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[1]Department of Agriculture, Payame Noor University (PNU), Tehran, Iran
[2]Department of Horticultural Sciences, Faculty of Agriculture, Shiraz University, Shiraz, Iran
[3]Department of Chemistry, Payame Noor University (PNU), Tehran, Iran

Biological and pharmacological activities of essential oils of *Ocimum basilicum* L. grown with Zn-salicylic acid nano-complex

Vahid Tavallali[1*], Hossein Gholami[2], Omid Espargham[3]

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Summary

A greenhouse study was conducted to investigate the impact of different rates of application of Zn-EDTA, salicylic acid (SA) and zinc-salicylic acid nano-complex (n[$Zn(SA)_2$]) on the antioxidant and antimicrobial activities of essential oil (EO) of sweet basil (*Ocimum basilicum* L.). Sixty-one compounds were detected in the EOs after Zn and SA sources were applied to the plants. GC-MS analysis showed that the main components of the EOs after the treatment were epi-α-Cadinol and trans-α-Bergamotene. The highest amount of epi-α-Cadinol (29.06±1.31%) and trans-α-Bergamotene (11.90±1.1%) in the EO were observed at 0.2% n[$Zn(SA)_2$] treatment. In general, the application of 0.2% n[$Zn(SA)_2$] significantly increased percentages of phenolic and flavonoid compounds of extract. HPLC analysis showed that the predominant phenolic compound after treatments with different Zn and SA sources were rosmarinic acid and quercetin, respectively. The lowest IC_{50} values for RNS, ROS, TBARS and H_2O_2, scavenging activities were obtained in EOs of basils which were treated with 0.2% n[$Zn(SA)_2$]. Zinc-salicylic nano-complex was the most effective treatment to inhibit fungal and bacterial growth. Our results are quite encouraging since the Eos of n[$Zn(SA)_2$] treated basil exhibited potent antioxidant effect, antimicrobial activities comparable with synthetic drugs.

Keywords: Antimicrobial activity, Flavonoids, Nanoparticle, Salicylic acid, Sweet basil, Zinc.

Introduction

The use of nanotechnology in all fields, including agriculture is expanding. One of the most important applications of this technology in agriculture is the use of nanofertilizers for plant nutrition (Zhu et al., 2008). Nanofertilizers have recently attracted much attention due to their increased absorption by the plant, which is due to its small size and high penetration through cell membranes (Panwar et al., 2012). Nanoparticles are highly reactivity due to special and higher surfaces, higher density and many reactive regions on the particle surfaces. These characteristics make nanofertilizers and pesticides in nano-scale easy to absorb (Chinnamuthu and Boopathi, 2009). Nanoparticles can bind to specific sites within biomolecules including proteins, nucleic acids and subcellular structures. In this way, they can pass through the cell membrane (Krystofova et al., 2013). Nanotechnology is gradually moving from the experimental stage to the operational stage, which has led to a more visible presence of this technology in the agricultural sector (Baruah and Dutta, 2009).
In this study, we focus on *Ocimum basilicum*, which is highly diverse in its morphological characteristics and secondary compounds, especially essential oil (Telci et al., 2006). Basil has been used as herbal medicine to treat headaches, coughs, diarrhea, parasites, warts and kidney diseases (Labra et al., 2004). Terpenoids constitute a significant proportion of basil essential oil which their type and amount is different in chemotypes, different climatic conditions and stages of plant development (Sajjadi, 2006). Synthesis of essential oils in plants changes affected by factors such as nutrition, light intensity, photosynthesis, photoperiodic changes, climatic conditions, seasonal variation, plant growth regulators, and environmental stresses such as drought, salinity and temperature (Werker et al., 1993).
Salicylic acid or 2-hydroxybenzoic acid is a phenolic compound in plants that affect growth, respiration, photosynthesis, absorption and ion transport, and create some changes in leaf morphology and chlorophyll structure (Popova et al., 2003). The application of salicylic acid can affect a range of different processes in plants such as germination of seeds (Wang et al., 2006), resistance to pathogens (Cag et al., 2009), flowering (Koda et al., 1992), exchange and transmission of ions (Cag et al., 2009), guard cells function and transpiration regulation (Metwally et al., 2003), membrane permeability, photosynthesis and growth rate (Khan et al., 2003).
Zinc is one of the micronutrient elements in the plant that is involved in various plant processes. Deficiency of zinc prevents the growth of plants (Gurmani et al., 2012). Although a plant's need for zinc is small, zinc deficiency results in severe physiological tensions such as inefficiency of many enzymatic systems and other metabolic actions (Sadeghzadeh and Rengel, 2006). Zinc plays a role in carbohydrate and protein metabolism and is indirectly controlling water-plant relationships. There also is a close relationship between zinc and the amount of auxin in plants. A lack of auxin due to zinc deficiency causes reduced cell wall growth due to high osmotic pressure and limited water absorption by the plant (Marschner, 2012).
The purpose of this study is to investigate the effect of various zinc and salicylic acid sources on growth characteristics, essential oil yield, antioxidant and antimicrobial properties of sweet basil.

Materials and methods

Soil analysis

Physico-chemical characteristics of the soil were: texture- sandy loam, pH 7.1, ECe 1.3 dS m^{-1}, CEC 11 Cmc kg^{-1}, organic matter 8.8 g kg^{-1}, N 0.08%, available K, P, Zn and Fe 61, 12, 1.5 and 1.01 mg kg^{-1}, respectively. 5 mg kg^{-1} Zn, Mn and Cu as $ZnSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$ and $CuSO_4 \cdot 5H_2O$ were applied, respectively. Also, 50 mg kg^{-1} N and P as NH_4NO_3 and KH_2PO_4 were applied to the soil, respectively.

Plant material

The seeds of sweet basil (*Ocimum basilicum* L.) were provided from an herbal garden of Bazrco Company in Tehran, Iran. A 2×2×2 factorial experiment, arranged in a randomized complete design (RCD) with eight replications, was conducted in a greenhouse located at the 29° 31´ N; 52° 31´ E in Shiraz, Iran (approximately 800-1000 µmol m^{-2} s^{-1} PAR-photosynthetically active radiation, with a 12 h photo-

* Corresponding author

period and temperature between 22-25 °C.). The experimental units were 7 L plastic pots. Treatments were Zn-EDTA (0.1 and 0.2%), salicylic acid (0.1 and 0.2%), n[$Zn(SA)_2$] nano-complex (0.1 and 0.2%) and control (deionized water). Salicylic acid and zinc sources were added in two steps. The first one after thinning and the second step was applied previous the flowering inception of plants. Basils were collected at full bloom stage.

Nanoparticles used

Zn-salicylic acid nanoparticles (n[$Zn(SA)_2$]) were purchased from "Zist Nano Fanavaran Atiye Pajooh" company in Fars Science and Technology Park, Iran. The size of n[$Zn(SA)_2$] nanoparticles were determined by the transmission electron micrographs (TEM) (100 kV Philips, EM208) (Fig. 1).

Fig. 1: The TEM image of Zn-salicylic acid nano-complexes.

Essential oil isolation

The aerial parts were air-dried and then hydrodistilled for 3 h using a Clevenger type device, according to the method recommended by the British Pharmacopoeia (1998). Eventually, the samples were then dried over anhydrous Na_2SO_4 and kept in sealed vials at low temperature until further analysis.

High Performance Liquid Chromatography (HPLC) analysis

Agilent HPLC 1200 series was applied to obtain phenolic acids (gallic acid, cinnamic acid, carvacrol, rosmarinic acid and ferulic acid were purchased from Sigma-Aldrich company for HPLC analysis and flavonoids (quercetin, kaempferol, catechin, luteolin and rutin were purchased from Sigma-Aldrich company for HPLC analysis) with the following method; 20 microliter of the dissolved extract was injected to Zorbax eclipse (XDB) C18, 4.6 × 5 µm (ID) × 150 mm column while column temperature was set on 30 °C. The gradient programming was applied to separate the constituents during 40 min. The mobile phase was a mixture of methanol: formic acid 1% with the flow rate of 1ml min^{-1}, started from (10:90); then it programmed to (25:75) at the time of 10min; changing to (60:40) at 20 min; at the time of 30 min the ratio was set on (70:30) which was held isocratic till 40 min. Photodiode array detector was set on 280 and 320 nm and Chemstation Software was used for data analysis. The standard solutions were all dissolved in methanol. The calibration curves with standard phenolic acids and flavonoids were obtained with good correlation. Concentrations of phenolic acids and flavonoids are calculated as as milligrams per gram of dry matter (mg g^{-1} DM).

Essential oil analysis

The experiment was carried out using Agilent gas chromatograph series 7890-A with an FID (flame ionization detector) on fused silica capillary HP-5 column (30 m × 0.32 mm i.d.; film thickness 0.25 µm) for quantitation analysis. The injector and detector temperatures were kept at 250 °C and 280 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min; the oven temperature program was 60-210 °C at the rate of 4 °C/min and then programmed to 240 °C at the rate of 20 °C/min and finally held isothermally for 8.5 min; the split ratio was 1:50. GC-MS analysis was carried out for quantification analysis by use of Agilent gas chromatograph equipped with fused silica capillary HP-5MS column (30 m × 0.25 mm i.d.; film thickness 0.25 µm) coupled with an Agilent mass spectrometer series 5975-C. Helium was used as carrier gas. The quadrupole mass spectrometer was scanned over 50-550 amu with an ionizing voltage of 70 eV. Ion source and interface temperatures were 230 °C and 280 °C, respectively. Mass range was from 45 to 550 amu. The oven temperature program was the same as the GC. The injection volume was 0.1 µl in two mothods. Retention indices of EO were determined based on retention times of alkanes (C_8-C_{25}) under the same chromatographic conditions. Eventually, identification of chemical components of EO was done by correspondence of their retention profiles with those reported in previous studies (Adams, 2007).

Antimicrobial activity of essential oil

Bacteria and fungi were obtained from the Persian Type Culture Collection (PTCC), Tehran, Iran. Those were *Escherichia coli* PTCC 1330 (ATCC 8739) and *Salmonella typhimurium* PTCC 1609 (Iran isolate) as Gram-negative bacteria and *Staphylococcus aureus* PTCC 1112 (ATCC 6538) and *Bacillus subtilis* PTCC 1023 (ATCC 6633) as Gram-positive bacteria. Also, the fungi used were fungi *Candida albicans* PTCC 5027 (ATCC 10231) and *Aspergillus niger* PTCC 5010 (ATCC 9142). The MIC (minimum inhibitory concentration) values of the EO were characterized by means of the microdilution method in accordance with the Clinical and Laboratory Standards Institute procedures (CLSI, 2012). Suspended bacteria and fungi strains in Luria-Bertani (LB) media were tuned to 0.6 McFarland standards at 640 nm (10^8 CFU/ml). Then, densities were diluted to 10^5 (CFU/ml) with Luria-Bertani. The test mixture (600 µL) contained 300 µL of suspensions of bacteria and fungi and 300 µL EO. In the next step, the samples were shaken in incubator (for 24 h at 36 °C). Positive control included in Ketoconazole, Ampicillin and Gentamicin for fungi, Gram-positive and Gram-negative bacteria, respectively. A medium without fungi and bacteria and a medium without essential oils but with bacteria as sterile and growth control were considered, respectively. MIC values were used for measuring the antibacterial and antifungal activities by using the formula MIC value = $[(A640_{blank} - A640_{sample}/A640_{blank}] \times 100$.

Antioxidant activity of the essential oil

Antioxidant activities of the EO were evaluated based on IC_{50}, applying $NaNO_2$, MDA, DPPH and H_2O_2 scavenging effects, shown for RNS, TBARS, ROS and H_2O_2 scavenging activities, respectively (Kavoosi and Rowshan, 2013; Burits et al., 2001). In ROS assay,

the reaction mixture (260 µL) contained 30 µL EO (0-0.5 mg mL^{-1} in DMSO) and 230 µL DPPH (120 mmol L^{-1} in methanol). In order to measure of RNS scavenging effect, to 100 µL of the EO (0-0.5 mg mL^{-1} in DMSO), 250 µL of sodium nitrite (0.02 mg mL^{-1} in 100 mM sodium citrate) was added. Then, the reaction mixture was kept for 120 min at 36 °C. Eventually, to this mixture, 600 µL of Griess reagent was added. The reaction mixture of 50 µL of the EO (0-0.5 mg mL^{-1} in DMSO) and 50 µL of the MDA (0.1 mM in acetic acid pH=4) was used in order to measure of TBARS scavenging effect. The, the solutions were kept at a 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 minutes. In H_2O_2 assay, the reaction mixture (100 µL) contained 50 µL of the EO (0-0.5 mg mL^{-1} in DMSO) and 50 µL of the H_2O_2 (100 mM in 200 mM phosphate buffer, pH=7.4). The solutions were incubated at 36 °C for 75 minutes. Eventually, samples' absorptions were read with EL×808 absorbance microplate reader (BioTek Instruments, Inc., USA) at wavelengths of 540, 532, 515 and 230 nm for $NaNO_2$, MDA, DPPH and H_2O_2 tests, respectively. The percentage of RNS, TBARS, H_2O_2 and ROS scavenging were then calculated by using the following formulas:

$$\text{RNS scavenging effect (\%)} = [(A540_{blank} - A540_{sample})/A540_{blank}] \times 100$$

$$\text{TBARS scavenging effect (\%)} = [(A532_{blank} - A532_{sample})/A532_{blank}] \times 100$$

$$H_2O_2 \text{ scavenging effect (\%)} = [(A230_{blank} - A230_{sample})/A230_{blank}] \times 100$$

$$\text{ROS scavenging effect (\%)} = [(A515_{sample} - A515_{blank})/A515_{control}] \times 100$$

Statistical analysis

Data are presented as mean values ±standard deviation of eight replications. Data were statistically analyzed using one-way ANOVA and Duncan's multiple range test (P<0.05) with SPSS (20.0).

Results and discussion

Growth characteristics and EO yield

Fig. 2a shows that the Zn-salicylic acid nano-complex application significantly stimulated most of the essential oil production. The highest EO yields were achieved at 0.2% n[$Zn(SA)_2$] addition and lowest in the untreated control.

Carbon dioxide and glucose are precursors of biosynthesis for monoterpenes. Saccharides are the energy source for the synthesis of terpenoids, which due to the role of zinc in the activity of the chloroplasts, photosynthesis, stomatal conductance and saccharides metabolism, the importance of this element in the production and accumulation of essential oil in plants can be significant. Also, increasing leaf area due to zinc application, and consequently increasing photosynthesis, CO_2 stabilization and higher carbohydrate production, is another reason for the increase of secretory glands of essential oil (Derakhshani et al., 2011b). Zehtab-salmasi et al., 2008, reported that vegetative growth and essential oil yield of peppermint with application of 250 mg L^{-1} zinc sulfate were significantly higher than control treatment. Also, research by Grejtovský et al., 2006 showed that the application of 50 mg L^{-1} of zinc, increases the growth and essential oil yield in chamomile. Increasing the essential oil yield after zinc application has also been reported for basil (Said-Al Ahl and Mahmoud, 2010).

The positive role of spraying salicylic acid in increasing the essential oil yield may be due to increased vegetative growth, nutrient absorption, photosynthetic activity and also the change in the number of secretory glands of essential oil in leaves and flowers. These results are consistent with the findings of another study (Gharib, 2007) on the positive role of salicylic acid in increasing the weight percent of essential oil of basil. One reason for increasing the secretory glands of essential oil may be the role of salicylic acid in enhancing the rubisco activity, photosynthesis, chlorophyll content and thereby increasing the performance of dry matter (Singh and Usha, 2003).

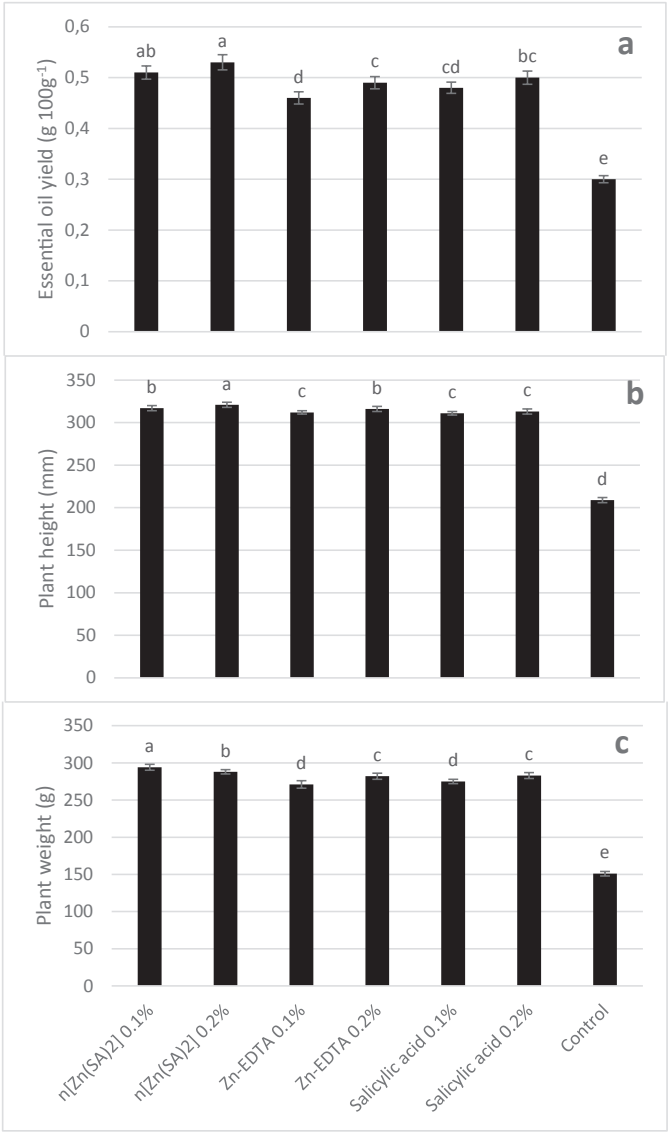

Fig. 2: Essential oil yield (a), plant height (b) and plant weight (c) of sweet basil under different zinc and salicylic acid sources. Values are means ± SE (n = 8). Bars having different letters are significantly different at the 5% level by Duncan's multiple range test.

The positive effect of Zn-salicylic acid nano-complexes spraying on growth and essential oil yield can be attributed to the specific properties such as specific surface area, surface energy and increased surface activity compared to conventional particles (Panwar et al., 2012).

Results indicated that the application of zinc and salicylic acid sources have a significant effect on the growth parameters of sweet basil ($P \leq 0.05$). The highest plant height and plant weight were found in sweet basil treated with 0.2% and 0.1% n[$Zn(SA)_2$], respectively (Fig. 2b and c).

In this investigation, the use of salicylic acid and zinc in the form of nano-complex had a higher efficiency on growth characteristics than their non-nano forms. Probably, the use of Zn-salicylic acid nano-complexes due to small size and their high penetration into cell membranes can justify their positive role (Panwar et al., 2012).

Chemical components of EO

The quantitative and qualitative compositions of the EOs of the sweet basil plants that were supplied with zinc and salicylic acid sources are presented in Tab. 1. Thirty-eight compounds were identified in the essential oil of basil at control treatment. Epi-α-Cadinol, Eugenol, Linalool, and trans-α-Bergamotene were the major components in the EO with the value of 24.67, 12.7, 10.85 and 8.36%, respectively. Sixty-one compounds were detected in the EOs after Zn and SA sources were applied to the plants. GC-MS analysis showed that the main components of the EOs after treatment of different Zn and SA fertilizer sources were epi-α-Cadinol and trans-α-Bergamotene. The highest amount of epi-α-Cadinol (29.06±1.31%) and trans-α-Bergamotene (11.90±1.1%) were observed in the essential oil of 0.2% n[$Zn(SA)_2$] treated plants and the lowest (24.67±1.7 and 8.36±0.86%, respectively) in control plants.

The effect of salicylic acid on the variation of chemical composition of essential oils in savory has been reported by Haiati and Rowshan (2013). Hassanpouraghdam et al. (2011) reported that foliar application of Zn influences the primary metabolic pathways, which ultimately causes the synthesis of essential oil compounds in basil leaves. In a study on basil, foliar application of zinc chelate improved growth characteristics and essential oil yield, as well as increasing the percentage of linalool and methyl chavicol as the dominant components of essential oil (Said-Al Ahl and Mahmoud, 2010).

Srivastava et al. (2006) observed a significant positive correlation between carbon assimilation pathways and the accumulation of secondary metabolites in turmeric. They consider several internal and external factors to be effective in the production of secondary metabolites, which have identified the most important factor in the supply of micronutrients such as Zn; since the Zn has been very effective on the biosynthesis of sesquiterpenes. Increased Zn levels have been associated with increased percentage of propyl 1-propenyl disulfide and reduced percentage of dimethylthiophene in onion (El-Tohamy et al., 2009). Chand et al., 2007 reported that Zn spraying has significantly increased the percentage of essential oil of geranium, and also changes the main components of the essential oil such as rose oxide, linalool, and isomenthone. Also, the positive effect of Zn on the increase of essential oil yield and methyl chavicol as the dominant component of anise essential oil has been reported (Pirzad et al., 2013). Changes in the compounds of the essential oil by Zn spraying are related to the effect of this element on divalent cations, enzyme activity and also carbon metabolism. Some enzymes that have metal ions in their building play an important role in the biosynthesis of monoterpene compounds (Prasad et al., 2008). Tavallali et al. (2018) reported changes in the essential oil components of sweet basil after the use of green synthesized zinc-amino nano-complexes. Methyl chavicol was identified as the dominant component of the essential oil.

Phenolic acids and flavonoids profiles

Identification of phenolic and flavonoid compounds of extract according to their spectral properties was performed out by HPLC. Calibration curves were prepared by analysis of calibration solutions of investigated compounds in the concentration range from 1 to 500 mg L^{-1}. In this study, carvacrol, rutin, ferulic acid and luteolin were not identified in control treatment. The results showed that the application of Zn and SA sources increased the number of phenolic and flavonoid compounds detected in the extract. A total of 10 phenolic compounds, including five phenolic acids and five flavonoids, were identified from the extract of treated plants with Zn and SA sources (Tab. 2). In general, the application of 0.2% n[$Zn(SA)_2$] fertilizer significantly increased percentages of phenolic and flavonoid compounds of extract. HPLC analysis showed that the predominant phenolic compound of the extract after treatment of different Zn and SA fertilizer sources were rosmarinic acid and quercetin, respectively.

In a study by Derakhshani et al. (2011a), the application of zinc sulfate increased the phenolic compounds in costmary. An increase in the amount of phenolic compounds may be due to the role of zinc in the expression of genes associated with the biosynthesis of phenolic and flavonoid compounds (Karabourniotis and Liakopoulos, 2005). Song et al. (2015) have shown that zinc inhibits the reduction of VvPAL gene expression, thereby increasing the content of phenolic compounds. Additionally, zinc with the direct effect on the activation of key enzymes in the biosynthetic pathways of flavonoids, such as chalcone synthase (CHS) and chalcone isomerase (CHI) increase the production of these compounds in the plants. It also increases the expression of VvCHS, VvMYBF1 and VvFLS4 genes, and stimulates the production of flavonoid compounds in the plant (Song et al., 2015).

Our results showed that the use of Zn-salicylic acid nano-complexes has a greater effect than their non-nano form. Increasing the transfer velocity, absorption efficiency, and specific surface of nanoparticles compared with conventional particles, can justify the greater effect of these particles (Monica and Cremonini, 2009). Possibly, the entry of nanoparticles in plant cells is done through stomatal opening and natural nanopores which may enhance plant cell metabolic activities that lead to higher phenolic compounds production (Tarafdar et al., 2014).

Antioxidant activity

The EO from sweet basil aerial parts was investigated for radical scavenging activity using four different assay methods (RNS, ROS, TBARS and H_2O_2, scavenging activities). The antioxidant activity of samples as milligrams ascorbic acid equivalents per gram of essential oil (mg AAE g^{-1}) was calculated and reported in Tab. 3. The highest antioxidant activity (22.94±1.7 mg AAE g^{-1}) was found in 0.2% n[$Zn(SA)_2$] nano-complex treatment followed by 0.1% n[$Zn(SA)_2$] nano-complex treatment, which increased the antioxidant activity to 21.44±1.5 mg AAE g^{-1}. The lowest amount (8.82±1.2 mg AAE g^{-1}) was obtained in untreated plants. On 0.2% n[$Zn(SA)_2$] nano-complex treatment, all four different assay methods showed better antioxidant activity than other treatments. Therefore, the lowest IC_{50} values for RNS, ROS, TBARS and H_2O_2, scavenging activities were obtained in EO of basil plants, which were treated with 0.2% [$Zn(SA)_2$] nano-complex.

The comparison of the treatments used in this experiment showed that the use of zinc and salicylic acid in the form of nano has a higher effect than their non-nano form. Probably, some of the properties of nanoparticles, such as higher transfer velocities, solubility and greater stability, can justify this high effect (Monica and Cremonini, 2009).

Antimicrobial activity

In the study, the antimicrobial activities of EO from the aerial parts of sweet basil were tested against Gram-negative (*Salmonella typhimurium* and *Escherichia coli*) and Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) bacteria and fungi (*Candida albicans* and *Aspergillus niger*) growth by Minimal Inhibitory Concentration (MIC) method. The results showed that the application of zinc and salicylic acid sources significantly increased the antifungal and antibacterial activity of EO from sweet basil compared to the control treatment (Tab. 4). The lowest MIC for *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger* growth were 0.180±0.011, 0.229±0.018, 0.007±0.0005, 0.031±0.003, 0.050±0.005 and 0.068±0.045 mg mL^{-1} of EO derived from treated basils with 0.2% n[$Zn(SA)_2$] nano-complex, respectively.

Tab. 1: Phytochemical profile (%) identified in essential oil of sweet basil supplied with diverse zinc and salicylic acid sources.

No.	Compound	Retention Index	n[Zn(SA)$_2$] 0.1%	n[Zn(SA)$_2$] 0.2%	Zn-EDTA 0.1%	Zn-EDTA 0.2%	Salicylic acid 0.1%	Salicylic acid 0.2%	Control
1	α-Pinene	932	0.258±0.07c	0.251±0.02c	0.212±0.01d	0.238±0.02cd	0.377±0.03b	0.422±0.05a	----------
2	Sabinene	972	0.576±0.03b	0.602±0.04b	0.571±0.05b	0.583±0.06b	0.580±0.06b	0.631±0.07a	----------
3	β-Pinene	976	0.286±0.04b	0.208±0.01c	0.224±0.02c	0.271±0.03b	1.084±0.23a	1.167±0.27a	----------
4	Myrcene	990	0.469±0.02c	0.510±0.03b	0.508±0.04b	0.416±0.03d	3.110±0.52a	3.224±0.59a	----------
5	n-Decane	999	0.606±0.04d	0.425±0.03e	3.587±0.45a	0.611±0.05d	0.884±0.07c	0.966±0.07b	3.357±0.43a
6	α-Phellandrene	1005	0.105±0.02cd	0.132±0.01ab	0.091±0.006d	0.121±0.03bc	0.158±0.03a	0.127±0.02b	----------
7	α-Terpinene	1016	0.081±0.007de	0.115±0.02b	0.069±0.005e	0.102±0.01bc	0.092±0.007cd	0.167±0.01a	----------
8	p-Cymene	1024	0.048±0.003c	0.093±0.007a	0.058±0.006c	0.082±0.005b	0.015±0.002e	0.029±0.004d	----------
9	Limonene	1028	0.134±0.01d	0.150±0.01c	0.127±0.02d	0.142±0.02cd	0.754±0.06a	0.796±0.07a	0.289±0.02b
10	1,8-Cineole	1030	1.088±0.11b	0.614±0.05c	3.523±0.50a	0.602±0.05c	1.074±0.20b	1.121±0.31b	3.463±0.66a
11	(Z)-β-Ocimene	1036	0.137±0.02bc	0.153±0.01b	0.126±0.02c	0.145±0.03bc	0.101±0.01d	0.125±0.01c	0.397±0.04a
12	Benzene acetaldehyde	1041	0.075±0.003b	0.094±0.006a	0.062±0.004c	0.085±0.006ab	0.018±0.002e	0.039±0.004d	----------
13	(E)-β-Ocimene	1046	0.566±0.03ab	0.567±0.04ab	0.549±0.05b	0.561±0.04ab	0.581±0.05ab	0.602±0.07a	----------
14	γ-Terpinene	1057	0.139±0.01a	0.123±0.01b	0.128±0.01b	0.142±0.02a	0.118±0.02b	0.126±0.02b	----------
15	cis-Sabinene hydrate	1066	0.151±0. 01ab	0.120±0.01c	0.137±0.02b	0.145±0.03b	0.150±0.02ab	0.163±0.03a	----------
16	Terpinolene	1088	0.309±0.02c	0.332±0.02b	0.281±0.03d	0.319±0.04bc	0.324±0.03bc	0.371±0.04a	----------
17	Linalool	1099	0.590±0.04c	0.516±0.04d	0.584±0.06c	0.593±0.05c	5.696±0.45b	5.733±0.51b	10.850±1.89a
18	1-Octen-3-yl acetate	1112	0.062±0.004c	0.079±0.005b	0.061±0.004c	0.072±0.007b	0.129±0.02a	0.136±0.02a	----------
19	Camphor	1144	0.114±0.01b	0.128±0.01a	0.102±0.03c	0.119±0.01ab	0.058±0.006e	0.089±0.009d	----------
20	δ-Terpineol	1166	0.170±0.02c	0.198±0.02b	0.132±0.02d	0.161±0.02c	0.190±0.03b	0.220±0.02a	----------
21	Terpinen-4-ol	1177	0.090±0.002a	0.097±0.007a	0.066±0.006c	0.076±0.006b	0.077±0.005b	0.097±0.007a	----------
22	α-Terpineol	1190	0.761±0.04c	0.664±0.04e	0.682±0.05de	0.703±0.05d	2.110±0.26a	1.360±0.18b	0.483±0.05f
23	n-Dodecane	1199	0.565±0.03c	0.414±0.03d	0.512±0.04c	0.690±0.06b	0.177±0.03e	0.203±0.04e	0.783±0.07a
24	Octanol acetate	1212	0.605±0.04c	0.604±0.05c	0.346±0.04d	0.589±0.06c	0.798±0.07b	0.821±0.08b	0.965±0.07a
25	Trans-Carveol	1222	0.250±0.02a	0.199±0.02b	0.101±0.009c	0.195±0.01b	0.088±0.007c	0.098±0.009c	0.248±0.01a
26	Linalyl acetate	1256	0.036±0.003c	0.043±0.003c	0.753±0.06a	0.791±0.06a	0.108±0.02b	0.129±0.02b	----------
27	Bornyl acetate	1286	1.332±0.12e	1.005±0.08f	2.395±0.18c	1.692±0.23	2.644±0.36b	1.843±0.28d	6.657±1.03a
28	δ-Elemene	1337	0.444±0.03c	0.497±0.03b	0.365±0.03d	0.562±0.05a	0.184±0.03e	0.219±0.03e	0.146±0.01f
29	α-Terpinyl acetate	1349	0.146±0.01b	0.135±0.01b	0.135±0.02b	0.154±0.02b	0.199±0.04a	0.224±0.03a	0.095±0.007c
30	Eugenol	1358	0.741±0.05f	0.403±0.03g	1.389±0.09d	1.711±0.12c	2.288±0.27b	1.140±0.21e	12.704±1.77a
31	α-Copaene	1375	0.297±0.02a	0.292±0.02a	0.291±0.04a	0.314±0.04a	0.091±0.008c	0.121±0.01b	0.056±0.006d
32	β-Cubebene	1390	0.168±0.01b	0.222±0.02a	0.130±0.02c	0.214±0.02a	0.060±0.007e	0.130±0.01c	0.084±0.009d
33	β-Elemene	1392	7.877±0.21a	7.970±0.18a	7.245±0.25b	7.520±0.30b	6.083±0.41c	6.117±0.49c	4.894±0.42d
34	n-Tetradeane	1400	0.288±0.02b	0.256±0.01c	0.253±0.03c	0.339±0.04a	0.078±0.008d	0.092±0.009d	----------
35	Methyl eugenol	1405	0.341±0.02c	0.309±0.02d	0.345±0.03c	0.392±0.04b	0.702±0.06a	0.715±0.05a	0.330±0.02cd
36	Cis-α-Bergamotene	1415	0.053±0.006b	0.076±0.005a	0.051±0.004b	0.072±0.005a	0.051±0.006b	0.072±0.007a	----------
37	(E)-Caryophyllene	1418	0.587±0.04ab	0.563±0.04b	0.469±0.03c	0.597±0.04a	0.308±0.05d	0.338±0.04d	0.196±0.02e
38	β-Gurjunene	1428	0.059±0.005c	0.070±0.004b	0.039±0.003e	0.077±0.005a	0.036±0.004e	0.051±0.005d	----------
39	**trans-α-Bergamotene**	**1436**	**11.838±1.02a**	**11.903±1.10a**	**10.920±1.06b**	**11.150±1.08b**	**9.093±0.87c**	**9.222±0.92c**	**8.369±0.86d**
40	α-Guaiene	1438	0.938±0.06b	1.108±0.09a	0.943±0.07b	1.148±0.11a	0.565±0.05c	0.606±0.07c	0.514±0.06d
41	(Z)-β-Farnesene	1443	0.196±0.01bc	0.219±0.02ab	0.132±0.01d	0.207±0.01abc	0.186±0.02c	0.233±0.03a	0.063±0.006e
42	α-Humulene	1453	2.912±0.17a	2.897±0.18a	2.752±0.18c	2.814±0.19b	1.968±0.17d	2.157±0.15d	1.323±0.12e
43	(E)-β-Farnesene	1457	0.488±0.03b	0.560±0.04a	0.392±0.04	0.459±0.05bc	0.403±0.04d	0.448±0.05c	0.247±0.01e
44	Allo-Aromadendrene	1462	1.860±0.08b	1.007±0.07f	1.649±0.09c	1.992±0.12a	1.406±0.14e	1.521±0.17d	0.993±0.11f
45	Germacrene D	1480	7.453±0.19b	7.561±0.41a	7.007±0.52d	7.254±0.55c	6.098±0.64e	6.133±0.60e	4.026±0.47f
46	Bicyclogermacrene	1496	4.474±0.19a	4.534±0.21a	4.071±0.34b	4.195±0.37b	2.913±0.21c	3.197±0.30c	1.901±0.32d
47	Trans-β-Guaiene	1498	0.467±0.03ab	0.485±0.03ab	0.397±0.05c	0.451±0.04	0.471±0.04ab	0.511±0.04a	0.190±0.01d
48	α-Bulnesene	1508	2.565±0.07c	2.795±0.08a	2.519±0.09c	2.682±0.09b	1.927±0.08d	2.010±0.07d	1.191±0.06e
49	γ-Cadinene	1514	6.085±0.15b	6.972±0.16a	5.677±0.26cd	5.741±0.31c	5.343±0.40e	5.423±0.48de	3.323±0.37f
50	β-Sesquiphellandrene	1521	1.228±0.06b	1.281±0.07a	1.097±0.09d	1.150±0.12c	1.146±0.10cd	1.214±0.13b	0.859±0.07e
51	δ-Cadinene	1524	0.731±0.04ab	0.748±0.06a	0.683±0.07cd	0.698±0.05bc	0.651±0.07d	0.612±0.05d	0.486±0.05e
52	α-Cadinene	1537	0.478±0.03bc	0.504±0.04b	0.461±0.06c	0.582±0.04a	0.376±0.03d	0.402±0.03d	0.150±0.01e
53	(E)-Nerolidol	1564	0.880±0.06c	0.990±0.07a	0.799±0.06d	0.944±0.07b	0.635±0.06e	0.659±0.05e	0.394±0.02f
54	Germacrene D-4-ol	1575	0.230±0.01ab	0.240±0.02a	0.215±0.04bc	0.223±0.01ab	0.197±0.02c	0.226±0.01ab	----------
55	Spathulenol	1577	0.195±0.01bc	0.228±0.01a	0.190±0.02c	0.215±0.02ab	0.182±0.02c	0.214±0.02ab	----------
56	n-Hexadecane	1599	0.516±0.04b	0.556±0.05a	0.485±0.04c	0.529±0.05b	0.494±0.04c	0.523±0.05b	----------
57	1,10-di-epi-Cubenol	1614	3.680±0.11a	3.566±0.10a	3.055±0.17c	3.510±0.22a	3.205±0.19bc	3.301±0.22b	2.65±0.20d
58	**epi-α-Cadinol**	**1642**	**28.737±1.22b**	**29.062±1.31a**	**26.620±1.55d**	**28.510±1.64b**	**27.402±1.70c**	**27.520±1.58c**	**24.679±1.78e**
59	β-Eudesmol	1649	1.399±0.07c	1.461±0.06b	1.391±0.08c	1.404±0.10bc	1.611±0.09a	1.663±0.09a	1.039±0.10d
60	α-Cadinol	1654	1.456±0.07c	1.508±0.06b	1.409±0.09c	1.421±0.09c	1.574±0.11a	1.588±0.09a	1.353±0.10d
61	β-Bisabolol	1685	0.536±0.04b	0.592±0.04a	0.419±0.05c	0.462±0.05c	0.544±0.04ab	0.543±0.03ab	0.248±0.01d

Data are mean ±standard deviation of eight replications. Means followed by the same letter within a row are not significantly different according to Duncan's multiple range test at $P \leq 0.05$.

Tab. 2: Effect of different zinc and salicylic acid sources on concentrations of phenolic compounds and flavonoids in extract of sweet basil.

Treatments	Gallic acid	Carvacrol	Kaempferol	Rosmarinc acid	Rutin	Catechin	Cinnamic acid	Ferulic acid	Luteolin	Quercetin
n[Zn(SA)$_2$] 0.1%	3.77±0.18b	2.41±0.10b	3.10±0.21b	5.29±0.23b	2.13±0.09bc	3.51±0.15b	3.71±0.17b	4.06±0.19b	0.91±0.06b	3.97±0.16b
n[Zn(SA)$_2$] 0.2%	4.37±0.20a	2.65±0.12a	3.81±0.17a	5.70±0.25a	2.74±0.11a	4.22±0.14a	4.04±0.16a	4.54±0.18a	1.13±0.09a	4.21±0.17a
Zn-EDTA 0.1%	3.20±0.17cd	2.16±0.12c	2.67±0.19d	4.85±0.21c	1.81±0.10d	3.48±0.11b	3.19±0.20d	4.10±0.16b	0.71±0.08b	3.68±0.19c
Zn-EDTA 0.2%	3.83±0.14b	2.38±0.14b	2.92±0.20c	5.10±0.19b	2.25±0.08b	4.05±0.13a	3.41±0.17c	3.43±0.16c	0.83±0.07b	3.97±0.16b
Salicylic acid 0.1%	3.03±0.18d	2.01±0.11c	2.82±0.16cd	2.88±0.20e	1.56±0.09e	3.50±0.13b	2.81±0.16e	2.11±0.13d	ND	3.97±0.16b
Salicylic acid 0.2%	3.44±0.16c	2.17±0.10c	3.12±0.18b	3.11±0.18d	1.99±0.08cd	3.66±0.12b	3.01±0.17de	2.21±0.14d	ND	4.14±0.17a
Control	2.22±0.12e	ND	1.54±0.10e	2.11±0.14f	ND	2.32±0.10c	1.92±0.09f	ND	ND	2.87±0.16d

ND: Not detected
*Calculated mean amount of the flavonoids and polyphenols (mg g^{-1} DM) based on the weight of the ground dry plant in eight replicates ± standard deviation. Means followed by the same letter within a column are not significantly different according to Duncan's multiple range test at $P \leq 0.05$.

Tab. 3: Radical scavenging activity of sweet basil's essential oils affected by different zinc and salicylic acid sources.

Properties	n[Zn(SA)$_2$] 0.1%	n[Zn(SA)$_2$] 0.2%	Zn-EDTA 0.1%	Zn-EDTA 0.2%	Salicylic acid 0.1%	Salicylic acid 0.2%	Control
Antioxidant (mg AAE g^{-1})[a]	21.44±1.5	22.94±1.7	16.30±1.4	18.78±1.6	15.08±1.3	16.71±1.4	8.82±1.2
IC_{50} for RNS scavenging (mg mL^{-1})[b]	1.59±0.1	1.47±0.1	2.62±0.2	2.44±0.3	2.19±0.3	1.94±0.1	5.12±0.5
IC_{50} for ROS scavenging (mg mL^{-1})[c]	1.24±0.3	1.03±0.2	1.43±0.3	1.31±0.4	1.76±0.4	1.59±0.3	3.56±0.7
IC_{50} for TBARS scavenging (mg mL^{-1})[d]	3.16±0.3	2.87±0.2	4.09±0.3	3.64±0.3	3.84±0.2	3.61±0.3	5.99±0.4
IC_{50} for H_2O_2 scavenging (mg mL^{-1})[c]	2.33±0.3	2.14±0.2	2.54±0.3	2.41±0.4	3.12±0.3	2.81±0.5	5.43±0.6

[a]Data are presented as milligrams ascorbic acid equivalents per gram of essential oil.
[b]IC_{50} is concentration of essential oil to scavenge RNS (reactive nitrogen species) by 50%.
[c]IC_{50} is concentration of essential oil required to scavenge ROS (reactive oxygen species) by 50%.
[d]IC_{50} is concentration of essential oil to scavenge TBARS (thiobarbituric acid reactive substances) by 50%.
[e]IC_{50} is concentration of essential oil to scavenge H_2O_2 by 50%.

Tab. 4: Antimicrobial activity of sweet basil's essential oils affected by different zinc and salicylic acid sources.

	MIC (mg mL^{-1})						
Species	n[Zn(SA)$_2$] 0.1%	n[Zn(SA)$_2$] 0.2%	Zn-EDTA 0.1%	Zn-EDTA 0.2%	Salicylic acid 0.1%	Salicylic acid 0.2%	Control
B. subtilis	0.053±0.004	0.031±0.003	0.111±0.009	0.095±0.008	0.127±0.011	0.116±0.011	0.149±0.013
S. aureus	0.011±0.002	0.007±0.0005	0.041±0.004	0.030±0.003	0.049±0.003	0.043±0.002	0.069±0.005
E. coli	0.298±0.021	0.229±0.018	0.499±0.025	0.419±0.024	0.536±0.030	0.521±0.031	0.787±0.047
S. typhimurium	0.261±0.015	0.180±0.011	0.453±0.027	0.410±0.025	0.514±0.037	0.488±0.035	0.801±0.052
A. niger	0.079±0.008	0.068±0.045	0.179±0.008	0.154±0.021	0.195±0.028	0.183±0.032	0.230±0.040
C. albicans	0.069±0.005	0.050±0.005	0.140±0.016	0.117±0.011	0.186±0.018	0.159±0.016	0.287±0.022

Values are means of MIC in eight replicates ± sd (standard deviation).
MIC: Minimal Inhibitory Concentration.

The application of zinc and plant growth regulator such as salicylic acid can affect the antimicrobial activity of the essential oils. Due to the complexity of the chemical structure of the essential oils, their volatility and their insolubility in water, investigating their antimicrobial activity is complicated. Hence, it is difficult to identify the molecular pathways involved in their actions. It is therefore assumed that each of the essential oil components has a separate mechanism for itself. In other words, because of the wide range of essential oil components, their antimicrobial properties do not depend on a single mechanism, but various mechanisms play a role at the molecular level in this regard.

One possible mechanism is to irreversibly damage the cell membrane of bacteria, which ultimately causes the leakage of the plasma membrane ions, cytoplasmic material, and energy substrates deficiency, such as glucose. This action will eventually lead to lysis of bacterial cells and death. Many researchers focus on the antimicrobial activity of essential oils and test some of the various components of essential oils to find possible synergistic effects (KALEMBA and KUNICKA, 2003; BAKKALI et al., 2008).

Our results showed that the application of 0.2% n[Zn(SA)$_2$] has a significant effect on *staphylococcus aureus* growth in comparison with other foodborne microbes. In other words, among the studied

microbes, this bacterium was the most sensitive to the sweet basil essential oil. *Staphylococcus aureus* threatens people's health through infectious diseases and food poisoning. In addition, it has the ability to simultaneously resist multiple antibiotics (Dallal et al., 2010; Normanno et al., 2007). The results of our study show that essential oil of basils that have been sprayed with Zn-salicylic acid nano-complexes have good antimicrobial properties and can therefore be used in the food and pharmaceutical industry.

The importance of the phenolic compounds, such as carvacrol, has been proved in improving the antimicrobial properties of essential oils. Previous studies have shown that carvacrol inhibits the growth of some bacteria, including *E. coli* and *Bacillus cereus* (Du et al., 2008). In another study by Karaman et al. (2001), the strong bacteriostatic effects of *Thymus revolatus* essential oil were shown against *Staphylococcus aureus* and *Escherichia coli*. They expressed cause of these effects the high amount of carvacrol in the essential oil. The mechanisms by which phenolic compounds create toxicity for microorganisms include surface absorption and cell membrane breakdown, interference in the cytoplasmic membrane and membrane proteins function, reaction with enzymes and reduction of metal ions (Negi, 2012). In general, it is difficult to compare the reported results about the antibacterial properties of essential oils. The reason for this is the difference in the methods used, their sources of preparation and bacterial strains (Basti et al., 2004).

Conclusion

Secondary metabolites in plants are of particular importance for food and medical purposes. Therefore, any factor that can increase these compounds without changes in the genetic structure of the plant are valuable. In this study, the application of Zn and salicylic acid nano-complexes improved growth characteristics, essential oil yield, antimicrobial and antioxidant activities of sweet basil essential oil. Improvement of the antimicrobial and antioxidant properties of sweet basil essential oil can increase its application in the food, medical, pharmaceutical, veterinary, cosmetic and sanitary industries. Application of nanofertilizers is more advantageous than conventional forms of fertilizers due to stable physical properties, small particle size, higher surface-area-to-volume ratio, high density and high reactivity. In addition, nanofertilizers can be considered as controlling agents for the release of fertilizers in order to produce intelligent nanofertilizers to overcome the technical constraints on the slow and controlled release of elements of nutrition.

Authors contributions

Study conception and design: Vahid Tavallali, Acquisition of data: Omid Espargham, Analysis and interpretation of data: Vahid Tavallali, Drafting of manuscript: Hossein Gholami, Critical revision: Vahid Tavallali.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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ORCID
Vahid Tavallali https://orcid.org/0000-0002-6819-2008

Address of the corresponding author:
Vahid Tavallali, Department of Agriculture, Payame Noor University (PNU), P.O. Box: 19395-3697 Tehran, Iran.
E-mail: vtavallali@gmail.com; v.tavalali@pnu.ac.ir