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Research article

Iron nano-complexes and iron chelate improve biological activities of sweet basil (*Ocimum basilicum* L.)

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ABSTRACT

In this study, antioxidant and antimicrobial activities of basil (*Ocimum basilicum* L.) essential oil (EO) in response to different Fe sources (Fe-arginine, Fe-glycine, and Fe-histidine nano-complexes and Fe-EDDHA) were examined. EO samples were predominantly constituted by the phenylpropanoid methyl chavicol (53–89.5%). Application of Fe nano-complexes significantly increased the occurrence and concentration of sesquiterpenes, while decreased the content of oxygenated monoterpenes. Antioxidant activity of basil EOs was evaluated using free radical 2,2-diphenyl-1-picrylhydrazyl, Nitric oxide, H_2O_2 and Thiobarbituric acid reactive substances scavenging assays, and in all assays the highest and the lowest activities were recorded in basils supplied with Fe-histidine nano-complex (1.02, 1.62, 2.21, 3.22 mg mL-1) and control (3.89, 4.89, 5.52, 6.79 mg mL-1), respectively. Fe-histidine nano-complex was the most effective treatment to inhibit fungal (*C. albicans*: 0.058 mg mL-1; *A. niger*: 0.066 mg mL-1), Gram-negative (*E. coli*: 0.181 mg mL-1; *S. typhimurium*: 0.163 mg mL-1) and Gram-positive (*B. subtilis*: 0.033 mg mL-1; *S. aureus:* 0.002 mg mL-1) growth. In conclusion, application of iron nano-complexes significantly altered biological and pharmacological characteristics of basil EOs. Our results are quite encouraging since EOs exhibited potent antioxidant effect and antimicrobial activities.

1. Introduction

Basil (*Ocimum basilicum* L.) is an annual herb in the family Lamiaceae, native to India, Africa, and southern Asia, and is commercially cultivated in different parts of the world. Basil is a popular culinary herb, and its essential oils (EOs) have been used extensively for many years in the flavoring of food products, perfumery, and dental and oral products. Basil EOs (BEOs) and their principal constituents have recently been used for increasing the shelf life of food products due to their antimicrobial activity against a wide range of bacteria, yeast, and mold (Suppakul et al., 2003). Additionally, drugs derived from basil have been consumed traditionally for the treatment of various disorders and diseases such as warts, inflammations, colds, and headaches.

Ocimum basilicum is characterized by a great variability in its chemotypes, with the major compounds being linalool, eugenol, methyl chavicol, methyl eugenol, geraniol, geranial and neral, methyl cinnamate, which is to a large extent genotype-specific (Grayer et al., 1996; Burducea et al., 2018; Zheljazkov et al., 2008). In addition to genetic makeup, extrinsic factors such as environmental conditions and agronomic practices (nutrient management) have also been evidenced to play a part.

Iron (Fe) is an essential micronutrient for plants and plays a key role in regulating numerous cellular processes, including chlorophyll biosynthesis, photosynthesis, and mitochondrial respiration (Ghasemi et al., 2014). While Fe is abundant in soil, the available Fe in soil for plants is often insufficient due to low iron solubility. Iron deficiency is one of the most important factors limiting crop production in the world. Fe deficiency-induced chlorosis is a major nutritional disorder in crops growing in alkaline and calcareous soils. A large proportion of the total land area of Iran is dominated by highly calcareous soils (Ziaeian and Malakouti, 2001). Application of synthetic iron chelates is effective in counteracting Fe deficiency symptoms of plants grown on calcareous soils and is the most commonly applied technique in agriculture (Vadas et al., 2007). However, because of some serious drawbacks e.g. cost, environmental side effects, susceptibility to photodegradation, their application is not regarded as a sustainable practice (Metsarinne et al., 2004; Vadas et al., 2007; Souri and Hatamian, 2019).

Nanomaterials are used in practically every aspect of modern life. Nanoparticles (NPs) display unique size-dependent optical, physicochemical, and biological properties that are extremely looked-for in many disciplines including agriculture, medicine, environment, etc. (Hao et al., 2019). Nowadays, it is widely believed that NPs should be

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used in the field of soil–plant nutrition to achieve sustainable development of agricultural production with minimal environmental impacts (Rui et al., 2016). Nano-complexes increase crop production via enhancing a series of physiological processes including seed germination rate, photosynthetic activity, seedling growth, protein and carbohydrate synthesis, and nitrogen metabolism (Huang et al., 2015; Rui et al., 2016). Nano-fertilizers have also exhibited promise for increasing nutrient use efficiency, declining nutrients deprivation, and reducing salinity stress in plants (Huang et al., 2015; Zia-ur-Rehman et al., 2018).

Practically, the majority of Iranian farmers in arid and semi-arid climates use different types of Fe chelates; however, the application of these fertilizers still remains questionable. NPs are expected to be the ideal candidates for use in these regions. The effects of chelated Fe on growth and development of medicinal plants have been extensively studied. However, plant responses to Fe-nano complexes remain poorly understood. To bridge this information gap, we examined the effectiveness of foliar application of iron-amino acid nano-complexes in comparison with a commercial chelated Fe on some biological activities of BEOs. This was an attempt to generate new information on the efficacy of nanoscale iron fertilizers on the growth and development of basil, and to develop a technical approach for the agricultural application of nanomaterials. It is to be noted that the increased efficiency of a product may encourage the farmers to use the product more profitably.

2. Materials and methods

2.1. Plant material and treatments

The experiment was conducted at the greenhouse of Shiraz Payame Noor University through spring 2018 (Shiraz, Iran). The soil texture was characterized as sandy loamy (Alloway, 2004). Other chemical and physical characteristics of the soil are as follows:

ECe (1.3 dS m^{-1}), pH (7.1), organic (8.8 g kg^{-1}), CEC (11 Cmc kg^{-1}), K (61 mg kg^{-1}), P (12 mg kg^{-1}), N (0.08%) and Zn (1.5 mg kg^{-1}) and Fe (1.01 mg kg^{-1}).

50 mg N and P kg^{-1} soil (as NH_4NO_3 and KH_2PO_4), and 5 mg Cu, Zn and Mn kg^{-1} soil (as $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$, and $MnSO_4.H_2O$) were applied to the soil, respectively. Seven-liter plastic pots were used, and for each pot, 6 kg of soil was weighed. In each pot, 20 selected *Ocimum basilicum* L. seeds purchased from an authenticated company (Shiraz, Iran) were sown. Pots were irrigated twice a week with deionized water. After 15 days, in each pot, the plants were thinned to ten steady and similar stands. Each treatment included 8 pots containing 10 seedlings in each pot. Since thinning and prior to the flowering inception, the following treatments were foliarly applied: Treatment 1 (T1): Fe-Arginine (n[$Fe(Arg)_3$]), Treatment 2 (T2): Fe-Glycine (n[$Fe(Gly)_3$]), Treatment 3 (T3): Fe-Histidine (n[$Fe(His)_3$]), and Treatment 4 (T4): Fe-EDDHA. One liter of each Fe source (0.2% (w/v)) was applied. Deionized water was sprayed as control. Plants were collected at full bloom stage. The nano-complex fertilizers were obtained from *Zist Nano Fanavarn Atiye Pajooh* (Shiraz, Iran). All Fe-amino acid nano complexes were visually similar and were produced as yellow-orange fine powders (Fig. 1).

2.2. Instrumentation and characterization

The following techniques were used to verify the successful biosynthesis of Fe-amino acid nano-complexes. The size of the nano-complexes was observed using a transmission electron microscope (TEM) (100 kV Philips, EM208). The energy dispersive X-ray (EDX) spectroscopy analysis (Tescan Vega II, with a Rontec detector) was used to confirm the presence of pure elemental Fe. FT-IR analysis was carried out using a Tensor II FT-IR spectrometer set to 500–4000 cm^{-1}.

Fig. 1. A sample of Fe-Histidine (n[$Fe(His)_3$]) nano complex used in this study.

2.3. EO extraction

The plant samples were shaded at ambient temperature, and then 50 g of each sample was hydro-distilled using a Clevenger-type apparatus for 3 h (British Pharmacopoeia, 1988). EO samples were dehydrated over anhydrous sodium sulfate and stored at 4 °C until analysis. The BEO yields were calculated based on g oil content 100 g^{-1} dried herb.

2.4. GC & GC/MS analysis

Gas chromatography (GC) and Gas chromatography-mass spectrometry (GC-MS) analyses were performed using an autosampler Agilent 7683B, fitted with a flame ionization detector (FID), HP-5 fused silica column (30 m × 0.32 mm i.d. × 0.25 μm). GC/MS analysis was performed using an Agilent gas chromatograph fitted with 5975-C mass spectrometer joint with a capillary HP-5MS column (30 m × 0.25 mm i.d. × 0.25 μm). The carrier gas was Helium at the ionization voltage of 70 eV. Oven temperature program was as follows: 60 °C for 3 min, then an increase of 3 °C min^{-1} until 150 °C, afterward, a further increase of 3 °C min^{-1} until 260 °C, and this temperature was maintained for 3 min. The injector and detector temperatures were set to 230 and 250 °C, respectively. Identification of oil components was made by comparison of their mass spectra and retention indices with those of the mass spectral reference library and with those given in the literature (Adams, 2007). The retention indices were determined in relation to a homologous series of *n*-alkanes (C_8–C_{24}) under the same operating conditions. Component relative percentages were calculated based on GC peak areas without using correction factors.

2.5. Antioxidant activity

Four antioxidant assays i.e. free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), Nitric oxide (NO), hydrogen peroxide (H_2O_2) and Thiobarbituric acid reactive substances (TBARS) scavenging assays were used for determining the antioxidant capacity of BEOs.

2.5.1. DPPH assay

The antioxidant activity of EO samples was determined by free radical (DPPH) scavenging assay method, as previously described (Burits

et al., 2001). DPPH radical is a stable organic free radical with an absorption band at 517 nm. It loses this absorption when accepting an electron or a free radical species, which results in a visually noticeable discoloration from purple to yellow. It can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentrations (Do et al., 2014). In short, 20 μL of EOs (0–0.4 mg mL-1 in DMSO) was mixed with 210 μL of 125 mmol L-1 radical dilution of DPPH in methanol at room temperature for 30 min using an absorbance microplate reader (EL × 808, BioTek, USA) at 515 nm wavelength, the absorbance of solutions was assessed. The values of samples providing 50% inhibition to restrain DPPH radical formation (IC_{50}) were assigned by the nonlinear regression plots using MATLAB (MathWorks®, USA). The percentage of ROS scavenging was assessed based on the following formula, where Asample and Ablank correspond to the absorption values of the test solution and the blank solution, respectively. For control and blank, DPPH (without plant extract) and methanol were used:

$$[(A515_{sample}–A515_{blank})/A515_{control}] \times 100$$

2.5.2. NO radical scavenging assay

Reactive nitrogen species (RNS) are a family of free radicals derived from the interaction of nitric oxide (NO) with oxygen or reactive oxygen species. NO is classified as a free radical because of its unpaired electron and displays important reactivity with certain types of proteins and other free radicals such as superoxide. Generally, *in vitro* quenching of NO radical is one of the methods that can be used to determine antioxidant activity in food and physiological systems. The procedure is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions (Boora et al., 2014).

NO radical scavenging activity of EOs were assessed following the previously described method by Kavoosi and Rowshan (2013). In short, 0.2 mL of the EOs (0–0.4 mg mL^{-1} in DMSO) was mixed with 0.5 mL of $NaNO_2$ (0.02 mg mL^{-1} in 100 mM $Na_3C_6H_5O_7$ (sodium citrate)). Then, at 36 °C, the combination was kept for 120 min. Then, 0.6 mL of Griess reagent was added to the mixture. The absorbance of solutions was measured at 540 nm using a spectrophotometer (Shimadzu 1601, Japan). The following equation was employed to obtain the percentage of RNS scavenging:

$$[(A540_{blank} - A540_{sample})/A540_{blank}]$$

2.5.3. H_2O_2 scavenging assay

Hydrogen peroxide (H_2O_2) can be formed *in vivo* by many enzymes such as superoxide dismutase. It can cross membranes and may slowly oxidize a wide range of compounds (Gülçin et al., 2007). H_2O_2 scavenging activity of BEOs was assessed following the method proposed by Kavoosi and Rowshan (2013). In detail, 0.2 mL of EOs (0–0.4 mg mL^{-1} in DMSO) was blended with 2.0 mL of H_2O_2 (100 mM in 200 mM phosphate buffer, pH = 7.4). The mixtures were then incubated at 36 °C for 75 min. The absorbance (A) of solutions was recorded at 230 nm using a spectrophotometer (Shimadzu 1601, Japan). A solution containing phosphate buffer without H_2O_2 was used as blank. The following equation was used to obtain the percentage of H_2O_2 scavenging of each sample:

$$[(A230_{blank} - A230_{sample})/A230_{blank}]$$

2.5.4. TBARS scavenging assay

The thiobarbituric acid reactive substances (TBARS) are formed as a byproduct of lipid peroxidation, which can be detected by the TBARS assay using thiobarbituric acid as a reagent (Ghani et al., 2017). TBARS scavenging activity assay is widely used to measure antioxidant activity in food and physiological systems. TBARS scavenging activities of BEOs were carried out according to the previously described method proposed by Kavoosi and Rowshan (2013). Briefly, 0.2 mL of the EOs (0–0.4 mg mL^{-1} in DMSO) was mixed with MDA (0.1 mM in acetic acid, pH = 4). Solutions were kept at 36 °C for 120 min. After adding one volume of thiobarbituric acid (0.3 mM in acetic acid pH = 4), the solutions were incubated at 90 °C for 60 min. Then, the mixtures were chilled at ambient temperature. Then, the absorbance of solutions was recorded at 532 nm using a spectrophotometer (Shimadzu 1601, Japan). The following equation was used to measure the percentage of TBARS scavenging activity:

$$[(A532_{blank} - A532_{sample})/A532_{blank}]$$

2.6. Antifungal and antibacterial assays

Bacteria and fungi were collected from the Persian Type Culture Collection (PTCC), Tehran, Iran. Two foodborne gram-positive bacteria [*Bacillus subtilis* PTCC 1023 (ATCC 6633) and *Staphylococcus aureus* PTCC 1112 (ATCC 6538)], two foodborne Gram-negative bacteria [*Escherichia coli* PTCC 1330 (ATCC 8739) and *Salmonella typhimurium* PTCC 1609 (Iran isolate) and two foodborne fungi [*Candida albicans* PTCC 5027 (ATCC 10231) and *Aspergillus niger* PTCC 5010 (ATCC 9142)] were tested. The minimum inhibitory concentration (MIC) of serial dilutions of EOs (0–0.3 mg mL^{-1}) against the pathogens was measured according to the microdilution procedure proposed by Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2012). Suspended fungi and bacteria strains in Luria–Bertani media were tuned to 0.6 McFarland standards at 640 nm (10^8 CFU/mL). Densities were diluted to 10^5 (CFU/ml) with Luria–Bertani. The EOs (0.5 mL) and suspensions of fungi and bacteria (0.5 mL) were added to 1.5 mL microtubes. The mixtures were then shaken with incubating at 36 °C for 24 h. In control (blank), EOs were excluded from the medium containing bacteria and fungi. Sterile control was a medium without fungi and bacteria. Positive control contained Ketoconazole, Ampicillin and Gentamicin (Pourateb, Iran, 0.015 mg mL^{-1}) for fungi, Gram-positive and Gram-negative bacteria, respectively. To show the growth inhibition, absorbance (A) of the culture medium was measured using a spectrophotometer (Shimadzu 1601, Japan). The following equation was employed to obtain the percentage of growth inhibition:

$$[(A640_{blank} - A640_{sample})/A640_{blank}] \times 100$$

It should be noted that during incubation, EO samples caused discoloration of the culture media, which may result in an inaccurate estimation of percent inhibition. This discoloration was accounted for by incubating EOs in uninoculated media for 24 h, measuring the change in turbidity, and adding this correction factor to final OD values (Donaldson et al., 2005).

2.7. Statistical analysis

All experiments were carried out in eight replications (eight pots), and data were analyzed using analysis of variance (ANOVA), and significant differences among means at ($p < 0.05$) were determined by Duncan's multiple range test using SPSS 20 (SPSS Inc., USA) software.

3. Results and discussion

3.1. Synthesis of Fe-amino acid nano complexes

3.1.1. TEM

The particle size and shape of biosynthesized Fe amino acid nano complexes were analyzed by using TEM technology (Fig. 2). The TEM images of nano-complexes revealed that they are in the nanoscale range

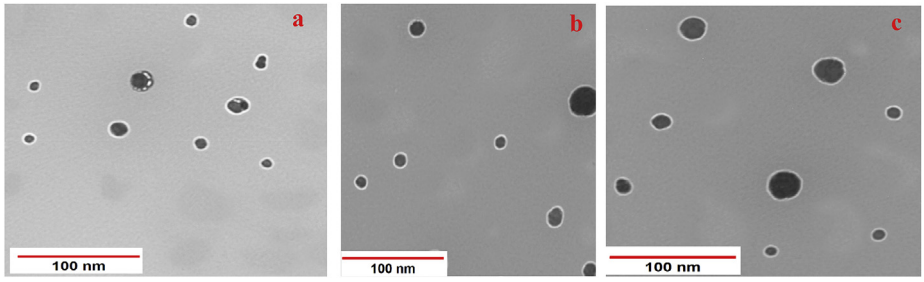

Fig. 2. Transmission electron microscopy images of (a) Fe-Histidine (n[Fe(His)$_3$]), (b) Fe-Glycine (n[Fe(Gly)$_3$]) and (c) Fe-Arginine (n[Fe(Arg)$_3$]) nano-complexes.

(~5–20 nm) and have a relatively uniform distribution with spherical shapes.

3.1.2. EDX

Energy-dispersive X-ray spectroscopy (EDX) is an analytical technique used for the elemental analysis or chemical characterization of a sample. Results of EDX analysis confirmed the presence of Fe NPs, along with three other elements including C, N, and O (Fig. 3).

3.1.3. FT-IR

Fourier Transform-Infrared Spectroscopy (FT-IR) is an analytical technique used to identify organic (and in some cases inorganic) materials. We performed an FT-IR analysis of free amino acids (histidine, glycine, and arginine) and biosynthesized Fe-amino acid nano complexes in order to identify their organic species (Table 1).

3.2. EO yield & composition

BEO yields under the influence of different iron sources were evaluated and results are illustrated in Fig. 4. Regardless of fertilizer type, foliar application of Fe contributed to a considerable increase in the EO yield in comparison to that of control. EO yield values ranged from 0.29 ± 0.007 g 100 g^{-1} in control to 0.53 ± 0.016 g 100 g^{-1} in n[Fe(His)$_3$], respectively. However, differences between treatments containing Fe nano-complexes (T1, T2 and T3) and Fe-EDDHA (0.39 ± 0.009 g 100 g^{-1}) were significant ($P < 0.05$). The highest EO yield was recorded in T3 (0.53 ± 0.016 g 100 g^{-1}), followed by T1 (0.51 ± 0.014 g 100 g^{-1}) and T2 (0.43 ± 0.017 g 100 g^{-1}); no significant difference was observed between T3 and T1. Although the lowest value among Fe-containing fertilizers was achieved in T4 (0.39 ± 0.009 g 100 g^{-1}), the yield was significantly higher than that of control (0.29 ± 0.007 g 100 g^{-1}).

The impact of Fe fertilizers (e.g. Tavallali, 2018), and Fe nano-complexes (e.g. Amuamuha et al., 2012; Tavallali, 2018; Yousefzadeh and Sabaghnia, 2016) on EO yield in some EO bearing crops have been well documented. In line with previous studies, we found evidence for the importance of the Fe source on EO yield in *O. basilicum*. On the other hand, the kind of the amino acid present in the iron nano-complex fertilizers also seemed to be a determinant factor. Fe-amino acid nano-complexes (T1, T2, T3) were more effective in enhancing the sesquiterpene content of BEO than Fe-EDDHA (T4). Apparently, the amino acids attached to Fe might have a part to play in modifying the constituents of BEO. The effects of amino acids on the quality and quantity of EO in e.g. *Rosmarinus officinalis*, *Satureja hortensis* and *Matricaria recutita* (Foroutan nia et al., 2016; Mehrabi et al., 2013; Omer et al., 2013; Gamal El-Din and Abd El-Wahed, 2005) have been reported. The decisive role of T3 and T2 on BEO, to some extent, could be attributed to the stimulating action of the amino acids histidine and arginine (Ortiz-Lopez et al., 2000). Histidine and arginine play a vital role in plant growth and reproduction, chelation and transport of metal ions, and biosynthesis regulation of other amino acids (Stepansky and Leustek, 2006; Abdul-Qados, 2009).

The composition of BEOs was analyzed and qualitative and quantitative differences were observed (Table 2). The minor and major EO components were variable in occurrence and concentration among the tested samples. In total, 72 compounds were identified accounting for over 99% of the total oils. A number of compounds belonging to different chemical classes have been reported, among which phenylpropanoids have been shown to play a significant role in the establishment of chemotypes in *O. basilicum*: (a) linalool, (b) methyl chavicol, (c) linalool-methyl chavicol, (d) linalool-eugenol, and (e) methyl chavicol-methyl eugenol chemotypes are common ones. Although phenylpropanoids are not common in EO-bearing plants, *O. basilicum* EO commonly contains abundant proportions of these compounds. In the current study, all of the EO samples (notably control) were predominantly constituted by the phenylpropanoid methyl chavicol, ranging from 52.94 ± 1.28% (T3) to 89.60 ± 1.75% (control). It is worth noting that the other constituents of BEOs constantly remained under 10%. According to these results, it can be concluded that the genotype used here is definitely a methyl chavicol chemotype. Given that, the content of the compound showed a decrescent trend by the application of Fe sources in comparison to control, though constantly remained over 50%.

As mentioned above, we identified 72 different compounds; the highest number of compounds was recorded in T1, T3 and T4 with 72 compounds, followed by T2 with 69 and control with 56 compounds; Spathulenol, (E)-Nerolidol, (Z)-β-Farnesene, β-copaene, cis-α-bergamotene, (E)-methyl cinnamate, neryl acetate, citronellyl acetate, α-terpinyl acetate, δ-Elemene, methyl geranate, (Z)-methyl cinnamate, carvacrol, thymol, chavicol and neral were those 16 compounds not identified in control, of which 10 were monoterpene derivatives, 5 were sesquiterpene, and the remaining one was the phenylpropene chavicol. In T3, two sesquiterpenes (Elemol and Spathulenol) and one monoterpene ester (Neryl acetate) were missing.

In general, the compounds identified belonged to different classes of (a) terpenes including monoterpenes (15 compounds), oxygenated monoterpenes (22 compounds), sesquiterpenes (18 compounds) and oxygenated sesquiterpenes (8 compounds), and (b) either non-terpene hydrocarbons or their derivatives (4 compounds), and (c) phenylpropanoids (5 compounds), of which the latter class boasted the highest contribution percentage of the total oils (from 53.93% to 92.24%).

The major constituents (over 1%) of EOs in control were specified by a great proportion of the phenylpropanoid methyl chavicol (89.60%) followed by methyl eugenol (2.57%), n-decane (1.41%), 1,8-cineole (1.06%). Other than methyl chavicol, the major constituents (> 2%) in the remaining EO samples were as follows:

1,8-cineole (9.4, 9.4 and 12%), linalool (2.30%) and (E)-caryophyllene (2.01%) were the main compounds found in the plants receiving T1, respectively. The main volatile components of the plants receiving T2 were (E)-caryophyllene (6.13 ± 0.07%), (E)-γ-bisabolene (3.75 ± 0.04%), α-humulene (2.60 ± 0.15%), germacrene D (2.44 ± 0.03%) and trans-α-bergamotene (2.27 ± 0.05%), respectively. (E)-caryophyllene (9.24 ± 0.08%), (E)-γ-bisabolene (7.33 ± 0.3%), linalool (5.30 ± 0.06%), α-humulene (4.79 ± 0.23%), germacrene D (4.52 ± 0.25%) and trans-α-bergamotene (2.80 ± 0.02%) were the main components in the EOs treated

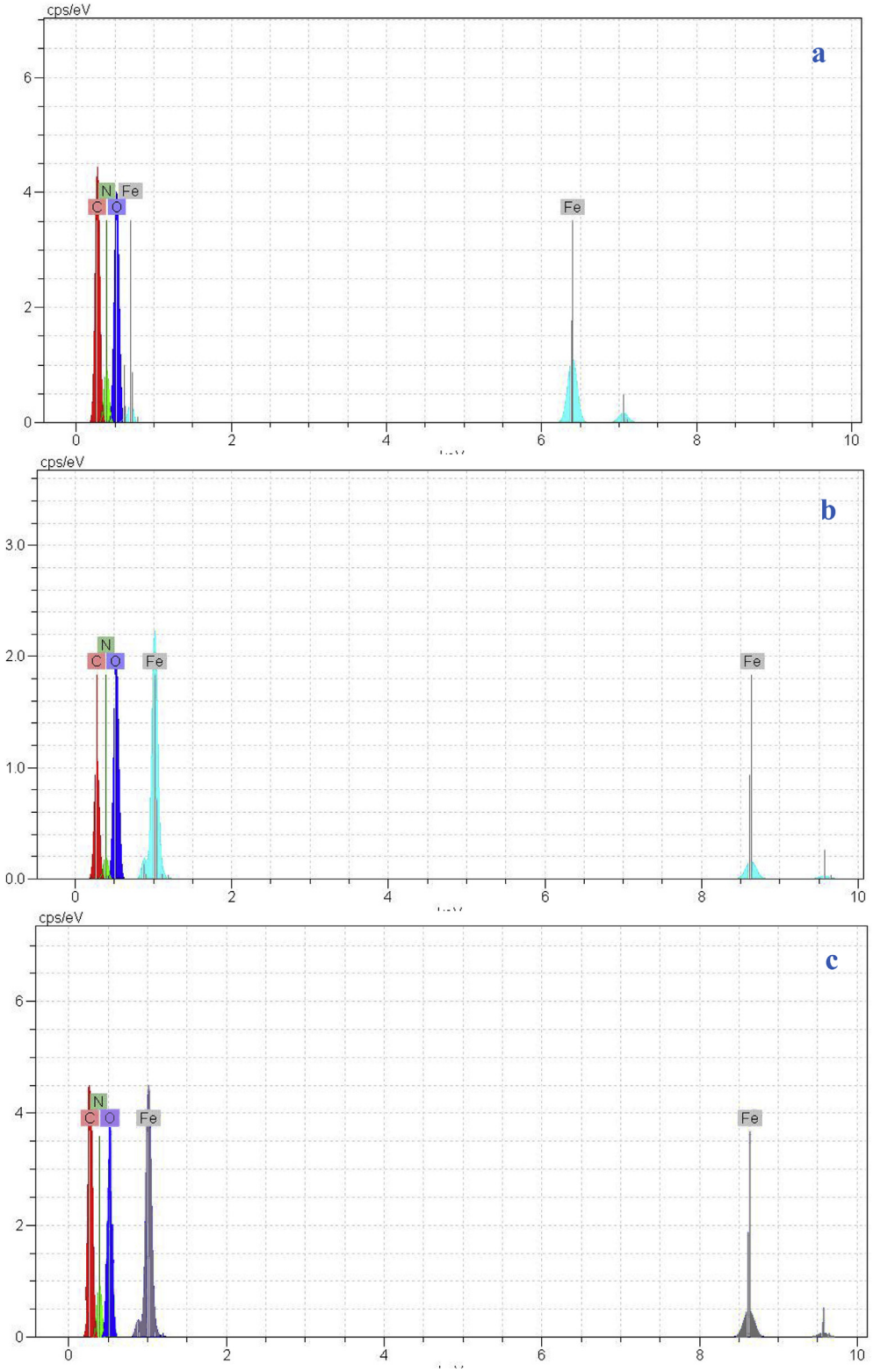

Fig. 3. EDX spectra of (a) Fe-Histidine (n[Fe(His)$_3$]), (b) Fe-Glycine (n[Fe(Gly)$_3$]) and (c) Fe-Arginine (n[Fe(Arg)$_3$]) nano-complexes.

by T3, while the major constituents of plants receiving T4 were 1,8-cineole (2.88 ± 0.4%), (E)-caryophyllene (2.53 ± 0.05%) and trans-α-bergamotene (2.20 ± 0.07%).

According to the results, foliar application of Fe sources led to a significant decrease in the content of phenylpropanoids in EO samples ($p < 0.05$), notably methyl eugenol and methyl chavicol. As opposed to monoterpenes, the application of iron sources boosted the proportion of sesquiterpenes (e.g. germacrene, α-bergamotene, β-farnesene, α-humulene, γ-bisabolene, and caryophyllene) in EO samples (Table 2), the greatest quantity of which was recorded in response to T3.

While biosynthesis of EO in plants has genetic determination, environmental conditions have also been evidenced to play a significant part. Growing conditions (e.g. cultivation method, fertilization, irrigation) which largely determine raw material yield, also determine raw

Table 1
FT-IR bands (cm^{-1}) of amino acids (histidine, glycine, and arginine) and Fe-amino acid nano-complexes (KBr disk).

	ν(C–O)	ν(C=O)	ν(NH_2)	δ(C=O)	δ(NH_2)
Histidine	1475	1588	3035	1100,500	1100,500
Arginine	1477	1585	3011, 3195	500,1100	1763
Glycine	1389	1622	2044, 3225	559	1581
n[Fe(His)$_3$]	1395	1560	3519	1092	–
n[Fe(Gly)$_3$]	1408	1651	3379, 3564	804	–
n[Fe(Arg)$_3$]	1411	1702	3201, 3262	682	–

The ultrasound-assisted reaction of $Fe(NO_3)_3$ and His, Gly, or Arg led to the synthesis of iron-amino acid nano-complexes. FT-IR bands of free amino acids and nano complexes both displayed an absorption template in the region of 400–4000 cm^{-1}. In nano-complexes, ν(C–O), ν(C=O), ν(NH_2), δ(C=O) and δ(NH_2) were the leading vibrations. Compared to the free amino acids, the NH_2 vibrational bands in nano-complexes are very broad and delicate and move toward higher frequencies about 3200–3600 cm^{-1}. The absorption bands for C=O are around 1550-1630 cm^{-1} in amino acids, which is transferred to higher spectra in nano-complexes.

material quality. In chemical terms, EOs are very diverse compounds, hence their biosynthesis in the plant occurs along different metabolic pathways, which are differently modified by the presence and availability of nutrients in the surrounding environment (Nurzyńska-Wierdak, 2013). It may be concluded that Fe sources caused the terpenoid synthesis pathways in *O. basilicum* to be transferred from phenylpropanoids and monoterpenes to sesquiterpenes. The positive influence of Fe NPs on certain sesquiterpenes in e.g. *Mentha piperita*, *Carum copticum*, *Ocimum basilicum* and *Satureja hortensis* (Askary et al., 2016; Abdossi and Kazemi, 2015; Zahedifar and Najafian, 2015) has also been reported.

3.3. Antioxidant activity

Oxidation is known to have negative effects, especially in the industrial context. Oxidation reactions occur in foods and cosmetics, often because of prolonged exposure to oxygen. Food preservation represents an issue of concern and requires assurance of protection from microbial spoilage and prolongation of shelf life (Mimica-Dukić et al., 2003). The antioxidants from natural sources could be the alternative to the problematic synthetic antioxidants in counteracting the oxidative stress associated diseases.

The antioxidant capacities of natural sources cannot be fully described with only one method; it is necessary to perform more than one type of antioxidant activity measurement to consider various mechanisms of antioxidant activity (Song et al., 2010). In this study, the antioxidant activity of BEO in response to different iron sources was evaluated in a series of *in vitro* tests. Four antioxidant assays i.e. DPPH, NO, H_2O_2 and TBARS scavenging assays were carried out for determining the antioxidant capacity of EO samples with reference to the concentrations providing 50% inhibition (the IC_{50} value) (Table 3). The antioxidant capacity of a given compound is inversely related to its IC_{50}; the lower IC_{50} of a certain chemical or compound, the greater antioxidant activity of the compound (Do et al., 2014).

DPPH radical scavenging activity of BEOs are shown in Table 3. In all samples, the order of the scavenging activity was as follows: Fe nano-complexes > Fe-EDDHA > control. The addition of EO to DPPH solution induced a rapid decrease in the optical density at 517 nm, and resulted in the significantly better free radical scavenging activity (lower IC50 value) about 1.5-4-fold, as compared to control (3.89 ± 0.5 mg mL^{-1}).

In this assay, the plants receiving T3 were the most active samples, whereas control showed the least activity. Furthermore, all EOs obtained by using nano-complexes (T3: 1.02 ± 0.09; T1: 1.35 ± 0.08; T2: 1.63 ± 0.2, respectively) mg mL^{-1} gave stronger radical scavenging capacity than those receiving T4 (2.36 ± 0.4 mg mL^{-1}). In the other three antioxidant assays, the same order of efficacy for all samples was recorded:

The ability of BEO treated by different Fe sources to scavenge hydrogen peroxide (H_2O_2) was determined (Table 3). The effectivity of the plants receiving nano-complexes (T3 (2.21 ± 0.3 mg mL-1), T1 (2.55 ± 0.2 mg mL-1), T2 (3.33 ± 0.3 mg mL-1), respectively) to scavenge H_2O_2 was significantly higher than that observed in T4 (3.77 ± 0.4 mg mL-1) and control (5.52 ± 0.5 mg mL-1).

TBARS radical scavenging activity of BEOs are illustrated in Table 3. While those plants treated by T3 showed a nonsignificant superiority (3.22 ± 0.2 mg mL-1) over other Fe nano-complex treatments (T1: 3.51 ± 0.2 mg mL-1; T2: 3.88 ± 0.3 mg mL-1), their TBARS scavenging activity was markedly higher in comparison to T4 (4.57 ± 0.3 mg mL-1), and control (6.79 ± 0.6 mg mL-1). The difference in the activity of the latter treatments was also significant.

NO radical scavenging activity of BEOs are shown in Table 3. The plants supplied with T3 were the most potent NO scavengers (1.62 ± 0.2 mg mL-1) in comparison to other treatments. However, T1 & T2, showed stronger scavenging activity compared to T4 (2.99 ± 0.08 mg mL-1). In this assay, control had a minimal scavenging activity, as it removed the nitrite radical at the highest concentration (4.89 ± 0.4 mg mL-1).

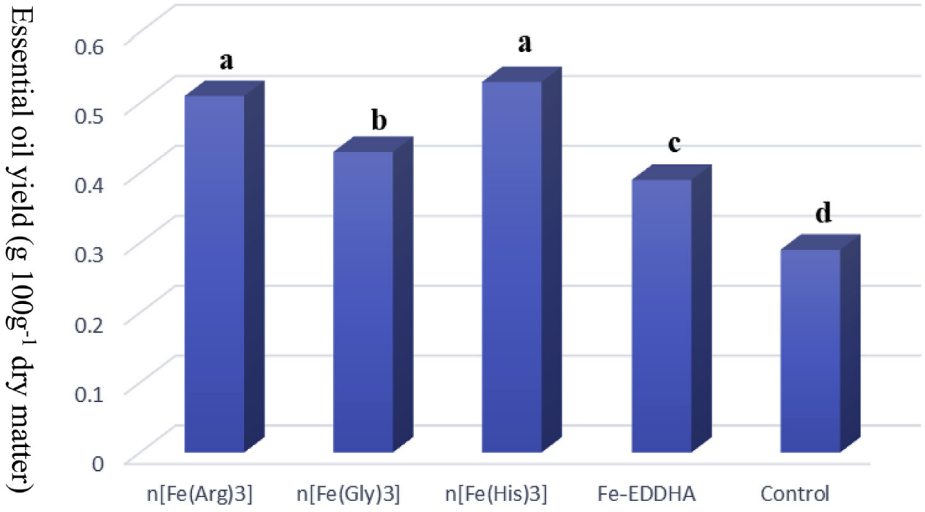

Fig. 4. Basil (*Ocimum basilicum*) Essential oil yield affected by different Fe sources.
Note: Different letters are significantly different at $p < 0.05$.

Table 2
Effect of different Fe sources on chemical composition of basil essential oils.

No.	Compound	Retention index	n[Fe(Arg)$_3$]	n[Fe(Gly)$_2$]	n[Fe(His)$_3$]	Fe-EDDHA	Control
1	α-Thujene	926	0.004 ± 0.0007d	0.017 ± 0.002b	0.006 ± 0.001c	0.002 ± 0.0006e	0.08 ± 0.008a
2	α-Pinene	933	0.26 ± 0.03a	0.11 ± 0.02bc	0.11 ± 0.01bc	0.15 ± 0.03b	0.08 ± 0.007c
3	Camphene	948	0.02 ± 0.005b	0.02 ± 0.005b	0.01 ± 0.008c	0.02 ± 0.007b	0.10 ± 0.03a
4	Sabinene	972	0.13 ± 0.02a	0.10 ± 0.03bc	0.08 ± 0.006c	0.10 ± 0.03bc	0.11 ± 0.03 ab
5	β-Pinene	976	0.29 ± 0.04a	0.18 ± 0.05c	0.19 ± 0.03c	0.22 ± 0.05b	0.11 ± 0.03d
6	Myrcene	990	0.14 ± 0.05bc	0.15 ± 0.03 ab	0.15 ± 0.02 ab	0.17 ± 0.03a	0.12 ± 0.01c
7	n-Decane	999	0.09 ± 0.007c	0.55 ± 0.02b	0.09 ± 0.009c	0.07 ± 0.01c	1.41 ± 0.13a
8	α-Phellandrene	1005	0.009 ± 0.003a	0.01 ± 0.007a	0.01 ± 0.006a	0.007 ± 0.002b	0.01 ± 0.007a
9	(3Z)-Hexenyl acetate	1006	0.02 ± 0.006a	0.02 ± 0.003a	0.01 ± 0.004b	0.01 ± 0.007b	0.01 ± 0.008b
10	δ-3-Carene	1010	0.001 ± 0.0007c	0.02 ± 0.0008a	0.005 ± 0.001b	0.001 ± 0.0005c	0.02 ± 0.006a
11	α-Terpinene	1016	0.02 ± 0.004a	0.01 ± 0.005b	0.02 ± 0.006a	0.01 ± 0.003b	0.02 ± 0.004a
12	*p*-Cymene	1023	0.001 ± 0.0005d	0.007 ± 0.0006b	0.004 ± 0.0007c	0.003 ± 0.0006cd	0.01 ± 0.005a
13	Limonene	1027	0.31 ± 0.06 ab	0.28 ± 0.04bc	0.34 ± 0.05a	0.25 ± 0.04c	0.29 ± 0.05b
14	1,8-Cineole	1029	2.49 ± 0.2 ab	2.16 ± 0.2bc	1.99 ± 0.3c	2.88 ± 0.4a	1.06 ± 0.12d
15	(Z)-β-Ocimene	1035	0.012 ± 0.007d	0.031 ± 0.003b	0.024 ± 0.006c	0.011 ± 0.005d	0.04 ± 0.004a
16	Benzene acetaldehyde	1041	0.03 ± 0.004bc	0.02 ± 0.003c	0.05 ± 0.004b	0.7 ± 0.06a	0.02 ± 0.005c
17	(E)-β-Ocimene	1045	0.28 ± 0.07c	0.48 ± 0.06a	0.45 ± 0.06 ab	0.34 ± 0.05bc	0.02 ± 0.009d
18	γ-Terpinene	1056	0.03 ± 0.007b	0.03 ± 0.005b	0.05 ± 0.006a	0.03 ± 0.007b	0.03 ± 0.008b
19	cis-Sabinene hydrate	1065	0.05 ± 0.006b	0.04 ± 0.004bc	0.10 ± 0.03a	0.05 ± 0.005b	0.03 ± 0.007c
20	Fenchone	1087	0.40 ± 0.03b	0.40 ± 0.02b	0.70 ± 0.03a	0.67 ± 0.08a	0.46 ± 0.07b
21	Linalool	1099	2.30 ± 0.13b	1.01 ± 0.03c	5.30 ± 0.06a	1.14 ± 0.06c	0.30 ± 0.07d
22	n-Nonanal	1103	0.02 ± 0.004b	0.02 ± 0.004b	0.02 ± 0.003b	0.01 ± 0.005c	0.05 ± 0.008a
23	endo-Fenchol	1112	0.07 ± 0.006c	0.16 ± 0.04b	0.22 ± 0.05a	0.05 ± 0.004c	0.20 ± 0.07 ab
24	Cis-p-Menth-2-en-l-ol	1120	0.01 ± 0.007c	0.02 ± 0.003bc	0.01 ± 0.004c	0.004 ± 0.0006d	0.03 ± 0.005a
25	Camphor	1142	0.04 ± 0.005c	0.25 ± 0.05a	0.18 ± 0.03b	0.17 ± 0.04b	0.03 ± 0.004c
26	δ-Terpineol	1165	0.05 ± 0.005b	0.04 ± 0.004b	0.12 ± 0.03a	0.03 ± 0.006b	0.01 ± 0.005c
27	Terpinen-4-ol	1175	0.04 ± 0.004c	0.04 ± 0.004c	0.16 ± 0.005a	0.07 ± 0.005b	0.02 ± 0.003d
28	α-Terpineol	1190	0.26 ± 0.03a	0.18 ± 0.03c	0.27 ± 0.03a	0.22 ± 0.02b	0.08 ± 0.005d
29	Methyl chavicol	1196	79.05 ± 1.34c	74.22 ± 1.42c	52.94 ± 1.28d	82.14 ± 1.55b	89.60 ± 1.75a
30	endo-Fenchyl acetate	1219	0.08 ± 0.005b	0.09 ± 0.003b	0.17 ± 0.04a	0.15 ± 0.04a	0.09 ± 0.007b
31	Nerol	1226	0.02 ± 0.006c	0.07 ± 0.005b	0.03 ± 0.004c	0.006 ± 0.0005d	0.12 ± 0.06a
32	Neral	1239	1.37 ± 0.05a	0.008 ± 0.0005d	1.16 ± 0.07b	0.02 ± 0.004c	n.d.
33	Chavicol	1251	0.02 ± 0.007a	0.02 ± 0.007a	0.005 ± 0.0006b	0.002 ± 0.0006b	n.d.
34	Geranial	1269	1.81 ± 0.06a	0.02 ± 0.006c	1.56 ± 0.08b	0.03 ± 0.005c	0.003 ± 0.0006d
35	(E)-Anethole	1284	0.19 ± 0.07b	0.30 ± 0.06d	0.20 ± 0.07b	0.27 ± 0.04a	0.07 ± 0.006c
36	Thymol	1291	0.002 ± 0.0009b	0.004 ± 0.001a	0.005 ± 0.0009a	0.001 ± 0.0005	n.d.
37	Carvacrol	1298	0.003 ± 0.0008 ab	0.002 ± 0.001bc	0.004 ± 0.001a	0.001 ± 0.0004c	n.d.
38	(Z)-Methyl cinnamate	1304	0.001 ± 0.0007c	0.007 ± 0.0008a	0.005 ± 0.0006b	0.002 ± 0.0007c	n.d.
39	Methyl geranate	1323	0.003 ± 0.0009c	0.001 ± 0.0006d	0.03 ± 0.006a	0.006 ± 0.0008b	n.d.
40	δ-Elemene	1335	0.009 ± 0.0005b	0.002 ± 0.0007c	0.03 ± 0.004a	0.001 ± 0.0007c	n.d.
41	α-Terpinyl acetate	1347	0.008 ± 0.0006b	0.004 ± 0.0008c	0.02 ± 0.005a	0.005 ± 0.0006c	n.d.
42	Citronellyl acetate	1352	0.006 ± 0.0007c	0.001 ± 0.0005d	0.02 ± 0.006a	0.01 ± 0.006b	n.d.
43	Eugenol	1355	0.02 ± 0.004c	0.02 ± 0.007c	0.10 ± 0.08b	0.30 ± 0.06a	0.008 ± 0.0006d
44	Neryl acetate	1363	0.009 ± 0.0005b	n.d.	0.04 ± 0.005a	0.009 ± 0.0004b	n.d.
45	α-Copaene	1373	0.03 ± 0.009b	0.009 ± 0.0007c	0.06 ± 0.006a	0.004 ± 0.0008d	0.004 ± 0.0007d
46	(E)-Methyl cinnamate	1381	0.04 ± 0.006b	0.01 ± 0.006c	0.12 ± 0.04a	0.04 ± 0.007b	n.d.
47	β-Elemene	1390	0.12 ± 0.05b	0.11 ± 0.06b	0.41 ± 0.06a	0.04 ± 0.004c	0.007 ± 0.001d
48	(Z)-Jasmone	1397	0.02 ± 0.004c	0.06 ± 0.006b	0.03 ± 0.005c	0.009 ± 0.0006d	0.08 ± 0.005a
49	Methyl eugenol	1404	0.50 ± 0.05c	0.38 ± 0.4d	0.69 ± 0.3b	0.47 ± 0.05c	2.57 ± 0.07a
50	Cis-α-Bergamotene	1413	0.01 ± 0.006b	0.008 ± 0.0007c	0.03 ± 0.005a	0.003 ± 0.0007d	n.d.
51	(E)-Caryophyllene	1416	2.02 ± 0.02c	6.13 ± 0.07b	9.24 ± 0.08a	2.53 ± 0.05c	0.73 ± 0.08d
52	β-Copaene	1426	0.01 ± 0.006a	0.003 ± 0.0008b	0.02 ± 0.006a	0.002 ± 0.0009b	n.d.
53	trans-α-Bergamotene	1433	1.42 ± 0.04c	2.27 ± 0.05b	2.80 ± 0.02a	2.20 ± 0.07b	0.06 ± 0.01d
54	(Z)-β-Farnesene	1441	0.05 ± 0.003b	0.02 ± 0.004c	0.08 ± 0.006a	0.006 ± 0.0007d	n.d.
55	α-Humulene	1450	0.98 ± 0.19d	2.60 ± 0.15b	4.79 ± 0.23a	1.33 ± 0.14c	0.17 ± 0.07e
56	(E)-β-Farnesene	1455	0.44 ± 0.05b	0.16 ± 0.08c	0.60 ± 0.04a	0.06 ± 0.008d	0.07 ± 0.006d
57	Allo-Aromadendrene	1460	0.02 ± 0.009b	0.02 ± 0.007b	0.04 ± 0.007a	0.006 ± 0.0007c	0.01 ± 0.006b
58	Germacrene D	1478	1.10 ± 0.22c	2.44 ± 0.03b	4.52 ± 0.25a	1.17 ± 0.04e	0.28 ± 0.08d
59	β-Selinene	1482	0.14 ± 0.07a	0.08 ± 0.006b	0.20 ± 0.08a	0.03 ± 0.005bc	0.01 ± 0.005c
60	α-Zingiberene	1493	0.31 ± 0.07b	0.13 ± 0.05c	0.52 ± 0.09a	0.05 ± 0.005d	0.03 ± 0.006d
61	β-Bisabolene	1506	0.09 ± 0.006b	0.03 ± 0.009c	0.14 ± 0.05a	0.01 ± 0.006d	0.007 ± 0.001e
62	γ-Cadinene	1511	0.13 ± 0.02b	0.11 ± 0.03b	0.19 ± 0.04a	0.04 ± 0.005d	0.09 ± 0.005c
63	δ-Cadinene	1521	0.05 ± 0.006b	0.02 ± 0.005c	0.10 ± 0.05a	0.006 ± 0.0008d	0.006 ± 0.0006d
64	(E)-γ-Bisabolene	1537	1.74 ± 0.06c	3.75 ± 0.04b	7.33 ± 0.3a	1.28 ± 0.04c	0.35 ± 0.04d
65	Elemol	1546	0.004 ± 0.0005c	n.d.	0.02 ± 0.007a	0.001 ± 0.0006d	0.007 ± 0.0006b
66	(E)-Nerolidol	1561	0.06 ± 0.007a	0.02 ± 0.006b	0.07 ± 0.006a	0.01 ± 0.006b	n.d.
67	Spathulenol	1577	0.01 ± 0.004b	n.d.	0.02 ± 0.006a	0.001 ± 0.0005c	n.d.
68	Caryophyllene oxide	1579	0.04 ± 0.005b	0.01 ± 0.006c	0.07 ± 0.005a	0.006 ± 0.0005d	0.005 ± 0.0008d
69	epi-α-Cadinol	1638	0.46 ± 0.05b	0.37 ± 0.02c	0.50 ± 0.09b	0.23 ± 0.07d	0.60 ± 0.08a
70	β-Eudesmol	1646	0.01 ± 0.007b	0.009 ± 0.0007c	0.05 ± 0.006a	0.01 ± 0.006b	0.007 ± 0.0007c
71	α-Cadinol	1651	0.05 ± 0.006b	0.06 ± 0.007 ab	0.06 ± 0.007 ab	0.01 ± 0.005c	0.08 ± 0.006a
72	α-Bisabolol	1681	0.11 ± 0.06a	0.03 ± 0.005b	0.13 ± 0.07a	0.02 ± 0.004b	0.02 ± 0.005b

Data are mean ± standard deviation of eight replications (pots).
Note: Different letters are significantly different at $p < 0.05$.

Table 3
Radical scavenging activity of basil essential oils in response to different Fe sources.

Properties	Fe sources (0.2% w/v)				
	$n[Fe(Arg)_3]$	$n[Fe(Gly)_3]$	$n[Fe(His)_3]$	Fe-EDDHA	Control
DPPH	1.35 ± 0.08b	1.63 ± 0.2b	1.02 ± 0.09a	2.36 ± 0.4c	3.89 ± 0.5d
H_2O_2	2.55 ± 0.2a	3.33 ± 0.3b	2.21 ± 0.3a	3.77 ± 0.4c	5.52 ± 0.5d
TBARS	3.51 ± 0.2b	3.88 ± 0.3c	3.22 ± 0.2a	4.57 ± 0.3d	6.79 ± 0.6e
NO	1.89 ± 0.08b	2.07 ± 0.3c	1.62 ± 0.2a	2.99 ± 0.08d	4.89 ± 0.4e

Note: In each row, different letters are significantly different at $p < 0.05$.

A large number of naturally occurring substances have been recognized to have antioxidant activities, among which phenolics and their derivatives are receiving particular attention (Ibrahim and Jaafar, 2013). A highly positive relationship between total phenols and antioxidant activity has been found in many plant taxa (Gülçin et al., 2004). Although the exact mode of action of phenolics has not been well understood, such compounds may exert their effect on biological systems by different mechanisms including the sequestration of free radicals, hydrogen donation and metallic ion chelation, etc. Moreover, today we know that additive and synergistic effects of phytochemicals in plants are responsible for their potent antioxidant activity, not a single compound (Liu, 2003). BEOs tested here were multi-component mixtures consisted of a variety of highly functionalized chemical entities, belonging to different chemical classes. BEOs contained surprisingly high proportions of the phenolic derivative (phenylpropanoid) methyl chavicol (~53–90%), and, in minor quantities, other phenylpropanoids such as methyl eugenol (~0.4–2.5%), anethole (~0.1–0.3), eugenol (~0.01–0.3%) and chavicol (~0–0.02%). In addition, two phenolics (carvacrol and thymol) and three monoterpene hydrocarbons (α-terpinene, γ-terpinene ad sabinene), claimed to be potent antioxidant agents, also played their parts.

To sum up, various Fe sources, $n[Fe(His)_3]$ in particular, markedly boosted the antioxidant activity of *O. basilicum* ($p < 0.05$). This is in agreement with several other researchers who came to a similar conclusion: Antioxidant activity in *Pimpinella anisum*, *Ocimum basilicum* and *Portulaca oleracea* has been shown to be increased by the application of Zn-ALA, Zn-amino acids and Fe-ALA nano-complexes, respectively (Tavallali et al., 2017, 2018; Tavallali, 2018). The greater influence of $n[Fe(His)_3]$ on antioxidant activity of *O. basilicum* could be attributed to antioxidant properties of histidine (Ghasemi et al., 2014; Ortiz-Lopez et al., 2000). Histidine has been known as a potent scavenger of the hydroxyl radical and singlet oxygen (Wade and Tucker, 1998).

3.4. Antimicrobial activity

The minimum inhibitory concentration (MIC) derived from serial dilutions (0–0.3 mg mL^{-1}) of BEOs against the pathogens was characterized. MIC is the lowest concentration of a drug which prevents visible growth of the pathogen(s) in a certain medium. MIC of ketoconazole, a synthetic reference antibiotic drug, against two fungi strains, *C. albicans* (0.008 mg mL^{-1}) and *A. niger* (0.009 mg mL^{-1}) was recorded. Although, antifungal activity of the synthetic drug was markedly higher than that of BEOs, EOs were still highly active against the fungi strains. Regardless of the iron sources applied, the viability of the pathogens was significantly affected by foliar application of fertilizers as compared to control. On the other hand, the performance of nano-complexes was particularly stronger in comparison to Fe-EDDHA. Generally, the trend for the activity towards six examined strains of pathogens followed similar order of efficacy (Table 4). The MICs ranged from 0.058 to 0.299 mg mL^{-1}, indicating different susceptibility of the pathogens to BEOs. In detail, the application of T3 presented the lowest MIC against two foodborne fungi *C. albicans* and *A. niger* (0.058 ± 0.006, 0.066 ± 0.008 mg mL^{-1}), followed by T1 (0.087 ± 0.021, 0.071 ± 0.008 mg mL^{-1}) and T2 (0.145 ± 0.031, 0.111 ± 0.045 mg mL^{-1}). The least activity among Fe fertilizers was recorded in T4 (0.200 ± 0.101, 0.181 ± 0.021), which was markedly higher than that in control (0.299 ± 0.112, 0.221 ± 0.102).

T3 yielded the most potent EO against 2 g-positive bacteria *B. subtilis* and *S. aureus* (0.033 ± 0.009, 0.002 ± 0.0007 mg mL^{-1}), and two foodborne gram-negative bacteria *E. coli* and *S. typhimurium* (0.181 ± 0.011, 0.163 ± 0.077 mg mL^{-1}), respectively. Conversely, the weakest bactericidal activity against both 2 g-positive bacteria *B. subtilis* (0.130 ± 0.033 mg mL^{-1}) and *S. aureus* (0.072 ± 0.015 mg mL^{-1}), and two foodborne gram-negative bacteria *E. coli* (0.833 ± 0.077 mg mL^{-1}) and *S. typhimurium* (0.781 ± 0.102 mg mL^{-1}) was recorded in control. T4 showed an intermediary activity between Fe nano-complexes and control, in terms of the ability to disturb bacterial growth (Table 4). MIC of ampicillin and gentamicin, the reference antibiotic drugs, were determined against gram-positive (*B. subtilis*: 0.018 mg mL^{-1}; *S. aureus*: 0.020 mg mL^{-1}) and gram-negative bacteria (*E. coli*: 0.012 mg mL^{-1}; *S. typhimurium*: 0.013 mg mL^{-1}), respectively, and results indicated that in most cases both are the most active agents against the pathogens. Exceptions include T3 and T1, in which the activity against *S. aureus* was dramatically higher as compared to antibiotics. The activity of BEOs against *B. subtilis* was very close to the antibiotic. It can be concluded that the combination of antibiotics/BEOs administered against the microbial strains is likely to augment the antimicrobial activity of the drugs.

If a certain drug of plant origin is active in concentrations between 0.75 and 1.5 mg mL^{-1} and 2–2.5 mg mL^{-1}, we assume it as a highly active and moderately active agent, respectively. The activity beyond the latter range is considered as weak activity. Taking this into consideration, BEOs were very active against the examined pathogens as very low concentrations of oils were sufficient to prevent microbial growth. Although, control groups were very active against the fungi, the plants treated by T3 yielded EOs with 5- and 3.3-fold increase in the activity against C. *albicans* and *A. niger*, respectively. This comes to mean that C. *albicans* was considerably more sensitive to the drug than *A. niger*.

All samples including control were very effective against the bacteria examined; compared to control, plants receiving T3 showed a ~4-fold increase in the activity against *B. subtilis*, while the sensitivity of *S. aureus* to this treatment was 36-fold larger than control. The susceptibility of 2 g-negative bacteria, *E. coli* and *S. typhimurium*, to this treatment was also significant and for both pathogens showed about 4.5-fold increase. It was evident that 2 g-positive bacteria (*B. subtilis* and *S. aureus*) were particularly more sensitive to the action of EOs than gram-negative bacteria. According to Burt (2004), EOs are slightly more active against gram-positive than gram-negative bacteria, since gram-negative organisms possess an outer membrane surrounding the cell wall, relatively effective in protecting the organism against antibacterial drugs (Ratledge and Wilkinson, 1988).

A number of compounds belonging to different chemical classes are recorded in BEOs. Therefore, antimicrobial activity of BEOs is not attributable to one particular mechanism; it is likely that there are several targets in the cell (Burt, 2004). Lipophilicity plays an especially vital role in antimicrobial activity of EOs, as it enables them to distribute in the lipids of the organism cell membrane and mitochondria. The most

Table 4
Antimicrobial activity of basil essential oils in response to different Fe sources.

	Species	MIC (mg mL^{-1})				
		$n[Fe(Arg)_3]$	$n[Fe(Gly)_3]$	$n[Fe(His)_3]$	Fe-EDDHA	Control
Fungi	*C. albicans*	0.087 ± 0.021a	0.145 ± 0.031b	0.058 ± 0.006a	0.200 ± 0.101c	0.299 ± 0.112c
	A. niger	0.071 ± 0.008a	0.111 ± 0.045c	0.066 ± 0.008a	0.181 ± 0.021b	0.221 ± 0.102d
Bacteria	*B. subtilis*	0.045 ± 0.004a	0.071 ± 0.006b	0.033 ± 0.009a	0.097 ± 0.020c	0.130 ± 0.033d
	S. aureus	0.008 ± 0.0006a	0.033 ± 0.011b	0.002 ± 0.0007a	0.061 ± 0.009c	0.072 ± 0.015d
	E. coli	0.309 ± 0.061b	0.411 ± 0.101c	0.181 ± 0.011a	0.582 ± 0.111d	0.833 ± 0.077e
	S. typhimurium	0.322 ± 0.021b	0.512 ± 0.120c	0.163 ± 0.077a	0.604 ± 0.115c	0.781 ± 0.102d

Values are means of Minimal Inhibitory Concentration (MIC) in eight replications (pots) ± sd. (standard deviation).
Note: In each row, different letters represent significant differences among treatments ($p < 0.05$).

important mechanisms are thought to be the disturbance of cytoplasmic membrane, damage to membrane proteins, leakage of cell contents, coagulation of cytoplasm, depletion of the proton motive force and most likely preventing enzyme action via binding to proteins (Devi et al., 2010).

The inherent biological and pharmacological activities of a given oil is related to the chemical configuration of the components, the proportions in which they are present and to interactions between them. The EOs possessing the strongest antibacterial properties against food borne pathogens mainly contain a high percentage of oxygenated monoterpenes and phenolic compounds (Aggarwal et al., 2002). BEOs examined here were particularly rich in such compounds, including the phenylpropanoids methyl chavicol (~53–90%), methyl eugenol (~0.4–2.5%), anethole (~0.1–0.3), eugenol (~0.01–0.3%) and chavicol (~0–0.02%). Phenylpropenes, notably eugenol, methyl chavicol and anethole, have a role in plant defense given they show activity against a wide range of pathogens (Atkinson, 2016). Additionally, two phenolic compounds of BEOs, carvacrol and its isomer thymol, are well-known for their strong antimicrobial activity (Memar et al., 2017). Antimicrobial activity of a number of nonphenolic monoterpenes e.g. α-pinene, limonene, myrcene, sabinene, linalool, terpineol, 1,8-cineole, elemene, has been reported (Zengin and Baysal, 2014; Sieniawska et al., 2017). On the other hand, the application of iron sources markedly enhanced the quantity of sesquiterpenes (e.g. germacrene, α-bergamotene, β-farnesene, α-humulene, γ-bisabolene and caryophyllene) in EO samples; strong antimicrobial activity of sesquiterpenes (e.g. β-caryophyllene and β-farnesene) has been well documented (e.g. Vieira et al., 2014; Siddiqui et al., 2017).

As mentioned earlier, the overall activity cannot always be attributed to any of the major constituents, and additive or even synergistic effects in the presence of other compounds within a mixture modifies the activity to exert significant effect (Raut and Karuppayil, 2014). For example, synergistic effects have been found between thymol-carvacrol, cinnamaldehyde-eugenol, thymol-eugenol, carvacrol-eugenol (Pei et al., 2009). In this regard, the specific composition of BEOs obtained from the plants treated by T3 may played an important role in its potent antimicrobial activity. These plants showed a very special EO profile with respect to the interplay of major and minor compounds; while the quantity of methyl chavicol, the major compound in all samples, showed the maximum decrease (~17% fall) in this treatment, the concentration of up to 44 minor compounds (notably 20 sesquiterpenes out of 23), surpassed other treatments. The proportion of oxygenated monoterpenes, notably phenolic monoterpenoids eugenol, thymol and carvacrol, in the total oil also exhibited the maximum increase among all treatments. Moreover, several studies have noted that histidine (Kacprzyk et al., 2007) and arginine (Cutrona et al., 2015) enhance the antimicrobial activity of the peptides. The cationic chains like guanidinium group in arginine (Hristova and Wimley, 2011) and imidazole in histidine (Kohn et al., 2018) are responsible for their antibacterial activities.

To sum up, the current study is an attempt to determine the antioxidant activity of basil, and to screen out a new source of natural and effective drugs against some common strains of foodborne fungi and bacteria. The initial results obtained are quite encouraging since EOs tested exhibited potent antioxidant effect, and antifungal and antibacterial activities.

Declaration of competing interest

The manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed.

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