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The effects of foliar application of some important micronutrient elements on essential oil content and components of *Dracocephalum kotschyi* Boiss.

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ABSTRACT

Purpose: *Dracocephalum kotschyi* Boiss. belongs to the Lamiaceae family, is one of the important and endangered endemic species in Iran. The present study was conducted to investigate the effects of micronutrient elements application on the content and composition of essential oil of *D.kotschyi* Boiss. shoots in southwestern Iran (Shahrekord) in 2022 and 2023. **Research Methods:** Four foliar fertilizers including Fe, Cu, Zn and Mn were applied in 0, 20, 40 and 60 $mg.l^{-1}$ in RCBD design by factorial layout and 3 replications. **Findings:** Results obtained from gas chromatography/mass spectrometry (GC-MS) showed 14 essential oil components. According to obtained results, applied micronutrients significantly influenced the essential oil content/ composition of *D.kotschyi.* In both years, the highest content of essential oil (0.98-0.99 %) was obtained in plants treated with 40 $mg.l^{-1}$of micronutrients ($Fe_2Cu_2Zn_2Mn_2$) and the lowest content (0.59-0.66%) made by control plants (0 $mg.l^{-1}$), however the plants treated by 60 $mg.l^{-1}$of micronutrients in most characters were in a same group with the control plants. The most important chemical compounds that determine the quality of *D. kotschyi* essential oil including Neral (9.02-16.31%), Limonene (25.4-35.6%), Geranial (8.6-16.5%), Eucalyptol (3.89-8.01%) and Myrtenol (22.5-32.1%) were identified alcoholic monoterpenes. Limonene belonging to monoterpene hydrocarbons was the predominant constituent of the *D.kotschyi.* **Limitations**: There were no limitations to the report. **Originality/Value**: The foliar application of micronutrients at 40 $mg.l^{-1}$ (Fe, Cu, Zn and Mn) can be a good strategy to improve the essential oil quantity and quality of *D.kotschyi* in cold and semi-arid climates.

INTRODUCTION

Dracocephalum kotschyi Boiss., locally known as Badrandjboie-Dennaie and Zarrin-Giah in Persian, belongs to the Lamiaceae family, is one of the endangered endemic species in Iran (Mozaffarian, 2008). The extreme harvesting, limited distribution, and no commercial farming of *D.kotachyi* have led to the danger of its extinction which is one of the most important challenges. The essential oil and extract from *D. kotachyi* have medicinal properties including anti-hyperlipidemic, immunomodulatory, anti-spasmodic, antinociceptive, cytotoxic (Ashrafi et al., 2017) and antimicrobial (Fallah et al., 2020) effects. Furthermore, the aerial parts and inflorescences of *D.kotachyi* are used for the treatment of pain, fever, headache, inflammation, congestion, stomach, and also seizures. In traditional Iranian medicine, the boiled aerial parts and inflorescences of the herb is used to heal wounds and relieve rheumatic pain (Golparvar et al., 2016; Samadi et al., 2018). This plant is rich in essential oil, flavonoids, monoterpene glycosides, trypanocidal terpenoids, rosmarinic acid, and linolenic acid. The main compounds of the essential oil of *D.kotschyi* are α-pinene, limonene, carvacrol, geranyl acetate, myrtenol, methyl geranate, γ-terpinene, perilla aldehyde, eucalyptol, nerol, and germinal (Taghizadeh et al., 2020; Sonboli et al., 2019), while those found in naturally grown plants were two main compounds: cyclohexylallene (52.63%) and limonene (35.88%) (Ghavam et al., 2021). The environmental factors cause changes in the growth, quantity, and quality of active substances (such as alkaloids, glycosides, steroids and volatile oils) in the medicinal and aromatic plants and by increasing of dry matter, the essential oil percentage increased (Shaykh-Samani et al., 2023a, b; Cham et al., 2022). Aromatic and volatile products of plant secondary metabolism are used in the pharmaceutical, chemical, cosmetic, and food industries. In recent years, there has been an increasing interest in the use of natural substances due to concerns about the safety of some synthetic compounds, which have encouraged more detailed studies on originated substances (Fallah et al., 2020; Pradhan et al., 2017).

When nutrient deficiency cannot be corrected through soil application, foliar nutrition is adopted as an alternate method (Marschner, 1995). It has been shown that micronutrients such as Fe, Mn, Zn and Cu are necessary for plant intensification in much lower amounts for plant intensification than those of the primary nutrients (Bilal et al., 2020).

Four important micronutrients used in medicinal plants are Fe, Cu, Mn and Cu. Iron (Fe) is one of the essential nutrient elements needed by plants and is a key element in cytochrome structure. In addition, plants treated with this micronutrient produce more yield (Majeed et al., 2020). Copper (Cu) is another essential microelement in higher plants as it occurs as part of the prosthetic groups of several enzymes. Zinc (Zn) is a building block of many proteins and an important chemical element in biological activity. Zn acts on enzymatic activation and cell division, so it has been shown that its deficiency causes cell damage, low protein and carbohydrate synthesis, impaired growth and development, and low crop yields (Alamer et al., 2020; Cakmak et al., 2017; Figueiredo et al., 2017). Manganese (Mn) is involved in many biochemical functions, primarily acting as an activator of enzymes such as dehydrogenases and decarboxylases involved in respiration, amino acid and lignin synthesis, and hormone concentrations (Alejandro et al., 2020). Foliar application of these micronutrients have important effects in morpho-physiological attributes such as chlorophyll, phenol and relative water content that made more essential oil content and composition in *Rosa damascena* (Yadegari, 2023), *Thymus* (Yadegari, 2022), *Satureja* (Bani Taba et al., 2022), *Dracocephalum moldavica* (Yadegari, 2021), *Carthamus tinctorius* (Galavi et al., 2012), *Calendula officinalis* L. *Borago officinalis*, *Alyssum desertorum* and *Thymus vulgaris*

(Yadegari, 2015, 2017a), *Anethum graveolens* (Rostaei et al., 2018), *Matricaria chamomilla* (Nasiri et al., 2010) and *Coriandrum sativum* (Said-Al Ahl & Omer, 2009).

Foliar fertilization is particularly useful to meet the basic needs of plants for one or more micro- or macro- nutrients, especially trace minerals. It also helps correct deficiencies, strengthen weak or damaged crops, and enhance growth (Aziz et al., 2019). The aim of this research was to determine the effects of foliar applications of iron, zinc, copper and manganese on essential oil content and composition in *D.kotschyi* Boiss. To introduce the best combination of these micronutrients for better yield in this multipurpose plant.

MATERIALS AND METHODS

Plant material and fertilizers

Four foliar fertilizers including Librel Fe-Lo, Librel Cu, Librel Zn and Librel Mn were applied and all of them are mineral fertilizers. Librel Fe-Lo contains 13.2% chelated iron, Librel Zn is a foliar fertilizer that contains 14% Zinc in chelated form, Librel Cu has 14% copper in chelated form and Librel Mn contains of 13% Mn chelated with EDTA obtained from the chemical company of Germany (BASF). Theses fertilizers were sprayed at three concentrations (for example Fe_1, Fe_2 and Fe_3 were concentrations of Fe which had 20, 40 and 60 $mg.l^{-1}$of Fe, respectively (Yadegari, 2023, 2017a, b). The concentrations were similar in other micronutrients). The foliar application was done in three stages once every 10 days (before harvest) in the early morning. The control plants had no any micronutrient and distilled water foliar application. For soil analysis, the soil samples were taken from three randomly selected sites in each plot from 0-15 and 15-30 cm of depth. The samples were homogenized, mixed and passed through a 2 mm filter for determination of soil physical and chemical characteristics (Table 1). Soil moisture was measured by a TDR device (PMS-714, Lutron, Taiwan) following the manufacturers' protocol.

Experimental conditions

This investigation was done and repeated in two years from spring (May) 2022 to fall (September) 2023 at the Research Farm of Islamic Azad University, Shahrekord Branch, Iran. Based on the Köppen climate classification, the climate of the area of study is classified as cold and semiarid. The present study was conducted based on a randomized complete block design (RCBD) with three replications and factorial layout. In each year, treatments were applied in stage of plants had 4-8 leaves in 3 steps by 1 week interval. The soil (typic calci xerocrepts) physical and chemical properties and climatic properties of the region are listed in Table 1 and 2 respectively. The top-soil of the experimental plot area was kept moist throughout the growing season when necessary.

Plant material and agronomic practices

Seeds of *D.kotschyi* Boiss. (Lamiaceae) were obtained from Forest and Rangeland Institute, Iran. Firstly, the seeds were sterilized and sown on May 2022 and 2023 under greenhouse condition (25°C, light 12/12 day/night, 65% RH). After about 45-50 days from sowing, when the seedlings had 4-6 true leaves with 8-10 cm tall, were transplanted in the experimental field on 20 July. The dimensions of each experimental plot were 4.0 × 3.0 m and the distance between plants on row and replicates were 20 cm and 2 m respectively. No inorganic fertilizer and systemic pesticide were used during the experiment, and weeds control was done manually. Irrigation (in 75-80% F.C., field capacity) was done every 5 to 7 days.

Table 1. Physico-chemical soil properties of research farm in two years.

Characters	Year	
	2022	2023
N_{total} (%)	0.18	0.25
Organic matter (%)	0.75	0.83
pH	7.82	7.73
P (mg·kg^{-1})	16.44	15.55
K (mg·kg^{-1})	312	308
Ca (mg·kg^{-1})	3.45	3.78
Mn (mg·kg^{-1})	8.44	8.34
Fe (mg·kg^{-1})	1.02	1.91
Cu (mg·kg^{-1})	0.55	0.43
Zn (mg·kg^{-1})	0.82	0.79
EC (dS·m^{-1})	0.62	0.65
Texture	Clay loam	Clay loam

Table 2. Climatic properties of research farm.

Average annual precipitation (mm)	Average of annual temperature (ºC)	Average maximum temperature (ºC)	Average minimum temperature (ºC)	Elevation (m)	Latitude and longitude
331	11.7	23.9	-1.8	2060	32˚19/N-50˚51/E

Preparation of essential oil (EO) extraction

The aerial parts and inflorescences of *D.kotschyi* were hand-harvested at the flowering and then were dried in the shade at room temperature (25±4°C) for two weeks with the moisture content fixed at around 14 to 16%. The samples were ground to fine a powder using a micro hammer cutter mill and passed through a sieve (mesh 20). The essential oil was extracted from 100 g of powdered tissue by hydro-distillation method using the Clevenger-type (made by Glass Fabricating of Ashk-e-Shishe Co., Tehran, Iran) with 500 mL water for 3 h according to the British Pharmacopoeia. Essential oil content was determined by distilling shoots in the Clevenger apparatus. The content of 100 g of deried shoots was placed in 6 L flask capacity Clevenger type distillation apparatus. And distilled for 5 h with 3 L of pure water. The oil content of *D.kotschyi* Boiss. obtained at the end of distillation and measured as mL and % ratios (w/w) then determined by multiplying the oil content with oil density i.e., 0.858. All the essential oil samples were dehydrated over hydrous sodium sulphate and stored at 4°C until analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) analysis. Ground GC analysis was done on an Agilent Technologies 7890 GC equipped with FID and a HP-5MS 5% capillary column. The carrier gas was helium at a flow of 0.8 mL/min. Initial column temperature was 60°C and programmed to increase at 4°C/min to 280°C. The split ratio was 40:1. The injector temperature was set at 300°C. The purity of helium gas was 99.99% and 0.1 mL of each sample was injected manually in the split mode. GC–MS analyses were carried out on a Thermo Finnigan Trace 2000 GC-MS system equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The oven temperature was held at 120°C for 5 min and then programmed to reach 280°C at a rate of 10°C/min. The detector temperature was 260°C and the injector temperature was 260°C. The compositions of the essential oil were identified by comparison of their retention indices relative to a series of *n*-alkanes (C7-C24), retention times and mass spectra with those of authentic samples in Wiley library (Adams, 2007).

Data analysis

After Bartlett test, all data were subjected to complex ANOVA and simple Pearson correlation indices using the statistical computer package SAS v.9 and treatment means separated using LSD's multiple range test at P≤0.05 and P≤0.01 levels.

RESULTS AND DISCUSSION

Essential oil content

The results showed that the effects of foliar-spraying on essential oil content were significant ($p \leq 0.01$) (Table 3). The maximum essential oil content was obtained in the foliar-sprayings of 40 mg.l^{-1} (0.98-0.99%), whereas the minimum (0.59-0.66%) was achieved in the control (Tables 4-6). In this investigation, the use of 40 mg.l^{-1} improved the content of *D.kotschyi* essential oil.

Chemical composition of the essential oil

According to the results of the chemical analysis of the essential oils from *D.kotschyi* by GC/MS, the most important chemical compounds that determine the quality of *D.kotschyi* essential oil including neral (9.02-14.11%), limonene (25.4-31.6%), geranial (8.6-15.2%), eucalyptol (3.89-6.32%), myrtenol (22.5-26.95%), α-pinene (1.34-3.08%), α-campholene aldehyde (1.16-2.99%) and geranic acid methyl ester (0.67-1.6%) were identified (Tables 4-6). Limonene belonging to monoterpene hydrocarbons was the predominant constituent of the *D.kotschyi* essential oil (Tables 4-6). The simple effects of the foliar application of each micronutrient were significant on the percentages of some of the major compounds of the essential oil (Table 3). In general, the foliar application of micronutrients in 40 mg.l^{-1} increased the percentage of all compounds compared to the control (Tables 4-6). The interaction effects of micronutrients (Fe, Zn, Cu and Mn) on the concentrations of constituents of essential oil such as neral, geranial, limonene, Myrtenol and eucalyptol ($p \leq 0.01$) was significant (Table 3). The highest levels of neral, limonene, eucalyptol, myrtenol and geranial were obtained under 40 mg.l^{-1} treatments (14.11, 31.6, 15.2, 6.32 and 26.95%, respectively) (Tables 4-6). In this study, the quality of essential oil, which is expressed as a percentage of the chemical compounds, showed a significant increase under concentration of micronutrients such as higher concentration of micronutrients than 40 mg.l^{-1} in most components, had decreasing effects. According to obtained results, application of micronutrients significantly influenced the essential oil content/composition of *D.kotschyi*, however the assessed traits showed slightly variation during the studied years. In most of measured characters, plants treated with 20 and 40 mg.l^{-1} were in the same group. Amounts of some characters in plants treated with 60 mg.l^{-1} were less than control plants. The main constituents of essential oil were neral, limonene, myrtenol, geranial and eucalyptol (alcoholic monoterpenes) that made the most components of essential oil plants. Applied combination of micronutrients showed better effectiveness on essential oil content and composition of *D.kotschyi* and the highest essential oil content was obtained from the treated plants by 20 and 40 mg.l^{-1} of these micronutrients. According to the biennial results of the chemical analysis of the essential oils from *D.kotschyi* by GC/MS, the most important chemical compounds that determine the quality of *D.kotschyi* essential oil including neral, limonene, geranial, eucalyptol and myrtenol were identified. Finally, the application of micronutrients at 40 mg.l^{-1} can be a good strategy to improve morpho-physiological characters and essential oil quantity and quality of *D.kotschyi* in cold and semi-arid climates. The results obtained from GC-MS indicated the presence of 14 components in the essential oil of *D.kotschyi* and in this regard, significant differences in chemical compositions of essential oil were observed between treatments (Table 3). Although in some main treatments such as foliar application of Cu and Mn, no significant difference in chemical compositions of *D.kotschyi* essential oil was found, however, in combined treatments there were differences in most compositions of essential oil and in this regard, the $Fe_2Cu_2Mn_2Zn_2$ treatment was the best treatment (Tables 4-6). The main components in the essential oil of all plants treated by micronutrients included α-pinene, P-cymene, limonene, eucalyptol, δ-terpinene, myrtenol, neral, geraniol and α.campholene aldehyde. The most amounts in some of constituents such as eucalyptol, p-cymene, carvacrol and geranyl acetate were made by plants that treated by 40 $mg.l^{-1}$ of Fe, Zn and Mn but in another components of essential oil, the most amounts were obtained by 40 $mg.l^{-1}$ of Fe, Zn, Mn and Cu (Tables 4-6). Treatments of 60 $mg.l^{-1}$ of micronutrients in many of constituents had the same group with control plants and the lowest amount of components made by this treatment. In some cases such as the lowest amounts of geranic acid, neral, geraniol, and geranial made by Mn or Cu in 20 $mg.l^{-1}$, but generally the control plants and plants treated with combination of 60 $mg.l^{-1}$of Fe, Mn, Zn and Cu in two cultivation seasons made the lowest levels of essential oil content and compositions (Tables 4-6). By comparing the data of two years, there were positive correlations between essential oil content and main components and a high correlation was observed between essential oil content and α-pinene, p-cymene, limonene, eucalyptol, δ-terpinene, myrtenol, neral, geraniol and α.campholene aldehyde (Table 7). The application of concentration of 20 $mg.l^{-1}$micronutrients improved most of the content and compositions of components but at higher concentrations (i.e. 60 $mg.l^{-1}$), the content and compositions in all treated plants were decreased. The mean content (%) of many chemical compositions in *D.kotschyi* were lower than those of the control treatment when the plants were sprayed with concentrations of 60 $mg.l^{-1}$. It seems that the content and composition of essential oil were more affected by Zn and Fe compared to other micronutrients (Tables 4-6).

Table 3. Complex ANOVA of variation of essential oil content and main compositions in *D.kotschyi* by different micronutrients.

SOV^{z}	df^{y}	Neral	Limonene	α.Pinene	Carvacrol	Caryophyllene	δ-Terpinene	P-Cymene
Year(Y)	1	14.1**	0.008^{ns}	27.2**	7.7^{ns}	1.4^{ns}	2.72**	15.1**
R/Y	4	0.4	0.009	1.3	8.7	3.1	0.8	0.2
Copper (Cu)	3	0.5^{ns}	0.11**	1.1^{ns}	32.5**	21.9**	24.8**	21.3**
Manganese (Mn)	3	5.2**	0.03^{ns}	14.4**	2.6^{ns}	21.6**	12.8**	31.1**
Iron (Fe)	3	4.9**	0.4**	2.7^{ns}	32.8**	9.8**	22.9**	21.4**
Zinc (Zn)	3	22.2**	0.29**	2.9^{ns}	22.6**	21.5**	12.3**	11.9**
Cu × Mn	9	54.2**	0.9**	14.4**	12.9**	21.1**	23.8**	32.8**
Cu × Fe	9	32.4**	0.7**	8.9**	16.8**	11.4**	23.9**	25.4**
Cu × Zn	9	23.8**	0.21**	8.7**	13.1**	13.3**	17.8**	22.1**
Mn × Fe	9	27.1**	0.51**	9.1**	19.9**	11.9**	27.7**	14.1**
Mn × Zn	9	3.1**	0.3**	10.7**	12.8**	14.9**	23.7**	24.9**
Fe × Zn	9	7.4**	0.4**	7.8**	10.1**	13.9**	23.5**	23.2*
Cu × Mn × Fe	27	2.1**	0.2**	8.91**	12.5**	21.4**	0.94^{ns}	27.8**
Cu × Mn × Zn	27	1.6**	0.8**	14.6**	10.8**	6.1**	1.2^{ns}	21.9**
Cu × Fe × Zn	27	2.22**	0.9**	1.2^{ns}	11.4**	0.9^{ns}	0.2^{ns}	0.7^{ns}
Fe × Zn × Mn	27	2.6**	0.011^{ns}	1.9^{ns}	15.6**	0.7^{ns}	0.1^{ns}	0.8^{ns}
Cu × Zn × Mn × Fe	81	0.32^{ns}	0.01^{ns}	1.7^{ns}	1.9^{ns}	0.1^{ns}	0.8^{ns}	0.9^{ns}
T(Cu,Zn,Mn,Fe) × Y	255	0.18^{ns}	0.021^{ns}	0.5^{ns}	0.42^{ns}	0.22^{ns}	0.3^{ns}	0.28^{ns}
E	1020	0.37	0.02	1.5	1.3	0.88	1.1	1.4
CV^{x}		2.2	3.3	7.2	4.7	6.5	4.5	3.1

^{z} SOV: source of variation, ^{y}df: degree of freedom, ^{x}CV: coefficient of variation, *, ** significant at P=0.05 and P=0.01 levels of probability respectively.

Table 3. (*Continued*).

SOV^{z}	df^{y}	Myrtenol	Eucalyptol	α.campholene aldehyde	Geranic acid	Geranyl acetate	Geraniol	Geranial	Essential oil
Year(Y)	1	3.3^{ns}	0.006^{ns}	18.2**	24.2**	0.88^{ns}	12.55**	0.99^{ns}	1.7**
R/Y	4	2.2	0.007	1.3	1.2	1.7	1.5	1.1	0.1
Copper (Cu)	3	1.1^{ns}	0.71**	25.1**	17.5**	33.6**	31.5**	0.32^{ns}	4.1**
Manganese (Mn)	3	15.2**	0.65**	14.4**	19.1**	21.8**	12.8**	26.1**	3.8**
Iron (Fe)	3	24.9**	0.25**	21.4**	42.8**	9.9**	18.9**	17.4**	4.5**
Zinc (Zn)	3	33.2**	0.36**	31.1**	31.6**	17.5**	12.9**	20.9**	3.3**
Cu × Mn	9	24.2**	0.44**	11.4**	11.9**	14.1**	31.8**	22.8**	8.9**
Cu × Fe	9	22.4**	0.87**	15.9**	13.8**	9.4**	25.9**	19.4**	14.8**
Cu × Zn	9	14.8**	0.29**	14.7**	15.1**	8.3**	18.8**	27.1**	11.1**
Mn × Fe	9	10.1**	0.8**	12.1**	22.9**	26.9**	17.7**	15.1**	4.7**
Mn × Zn	9	11.1**	0.8**	8.7**	12.8**	22.9**	14.7**	18.9**	8.8**
Fe × Zn	9	27.4**	1.4**	9.8**	14.1**	14.9**	8.5**	29.2**	12.1**
Cu × Mn × Fe	27	32.1**	1.2**	9.9**	9.5**	15.4**	0.31^{ns}	17.8**	4.5**
Cu × Mn × Zn	27	0.6^{ns}	2.8**	24.6**	13.8**	11.1**	0.58^{ns}	14.9**	8.1**
Cu × Fe × Zn	27	0.22^{ns}	1.8**	0.2^{ns}	14.4**	0.14^{ns}	0.42^{ns}	0.88^{ns}	9.4**
Fe × Zn × Mn	27	0.66^{ns}	0.015^{ns}	1.02^{ns}	12.6**	0.8^{ns}	0.21^{ns}	0.55^{ns}	1.4**
Cu × Zn × Mn × Fe	81	0.72^{ns}	0.014^{ns}	0.7^{ns}	0.8^{ns}	0.9^{ns}	0.18^{ns}	0.44^{ns}	0.008^{ns}
T(Cu,Zn,Mn,Fe)×Y	255	0.55^{ns}	0.011^{ns}	0.8^{ns}	0.66^{ns}	0.41^{ns}	0.44^{ns}	0.21^{ns}	0.042^{ns}
E	1020	0.88	0.01	1.1	1.3	1.02	0.88	0.91	0.072
CV^{x}		5.8	7.9	4.3	3.7	2.8	4.5	3.9	2.8

^{z} SOV: source of variation, ^{y}df: degree of freedom, ^{x}CV: coefficient of variation, *, ** significant at P=0.05 and P=0.01 levels of probability respectively.

Table 4. Means of essential oil content and composition (%) in *D.kotschyi* plants affected by micronutrients (20 mg.l^{-1}).

Year	Compound	RI^{z}	Fe^{y}	Zn	Cu	Mn	Fe×Zn	Cu×Fe	Mn×Fe	Cu×Zn
2022	α-Pinene	940	**1.8±0.01^{d}	1.4±0.02^{e}	1.37±0.03^{e}	1.39±0.02^{e}	1.41±0.01^{e}	1.4±0.02^{e}	2.9±0.02^{a}	2.4±0.02^{c}
	P-Cymene	1026	1.41±0.1^{e}	1.42±0.01^{e}	1.55±0.02^{e}	1.53±0.01^{e}	1.44±0.04^{e}	1.6±0.01^{e}	1.43±0.02^{e}	1.8±0.01^{d}
	Limonene	1033	28.8±0.8^{b}	27.7±0.9^{c}	29.8±0.5^{c}	27.7±0.8^{c}	28.7±0.9^{b}	28.4±0.9^{b}	30.1±0.8^{a}	30.5±1.1^{a}
	Eucalyptol	1035	4.7±0.1^{c}	3.94±0.1^{d}	3.95±0.1^{d}	3.99±0.2^{cd}	4.6±0.08^{c}	4.01±0.1^{c}	5.1±0.1^{b}	4.04±0.1^{c}
	δ-Terpinene	1062	1.57±0.1^{c}	1.59±0.2^{c}	1.61±0.2^{c}	1.63±0.2^{c}	1.64±0.3^{c}	1.59±0.31^{c}	1.9±0.3^{c}	1.59±0.2^{c}
	α campholene	1110	1.18±0.1^{d}	1.21±0.01^{d}	1.22±0.01^{d}	1.25±0.02^{d}	1.77±0.01^{c}	1.9±0.02^{c}	1.22±0.01^{d}	1.7±0.03^{c}
	Myrtenol	1202	22.66±1.1^{d}	22.76±0.9^{d}	23.03±0.8^{cd}	24.1±0.5^{b}	25.1±0.5^{b}	26.2±0.4^{ab}	25.1±0.7^{b}	23.1±0.5^{c}
	Neral	1239	11.7±0.1^{c}	10.7±0.8^{d}	9.02±0.7^{e}	10.44±0.6^{d}	11.66±0.8^{c}	11.1±0.8^{cd}	11.1±0.9^{cd}	10.5±0.5^{d}
	Geraniol	1257	0.8±0.01^{c}	0.77±0.01^{c}	0.66±0.03^{d}	0.7±0.02^{cd}	0.9±0.01^{c}	0.8±0.01^{c}	1.2±0.02^{b}	1.3±0.02^{b}
	Geranial	1270	11.1±0.8^{c}	8.66±0.9^{e}	8.66±0.7^{e}	8.6±0.5^{e}	8.82±0.1^{e}	11.3±0.2^{c}	11.5±0.3^{c}	12.5±0.5^{b}
	Carvacrol	1298	0.8±0.01^{c}	0.8±0.01^{c}	0.7±0.02^{c}	0.2±0.02^{d}	0.7±0.01^{c}	0.52±0.01^{d}	0.9±0.02^{c}	1.1±0.03^{b}
	Geranic acid	1320	0.67±0.1^{c}	0.69±0.01^{c}	0.7±0.02^{c}	0.72±0.01^{c}	0.73±0.02^{c}	1.1±0.01^{b}	0.9±0.02^{b}	1.3±0.03^{ab}
	Geranyl acetate	1385	0.88±0.1^{cd}	0.9±0.02^{c}	0.91±0.01^{c}	0.92±0.01^{c}	0.93±0.04^{c}	0.94±0.03^{c}	0.9±0.01^{c}	0.95±0.1^{c}
	Caryophyllene	1418	1.56±0.1^{b}	1.6±0.01^{b}	1.61±0.02^{b}	1.7±0.01^{b}	1.72±0.02^{b}	1.81±0.01^{b}	1.71±0.01^{b}	1.82±0.1^{b}
	Monoterpene hydrocarbons		33.58±0.9^{c}	32.11±0.8^{c}	34.33±1.2^{c}	32.25±1.3^{c}	33.19±0.9^{c}	32.99±1.1^{c}	36.33±1.2^{b}	36.29±0.9^{b}
	Oxygenated monoterpenes		52.94±1.5^{d}	48.84±1.2^{f}	47.24±1.4^{f}	49.28±1.6^{f}	53.55±1.8^{d}	55.83±1.7^{c}	56.12±1.6^{c}	54.24±1.5^{d}
	Sesquiterpenes		1.56±0.1^{c}	1.6±0.01^{c}	1.61±0.02^{c}	1.7±0.01^{bc}	1.72±0.02^{b}	1.81±0.01^{b}	1.71±0.01^{b}	1.82±0.1^{b}
	Essential oil content (w/w%, g/100g fresh weight basis)		0.69±0.1^{c}	0.71±0.02^{c}	0.73±0.01^{c}	0.7±0.02^{c}	0.68±0.04^{c}	0.81±0.01^{b}	0.83±0.02^{b}	0.8±0.01^{b}

Year	Compound	Mn×Zn	Cu×Mn	Cu×Fe×Zn	Fe×Zn×Mn	Cu×Mn×Fe	Cu×Mn×Zn	Cu×Zn×Mn×Fe	Control
2022	α-Pinene	2.5±0.01^{b}	2.1±0.02^{c}	3.2±0.02^{a}	3.1±0.01^{a}	3.04±0.02^{a}	3.03±0.01^{a}	2.1±0.02^{c}	1.34±0.01^{e}
	P-Cymene	1.9±0.03^{d}	1.5±0.01^{e}	2.5±0.02^{c}	2.5±0.02^{c}	2.4±0.04^{c}	2.8±0.01^{b}	3.1±0.02^{b}	1.41±0.01^{e}
	Limonene	30.2±0.9^{a}	30.5±0.8^{a}	31.6±0.8^{a}	30.6±0.7^{a}	28.8±0.9^{b}	28.8±0.8^{b}	29.7±0.7^{b}	25.5±0.8^{d}
	Eucalyptol	5.4±0.02^{b}	4.4±0.01^{c}	6.6±0.02^{a}	4.5±0.02^{c}	4.9±0.03^{c}	4.7±0.01^{c}	5.7±0.02^{b}	3.93±0.04^{d}
	δ-Terpinene	1.9±0.2^{c}	1.9±0.3^{c}	2.4±0.2^{c}	2.3±0.2^{c}	3.3±0.3^{b}	2.5±0.2^{bc}	2.73±0.2^{b}	1.55±0.2^{cd}
	α campholene	1.8±0.2^{c}	1.3±0.2^{d}	1.6±0.3^{c}	1.2±0.2^{d}	1.8±0.2^{c}	2.7±0.21^{a}	1.81±0.2^{c}	1.16±0.1^{d}
	Myrtenol	23.9±0.6^{bc}	22.7±0.9^{cd}	22.7±0.8^{cd}	23.9±0.7^{c}	24.7±0.8^{b}	23.1±0.9^{c}	24.1±0.8^{b}	22.5±0.7^{d}
	Neral	11.8±0.3^{c}	9.2±0.4^{e}	10.5±0.5^{d}	12.8±0.4^{b}	12.7±0.8^{b}	11.7±0.9^{c}	11.6±0.8^{c}	9.3±0.1^{e}
	Geraniol	0.8±0.01^{c}	1.2±0.02^{b}	1.7±0.02^{a}	1.2±0.01^{b}	0.9±0.02^{c}	0.8±0.01^{c}	0.81±0.02^{c}	0.6±0.01^{d}
	Geranial	11.9±0.7^{c}	13.7±0.8^{a}	12.7±0.6^{b}	12.9±0.5^{b}	13.7±0.7^{a}	13.1±0.7^{b}	12.1±0.6^{bc}	9.9±0.8^{d}
	Carvacrol	0.8±0.03^{c}	0.7±0.02^{c}	0.7±0.04^{c}	0.7±0.02^{c}	0.51±0.01^{c}	1.1±0.01^{b}	1.5±0.01^{ab}	0.23±0.03^{d}
	Geranic acid	0.71±0.01^{c}	0.73±0.03^{c}	0.97±0.02^{bc}	0.9±0.01^{c}	1.1±0.01^{b}	1.6±0.02^{a}	0.11±0.01^{e}	0.68±0.01^{c}
	Geranyl acetate	0.89±0.02^{c}	0.91±0.02^{c}	1.1±0.01^{bc}	0.6±0.01^{d}	0.94±0.02^{c}	0.7±0.01^{d}	0.93±0.01^{c}	0.87±0.02^{cd}
	Caryophyllene	1.86±0.01^{b}	1.89±0.03^{b}	1.11±0.01^{c}	1.93±0.01^{ab}	0.34±0.01^{e}	1.2±0.01^{c}	0.4±0.03^{e}	1.55±0.02^{b}
	Monoterpene hydrocarbons	36.5±0.9^{b}	36±0.81^{b}	39.7±1.1^{a}	38.5±0.85^{a}	39.54±0.76^{a}	37.13±0.9^{b}	37.63±1.1^{b}	29.8±0.65^{e}
	Oxygenated monoterpenes	56.4±1.5^{c}	53.2±1.2^{d}	56.5±1.6^{c}	57.2±1.7^{c}	57.21±1.4^{c}	57.2±1.3^{c}	57.62±1.4^{c}	47.62±0.9^{g}
	Sesquiterpenes	1.86±0.01^{b}	1.89±0.03^{b}	1.11±0.01^{d}	1.93±0.01^{b}	1.34±0.01^{d}	1.2±0.01^{d}	1.4±0.03^{c}	1.55±0.02^{c}
	Essential oil content (w/w%, g/100g fresh weight basis)	0.8±0.03^{b}	0.7±0.01^{b}	0.9±0.02^{a}	0.81±0.01^{b}	0.8±0.01^{b}	0.83±0.01^{b}	0.91±0.01^{a}	0.66±0.01^{cd}

Year	Compound	Fe	Zn	Cu	Mn	Fe×Zn	Cu×Fe	Mn×Fe	Cu×Zn
2023	α-Pinene	2.66±0.02^{b}	2.32±0.1^{c}	2.25±0.1^{c}	2.33±0.08^{c}	2.14±0.01^{c}	2.22±0.1^{c}	2.65±0.1^{b}	2.53±0.02^{b}
	P-Cymene	1.85±0.01^{d}	1.99±0.1^{d}	1.66±0.1^{e}	1.88±0.02^{d}	1.78±0.08^{d}	1.71±0.1^{d}	1.81±0.1^{d}	1.92±0.03^{d}
	Limonene	27.7±0.9^{c}	26.5±0.8^{c}	29.8±0.7^{b}	26.6±0.8^{c}	28.9±0.9^{b}	27.9±0.8^{c}	29.9±0.8^{b}	30.68±0.7^{a}
	Eucalyptol	3.92±0.1^{d}	4.01±0.1^{c}	4.03±0.1^{c}	3.89±0.1^{d}	4.55±0.12^{c}	3.91±0.1^{d}	4.92±0.1^{c}	4.03±0.11^{c}
	δ-Terpinene	2.66±0.3^{b}	2.88±0.2^{b}	2.82±0^{b}	2.78±0.3^{b}	3.02±0.2^{b}	2.98±0.3^{b}	3.14±0.2^{ab}	3.44±0.3^{ab}
	α campholene	1.7±0.2^{c}	1.8±0.2^{c}	1.6±0.3^{c}	1.5±0.2^{cd}	1.99±0.2^{bc}	2.4±0.3^{b}	2.1±0.2^{b}	2.2±0.3^{b}
	Myrtenol	24.15±0.9^{b}	24.6±0.8^{b}	25.6±0.9^{ab}	26.1±0.8^{a}	26.6±0.7^{a}	27.3±0.8^{a}	26.5±0.9^{a}	24.5±0.8^{b}
	Neral	11.7±0.5^{c}	10.7±0.6^{d}	10.6±0.7^{d}	10.1±0.8^{d}	11.66±0.6^{c}	11.1±0.7^{c}	11.1±0.4^{c}	10.5±0.8^{d}

	Geraniol	0.8±0.01^{c}	0.7±0.01^{c}	0.62±0.1^{d}	0.83±0.02^{c}	0.92±0.01^{c}	0.8±0.01^{c}	1.2±0.02^{b}	1.3±0.01^{b}
	Geranial	11.05±0.9^{c}	12.6±0.7^{b}	12.3±0.7^{b}	12.8±0.4^{b}	11.16±0.6^{c}	11.3±0.5^{c}	12.5±0.4^{b}	12.5±0.6^{b}
	Carvacrol	0.93±0.01^{c}	0.94±0.1^{c}	0.95±0.1^{c}	0.97±0.01^{c}	0.99±0.01^{bc}	1.01±0.1^{b}	1.02±0.2^{b}	1.1±0.01^{b}
	Geranic acid	0.9±0.02^{c}	0.92±0.1^{c}	0.94±0.1^{c}	0.96±0.01^{c}	0.97±0.01^{c}	0.98±0.1^{c}	1.01±0.1^{b}	1.03±0.02^{b}
	Geranyl acetate	1.5±0.01^{ab}	1.6±0.01^{a}	1.44±0.01^{b}	0.5±0.01^{d}	1.43±0.03^{b}	1.33±0.1^{b}	0.9±0.02^{c}	1.3±0.03^{b}
	Caryophyllene	0.8±0.02^{d}	0.6±0.01^{d}	1.61±0.01^{b}	0.7±0.01^{d}	0.66±0.01^{d}	0.9±0.02^{d}	0.1±0.01^{e}	0.7±0.02^{d}
	Monoterpene hydrocarbons	34.87±0.9^{c}	33.69±0.8^{c}	36.53±0.9^{b}	33.59±0.8^{b}	35.84±0.76^{b}	34.81±0.9^{b}	37.5±0.82^{b}	38.57±1.1^{a}
	Oxygenated monoterpenes	54.25±1.5^{cd}	55.35±1.4^{c}	55.7±1.2^{c}	56.19±1.3^{c}	57.87±1.2^{bc}	57.82±1.1^{bc}	59.34±1.4^{b}	56.13±0.9^{c}
	Sesquiterpenes	0.8±0.02^{e}	0.6±0.01^{f}	1.61±0.01^{c}	0.7±0.01^{e}	0.66±0.01^{f}	0.9±0.02^{e}	1.1±0.01^{d}	0.7±0.02^{e}
	Essential oil content (w/w%, g/100g fresh weight basis)	0.5±0.01^{e}	0.41±0.01^{f}	0.4±0.02^{f}	0.58±0.01^{d}	0.7±0.04^{c}	0.7±0.01^{c}	0.81±0.02^{b}	0.9±0.02^{a}
Year	Compound	Mn×Zn	Cu×Mn	Cu×Fe×Zn	Fe×Zn×Mn	Cu×Mn×Fe	Cu×Mn×Zn	Cu×Zn×Mn×Fe	Control
2023	α-Pinene	2.47±0.03^{c}	2.25±0.1^{c}	3.08±0.01^{a}	3.06±0.02^{a}	2.11±0.01^{c}	2.55±0.02^{b}	2.99±0.03^{a}	2.01±0.1^{c}
	P-Cymene	1.77±0.1^{d}	1.79±0.2^{d}	1.44±0.03^{e}	2.33±0.1^{c}	2.66±0.1^{c}	1.43±0.02^{e}	1.91±0.01^{d}	1.55±0.01^{e}
	Limonene	29.98±0.8^{ab}	31.1±0.65^{a}	30.65±1.2^{a}	31.01±0.64^{a}	30.8±0.98^{a}	29.9±0.69^{b}	30.09±0.9^{a}	26.01±0.8^{c}
	Eucalyptol	5.22±0.01^{b}	4.66±0.02^{c}	5.54±0.03^{b}	5.45±0.01^{b}	4.76±0.02^{c}	4.81±0.01^{c}	4.44±0.03^{c}	4.49±0.01^{c}
	δ-Terpinene	3.77±0.3^{a}	3.88±0.3^{a}	3.66±0.3^{a}	2.44±0.2^{c}	2.12±0.1^{c}	2.44±0.2^{c}	2.12±0.2^{c}	2.61±0.3^{b}
	α campholene	1.8±0.2^{c}	1.55±0.2^{c}	1.61±0.1^{c}	1.91±0.2^{c}	2.1±0.2^{b}	2.12±0.2^{b}	2.55±0.3^{ab}	1.43±0.2^{d}
	Myrtenol	24.9±0.4^{b}	25.7±0.5^{a}	24.8±0.7^{b}	25.1±0.8^{ab}	25.5±0.7^{ab}	25.1±0.8^{b}	24.1±0.7^{b}	23.1±0.6^{c}
	Neral	11.1±0.8^{c}	11.4±0.5^{c}	11.8±0.8^{c}	10.1±0.5^{d}	10.3±0.8^{d}	10.7±0.6^{d}	10.9±0.9^{d}	10.12±0.9^{d}
	Geraniol	0.96±0.01^{c}	1.2±0.02^{b}	1.6±0.02^{a}	1.85±0.01^{a}	1.83±0.02^{a}	1.77±0.01^{a}	1.79±0.02^{a}	0.99±0.01^{bc}
	Geranial	11.6±0.6	11.9±0.8	12.1±0.7	12.4±0.8	12.3±0.9	12.6±0.8	12.81±0.7	10.99±0.8
	Carvacrol	1.1±0.1^{b}	1.2±0.02^{b}	1.1±0.01^{b}	1.66±0.02^{a}	0.99±0.01^{bc}	1.3±0.01^{a}	1.91±0.01^{a}	0.91±0.02^{c}
	Geranic acid	1.1±0.01^{b}	0.14±0.03^{e}	0.91±0.02^{c}	0.77±0.01^{c}	0.4±0.01^{d}	0.32±0.02^{e}	0.41±0.01^{d}	0.89±0.01^{c}
	Geranyl acetate	1.87±0.02^{a}	0.88±0.02^{c}	0.44±0.01^{e}	0.32±0.01^{e}	0.25±0.02^{e}	0.41±0.01^{e}	0.01±0.01^{f}	1.21±0.02^{b}
	Caryophyllene	0.88±0.01^{d}	1.91±0.03^{b}	0.81±0.01^{d}	0.91±0.01^{d}	0.69±0.01^{d}	0.77±0.01^{d}	0.88±0.03^{d}	0.97±0.02^{d}
	Monoterpene hydrocarbons	37.99±0.9^{ab}	39.02±0.85^{a}	38.83±1.1^{a}	38.84±1.2^{a}	37.69±1.3^{b}	36.32±1.1^{b}	37.11±1.4^{b}	32.18±0.9^{c}
	Oxygenated monoterpenes	56.58±1.4^{c}	57.61±1.2^{bc}	58.55±1.6^{b}	58.38±1.5^{b}	57.78±1.2^{b}	58.4±1.5^{b}	58.5±1.6^{b}	52.03±0.9^{d}
	Sesquiterpenes	1.88±0.01^{b}	1.91±0.03^{b}	0.81±0.01^{e}	0.91±0.01^{e}	0.69±0.01^{f}	0.77±0.01^{e}	0.88±0.03^{e}	0.97±0.02^{e}
	Essential oil content (w/w%, g/100g fresh weight basis)	0.8±0.03^{b}	0.7±0.01^{c}	0.9±0.02^{a}	0.81±0.01^{b}	0.8±0.01^{b}	0.83±0.01^{b}	0.85±0.01^{b}	0.66±0.01^{cd}

^{z} RI: Retention Indices, as determined with FID and HP-5MS 5% capillary column using a series of the standards of C7-C30 saturated n-alkanes. ^{y} Values are means of triplicates ± standard deviation (p <0.05)

**Numbers in each row that have same letter, have same group.

Table 5. Means of essential oil content and composition (%) in *D.kotschyi* plants affected by micronutrients (40 mg.l^{-1}).

Year	Compound	RI ^{z}	Fe ^{y}	Zn	Cu	Mn	Fe×Zn	Cu×Fe	Mn×Fe	Cu×Zn
2022	α-Pinene	940	**1.81±0.01^{d}	1.44±0.02^{e}	1.45±0.03^{e}	1.48±0.02^{e}	1.55±0.08^{d}	1.66±0.02^{d}	2.15±0.02^{c}	1.38±0.01^{e}
	P-Cymene	1026	1.9±0.01^{d}	1.71±0.01^{d}	1.82±0.02^{d}	1.94±0.01^{d}	2.1±0.04^{d}	2.41±0.01^{c}	2.38±0.02^{c}	1.91±0.02^{d}
	Limonene	1033	26.2±0.8^{c}	27.9±0.9^{c}	28.82±0.5^{c}	28.99±0.8^{c}	29.9±0.6^{ab}	28.87±0.9^{b}	27.67±0.8^{c}	28.44±0.8^{b}
	Eucalyptol	1035	5.11±0.1^{b}	4.41±0.1^{c}	4.45±0.08^{c}	4.71±0.06^{c}	5.81±0.08^{ab}	5.91±0.02^{a}	5.01±0.02^{b}	5.12±0.02^{b}
	δ-Terpinene	1062	0.9±0.01^{d}	1.1±0.07^{d}	1.12±0.08 ^{d}	1.13±0.06 ^{d}	0.91±0.09 ^{d}	1.14±0.2 ^{d}	2.11±0.2^{c}	1.41±0.2^{d}
	α campholene	1110	1.88±0.2^{c}	1.91±0.2^{c}	1.77±0.2^{c}	1.67±0.2^{c}	2.99±0.3^{a}	2.99±0.1^{a}	2.51±0.2^{ab}	2.81±0.3^{a}
	Myrtenol	1202	23.1±1.1^{c}	24.91±0.9^{b}	25.9±0.8^{ab}	24.8±0.7^{b}	25.21±0.8^{b}	26.3±0.7^{a}	26.65±0.6^{a}	26.88±0.8^{a}
	Neral	1239	12.17±0.6^{b}	11.1±0.9^{c}	11.45±0.8^{c}	11.21±0.8^{c}	11.8±0.7^{c}	12.1±0.6^{b}	12.21±0.5^{b}	12.32±0.9^{b}
	Geraniol	1257	0.9±0.01^{c}	0.92±0.01^{c}	0.95±0.03^{c}	0.97±0.02^{c}	1.01±0.01^{b}	1.03±0.02^{b}	1.05±0.02^{b}	1.16±0.03^{b}
	Geranial	1270	11.1±0.5^{c}	9.6±0.8^{d}	10.6±0.9^{c}	11.4±0.7^{c}	11.6±0.6^{c}	12.3±0.9^{b}	14.5±0.8^{a}	13.5±0.7^{ab}
	Carvacrol	1298	0.99±0.01^{bc}	0.89±0.01^{c}	0.69±0.02^{c}	0.83±0.02^{c}	0.87±0.01^{c}	0.76±0.01^{c}	0.92±0.02^{c}	0.99±0.01^{c}
	Geranic acid	1320	0.88±0.01^{c}	0.99±0.01^{bc}	0.14±0.02^{e}	0.11±0.01^{e}	0.88±0.03^{c}	0.99±0.01^{c}	0.98±0.02^{c}	0.26±0.03^{e}
	Geranyl acetate	1385	0.96±0.02^{c}	0.99±0.01^{c}	0.96±0.02^{c}	0.99±0.02^{c}	0.81±0.01^{d}	0.1±0.03^{e}	0.45±0.01^{e}	0.78±0.02^{d}
	Caryophyllene	1418	0.97±0.02^{d}	0.77±0.01^{d}	0.79±0.02^{d}	0.65±0.03^{d}	0.89±0.01^{d}	0.77±0.02^{d}	0.51±0.01^{cd}	0.62±0.02^{d}
	Monoterpene hydrocarbons		27.1±0.8^{f}	32.15±0.9^{c}	33.21±0.75^{c}	35.54±0.8^{b}	34.46±1.1^{c}	34.08±0.9^{c}	34.31±0.8^{c}	33.14±0.7^{c}
	Oxygenated monoterpenes		55.25±1.2^{c}	53.74±1.1^{d}	55.81±1.3^{c}	55.59±1.1^{c}	59.29±1.5^{ab}	61.39±1.4^{a}	62.85±1.8^{a}	62.78±1.5^{a}
	Sesquiterpenes		0.97±0.02^{e}	0.77±0.01^{e}	0.79±0.02^{e}	0.65±0.03^{f}	0.89±0.01^{e}	0.77±0.02^{e}	0.51±0.01^{fg}	0.62±0.02^{f}
	Essential oil content (w/w%, g/100g fresh weight basis)		0.77±0.01^{bc}	0.76±0.01^{c}	0.75±0.01^{c}	0.79±0.01^{b}	0.78±0.02^{b}	0.81±0.03^{b}	0.93±0.04^{a}	0.71±0.01^{c}
Year	Compound	RI	Mn×Zn	Cu×Mn	Cu×Fe×Zn	Fe×Zn×Mn	Cu×Mn×Fe	Cu×Mn×Zn	Cu×Zn×Mn×Fe	
2022	α-Pinene	940	2.91±0.01^{a}	1.38±0.02^{e}	2.55±0.03^{b}	2.35±0.02^{c}	2.63±0.03^{b}	2.71±0.02^{b}	0.3±0.02^{g}	
	P-Cymene	1026	2.1±0.01^{d}	2.99±0.01^{b}	2.01±0.02^{d}	2.53±0.09^{c}	2.21±0.04^{c}	2.39±0.1^{c}	1.51±0.1^{e}	
	Limonene	1033	28.87±0.8^{b}	28.1±0.9^{b}	28.11±0.5^{b}	28.14±0.8^{b}	28.88±0.8^{b}	29.1±0.9^{b}	29.12±0.8^{b}	
	Eucalyptol	1035	4.43±0.1^{c}	4.55±0.2^{c}	4.77±0.1^{c}	4.93±0.1^{c}	4.89±0.08^{c}	4.78±0.02^{c}	5.91±0.06^{b}	
	δ-Terpinene	1062	2.78±0.3^{b}	2.99±0.2^{b}	3.04±0.2^{b}	3.15±0.3^{b}	3.14±0.2^{b}	2.36±0.3^{c}	2.01±0.2^{c}	
	α campholene	1110	2.98±0.2^{a}	2.99±0.2^{a}	2.96±0.3^{a}	2.87±0.2^{a}	2.19±0.2^{b}	2.33±0.2^{b}	2.5±0.3^{ab}	
	Myrtenol	1202	25.02±1.1^{b}	25.12±0.9^{b}	25.1±0.6^{b}	25.32±0.8^{b}	25.55±0.9^{b}	25.19±0.8^{b}	26.95±0.9^{a}	
	Neral	1239	12.55±0.7^{b}	12.65±0.8^{b}	12.88±0.9^{b}	12.01±0.8^{b}	12.44±0.9^{b}	12.32±0.6^{b}	13.11±0.6^{a}	
	Geraniol	1257	1.23±0.01^{b}	1.43±0.01^{b}	1.55±0.03^{ab}	1.67±0.02^{a}	1.59±0.01^{a}	1.71±0.01^{a}	1.01±0.02^{b}	
	Geranial	1270	12.3±0.5^{b}	12.41±0.7^{b}	11.02±0.6^{c}	12.3±0.8^{b}	12.91±0.9^{b}	12.88±0.8^{b}	15.2±0.2^{a}	
	Carvacrol	1298	1.87±0.01^{a}	1.78±0.02^{a}	0.91±0.01^{c}	1.02±0.01^{b}	1.17±0.02^{b}	1.21±0.01^{b}	1.34±0.02^{b}	
	Geranic acid	1320	0.89±0.01^{c}	0.91±0.03^{c}	0.99±0.01^{bc}	0.89±0.03^{bc}	0.77±0.02^{c}	0.11±0.05^{e}	0.88±0.02^{c}	
	Geranyl acetate	1385	0.99±0.02^{c}	0.89±0.01^{c}	0.93±0.02^{c}	0.27±0.01^{e}	0.99±0.02^{c}	0.88±0.03^{cd}	0.17±0.01^{e}	
	Caryophyllene	1418	0.89±0.02^{d}	0.91±0.01^{d}	0.77±0.01^{d}	0.88±0.02^{d}	0.93±0.01^{d}	0.3±0.03^{e}	0.77±0.01^{d}	
	Monoterpene hydrocarbons		36.66±0.8^{b}	35.46±0.9^{b}	35.71±0.8^{b}	36.17±1.1^{b}	36.86±1.2^{b}	36.56±11.1^{b}	35.94±1.2^{b}	
	Oxygenated monoterpenes		60.38±1.4^{ab}	60.93±1.2^{a}	59.19±1.3^{b}	59.12±1.7^{b}	60.74±1.8^{a}	60.42±1.4^{a}	62.96±1.6^{a}	
	Sesquiterpenes		0.89±0.02^{e}	0.91±0.01^{e}	0.77±0.01^{e}	0.88±0.02^{e}	0.93±0.01^{e}	0.3±0.03^{g}	0.77±0.01^{e}	
	Essential oil content		0.81±0.03^{b}	0.82±0.01^{b}	0.77±0.01^{bc}	0.81±0.02^{b}	0.91±0.01^{a}	0.88±0.01^{ab}	0.98±0.03^{a}	

(w/w%, g/100g fresh weight basis)

Year	Compound	Fe	Zn	Cu	Mn	Fe×Zn	Cu×Fe	Mn×Fe	Cu×Zn
2023	α-Pinene	2.85±0.01^{b}	2.66±0.02^{b}	2.29±0.03^{c}	2.43±0.02^{c}	2.22±0.08^{c}	2.44±0.02^{c}	1.39±0.02^{e}	1.77±0.01^{d}
	P-Cymene	1.88±0.01^{d}	1.82±0.01^{d}	1.88±0.02^{d}	1.99±0.01^{d}	1.04±0.04^{e}	1.33±0.01^{e}	1.41±0.02^{e}	1.87±0.02^{d}
	Limonene	28.83±0.8^{b}	28.12±0.6^{b}	29.01±0.5^{b}	27.14±0.8^{c}	27.88±0.9^{c}	27.73±0.8^{c}	27.88±0.7^{c}	28.05±0.8^{c}
	Eucalyptol	6.02±0.5^{a}	5.57±0.3^{b}	5.39±0.1^{b}	5.88±0.2^{b}	6.88±0.1^{a}	5.02±0.2^{b}	6.12±0.1^{a}	6.32±0.1^{a}
	δ-Terpinene	2.88±0.3^{b}	2.99±0.2^{b}	2.97±0.2^{b}	3.02±0.2^{b}	3.14±0.3^{b}	3.21±0.2^{b}	3.88±0.2^{a}	3.91±0.3^{a}
	α campholene	1.99±0.2^{bc}	1.88±0.2^{c}	1.85±0.3^{c}	1.79±0.2^{c}	0.84±0.1^{e}	1.66±0.3^{c}	0.77±0.08^{e}	1.99±0.3^{bc}
	Myrtenol	24.12±1.1^{b}	25.16±0.9^{b}	26.1±0.8^{a}	25.91±0.7^{ab}	26.99±0.6^{a}	27.98±0.7^{a}	25.78±0.9^{b}	26.98±0.8^{a}
	Neral	13.12±0.5^{a}	14.11±0.6^{a}	13.44±0.8^{a}	12.23±0.6^{b}	12.55±0.7^{b}	12.82±0.8^{b}	12.99±0.6^{ab}	11.41±0.8^{c}
	Geraniol	0.99±0.01^{bc}	0.91±0.02^{c}	0.96±0.04^{c}	0.88±0.02^{c}	0.78±0.01^{c}	0.91±0.01^{c}	1.11±0.02^{b}	1.19±0.02^{b}
	Geranial	12.03±0.5^{b}	12.33±0.8^{b}	11.9±0.9^{c}	12.88±0.7^{b}	12.44±0.3^{b}	12.98±0.5^{b}	12.99±0.6^{b}	11.44±0.7^{c}
	Carvacrol	1.01±0.01^{b}	1.02±0.02^{b}	0.96±0.01^{c}	0.98±0.03^{c}	0.99±0.01^{bc}	0.97±0.02^{c}	1.1±0.04^{b}	1.03±0.01^{b}
	Geranic acid	0.99±0.01^{bc}	0.95±0.01^{c}	0.97±0.02^{c}	0.02±0.01^{f}	0.12±0.03^{e}	0.15±0.01^{e}	0.21±0.02^{e}	0.27±0.03^{e}
	Geranyl acetate	0.99±0.02^{c}	0.89±0.01^{c}	0.75±0.02^{c}	0.55±0.01^{d}	0.75±0.01^{d}	0.63±0.03^{d}	0.63±0.01^{d}	0.93±0.02^{c}
	Caryophyllene	0.83±0.02^{d}	0.73±0.01^{d}	0.88±0.01^{d}	0.82±0.01^{d}	0.71±0.01^{d}	0.88±0.02^{d}	0.77±0.01^{d}	0.91±0.02^{d}
	Monoterpene hydrocarbons	36.44±0.8^{b}	35.59±0.9^{b}	36.15±0.7^{b}	34.58±0.9^{c}	34.28±0.8^{c}	34.71±0.9^{c}	34.56±0.8^{c}	35.6±0.7^{b}
	Oxygenated monoterpenes	59.28±1.2^{ab}	60.98±1.1^{a}	60.6±1.4^{a}	60.55±1.2^{a}	63.47±1.4^{a}	62.34±1.2^{a}	60.86±1.1^{a}	60.36±1.2^{a}
	Sesquiterpenes	0.83±0.02^{e}	0.73±0.01^{e}	0.88±0.01^{e}	0.82±0.01^{e}	0.71±0.01^{e}	0.88±0.02^{e}	0.77±0.01^{e}	0.91±0.02^{e}
	Essential oil content (w/w%, g/100g fresh weight basis)	0.77±0.01^{b}	0.62±0.01^{d}	0.63±0.01^{d}	0.66±0.02^{cd}	0.79±0.01^{b}	0.88±0.01^{ab}	0.65±0.03^{cd}	0.82±0.01^{b}

Year	Compound	RI	Mn×Zn	Cu×Mn	Cu×Fe×Zn	Fe×Zn×Mn	Cu×Mn×Fe	Cu×Mn×Zn	Cu×Zn×Mn×Fe
2023	α-Pinene	940	2.12±0.01^{c}	2.54±0.02^{b}	2.44±0.03^{c}	2.56±0.02^{b}	2.54±0.08^{b}	2.39±0.02^{c}	2.55±0.02^{b}
	P-Cymene	1026	2.83±0.01^{b}	3.75±0.01^{a}	2.99±0.02^{a}	3.54±0.01^{a}	3.04±0.04^{b}	2.88±0.01^{b}	2.44±0.02^{c}
	Limonene	1033	29.02±0.8^{b}	30.14±0.5^{a}	30.78±0.5^{a}	30.55±0.8^{a}	29.04±0.9^{b}	30.98±0.8^{a}	31.6±0.7^{a}
	Eucalyptol	1035	5.55±0.1^{b}	5.03±0.1^{b}	5.66±0.06^{b}	5.01±0.07^{b}	5.82±0.09^{b}	5.49±0.08^{b}	5.78±0.06^{b}
	δ-Terpinene	1062	3.91±0.31^{a}	3.86±0.2^{a}	2.68±0.2^{b}	3.11±0.2^{b}	3.99±0.3^{a}	3.18±0.3^{b}	3.55±0.3^{ab}
	α campholene	1110	1.87±0.2^{c}	0.71±0.08^{e}	1.84±0.2^{c}	1.91±0.2^{c}	1.97±0.2^{c}	2.22±0.3^{b}	2.12±0.2^{b}
	Myrtenol	1202	24.01±1.1^{b}	24.11±0.9^{b}	24.91±0.8^{b}	24.03±0.7^{b}	24.15±0.5^{b}	24.27±0.89^{b}	25.1±0.5^{ab}
	Neral	1239	12.12±0.6^{b}	11.14±0.7^{c}	11.99±0.6^{bc}	12.31±0.8^{b}	12.51±0.9^{b}	12.78±0.7^{b}	11.31±0.8^{c}
	Geraniol	1257	0.82±0.01^{c}	0.99±0.02^{bc}	1.66±0.02^{a}	1.99±0.01^{a}	1.43±0.01^{b}	1.11±0.01^{b}	1.96±0.01^{a}
	Geranial	1270	12.11±0.7^{b}	12.55±0.8^{b}	12.14±0.9^{b}	12.31±0.7^{b}	12.25±0.8^{b}	11.32±0.7^{c}	12.5±0.9^{b}
	Carvacrol	1298	1.04±0.01^{b}	0.12±0.01^{d}	0.99±0.02^{bc}	0.33±0.02^{d}	0.44±0.01^{d}	0.55±0.01^{c}	0.22±0.02^{d}
	Geranic acid	1320	0.09±0.01^{ef}	1.21±0.01^{b}	0.91±0.02^{c}	0.92±0.01^{c}	0.55±0.03^{d}	0.11±0.01^{e}	0.09±0.01^{ef}
	Geranyl acetate	1385	0.81±0.02^{d}	1.72±0.01^{a}	0.22±0.02^{e}	0.23±0.01^{e}	0.83±0.01^{d}	0.72±0.03^{d}	0.32±0.01^{e}
	Caryophyllene	1418	0.92±0.02^{d}	1.66±0.01^{b}	0.65±0.01^{d}	0.77±0.01^{d}	0.66±0.01^{d}	0.99±0.02^{cd}	0.44±0.1^{e}
	Monoterpene hydrocarbons		37.88±0.8^{ab}	40.29±1.1^{a}	38.89±0.92^{a}	39.76±1.1^{a}	38.61±1.4^{a}	39.43±1.4^{a}	40.14±1.2^{a}
	Oxygenated monoterpenes		57.52±1.4^{c}	54.65±1.5^{cd}	59.19±1.7^{ab}	56.89±1.6^{c}	58.57±1.5^{b}	57.74±1.4^{bc}	58.99±1.6^{b}
	Sesquiterpenes		0.92±0.02^{e}	1.66±0.01^{c}	0.65±0.01^{f}	0.77±0.01^{e}	0.66±0.01^{f}	0.99±0.02^{de}	0.44±0.1^{g}
	Essential oil content (w/w%, g/100g fresh weight basis)		0.81±0.04^{b}	0.81±0.02^{b}	0.88±0.01^{ab}	0.91±0.04^{a}	0.93±0.01^{a}	0.87±0.02^{ab}	0.99±0.01^{a}

[z] RI: Retention Indices, as determined with FID and HP-5MS 5% capillary column using a series of the standards of C7-C30 saturated n-alkanes. [y] Values are means of triplicates ± standard deviation (p <0.05)

**Numbers in each row that have same letter, have same group.

Table 6. Means of essential oil content and composition (%) in *D.kotschyi* plants affected by micronutrients (60 mg.l^{-1}).

Year	Compound	RI [z]	Fe [y]	Zn	Cu	Mn	Fe×Zn	Cu×Fe	Mn×Fe	Cu×Zn
2022	α-Pinene	940	**1.55±0.01^{d}	1.66±0.02^{d}	1.77±0.03^{d}	1.84±0.02^{d}	1.99±0.08^{d}	1.88±0.02^{d}	2.06±0.02^{c}	2.21±0.01^{c}
	P-Cymene	1026	1.77±0.01^{d}	2.85±0.01^{b}	2.67±0.02^{c}	2.81±0.01^{b}	3.39±0.04^{a}	2.98±0.01^{b}	2.96±0.02^{b}	2.11±0.02^{d}
	Limonene	1033	26.56±0.8^{c}	26.6±0.5^{c}	28.41±0.5^{b}	29.62±0.8^{b}	30.12±0.9^{a}	29.21±0.8^{b}	30.31±0.7^{a}	30.44±0.8^{a}
	Eucalyptol	1035	4.48±0.1^{c}	4.39±0.05^{c}	4.18±0.07^{c}	4.34±0.08^{c}	4.31±0.09^{c}	4.31±0.02^{c}	4.71±0.08^{c}	4.39±0.1^{c}
	δ-Terpinene	1062	2.99±0.01^{b}	3.66±0.01^{a}	2.81±0.02^{b}	2.82±0.02^{b}	3.01±0.01^{b}	2.91±0.09^{b}	3.22±0.01^{b}	2.44±0.01^{c}
	α campholene	1110	2.33±0.2^{b}	2.72±0.2^{a}	2.69±0.3^{a}	2.57±0.2^{a}	2.81±0.3^{a}	2.12±0.2^{b}	2.33±0.2^{b}	2.41±0.3^{b}
	Myrtenol	1202	23.02±1.1^{c}	25.16±0.9^{b}	24.88±0.8^{b}	24.88±0.7^{b}	24.9±0.6^{b}	24.02±0.9^{b}	24.31±0.8^{b}	23.77±0.9^{c}
	Neral	1239	10.88±0.8^{d}	10.98±0.5^{cd}	10.33±0.6^{d}	10.08±0.8^{d}	10.88±0.7^{d}	10.82±0.6^{d}	10.84±0.5^{d}	10.06±0.8^{d}
	Geraniol	1257	1.77±0.01^{a}	1.72±0.01^{a}	0.88±0.02^{c}	0.91±0.02^{c}	0.93±0.01^{c}	0.95±0.01^{c}	0.98±0.02^{c}	1.01±0.03^{bc}
	Geranial	1270	12.99±0.7^{b}	12.88±0.5^{b}	12.86±0.5^{b}	12.18±0.7^{b}	11.98±0.8^{c}	11.99±0.9^{c}	10.33±0.8^{d}	11.93±0.7^{b}
	Carvacrol	1298	1.22±0.01^{b}	1.31±0.02^{b}	1.61±0.01^{a}	1.55±0.02^{a}	1.22±0.01^{b}	0.88±0.02^{c}	0.93±0.02^{c}	0.94±0.01^{c}
	Geranic acid	1320	0.99±0.01^{bc}	0.82±0.01^{c}	0.27±0.02^{e}	0.65±0.01^{cd}	0.44±0.03^{d}	0.29±0.01^{e}	0.99±0.02^{bc}	0.01±0.03^{f}
	Geranyl acetate	1385	0.91±0.02^{c}	0.88±0.01^{c}	0.15±0.01^{e}	0.66±0.02^{d}	0.54±0.01^{d}	0.32±0.03^{e}	0.41±0.01^{e}	0.99±0.02^{c}
	Caryophyllene	1418	0.61±0.01^{d}	0.66±0.01^{d}	0.12±0.02^{e}	0.77±0.03^{d}	0.69±0.04^{d}	0.99±0.02^{cd}	0.77±0.04^{d}	0.88±0.02^{d}
	Monoterpene hydrocarbons		31.87±0.8^{d}	34.77±0.9^{c}	35.66±0.6^{b}	37.09±0.8^{b}	38.51±0.7^{a}	36.98±0.8^{b}	37.77±0.7^{ab}	37.2±0.8^{b}
	Oxygenated monoterpenes		57.69±1.4^{bc}	59.16±1.2^{b}	57.43±1.3^{b}	56.51±1.5^{b}	57.03±1.4^{b}	55.09±1.1^{c}	54.43±1.3^{d}	54.51±1.2^{d}
	Sesquiterpenes		0.61±0.01^{f}	0.66±0.01^{f}	0.12±0.02^{g}	0.77±0.03^{f}	0.69±0.04^{f}	0.99±0.02^{e}	0.77±0.04^{e}	0.88±0.02^{e}
	Essential oil content (w/w%, g/100g fresh weight basis)		0.77±0.01^{bc}	0.69±0.02^{c}	0.67±0.01^{c}	0.8±0.02^{b}	0.81±0.03^{b}	0.68±0.01^{c}	0.69±0.02^{c}	0.77±0.01^{bc}

Year	Compound	RI	Mn×Zn	Cu×Mn	Cu×Fe×Zn	Fe×Zn×Mn	Cu×Mn×Fe	Cu×Mn×Zn	Cu×Zn×Mn×Fe
2022	α-Pinene	940	2.55±0.01^{b}	2.1±0.02^{c}	2.01±0.03^{c}	1.55±0.02^{d}	2.21±0.08^{c}	3.08±0.02^{a}	2.21±0.02^{c}
	P-Cymene	1026	2.88±0.01^{b}	2.71±0.01^{b}	1.61±0.02^{e}	1.72±0.01^{d}	1.92±0.04^{d}	1.99±0.01^{d}	1.39±0.02^{e}
	Limonene	1033	29.62±0.7^{b}	29.14±0.5^{b}	28.55±0.5^{b}	27.63±0.8^{c}	26.83±0.9^{c}	26.64±0.8^{c}	25.4±0.8^{d}
	Eucalyptol	1035	4.66±0.1^{c}	5.88±0.2^{b}	5.33±0.06^{b}	5.22±0.07^{b}	5.28±0.08^{b}	5.37±0.07^{b}	3.95±0.09^{d}
	δ-Terpinene	1062	2.44±0.1^{c}	2.57±0.3^{c}	1.93±0.2^{c}	1.57±0.2^{c}	1.88±0.2^{c}	1.99±0.3^{c}	2.21±0.2^{c}

	α campholene	1110	2.61±0.2^{a}	2.56±0.2^{ab}	2.72±0.2^{a}	2.66±0.3^{a}	1.88±0.1^{c}	1.25±0.1^{d}	1.21±0.1^{d}
	Myrtenol	1202	24.91±1.1^{b}	26.11±0.5^{a}	25.88±0.6^{ab}	25.01±0.7^{b}	24.83±0.8^{b}	24.21±0.9^{b}	23.1±0.8^{c}
	Neral	1239	10.88±0.5^{d}	10.99±0.6^{cd}	11.93±0.7^{c}	9.12±0.8^{e}	12.88±0.9^{b}	12.04±0.5^{b}	13.14±0.9^{a}
	Geraniol	1257	0.99±0.01^{bc}	0.95±0.01^{c}	0.91±0.03^{c}	0.85±0.02^{c}	0.77±0.01^{c}	0.67±0.02^{cd}	0.66±0.03^{cd}
	Geranial	1270	10.82±0.5^{c}	10.88±0.6^{c}	9.88±0.7^{d}	8.7±0.8^{e}	9.08±0.9^{d}	9.94±0.7^{d}	10.11±0.8^{d}
	Carvacrol	1298	0.55±0.01^{c}	0.61±0.02^{c}	0.71±0.02^{c}	0.24±0.01^{d}	0.52±0.01^{c}	0.44±0.03^{d}	0.34±0.02^{d}
	Geranic acid	1320	0.95±0.01^{c}	0.97±0.02^{c}	0.99±0.01^{c}	0.02±0.02^{f}	0.13±0.04^{e}	0.01±0.01^{f}	0.22±0.02^{f}
	Geranyl acetate	1385	0.99±0.02^{c}	0.05±0.03^{f}	0.12±0.02^{e}	0.44±0.01^{d}	0.44±0.02^{d}	0.91±0.03^{c}	0.85±0.01^{c}
	Caryophyllene	1418	0.66±0.02^{d}	0.75±0.01^{d}	0.81±0.01^{d}	0.73±0.02^{d}	0.71±0.01^{d}	0.99±0.02^{cd}	0.88±0.01^{d}
	Monoterpene hydrocarbons		37.49±0.7^{b}	36.52±0.8^{b}	34.1±0.6^{c}	32.47±0.5^{c}	32.88±0.7^{c}	33.7±0.65^{c}	31.21±0.8^{d}
	Oxygenated monoterpenes		57.42±1.2^{c}	57.98±1.3^{bc}	57.36±1.1^{c}	51.8±0.9^{de}	55.24±1.2^{c}	53.92±1.1^{d}	52.51±1.4^{d}
	Sesquiterpenes		0.66±0.02^{f}	0.75±0.01^{e}	0.81±0.01^{e}	0.73±0.02^{e}	0.71±0.01^{e}	0.99±0.02^{de}	0.88±0.01^{e}
	Essential oil content (w/w%, g/100g fresh weight basis)		0.76±0.03^{c}	0.74±0.02^{c}	0.71±0.01^{c}	0.73±0.01^{c}	0.8±0.02^{b}	0.77±0.01^{bc}	0.69±0.02^{c}

Year	Compound	Fe	Zn	Cu	Mn	Fe×Zn	Cu×Fe	Mn×Fe	Cu×Zn
2023	α-Pinene	2.44±0.02^{c}	2.2±0.01^{c}	2.55±0.1^{b}	2.77±0.1^{b}	1.83±0.09^{b}	3.34±0.1^{a}	2.99±0.1^{a}	2.89±0.09^{b}
	P-Cymene	1.99±0.01^{d}	1.7±0.02^{de}	1.81±0.1^{d}	1.77±0.02^{d}	1.99±0.08^{d}	1.77±0.1^{d}	2.61±0.1^{c}	1.69±0.03^{e}
	Limonene	28.02±0.5^{b}	28.9±0.6^{ab}	28.4±0.7^{b}	28.5±0.8^{b}	27.7±0.9^{c}	26.6±0.9^{c}	28.8±0.8^{b}	28.5±0.7^{b}
	Eucalyptol	3.21±0.1^{d}	3.33±0.1^{d}	3.22±0.2^{cd}	3.99±0.2^{d}	3.22±0.12^{d}	3.21±0.1^{d}	3.33±0.1^{d}	3.05±0.11^{d}
	δ-Terpinene	3.44±0.2^{ab}	3.7±0.2^{a}	2.66±03^{b}	2.55±0.2^{bc}	4.15±0.2^{a}	3.74±03^{a}	3.85±02^{a}	2.99±0.1^{b}
	α campholene	2.51±0.2^{ab}	2.24±0.2^{b}	2.4±0.3^{a}	1.3±0.1^{d}	1.8±0.1^{c}	1.9±0.2^{c}	1.9±0.2^{c}	1.1±0.1^{d}
	Myrtenol	23.1±0.5^{c}	23.8±0.6^{c}	23.7±0.3^{c}	23.2±0.4^{c}	23.1±0.3^{c}	23.2±0.5^{c}	23.4±0.4^{c}	23.5±0.6^{c}
	Neral	13.3±0.5^{a}	13.9±0.3^{a}	13.8±0.5^{a}	13.87±0.4^{a}	13.69±0.6^{a}	12.8±0.7^{b}	11.8±0.4^{c}	11.9±0.6^{c}
	Geraniol	1.56±0.01^{a}	1.4±0.01^{b}	1.3±0.02^{b}	1.4±0.01^{b}	1.81±0.01^{a}	1.7±0.02^{a}	0.9±0.02^{c}	1.1±0.03^{b}
	Geranial	11.88±0.5^{c}	12.3±0.6^{b}	11.9±0.7^{c}	11.8±0.5^{c}	11.7±0.4^{c}	11.1±0.6^{c}	13.4±0.4^{b}	11.3±0.5^{b}
	Carvacrol	1.33±0.01^{a}	1.44±0.2^{a}	1.88±0.1^{a}	1.33±0.01^{a}	0.99±0.01^{bc}	0.97±0.1^{c}	1.12±0.1^{a}	1.23±0.01^{b}
	Geranic acid	0.99±0.02^{bc}	0.85±0.1^{c}	0.12±0.1^{e}	0.71±0.02^{c}	0.4±0.02^{d}	0.5±0.03^{d}	0.3±0.01^{e}	0.4±0.02^{d}
	Geranyl acetate	0.99±0.01^{c}	0.92±0.1^{c}	0.98±0.01^{c}	0.7±0.01^{d}	0.1±0.01^{e}	0.5±0.01^{d}	0.6±0.02^{d}	0.1±0.03^{e}
	Caryophyllene	1.77±0.02^{b}	1.88±0.1^{b}	2.32±0.01^{a}	1.72±0.02^{b}	2.1±0.01^{a}	1.91±0.1^{b}	1.9±0.01^{b}	1.99±0.02^{ab}
	Monoterpene hydrocarbons	35.89±0.8^{b}	36.5±0.9^{b}	35.42±0.7^{b}	35.59±0.6^{b}	35.67±0.8^{b}	35.45±0.7^{b}	38.25±0.6^{a}	36.07±0.5^{b}
	Oxygenated monoterpenes	56.89±1.4^{c}	58.41±1.2^{b}	58.2±1.1^{b}	56.89±1.2^{c}	56.31±1.4^{c}	54.88±1.1^{d}	55.85±1.5^{c}	53.18±1.4^{d}
	Sesquiterpenes	1.77±0.02^{b}	1.88±0.1^{b}	2.32±0.01^{a}	1.72±0.02^{b}	2.1±0.01^{a}	1.91±0.1^{b}	1.9±0.01^{b}	1.99±0.02^{ab}
	Essential oil content (w/w%, g/100g fresh weight basis)	0.77±0.01^{bc}	0.65±0.1^{d}	0.62±0.1^{d}	0.81±0.01^{b}	0.85±0.04^{b}	0.79±0.1^{b}	0.83±0.2^{b}	0.8±0.02^{b}

Year	Compound	RI	Mn×Zn	Cu×Mn	Cu×Fe×Zn	Fe×Zn×Mn	Cu×Mn×Fe	Cu×Mn×Zn	Cu×Zn×Mn×Fe
2023	α-Pinene	940	2.99±0.01^{a}	2.42±0.02^{c}	2.38±0.03^{c}	2.23±0.02^{c}	2.44±0.01^{c}	2.55±0.02^{b}	2.44±0.01^{c}
	P-Cymene	1026	1.77±0.01^{d}	1.71±0.02^{d}	1.6±0.01^{e}	1.55±0.01^{e}	1.47±0.04^{e}	1.44±0.01^{e}	1.39±0.02^{e}
	Limonene	1033	28.5±0.65^{b}	27.88±0.5^{c}	27.5±0.6^{c}	27.4±0.8^{c}	28.7±0.5^{b}	27.6±0.9^{c}	25.98±0.8^{cd}
	Eucalyptol	1035	4.33±0.1^{c}	3.55±0.1^{d}	5.12±0.2^{b}	4.88±0.1^{c}	5.15±0.2^{b}	5.01±0.2^{b}	3.93±0.5^{d}
	δ-Terpinene	1062	3.88±0.2^{a}	3.77±0.2^{a}	3.55±0.3^{ab}	2.44±0.3^{b}	2.87±0.2^{b}	2.93±0.2^{b}	3.22±0.3^{ab}
	α campholene	1110	1.74±0.2^{c}	1.68±0.2^{c}	1.78±0.2^{c}	1.88±0.2^{c}	1.84±0.2^{c}	1.66±0.1^{c}	1.55±0.1^{cd}
	Myrtenol	120	24.88±1.1^{b}	24.9±0.9^{b}	25.8±0.8^{ab}	24.88±0.6^{b}	23.2±0.7^{c}	23.2±0.6^{c}	22.9±0.6^{cd}
	Neral	1239	11.3±0.5^{c}	12.8±0.6^{b}	12.4±0.7^{b}	10.12±0.8^{d}	11.1±0.9^{c}	11.04±0.5^{c}	11.9±0.5^{c}
	Geraniol	1257	1.23±0.01^{b}	1.01±0.02^{c}	0.96±0.1^{c}	0.99±0.02^{c}	0.94±0.01^{c}	0.88±0.01^{c}	0.77±0.02^{c}
	Geranial	1270	11.8±0.5^{c}	10.95±0.6^{c}	10.1±0.7^{d}	9.1±0.8^{d}	10.4±0.9^{d}	10.9±0.6^{c}	10.9±0.6^{c}
	Carvacrol	1298	1.04±0.01^{b}	1.01±0.02^{b}	0.97±0.03^{c}	0.87±0.02^{c}	0.99±0.02^{bc}	0.9±0.02^{c}	0.99±0.02^{bc}
	Geranic acid	1320	0.21±0.01^{e}	0.96±0.01^{c}	0.89±0.02^{c}	0.95±0.01^{c}	0.91±0.03^{c}	0.88±0.01^{c}	0.98±0.02^{c}
	Geranyl acetate	1385	0.66±0.02^{d}	0.99±0.01^{c}	0.98±0.02^{c}	0.99±0.01^{c}	0.11±0.01^{e}	0.2±0.03^{e}	0.99±0.01^{c}
	Caryophyllene	1418	1.99±0.02^{ab}	2.01±0.01^{a}	1.88±0.01^{a}	2.12±0.01^{a}	2.22±0.02^{a}	1.82±0.02^{b}	1.88±0.01^{b}
	Monoterpene hydrocarbons		37.14±0.65^{b}	35.78±0.8^{b}	35.03±0.5^{b}	33.62±0.7^{c}	35.48±0.8^{b}	34.52±0.7^{bc}	33.03±0.6^{c}
	Oxygenated monoterpenes		56.32±1.2^{c}	55.9±1.1^{c}	57.13±1.2^{c}	52.72±0.9^{c}	53.62±0.9^{c}	53.59±1.1^{d}	52.94±0.9^{d}
	Sesquiterpenes		1.99±0.02^{ab}	2.01±0.01^{a}	1.88±0.01^{b}	2.12±0.01^{a}	2.22±0.02^{a}	1.82±0.02^{b}	1.88±0.01^{b}
	Essential oil content (w/w%, g/100g fresh weight basis)		0.77±0.04^{bc}	0.69±0.02^{c}	0.78±0.01^{b}	0.82±0.01^{b}	0.7±0.01^{c}	0.69±0.02^{c}	0.63±0.01^{d}

[z] RI: Retention Indices, as determined with FID and HP-5MS 5% capillary column using a series of the standards of C7-C30 saturated n-alkanes. [y] Values are means of triplicates ± standard deviation (p <0.05)

**Numbers in each row that have same letter, have same group.

Table 7. Results of simple correlation between essential oil content and main compositions in *D.kotschyi* plants under application of tested micronutrients in two years.

Year	Eucalyptol (10)	α.campholene (9)	Geranic acid (8)	Geranyl acetate(7)	Geraniol (6)	Geranial (5)	Neral (4)	Limonene (3)	Myrtenol (2)	Essential oil (1)	
	-	-	-	-	-		-	-	-	1	1
	-	-	-	-	-		-	-	1	0.95**	2
	-	-	-	-	-		-	1	0.75**	0.64*	3
	-	-	-	-	-		1	0.8**	0.52**	0.5**	4
2022	-	-	-	-	-	1	0.66**	0.71**	0.55**	0.9**	5
	-	-	-	-	1	0.54**	0.95**	0.47**	0.64**	0.68*	6
	-	-	-	1	0.95**	0.78**	0.66**	0.82**	0.6**	0.4*	7
	-	-	1	0.69**	0.48**	0.48**	0.86**	0.67**	0.72**	0.88**	8
	-	1	0.86**	0.53**	0.81**	0.81**	0.68**	0.79**	0.65**	0.75**	9
	1	0.81**	0.95**	0.9**	0.92**	0.95**	0.77**	0.95**	0.65**	0.85**	10
	-	-	-	-	-	-	-	-	-	1	1
	-	-	-	-	-	-	-	-	1	0.84**	2
	-	-	-	-	-	-	-	1	0.9**	0.66**	3
	-	-	-	-	-	-	1	0.62**	0.59**	0.51**	4
2023	-	-	-	-	-	1	0.3	0.75**	0.55**	0.82**	5
	-	-	-	-	1	0.79**	0.42**	0.45**	0.71**	0.5**	6
	-	-	-	1	0.68**	0.95**	0.76**	0.73**	0.62**	0.4**	7
	-	-	1	0.77**	0.95**	0.77**	0.66**	0.63**	0.7**	0.8**	8
	-	1	0.67**	0.72**	0.88**	0.81**	0.88**	0.65**	0.66**	0.45**	9
	1	0.81**	0.86**	0.53**	0.81**	0.86**	0.66**	0.79**	0.65**	0.45**	10

*, ** significant at P=0.05 and P=0.01 levels of probability respectively.

In the current study it was found that the essential oil content is in a yield ranging from 0.59 to 0.99% (w/w) made by control plants and plants treated with 40 mg.l^{-1} of micronutrients ($Fe_2Zn_2Mn_2Cu_2$) respictively (Tables 4-6). The main reasons for this enhancement in the essential oil content by applying micronutrients can be related to the balance between absorption of the essential elements in the root environment, increasing the rate of photosynthesis, stimulating the vital enzymes, activating plant growth regulators (PGR) production as inducing signal for terpenes biosynthesis (Pradhan et al., 2017). In this research use of 40 mg.l^{-1} of Fe, Zn, Mn and Cu increased essential oil content from 0.59% to 99% with an increase of 67%. It has been shown those micronutrients of Fe, Cu, Zn and Mn help to increase better and more absorption of nutrients by influencing enzyme activities (Marschner, 1995; Pradhan et al., 2017). Copper deficiency limits the activity of many plant enzymes, including ascorbate oxidase, phenolase, cytochrome oxidase, diamine oxidase, plastocyanin, and superoxide dismutase. Oxidation–reduction cycling between Cu(I) and Cu(II) oxidation states is required during single electron transfer reactions in copper-containing enzymes and proteins (Barker & Pilbeam, 2007). Iron is limited largely by diffusion in the soil solution, and thus the absorption is highly dependent on root activity and growth. In terms of fertilizers for terrestrial plants, iron deficiency usually comes about because of alkaline pH in the soil, and supply of iron salts to the soil would have no effect. Foliar application of iron-cheletes can be effective. Therefore, the usual way in which lime-induced chlorosis is alleviated is by supply of iron chelates to the foliage (Barker & Pilbeam, 2007; Pradhan et al., 2017). Manganese involved in many biochemical functions, primarily acting as an activator of enzymes such as dehydrogenases, transferases, hydroxylases, and decarboxylases involved in respiration, amino acid and lignin synthesis, and hormone concentrations, but in some cases it may be replaced by other metal ions (Marschner, 1995). Zinc is an integral component of enzyme structures and coordinated to four ligands in enzymes with catalytic functions. Three of them are amino acids, with histidine being the most frequent, followed by glutamine and asparagine (Marschner, 1995; Pradhan et al., 2017). As a result, these positive impacts of the micronutrients could lead to the improvement of photosynthetic rate, biomass production and yield of aerial parts of medicinal plants (Hamedi et al., 2020; Yadegari, 2023). In the current study it was found that the EOs content is in a yield ranging from 0.37 to 0.68% (w/w) made by control plants and plants treated with 40 mg.l-1 of micronutrients (Fe2Zn2Mn2Cu2) respectively. The main reasons for this enhancement in the EOs content by applying micronutrients can be related to the balance between absorption of the essential elements in the root environment, increasing the rate of photosynthesis, stimulating the vital enzymes, activating plant growth regulators (PGR) production as inducing signal for terpenes biosynthesis (Pradhan et al., 2017).

The findings of this research showed that by increasing essential oil content, the main compositions such as limonene, geranial, neral, myrtenol and eucalyptol in *D.kotschyi* plants treated with micronutrients in two years, increased (Table 7). Also, in the current study, 14 chemical components of the essential oils of *D.kotschyi* were recognized. It has been shown that the percentage of the main compounds of essential oil is the main factor determining the quality of the essential oil. It has been reported that among these compounds, monoterpene alcohols such as limonene, geranial, eucalyptol and myrtenol improve the quality of the essential oil of *D.kotschyi* (Shaykh-Samani et al., 2023a, b; Ashrafi et al., 2017, Golparvar et al., 2016). Maintaining the balance between nutrients and soil fertility is critical in sustainable soil management. Organic, biological and chemical fertilizers return nutrients consumed by plants to the soil. According to the results of the present study, the availability of optimum amounts of micronutrients provided the necessary nutrients for producing higher content and composition of essential oil of *D.kotschyi*. The essential oil composition of *D.kotschyi* varies depending on the variety, climatic conditions, and nutritional status of the plant and soil. According to reported literature myrtenol, limonene, beta-pinene, neral, geranial, geraniol, geranyl acetate, geranic acid, alpha campholene, eucalyptol and alpha pinene, were the identified constituents in the extracted essential oils from aerial parts of *D.kotschyi* (Shaykh-Samani et al., 2023; Cham et al., 2022; Ghavam et al., 2021; Fallah et al., 2020). A combination of the four micronutrients had a greater effect than a single micronutrient. Thus, the foliar application of 40 $mg.l^{-1}$ Fe, Cu, Mn and Zn was the most effective treatment compared to other treatments. The amount (percentage) of the main components in plants treated with 40 $mg.l^{-1}$ of micronutrients was produced twice more than those of the control plants (Tables 4-6).

Exogenous micronutrients affect respiration, photosynthesis, carbohydrate assimilation, and amino acids biosynthesis. These processes usually are simultaneous with the changes in the content of intermediate compounds and the activity of involved enzymes in the primary and secondary metabolism of plants. Therefore, induced variations in the plant's physiological behavior by micronutrients determine the quality of produced secondary metabolites. Essential oils belong to the group of terpenes and glucose is an essential precursor in the synthesis of terpenoids, especially monoterpenes. Therefore photosynthesis and photosynthetic products directly determine the biosynthesis of essential oils. A sufficient supply of nutrient elements in plant in response to exogenous micronutrients affects the biosynthesis of involved substrates and enzymes in terpenoids biosynthesis (Aghaei et al., 2021; Bohlman & Keeling, 2008; Pradhan et al., 2017). For instance, providing a sufficient amount of magnesium may affect the activity of geranyl diphosphate synthase, which requires this element for its activity (Chiyaneh et al., 2022). Essential oil content is directly correlated with the main compounds such as myrtenol and limonene. In the present study based on GC and GC-MS results, monoterpene hydrocarbons represented by alpha-pinene, p-cymene, limonene and gamma-terpinene, made more 35% of compounds of essential oil and oxygenated monoterpenes represented by eucalyptol, alpha-campholene, myrtenol, neral, geraniol, geranial and carvacrol made more than 52% of compounds of essential oil of treated plants. In all of treated plants, the most compound belongs of oxygenated monoterpenes and monoterpene hydrocarbons, were myrtenol and limonene respectively. The main of compounds of essential oil of treated plants by 40- $mg.l^{-1}$ of micronutrients are more than other treated plants, especially oxygenated monoterpenes in compounds were more than other categories and otherwise the sesquiterpenes in plants treated by 60 $mg.l^{-1}$ of micronutrients increased. Treatment of $Fe_2Cu_2Zn_2Mn_2$ in this research made the most myrtenol such as oxygenated monoterpenes and limonene belongs to the category of monoterpene hydrocarbons. Also treatment of $Fe_2Cu_2Zn_2Mn_1$ and $Fe_2Cu_1Zn_2Mn_2$ in most of main compounds were in the same group with $Fe_2Cu_2Zn_2Mn_2$. It seems that the role of iron and zinc are more important than other micronutrients that reported in pervious researches (Yadegari, 2023; Bilal et al., 2020; Hamedi et al., 2020). The results of previous investigation by Shaabani et al. (2020) showed that the essential oil of *D.kotschyi* mainly consisted of β-caryophellene, α-pinene, and limonene. The results of another study indicated that the major components of volatile oil from *D.kotschyi* were limonene, carvacrol, γ-terpinene, and α-pinene. Numerous studies have reported that the chemical compounds of *D.kotschyi* have antibacterial and antiseptic properties. Terpenoids, such as geranial, lower blood triglycerides and cholesterol, and ultimately reduce cardiovascular disease (Ashrafi et al., 2017; Golparvar et al., 2016; Samadi et al., 2018). Compounds such as geranial and neral cause the antioxidant activity of *D.kotschyi*. The upper range of micronutrients (i.e. 60 $mg.l^{-1}$) decreased the content of the essential oil of *D.kotschyi* but the composition of the essential oil was similar in all treatments. Control plants made better amounts of many essential oil components of *D.kotschyi* than those treated plants with 60- $mg.l^{-1}$ concentrations of Fe, Cu, Mn and Zn. In most treatments, the combination of $Fe_2Cu_2Mn_2Zn_2$, $Fe_2Cu_3Mn_3Zn_2$ and $Fe_2Cu_2Mn_2Zn_2$ made the maximum amount of essential oil. However, $Fe_2Cu_2Mn_2Zn_2$ was the best combination. The increase in the essential oil content in the plants treated with 20 and 40 $mg.l^{-1}$ may be due to its role in enhancing the overall growth of aerial parts. In addition, active substances such as volatile oils are made by plants due to the plant's adaptation to biological and abiotic stresses, and the signals of these stresses act as elicitors for the plant cell (Sonboli et al., 2019; Ashrafi et al., 2017).

In some essential oil compositions, control plants were similar to plants treated with $Fe_3Cu_3Mn_3Zn_3$, $Fe_3Cu_3Mn_2Zn_3$ and $Fe_2Cu_2Mn_3Zn_3$. It seems for toxicities of upper concentrations of Fe, Cu, Mn and Zn (i.e. 60 $mg.l^{-1}$), the content of components obtained at higher concentrations of micronutrients was similar to control plants. It was clear from the presented data that the highest levels of the four foliar fertilizers were more effective than the lower levels, and Librel Zn and Fe fertilizers were superior over other micronutrients. However, the highest essential oil percentage was found with $Fe_2Cu_2Mn_2Zn_2$ (Tables 4-6). Similar to the results obtained in this study regarding *D.kotschyi*, the beneficial effect of micronutrients (Fe, Zn, Cu and Mn) and production of higher essential oil content were reported by other researchers in *Rosa damascena* (Yadegari, 2023), *Thymus* (Yadegari, 2022), *Satureja* (Bani Taba et al., 2022), *Dracocephalum moldavica* (Yadegari, 2021), Safflower (*Carthamus tinctorius*) (Galavi et al., 2012), marigold (*Calendula officinalis* L.), borage (*Borago officinalis*), alyssum (*Alyssum desertorum*) and thyme (*Thymus vulgaris*) (Yadegari, 2015, 2017a), lemon balm (*Melissa officinalis* L.) (Yadegari, 2017b), dill (*Anethum graveolens*) (Rostaei et al., 2018), *Matricaria chamomilla* (Nasiri et al., 2010) and *Coriandrum sativum* (Said-Al Ahl & Omer, 2009).

Results of this research indicated that foliar application of micronutrients resulted in higher essential oil content in the shoots of *D.kotschyi* plants than the control plants. In the present study, the effect of micronutrients on the essential oil content and composition was determined over two consecutive years. The essential oil yield increased with Fe, Cu, Zn and Mn applications because of a significant rise in dry matter and the number of flowers (data not published). It was revealed that Fe, Cu, Zn and Mn are beneficial for *D.kotschyi* plants with concentrations of 40 mg.l^{-1}or lower, and can result in more content of essential oil up to 40%. Fe, Cu, Zn and Mn have immediate impacts on the growth and development of plants. There are still many unanswered questions about the mechanism of Fe, Cu, Zn and Mn in enhancing yield and its components for *D.kotschyi* plants. One possibility is that the foliar applied Fe, Cu, Zn and Mn can affect absorbtion of other minerals and then increase shoot dry matter and finally the essential oil yield in plants increased (Alamer et al., 2020; Alejandro et al., 2020; Aziz et al., 2019). It was determined in this study that control plants with no foliar treatment experienced better growth than the plants with a higher concentration of the micronutrients. Combinations of micronutrients with an optimum concentration (i.e. 40 mg.l^{-1}) had the best effect. However, combinations of micronutrients of a concentration higher than 40 mg.l^{-1} (i.e. 60 mg.l^{-1}) had more diminishing effects than single micronutrients. These results reflect the role of the simultaneous application of an optimum concentration of the four foliar fertilizers in improving the total essential oils in medicinal plants. Micronutrients of higher than 40 mg.l^{-1} concentration especially in three micronutrient- or in four micronutrient-combinations reduced the content and composition of the essential oil (Tables 4-6). Generally, the production of the secondary metabolites and the chemical compositions of the plant essential oils is influenced by genetic factors, ecological, soil conditions, management (sowing to harvesting and post-harvesting processes) and their interactions (Golparvar et al., 2016, Ashrafi et al., 2017, Shaykh-Samani et al., 2023a, b).

CONCLUSION

D.kotschyi plants treated with 40 mg.l^{-1} of iron, zinc, manganese and copper in the chelate formula, produced higher content and composition of the essential oil. It could be concluded from the results that iron, zinc, manganese and copper fertilization had significant effects on the measured characters as well as the chemical composition of the essential oil of *D.kotschyi* plants. Also, the combined application of micronutrient fertilizers had a more pronounced effect in comparison with the individual use of the micronutrients. This study provides some useful information about the impact of foliar application of micronutrients where soils have undesirable characteristics and chemical properties in particular. The main constituents of the volatile oils of *D.kotschyi* were limonene, neral, geranial, myrtenol and eucalyptol (45-65%) were identified in the essential oil. In this study, the highest content of essential oil and the percentages of neral, geranial, myrtenol, eucalyptol and limonene in the essential oil were observed in the herbs under treatments of 40 mg.l^{-1} of micronutrients however the combination of 40 mg.l^{-1} of iron, zinc and copper was in the same goup. Finally, the use of 40 mg.l^{-1} of micronutrients (Fe, Zn, Cu and Mn) is recommended for stabilizing the quantitative and qualitative yield of *D.kotschyi* in same climates.

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Conflict of Interest
The author declares that he has no conflict of interest.

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