



The efficacy of solid and enriched biochars with magnesium and iron nanoparticles on growth and essential oil composition of German chamomile under salt stress

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Article Info

Article type:

Research article

Article history:

Received December 10, 2024

Revised January 5, 2025

Accepted January 29, 2025

Published online June 15, 2025

Keywords:

Essence,
Root mass,
Salinity,
Shoot mass

Abstract

Objective: This research was aimed to observe the effects of solid and enriched biochars with magnesium and iron nanoparticles on the growth and essential oil composition of German chamomile.

Methods: A factorial experiment based on a randomized complete block design with three replications was conducted to study the potential effects of solid biochar (25 g kg⁻¹ soil) and biochar-based nanoparticles (BNPs) of magnesium oxide (25 g BNP-MgO kg⁻¹ soil), iron oxide (25 g BNP-Fe₃O₄ kg⁻¹ soil), and their combined form (12.5 g BNP-MgO + 12.5 g BNP-Fe₃O₄ kg⁻¹ soil), on root and shoot masses and essential oil composition of chamomile under non-saline and saline (6 and 12 dSm⁻¹) conditions in a greenhouse at the University of Tabriz, Iran during 2021.

Results: Salinity caused a decrease in root and shoot masses and root/shoot ratio, but the application of biochar, especially BNPs, improved the root and shoot growth. The BNP-Fe₃O₄ under both salinity levels and BNP-MgO + BNP-Fe₃O₄ only under high salinity (12 dSm⁻¹) increased the root/shoot ratio. Enriched biochars also enhanced most of the essential constituents of chamomile flowers, compared to the solid biochar. The BNPs were superior treatments in reducing the adverse effects of salinity on plants. Forty-eight constituents were identified in the essential oil, some of which were only produced by BNPs and salinity treatments. Azoline, trans-β-farnesene, bisabolol oxide A, and bisabolone oxide were the major constituents of essential oil. Production of oleic and hexadecanoic acids was only induced by salt stress. However, the juniper camphor, E-citral, Z-citral, geranic acid, aromadendrene, and sesquibabinene were distinctly synthesized under enriched biochars.

Conclusion: The application of BNPs can boost the essence production of chamomile by enhancing plant growth and most of the essential oil constituents under various salinity levels.

Cite this article: Solhi-Khajehmarjan R, Ghassemi-Golezani K, Alizadeh Salteh S. 2025. The efficacy of solid and enriched biochars with magnesium and iron nanoparticles on growth and essential oil composition of German chamomile under salt stress. J Plant Physiol Breed. 15(1): 1-15.



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Introduction

Salinity imposes ionic and osmotic stresses on plants, thereby reducing their growth and productivity (Zhang *et al.* 2024). Soil salinization is a significant problem in soil degradation, for which organic matter such as biochar can be a potential solution. The pyrolysis of agricultural waste biomass produces biochar, which is considered a potential strategy for improving soil properties and mitigating environmental risks (Kung *et al.* 2015). Biochar can improve soil fertility and plant access to nitrogen, phosphorus, and potassium by increasing the accumulation of organic carbon (Khan *et al.* 2020). In comparison to traditional chemical fertilizers, enriched biochar may be more advantageous in the long term for preserving soil fertility due to its high cation exchange capacity, large surface area, and highly porous structure, which results in a high adsorption capacity (Rahimzadeh and Ghassemi-Golezani 2024). The porous structure of biochar provides an excellent habitat for soil microbes to grow and multiply, thereby enhancing soil health and plant performance (Yuan *et al.* 2023). The role of biochar in increasing plant tolerance against various stresses such as salinity by maintaining water potential at an ideal level, changing soil characteristics, reducing sodium uptake, and increasing potassium intake was previously reported for various crops (Ghassemi-Golezani *et al.* 2024).

Enriching biochar with essential nutrients such as magnesium and iron may improve its potential to overcome salt toxicity and improve plant performance. Magnesium (Mg) as an integral component of chlorophyll, enhances the growth and productivity of plants (Ahmed *et al.* 2023; Ghassemi-Golezani *et al.* 2023). In addition, Mg fortifies plants against environmental stressors by reinforcing cell walls and stimulating the synthesis of defense substances (Ayyaswamy 2023). Iron (Fe) is also essential for the growth of plants and other organisms. It is insoluble in neutral and alkaline soils, which affects the productivity and quality of crops. Iron is necessary for regulating several fundamental plant functions, including respiration, nitrogen fixation, chlorophyll synthesis, photosynthesis, assimilation, nucleotide synthesis and repair, metal homeostasis, and hormonal regulation (Ghassemi-Golezani *et al.* 2022; Bhatla and Kathpalia 2023).

The biochar-based nanoparticles (BNPs) combine the benefits of nanomaterials with the presence of many functional groups in pyrolyzed biochar (Ghassemi-Golezani *et al.* 2023). In addition, the BNPs are more effective due to their intrinsic large specific surface area, which results from the

properties of nanomaterials and biochar. The surface of biochar becomes more negative with oxygenated groups, which attracts more cations instead of anions. Therefore, enriched biochar with cations such as MgO and Fe₃O₄ creates more surface sites for element adsorption. According to Ghassemi-Golezani and Farhangi-Abriz (2022), the resulting nanocomposites often show a significant improvement in physicochemical characteristics such as number of pores, functional groups, and cation exchange capacity. These characteristics may improve the performance of medicinal plants such as chamomile under salt stress.

Increasing essential oil production of medicinal plants is a strategy to overcome oxidative stress under drought (Ghassemi-Golezani and Solhi-Khajemarjan 2021) and salt (Ghassemi-Golezani and Rahimzadeh 2022) stresses. The essential oil of German chamomile is used in traditional and industrial medicines. Galenic products of German chamomile have been used to treat mild skin diseases, spasms, and inflammation (Sepp *et al.* 2024). The major compounds of chamomile essential oil are terpenoids (28 types) and flavonoids (36 types) (Hazrati *et al.* 2020). The main constituents of its essential oil are bisabolone oxide, bisabolol oxide A, bisabolol oxide B, α -bisabolol, chamazulene, α -farnesene, β -farnesene, cis-trans-en-dicycloether, germacrene D, and bicyclogermacrene (Katsoulis *et al.* 2022). Today, consumers of medicinal plants show an increasing appetite for superior quality products, which makes it not only a scientific endeavor but also an economic necessity to explore the depth of the role of enriched biochars on essential oil production. Thus, this research was oriented to find out the potential effects of solid and enriched biochars with magnesium and iron nanoparticles on the growth and essential oil composition of German chamomile.

Materials and Methods

Preparation of enriched biochars

Pomegranate waste wood biochar and nano oxides (MgO and Fe₃O₄) were purchased from Shiraz Company and Sigma-Aldrich Chemicals Company (Missouri, USA), respectively. Biochar was mixed with magnesium oxide (MgO, 10 mM) and iron oxide (III) (Fe₃O₄, 10 mM) nanoparticles at a rate of 1 g of biochar in 5 ml of distilled water and kept for 24 hours at about 25 °C, and then it was dried in an oven at 80 °C for half an hour. The properties of biochar are listed in Table 1.

Table 1. Some chemical characteristics of the biochar used in this experiment.

pH	EC (dS m ⁻¹)	OM (%)	OC (%)	C/N	N (%)	P (%)	K (%)	Ca (%)	Ash (%)
8.2	0.2	13.89	8.06	10.20	6.79	6.14	0.17	4.26	24.6

EC: Electrical conductivity; OM: Organic matter; OC: Organic carbon; pH: Potential of hydrogen

Treatments

This experiment was conducted in a greenhouse at the Faculty of Agriculture, University of Tabriz, Iran in 2021 with an approximate photoperiod of 13 h, light intensity of 140 W m^{-2} , and day and night average temperature of 24°C as a factorial arrangement based on a randomized complete block design with three replications. The magnesium sulfate, calcium chloride, sodium sulfate, and sodium chloride salts in a ratio of 1:2:2:4 were used to provide saline solutions (6, and 12 dS m^{-1} as moderate and high salinities, respectively) (Askari-Khorasgani *et al.* 2017). Tap water was used for non-saline treatment. Biochar-based treatments were non-biochar, 25 g kg^{-1} solid biochar, enriched biochar with 25 g kg^{-1} nano magnesium oxide, enriched biochar with 25 g kg^{-1} nano-iron oxide, and combination of both ($12.5 \text{ g kg}^{-1} + 12.5 \text{ g kg}^{-1}$). Before starting the experiment, the soil was analyzed (Table 2), and then the pots ($23 \times 26 \text{ cm}$ with a capacity of 6 kg) were filled with silty loam soil mixed with solid and enriched biochars according to the treatments. German chamomile (*Matricaria chamomilla* L.) seeds were purchased from Pakan Bazr of Isfahan, Iran, and sown in 0.5 cm depth of soil at each pot. The germination percentage of these seeds was 99%. The pots were first irrigated with tap water (non-saline) and saline solutions to achieve 100% field capacity (FC), but the following irrigations of all pots were only carried up by tap water. The water loss in the pots was compensated by regular weighing of the four unsown pots (with non-biochar and biochar-related treatments). The emerged seedlings were thinned to keep eight plants per pot.

Table 2. Physical and chemical properties of the experimental soil.

Texture	pH	EC (dS m^{-1})	CEC (cMol Kg^{-1})	OM (mg Kg^{-1})	N (%)	P (mg Kg^{-1})	K (mg Kg^{-1})	Mg (mg Kg^{-1})	Fe (mg Kg^{-1})
Silty loam	6.5	1.8	19.3	15	0.05	20.3	169.5	45	5

EC: Electrical conductivity; CEC: Cation exchange capacity; OM: Organic matter

Root and shoot masses

Four plants were randomly removed from each pot at the flowering stage, and roots and shoots were separately dried in an oven at 75°C for 48 h and their masses were determined as g per plant. Then, root/shoot ratio was calculated.

Essential oil extraction

Flowers of four plants were removed and dried at about 25°C (Solhi-Khajehmarjan and Ghassemi-Golezani 2023). A sample of 5 g powdered flowers from each pot was used to extract the essential

oil by micro-Clevenger. Each sample was mixed with 250 ml of distilled water and boiled at 250 °C for 3 h. A hexane solution was used to extract the essential oil.

Essential oil composition

The essential oil constituents were determined by GC-MS method (Quadrupole mass spectrometer with Agilent 6890N model coupled with Agilent 5973 mass spectrometer). The Agilent with HP-5MS capillary column (length 30 m, inner diameter 0.25 mm, and resident layer thickness 0.25 µm) with the stationary phase of 5% methyl phenyl siloxane and ionization energy of 70 electron volts was used (Ghassemi-Golezani and Rahimzadeh 2022). The helium gas with 1 mL min⁻¹ flow rate was used as a carrier. The temperature of the device was initially started from 45 °C (held for 3 min) and then increased to 250 °C and then kept at this temperature for 10 minutes. The chemical components of the essential oil were determined by comparison of their mass spectra with those reported in the Wiley 5 library or with mass spectra from literature (Kobayashi and Nishizawa 2012).

Statistical analysis

The MSTAT-C software was applied for data analysis and Excel-2019 was used for drawing figures. Comparison of means for different traits was carried out by Tukey's test at $p \leq 0.05$.

Results

Analysis of the data indicated that salinity and biochar treatments had a significant effect on root mass, shoot mass, and root/shoot ratio (R/S). The salinity \times biochar interaction was significant for shoot mass and R/S ratio, but not for root mass (Table 3).

Table 3. Analysis of variance of root mass, shoot mass, and root/shoot ratio in chamomile affected by salinity, and solid and enriched biochars.

Source of variation	df	Mean squares		
		Root mass	Shoot mass	Root/shoot
Replication	2	0.0001	0.15	0.00001
Biochar	4	0.01**	11.49**	0.001**
Salinity	2	0.002**	1.47**	0.001**
Biochar \times Salinity	8	0.0001	0.12**	0.0001**
Error	28	0.00001	0.02	0.00003
CV (%)		5.31	4.72	8.61

CV: Coefficient of variation; df: Degrees of freedom; **: Significant at $p \leq 0.01$.

Root mass

Increasing salinity significantly decreased root mass under both moderate and high salinities. The increment in root mass by solid biochar was not significant, compared to the non-biochar treatment. However, the application of enriched biochars increased root mass (Figure 1). The lack of significant interaction between salinity and biochar-related treatments indicates that improving root mass by enriched biochars was similar under all salinity levels. BNP-MgO + BNP- Fe₃O₄ treatment increased the root mass by 19% compared to the non-saline condition.

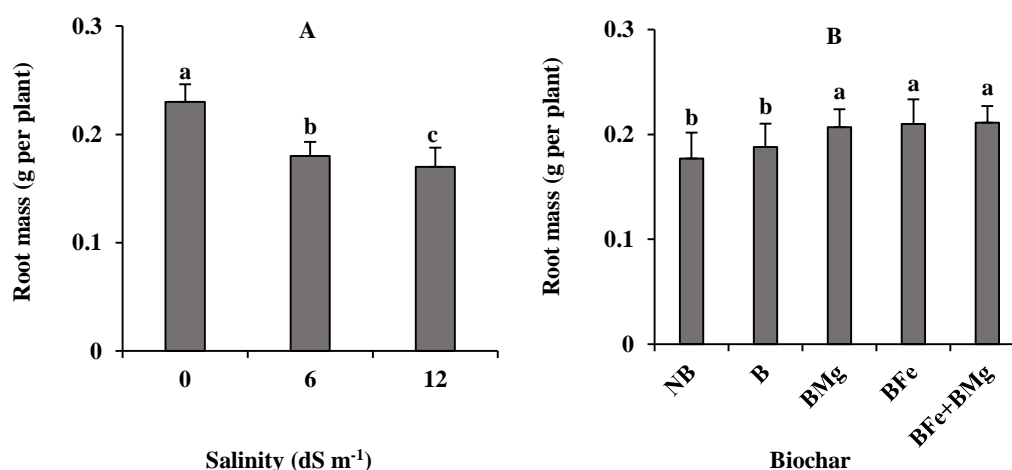


Figure 1. Variation of root mass of chamomile in response to salinity (A) and solid and enriched biochars (B); Means with different letters indicate significant difference at $p \leq 0.05$ (Tukey's test); NB: No biochar; B: biochar.

Shoot mass

Shoot mass was generally decreased with increasing salinity in BNPs treated and untreated pots. The application of solid and especially enriched biochars increased the shoot mass of chamomile under non-saline and all saline conditions. The improvement in shoot mass due to enriched biochars was more pronounced under non-saline treatment, compared to salinity treatments. However, there was no significant difference between solid biochar and non-biochar treatment in shoot mass under all salinity levels (Figure 2). Nanocomposite treatments increased shoot mass by 43% and 39% in non-saline and high-salinity conditions, respectively.

Root/shoot ratio

The increase in salt toxicity caused a decrease in the R/S ratio, which was more evident in high salinity. The BNP-Fe₃O₄ and combined form of enriched biochar (BNP-MgO + BNP- Fe₃O₄) were the superior treatments for improving the root/shoot ratio of chamomile under high salinity. The BNP-

Fe_3O_4 was also the best treatment for enhancing root/shoot ratio under non-saline and moderate salinity conditions (Figure 3).

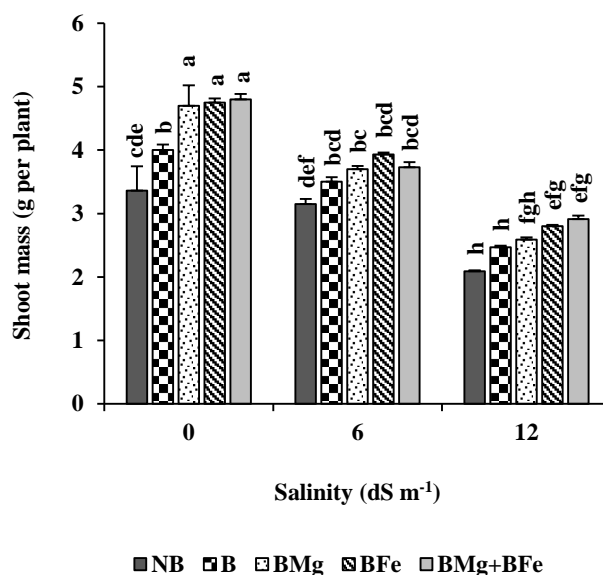


Figure 2. Changes in shoot mass of chamomile in response to salinity and enriched biochars; Different letters indicate significant difference at $p \leq 0.05$ (Tukey's test); NB: No biochar; B: biochar.

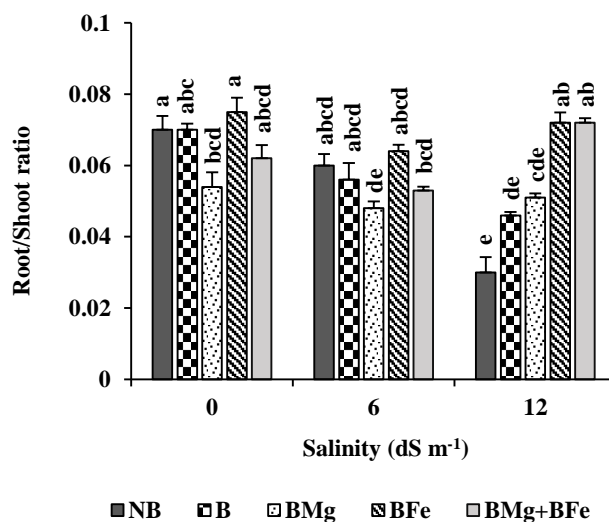


Figure 3. Changes in root/shoot ratio of chamomile in response to salinity and solid and enriched biochars; Different letters indicate significant difference at $p \leq 0.05$ (Tukey's test); NB: No biochar; B: biochar.

Essential oil composition

About 48 compounds were identified in the essential oil of chamomile flowers. The main components are listed in Table 4. The main ingredients were azulene (chamazulene) ranging from 0.12 to 11.88%, trans- β -farnesene (known as (E)- β -farnesene) in the range of 2.84% to 35.99%, bisabolol oxide A with a range 7.19 to 55.22%, and bisabolone oxide from 1.18 to 16.42%. The content of chamomile

Elemol	-	-	-	-	-	-	-	-	-	-	-	-	0.68	-	-
α Bisabolene	-	-	-	-	-	-	-	0.30	-	-	-	-	2.84	-	-
Octadecadienoic acid	-	-	-	-	-	-	-	-	0.49	-	-	-	0.57	-	-
DI-Limonene	0.83	-	-	-	-	-	0.21	-	0.18	-	-	-	-	-	-
Decanol	-	-	-	-	-	-	-	-	0.21	-	-	-	-	-	0.63
Cis-dihydrocarvone	-	-	-	-	-	-	0.32	-	0.16	-	-	-	-	-	-
Naphthalene	0.34	-	-	-	-	-	0.22	9.54	1.05	-	2.17	2.33	-	0.31	-
Z-citral	-	-	-	-	-	-	0.21	4.36	2.42	-	-	-	-	-	-
E-citral	-	-	-	-	-	-	1.60	3.70	2.74	-	-	-	-	-	-
Geranic acid	-	-	-	-	-	-	-	4.56	0.14	-	-	-	-	-	-
Bi cyclo	0.57	-	-	-	-	-	0.55	-	0.22	-	-	-	-	-	-
Isospathulenol	-	-	-	-	-	-	0.57	-	0.36	-	-	-	-	-	-
Amino salicylic acid	-	-	-	-	-	-	-	-	0.80	-	-	-	-	-	-
Farnesol	-	-	-	-	-	-	-	1.62	1	-	-	-	-	-	1.66
Farnesyl acetate	-	-	-	-	-	-	-	0.99	1.2	-	-	-	-	-	-
β citronellol	-	-	-	-	-	-	-	0.66	0.30	-	0.24	-	-	-	-
Geranyl linalool	-	-	-	-	-	-	-	-	0.72	-	-	-	-	-	-
Heptanoic acid	-	-	-	-	-	-	-	0.49	-	-	-	-	-	-	-
Calarene	-	-	-	-	-	-	-	0.69	-	-	-	0.25	-	-	0.72
Junipercamphor	-	-	-	-	-	-	-	1.01	-	-	-	-	-	-	-
Sesquisabinene hydrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.29

BNP: Biochar enriched nanoparticles; B₀, B₁, B₂, B₃, B₄: Non-biochar, biochar, BNP-MgO, BNP-Fe₃O₄, and BNP-MgO+Fe₃O₄, respectively; S₀, S₁, S₂: Non saline, moderate, and high salinities, respectively.

Discussion

Chamomile root and shoot growth reduction under salinity (Figures 1 and 2) could be related to the toxic and osmotic impacts of this stress (Ghassemi-Golezani and Farhangi-Abriz 2022). Salt stress can damage roots and inhibit cell and plant growth by limiting the ability of plants to absorb nutrients and water (Kamran *et al.* 2020). High sodium ions in soil compete with potassium absorption by roots disrupt the integrity of the cell membrane, and change the selectivity of ions (Isayenkov and Maathuis 2019). High Na and low K contents of plant tissues have negative impacts on root and shoot growth (Figures 1 and 2), due to oxidative damage, stomatal closure, thylakoid membrane damage, and ionic and osmotic stresses (Yuan *et al.* 2023). Reduction of root growth and the ratio of R/S is a suitable strategy for plants under non-enriched biochar (Figures 1 and 3) to prevent excess sodium absorption.

The changes observed in the composition of chamomile flower essential oil in saline conditions (Table 4) can be related to spending more energy to reduce oxidative stress induced by salinity (Ghassemi-Golezani and Rahimzadeh 2022). The content of bisabolol increases at the flowering stage due to a decrease in dicyclopentadiene content (Omran *et al.* 2023). The production of more oxygenated compounds, for example, α -bisabolone oxide, α -bisabolol oxide A, and α -bisabolol oxide B (Table

4) may be attributed to the oxidative effects of salinity on plants, which is also supported by a previous report on chamomile plants (El Mihaoui *et al.* 2022). Production of oleic and hexadecanoic acids only under high salinity without biochar treatments (Table 4) helps to safeguard the photosynthetic system of plants under saline conditions (Wang *et al.* 2024). Oleic acid accumulation in response to saline conditions is probably caused by either a shorter half-life or total inhibition of oleate desaturase. This is the main enzyme responsible for adding the double bond to oleic acid at position 12 (Zhou *et al.* 2023).

The enriched biochars facilitate root and shoot growth (Figures 1, 2, and 3) by decreasing the absorption of Na and Cl and improving the nutrient status of plants (Ghassemi-Golezani and Rahimzadeh 2024). In addition, the presence of different nutrients such as magnesium (Mg), calcium (Ca), and phosphorus (P) on the surface of BNP increases the cation exchange and water-holding capacity in the soil (Ghassemi-Golezani and Farhangi-Abriz 2022; Yang *et al.* 2024). Solid and enriched biochars with nanoparticles protect plants against reactive oxygen species (H_2O_2), membrane damage, and chlorophyll degradation (Hasnain *et al.* 2023), which may be the reason for balanced growth of plants, leading to induction and increment of most of the essential oil compounds in chamomile flowers.

Essential oils are made of components belonging to families of terpenes and phenylpropanoids, such as monoterpenes and sesquiterpenes, which are the primary constituents that generally dictate the biological qualities of the essential oil (Bhatla and Kathpalia 2023). The biosynthesis of terpenoids (monoterpenes and sesquiterpenes) occurs as the main defense compounds of the plants from the methyl erythritol phosphate (MEP) and mevalonic acid (MVA) pathways (Sinha *et al.* 2024). Monoterpenes such as citronella, E-citral, Z-citral, and geranic acid are synthesized through the MEP pathway in plastids, and sesquiterpenes such as juniper camphor, aromadendrene, and sesquisabinene (Table 4) are produced through the MVA pathway in the cytosol. The activities of enzymes involved in these pathways lead to changes in the ratio of essential oil composition and the production of new compounds. The activity of the geranyl diphosphate synthesis enzyme was increased by Mg content, which is effective in the synthesis of secondary metabolites. Monoterpene synthase enzymes require a +2-metal ion (usually Mg or Mn) as a cofactor for catalysis (Farzadfar *et al.* 2017). Mg and Mn (or Fe) are the two main cofactors of terpene synthase enzymes. Sesquiterpene synthases prefer Mg, but monoterpene synthases are less selective in their divalent cation requirements. Therefore, changes in the balance between Mg and Mn can activate different groups of terpene synthases, which in turn can change the composition of terpenes (Ruan *et al.* 2016). Iron plays an important role in the metabolism of plants, such as the activity of catalytic enzymes and enzymes of the photorespiratory pathway and

glycolate (Rout and Sahoo 2015). Since monoterpenes accumulate in chloroplasts, CO₂ stabilization, and photosynthesis are closely related to the accumulation of monoterpenes in the plant. The essential oil of medicinal plants is influenced by plant nutrition as an environmental factor. The production of monoterpenes starts with CO₂ and glucose. Terpenoid synthesis uses carbohydrates as a source of energy and reducing power. The accumulation of essential oils is intimately related to CO₂ fixation, primary metabolite concentration, and sucrose metabolism (Emami Bistgani *et al.* 2024). The magnesium and iron-enriched biochars increase photosynthesis by improving chlorophyll content, thereby inducing the production of the most common and specific compounds of essential oil (Table 4). Increasing most of the essential oil constituents and decreasing some of those under biochar-related treatments can be related to the regulation and activity of some proteins, enzymes, and expression of related genes (Ghassemi-Golezani and Rahimzadeh 2022).

Conclusion

Salt osmotic effect reduced root and shoot masses and root/shoot ratio in chamomilla plants. However, biochar and especially the enriched biochars with MgO and Fe₃O₄ nanoparticles improved root and shoot growth under both levels of salinity. The enriched biochars with Fe₃O₄ and MgO + Fe₃O₄ nanoparticles were the best treatments for improving plant performance under salt stress. Forty-eight constituents were identified in the essential oil of chamomile, some of which were only produced under enriched biochars such as juniper camphor, E-citral, Z-citral, geranic acid, aromadendrene, and sesquisabinene. More research is required to upgrade our knowledge of mechanistic responses of medicinal plants to enriched biochars under different environmental conditions.

Acknowledgments

We appreciate the financial support of this research by the University of Tabriz.

Conflict of Interest

The authors declare that they have no conflict of interest.

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