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Optimization of Growth and Biochemical Production in *Dracocephalum moldavica* L. through Biochar and Salicylic Acid Application in a Pot Experiment

Sudabeh Mafakheri[1*] and Behvar Asghari [1, 2]

[1] *Department of Horticultural science engineering, Faculty of Agriculture and Natural Resources, Imam Khomeini International University, Qazvin Iran*

2 Department of Chemistry, Faculty of Basic Sciences, Azarbaijan Shahid Madani University, Tabriz, Iran

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***Corresponding author**
Mafakheri@ikiu.ac.ir;
smafakheri@gmail.com

ABSTRACT

This study examines the impact of biochar and salicylic acid on the morphological and biochemical characteristics of Moldavian balm (*Dracocephalum moldavica* L). A pot experiment was conducted usig a factorial experiment basd on CRD with two factors: salicylic acid applied as a foliar spray at concentrations of 0, 0.5, and 1 mM, and biochar incorporated into the soil at 0, 50, and 100 g/kg in 3 replication at scientific greenhouse at Imam Khomeini International University. The findings reveal that salicylic acid significantly promotes plant growth, with notable increases in height, branching, and biomass, and boosts chlorophyll a and b, essential oil percentage, carotenoids, total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical scavenging activity, especially at the 1 mM concentration. Biochar also positively influences plant height, branching, and biomass, and increases chlorophyll, essential oil percentage, carotenoid content, and TFC, although it has no significant effect on TPC or essential oil composition. The combined application of biochar and salicylic acid produces a synergistic effect, with the highest treatment combinations (1 mM & 100 g/kg) yielding the most significant improvements. Essential oil analysis indicates that salicylic acid markedly enhanced main components such as geraniol, geranial, geranyl acetate, and borneol, while biochar's influence on these compounds is minimal. The interaction between biochar and salicylic acid is particularly effective in boosting chlorophyll content and essential oil composition. These results underscore the potential of integrating biochar and salicylic acid to optimize plant growth and secondary metabolite production in Moldavian balm.

Keywords: Essential oil, Flavonoid, Geranial, Leaf nutrition, Phenolic compounds

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INTRODUCTION

Dracocephalum moldavica, commonly known as Moldavian balm or dragonhead, is an aromatic and medicinal herb from the Lamiaceae family, renowned for its therapeutic applications and fragrant essential oils. Native to Central Asia, *D. moldavica* has been extensively used in traditional medicine, particularly for its anti-inflammatory, antimicrobial, and antioxidant properties [1, 2]. The essential oil of *D. moldavica* contains bioactive compounds such as geraniol, geranial, and borneol, which contribute to its medicinal and aromatic value [3]. Given the increasing demand for natural products in the pharmaceutical, cosmetic, and food industries, enhancing the cultivation and phytochemical profile of *D. moldavica* has become a subject of significant research interest. Due to its popularity and economic potential, large-scale cultivation of *D. moldavica* has become prominent in Iran, with more than 300 hectares dedicated to its growth in the West Azarbayjan province alone.

In recent years, sustainable agricultural practices have gained prominence in the cultivation of medicinal plants, with a focus on improving both the quantitative and qualitative aspects of plant production. Among these practices, the application of biochar and salicylic acid (SA) has emerged as a potential strategy for enhancing plant growth, resilience, and secondary metabolite production.

Biochar, a carbon-rich byproduct of pyrolysis, is derived from the thermal decomposition of organic materials such as crop residues, wood chips, or manure under oxygen-limited conditions [4]. The application of biochar to soil has been shown to improve soil structure, increase water retention, and enhance nutrient availability [5]. These improvements in soil properties can have a direct impact on plant growth parameters, including plant height, biomass accumulation, and the number of branches per plant, which are crucial for maximizing yield in medicinal plants [6]. Moreover, biochar has been reported to influence the soil microbial community, promoting beneficial microbes that can enhance nutrient uptake and plant health [7]. The influence of biochar on plant growth is also linked to its ability to mitigate environmental stresses. For instance, biochar can reduce soil salinity, improve soil pH, and sequester carbon, thereby creating a more favorable environment for plant growth [4]. In medicinal plants like *D. moldavica*, these effects can be particularly beneficial, as the quality and quantity of secondary metabolites are often sensitive to environmental conditions [8]. By improving the overall health and growth of the plant, biochar application can indirectly contribute to higher concentrations of valuable phytochemicals, such as chlorophylls, carotenoids, phenolic compounds, and essential oils.

Salicylic acid (SA), on the other hand, is a naturally occurring phenolic compound that functions as a vital plant hormone, playing a key role in regulating plant responses to both abiotic and biotic stresses [9]. SA is involved in various physiological processes, including photosynthesis, transpiration, ion uptake, and transport, and it plays a critical role in the systemic acquired resistance (SAR) mechanism, which enhances the plant's defense against pathogens [10, 11]. The exogenous application of SA, particularly through foliar sprays, has been widely studied for its ability to enhance plant growth, yield, and secondary metabolite production under various stress conditions [12].

In *D. moldavica*, the foliar application of SA has the potential to modulate several key traits, including plant height, fresh and dry biomass, number of branches, and number of inflorescences per plant. Furthermore, SA has been shown to influence the biosynthesis of chlorophylls (a and b) and carotenoids, which are essential for the photosynthetic efficiency of the plant [13]. Additionally, SA can enhance the accumulation of secondary metabolites such as essential oil, phenolic compounds and flavonoids, which are known for their antioxidant properties and play a significant role in the plant's defense mechanisms [11]. The interaction between biochar and salicylic acid in enhancing plant growth and secondary metabolite production is an area of growing interest. Studies have suggested that biochar can serve as a reservoir for plant nutrients and bioactive compounds, including SA, thereby prolonging their availability and effectiveness [14]. This synergy between biochar and SA could lead to more sustained and pronounced improvements in both morphological and phytochemical traits in *D. moldavica*. For instance, Zhang et al. (2019) demonstrated that the combined application of biochar and SA significantly improved the growth and essential oil content of *Lavandula angustifolia*, another member of the Lamiaceae family [8], suggesting that similar benefits could be expected in *D. moldavica*.

The current study aims to investigate the effects of different levels of biochar application and salicylic acid foliar application on the morphological and phytochemical traits of *D. moldavica*. Understanding how these treatments influence the growth and chemical composition of *D. moldavica* will not only contribute to the optimization of its cultivation but also provide insights into sustainable agricultural practices that can be applied to other medicinal plants.

MATERIAL AND METHODS

Plant Material, Growth Conditions and Research Methods

This study was conducted in 2024 at the research greenhouse of the Faculty of Agriculture and Natural Resources, Imam Khomeini International University, Qazvin, Iran. Seeds of *D. moldavica* L., obtained from Pakan Bazr Company, Isfahan, Iran, were sown at a depth of 2 cm in 27 pots filled with a standard potting mix and evenly distributed across five sections. The plants were cultivated under controlled greenhouse conditions, with a temperature of 25 ± 2 °C, relative humidity between 60-70%, and a natural daylight photoperiod. The soil used was loamy-sand in texture, with a pH of 7.1, organic matter content of 1.7%, total nitrogen of 0.11%, available phosphorus at 16 mg/kg, and available potassium at 175 mg/kg.

A factorial experiment with three replicates was conducted, examining two main factors: biochar application and salicylic acid (SA) foliar spray. Biochar was applied at three levels: 0 g/kg (control, B_0), 50 g/kg (B_1), and 100 g/kg (B_2). The biochar used in this study was sourced from Fasle Panjom Knowledge-Based Company, Shiraz, Iran and was thoroughly mixed with the soil before potting.

Salicylic acid was applied as a foliar spray at three concentrations: 0 mM (S_0), 0.5 mM (S_1), and 1 mM (S_2). The first SA application was performed at the 4-5 leaf stage, followed by two additional applications at two-week intervals. The SA solution was prepared by dissolving the appropriate amount of salicylic acid in distilled water, with 0.01% Tween 20 added as a surfactant to ensure even distribution. Foliar sprays were administered in the early morning to reduce rapid evaporation, ensuring complete coverage by spraying until runoff. The control plants were sprayed with distilled water.

Morphological Measurements

At the full bloom stage, plants were harvested, and various morphological parameters were recorded. Plant height was measured from the base of the stem to the tip of the tallest point using a ruler. The fresh weight of the harvested plants was recorded before transferring them to a drying room for desiccation at room temperature in complete shade. Dry weight was then determined and documented. The total number of lateral branches emerging from the main stem was also counted.

Photosynthetic Pigments

Prior to harvest, samples were collected from the aerial parts of the plants, and 0.25 g of young leaves were extracted in 10 ml of 80% acetone. The concentrations of chlorophyll a and b were determined by measuring absorbance at 663 and 645 nm wavelengths, respectively, using a UNICO 2100 spectrophotometer. The concentrations were calculated in milligrams per gram of fresh leaf weight following the formulas provided by Wellburn [15]:

- Chlorophyll a = $(19.3 \times A663 - 0.86 \times A645)\ V / 100W$
- Chlorophyll b = $(19.3 \times A645 - 3.6 \times A663)\ V / 100W$

where V is the volume of the supernatant obtained from centrifugation, A is the absorbance at 663 and 645 nm wavelengths, and W is the sample weight in grams.

Total Phenolic Content Assay (TPC)

The total phenolic content of the plant samples was measured using the Folin-Ciocalteu method [16]. A 10% Folin reagent solution was prepared, and 100 µl of this solution was mixed with 20 µl of plant extract. After a 10-minute incubation in darkness, 80 µl of 5.7% w/v sodium carbonate solution was added. The absorbance was measured at 760 nm. Gallic acid was used as the standard, and phenolic content was expressed as milligrams of gallic acid equivalent per gram of extract.

Total Flavonoid Content Assay (TFC)

The total flavonoid content in the plant samples was determined using an aluminum chloride reagent. Ten microliters of 5% w/v aluminum chloride solution were mixed with 20 µl of plant extract. The mixture was diluted with 60 µl of methanol, and the final reaction volume was adjusted to 200 µl by adding 10 µl of 0.5 M potassium acetate solution and distilled water. After a 30-minute incubation, absorbance was measured at 415 nm. Quercetin was used as the reference standard, and results were expressed as milligrams of quercetin equivalent per gram of dry extract [17].

DPPH Radical Scavenging Activity Assay

The antioxidant activity of plant extracts was assessed using the DPPH assay. Twenty microliters of plant extracts at known concentrations were mixed with 180 µl of 1.0 mM DPPH solution.

After a 30-minute incubation, absorbance was measured at 517 nm, and the results were expressed as the percentage of DPPH radical inhibition.

Essential Oil Analysis

Essential oil was extracted from dried leaf samples via hydrodistillation using a Clevenger apparatus for 3 hours. The extracted oil was dried over anhydrous sodium sulfate and stored in sealed vials at 4 °C until analysis. The composition of the essential oil was determined using gas chromatography-mass spectrometry (GC-MS), focusing on major constituents such as geraniol, geranial, geranyl acetate, and borneol. GC/MS analyses were carried out using a system equipped with DB-5 fused silica column (30 m × 0.25 mm i.d.) on a Varian 3400 GC/MS system. Oven temperature was 50°C increasing to 280 °C at a rate of 10°C, transfer line temperature 260 °C. The carrier gas was helium with a linear velocity of 32 cm3s-1, split ratio 1:60, Ionization energy 70 eV, scan time1 s and mass range of 40-300 amu. The percentages of compounds were calculated by the area normalization method, without considering response factors. The constituents of the essential oils were identified by matching of their mass spectra with those of a computer library or with authentic compounds, and confirmed by comparison of their retention indices either with those of valid compounds or with data published in the literature.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) with SPSS software (version 26.0). Differences between treatment means were assessed using Duncan's multiple range test at a 5% significance level. Correlation analysis was also performed to evaluate the relationships between morphological and phytochemical traits.

RESULTS

The application of biochar and salicylic acid (SA) had significant effects on the growth, physiological, and biochemical traits of *D. moldavica* L. (Tables 1 and 2).

Table 1 Variance analysis of effects of Biochar and Salicylic acid application on morphological and biochemical traits of *D. moldavica*

Source	df	M.S.						
		Plant Height	Number of Branches/Plant	Fresh weight	Dry weight	Ch a	Cl b	Carotenoid
Biochar (B)	2	122.324 **	18.810 **	34.437 **	2.928 **	25.792 **	6.218 **	4.694 *
Salicylic acid (S)	2	141.415 **	9.898 **	43.570 **	3.923 **	75.859 **	7.083 **	10.912 **
B ×S	4	9.730 *	1.407 ns	5.063 **	0.349 **	7.980 **	0.538 ns	0.812 ns
Error	18	3.120	1.534	0.812	0.069	0.800	1.047	1.125
Total	26							
C.V.		19.42	8.12	10.01	13.00	11.52	5.98	5.67

*, **, ns: Significantly difference at the 5 and 1 of probability level\, and non-significantly difference, respectively.
B: Biochar; S: Salicylic acid.

Table 2 Variance analysis of effects of Biochar and Salicylic acid application on biochemical traits of *D. moldavica*

Source	df	M.S							
		Phenol	Flavonoid	DPPH	Essential oil %	Geraniol	Geranial	Geranyl acetate	Borneol
Biochar (B)	2	0.897 ns	25.104 **	27.132 **	0.44 **	1.959 ns	0.487 ns	4.507 ns	1.229 ns
Salicylic acid (S)	2	854.629 **	128.464 **	593.620 **	0.010 **	11.255 **	23.598 **	13.676 *	10.707 **
B ×S	4	0.627 ns	2.221 ns	2.189 ns	0.001 ns	5.741 *	2.483 ns	8.211 *	1.473 *
Error	18	7.723	3.024	1.676	0.0001	1.676	1.706	2.484	0.483
Total	26								
C.V.		26.37	13.69	21.78	14.2	6.90	7.16	9.60	5.18

*, **, ns: Significantly difference at the 5 and 1 of probability level\, and non-significantly difference, respectively.
B: Biochar; S: Salicylic acid.

Plant Growth Parameters

The application of salicylic acid (SA) significantly enhanced plant growth parameters in *D. moldavica* L. Increasing the SA concentration from 0 mM (S_0) to 1 mM (S_2) led to a marked improvement in plant height. Plants treated with S_2 reached an average height of 46.84 cm, representing a 20% increase compared to the control (S_0 = 38.93 cm), while those treated with S_1 (0.5 mM) achieved a height of 43.30 cm, an 11.22% increase (Table 3). The number of branches per plant also increased with SA treatment. S_2-treated plants exhibited an average of 16.22 branches, reflecting a 14.89% increase over the control (S_0 = 14.12), while S1-treated plants showed an 8.00% increase with 15.25 branches per plant. Fresh biomass was significantly enhanced in S_2-treated plants, reaching 22.85 g, which was 23.23% higher than the control (S_0 = 18.54 g). S_1 treatment resulted in a fresh weight of 21.46 g, indicating a 15.76% increase. Similarly, the dry weight was highest in S_2-treated plants (6.04 g), representing a 27.63% increase over the control (S_0 = 4.74 g), while S1 treatment led to an 18.07% increase in dry weight (Table 3).

Biochar application also had a significant positive impact on plant growth, branching, and biomass production. The highest biochar concentration (B_2) produced the tallest plants, with an average height of 46.72 cm, an 18.75% increase compared to the control (B_0 = 39.34 cm). Plants treated with B_1 (50 g/kg) reached a height of 43.02 cm, showing a 9.36% increase. The number of branches per plant also increased with biochar treatment, with B_2-treated plants averaging 16.44 branches, a 20.76% increase over the control (B_0 = 13.61). Fresh and dry biomass were notably higher in B_2-treated plants, with fresh weight reaching 23 g and dry weight 6.06 g, representing increases of 20.34% and 27.85%, respectively, over the control (B_0 = 19.11 g fresh weight, 4.74 g dry weight). B_1 treatment resulted in increases of 8.55% in fresh weight and 18.10% in dry weight compared to the control (Table 3).

The combination of biochar and salicylic acid had a profound impact on plant growth. The tallest plants were observed in the

B_2S_2 and B_1S_2 treatments, reaching heights of 49.37 cm and 47.51 cm, respectively. In contrast, the shortest plants were recorded in the control treatment (B_0S_0), with a height of 33.39 cm (Fig. 1). Fresh weight followed a similar trend, with the highest values observed in B_2S_2 (24.10 g), B_1S_2 (22.90 g), and B_2S_1 (22.71 g) treatments. The lowest fresh weight was seen in the B_0S_0 treatment (15.33 g), further emphasizing the importance of these amendments (Fig. 2). Dry weight was also highest in the B_2S_2 treatment (6.47 g) and the lowest dry weight was recorded in the B_0S_0 treatment (3.85 g), highlighting the limited growth potential without these amendments (Fig. 3).

Table 3 Mean comparison of simple effects of salicylic acid foliar application on morpho-physiological traits of *D. moldavica*.

Treatment	Plant Height (cm)	Number of Branches Per Plant	Fresh weigh Per Plant (g)	Dry weight per/Plant (g)	Ch a (mg/gFW)	Cl b (mg/gFW)	Carotenoid (mg/gFW)
Control	38.93 c	14.12 b	18.54 c	4.74 c	18.59 c	12.83 b	8.30 b
SA 0.5 mM	43.30 b	15.25 ab	21.46 b	5.60 b	23.01 b	14.40 a	9.27 b
SA 1 mM	46.84 a	16.22 a	22.85 a	6.04 a	24.06 a	14.34 a	10.49 a
Control	39.34 c	13.61 b	19.11 c	4.93 c	20.24 c	12.90 b	8.65 b
Biochar (50 g/kg)	43.02 b	15.53 a	20.74 b	5.38 b	21.80 b	14.27 a	9.33 ab
Biochar (100 g/kg)	46.72 a	16.44 a	23.00 a	6.06 a	23.62 a	14.41 a	10.09 a

Common letters within each factor indicate the absence of a significant difference at a 5% probability level, as per the Duncan test.
S_0, S_1, and S_2: Salicylic acid foliar application with 0, 0.5 and 1 mM. respectively. B_0, B_1 and B_2: Biochar application with 0, 50 and 100 g/kg soil respectively.

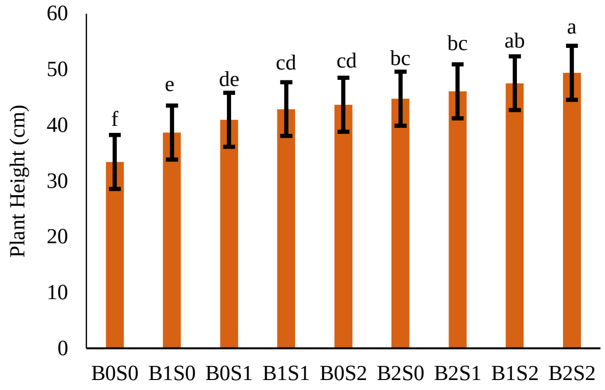

Fig. 1 Interaction effects of Biochar and Salicylic foliar application on plant height. S_0, S_1, and S_2: Salicylic acid foliar application with 0, 0.5 and 1 mM. respectively. B_0, B_1 and B_2: Biochar application with 0, 50 and 100 g/kg soil respectively

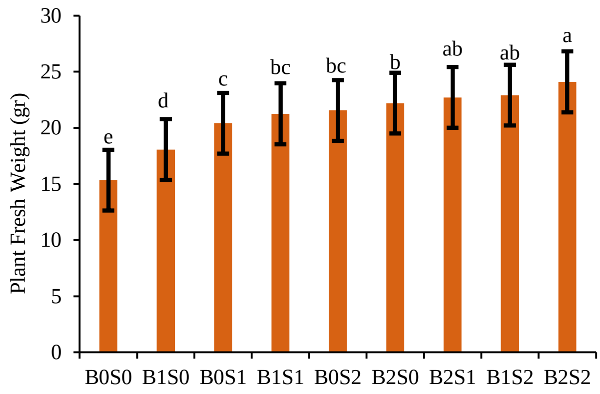

Fig. 2 Interaction effects of Biochar and Salicylic foliar application on plant fresh weight. S_0, S_1, and S_2: Salicylic acid foliar application with 0, 0.5 and 1 mM. respectively. B_0, B_1 and B_2: Biochar application with 0, 50 and 100 g/kg soil respectively

Photosynthetic Pigments

Both biochar and salicylic acid (SA) significantly influenced chlorophyll and carotenoid content in *D. moldavica* L. The highest chlorophyll a (Chl a) content was observed in plants treated with the highest biochar concentration (B_2), reaching 23.62 mg/g FW, which represents a 17% increase compared to the control (B_0 = 20.24 mg/g FW). Plants treated with B_1 (21.80 mg/g FW) showed a 7.69% increase. Chlorophyll b (Chl b) content followed a similar trend, with B_2-treated plants recording 14.41 mg/g FW, an 11.70% increase over the control (B_0 = 12.90 mg/g FW), and B_1-treated plants showing a 10.62% increase (14.27 mg/g FW). Carotenoid content was also maximized in B_2-treated plants (10.09 mg/g FW), marking a 16.67% increase over the control (B_0 = 8.65 mg/g FW), followed by B_1 (9.33 mg/g FW), a 7.87% increase (Table 3).

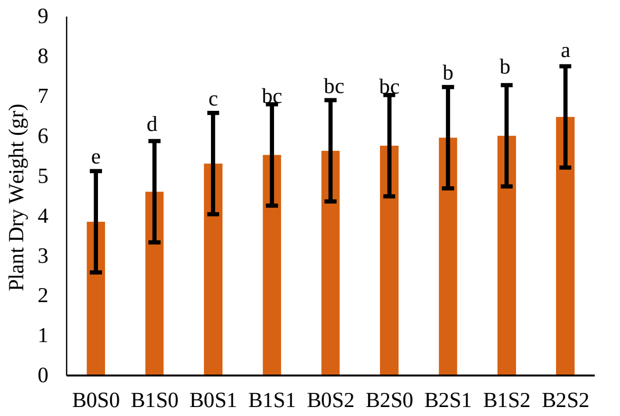

Fig. 3 Interaction effects of Biochar and Salicylic foliar application on plant dry weight. S_0, S_1, and S_2: Salicylic acid foliar application with 0, 0.5 and 1 mM. respectively. B_0, B_1 and B_2: Biochar application with 0, 50 and 100 g/kg soil respectively

Salicylic acid application also led to significant enhancements in chlorophyll and carotenoid content. Plants treated with 1 mM SA (S_2) exhibited the highest Chl a content (24.06 mg/g FW), representing a 29.46% increase compared to the control (S_0 = 18.59 mg/g FW). Plants treated with 0.5 mM SA (S_1) showed a Chl content of 23.01 mg/g FW, indicating a 23.77% increase. Chl b content was similarly increased with SA treatment, with S_2 and S_1 treatments resulting in 14.34 mg/g FW and 14.40 mg/g FW, respectively, reflecting increases of 11.78% and 12.23% over the control (S_0 = 12.83 mg/g FW). The carotenoid content peaked in S_2-treated plants (10.49 mg/g FW), showing a 26.39% increase compared to the control (S_0 = 8.30 mg/g FW), while S1-treated plants displayed an 11.69% increase (9.27 mg/g FW) (Table 3).

The interaction between biochar and salicylic acid significantly influenced Chl a content, with the highest levels observed in B_2S_1 (24.45 mg/g FW), B_2S_2 (24.34 mg/g FW), B_1S_2 (24.33 mg/g FW), and B_0S_2 (23.52 mg/g FW) treatments. The lowest chlorophyll a content was recorded in the control treatment (B_0S_0), which measured 15.12 mg/g FW (Fig. 4).

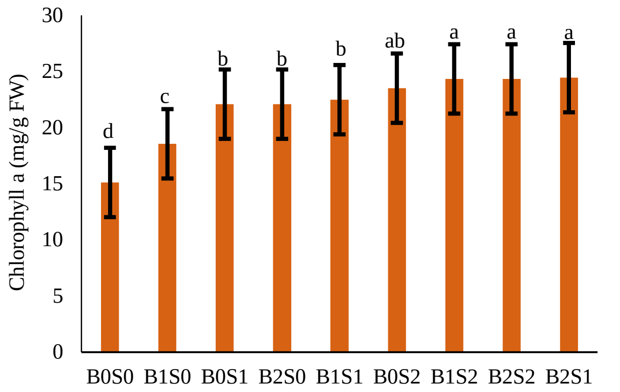

Fig. 4 Interaction effects of Biochar and Salicylic foliar application on chlorophyll a. S_0, S_1, and S_2: Salicylic acid foliar application with 0, 0.5 and 1 mM. respectively. B_0, B_1 and B_2: Biochar application with 0, 50 and 100 g/kg soil respectively

Phenolic and Flavonoid Content

Total phenolic content (TPC) and total flavonoid content (TFC) were significantly influenced by salicylic acid, while biochar primarily affected TFC. The highest TPC was observed in S_2-treated plants (47.94 mg GAEs/g FW), representing an increase of over 130% compared to the control (S_0 = 20.51 mg GAEs/g FW) (Table 4). TFC was also highest in S_2-treated plants (27.56 mg QEs/g DW), reflecting a 34.39% increase over the control (S_0 = 20.51 mg QEs/g DW), followed by S_1-treated plants (26.39 mg QEs/g DW), which showed a 28.62% increase (Table 4).

Biochar significantly impacted TFC, with the highest value observed in B_2-treated plants (26.70 mg QEs/g DW), representing a 13.62% increase compared to the control (B_0 = 23.50 mg QEs/g DW). B1-treated plants showed a TFC of 24.27 mg QEs/g DW, marking a 3.28% increase over the control. However, biochar did not significantly affect TPC (Table 4).

Antioxidant Activity (DPPH)

The DPPH radical scavenging activity increased significantly with both biochar and salicylic acid application. The highest DPPH activity was observed in B_2-treated plants (52.57%), representing a 6.18% increase over the control (B_0 = 49.50%). Salicylic acid also significantly enhanced DPPH activity, with S_2-treated plants showing the highest activity (59.86%), representing a 35.32% increase over the control (S_0 = 44.23%) (Table 4).

Table 4 Mean comparison of simple effects of Salicylic acid foliar application on Biochemical traits of *D. moldavica*

Treatment	Total phenol (mg GAEs/g FW)	Total flavonoid (mg QEs/g D)	DPPH (%)	Essential oil (%)	Geraniol (%)	Gerانial (%)	Geranyl Acetate (%)	Borneol (%)
Control	28.70 c	20.51 b	44.23 c	0.45 b	14.41 b	10.33 c	24.46 b	7.57 c
SA 0.5 mM	35.62 b	26.39 a	48.22 b	0.46 b	14.79 b	12.19 b	26.48 a	8.69 b
SA 1 mM	47.94 a	27.56 a	59.86 a	0.52 a	16.51 a	13.55 a	26.69 a	9.75 a
Control	37.32 a	23.50 b	49.50 b	0.45 b	15.34 a	12.11 a	25.41 a	9.09 a
Biochar (50 g/kg)	37.17 a	24.27 b	50.07 b	0.43 b	14.95 a	12.20 a	25.54 a	8.52 a
Biochar (100 g/kg)	37.77 a	26.70 a	52.75 a	0.56 a	14.75 a	11.76 a	26.69 a	8.41 a

Common letters within each factor indicate the absence of a significant difference at a 5% probability level, as per the Duncan test.
S_0, S_1, and S_2: Salicylic acid foliar application with 0, 0.5 and 1 mM. respectively. B_0, B_1 and B_2: Biochar application with 0, 50 and 100 g/kg soil respectively

Essential Oil Percentage and Components

Table 5 Essential oil constituents of *D. moldavica* L.

No	Retention time	Compound	Percentage
1	3.32	α-Pinene	2.78
2	3.64	Camphene	0.79
3	3.80	2,4-Thujadiene	0.44
4	3.95	β-Pinene	0.52
5	4.19	β-Myrcene	1.42
6	4.35	*p*-Mentha-1(7),8-diene	0.82
7	4.45	4-terpinenyl acetate	0.64
8	4.60	Limonene	1.9
9	4.74	1,8-Cineol	1.84
10	4.96	γ-Terpinene	1.96
11	5.22	*p*-Mentha-2,4(8)-diene	2.92
12	5.43	Linalool	1.45
13	6.21	Borneol	8.62
14	6.36	Decanal	2.21
15	6.44	α-terpineol	1.98
16	6.48	Myrtenol	0.51
17	6.81	Geraniol	16.56
18	6.92	Geranial	12.2
19	7.71	Geranyl acetate	26.51
20	8.16	Humulene	0.43
21	8.43	Methyl-eugenol	2.52
22	9.41	Caryophyllene oxide	1.97
			90.99

A total of 22 compounds were identified in the essential oil of Moldavian balm (Table 5). For statistical analysis, we selected the four compounds with the highest percentages: geraniol, geranial, geranyl acetate, and borneol. Salicylic acid (SA) application significantly affected both the essential oil percentage and the composition of key components in *D. moldavica* L. The highest essential oil percentages were observed in the B_2 treatment, reaching 0.56%, and in the S_2 treatment, at 0.52% (Table 4). The highest levels of geraniol (16.51%), geranial (13.55%), and borneol (9.75%) were observed in S_2-treated plants, representing increases of 22.46%, 17.34%, and 14.39%, respectively, compared to the control (S_0 = 13.48% for geraniol, 11.55% for geranial, and 8.52% for borneol) (Table 4). Geranyl acetate content was also significantly increased by SA, with S_1 (25.71%) and S_2 (28.49%) showing increases of 5.12% and 16.47%, respectively, over the control. In contrast, biochar application did not have a significant effect on the content of geraniol, geranial, geranyl acetate, or borneol (Table 4).

The interaction between biochar and salicylic acid did not significantly impact the essential oil percentage; however, it did noticeably affect the composition of essential oil components (Table 2). Geraniol content was highest in the B_0S_1, B_0S_2, B_2S_2, B_1S_2, and B_1S_0 treatments, with percentages ranging from 16.85% to 15.63%. The lowest geraniol content was recorded in the B_0S_0 and B_2S_1 treatments (Fig. 5). Geranyl acetate content showed a statistically significant reduction in the B_0S_0 treatment, while other treatments, except B_1S_2, were statistically similar (Fig. 6). Lastly, the highest borneol percentages were observed in the B_0S_2, B_0S_1, B_1S_2, and B_2S_2 treatments (Fig. 7).

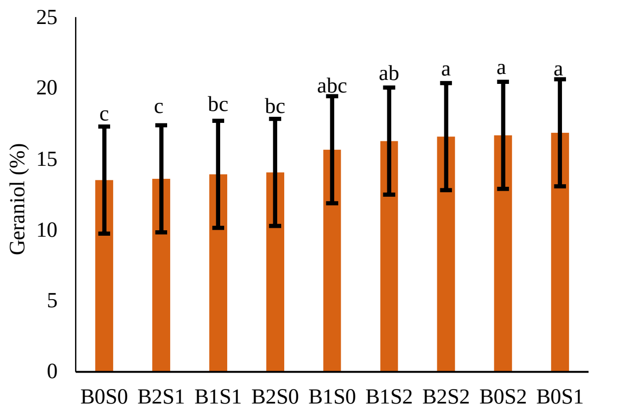

Fig. 5 Interaction effects of Biochar and Salicylic foliar application on Geraniol%. S_0, S_1, and S_2: Salicylic acid foliar application with 0, 0.5 and 1 mM. respectively. B_0, B_1 and B_2: Biochar application with 0, 50 and 100 g/kg soil respectively.

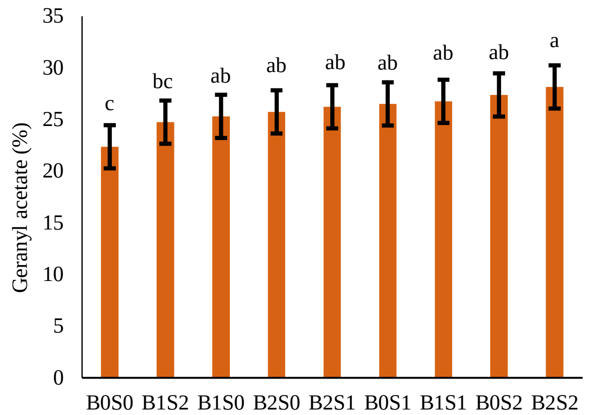

Fig. 6 Interaction effects of Biochar and Salicylic foliar application on Geranyl acetate.

S_0, S_1, and S_2: Salicylic acid foliar application with 0, 0.5 and 1 mM. respectively. B_0, B_1 and B_2: Biochar application with 0, 50 and 100 g/kg soil respectively.

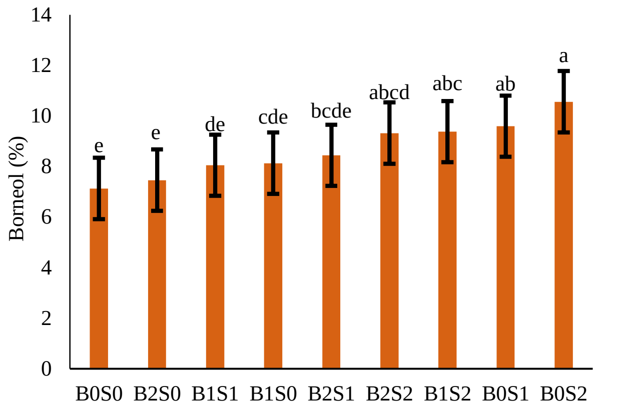

Fig. 7 Interaction effects of Biochar and Salicylic foliar application on Borneol%.

S_0, S_1, and S_2: Salicylic acid foliar application with 0, 0.5 and 1 mM. respectively. B_0, B_1 and B_2: Biochar application with 0, 50 and 100 g/kg soil respectively.

DISCUSSION

This study investigated the effects of biochar application at varying concentrations (0, 50, and 100 g/kg soil) and salicylic acid (SA) foliar application at different levels (0, 0.5, and 1 mM) on the morphological and phytochemical traits of *D. moldavica* L. The findings revealed significant enhancements in both growth parameters and secondary metabolite content, demonstrating the potential of biochar and SA as effective tools for optimizing the cultivation of this valuable medicinal plant.

The application of biochar and SA significantly improved growth, physiological, and biochemical traits, aligning with previous research highlighting the beneficial effects of these treatments in enhancing plant performance under various conditions. The observed increases in plant height, branch number, and biomass due to biochar application are consistent with prior studies, which have reported biochar's positive impact on plant growth. Biochar is known to enhance soil structure, water retention, and nutrient availability, all contributing to improved plant growth [18]. The substantial increases in fresh and dry biomass, particularly in the B2 treatment, suggest that higher biochar concentrations provide more significant benefits, likely due to improved nutrient uptake and enhanced microbial activity in the rhizosphere [4].

Salicylic acid's role in promoting plant growth is well-established. As a signaling molecule, SA enhances plant defense mechanisms, leading to improved growth and development [10]. Rithichai et al. [19] demonstrated that foliar application of SA significantly increased plant height, shoot fresh and dry weight, branch number, and leaf area in holy basil. These findings are in line with those of Batista et al. [20], who reported that SA application enhanced plant growth by improving photosynthetic efficiency and modulating hormonal balance. Similarly, Gorni et al. [21] found that SA application in *Achillea millefolium* cultivation increased plant biomass and primary metabolite levels.

The enhancement of chlorophyll and carotenoid content with biochar application suggests an improvement in photosynthetic capacity. Biochar's ability to enrich soil nutrient content, particularly nitrogen and phosphorus, likely contributed to the observed increases in chlorophyll a and b, as these nutrients are essential for chlorophyll synthesis [22] The positive effects on carotenoid content indicate that biochar may help protect plants from oxidative stress by enhancing antioxidant levels, as carotenoids play a crucial role in quenching reactive oxygen species (ROS) [8].

Salicylic acid's impact on photosynthetic pigments is also noteworthy. The significant increase in chlorophyll and carotenoid content with SA treatment aligns with studies showing that SA enhances photosynthetic efficiency by modulating the expression of genes involved in chlorophyll biosynthesis and stabilizing chloroplast membranes [23]. The increased pigment content likely contributed to the improved growth parameters observed in SA-treated plants, as higher chlorophyll levels are associated with greater photosynthetic capacity and better growth [19, 24].

The significant increase in total phenolic content (TPC) and total flavonoid content (TFC) with SA application suggests that SA plays a crucial role in enhancing the antioxidant defense system in plants. Phenolics and flavonoids are key secondary metabolites that contribute to plant defense against biotic and abiotic stresses by scavenging free radicals and reducing oxidative damage [25]. The substantial increase in TPC and TFC in S2-treated plants indicates that higher concentrations of SA effectively boost the production of these metabolites, enhancing the plant's stress tolerance. These findings are consistent with those of Grzeszczuk et al. [26], who reported that SA application increased phenolic compound accumulation, leading to improved antioxidant activity in plants. This study's findings also align with earlier research showing that exogenous SA application at low doses significantly induces the accumulation of bioactive compounds [9, 19].

Foliar SA treatment significantly increased total phenol and flavonoid contents in various Lamiaceae plants, including lavender (*Lavandula angustifolia* Mill.), sage (*Salvia officinalis* L.), hyssop (*Hyssopus officinalis* L.), anise hyssop (*Agastache foeniculum* (Pursh) Kuntze), peppermint (*Mentha × piperita* L.), oregano (*Origanum vulgare* L.), and scarlet beebalm (*Monarda didyma* L.) [27]. It is well established that SA increases the activity of phenylalanine ammonia-lyase (PAL), a key enzyme involved in the initial stages of phenolic compound biosynthesis [28]. In plants from the *Salvia* genus (*Salvia officinalis*, *Salvia virgate*, *Salvia miltiorrhiza*), exogenous SA use has been shown to activate PAL expression [29]. In some plants from other families, the activation of genes involved in stilbene and flavonoid biosynthesis has also been observed [27].

Biochar's effect on TFC, although less pronounced than that of SA, still shows significant enhancement, particularly in B2-treated plants. This suggests that biochar may contribute to flavonoid synthesis by improving nutrient availability and stimulating microbial activity in the soil, which in turn influences the plant's metabolic pathways [30].

The increase in DPPH radical scavenging activity with both biochar and SA application indicates enhanced antioxidant capacity in the treated plants. The higher antioxidant activity observed in S2 and B2 treatments suggests that these amendments not only improve growth and physiological traits but also strengthen the plant's defense mechanisms against oxidative stress. The correlation between increased phenolic and flavonoid content and higher DPPH activity supports the notion that these secondary metabolites play a vital role in the plant's antioxidant defense [31]. Similar findings have been reported by He *et al.* [32], who observed that biochar and SA treatments increased antioxidant enzyme activities and reduced oxidative stress in plants.

Our research demonstrated the positive effect of high concentrations of putrescine on the essential oil percentage in Moldavian balm. This aligns with findings by researchers, who observed that biochar amendments increased essential oil yield in *Ocimum basilicum*, attributing this effect to enhanced nutrient availability and microbial activity, which stimulated pathways involved in oil production [33]. Similarly, Farhangi-Abriz and Torabian (2017) reported a significant increase in essential oil percentage in *Thymus vulgaris* with biochar application, likely due to improved nutrient and water uptake efficiency. Biochar's influence on soil pH and microbial communities may further enhance essential oil content [34]. For instance, the alkalizing effect of biochar can adjust soil pH to optimal levels for medicinal plants, promoting growth and essential oil production. Additionally, biochar-induced enhancements in soil microbial activity and enzyme production can improve nutrient cycling and availability, both of which have been linked to increased essential oil synthesis in plants such as *Lavandula angustifolia* [36].

Foliar application of salicylic acid at high concentrations has shown promising effects in enhancing the essential oil percentage in *D. moldavica* L. For instance, in *Ocimum basilicum*, SA foliar application increased essential oil yield by activating metabolic pathways associated with oil biosynthesis, particularly under mild stress conditions [37]. Similarly, in *Mentha arvensis*, SA treatment significantly boosted essential oil content by enhancing enzymatic activities involved in the biosynthesis of monoterpenes, which are key components of mint essential oil [38].

Salicylic acid significantly influenced the content of key essential oil components, such as geraniol, geranial, and borneol, in a manner consistent with previous research. SA has been shown to enhance essential oil production by modulating the expression of genes involved in terpenoid biosynthesis [39]. The increased levels of these compounds in S2-treated plants suggest that higher SA concentrations can stimulate essential oil production, likely through its effects on metabolic pathways and stress response mechanisms. This finding is consistent with those of Bistgani et al [40], Haydari et al. [41], Gorni et al. [21], and Jahani et al. [24], who reported that SA application enhanced essential oil yield and quality in medicinal plants. For example, in *Cuminum cyminum*, essential oil analysis showed that the amounts of β-pinene (2.36-3.48%), ρ-cymene (7.55-9.19%), γ-terpinene (9.61-12.37%), γ-terpinene-7-al (24.47-31.96%), and cumin aldehyde (24.60-29.19%) as major oil components increased gradually with rising SA levels [42]. The lack of significant effects of biochar on essential oil components suggests that while biochar may improve overall plant growth and antioxidant capacity, it may not directly influence the biosynthesis of specific secondary metabolites like essential oils. This observation is consistent with previous studies reporting variable effects of biochar on essential oil production, depending on the plant species and the specific components measured [43].

CONCLUSION

This study highlights the significant positive effects of biochar and salicylic acid on plant growth, physiological traits, and biochemical composition. The application of biochar, particularly at higher concentrations, significantly enhanced plant height, biomass production, and the accumulation of photosynthetic pigments. These improvements are likely due to biochar's ability to enhance soil properties, such as nutrient availability and water retention, which collectively contribute to better plant growth and health. Salicylic acid, on the other hand, demonstrated a strong influence on the enhancement of secondary metabolites, including phenolics, flavonoids, and essential oil components, along with improvements in antioxidant activity. SA's role in modulating stress responses and enhancing photosynthetic efficiency appears to be a key factor in its effectiveness in improving plant performance under the conditions studied. The combined application of biochar and SA offers a promising approach for enhancing plant growth and metabolic activity, making these amendments valuable tools in sustainable agricultural practices. Future studies should further explore the synergistic effects of these treatments under different environmental conditions and in various plant species to better understand their potential in optimizing crop production and quality.

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