280 °C.

Gaschromatography–mass spectrometry (GC–MS) analysis was carried out using an Agilent 7890A/5975C GC–MS system equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Again, the oven temperature was started at 60 °C, and then was enhanced from 60 to 220 °C at a rate of 3 °C/min; subsequently, the temperature was raised up to 240 °C at 20 °C/min and was kept at this temperature for three min. The temperature of injector and transfer line were maintained at 260 °C and 280 °C, respectively. The split ratio was adjusted to 100:1. Helium served as the carrier gas with a linear velocity of 30.6 cm/s. The mass spectra were recorded at 70 eV of ionization voltage; where, the mass range was 40-300 m/z.

Essential oil chemical compounds were identified by comparing their mass spectra with the ones held in the computer library or achieved through the use of authentic components. Retention indices (RI) were calculated using the retention times of *n*-alkanes (C8-C24) injected after the essential oil, under similar conditions. Chemical components' identities were confirmed by the comparison of their relative retention indices, either with those of authentic components or with the data published in the literature (Adams, 2007; Samadi et al., 2018).

2.4. Antioxidant activity

The antioxidant capacity is most commonly determined by the 2,2 diphenyl-1-picrylhydrazyl (DPPH) scavenging activity test (Baharfar et al., 2015). DPPH radical scavenging activity was measured according to the method introduced by Fallah et al., 2018. In order to do that, 20 μl of various samples' essential oil were weighted and added into 100 μl of 0.5 mM DPPH solution in methanol and final volume was made up to 200 μl. The mixtures were shaken and incubated in the dark condition at the room temperature for 15 min. The absorbance of the solutions including the blank (without sample) and diff ;erent concentrations were recorded at 517 nm using an Elisa reader (Awareness Technology, Inc). The inhibition percentage of the samples was calculated as:

$$\text{Inhibition} = \{(\text{AB-AA})/\text{AB})\} \times 100 \quad (2)$$

where AA and AB are the absorbance values of the DPPH radical in the presence of the plant essential oil sample and the control treatment, respectively. A less absorbance of the mixtures indicated a higher DPPH radical scavenging ability. IC$_{50}$ value (μg/mL) is the concentration in

Table 1
Collection site, climate, type of ecosystem plus its code, geographical attributes, applied fertilizer, harvesting time and water supply of sample of *D. kotschyi* in different ecosystems.

Collection site	province	Climate	Type of ecosystem	Code	Altitude above sea level (m)	Longitude	Latitude	Applied fertilizer	Harvesting time	Water supply
Fereydoun shahr	Esfahan	cold semi-arid	Natural	Nat. 1	3040 m	49°79´E	32°93´N	No fertilizer	6 June 2018	Rainfall
Fereydoun shahr	Esfahan	cold semi-arid	Natural	Nat. 2	3646 m	49°98´E	32°80´N	No fertilizer	10 June 2018	Rainfall
Fereydoun shahr	Esfahan	cold semi-arid	Controlled	Con. 1	2393 m	50°18´E	32°93´N	Broiler liter (1.7 Mg/ha)	30 June 2018	Irrigation + rainfall
Fereydoun shahr	Esfahan	cold semi-arid	Controlled	Con. 2	2393 m	50°18´E	32°93´N	Sheep manure (1.7 Mg/ha)	30 June 2018	Irrigation + rainfall
Fereydoun shahr	Esfahan	cold semi-arid	Agricultural	Agr. 1	2539 m	49°96´E	32°93´N	No fertilizer	14May 2018	Irrigation + rainfall
Fereydoun shahr	Esfahan	cold semi-arid	Agricultural	Agr. 2	2589 m	50°12´E	32°87´N	No fertilizer	4 June 2018	Irrigation + rainfall

which 50% of DPPH radicals are scavenged and is determined by interpolation from linear regression analysis.

2.5. Statistical analysis

The results reported in this study are the averages of the samples collected from different ecosystems. The data was statistically analyzed using one-way ANOVA by the program SPSS (16.0). Each data point was the mean of three replications and the means were compared through the least significant difference (LSD) test at 5% probability level. To evaluate possible similarities among various chemical compounds of *D. kotschyi* samples collected from different ecosystems, according to Ward method, hierarchical cluster analysis was performed using the SPSS statistical software (ver.16).

3. Results and discussion

3.1. Aboveground biomass

Analysis of variance indicated that there were significant differences among the samples of *D. kotschyi* in various ecosystems in terms of aboveground biomass (Table 4). As presented in Fig. 1, the aboveground biomass of *D. kotschyi* was similar in the samples of Nat. 1 (23.09 g/plant), Nat. 2 (22.99 g/plant) and Con. 2 (20.89 g/plant). The aboveground biomass of these samples was significantly lower than other samples except the Con. 1. No significant difference was observed among the samples of Nat. 1 (23.09 g/plant), Nat. 2 (22.99 g/plant), and Con. 1 (24.94 g/plant). Also, the aboveground biomass of *D. kotschyi* was similar in the samples of Agr. 1 (28.11 g/plant) and Con. 1 (24.94 g/plant). The sample of Con. 2 had the lowest aboveground biomass in comparison with other samples. The highest aboveground biomass of *D. kotschyi* was found in the sample of Agr. 2 (29.21 g/plant) which had a insignificant difference with the sample of Agr. 1 (28.11 g/plant).

Table 3
Chemical properties of applied organic manures in controlled ecosystems.

Parameter	Broiler litter (Con. 1)	Sheep manure (Con. 2)
EC (dS/m)	4.73	2.43
pH	8.19	8.33
Nitrogen (g/kg)	29.1	28.6
Phosphorus (g/kg)	5.5	2.7
Potassium (g/kg)	16.3	23.6

EC: Electrical conductivity.
Con. 1 and Con. 2 are *D. kotschyi* grown in the controlled 1 and controlled 2 ecosystems, respectively.

Table 4
Analysis variance (mean square) for biomass, essential oil content, essential oil yield and antioxidant capacity (IC_{50}) of *D. kotschyi* in various ecosystems.

Source of variation	df	Biomass	Essential oil content	Essential oil yield	Antioxidant capacity (IC_{50})
Treatment	5	31.12**	0.26***	0.01*	52.90***
Error	12	4.21	0.029	0.0002	0.66
LSD ($P \leq 0.05$)	–	3.65	0.30	0.09	1.45

Ns,*,** and ***: non-significant and significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively.
LSD: Least significant diff ;erence at 5% level probability.

The magnitude of aboveground biomass enhancement, exhibited the following order: Agr. 2 (29.21 g/plant) > Agr. 1 (28.11 g/plant) > Con. 1 (24.94 g/plant) > Nat. 1 (23.09 g/plant) > Nat. 2 (22.99 g/plant) > Con. 2 (20.89 g/plant) (Fig. 1).

The growth and function of aromatic and medicinal plants in different (natural and cultivated) ecosystems could be affected by several factors such as solar radiation, nutrition, humidity, temperature, climate, soil, altitude and geographical area (Habibi et al., 2006; Ložienė

Table 2
Physical and chemical properties of soils (depth of 0–30 cm) of *various* ecosystems.

Parameter	Nat. 1	Nat. 2	Con. 1	Con. 2	Agr. 1	Agr. 2
Texture	Loam	Sandy loam	Silty clay loam	Silty clay loam	Clay loam	Clay loam
EC (dS/m)	0.20	0.15	0.22	0.22	0.40	0.40
pH	7.5	7.5	8.25	8.25	8.1	7.9
Nitrogen (g/kg)	0.32	0.21	0.7	0.7	0.7	0.9
Phosphorus (g/kg)	0.012	0.008	0.018	0.018	0.022	0.024
Potassium (g/kg)	0.29	0.32	0.49	0.49	0.38	0.37

EC: Electrical conductivity.
Nat. 1 and Nat. 2 are *D. kotschyi* grown in the natural 1 and natural 2 ecosystems, respectively.
Con. 1 and Con. 2 are *D. kotschyi* grown in the controlled 1 and controlled 2 ecosystems, respectively.
Agr. 1 and Agr. 2 are *D. kotschyi* grown in the agricultural 1 and agricultural 2 ecosystems, respectively.

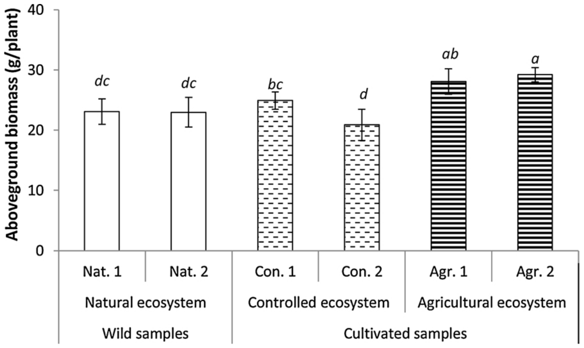

Fig. 1. Above ground biomass of *D. kotschyi* in various ecosystems. Means with different letters are significantly different, according to LSD test at $P < 0.05$. Error bars represent the mean ± SD.

and Venskutonis, 2005). Agricultural practices as well as seedbed preparation, irrigation, fertilization and weeding result in an increase in above ground biomass of *D. kotschyi* in both controlled and agricultural ecosystems. In this study, the plants grown in controlled ecosystem were transplanted. Hence, it seems that these plants may be allocated the more assimilation for their proper establishment at early stage of the growth season; while, they complete their growth and development (life cycle) at the next growth seasons. For this reason, they possessed less biomass than the plants grown in agricultural ecosystem. In addition, agricultural practices were not accomplished in natural ecosystems. On the other hand, it is obvious that the plants grown in natural ecosystems are normally confronted with many stressors during the growing season (Castro et al., 2017). However, stressors are partly controlled in agricultural and controlled ecosystems. Therefore, lower aboveground biomass of *D. kotschyi* in natural ecosystems can be due to the lack of agricultural practices and also stressors' intervention.

3.2. Essential oil content

As exhibited in Table 4, significant differences were observed in terms of essential oil content among the samples of *D. kotschyi* in various ecosystems ($P < 0.001$). This confirms environmental factors' effect on the production of secondary metabolites of medicinal and aromatic plants. Moreover, no significant difference was revealed in terms of essential oil content of *D. kotschyi* among the samples of Agr. 1 (0.37%), Agr. 2 (0.57%), and Nat. 2 (0.30%) (Fig. 2). Essential oil content in these samples was significantly lower than other samples except the sample of Con. 2. Again, no significant difference was recognized between the samples of Con. 2 (0.74%) and Agr. 2 (0.57%). Higher essential oil content was recorded in the samples of Nat. 1 (1.04%), Con. 1 (0.93%), and Con. 2 (0.74%). Also, essential oil content of these samples (Nat. 1, Con. 1, and Con. 2) was not significantly different. The highest essential oil content of *D. kotachyi* was obtained in the sample of Nat. 1. Oppositely, the sample of Nat. 2, possessed the lowest essential oil content of *D. kotschy*.

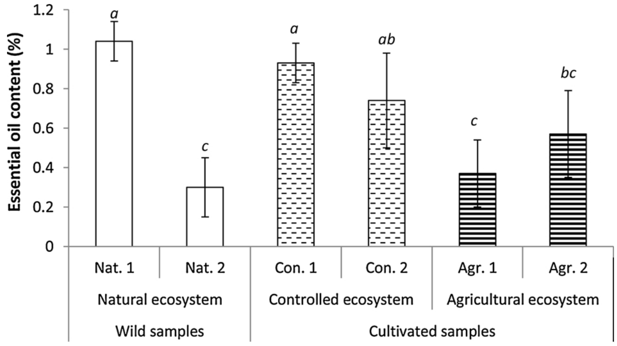

Fig. 2. Essential oil content of *D. kotschyi* in various ecosystems. Means with different letters are significantly different, according to LSD test at $P < 0.05$. Error bars represent the mean ± SD.

The Nat. 2 ecosystem located at the altitude of 3646 m possessed the lowest essential oil percentage. Many environmental factors affect essential oil content; where, the altitude is considered as the most important environmental factor influencing essential oil content (Vokou et al., 1993). Diurnal change between day and night temperature at the high altitude can lead to a decrease in essential oil content (Kizil, 2010). Haider et al. (2010) reported that the lowest oil yield (0.25%) of *Artemisia nilagirica* was obtained at the altitude of 2210 m among various altitudes (1044 m and 2050 m). Another research similarly revealed that essential oil content of the *Artemisia persica* plant is reduced at high altitudes (Mohamadi and Rajaei, 2016).

The results of current study indicated that the samples of Nat. 1 located at the altitude of 3040 m had the maximum essential oil content among various altitudes (2393 m, 2539 m, 2589 m, and 3646 m). This can be caused by alterations in the rate of temperature, wind, radiation, moisture and available water and daylight period (Mahdavi et al., 2013). It is worthy to underline that under natural conditions, the plants rarely experience single abiotic factors altogether; nevertheless, they are much more likely to be simultaneously exposed to multiple stressors (Castro et al., 2017). As said earlier, the plants grown in natural ecosystems may be allocated higher proportion of photosynthetic assimilates to produce secondary metabolites such as essential oil. The reason is that these secondary metabolites are necessary for plant-stress tolerance and defense (Tajkarimi et al., 2010). Essential oil content of *Tanacetum polycephalum* collected from the altitude of 3200 m, possessed higher essential oil percentage compared with those collected from the altitudes of 1600 m and 2400 m (Mahdavi et al., 2013). Ghasemi Pirbalouti et al. (2013b) investigated essential oil production of *Thymus caramanicus* Jalas in natural populations of three different altitudes (2000 m–2500 m, 2500 m–3000 m and 3000 m–3500 m) and reported that the highest essential oil production belonged to the altitude of 3000 m–3500 m.

The increase in essential oil content of the plants treated with organic manures in the samples of Con. 1 using broiler litter and Con. 2 using sheep manure, could be related to organic manures' capacity to supply much of essential metal and other mineral nutrients required for biosynthetic processes of secondary metabolites (Fallah et al., 2018; Rostaei et al., 2018a; Rostaei et al., 2018b). Nitrogen nutrient is one of the most important nutrients needed for crop production and this nutrient is essential for ensuring optimal dry matter production, suitable leaf surface area, and sufficient photosynthesis rates (Dordas and Sioulas, 2008). Phosphorus is also a vital nutrient which plays a significant role in metabolic processes and is regarded as a main constituent of energy compounds, nucleic acids, phospholipids and co-enzymes (Hafez and Mahmoud, 2009). Furthermore, micronutrients including zinc, iron, copper and manganese facilitate the enhancement of secondary metabolites' content. Manganese plays an important role in several physiological processes and acts as cofactor for oxidases, dehydrogenases and sugar transferases (Crowley et al., 2000; Culotta et al., 2005; Keen et al., 2000). Iron nutrient is a cofactor for a large number of enzymes that catalyze numerous biochemical processes within the plant (Brittenham, 1994; Marschner, 1995) and plays a key role in chlorophylls formation, thylakoid synthesis and chloroplast development and serves in energy transportation of the plant (Nasiri and Najafi, 2015). Zinc and copper are essential for the plants and play central roles in metabolism, photosynthesis and respiratory electron transport chain, chlorophyll and protein synthesis, auxin synthesis and cell division, and function as metal component of different enzymes and acts as regulatory cofactor and saccharide metabolism (El-Sawi and Mohamed, 2002). Many zinc-depended enzymes are involved in plant's carbohydrate metabolism in general and leaves' carbohydrate metabolism in particular. Beside mentioned functions,zinc is required in carbonic anhydrate reaction. For example, it is essential for the activity of

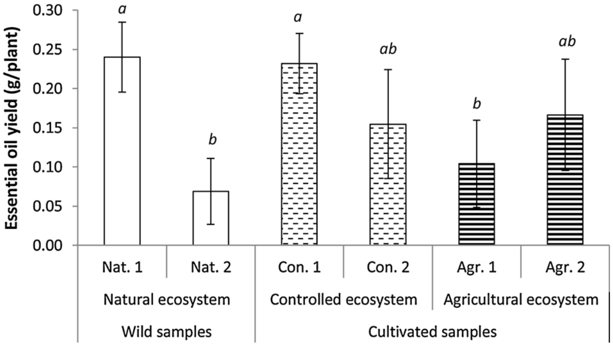

Fig. 3. Essential oil yield of *D. kotschyi* in various ecosystems. Means with different letters are significantly different, according to LSD test at $P < 0.05$. Error bars represent the mean ± SD.

two other key enzymes named: fructose 1.6-biphosphatase and aldolase (Marschner, 1995). Copper proteins represent peroxidases and oxidize monophenols to diphenols. Additionally, in the plants suffering from copper deficiency the content of soluble carbohydrates is considerably lower than the normal during the vegetative stage (Marschner, 1995). It has been revealed that essential oil content of Japanese mint (*Mentha arvensis* L.) increases with the application of organic manures (Bajeli et al., 2016). Supporting this result, Anwar et al. (2005), examined the impact of vermicompost at 10 Mg/ha on essential oil content of dill (*Anethum graveolens*) and uncovered that its application improves essential oil content by 15% and 13% as compared with the control treatment and chemical fertilizer treatment, respectively.

3.3. Essential oil yield

Statistical analysis showed that there was significant difference among the samples of *D. kotschyi* in various ecosystems ($P < 0.05$) in terms of essential oil yield (Table 4). The essential oil yield of *D. kotschyi* was similar in the samples of Agr. 2 (0.16 g/plant) and Con. 2 (0.15 g/plant), Agr. 1 (0.10 g/plant) and Nat. 2 (0.07 g/plant) (Fig. 3). In an opposite manner, no significant difference was achieved in terms of essential oil yield of *D. kotschyi* among the samples of Nat. 1 (0.24 g/plant), Con. 1 (0.23 g/plant), Agr. 2 (0.16 g/plant) and Con. 2 (0.15 g/plant) (Fig. 3). The lowest and highest essential oil yield was obtained in the sample of Nat. 2 and Nat. 1, respectively.

Increased essential oil yield of *D. kotschyi* recorded in Nat. 1 ecosystems at the altitude of 3040 m, can be attributed to moderate aboveground biomass (Fig. 1) and the highest essential oil content (Fig. 2). It has been proved that the altitude plays a fundamental role in the growth, production and the quality of medicinal plants in a variety of natural ecosystems (Alonso-Amelot et al., 2007; Habibi et al., 2006; Haider et al., 2009; Mahdavi et al., 2013). Additionally, the altitude level can change the temperature, relative humidity, solar radiation, wind and available water to the plant's root. Consequently, along with altitudinal level changes, ecophysiological reactions of the plant will also change (Ebrahimi and Ranjbar, 2016). Based on the results, essential oil yield of *D. kotschyi* in the Con. 1 ecosystem (0.23 g/plant) was comparable with the Nat. 1 ecosystem (0.24 g/plant). Adding organic manure to soil improves both soil chemical and physical properties, increases microbial biomass, water holding capacity and cation exchange capacity and ultimately enhances growth, production and quality (Pandey et al., 2016; Schlegel et al., 2015). Rostaei et al. (2018b) reported that the usage of broiler litter did enhance the biomass and essential oil yield of dill (*Anethum graveolens*). Singh et al. (2014) similarly observed that the application of farmyard increased biomass and essential oil yield of basil (*Ocimum basilicum*). Besides, the biomass and essential oil content of the leaves of Dysphania ambrosioides L. enhanced through applying chicken manure (Bibiano et al., 2019).

Table 5
The chemical compositions of *D. kotschyi* essential oil in the natural ecosystems.

Compound (%)	KI^a	KI^b	Empirical formula	Nat.1	Nat. 2	Mean
α-Pinene	943	939	C10H16	14.21	0.98	7.595
Sabinene	978	975	C10H16	0.52	–	0.26
β-Pinene	986	979	C10H16	1.86	–	0.93
Myrcene	998	991	C10H16	0.29	–	0.145
α-Terpinene	1013	1017	C10H16	0.33	0.25	0.29
p-Cymene	1028	1025	C10H14	0.31	–	0.155
Limonene	1035	1029	C10H16	6.44	20.13	13.285
1,8-Cineole	1038	1031	C10H18O	0.86	–	0.43
Trans-β-ocimene	1040	1037	C10H16	0.28	0.20	0.24
Unknown	–	–	–	0.38	–	0.19
Linalool	1100	1097	C10H18O	0.28	–	0.14
α-Campholenal	1132	1126	C10H16O	1.04	0.37	0.705
Trans-limonen oxide	1146	1142	C10H16O	1.53	0.87	1.2
Unknown	–	–	–	0.73	–	0.365
p-Mentha-1,5-dien-8-ol	1175	1170	C10H16O	1.76	1.54	1.65
Unknown	–	–	–	1.13	–	0.565
α-Terpineol	1191	1189	C10H18O	0.39	–	0.195
Myrtenol	1198	1196	C10H16O	0.45	0.73	0.59
Trans-carveol	1210	1217	C10H16O	0.64	52.65	26.645
Neral	1242	1238	C10H16O	20.29	3.92	12.105
Geraniol	1250	1253	C10H18O	1.19	2.63	1.91
Geranial	1265	1267	C10H16O	22.89	4.13	13.51
Methyl geranate	1328	1325	C11H18O2	6.74	1.24	3.99
α-Cubebene	1360	1351	C15H24	0.54	0.41	0.475
Geranyl acetate	1376	1381	C12H20O2	14.11	9.52	11.815
Germacrene D	1480	1485	C15H24	0.60	0.30	0.45
Monoterpene hydrocarbons	–	–	–	24.24	21.56	22.9
Oxygenated monoterpenes	–	–	–	72.17	77.6	74.88
Sesquiterpene hydrocarbons	–	–	–	1.14	0.71	0.92
Total identified (%)	–	–	–	97.55	99.87	98.71
Unidentified	–	–	–	2.24	0	1.12

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.
Nat.1 and Nat. 2 are *D. kotschyi* grown in the natural 1 and natural 2 ecosystems, respectively.

Lower essential oil production (Fig. 2) in the Nat. 2 ecosystem at the altitude of 3646 m, resulted in essential oil yield decrease (Fig. 3). The plants grown under environmental stress are less to the attacks by pests, pathogens and herbivores due to adverse weather conditions and limited available nutrients. Likewise, less temperature which is common at high altitude, may reduce the oil production (Ghasemi Pirbalouti et al., 2013a). Also, agricultural practices such as seedbed preparation, irrigation, fertilization and weeding in controlled and agricultural ecosystems result in dry matter accumulation increase. In this regard, Ghasemi Pirbalouti et al. (2013a) indicated that wild sample of *Tymus daenensis* had lowest dry matter (92.11 g/plant) and oil yield (0.85%) compared with cultivated samples.

3.4. Chemical compounds of essential oil

Essential oils' chemical compositions of *D. kotschyi* aerial parts in various ecosystems were established by GC–MS and are presented in Tables 5–7. The mass chromatogram of *D. kotschyi* essential oil is indicated in Fig. 4. According to GC–MS analysis, twenty-three chemical compositions were detected in the Con. 1 and Con. 2 ecosystems. At the end, the numbers of twenty-two, twenty-four, twenty-three and sixteen chemical compounds were identified in the Agr. 1, Agr. 2, Nat. 1 and Nat. 2 ecosystems, respectively. Neral (20.29%–28.24%), geranial (17.32%–26.36%), geranyl acetate (7.16%–20.82%) and α-pinene (11%–15.09%) were recognized as major chemical compositions of *D. kotschyi* in all samples with the exception of Nat. 2 (Tables 5–7). The

Table 6
The chemical compositions of *D. kotschyi* essential oil in controlled ecosystems.

Compound (%)	KI^a	KI^b	Empirical formula	Con. 1	Con. 2	Mean
α-Pinene	943	939	C10H16	15.09	12.36	13.725
Sabinene	978	975	C10H16	0.46	0.36	0.41
β-Pinene	986	979	C10H16	1.9	2.09	1.995
Myrcene	998	991	C10H16	0.26	0.22	0.24
α-Terpinene	1013	1017	C10H16	0.44	0.49	0.465
p-Cymene	1028	1025	C10H14	0.14	0.13	0.135
Limonene	1035	1029	C10H16	6.16	7.76	6.96
1,8-Cineole	1038	1031	C10H18O	0.91	0.5	0.705
Trans-β-ocimene	1040	1037	C10H16	0.34	0.52	0.43
Unknown	–	–	–	0.19	0.06	0.125
Linalool	1100	1097	C10H18O	0.22	0.21	0.215
α-Campholenal	1132	1126	C10H16O	1.2	1.19	1.195
Trans-limonen oxide	1146	1142	C10H16O	1.38	0.98	1.18
Unknown	–	–	–	0.81	0.7	0.755
p-Mentha-1,5-dien-8-ol	1175	1170	C10H16O	1.24	0.93	1.085
Unknown	–	–	–	1.17	1.06	1.115
α-Terpineol	1191	1189	C10H18O	0.37	0.52	0.445
Myrtenol	1198	1196	C10H16O	0.24	0.62	0.43
Trans-carveol	1210	1217	C10H16O	0.56	0.43	0.495
Neral	1242	1238	C10H16O	26.4	28.24	27.32
Geraniol	1250	1253	C10H18O	0.13	0.51	0.32
Geranial	1265	1267	C10H16O	26.36	24.75	25.555
Methyl geranate	1328	1325	C11H18O2	5.9	6.32	6.11
α-Cubebene	1360	1351	C15H24	0.45	0.53	0.49
Geranyl acetate	1376	1381	C12H20O2	7.16	7.95	7.555
Germacrene D	1480	1485	C15H24	0.28	0.34	0.31
Monoterpene hydrocarbons	–	–	–	24.79	23.93	24.36
Oxygenated monoterpenes	–	–	–	72.07	73.15	72.61
Sesquiterpene hydrocarbons	–	–	–	0.73	0.87	0.8
Total identified (%)	–	–	–	97.59	97.95	99.765
Unidentified	–	–	–	2.17	1.82	1.995

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.
Con. 1 and Con. 2 are *D. kotschyi* grown in the controlled 1 and controlled 2 ecosystems, respectively.

Table 7
The chemical compositions of *D. kotschyi* essential oil in the agricultural ecosystem.

Compound (%)	KI^a	KI^b	Empirical formula	Agr. 1	Agr. 2	Mean
α-Pinene	943	939	C10H16	12.07	11	11.54
Camphene	960	954	C10H16	0.29	0.19	0.24
Sabinene	978	975	C10H16	0.17	0.18	0.175
β-Pinene	986	979	C10H16	0.84	0.9	0.87
Myrcene	998	991	C10H16	0.22	0.18	0.2
α-Terpinene	1013	1017	C10H16	0.35	0.7	0.525
p-Cymene	1028	1025	C10H14	0.25	0.17	0.21
Limonene	1035	1029	C10H16	3.90	10.49	7.195
1,8-Cineole	1038	1031	C10H18O	–	0.22	0.11
Trans-β-ocimene	1040	1037	C10H16	0.79	0.63	0.71
Unknown	–	–	–	–	–	–
Linalool	1100	1097	C10H18O	–	0.14	0.07
α-Campholenal	1132	1126	C10H16O	1.77	1.24	1.505
Trans-limonen oxide	1146	1142	C10H16O	4.77	2.48	3.625
Unknown	–	–	–	0.89	0.56	0.725
p-Mentha-1,5-dien-8-ol	1175	1170	C10H16O	3.39	1.68	2.535
Unknown	–	–	–	1.06	0.71	0.885
α-Terpineol	1191	1189	C10H18O	0.59	0.51	0.55
Myrtenol	1198	1196	C10H16O	0.33	0.45	0.39
Trans-carveol	1210	1217	C10H16O	1.24	1.24	1.24
Neral	1242	1238	C10H16O	28.03	21.37	24.7
Geraniol	1250	1253	C10H18O	0.50	1.1	0.8
Geranial	1265	1267	C10H16O	23.43	17.32	20.375
Methyl geranate	1328	1325	C11H18O2	6.20	4.57	5.385
α-Cubebene	1360	1351	C15H24	0.74	0.72	0.73
Geranyl acetate	1376	1381	C12H20O2	7.93	20.82	14.375
Germacrene D	1480	1485	C15H24	0.10	0.29	0.195
Monoterpene hydrocarbons	–	–	–	18.88	24.44	21.66
Oxygenated monoterpenes	–	–	–	78.18	73.14	75.66
Sesquiterpene hydrocarbons	–	–	–	0.84	1.01	0.92
Total identified (%)	–	–	–	97.90	98.59	98.25
Unidentified	–	–	–	1.95	1.27	1.61

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.
Agr. 1 and Agr. 2 are *D. kotschyi* grown in the agricultural 1 and agricultural 2 ecosystems, respectively.

maximum neral (28.24%), geranial (26.36%), gernyl acetate (20.82%) and α-pinene (15.09%) were achieved in Con. 2, Con. 1, Agr. 2 and Con. 1 respectively (Tables 6 and 7). Major chemical compounds in Nat. 2 ecosystem were *trans*-carveol (52.65%), limonene (20.13%), geranyl acetate (9.52%) and geranial (4.13%) (Table 5).

Several studies have been implemented on essential oils' chemical compounds of *D. kotschyi*. One or more than one major chemical compositions identified from different samples of this study, were detected as major chemical compounds of the oils of *D. kotschyi* in previous studies; However, the extent of these chemical compositions was different (Asharfi et al., 2017; Golparvar et al., 2016; Fattahi et al., 2016; Javidnia et al., 2005; Monsef-Esfahani et al., 2007; Moridi Farimani et al., 2017; Saeidnia et al., 2007; Samadi et al., 2018). Trans-carveol (52.65%) and limonene (20.13%) were characterized as the main chemical compositions in the Nat. 2 at the altitude of 3646 m, and were not identified as major chemical components in other altitudes of different ecosystems. Trans-carveol presented the highest variation among other chemical compositions. It was varied from 0.43 in Con. 2 ecosystem up to 52.65% in Nat. 2 ecosystem. Camphene was found in agricultural ecosystem and was not detected in controlled and natural ecosystems. Linalool and 1,8-cineole were not identified in ecosystems of Agr. 1 and Nat. 2. Also, chemical compositions such as sabinene, β-pinene, myrcene, p-cymene and myrtenol were not detected in the plants collected from high altitude (Table 2); instead, they were obtained in essential oil of the plants collected from lower altitude. These results confirm the fact that the altitude plays a significant role in essential oil' chemical constituents of the wild *D. kotschyi* samples (Haider et al., 2009, 2010). Furthermore, the plants survive at lower altitude would receive a minimum rate of of direct sunlight, rainfall, nutrients, wind, and coldness compared with plants which grow in higher altitudes (Kalaiselvi et al., 2019). While, the role of altitude is less important in cultivated samples due to complementary energy presence. Kalaiselvi et al. (2019) reported that major chemical compositions of *Artemisia nilagirica* essential oil have variations in deferent altitudes. For instance, main chemical compounds of essential oil at lower altitude (510 m) are camphor, caryophyllene oxide and eucalyptol. While, α-thujone, α-myrcene and linalyl isovalerate were identified as major chemical components of this plant at high altitude (1780 m). Dillapiole (66.14%) and nothoapiole (62.81%) were recognized as major chemical compounds of *Angelica archangelica* L. at the altitude of 3300 m and 3500 m, respectively (Chauhan et al., 2016).

The differences in chemical compounds of essential oil among various production regions may be due to variations in cultivation conditions or physiological /structural modification of the plant. This could be occurred as a result of specific environmental factors (Gong et al., 2014) and agronomic management including nutrients, irrigation, plant density and soil tillage (Lubbe and Verpoorte, 2011). Moreover, plants' age and genetic variability may impact on chemical compounds of essential oil (Chauhan et al., 2016).

Oils' chemical compositions in various samples of *D. kotschyi* consisted of mainly monoterpene hydrocarbons and oxygenated

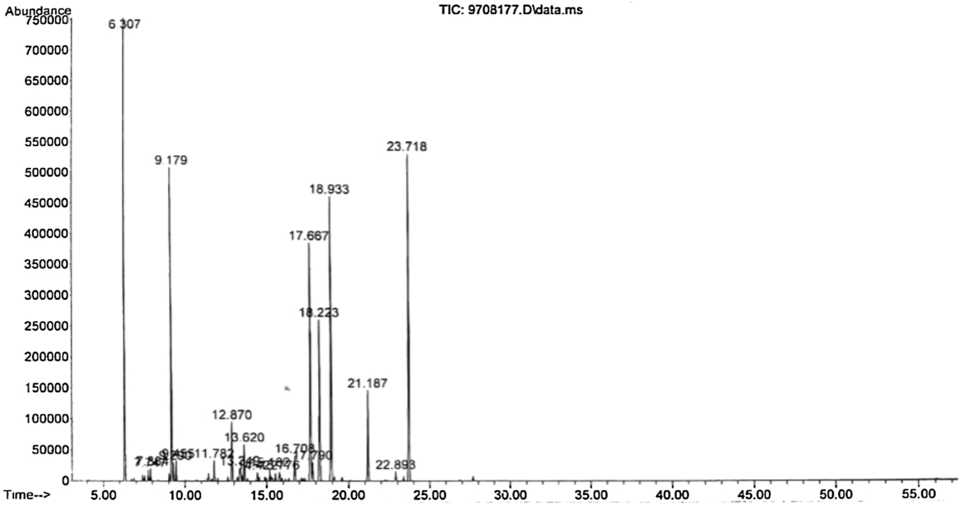

Fig. 4. *D. kotschyi* essential oil chromatogram carried out using a gas chromatograph mass spectrometry (DB-5; 30 m × 0.25 mm i.d., film thickness 0.25 μm).

monoterpenes (Tables 5–7). Generally, essential oils of *D. kotschyi* have been mostly reported as monoterpene hydrocarbons and oxygenated monoterpenes (Ashrafi et al., 2017; Moridi Farimani et al., 2017). The magnitude of total oxygenated monoterpenes enhancement, exhibited the following order: Agr. 1 (78.18%) > Nat. 2 (77.60%) > Con. 2 (73.15%) > Agr. 2 (73.14%) > Nat. 1 (72.17%) > Con. 1 (72.07%).

Cluster analysis of essential oils' chemical compositions of *D. kotschyi* samples in various ecosystems, was accomplished and they were classified into three different clusters (Fig. 5). The first cluster comprised of the plants grown in the ecosystems of Con. 1, Con. 2 and Agr. 1; which possessed high content of neral (26.4%-28.24%), geranial (23.43%–26.36%) and α-pinene (12.07%–15.09%) and oppositely low content of geranyl acetate (7.16%–7.93%). The plants grown in Nat. 1 and Agr. 2 ecosystems with high content of geranyl acetate (14.11%–20.82%) and moderate amounts of neral (20.29%–21.37%), geranial (17.32%–22.89%) and α-pinene (11%–14.21%) were classified as the second cluster. Eventually, the third cluster consisted of the plants grown in ecosystem of Nat. 2 and was extremely rich in terms of *trans*-carveol (52.65%) and limonene (20.13%). Also this cluster contained a moderate content of geranyl acetate (9.52%) and very low content of neral (3.92%), geranial (4.13%) and α-pinene (0.98%).

3.5. Antioxidant activity

Significant differences were observed in samples' antioxidant activity of *D. kotschyi* in various ecosystems (Table 4). All studied samples had an acceptable antioxidant activity. As presented in Fig. 6, the IC_{50} value ranged from 3.17 μg/mL to 13.68 μg/mL in the samples of various ecosystems. The sample of Nat. 1 exhibited the lowest antioxidant activity (the highest IC_{50} value = 13.68 μg/mL). The antioxidant activity of *D. kotschyi* was similar in the samples of Agr. 2 (IC_{50} value = 11.33 μg/mL), Agr. 1 (IC_{50} value = 11.78 μg/mL) and Nat. 2 (IC_{50} value = 12.18 μg/mL). The antioxidant activity in three samples showed a significant increase in comparison with the sample of Nat. 1.

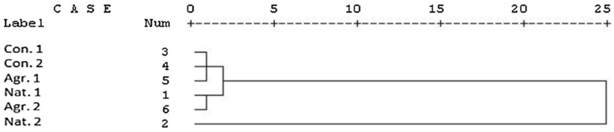

Fig. 5. Dendrogram achieved by cluster analysis based on the chemical compounds of essentials oil of six samples of *D. kotschyi* grown in various ecosystems using Ward method. Nat.1, Nat. 2, Con. 1, Con. 2, Agr. 1 and Agr. 2 are *D. kotschyi* grown in the natural 1, natural 2, controlled 1, controlled 2, agricultural 1 and agricultural ecosystems, respectively.

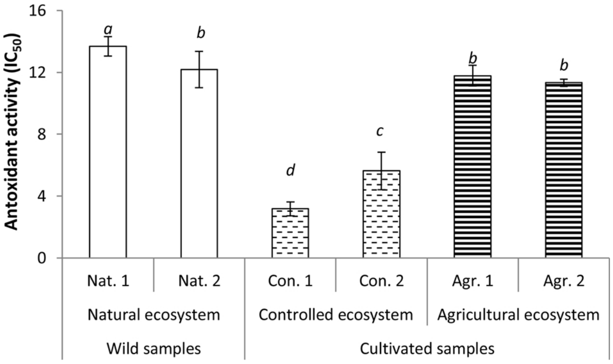

Fig. 6. Antioxidant activity of *D. kotschyi* in various ecosystems. Means with different letters are significantly different, according to LSD test at $P < 0.05$. Error bars represent the mean ± SD.

The higher antioxidant activity (the lower IC_{50} value = 5.63 μg/mL) was recorded in the sample of Con. 2. The antioxidant activity significantly enhanced in this sample as compared with the samples of Nat. 1, Nat. 2, Agr. 1, and Agr. 2. The sample of Con. 1 had the highest antioxidant activity (the lowest IC_{50} value = 3.17 μg/mL). In total, following increasing order was achieved concerning antioxidant capacity (IC_{50} value) in studied samples: Con. 1 (IC_{50} value = 3.17 μg/mL) > Con. 2 (IC_{50} value = 5.63 μg/mL) > Agr. 2 (IC_{50} value = 11.33 μg/mL) > Agr. 1 (IC_{50} value = 11.78 μg/mL) > Nat. 2 (IC_{50} value = 12.18 μg/mL) > Nat. 1 (IC_{50} value = 13.68 μg/mL).

With regard to IC_{50} values among various samples, the samples of Con. 1 and Con. 2 with lower IC_{50} (3.17 and 5.63 μg/mL, respectively), exhibited considerable DPPH radical scavenging activity compared with other ecosystems (Fig. 6). The difference in antioxidant capacity among the samples of various ecosystems could be attributed to the differences in their polyphenolic compounds (Rostaei et al., 2018b). Higher oxygenated monoterpenes leads to higher antioxidant, antibacterial and antifungal activities (Deba et al., 2008; Sökmen et al., 2003; Tohidi et al., 2017). Although the samples of Con. 1 treated with broiler litter and Con. 2 treated with sheep manure were lower oxygenated monoterpenes, they had higher antioxidant capacity. The enhancement in secondary metabolites and antioxidant activity with organic amendments might be due to the ability of manures to supply a large number of the essential metal and other mineral nutrients in addition to usual N, P, and K (Fallah et al., 2018; Pandey et al., 2015; Ibrahim et al., 2013). Rostaei et al. (2018b) reported that the usage of broiler litter brings about the enhancement of dill plant' antioxidant activity. Another research revealed that antioxidant activity of dragonhead (*Dracocephalum moldavica*) was raised through applying broiler litter (Fallah et al., 2018). Pandey et al. (2016) also represented that poultry manure application increases basil antioxidant capacity compared with chemical fertilizers.

4. Conclusions

Biomass, oil essential content, chemical compositions and antioxidant activity of the samples of *D. kotschyi* were different in various ecosystems. Environmental conditions and agronomic management in various ecosystems result in the differences in the quantity and quality of these samples. The findings demonstrated that the greatest aboveground biomass of *D. kotschyi* was obtained in agricultural ecosystem. Also, the altitude was effective on essential oil content and chemical compositions of essential oils in different samples. The samples of Nat. 1 and Con. 1 possessed higher oil essential percentage than other ecosystems. The highest antioxidant activity was recorded in Con. 1 and Con. 2 which received the broiler litter and sheep manure, respectively. Chemical compounds of Nat. 1 samples (at the altitude of 3040 m), Agr. 1 (at the altitude of 2539 m), Agr. 2 (at the altitude of 2589 m), Con. 1 (at the altitude of 2393 m) and Con. 2 (at the altitude of 2393 m) were similar and neral, geranial, geranyl acetate and α-pinene were identified as major chemical compounds of these ecosystems. Major chemical compositions in Nat. 2 (at the altitude of 3464 m) were *trans*-carveol, limonene, geranyl acetate and geranial. Essential oil of Nat. 2 at the altitude of 3646 m was rich in terms of *trans*-carveol (more than 52%) and limonene (more than 20%). Major chemical compositions identified from the samples of different ecosystems can be used in various industries. So, different food and pharmaceutical industries can benefit from different samples with especial chemical compounds depending on their purpose.

According to present study, cultivation of *D. kotschyi* under the application of broiler litter leads to high essential oil content, main chemical compositions and greatest antioxidant activity. Therefore, broiler litter application could be considered as an alternative solution to protect this valuable and endangered medicinal plant in natural ecosystems.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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