Essential oil content and components of *Ferulago angulate* (Schltdl.) Boiss affected by foliar application of some important micronutrient

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Abstract

Ferulago angulata (Schltdl.) Boiss, belonging to the family Apiaceae, is one of the important and endangered endemic species in Iran. The present study was conducted to investigate the effects of micronutrient element application on the content and composition of essential oils (EOs) of *F. angulata* shoots in southwestern Iran (Shahrekord) during 2022 and 2023. Four foliar fertilizers—Fe, Cu, Zn, and Mn—were applied at concentrations of 20, 40, and 60 mg/L in a randomized complete block design (RCBD) with a factorial layout and three replications. Results obtained from gas chromatography-mass spectrometry (GC-MS) revealed 15 EO components. The applied micronutrients significantly influenced the EO content and composition of *F. angulata*. Over the two years, the highest EO content (0.59–0.68%) was obtained in plants treated with 40 mg/L of micronutrients (Fe, Cu, Zn, Mn), while the lowest content (0.37–0.41%) was observed in the control plants. However, plants treated with 60 mg/L of micronutrients were grouped similarly to the control plants in most characteristics. The most important chemical compounds that determine the quality of *F. angulata* EOs were identified as alpha-pinene (20.13–35.88%), alpha-thujene (12.67–18.14%), and cis-ocimene (11.41–22.01%) from the monoterpene hydrocarbons category, and 4-thujanol (1.01–10.54%) from the oxygenated monoterpenes category. Alpha-pinene, a monoterpene hydrocarbon, was the predominant constituent of the EOs of *F. angulata*. In conclusion, the application of micronutrients at a concentration of 40 mg/L can be a promising strategy to improve the quantity and quality of EOs in *F. angulata* under cold and semi-arid climates.

Keywords: Alpha-pinene, Medicinal plant, Iron, Zinc

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Introduction

The genus Ferulago is a member of the Apiaceae (Umbelliferae) family and consists of 49 species throughout the world. In traditional medicine, Ferulago species have been utilized as sedatives, carminatives, aphrodisiacs, for increasing milk secretion, and for the treatment of bronchitis, skin diseases, and eye (Süzgeç-Selçuk and Dikpınar, 2021). This genus is common throughout the Mediterranean area, particularly in western Iran, Turkey, Iraq, Greece, Serbia, and Macedonia. Plants of tahis genus have been used since ancient times as spices and in salads, as a tonic, and as

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remedies for digestive pains and hemorrhoids, mainly in Turkey and Iran. These properties have been confirmed by numerous pharmacological studies. These plants, therefore, possess antibacterial, antifungal, antioxidant, anticoagulant, and anti-inflammatory properties, as well as hepatoprotective and nephroprotective effects, and insecticidal activities (Badalamenti et al., 2021). Due to their multiple properties, the chemical compositions and biological activities of Ferulago species have been extensively studied, taking into consideration the different parts of the plants (roots, stems, fruits, and leaves). Most of the therapeutic effects may be due to the presence of various phytoconstituents such as coumarins, essential oils (EOs), terpenoids, and flavonoids (Razavi et al., 2015). The Ferulago genus, rich in secondary metabolites and various biological activities, seems to be a potential source for the development of new drugs and for application in the agro-food field (Süzgeç-Selçuk and Dikpınar, 2021). The major compounds in this plant, such as flavonoids, sesquiterpene aryl esters, phenolic acids, aromatics, and sitosterols, have been assessed phytochemically. Furanocoumarins are the major phytochemical group in the Ferulago genus. The antibacterial and antifungal activities of Ferulago spp. have been previously investigated, and their significant inhibitory effects against microorganisms have been reported (Badalamenti et al., 2021; Shahbazi, 2016).

Ferulago angulata, commonly known as "Chavil" in Persian, is an aromatic plant growing in the west of Iran. It is a perennial, glabrous herb with a height of 40–100 cm, a cylindrical, dichotomously branched stem, shortly petiolate, pinnate-sect, terminal segment, linear-oblong, acute leaves, and yellowish, synflorescence corymbose-paniculiform flowers (Mozaffarian, 2008). The aerial parts of *F. angulata* have been used as a food protectant and flavoring agent. In Iran, the aerial parts of *F. angulata* are used in meat, dairy, and ghee oil as a natural food preservative. Ferulago species have been used for treating ulcers, snakebites, intestinal worms, and hemorrhoids (Golfakhrabadi et al., 2015). The essential oils (EOs) extracted from *F. angulata* were traditionally used to treat bacterial and fungal infections in Iran for several centuries (Azarbani et al., 2023). Previous studies showed that the EOs of *F. angulata* are characterized by large amounts of monoterpene hydrocarbons. Research on the EOs of *F. angulata* showed that the major constituents were Z-beta-ocimene, bornyl acetate, δ-terpinolene, sabinene (Ghasemi Pirbalouti et al., 2016; Razavi et al., 2015); alpha-pinene, sabinene, (Z)-beta-ocimene, p-cymene, alpha-phellandrene, beta-phellandrene (Golfakhrabadi et al., 2015; Moghaddam et al., 2018); Cis-beta-ocimene, alpha-pinene, alpha-phellandrene (Mumivand et al., 2019); alpha-pinene, alpha-thujene, alpha-phellandrene, cis-ocimene, beta-phellandrene, beta-ocimene (Shahbazi, 2016; Safari et al., 2019); alpha-pinene, bornyl acetate, terpinolene, octane, beta-pinene, alpha-phellandrene, dodecane, germacrene-D, caryophyllene oxide (Azarbani et al., 2023).

When nutrient deficiency cannot be corrected through soil application, foliar nutrition is adopted as an alternative method (Marschner, 2011). It has been shown that micronutrients such as Fe, Mn, Zn, and Cu are necessary for plant growth and development in much lower amounts than primary nutrients (Bilal et al., 2020). Four important micronutrients used in medicinal plants are Fe, Cu, Mn, and Zn. Iron (Fe) is one of the four essential nutrient elements needed by plants and is a key component of the cytochrome structure. In addition, plants treated with this micronutrient produce higher yields (Majeed et al., 2020). Copper (Cu) is another essential microelement in higher plants, occurring 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; its deficiency causes cell damage, low protein and carbohydrate synthesis, impaired growth and development, and reduced crop yields (Alamer et al., 2020; Cakmak et al., 2017; de 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 has important effects on morphophysiological attributes such as chlorophyll, phenol, and relative water content, which result in increased EO content and composition in *Rosa damascena* (Yadegari, 2023), Satureja (Bani Taba and Naderi, 2022), *Melissa officinalis* (Yadegari, 2017a), *Carthamus tinctorius* (Galavi et al., 2012), *Calendula officinalis*, *Borago officinalis*, *Alyssum desertorum*, and *Thymus vulgaris* (Yadegari, 2015; Yadegari, 2017b), *Anethum graveolens* (Rostaei et al., 2018; Yadegari, 2017b), *Matricaria chamomilla* (Nasiri et al., 2010), and *Coriandrum sativum* (Said-Al Ahl and Omer, 2009).

Foliar fertilization is particularly useful to meet the basic needs of plants for one or more micro- or macronutrients, especially trace minerals. It also helps correct deficiencies, strengthen weak or damaged crops, and enhance growth (Aziz et al., 2019). As far as I have found, there has been no comprehensive study on the foliar application of important micronutrients on the content and compositions of *F. angulata* EOs grown in Iran. Hence, here I report the volatile oil compositions of *F. angulata* from western Iran. Additionally, I evaluate the comparisons between the content and the main compositions of EOs of this plant. The aim of this research was to determine the effects of foliar applications of iron, zinc, copper, and manganese on EO content and composition in *F. angulata* 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 contains 14% zinc in chelated form, Librel Cu has 14% copper in chelated form, and Librel Mn contains 13% manganese chelated with EDTA (obtained from The Chemical Company of England and Germany). These fertilizers were sprayed at three concentrations (for example, Fe1, Fe2, and Fe3 were concentrations of Fe which had 20, 40, and 60 mg.L^{-1} of Fe, respectively. The concentrations were similar for the other micronutrients. The foliar application was done in three stages, once every 10 days (before harvest) in the early morning. The control plants received no micronutrient foliar application. For soil analysis, soil samples were taken from three randomly selected sites in each plot from 0-15 and 15-30 cm depths. The samples were homogenized, mixed, and passed through a 2 mm filter for the determination of soil physical and chemical characteristics. Soil moisture was measured by a TDR device (PMS-714, Lutron, Taiwan) following the manufacturer’s protocol.

Experimental Conditions

This investigation was conducted 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 study area is classified as cold and semiarid. The study was conducted in a randomized complete block design (RCBD) with three replications. Each year, treatments were applied during the V4-V8 growth stages, and sampling was performed at the flowering stage. The soil (typic calci xerocrepts) physical and chemical properties and climatic properties of the region are listed in Table 1 and Table 2, respectively. The topsoil of the experimental plot area was kept moist throughout the growing season as necessary. The aerial parts and inflorescences of *F. angulata* were hand-harvested at flowering and then dried in the shade at room temperature (25 ± 4 °C) for two weeks, with the moisture content maintained at around 14 to 16%. The samples were ground to a fine powder using a micro hammer cutter mill and passed through a sieve (mesh 20). The EOs were extracted from 100 g of powdered tissue by the hydro-distillation method using a Clevenger-type apparatus (made by Glass Fabricating of Ashk-e-Shishe Co., Tehran, Iran) with 500 mL of water for 3 hours, according to the British Pharmacopoeia.

Plant Material and Agronomic Practices

Seeds of *F. angulate* Boiss. (Apiaceae) were obtained from the Forest and Rangeland Institute, Iran. First, the seeds were sterilized and sown in May 2022. After about 45-50 days from sowing, when the seedlings had 4-6 true leaves and were 8-10 cm tall, they were transplanted into the experimental field. Each experimental plot measured 4.0 × 3.0 m, and the distance between replicates was 2 m. No inorganic fertilizers or systemic pesticides were used during the experiment, and weed control was done manually.

Preparation of Essential Oils (EOs) Extraction

The EOs content was determined by distilling shoots using a Clevenger apparatus. A 100 g portion of shoots was placed in a 6 L Clevenger-type distillation apparatus and distilled for 5 hours with 3 L of pure water. The oil content of F. angulate was obtained at the end of distillation and measured in mL and % ratios (w/w), then determined by multiplying the oil content by the oil density (0.858). All the EOs samples were dried over anhydrous sodium sulfate and stored at 4°C until they were analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS).

GC and GC–MS Analysis

GC analysis was conducted using an Agilent Technologies 7890 GC equipped with an FID and an HP-5MS 5% capillary column. The carrier gas was helium at a flow rate of 0.8 mL/min. The initial column temperature was 60°C and was programmed to increase at 4 °C/min to 280 °C. The split ratio was 40:1, and 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 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 minutes 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 EOs were identified by comparing their retention indices relative to a series of n-alkanes (C7-C24), retention **times**, and mass spectra with those of authentic samples in the Wiley library (Adams, 2007).

Table 1
Physico-chemical 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 ($mg \cdot kg^{-1}$)	16.44	15.55
K ($mg \cdot kg^{-1}$)	312	308
Ca ($mg \cdot kg^{-1}$)	3.45	3.78
Mn ($mg \cdot kg^{-1}$)	8.44	8.34
Fe ($mg \cdot kg^{-1}$)	1.02	1.91
Cu ($mg \cdot kg^{-1}$)	0.55	0.43
Zn ($mg \cdot kg^{-1}$)	0.82	0.79
EC ($dS \cdot m^{-1}$)	0.62	0.65
Texture	Clay loam	Clay loam

Data Analysis

After the Bartlett test, all data were subjected to ANOVA and simple Pearson correlation indices using the statistical software package SAS v.11. Treatment means were separated using LSD's multiple range test at $P < 0.05$ and $P < 0.01$ levels.

Results

The results showed that the effects of foliar spraying on essential oil (EO) content were significant ($p \leq 0.01$) (Table 3). The results obtained from GC-MS indicated the presence of 15 components in the EOs of *F. angulate*, and significant differences in the chemical compositions of EOs were observed between treatments (Table 3). The maximum EO content

Table 2
Climatic properties of research farm.

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

Table 3
Complex analysis of variance of variation of EOs content and main compositions in *F.angulate* by different micronutrients

SOV^{z}	df^{y}	EOs	4-thujanol	Cis-ocimene	beta-phellandrene	alpha-phellandrene	beta-myrcene	alpha-pinene	alpha-Thujene
Year(Y)	1	6.62^{ns}	12.5**	0.007^{ns}	25.2**	5.56^{ns}	1.21**	3.44**	14.4**
R/Y	4	8.7	1.4	0.009	1.3	6.7	1.1	0.32	0.4
Copper (Cu)	3	27.5**	0.5^{ns}	1.11**	1.1^{ns}	14.4**	12.9**	1.8^{ns}	1.3^{ns}
Manganese (Mn)	3	4.1^{ns}	11.2**	0.02^{ns}	12.2**	12.6**	14.6**	1.8^{ns}	2.1^{ns}
Iron (Fe)	3	42.8**	12.9**	1.24**	14.4^{ns}	32.8**	16.8**	15.9**	22.4**
Zinc (Zn)	3	33.6**	14.2**	1.05**	11.1**	22.6**	8.5**	11.3**	11.9**
Cu × Mn	9	15.9**	25.2**	0.98**	0.84**	2.9**	7.1**	108**	12.8**
Cu × Fe	9	17.8**	32.4**	0.89**	0.92**	6.8**	6.4**	9.9**	10.4**
Cu × Zn	9	22.1**	21.8**	0.21**	0.77**	3.1**	5.3**	8.8**	12.1**
Mn × Fe	9	26.9**	27.1**	0.59**	0.92**	9.9**	7.9**	7.7**	14.1**
Mn × Zn	9	24.8**	12.1**	0.39**	0.88**	2.8**	8.9**	6.7**	10.9**
Fe × Zn	9	18.1**	8.4**	0.64**	17.8**	1.98**	5.9**	8.5**	9.2*
Cu × Mn × Fe	27	21.5**	5.1**	0.42**	0.91**	1.5**	3.4**	0.94^{ns}	10.8**
Cu × Mn × Zn	27	14.8**	4.6**	0.82**	0.99**	1.8**	4.1**	1.2^{ns}	1.9^{ns}
Cu × Fe × Zn	27	15.4**	4.22**	0.43**	0.2^{ns}	1.4**	0.44^{ns}	0.2^{ns}	0.7^{ns}
Fe × Zn × Mn	27	12.6**	5.6**	0.012^{ns}	0.1^{ns}	1.6**	0.57^{ns}	0.1^{ns}	0.8^{ns}
Cu × Zn × Mn × Fe	81	1.7^{ns}	0.28^{ns}	0.02^{ns}	0.11^{ns}	0.22^{ns}	0.21^{ns}	0.8^{ns}	0.9^{ns}
T(Cu,Zn,Mn,Fe)×Y	255	0.55^{ns}	0.33^{ns}	0.001^{ns}	0.12^{ns}	0.22^{ns}	0.32^{ns}	0.43^{ns}	0.28^{ns}
E	1020	1.9	0.45	0.02	0.11	0.3	0.55	0.88	2.23
CV^{x}		6.5	4.54	5.65	8.8	7.7	6.5	10.1	11.1

^{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 complex analysis of variance of variation of EOs content and main compositions in *F.angulate* by different micronutrients

SOV^{z}	df^{y}	Henicosane	Nonadecane	Bicyclo-germacrene	Naphthalenemethanol	Bornyl acetate	Verbenone	Sabinene	beta-ocimene
Year(Y)	1	0.005^{ns}	2.2**	24.2**	11.99^{ns}	0.88^{ns}	12.55**	0.99^{ns}	1.7**
R/Y	4	0.07	0.3	1.2	11.14	1.7	0.5	1.1	0.1
Copper (Cu)	3	1.71**	20.1**	12.5**	0.32^{ns}	1.6^{ns}	1.5^{ns}	0.69^{ns}	11.1**
Manganese (Mn)	3	1.65**	16.4**	13.1**	25.1**	1.8**	24.8**	1.1^{ns}	15.8**
Iron (Fe)	3	125**	21.4**	14.8**	26.4**	22.9**	22.9**	31.4**	14.5**
Zinc (Zn)	3	1.36**	18.1**	10.6**	20.9**	17.5**	26.9**	22.9**	15.3**
Cu × Mn	9	0.99**	11.4**	11.9**	16.8**	14.1**	14.8**	23.8**	10.9**
Cu × Fe	9	0.89**	10.9**	12.8**	13.4**	10.4**	15.9**	22.4**	9.8**
Cu × Zn	9	1.1**	12.7**	9.1**	14.1**	11.3**	12.8**	14.1**	11.1**
Mn × Fe	9	1.08**	8.1**	8.9**	12.1**	13.9**	17.7**	15.1**	10.7**
Mn × Zn	9	1.07**	7.7**	9.8**	10.9**	12.9**	13.7**	18.9**	8.8**
Fe × Zn	9	0.88**	6.8**	9.5**	9.2**	10.9**	10.5**	12.2**	9.1**
Cu × Mn × Fe	27	0.59**	5.9**	4.4**	8.8**	9.4**	0.61^{ns}	11.8**	4.5**
Cu × Mn × Zn	27	0.66**	6.6**	5.8**	7.9**	8.1**	0.58^{ns}	10.9**	8.1**
Cu × Fe × Zn	27	0.61**	0.2^{ns}	6.4**	0.48^{ns}	0.22^{ns}	0.52^{ns}	0.99^{ns}	7.4**
Fe × Zn × Mn	27	0.45^{ns}	0.41^{ns}	5.6**	0.35^{ns}	0.28^{ns}	0.41^{ns}	0.67^{ns}	6.4**
Cu × Zn × Mn × Fe	81	0.012^{ns}	0.32^{ns}	0.38^{ns}	0.24^{ns}	0.19^{ns}	0.48^{ns}	0.59^{ns}	0.68^{ns}
T(Cu,Zn,Mn,Fe)×Y	255	0.061^{ns}	0.11^{ns}	0.46^{ns}	0.41^{ns}	0.22^{ns}	0.44^{ns}	0.66^{ns}	0.42^{ns}
E	1020	0.11	0.55	1.1	0.91	1.02	1.44	1.53	0.72
CV^{x}		8.8	9.9	5.8	3.9	5.15	6.12	6.18	5.5

^{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.

was obtained with foliar spraying of 40 mg/L (0.59-0.68%), whereas the minimum (0.37-0.41%) was achieved in the control (Tables 4-9). In this investigation, the use of 40 mg/L improved the EO content of *F. angulate*.

According to the chemical analysis of the EOs from *F. angulate* by GC/MS, the most important chemical compounds that determine the quality of *F. angulate* EOs were identified as alpha-thujene (12.67-18.14%), alpha-pinene (20.13-35.88%), beta-phellandrene (1.11-7.12%), cis-ocimene (11.41-22.01%), and 4-thujanol (1.01-10.54%) (Tables 4-9). Alpha-pinene, belonging to monoterpene hydrocarbons, was the predominant constituent of the F. angulate EOs (Tables 4-9). The simple effects of the foliar application of each micronutrient were significant

Table 4
Means of EOs content and main constituents (%) in *F.angulate* plants affected by micronutrients (20 mg.l^{-1}) concentration (1st year)

Compound	RI[z]	Fe	Zn	Cu	Mn	Fe×Zn	Cu×Fe	Mn×Fe	Cu×Zn
alpha-Thujene	926	12.61±0.5[y]	12.43±0.4	12.22±0.3	12.55±0.2	12.81±0.2	13.01±0.3	13.09±0.2	13.02±0.2
alpha -pinene	937	22.21±0.5	21.42±0.4	21.55±0.4	21.66±0.5	22.33±0.6	22.41±0.7	23.66±0.5	23.55±0.6
beta-myrcene	986	1.81±0.01	1.75±0.1	1.66±0.01	1.63±0.02	1.93±0.08	1.91±0.01	1.88±0.01	1.91±0.01
alpha -phellandrene	1003	1.99±0.01	1.26±0.01	1.24±0.03	1.21±0.02	2.01±0.01	2.11±0.01	2.08±0.02	2.11±0.02
beta -phellandrene	1027	3.31±0.01	3.26±0.01	3.24±0.03	3.21±0.02	3.34±0.01	3.37±0.01	4.35±0.02	3.29±0.02
Cis-ocimene	1031	13.18±0.8	12.8±0.8	12.2±0.7	12.1±0.6	13.3±0.8	13.5±0.6	13.83±0.8	12.9±0.9
4-thujanol	1041	7.88±0.01	7.77±0.01	7.66±0.03	7.7±0.02	8.67±0.01	8.12±0.01	8.19±0.02	8.11±0.01
beta -ocimene	1053	3.88±0.01	3.77±0.01	3.65±0.02	3.58±0.02	4.01±0.01	4.11±0.01	4.14±0.02	4.19±0.3
Sabinene	1192	1.65±0.01	1.63±0.01	1.61±0.03	1.59±0.02	1.67±0.01	1.69±0.01	1.71±0.02	1.63±0.02
Verbenone	1204	6.69±0.01	6.65±0.03	6.61±0.02	6.51±0.1	6.71±0.06	6.73±0.03	6.74±0.02	6.69±0.02
Bornyl acetate	1219	3.29±0.01	3.26±0.01	3.25±0.02	3.21±0.02	3.36±0.01	3.39±0.01	3.45±0.02	3.41±0.03
Naphthalenemethanol	1468	1.11±0.01	1.09±0.01	1.07±0.02	1.02±0.01	1.13±0.02	1.14±0.01	1.16±0.02	1.15±0.03
Bicyclo-germacrene	1491	0.89±0.01	0.92±0.02	0.91±0.01	0.88±0.01	0.95±0.01	0.99±0.01	1.01±0.01	1.03±0.1
Nonadecane	1900	1.88±0.01	1.78±0.01	1.61±0.02	1.63±0.01	1.91±0.02	1.95±0.01	1.99±0.01	1.92±0.1
Henicosane	2109	0.88±0.02	0.78±0.01	0.61±0.02	0.63±0.01	0.91±0.02	0.95±0.01	1.02±0.01	1.14±0.1
Monoterpene hydrocarbons		60.64±1.8	58.32±1.9	57.37±1.7	57.53±1.5	61.4±1.6	62.11±1.4	64.74±1.8	62.6±1.4
Oxygenated monoterpenes		14.57±0.2	14.42±0.3	14.27±0.2	14.21±0.3	15.38±0.2	14.85±0.3	14.93±0.2	14.8±0.2
Sesquiterpenes		0.89±0.01	0.92±0.02	0.91±0.01	0.88±0.01	0.95±0.01	0.99±0.01	1.01±0.01	1.03±0.1
Acetate esters terpenoids		3.29±0.01	3.26±0.01	3.25±0.02	3.21±0.02	3.36±0.01	3.39±0.01	3.45±0.02	3.41±0.03
Polycyclic Aromatic Hydrocarbons		1.11±0.01	1.09±0.01	1.07±0.02	1.02±0.01	1.13±0.02	1.14±0.01	1.16±0.02	1.15±0.03
Hydrocarbons Alkanes		2.76±0.03	2.56±0.02	2.22±0.01	2.26±0.02	2.82±0.01	2.9±0.02	3.01±0.03	3.06±0.02
EOs content (w/w%, g/100g fresh weight basis)		0.41±0.01	0.40±0.01	0.40±0.02	0.39±0.01	0.42±0.02	0.42±0.01	0.41±0.01	0.43±0.01

[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)

Table 4
Continued means of EOs content and composition (%) in *F.angulate* plants affected by micronutrients (20 mg.l^{-1}) concentration (1st year)

Compound	RI[z]	Mn×Zn	Cu×Mn	Cu×Fe×Zn	Fe×Zn×Mn	Cu×Mn×Fe	Cu×Mn×Zn	Cu×Zn×Mn×Fe	Control
alpha -Thujene	926	13.61±0.5[y]	13.43±0.4	14.12±0.3	14.23±0.2	14.81±0.2	14.55±0.3	13.32±0.2	13.12±0.2
alpha -pinene	937	24.11±0.5	24.42±0.4	25.55±0.4	25.66±0.5	27.02±0.6	26.66±0.7	28.43±0.5	20.41±0.6
beta -myrcene	986	1.88±0.01	1.93±0.01	1.98±0.03	1.99±0.02	2.01±0.01	2.03±0.01	2.05±0.02	1.43±0.01
alpha -phellandrene	1003	1.88±0.01	1.94±0.01	2.11±0.01	2.14±0.02	2.22±0.01	2.15±0.01	2.31±0.02	1.51±0.01
beta -phellandrene	1027	3.12±0.01	3.26±0.01	3.33±0.04	3.32±0.02	3.36±0.01	3.29±0.01	4.01±0.01	3.12±0.02
Cis-ocimene	1031	13.18±0.6	13.21±0.5	14.22±0.5	13.25±0.6	14.12±0.6	13.89±0.5	15.22±0.6	11.41±0.6
4-thujanol	1041	8.21±0.01	8.12±0.01	8.66±0.03	8.73±0.02	8.92±0.01	8.45±0.01	9.11±0.02	8.03±0.01
beta -ocimene	1053	4.22±0.01	4.26±0.01	4.45±0.02	4.33±0.02	4.39±0.01	4.42±0.01	4.55±0.02	3.14±0.03
Sabinene	1192	1.66±0.01	1.69±0.01	1.71±0.03	1.73±0.02	1.81±0.01	1.84±0.01	1.99±0.02	1.58±0.01
Verbenone	1204	6.71±0.01	6.73±0.01	6.76±0.02	6.81±0.02	6.85±0.01	6.77±0.01	7.11±0.02	6.47±0.01
Bornyl acetate	1219	3.45±0.01	3.48±0.01	3.49±0.02	3.37±0.02	3.55±0.01	3.99±0.01	4.99±0.02	3.12±0.01
Naphthalenemethanol	1468	1.21±0.01	1.22±0.01	1.24±0.02	1.27±0.01	1.66±0.02	1.55±0.01	1.99±0.02	0.99±0.01
Bicyclo-germacrene	1491	1.07±0.01	1.09±0.02	1.11±0.01	1.15±0.01	1.21±0.04	1.23±0.03	2.11±0.01	0.79±0.01
Nonadecane	1900	1.98±0.01	2.01±0.01	2.05±0.02	2.08±0.01	2.39±0.02	2.15±0.01	0.71±0.01	5.45±0.01
Henicosane	2109	1.15±0.01	1.17±0.01	1.22±0.02	1.27±0.01	1.23±0.02	1.29±0.01	0.51±0.01	2.56±0.01
Monoterpene hydrocarbons		63.66±1.5	64.14±1.3	67.47±1.5	66.65±1.4	69.74±1.5	68.83±1.3	71.88±1.5	55.72±1.1
Oxygenated monoterpenes		14.92±0.04	14.85±0.05	15.42±0.03	15.54±0.04	15.77±0.05	15.22±0.05	16.22±0.04	14.5±0.02
Sesquiterpenes		1.07±0.01	1.09±0.02	1.11±0.01	1.15±0.01	1.21±0.04	1.23±0.03	2.11±0.01	0.79±0.01
Acetate esters terpenoids		3.45±0.01	3.48±0.01	3.49±0.02	3.37±0.02	3.55±0.01	3.99±0.01	4.99±0.02	3.12±0.01
Polycyclic Aromatic Hydrocarbons		1.21±0.01	1.22±0.01	1.24±0.02	1.27±0.01	1.66±0.02	1.55±0.01	1.99±0.02	0.99±0.01
Hydrocarbons Alkanes		3.13±0.02	3.18±0.03	3.27±0.02	3.35±0.03	3.62±0.02	3.44±0.04	1.22±0.02	8.01±0.01
EOs content (w/w%, g/100g fresh weight basis)		0.44±0.01	0.44±0.01	0.45±0.02	0.46±0.01	0.47±0.02	0.46±0.01	0.49±0.01	0.37±0.01

[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)

on the percentages of some of the major compounds in the EOs (Table 3). In general, the foliar application of micronutrients at 40 mg/L increased the percentage of most compounds compared to the control (Tables 4-9).

The interaction effects of micronutrients (Fe, Zn, Cu, and Mn) on the concentrations of constituents

Table 5
Means of EOs content and composition (%) in *F.angulate* plants affected by micronutrients (20 mg.l[-1]) concentration (2[nd] year)

Compound	RI [z]	Fe	Zn	Cu	Mn	Fe×Zn	Cu×Fe	Mn×Fe	Cu×Zn
alpha -Thujene	926	13.91±0.7[y]	13.84±0.5	13.82±0.6	13.77±0.6	15.99±0.5	14.85±0.5	14.12±0.6	14.14±0.5
alpha -pinene	937	22.11±0.9	21.9±0.8	21.5±0.7	21.53±0.9	23.44±0.6	24.6±0.5	24.43±0.5	23.8±0.6
beta -myrcene	986	1.65±0.01	1.63±0.01	1.62±0.03	1.58±0.02	1.75±0.01	1.82±0.01	1.86±0.02	1.89±0.02
alpha -phellandrene	1003	2.22±0.01	2.17±0.02	2.14±0.04	2.12±0.04	2.29±0.08	2.27±0.03	2.31±0.03	2.25±0.02
beta -phellandrene	1027	3.66±0.02	3.63±0.01	3.61±0.04	3.58±0.02	3.74±0.01	3.77±0.01	3.81±0.01	3.72±0.02
Cis-ocimene	1031	14.18±0.8	13.21±0.9	13.22±0.6	13.25±0.6	14.77±0.9	14.9±0.8	14.22±0.6	14.7±0.5
4-thujanol	1041	8.77±0.01	8.72±0.01	8.66±0.03	7.91±0.02	9.99±0.01	9.88±0.01	9.66±0.02	9.89±0.01
beta -ocimene	1053	4.66±0.01	4.71±0.01	4.12±0.02	4.33±0.02	4.44±0.01	4.52±0.01	4.99±0.02	4.71±0.03
Sabinene	1192	1.89±0.01	1.85±0.01	1.81±0.03	1.83±0.02	1.91±0.01	1.93±0.01	1.94±0.02	1.89±0.02
Verbenone	1204	6.94±0.01	6.92±0.01	7.89±0.02	6.81±0.02	7.96±0.01	6.98±0.01	7.99±0.02	6.94±0.01
Bornyl acetate	1219	3.61±0.01	3.58±0.01	3.55±0.02	3.49±0.02	3.65±0.01	3.68±0.01	3.71±0.02	3.61±0.03
Naphthalenemethanol	1468	1.19±0.01	1.17±0.01	1.14±0.02	1.15±0.01	1.21±0.02	1.23±0.01	1.26±0.02	1.31±0.03
Bicyclo-germacrene	1491	1.26±0.01	1.24±0.02	1.21±0.01	1.15±0.01	1.28±0.04	1.31±0.03	1.33±0.01	1.32±0.02
Nonadecane	1900	1.39±0.01	1.32±0.01	1.28±0.02	1.22±0.01	1.44±0.02	1.48±0.01	1.53±0.01	1.51±0.02
Henicosane	2109	0.88±0.01	0.78±0.01	0.66±0.02	0.81±0.01	0.91±0.02	0.97±0.01	0.91±0.01	0.92±0.02
Monoterpene hydrocarbons		64.28±1.6	62.94±1.4	61.84±1.5	61.99±1.3	68.33±1.5	68.66±1.6	67.68±1.4	67.11±1.3
Oxygenated monoterpenes		15.71±0.6	15.64±0.4	16.55±0.5	14.72±0.6	17.95±0.8	16.86±0.6	17.65±0.8	16.83±0.6
Sesquiterpenes		1.26±0.01	1.24±0.02	1.21±0.01	1.15±0.01	1.28±0.04	1.31±0.03	1.33±0.01	1.32±0.02
Acetate esters terpenoids		3.61±0.01	3.58±0.01	3.55±0.02	3.49±0.02	3.65±0.01	3.68±0.01	3.71±0.02	3.61±0.03
Polycyclic Aromatic Hydrocarbons		1.19±0.01	1.17±0.01	1.14±0.02	1.15±0.01	1.21±0.02	1.23±0.01	1.26±0.02	1.31±0.03
Hydrocarbons Alkanes		2.27±0.01	2.1±0.02	1.94±0.03	2.03±0.01	2.35±0.02	2.45±0.02	2.44±0.01	2.43±0.02
EOs content (w/w%, g/100g fresh weight basis)		0.44±0.02	0.43±0.01	0.42±0.02	0.42±0.01	0.45±0.02	0.45±0.01	0.45±0.01	0.46±0.01

[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)

Table 5
Continued means of EOs content and composition (%) in *F.angulate* plants affected by micronutrients (20 mg.l[-1]) concentration (2[nd] year)

Compound	RI [z]	Mn×Zn	Cu×Mn	Cu×Fe×Zn	Fe×Zn×Mn	Cu×Mn×Fe	Cu×Mn×Zn	Cu×Zn×Mn×Fe	Control
alpha -Thujene	926	14.17±0.6[y]	14.15±0.5	14.23±0.6	13.33±0.5	13.44±0.6	14.47±0.5	14.51±0.5	13.67±0.7
alpha -pinene	937	24.11±0.9	24.42±0.8	26.55±0.9	27.53±0.7	28.44±0.9	28.6±0.8	29.43±0.8	21.12±0.7
beta -myrcene	986	1.83±0.01	1.81±0.01	1.93±0.03	1.94±0.02	1.98±0.01	2.03±0.01	2.09±0.02	1.54±0.02
alpha -phellandrene	1003	2.23±0.01	2.21±0.01	2.25±0.03	2.27±0.02	2.29±0.01	2.31±0.01	2.33±0.02	1.66±0.02
beta -phellandrene	1027	3.57±0.04	3.59±0.01	3.81±0.04	3.83±0.02	3.79±0.01	3.89±0.01	4.15±0.01	3.55±0.02
Cis-ocimene	1031	14.18±0.9	13.21±0.7	13.22±0.5	14.25±0.5	14.77±0.6	15.11±0.7	15.32±0.8	11.71±0.8
4-thujanol	1041	8.89±0.01	8.91±0.01	8.66±0.03	9.01±0.02	9.03±0.01	8.99±0.01	9.22±0.02	8.29±0.01
beta -ocimene	1053	4.88±0.01	4.93±0.01	4.77±0.02	4.66±0.02	4.82±0.01	4.88±0.01	5.01±0.02	3.57±0.03
Sabinene	1192	1.91±0.01	1.93±0.01	1.95±0.03	1.99±0.02	2.01±0.01	2.06±0.01	2.14±0.02	1.71±0.02
Verbenone	1204	6.98±0.01	6.99±0.01	7.01±0.02	7.03±0.02	7.07±0.01	7.18±0.01	7.23±0.02	6.79±0.01
Bornyl acetate	1219	3.77±0.01	3.81±0.01	4.01±0.02	4.22±0.02	3.99±0.01	4.56±0.01	2.11±0.02	3.46±0.02
Naphthalenemethanol	1468	1.33±0.05	1.35±0.01	1.36±0.02	1.82±0.01	1.44±0.02	1.79±0.01	2.11±0.02	1.12±0.01
Bicyclo-germacrene	1491	1.44±0.04	1.55±0.02	1.69±0.01	1.78±0.01	1.71±0.04	0.31±0.03	0.21±0.01	1.12±0.01
Nonadecane	1900	1.56±0.06	1.62±0.01	1.61±0.02	2.17±0.01	2.01±0.02	0.81±0.01	0.98±0.01	5.99±0.02
Henicosane	2109	1.11±0.02	1.23±0.01	1.34±0.02	1.45±0.01	1.65±0.02	0.54±0.01	0.93±0.01	5.01±0.02
Monoterpene hydrocarbons		66.88±1.6	66.25±1.2	68.71±1.5	69.8±1.2	71.54±1.7	73.35±1.6	74.98±1.6	58.53±1.2
Oxygenated monoterpenes		15.87±0.2	15.9±0.1	15.67±0.3	16.04±0.2	17.01±0.1	16.17±0.3	16.45±0.2	15.08±0.1
Sesquiterpenes		1.44±0.04	1.55±0.02	1.69±0.01	1.78±0.01	1.71±0.04	0.31±0.03	0.21±0.01	1.12±0.01
Acetate esters terpenoids		3.77±0.01	3.81±0.01	4.01±0.02	4.22±0.02	3.99±0.01	4.56±0.01	2.11±0.02	3.46±0.02
Polycyclic Aromatic Hydrocarbons		1.33±0.05	1.35±0.01	1.36±0.02	1.82±0.01	1.44±0.02	1.79±0.01	2.11±0.02	1.12±0.01
Hydrocarbons Alkanes		3.01±0.01	2.85±0.02	2.95±0.01	3.62±0.03	3.66±0.04	1.35±0.03	1.91±0.04	11.01±0.06
EOs content (w/w%, g/100g fresh weight basis)		0.46±0.02	0.47±0.01	0.47±0.02	0.47±0.01	0.48±0.02	0.48±0.01	0.48±0.01	0.41±0.01

[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)

of EOs, such as alpha-thujene, alpha-pinene, beta-phellandrene, cis-ocimene, and 4-thujanol, were significant ($p \leq 0.01$) (Table 3). The highest levels of alpha-thujene, alpha-pinene, beta-phellandrene, cis-ocimene, and 4-thujanol were obtained under 40 mg/L treatments (18.14%,

Table 6
Means of EOs content and composition (%) in *F.angulate* plants affected by micronutrients (40 mg.l^{-1}) concentration and control plants (1st year)

Compound	RI^z	Fe	Zn	Cu	Mn	Fe×Zn	Cu×Fe	Mn×Fe	Cu×Zn
alpha -Thujene	926	14.33±0.5^y	14.09±0.8	14.08±0.5	14.12±0.6	13.33±0.7	13.39±0.5	13.44±0.6	14.22±0.5
alpha -pinene	937	29.11±0.9	27.9±0.9	27.6±0.8	27.1±0.9	30.1±0.8	30.3±0.9	30.4±0.7	29.8±0.8
beta -myrcene	986	2.23±0.01	2.19±0.01	2.18±0.03	2.15±0.02	2.26±0.01	2.29±0.01	2.33±0.02	2.25±0.02
alpha -phellandrene	1003	2.77±0.1	2.64±0.1	2.59±0.1	2.61±0.1	2.65±0.08	2.77±0.1	2.82±0.1	2.55±0.1
beta -phellandrene	1027	4.35±0.1	4.12±0.1	3.99±0.1	4.02±0.1	4.38±0.1	4.44±0.05	4.55±0.05	4.66±0.1
Cis-ocimene	1031	16.18±0.9	15.1±0.8	15.2±0.9	14.9±0.6	16.4±0.8	16.9±0.9	16.22±0.8	16.7±0.7
4-thujanol	1041	6.88±0.01	6.92±0.01	6.66±0.03	6.74±0.02	6.92±0.01	6.84±0.01	6.99±0.02	6.81±0.01
beta -ocimene	1053	4.98±0.01	4.77±0.01	4.65±0.02	4.51±0.02	4.99±0.01	5.05±0.01	5.09±0.02	5.01±0.03
Sabinene	1192	2.59±0.01	2.51±0.01	2.48±0.03	2.44±0.02	2.61±0.01	2.63±0.01	2.65±0.02	2.71±0.02
Verbenone	1204	5.45±0.01	5.41±0.01	5.33±0.02	5.22±0.02	4.55±0.01	4.61±0.01	4.65±0.02	4.71±0.01
Bornyl acetate	1219	3.99±0.01	3.88±0.01	3.74±0.02	3.58±0.02	4.01±0.01	4.04±0.01	4.19±0.02	4.05±0.03
Naphthalenemethanol	1468	1.19±0.1	1.14±0.05	1.12±0.05	1.09±0.07	1.32±0.07	1.38±0.08	1.42±0.07	1.39±0.07
Bicyclo-germacrene	1491	2.88±0.1	2.55±0.05	2.44±0.07	2.39±0.08	2.89±0.09	2.94±0.06	2.97±0.08	2.01±0.1
Nonadecane	1900	0.56±0.1	0.33±0.06	0.21±0.07	0.59±0.06	0.61±0.03	0.81±0.08	0.71±0.07	0.82±0.1
Henicosane	2109	0.91±0.1	0.78±0.06	0.61±0.06	0.63±0.06	0.92±0.07	0.95±0.06	0.94±0.05	0.92±0.1
Monoterpene hydrocarbons		76.54±1.6	73.32±1.3	72.77±1.2	71.4±1.5	76.72±1.6	77.71±1.7	77.5±1.6	77.9±1.9
Oxygenated monoterpenes		12.33±0.5	12.33±0.4	13.99±0.7	12.96±0.6	13.47±0.8	13.45±0.5	11.64±0.4	11.52±0.5
Sesquiterpenes		2.88±0.1	2.55±0.05	2.44±0.07	2.39±0.08	2.89±0.09	2.94±0.06	2.97±0.08	2.01±0.1
Acetate esters terpenoids		3.99±0.01	3.88±0.01	3.74±0.02	3.58±0.02	4.01±0.01	4.04±0.01	4.19±0.02	4.05±0.03
Polycyclic Aromatic Hydrocarbons		1.19±0.1	1.14±0.05	1.12±0.05	1.09±0.07	1.32±0.07	1.38±0.08	1.42±0.07	1.39±0.07
Hydrocarbons Alkanes		1.47±0.1	1.11±0.2	0.82±0.1	1.22±0.2	1.53±0.3	1.76±0.2	1.65±0.1	1.74±0.5
EOs content (w/w%, g/100g fresh weight basis)		0.46±0.01	0.45±0.01	0.45±0.02	0.45±0.01	0.47±0.02	0.47±0.01	0.48±0.01	0.48±0.02

^{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)

Table 6
Continued means of EOs content and composition (%) in *F.angulate* plants affected by micronutrients (40 mg.l^{-1}) concentration and control plants (1st year)

Compound	RI^z	Mn×Zn	Cu×Mn	Cu×Fe×Zn	Fe×Zn×Mn	Cu×Mn×Fe	Cu×Mn×Zn	Cu×Zn×Mn×Fe
alpha -Thujene	926	14.44±0.8^y	14.31±0.9	14.29±0.8	14.33±0.9	14.54±0.7	14.31±0.6	16.12±0.6
alpha -pinene	937	31.11±0.9	30.42±0.9	32.55±0.8	33.53±0.8	33.44±0.9	33.11±0.9	34.51±0.8
beta -myrcene	986	0.56±0.01	0.63±0.01	0.78±0.03	0.73±0.02	0.69±0.01	0.65±0.01	0.81±0.02
alpha -phellandrene	1003	0.28±0.01	0.23±0.01	0.69±0.03	0.75±0.02	0.77±0.01	0.33±0.01	0.12±0.02
beta -phellandrene	1027	1.55±0.1	1.64±0.06	1.11±0.06	1.23±0.06	1.44±0.05	1.24±0.05	1.55±0.08
Cis-ocimene	1031	16.18±0.7	17.21±0.9	17.22±0.9	18.25±0.8	18.77±0.9	17.9±0.8	19.11±0.9
4-thujanol	1041	3.98±0.01	3.88±0.01	3.66±0.03	5.01±0.02	4.12±0.01	4.03±0.01	5.12±0.02
beta -ocimene	1053	5.07±0.01	5.11±0.01	5.15±0.02	5.99±0.02	5.31±0.01	5.77±0.01	5.41±0.02
Sabinene	1192	2.88±0.01	2.91±0.01	2.94±0.03	3.15±0.02	3.12±0.01	3.18±0.01	3.55±0.02
Verbenone	1204	4.78±0.01	4.81±0.01	4.85±0.02	5.11±0.02	5.11±0.01	5.01±0.01	5.44±0.02
Bornyl acetate	1219	5.11±0.01	5.16±0.01	5.19±0.02	5.99±0.02	5.11±0.01	5.78±0.01	6.51±0.02
Naphthalenemethanol	1468	2.42±0.1	2.45±0.05	2.49±0.05	2.72±0.06	2.73±0.06	2.66±0.05	3.21±0.05
Bicyclo-germacrene	1491	1.02±0.1	1.04±0.05	1.08±0.05	1.11±0.06	1.14±0.07	1.09±0.08	1.21±0.05
Nonadecane	1900	0.89±0.1	0.01±0.05	0.03±0.05	0.08±0.06	0.44±0.06	0.22±0.05	0.45±0.06
Henicosane	2109	0.01±0.1	0.07±0.06	0.09±0.08	0.11±0.08	0.22±0.05	0.09±0.06	0.56±0.05
Monoterpene hydrocarbons		72.07±1.5	72.46±1.2	74.73±1.5	77.96±1.1	78.08±1.2	76.49±1.5	81.18±1.8
Oxygenated monoterpenes		4.78±0.01	4.81±0.01	4.85±0.02	5.11±0.02	5.11±0.01	5.01±0.01	5.44±0.02
Sesquiterpenes		1.02±0.1	1.04±0.05	1.08±0.05	1.11±0.06	1.14±0.07	1.09±0.08	1.21±0.05
Acetate esters terpenoids		5.11±0.01	5.16±0.01	5.19±0.02	5.99±0.02	5.11±0.01	5.78±0.01	6.51±0.02
Polycyclic Aromatic Hydrocarbons		2.42±0.1	2.45±0.05	2.49±0.05	2.72±0.06	2.73±0.06	2.66±0.05	3.21±0.05
Hydrocarbons Alkanes		0.90±0.01	0.08±0.01	0.12±0.01	0.19±0.01	0.66±0.02	0.31±0.03	1.01±0.01
EOs content (w/w%, g/100g fresh weight basis)		0.49±0.02	0.48±0.01	0.50±0.02	0.51±0.01	0.57±0.02	0.55±0.01	0.59±0.01

^{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)

35.88%, 7.12%, 22.01%, and 10.54%, respectively) (Tables 4-9).

According to the biennial results of the chemical analysis of the EOs from *F. angulate* by GC/MS, the most important chemical compounds that

Table 7
Means of EOs content and composition (%) in *F.angulate* plants affected by micronutrients (40 $mg.l^{-1}$) concentration and control plants (2nd year)

Compound	RI [z]	Fe	Zn	Cu	Mn	Fe×Zn	Cu×Fe	Mn×Fe	Cu×Zn
alpha -Thujene	926	15.77±0.9[y]	15.12±0.8	14.22±0.8	14.31±0.8	14.26±0.9	15.39±0.7	15.51±0.6	16.22±0.9
alpha -pinene	937	32.11±0.9	31.9±0.9	31.5±0.8	31.3±0.8	33.4±0.9	33.6±0.9	32.43±1.1	31.8±0.9
beta -myrcene	986	2.77±0.01	2.65±0.01	2.61±0.03	2.55±0.02	2.81±0.01	2.83±0.01	2.84±0.02	2.73±0.02
alpha -phellandrene	1003	2.99±0.1	2.87±0.1	2.82±0.1	2.79±0.2	3.11±0.08	3.15±0.1	3.16±0.1	3.01±0.1
beta -phellandrene	1027	5.57±0.1	5.59±0.08	4.61±0.08	4.63±0.06	6.64±0.08	6.59±0.09	6.93±0.07	6.59±0.1
Cis-ocimene	1031	17.18±0.9	16.1±0.9	15.8±0.8	15.5±0.8	17.7±0.9	17.9±0.6	17.22±0.8	17.4±0.9
4-thujanol	1041	3.89±0.01	3.77±0.01	2.66±0.03	3.67±0.02	3.9±0.01	2.11±0.01	2.15±0.02	2.19±0.01
beta -ocimene	1053	5.01±0.01	4.88±0.01	4.66±0.02	4.71±0.02	5.11±0.01	5.52±0.01	5.33±0.02	5.21±0.03
Sabinene	1192	2.88±0.01	2.77±0.01	2.66±0.03	2.55±0.02	2.91±0.01	2.94±0.01	2.95±0.02	2.88±0.02
Verbenone	1204	2.66±0.01	3.55±0.01	2.34±0.02	2.31±0.02	3.69±0.01	2.73±0.01	2.75±0.02	2.71±0.01
Bornyl acetate	1219	2.99±0.01	2.81±0.01	2.73±0.02	2.55±0.02	1.01±0.01	1.09±0.01	1.19±0.02	1.02±0.03
Naphthalenemethanol	1468	0.14±0.02	0.18±0.01	0.15±0.02	0.14±0.01	0.15±0.02	0.19±0.01	0.22±0.02	0.18±0.03
Bicyclo-germacrene	1491	2.45±0.01	2.33±0.02	2.28±0.01	2.17±0.01	2.48±0.04	2.49±0.03	2.55±0.01	2.51±0.1
Nonadecane	1900	0.33±0.03	0.21±0.01	0.11±0.02	0.07±0.01	0.45±0.02	0.81±0.01	0.71±0.01	0.82±0.03
Henicosane	2109	0.34±0.01	0.25±0.01	0.12±0.02	0.09±0.01	0.44±0.02	0.47±0.01	0.55±0.01	0.59±0.01
Monoterpene hydrocarbons		84.28±1.6	81.88±1.2	78.88±1.4	78.34±1.2	85.94±1.6	87.92±1.2	86.37±1.6	85.84±1.2
Oxygenated monoterpenes		6.55±0.3	7.32±0.4	5.01±0.01	5.98±0.2	7.59±0.3	4.84±0.2	4.90±0.3	4.90±0.2
Sesquiterpenes		2.45±0.01	2.33±0.02	2.28±0.01	2.17±0.01	2.48±0.04	2.49±0.03	2.55±0.01	2.51±0.1
Acetate esters terpenoids		2.99±0.01	2.81±0.01	2.73±0.02	2.55±0.02	1.01±0.01	1.09±0.01	1.19±0.02	1.02±0.03
Polycyclic Aromatic Hydrocarbons		0.14±0.02	0.18±0.01	0.15±0.02	0.14±0.01	0.15±0.02	0.19±0.01	0.22±0.02	0.18±0.03
Hydrocarbons Alkanes		0.67±0.01	0.46±0.01	0.23±0.01	0.16±0.01	0.89±0.01	1.28±0.04	1.26±0.03	1.41±0.05
EOs content (w/w%, g/100g fresh weight basis)		0.59±0.02	0.58±0.01	0.58±0.02	0.58±0.01	0.60±0.02	0.61±0.01	0.61±0.01	0.61±0.02

[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)

Table 7
Continued means of EOs content and composition (%) in *F.angulate* plants affected by micronutrients (40 $mg.l^{-1}$) concentration and control plants (2nd year).

Compound	RI [z]	Mn×Zn	Cu×Mn	Cu×Fe×Zn	Fe×Zn×Mn	Cu×Mn×Fe	Cu×Mn×Zn	Cu×Zn×Mn×Fe
alpha -Thujene	926	16.11±0.6[y]	16.18±0.7	17.12±0.5	17.34±0.9	17.55±0.8	17.21±0.9	18.14±0.8
alpha -pinene	937	31.11±1.1	31.42±1.1	33.55±1.2	34.53±1.4	34.44±1.2	33.6±1.5	34.88±1.5
beta -myrcene	986	2.66±0.01	2.71±0.01	2.89±0.03	2.81±0.02	2.85±0.01	2.82±0.01	2.12±0.02
alpha -phellandrene	1003	3.03±0.01	3.06±0.01	3.19±0.03	3.22±0.02	3.31±0.01	3.25±0.01	3.44±0.02
beta -phellandrene	1027	5.57±0.1	6.11±0.1	6.61±0.08	6.63±0.09	6.64±0.08	6.23±0.08	6.12±0.07
Cis-ocimene	1031	17.18±1.1	18.21±1.2	18.22±0.9	18.25±0.9	19.77±1.1	18.9±0.9	19.01±1.1
4-thujanol	1041	3.15±0.01	3.22±0.01	3.36±0.03	2.66±0.02	1.91±0.01	1.11±0.01	1.54±0.02
beta -ocimene	1053	5.41±0.01	5.43±0.01	5.55±0.02	5.57±0.02	5.63±0.01	5.33±0.01	5.89±0.02
Sabinene	1192	2.93±0.01	2.91±0.01	3.01±0.03	3.09±0.02	3.18±0.01	3.29±0.01	3.69±0.02
Verbenone	1204	2.75±0.01	2.77±0.01	2.81±0.02	3.83±0.02	2.11±0.01	2.99±0.01	1.59±0.02
Bornyl acetate	1219	1.18±0.01	1.22±0.01	1.31±0.02	1.33±0.02	1.39±0.01	1.55±0.01	0.87±0.02
Naphthalenemethanol	1468	0.26±0.05	0.36±0.01	0.44±0.02	0.51±0.01	0.99±0.02	0.88±0.01	0.51±0.02
Bicyclo-germacrene	1491	0.53±0.05	0.56±0.02	0.62±0.01	0.72±0.01	0.81±0.04	0.76±0.03	0.01±0.01
Nonadecane	1900	0.56±0.07	0.61±0.01	0.64±0.02	0.99±0.01	0.88±0.02	0.31±0.01	0.99±0.01
Henicosane	2109	0.88±0.04	0.11±0.01	0.61±0.02	0.63±0.01	0.91±0.02	0.82±0.01	0.01±0.01
Monoterpene hydrocarbons		84.01±1.8	86.03±1.4	90.13±1.6	91.44±1.1	93.37±1.4	90.63±1.2	93.29±1.1
Oxygenated monoterpenes		5.9±0.2	5.99±0.3	6.17±0.2	5.49±0.4	5.02±0.3	5.1±0.4	3.13±0.2
Sesquiterpenes		0.53±0.05	0.56±0.02	0.62±0.01	0.72±0.01	0.81±0.04	0.76±0.03	0.11±0.01
Acetate esters terpenoids		1.18±0.01	1.22±0.01	1.31±0.02	1.33±0.02	1.39±0.01	1.55±0.01	0.87±0.02
Polycyclic Aromatic Hydrocarbons		0.26±0.05	0.36±0.01	0.44±0.02	0.51±0.01	0.99±0.02	0.88±0.01	0.51±0.02
Hydrocarbons Alkanes		1.44±0.05	0.77±0.02	1.25±0.06	1.62±0.05	1.79±0.06	1.13±0.04	1.01±0.01
EOs content (w/w%, g/100g fresh weight basis)		0.62±0.01	0.62±0.01	0.63±0.02	0.63±0.01	0.64±0.02	0.65±0.01	0.68±0.01

[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)

determine the quality of *F. angulate* EOs, including alpha-thujene, alpha-pinene, beta-phellandrene, cis-ocimene, and 4-thujanol, were identified. By comparing the data from the two years, there were positive correlations between EO content and the main components, and a high correlation was observed between EO content and alpha-thujene, alpha-pinene, beta-myrcene, alpha-

Table 8

Means of EOs content and composition (%) in *F.angulate* plants affected by micronutrients (60 mg.l^{-1}) concentration (1st year)

Compound	RI ^{z}	Fe	Zn	Cu	Mn	Fe×Zn	Cu×Fe	Mn×Fe	Cu×Zn
alpha -Thujene	926	18.9±1.2^y	18.7±1.4	17.5±1.1	18.2±0.9	18.1±0.8	17.6±0.9	18.39±0.8	19.4±0.9
alpha -pinene	937	23.4±1.1	23.2±1.2	22.5±1.1	22.3±1.2	21.9±0.9	21.6±0.8	20.43±0.9	21.8±1.1
beta -myrcene	986	1.88±0.01	1.77±0.01	1.66±0.03	1.58±0.02	1.82±0.01	1.81±0.01	1.79±0.02	1.78±0.02
alpha -phellandrene	1003	1.71±0.01	1.69±0.01	1.67±0.03	1.59±0.02	1.67±0.01	1.62±0.01	1.59±0.02	1.58±0.02
beta -phellandrene	1027	5.35±0.06	5.12±0.01	5.99±0.04	5.02±0.02	4.89±0.01	4.78±0.01	4.31±0.01	4.66±0.07
Cis-ocimene	1031	15.77±0.9	14.66±0.7	14.81±0.8	14.5±0.9	13.7±0.8	13.9±0.7	12.22±0.7	13.7±0.8
4-thujanol	1041	3.88±0.01	3.77±0.1	4.66±0.0	3.17±0.2	4.99±0.08	3.88±0.09	2.66±0.08	3.65±0.08
beta -ocimene	1053	5.03±0.01	4.88±0.01	4.73±0.02	4.61±0.02	4.55±0.01	4.66±0.01	3.15±0.02	3.55±0.03
Sabinene	1192	2.01±0.01	1.99±0.01	1.89±0.03	1.79±0.02	1.69±0.01	1.73±0.01	1.71±0.02	1.75±0.02
Verbenone	1204	3.15±0.01	3.14±0.01	3.11±0.02	2.08±0.02	3.17±0.01	2.13±0.01	3.12±0.02	3.17±0.01
Bornyl acetate	1219	4.43±0.01	4.35±0.01	4.22±0.02	4.15±0.02	4.11±0.01	4.05±0.01	4.01±0.02	4.08±0.03
Naphthalenemethanol	1468	1.67±0.05	1.69±0.01	1.71±0.02	1.72±0.01	1.55±0.02	1.51±0.01	1.49±0.02	1.52±0.03
Bicyclo-germacrene	1491	1.88±0.04	1.76±0.02	1.66±0.01	1.58±0.01	1.91±0.04	1.94±0.03	1.88±0.01	1.82±0.03
Nonadecane	1900	1.56±0.03	1.63±0.01	1.61±0.02	1.63±0.01	1.72±0.02	1.81±0.01	1.75±0.01	1.73±0.02
Henicosane	2109	1.32±0.05	1.26±0.01	1.22±0.02	1.11±0.01	1.22±0.02	1.33±0.01	1.17±0.01	1.08±0.06
Monoterpene hydrocarbons		74.05±1.5	72.01±1.2	70.75±1.8	69.59±1.3	68.32±1.4	67.7±1.2	63.59±1.4	68.22±1.2
Oxygenated monoterpenes		7.03±0.3	6.91±0.4	7.77±0.2	5.25±0.3	8.16±0.5	6.01±0.3	5.78±0.2	6.82±0.3
Sesquiterpenes		1.88±0.04	1.76±0.02	1.66±0.01	1.58±0.01	1.91±0.04	1.94±0.03	1.88±0.01	1.82±0.03
Acetate esters terpenoids		4.43±0.01	4.35±0.01	4.22±0.02	4.15±0.02	4.11±0.01	4.05±0.01	4.01±0.02	4.08±0.03
Polycyclic Aromatic Hydrocarbons		1.67±0.05	1.69±0.01	1.71±0.02	1.72±0.01	1.55±0.02	1.51±0.01	1.49±0.02	1.52±0.03
Hydrocarbons Alkanes		2.88±0.05	2.89±0.04	2.83±0.03	2.74±0.02	2.94±0.04	3.14±0.05	2.92±0.02	2.81±0.01
EOs content (w/w%, g/100g fresh weight basis)		0.44±0.02	0.43±0.01	0.41±0.02	0.42±0.01	0.40±0.02	0.39±0.01	0.38±0.01	0.40±0.01

^{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)

Table 8

Continued means of EOs content and composition (%) in *F.angulate* plants affected by micronutrients (60 mg.l^{-1}) concentration (1st year)

Compound	RI ^{z}	Mn×Zn	Cu×Mn	Cu×Fe×Zn	Fe×Zn×Mn	Cu×Mn×Fe	Cu×Mn×Zn	Cu×Zn×Mn×Fe
alpha -Thujene	926	18.32±1.1^y	18.35±0.9	18.29±0.8	19.25±0.9	18.22±0.8	17.57±0.9	13.11±0.5
alpha -pinene	937	21.11±1.2	21.42±1.2	22.55±0.9	22.53±0.8	21.44±1.1	20.64±1.1	19.43±0.9
beta -myrcene	986	1.67±0.01	1.77±0.01	1.66±0.03	1.59±0.02	1.56±0.01	1.53±0.01	1.45±0.02
alpha -phellandrene	1003	1.63±0.01	1.65±0.01	1.62±0.03	1.58±0.02	1.56±0.01	1.57±0.01	1.52±0.02
beta -phellandrene	1027	4.45±0.06	4.33±0.01	4.23±0.04	4.23±0.02	3.44±0.01	3.49±0.01	3.13±0.01
Cis-ocimene	1031	13.18±0.9	12.21±0.8	13.22±0.9	12.25±0.9	12.77±1.1	12.9±1.2	11.43±1.1
4-thujanol	1041	3.51±0.01	3.33±0.01	1.22±0.03	1.11±0.02	1.09±0.01	2.01±0.01	1.01±0.02
beta -ocimene	1053	3.42±0.01	3.66±0.01	3.55±0.02	3.44±0.02	3.61±0.01	3.52±0.01	3.12±0.02
Sabinene	1192	2.01±0.01	1.92±0.01	1.89±0.03	1.83±0.02	1.77±0.01	1.66±0.01	1.56±0.02
Verbenone	1204	3.15±0.01	3.11±0.01	2.06±0.02	3.05±0.02	3.16±0.01	2.78±0.01	2.45±0.02
Bornyl acetate	1219	3.98±0.01	3.89±0.01	3.77±0.02	3.65±0.02	3.41±0.01	3.53±0.01	3.11±0.02
Naphthalenemethanol	1468	3.48±0.07	3.44±0.01	3.41±0.02	3.37±0.01	3.33±0.02	3.39±0.01	3.01±0.02
Bicyclo-germacrene	1491	1.55±0.07	1.44±0.02	1.37±0.01	1.22±0.01	0.99±0.04	1.01±0.03	0.78±0.01
Nonadecane	1900	4.56±0.05	4.62±0.01	3.59±0.02	3.61±0.01	4.44±0.02	3.12±0.01	3.99±0.01
Henicosane	2109	4.09±0.05	3.01±0.01	3.02±0.02	3.01±0.01	3.08±0.02	3.95±0.01	4.54±0.01
Monoterpene hydrocarbons		65.79±1.4	65.31±1.2	65.39±1.4	66.7±1.7	64.37±1.2	62.88±1.1	54.75±1.4
Oxygenated monoterpenes		6.66±0.3	6.44±0.2	3.28±0.3	4.16±0.2	4.25±0.1	4.79±0.2	3.46±0.1
Sesquiterpenes		1.55±0.07	1.44±0.02	1.37±0.01	1.22±0.01	0.99±0.04	1.01±0.03	0.78±0.01
Acetate esters terpenoids		3.98±0.01	3.89±0.01	3.77±0.02	3.65±0.02	3.41±0.01	3.53±0.01	3.11±0.02
Polycyclic Aromatic Hydrocarbons		4.48±0.07	3.44±0.01	3.41±0.02	3.37±0.01	3.33±0.02	3.39±0.01	3.01±0.02
Hydrocarbons Alkanes		8.65±0.3	7.63±0.2	6.61±0.1	6.62±0.2	7.52±0.2	7.07±0.3	8.53±0.2
EOs content (w/w%, g/100g fresh weight basis)		0.39±0.01	0.40±0.01	0.41±0.02	0.40±0.01	0.39±0.02	0.39±0.01	0.38±0.01

^{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)

phellandrene, beta-phellandrene, cis-ocimene, beta-ocimene, and sabinene (Table 10).

Discussion

In this study, the quality of essential oils (EOs), expressed as the percentage of chemical compounds, showed a significant increase with

Table 9
Means of EOs content and composition (%) in *F.angulate* plants affected by micronutrients (60 mg.l-1) concentration (2nd year)

Compound	RI z	Fe	Zn	Cu	Mn	Fe×Zn	Cu×Fe	Mn×Fe	Cu×Zn
alpha -Thujene	926	14.12±0.9y	14.03±0.8	14.05±0.9	13.09±0.8	13.02±0.9	13.99±0.7	14.82±0.7	14.75±0.8
alpha -pinene	937	23.11±1.1	23.1±1.2	23.5±1.1	23.3±1.1	22.9±1.2	22.6±1.1	22.43±1.2	22.8±0.9
beta -myrcene	986	1.91±0.01	1.88±0.01	1.76±0.03	1.87±0.02	1.77±0.01	1.72±0.01	1.71±0.02	1.69±0.02
alpha -phellandrene	1003	2.01±0.01	1.89±0.01	1.91±0.03	1.85±0.02	1.87±0.01	1.79±0.01	1.75±0.02	1.78±0.02
beta -phellandrene	1027	4.57±0.07	4.34±0.01	4.61±0.04	4.63±0.02	4.64±0.01	4.59±0.01	4.94±0.01	4.59±0.07
Cis-ocimene	1031	16.18±0.9	16.1±0.8	15.2±0.9	15.5±0.8	15.7±0.7	14.9±0.9	14.22±0.8	14.7±0.9
4-thujanol	1041	10.12±0.01	10.11±0.5	10.08±0.5	9.02±0.08	9.91±0.07	9.88±0.09	8.79±0.08	8.68±0.09
beta -ocimene	1053	5.11±0.01	5.08±0.01	5.01±0.02	5.04±0.02	4.88±0.01	4.79±0.01	4.66±0.02	4.82±0.03
Sabinene	1192	2.12±0.01	2.08±0.01	2.03±0.03	2.01±0.02	1.99±0.01	1.89±0.01	2.01±0.02	2.04±0.02
Verbenone	1204	7.15±0.01	7.13±0.01	7.08±0.02	8.03±0.02	7.19±0.01	7.11±0.01	7.07±0.02	8.01±0.01
Bornyl acetate	1219	5.22±0.01	5.12±0.01	5.06±0.02	5.02±0.02	5.14±0.01	5.12±0.01	4.99±0.02	5.01±0.03
Naphthalenemethanol	1468	1.89±0.05	1.78±0.01	1.77±0.02	1.69±0.01	1.65±0.02	1.61±0.01	1.58±0.02	1.62±0.03
Bicyclo-germacrene	1491	1.91±0.06	1.89±0.02	1.77±0.01	1.66±0.01	1.55±0.04	1.44±0.03	1.39±0.01	1.47±0.05
Nonadecane	1900	1.44±0.04	1.35±0.01	1.31±0.02	1.28±0.01	1.47±0.02	1.46±0.01	1.48±0.01	1.33±0.05
Henicosane	2109	1.33±0.04	1.21±0.01	1.19±0.02	1.12±0.01	1.09±0.02	1.11±0.01	1.13±0.01	1.17±0.04
Monoterpene hydrocarbons		69.13±1.3	68.5±1.1	68.07±1.2	67.29±1.1	66.77±1.3	66.27±1.1	66.54±1.2	67.17±1.1
Oxygenated monoterpenes		17.27±0.6	17.24±0.3	17.16±0.2	17.05±0.3	17.01±0.2	16.99±0.5	15.86±0.3	16.69±0.4
Sesquiterpenes		1.91±0.06	1.89±0.02	1.77±0.01	1.66±0.01	1.55±0.04	1.44±0.03	1.39±0.01	1.47±0.05
Acetate esters terpenoids		5.22±0.01	5.12±0.01	5.06±0.02	5.02±0.02	5.14±0.01	5.12±0.01	4.99±0.02	5.01±0.03
Polycyclic Aromatic Hydrocarbons		1.89±0.05	1.78±0.01	1.77±0.02	1.69±0.01	1.65±0.02	1.61±0.01	1.58±0.02	1.62±0.03
Hydrocarbons Alkanes		2.77±0.01	2.56±0.01	2.51±0.01	2.41±0.01	2.56±0.01	2.57±0.02	2.61±0.01	2.51±0.01
EOs content (w/w%, g/100g fresh weight basis)		0.51±0.01	0.50±0.01	0.50±0.02	0.49±0.01	0.48±0.02	0.50±0.01	0.48±0.01	0.48±0.01

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)

Table 9
Continued means of EOs content and composition (%) in *F.angulate* plants affected by micronutrients (60 mg.l-1) concentration (2nd year)

Compound	RI z	Mn×Zn	Cu×Mn	Cu×Fe×Zn	Fe×Zn×Mn	Cu×Mn×Fe	Cu×Mn×Zn	Cu×Zn×Mn×Fe
alpha -Thujene	926	13.71±0.6y	13.79±0.7	12.81±0.8	13.81±0.7	13.77±0.6	14.72±0.7	12.18±0.7
alpha -pinene	937	22.11±1.1	22.42±1.1	22.55±0.9	22.53±0.9	21.95±0.8	21.44±0.8	20.13±0.9
beta -myrcene	986	1.71±0.01	1.68±0.01	1.61±0.03	1.62±0.02	1.59±0.01	1.57±0.01	1.55±0.02
alpha -phellandrene	1003	1.89±0.01	1.84±0.01	1.82±0.03	1.75±0.02	1.73±0.01	1.74±0.01	1.65±0.02
beta -phellandrene	1027	4.57±0.04	4.59±0.01	4.11±0.04	4.16±0.02	4.12±0.01	4.09±0.01	3.56±0.01
Cis-ocimene	1031	14.18±1.2	13.21±1.1	13.22±1.2	12.25±0.9	12.77±0.8	12.9±0.9	11.72±0.8
4-thujanol	1041	9.55±0.01	9.33±0.01	9.22±0.03	8.12±0.02	8.99±0.01	8.81±0.01	8.28±0.02
beta -ocimene	1053	4.55±0.01	4.34±0.01	4.12±0.02	4.09±0.02	4.02±0.01	3.89±0.01	3.55±0.02
Sabinene	1192	2.02±0.01	2.01±0.01	1.99±0.03	1.89±0.02	1.88±0.01	1.91±0.01	1.69±0.02
Verbenone	1204	7.05±0.01	7.06±0.01	7.03±0.02	6.99±0.02	6.93±0.01	6.96±0.01	5.78±0.02
Bornyl acetate	1219	4.78±0.01	4.65±0.01	4.55±0.02	4.43±0.02	4.21±0.01	4.22±0.01	3.45±0.02
Naphthalenemethanol	1468	1.67±0.04	1.55±0.01	1.48±0.02	1.44±0.01	1.38±0.02	1.39±0.01	1.13±0.02
Bicyclo-germacrene	1491	1.33±0.05	1.25±0.02	1.22±0.01	1.17±0.01	1.11±0.04	1.15±0.03	1.01±0.01
Nonadecane	1900	5.31±0.03	5.21±0.01	5.27±0.02	5.25±0.01	5.22±0.02	5.19±0.01	5.81±0.01
Henicosane	2109	4.09±0.07	4.11±0.01	4.06±0.02	4.05±0.01	4.91±0.02	4.95±0.01	4.99±0.01
Monoterpene hydrocarbons		64.74±1.4	63.88±1.2	62.23±1.5	62.1±1.1	61.83±1.2	62.26±1.4	56.03±0.9
Oxygenated monoterpenes		16.6±0.8	16.39±0.4	16.25±0.4	15.11±0.3	15.92±0.5	15.77±0.4	14.06±0.5
Sesquiterpenes		1.33±0.05	1.25±0.02	1.22±0.01	1.17±0.01	1.11±0.04	1.15±0.03	1.01±0.01
Acetate esters terpenoids		4.78±0.01	4.65±0.01	4.55±0.02	4.43±0.02	4.21±0.01	4.22±0.01	3.45±0.02
Polycyclic Aromatic Hydrocarbons		1.67±0.04	1.55±0.01	1.48±0.02	1.44±0.01	1.38±0.02	1.39±0.01	1.13±0.02
Hydrocarbons Alkanes		9.40±0.04	9.32±0.03	9.33±0.04	9.30±0.05	10.13±0.03	10.14±0.04	10.81±0.03
EOs content (w/w%, g/100g fresh weight basis)		0.47±0.01	0.46±0.01	0.47±0.02	0.45±0.01	0.45±0.02	0.44±0.01	0.42±0.01

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)

the application of micronutrients. However, higher concentrations of micronutrients (greater than 20 mg L^{-1}) in most components led to decreased EO content. According to the results, the application of micronutrients significantly influenced the EO content and composition of F. angulate. The traits assessed showed slight variations over the study years. Plants treated with 20 and 40 mg L^{-1} were generally grouped together for most measured traits. In contrast, plants treated with 60 mg L^{-1} often had lower values than the control plants.

(oxygenated monoterpenes), bicyclo-germacrene (sesquiterpenes), bornyl acetate (acetate ester terpenoids), naphthalenemethanol (polycyclic aromatic hydrocarbons), and nonadecane and henicosane (alkane hydrocarbons).

Table 10
Results of simple correlation between content and main compositions of EOs of *F.angulate* plants under application of tested micronutrients in two year

Year	Verbenone (10)	4-thojanol (9)	Nonadecane (8)	Bornyl acetate (7)	beta - ocimene (6)	Cis-ocimene (5)	beta - phellandrene (4)	alpha - pinene (3)	alpha - Thujene (2)	EOs (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^{**}	-0.8^{**}	8
	-	1	0.86^{**}	0.53^{**}	0.81^{**}	0.81^{**}	0.68^{**}	0.79^{**}	0.65^{**}	0.75^{**}	9
	1	0.77^{**}	0.99^{**}	0.86^{**}	0.66^{**}	0.81^{**}	0.84^{**}	0.68^{**}	0.69^{**}	0.55^{**}	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^{**}	-0.9^{**}	8
	-	1	0.67^{**}	0.72^{**}	0.88^{**}	0.81^{**}	0.88^{**}	0.65^{**}	0.66^{**}	0.45^{**}	9
	1	0.81^{**}	0.77^{**}	0.86^{**}	0.53^{**}	0.73^{**}	0.66^{**}	0.55^{**}	0.83^{**}	0.65^{**}	10

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

The main EO constituents included alpha-thujene, alpha-pinene, beta-phellandrene, cis-ocimene (monoterpene hydrocarbons), and 4-thujanol (oxygenated monoterpenes), which were predominant in the EOs of treated plants. The combination of micronutrients was more effective in enhancing EO content and composition, with the highest EO content observed in plants treated with 20 and 40 mg L^{-1} of micronutrients. Although some main treatments, such as foliar application of Cu and Mn, did not show significant differences in the chemical composition of F. angulate EOs, combined treatments exhibited differences in most EO compositions. Among these, the Fe_2Cu_2 Mn_2 Zn_2 combination was the most effective.

The primary components in the EOs of all plants treated with micronutrients included alpha-thujene, alpha-pinene, beta-myrcene, alpha-phellandrene, beta-phellandrene, cis-ocimene, beta-ocimene, sabinene (monoterpene hydrocarbons), 4-thujanol, verbenone

Plants treated with 40 mg L^{-1} of Fe, Zn, and Mn produced the highest amounts of some constituents such as alpha-thujene, alpha-pinene, beta-myrcene, alpha-phellandrene, beta-phellandrene, cis-ocimene, beta-ocimene, and sabinene. For other EO components, the highest amounts were achieved with 40 mg L^{-1} of Fe, Zn, Mn, and Cu. Treatments at 60 mg L^{-1} often showed similar levels of constituents to control plants, with the lowest amounts observed for several components. In particular, the lowest levels of alpha-thujene, alpha-pinene, beta-myrcene, alpha-phellandrene, beta-phellandrene, cis-ocimene, beta-ocimene, and sabinene were recorded with 60 mg L^{-1} of Mn or Cu. Generally, control plants and those treated with 60 mg L^{-1} of Fe, Mn, Zn, and Cu had the lowest levels of EO content and composition across both cultivation seasons.

The highest amounts of constituents belonging to the hydrocarbons alkanes category were obtained from the combination of 60 mg L^{-1} of Fe, Mn, Zn, and Cu and the control treatment. The application of 20 mg L^{-1} of micronutrients improved the content and composition of most EO components, while higher concentrations (i.e., 60 mg L^{-1}) led to a decrease in these traits across all treated plants. The mean content (%) of many chemical components in *F. angulate* was lower than in the control treatment when plants were sprayed with 60 mg L^{-1}. It appears that the content and composition of EOs were more significantly affected by Zn and Fe compared to other micronutrients.

Micronutrients such as Fe, Cu, Zn, and Mn enhance nutrient absorption by influencing enzyme activities ((Marschner, 2011; Pradhan et al., 2017). Copper deficiency impairs the activity of various plant enzymes, including ascorbate oxidase, phenolase, cytochrome oxidase, diamine oxidase, plastocyanin, and superoxide dismutase. The oxidation-reduction cycling between Cu(I) and Cu(II) oxidation states is crucial for single-electron transfer reactions in copper-containing enzymes and proteins (Mengel et al., 2007). Iron absorption is limited by diffusion in the soil, making root activity and growth critical. Iron deficiency in terrestrial plants is often due to alkaline soil pH, which makes the addition of iron salts ineffective. Foliar application of iron chelates can be an effective solution for alleviating lime-induced chlorosis (Mengel et al., 2007; Pradhan et al., 2017). Manganese plays a role in various biochemical functions, acting as an activator of enzymes such as dehydrogenases, transferases, hydroxylases, and decarboxylases involved in respiration, amino acid and lignin synthesis, and hormone regulation. However, manganese can sometimes be substituted by other metal ions (Marschner, 2011). Zinc is an integral component of enzyme structures, coordinated to four ligands, with histidine being the most common, followed by glutamine and asparagine (Marschner, 2011; Pradhan et al., 2017).

The positive impacts of micronutrients can lead to improvements in photosynthetic rate, biomass production, and yield of aerial parts in medicinal plants (Hamedi et al., 2022; Yadegari, 2023). In the current study, the EO content ranged from 0.37% to 0.68% (w/w) in control plants and plants treated with 40 mg L^{-1} of micronutrients ($Fe_2Zn_2Mn_2Cu_2$), respectively. This enhancement in EO content is likely due to a balance in the absorption of essential elements in the root environment, increased photosynthesis rate, stimulation of vital enzymes, and activation of plant growth regulators (PGRs), which serve as signals for terpenes biosynthesis (Pradhan et al., 2017).

The application of 40 mg L^{-1} of Fe, Zn, Mn, and Cu increased EO content from 0.37% to 59% in the first year and from 0.41% to 68% in the second year, marking an increase of over 60%. The research findings indicated that with higher EO content, the main compositions such as alpha-thujene, alpha-pinene, beta-phellandrene, cis-ocimene, beta-ocimene, 4-thujanol, and verbenone in F. angulate plants treated with micronutrients increased over two years. The percentage of these main EO compounds is a key factor determining EO quality. Monoterpene hydrocarbons like alpha-thujene, alpha-pinene, beta-phellandrene, cis-ocimene, and beta-ocimene have been reported to improve the quality of F. angulate EOs (Azarbani et al., 2023; Ghasemi Pirbalouti et al., 2016).

Maintaining nutrient balance and soil fertility is critical for sustainable soil management. Organic, biological, and chemical fertilizers help return nutrients to the soil that plants consume. According to the results of this study, optimal micronutrient levels provided the necessary nutrients for producing higher EO content and composition in *F. angulate*. The composition of EOs in F. angulate varies depending on variety, climatic conditions, and nutritional status of the plant and soil. Literature reports suggest that key EO components include alpha-pinene, beta-pinene, beta-ocimene, bornyl acetate, thujanol, δ-terpinolene, sabinene, verbenone, alpha-phellandrene, beta-phellandrene, cis-beta-ocimene, and alpha-thujene (Golfakhrabadi et al., 2015; Hamedi et al., 2022; Moghaddam et al., 2018; Mumivand et al., 2019; Razavi et al., 2015; Safari et al., 2019).

The combination of four micronutrients was more effective than any single micronutrient. Therefore, the foliar application of 40 mg L^{-1} of Fe, Cu, Mn, and Zn was the most effective treatment compared to other concentrations. The main components in plants treated with 40 mg L^{-1} of micronutrients were produced at twice the percentage compared to those in control plants.

Exogenous micronutrients impact various physiological processes in plants, including respiration, photosynthesis, carbohydrate assimilation, and amino acid biosynthesis. These processes are often accompanied by changes in the content of intermediate compounds and the activity of enzymes involved in primary and secondary plant metabolism (Marschner, 2011). Consequently, micronutrient-induced variations in plant physiological behavior can affect the quality of produced secondary metabolites.

Essential oils (EOs), which are terpenes, have glucose as a critical precursor in their synthesis, particularly for monoterpenes. Thus, photosynthesis and its products directly influence EO biosynthesis (Bohlmann and Keeling, 2008). Adequate nutrient supply in response to exogenous micronutrients affects the biosynthesis of substrates and enzymes involved in terpenoid production (Aghaie et al., 2022; Pradhan et al., 2017). For example, sufficient magnesium may influence the activity of geranyl diphosphate synthase, an enzyme requiring this element for its function (Raz et al., 2020).

The EO content is closely correlated with key compounds such as alpha-thujene, alpha-pinene, beta-phellandrene, cis-ocimene, beta-ocimene, 4-thujanol, and verbenone. According to GC and GC-MS results from this study, monoterpene hydrocarbons (including alpha-thujene, alpha-pinene, beta-myrcene, alpha-phellandrene, beta-phellandrene, cis-ocimene, beta-ocimene, and sabinene) accounted for over 55% of the EO compounds, while oxygenated monoterpenes (such as 4-thujanol and verbenone) comprised more than 4% of the compounds in treated plants. Among all treatments, alpha-pinene and 4-thujanol were the predominant compounds of monoterpenes hydrocarbons and oxygenated monoterpenes, respectively.

Plants treated with 40 mg L^{-1} of micronutrients generally had higher levels of monoterpene hydrocarbons compared to other treatments. Conversely, the treatment with 60 mg L^{-1} of micronutrients resulted in increased levels of hydrocarbons alkanes like nonadecane and henicosane. The $Fe_2Cu_2Zn_2Mn_2$ treatment produced the highest levels of alpha-thujene, alpha-pinene, beta-phellandrene, cis-ocimene, beta-ocimene, 4-thujanol, and verbenone. Similarly, treatments of $Fe_2Cu_2Zn_1Mn_2$ and $Fe_2Cu_1Zn_2Mn_2$ yielded results comparable to $Fe_2Cu_2Zn_2Mn_2$. It appears that iron and zinc play more crucial roles than other micronutrients, as reported in previous studies (Bilal et al., 2020; Hamedi et al., 2022; Yadegari, 2023).

Previous research by Azarbani et al. (2023) found that the EOs of *F. angulate* mainly consisted of alpha-pinene, bornyl acetate, beta-pinene, and alpha-phellandrene. Another study identified major components of volatile oil in *F. angulate* as cis-beta-ocimene, alpha-pinene, alpha-phellandrene, alpha-thujene, cis-ocimene, and beta-phellandrene (Mumivand et al., 2019). Higher concentrations of micronutrients (i.e., 60 mg L^{-1}) reduced EO content but increased the proportion of hydrocarbons alkanes. Control plants generally produced better amounts of many EO components than those treated with 60 mg L^{-1} concentrations of Fe, Cu, Mn, and Zn. Among the treatments, the combinations $Fe_2Cu_2Mn_2Zn_2$, $Fe_2Cu_3Mn_3Zn_2$, and $Fe_2Cu_2Mn_2Zn_2$ were most effective, with $Fe_2Cu_2Mn_2Zn_2$ being the best. The increase in EO content with 20 and 40 mg L^{-1} treatments is likely due to enhanced overall growth of aerial parts. Additionally, the production of active substances such as volatile oils is a plant response to biological and abiotic stresses, with these stress signals acting as elicitors (Azarbani et al., 2023; Badalamenti et al., 2024).

In some EO compositions, control plants showed similar results to those treated with $Fe_3Cu_3Mn_3Zn_3$, $Fe_3Cu_3Mn_2Zn_3$, and $Fe_2Cu_2Mn_3Zn_3$. It appears that at higher concentrations of Fe, Cu, Mn, and Zn (i.e., 60 mg L^{-1}), the EO component content in treated plants resembled that of the control plants. The data indicate that the highest levels of foliar fertilizers were more effective than lower levels, with Librel Zn and Fe fertilizers proving superior to other micronutrients. However, the highest EO percentage was achieved with the $Fe_2Cu_2Mn_2Zn_2$ treatment.

Consistent with the results of this study on F. angulate, other researchers have reported beneficial effects of micronutrients (Fe, Zn, Cu, and Mn) on EO production in various plants, including *Rosa damascena* (Yadegari, 2023), *Satureja* sp. (Bani Taba and Naderi, 2022), *Melissa officinalis* (Yadegari, 2017a), *Carthamus tinctorius* (Galavi et al., 2012), *Calendula officinalis* L., *Borago officinalis, Alyssum desertorum*, *Thymus vulgaris* ((Yadegari, 2017a; Yadegari, 2015; Yadegari, 2017b), *Anethum* (Rostaei et al., 2018), and *Matricaria chamomilla* (Nasiri et al., 2010).

The results of this research indicate that foliar application of micronutrients resulted in higher EO content in the shoots of *F. angulate* plants compared to control plants. This effect was observed over two consecutive years. The application of Fe, Cu, Zn, and Mn led to an increase in EO yield due to a significant rise in dry matter and the number of flowers (data not published). It was found that Fe, Cu, Zn, and Mn are beneficial for *F. angulate* plants at concentrations of 40 mg L^{-1} or lower, potentially increasing EO content by up to 40%. These micronutrients have immediate impacts on plant growth and development.

There are still many unanswered questions about the mechanisms through which Fe, Cu, Zn, and Mn enhance yield and its components in F. angulate. One possibility is that foliar applications of these micronutrients could affect the absorption of other minerals, subsequently increasing shoot dry matter and EO yield (Alamer et al., 2020; Alejandro et al., 2020; Aziz et al., 2019). The study determined that control plants without foliar treatment generally exhibited better growth compared to plants treated with higher concentrations of micronutrients. Optimal combinations of micronutrients (i.e., 40 mg L^{-1}) had the most positive effect, while higher concentrations (i.e., 60 mg L^{-1}) had more detrimental effects than single micronutrients. These results highlight the importance of applying an optimal concentration of micronutrients to improve total EO content in medicinal plants. Micronutrient concentrations exceeding 40 mg L^{-1}, especially in three- or four-micronutrient combinations, tended to reduce EO content and composition.

Overall, the production of secondary metabolites and the chemical composition of plant EOs are influenced by genetic factors, ecological and soil conditions, management practices (from sowing to harvesting and post-harvesting), and their interactions (Ghasemi Pirbalouti et al., 2016; Hamedi et al., 2022; Shahbazi, 2016).

Conclusion

F. angulate plants treated with 40 mg L^{-1} of iron, zinc, manganese, and copper in a chelated formula produced higher content and improved composition of essential oils (EOs). The results indicate that the application of these micronutrients significantly affected both the measured traits and the chemical composition of the EOs in *F. angulate* plants. Furthermore, the combined application of micronutrient fertilizers had a more pronounced effect compared to individual micronutrient treatments. This study provides valuable insights into the impact of foliar application of micronutrients, particularly in soils with undesirable characteristics and chemical properties. The main constituents of the volatile oils in *F. angulate* were alpha-thujene, alpha-pinene, beta-myrcene, alpha-phellandrene, beta-phellandrene, cis-ocimene, beta-ocimene, and sabinene, which collectively accounted for 54-93% of the EOs. The highest content of EOs and the percentages of monoterpene hydrocarbons were observed in plants treated with 40 mg L^{-1} of micronutrients, with the combination of iron, zinc, and copper also showing comparable results.

In conclusion, the use of 40 mg L^{-1} of micronutrients (Fe, Zn, Cu, and Mn) is recommended for stabilizing both the quantitative and qualitative yield of *F. angulate* in similar climates.

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