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Exploring the impact of exogenous melatonin on agro-morphological characteristics, carvacrol, and rosmarinic acid production in *Satureja rechingeri* Jamzad under drought stress

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Abstract

Satureja rechingeri Jamzad (known as "*Jatra*" in Persian), which belongs to the Lamiaceae family, is a rich source of essential oil particularly carvacrol, and rosmarinic acid. Drought stress has a detrimental impact on the physiological and biochemical parameters of plants, leading to a decline in plant productivity. Melatonin (MT), a new plant growth regulator found abundantly in plants, has been found to enhance the plant's internal resistance to various environmental stresses. The present study aimed to examine the impact of exogenously applied MT on the agro-morphological, physio-biochemical, and phytochemical traits of *S. rechingeri* plants cultivated under different levels of drought stress. The results indicated that plants treated with 200 µM MT obtained the highest plant height, length and width of leaf, fresh, dry and drug weight under different drought stress levels. The highest values of relative water content (RWC) (93.5%) and chlorophyll content (15.4 mg/g FW) were recorded by MT 200 µM and 100 µM, respectively, in 100% FC. Drought stress treatments (40, 60, and 80% FC) without foliar spray of MT significantly enhanced the H_2O_2 content, electrolyte leakage, and malondialdehyde content in leaves, whereas MT treatment under drought stress significantly decreased the above parameters. The lowest H_2O_2 content (11.5 nmol/g), electrolyte leakage (3.08%), and malondialdehyde content (0.78 µM/g) were obtained by 200 µM MT at 100% FC. In contrast, drought stress treatment increased the total phenol content (TPC), rosmarinic acid (RA), essential oils (EOs) content and yield, and carvacrol. The maximum values of TPC (28.1 mg GAE/g DW), EOs content (3.63%) and yield (0.96%), and carvacrol (95.66%) were achieved by 200 µM MT at 40% FC. The highest RA content (7.43 mg GAE/g DW) was recorded in 100 µM MT at 40% FC. Thus, foliar spray MT has the potential to enhance plant growth through the mitigation of reactive oxygen species (ROS)-induced oxidative harm, as well as the augmentation of photosynthesis pigments, secondary metabolites such as phenolics, EOs levels, overall antioxidant scavenging capacity, and the preservation of RWC during periods of drought stress.

Keywords Water stress, Essential oil, Oxidative damage, Carvacrol, Rosmarinic acid

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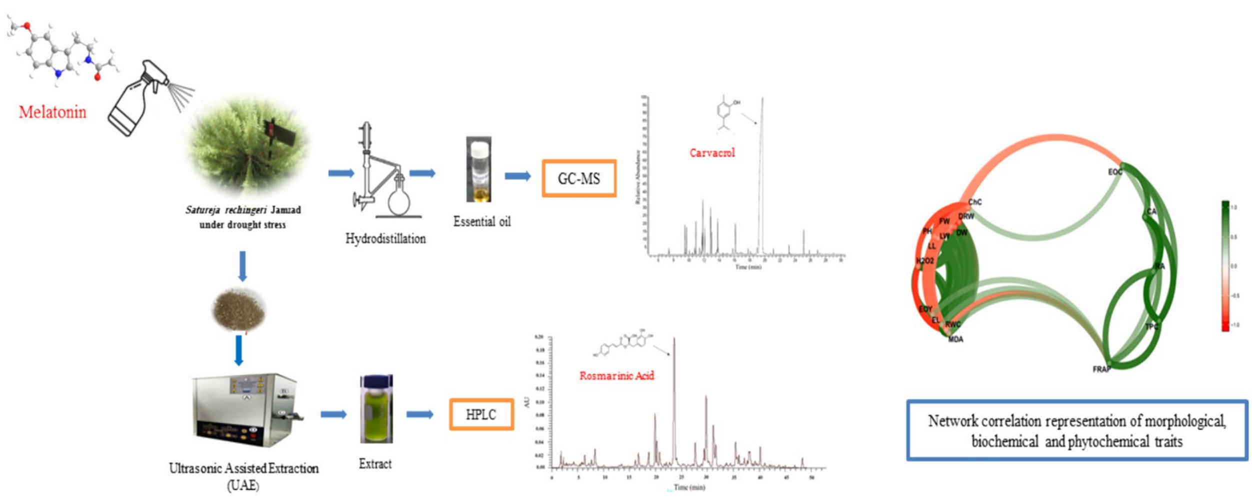
Graphical Abstract



Introduction

Today, global demand for the use of medicinal and aromatic plants due to their bioactive compounds in traditional and modern medicine as well as in various pharmaceutical, food, and cosmetic industries is expanding dramatically and has an annual commercial value of 1.3 billion dollars [1, 2]. The quantity and quality of bioactive compounds in plants are influenced by different components such as genetic background, weather conditions, soil type, harvest time, plant age, and environmental stresses [3]. Drought stress is one of the foremost environmental stresses, which is one of the major global concerns due to climate change and the reduction of underground water resources [4]. Drought stress limits plant growth, development, and performance [5]. Almost 60% of crops, including medicinal plants, are affected by drought stress [6]. Drought stress causes physiological disorders such as reduction of transpiration and photosynthesis by the closing of stomata, biochemical changes including production of ROS, protein denaturation, and increase of osmotic protectors and antioxidant enzymes [7, 8]. Also, drought stress can change the yield of secondary metabolites (phenolic compounds, flavonoids, alkaloids, EOs, and their compounds, etc.) in medicinal plants [6]. According to earlier studies, plant growth regulators exhibited fundamental and central roles in regulating stress signaling as well as the physiological and biochemical pathways of plants [9, 10]. Therefore, the foliar spray of these compounds can be a valuable method to improve crop defense and adaptation to environmental stresses, including drought stress [11].

Melatonin (MT) or N-acetyl-5-methoxytryptamine is a multifunctional phytohormone that has direct effects in enhancing ROS inhibition, improving the function of the electron transport chain function, increasing antioxidant activity, and protecting antioxidant enzymes against oxidative damage [11–14]. MT improves the adverse effects of abiotic stresses in mutant barley [15], rapeseed [16], cotton [17], tomato [18], *Dracocephalum kotschyi* [19], horticultural plants [20], and vegetable crops [21]. *Satureja rechingeri* Jamzad. is a perennial plant (Lamiaceae), which is an endemic species of Iran, whose EOs are rich in carvacrol (over 90%) and its extract is rich in phenolic compounds, including rosmarinic acid [22]. Due to having these bioactive compounds, this plant has biological properties such as antiseptic, analgesic, antimicrobial, antiviral, antifungal, anti-inflammatory, anticancer, and antioxidant activities [23–26]. Many medicines such as Dentol®, Saturex®, and Aortodntol® have been produced from this plant and new formulations have been prepared from this plant such as Zagrol® for use in the veterinary industry. Also, the plant waste is used as animal fodder after extracting EOs. So far, no study has been conducted to investigate the role of the exogenous application of MT on the morphological, functional, and phytochemical characters (EOs content, carvacrol, and rosmarinic acid) and enzyme activity of *Satureja rechingeri* under drought stress. Therefore, this study was conducted to investigate the effects of the exogenous application of MT on the agro-morphological, biochemical, and phytochemical characteristics of *Satureja rechingeri* under drought stress. The results of this research might be utilized for the cultivation of *Satureja rechingeri* in dry and low-yielding lands to supply raw materials for the pharmaceutical and food industries.

Materials and methods

Experimental design and plant materials

S. rechingeri seeds sourced from Pakanbazr Company in Isfahan, Iran, were planted in cultivation trays (61×40 mm, depth 76 mm and 200 cc) filled with coco peat: perlite (1:1). Seedlings were placed in plastic pots with a diameter of 15 cm and a height 20 cm of on sandy loam soil (Table 1) after 40 days. The experiment was performed in a greenhouse at the Medicinal Plants and Drug Research Institute, Shahid Beheshti University, Tehran, Iran (32° 43′ 13.7″ N and 51° 31′ 42.2″ E) for 60 days, with a photoperiod of 11 h, light/13 dark, relative humidity 55–65%, night temperature 16–18 °C and daytime temperatures 24–26 °C. The experiment followed a completely randomized design (RCD) with two factors: irrigation levels (100%, 80%, 60%, and 40% of field capacity) and foliar application of MT at concentrations of 0, 50, 100, and 200 µM. Each treatment consisted of four transplants with three replicates.

Irrigation regimes and MT treatments

Soil FC (field capacity) measurements were carried out following the pot experiment according to Pourmeidani et al., [27], to determine the amount of water required for each irrigation scheme. Five kilograms of soil were placed in the oven and dried at 105 °C for 72 h to obtain dry weight. The containers were then filled with dried soil from the oven and weighed every two hours for 36 h after watering and saturating the soil. A soil sample was taken from each container once the weight of the containers had stabilized. Equation (1) was utilized to calculate the percentage of soil water at field capacity.:

$$\text{Percentage of water in the soil} = (\text{soil fresh weight} - \text{soil dry weight} / \text{soil dry weight}) \times 100 \quad (1)$$

After obtaining the weighted soil moisture values in FC, different treatments (100, 80, 60, and 40% FC) were calculated. To administer the treatment, the pots were weighed every day and the necessary amount of water was added to each pot. For the MT exogenous treatment, MT was first dissolved in ethanol, then diluted with phosphate-buffered saline, and various concentrations of 0, 50, 100, and 200 µM were prepared. To enhance the consistency of the spray application, two drops of Tween-20 were included as a surfactant for every 100 ml of solution.

Measurement of agro-morphological traits

Morphological characteristics like plant height, leaf length, and leaf width were measured at the full flowering stage using a ruler and digital caliper. The fresh weight of aerial parts immediately after harvesting, was measured by a digital scale. Subsequently, the plant material was dried at room temperature. The digital scale was then employed to measure the dry weight of both the aerial parts and the leaves and flowers were used to record the drug weight of each plant.

Determination of physio-biochemical characteristics

Relative water content

To determine the relative water content (RWC) of the leaves, first, separated from the upper parts of the plant from each experimental unit, and their fresh weight was measured. To measure the turgor weight, these parts were placed in distilled water for 24 h under light intensity, and then, the turgor weight of the sample was read. The samples were placed in an oven at 75 °C for 48 h, and their dry weight was also measured and calculated using Eq. (2) [28]:

$$\text{RWC}(\%) = (Ww - Wd/Wt - Wd) \times 100 \quad (2)$$

Ww is wet weight, Wd is dry weight and Wt is turgor weight.

Electrolyte leakage

The method employed by Lutts et al. [29] was utilized to measure the electrolyte leakage (EL). To eliminate any potential ions present on the leaf surface, the leaves were thoroughly washed three times with distilled water. Subsequently, 0.3 g of fresh leaves were divided into smaller parts and placed in 10 ml of distilled water. The mixture was then incubated at room temperature for 24 h. The

Table 1 Soil physicochemical properties analyzed in this study

Physical texture (%)			pH	EC (ds/cm)	OC (%)	N (%)	P (mg/kg)	K (mg/kg)	Ca (meq/l)	Mg (meq/l)	Cl (meq/l)
Sand	Silt	Clay									
71.43	20.81	7.76	8.2	3.92	0.81	0.06	21.86	235	15.3	11	15

EC electrical conductivity, *OC* organic carbon initial electrical conductivity (EC_1) was determined using an EC meter. To ensure the release of all electrolytes, For 15 min, the samples were subjected to an autoclavation at 121 °C. Following the cooling of the solution, the final electrical conductivity of the samples (EC_2) was determined once again. The calculation of EL was performed using the formula (3).:

$$EL = \left(\frac{EC_1}{EC_2}\right) \times 100 \tag{3}$$

Chlorophyll content

To determine chlorophyll content, 0.5 g of freshly harvested leaves of *S. rechingeri* were finely ground with 80% acetone. The extract was then transferred to a Falcon tube, and its volume reached 10 ml with 80% acetone. The extracted solution was centrifuged and the absorbance of the extract was determined at 645 and 663 nm using a spectrophotometer. Total chlorophyll content was calculated based on Eq. 4 and expressed in mg/g of fresh weight [30]:

$$\text{Chlorophyll content} = [(20.2 \times A_{645}) + (8.02 \times A_{663})/1000W] \times V \tag{4}$$

V, volume of solution; A, absorption of light at wavelengths of 663 and 645 nm; and W, wet weight of the sample (g).

Hydrogen peroxide and malondialdehyde content

Hydrogen peroxide (H_2O_2) was quantified utilizing a spectrophotometer at 390 nm according to Velikova and Loreto method [31]. To measure the concentration of malondialdehyde (MAD), 0.2 g of fresh leaf was crushed with 0.5 ml of 0.1% trichloroacetic acid (TCA). The extract was centrifuged for 5 min. 4.5 ml of 20% TCA solution containing 5 g of thiobarbituric acid (TBA) per 100 g was mixed with 1 ml of the supernatant solution obtained from the centrifuge. Then, the mixture was centrifuged for 10 min at 4000 rpm. The absorbance of the solution was measured using a spectrophotometer at 532 nm, with a red compound (MAD-TBA) in the target material for absorption at this wavelength. The absorbance of any other non-specific pigments was assessed at 600 nm and subtracted from the initial value [32].

Phytochemical analysis

Extraction and determination of the total phenol content

To extract the total phenol concentration, 500 mg of the aerial parts of *S. rechingeri* were powdered and mixed in 20 ml of 80% methanol and the sonicator (SingenHtw Elmasonic-D 78224; Elma Germany) was extracted at 40 °C for 20 min. The obtained sample was placed in a refrigerated centrifuge (R5702; Eppendorf) at 3000 rpm for 10 min, and the supernatant was kept in the refrigerator until analysis. The total phenolic content of *S. rechingeri* extract was measured using the Folin–Ciocalteu method. In this test, 300 μl of the extract (1 mg/mL) was mixed with 450 μl of 1 M Folin reagent and 270 μL of sodium carbonate, deionized water was added, and the mixture was placed in a dark condition for 50 min at 25 °C. The absorbance of the samples at a wavelength of 765 nm was measured with a spectrophotometer. To draw the calibration curve, gallic acid was employed as a standard and, the total phenol content was quantified in mg gallic acid equivalent/g dry weight [33].

Measurement of rosmarinic acid by high-performance liquid chromatography (HPLC)

Analysis and evaluation of rosmarinic acid content using the device of Waters 2695 Alliance HPLC system (Wellchron-K1001) equipped with a PDA detector was performed. The separation was done using a RP-C18 chromatography column with an inner diameter of 4.6 mm and a length of 250 mm (Eurosphr). A mixture of acetonitrile (mobile phase A) and phosphoric acid (mobile phase B) was used as the mobile phase. The injection volume was 20 μL, and the temperature was fixed at 25 °C. The rosmarinic acid standard was prepared from Sigma Aldric. To determine the amount of rosmarinic acid compound, calibration curves were obtained by injecting different concentrations (5, 10, 20, 40, 80, 160, 380 ppm) of the standard compound. The amount of rosmarinic acid was expressed in mg/g dry weight.

Antioxidant activity

The antioxidant activity of *S. rechingeri* extracts using of ferric-reducing power (FRAP) was evaluated. The ability of extracts to reduce ferric ions (Fe^{3+}) in the presence of antioxidants was measured. Fe-TPTZ complex was formed by reducing Fe^{3+} to Fe^{2+} in acidic pH and the presence of TPTZ. $FeSO_4$ was used to draw the standard curve. 3 ml of FRAP reagent was placed in a Bain-Marie at 37 °C for 5 min. 100 μL of the sample was added and placed in a Bain-Marie at 37 °C for 10 min, and its absorbance was measured at 593 nm. The amount of antioxidant activity was expressed as the equivalent of mM Fe/g dry weight [34].

EOs content and yield

To extract the EO, the dried plants were cleaned to remove the excess material. For homogenizing the dried plants, the samples were ground and powdered. An amount of 30 g powdered plant material was mixed with water and distilled using a Clevenger apparatus for three hours. The EO content and yield were calculated using Eqs. 4 and 5, respectively:

$$\text{EO Content (\%)} = (\text{EO weight / dry weight}) \times 100 \quad (5)$$

$$\text{EO Yield (g/plant)} = \text{EO content} \times \text{Total dry weight} / 100 \quad (6)$$

Determination of carvacrol in EO by GC and GC–MS

Essential oil analysis was done with the Gas Chromatography (GC) model Shimadzu 15A. N_2 was used as the carrier gas with a velocity (1 ml/min) and DB-5 column (30 m length × 0.25 mm inner diameter × 0.25 µm film thickness). The column temperature was kept at 60 °C for 3 min and then increased to 220 °C at a rate of 5 °C/min and fixed at 220 °C for 5 min.

GC–MS analyzes were performed using a Hewlett-Packard 5973 machine equipped with a HP-5MS column (30 m length × 0.25 mm inner diameter × 0.25 µm film thickness). The column temperature was kept at 60 °C for 3 min and then increased to 220 °C at a rate of 5 °C/minute and fixed at 220 °C for 5 min. At a flow rate of 1.1 mL per minute, helium gas was used as the carrier gas, and 1 µL of EO samples were injected at separate ratios of 1:1. The ionization energy was set at 70 eV, the scan time was 0.4 s, and the mass range was adjusted between 40 and 460 amu. The EO compounds were identified using the retention index, mass spectra data, literature, and the National Institute of Standards and Technology (NIST) computer library. Normalizing the surface area and ignoring the response coefficients obtained the relative percentages of EO components.

Statistical analysis

The statistical analysis was performed using SAS 9.2 software, employing analysis of variance (ANOVA) as a completely randomized design (RCD). The means were compared using the least significant difference test (LSD test) at a significance level of $p \leq 0.05$. Data are presented as means ± SE ($n = 3$). To determine the correlation between the different traits, Pearson's correlation coefficient was calculated using R Software. All graphical representations were created using Microsoft Excel 2016.

Results

Agro-morphological traits

Plant height, leaf length, leaf width, fresh weight, dry weight, and drug weight of *S. rechingeri* were evaluated in MT-treated plants as compared with drought stress. The lowest values of these parameters were obtained in 40% FC. Exogenous MT treatments significantly increased all the evaluated morphological traits in *S. rechingeri* plants under drought stress. The results of ANOVA have shown significant interactions between MT foliar spray and drought stress on the plant height, leaf length, and leaf width, as well as the fresh weight, dry weight, and drug weight ($p < 0.01$). Exogenous MT application in drought conditions was effective on plant height, leaf length, and leaf width so that the characteristics were maximized following the I1M4 (65.16 cm, 18.00 mm, and 12.64 mm in order) (Table 2). The exogenous MT improved the plant height, leaf length, and leaf width by 83.54%, 133.76%, and 110.66%, respectively, compared to the conditions of drought stress (I1M4). Further, Exogenous MT treatments under the various levels of drought promoted the fresh weight, dry weight, and drug weight compared to the control (I1M1). In comparison to the control, treatment reduced the effect of drought stress and led to a lower reduction in soil characteristics. The highest fresh weight (186.66 g/plant) dry weight (89.00 g/plant), and drug weight (35.50 g/plant), were found after applying the I1M4 (100% FC + 200 µM MT). Exogenous MT (I1M4) increased the fresh weight, dry weight, and drug weight by 38.26%, 45.11%, and 30.99%, respectively, compared to the control (Table 2).

Physio-biochemical traits

Relative water content

Relative water content (RWC) serves as a crucial indicator of plants' survival capacity and the status of water in their leaves. The data in Fig. 1A demonstrates a significant decrease in RWC as water levels declined from 100% FC to 40% FC. The lowest values (51.40%) were achieved by the combined effect between 40% FC and the absence of MT. The highest values (93.50%) were recorded by 100% FC and foliar application of MT at 100 µM.

Electrolyte leakage

Electrolyte leakage (EL) is a crucial parameter for examining the plant's reaction to water deficiency, aiming to assess cellular membrane injury. As presented in Fig. 1B, an incremental rise in EL was observed as the level of drought stress increased from 100 to 40% FC. Without MT treatment, the irrigation control group at 100% FC exhibited a lower EL of 3.08%. Conversely, the irrigation treatment at 40% FC displayed the highest EL value of 39.40% without MT intervention. However, a conspicuous decline in EL proficiency was observed with the rise in MT concentration.

Chlorophyll content

The total chlorophyll content of *S. rechingeri* leaves was negatively impacted by drought stress, as presented in Fig. 2. Chlorophyll content varied from 6.80 to 15.40 mg/g FW. The chlorophyll content decreased when the leaves underwent drought stress (80–40% FC) in

Table 2 Mean comparison of interaction effects of drought stress and melatonin application on growth traits of *S. rechingeri*

Treatment	Plant height (cm)	Leaf length (mm)	Leaf width (mm)	Fresh w(g/plant)	Dry weight (g/plant)	Drug weight (g/plant)
I1M1	55.13[d]	12.66[f]	8.63[d]	135.00[e]	61.33[e]	27.10[d]
I2M1	46.50[g]	10.66[g]	7.65[e]	102.00[j]	47.00[h]	20.50[h]
I3M1	40.50[h]	8.65[h]	6.65[fg]	83.33[m]	38.66[j]	16.50[j]
I4M1	35.50[i]	7.70[i]	6.00[g]	65.00[o]	30.00[l]	13.30[l]
I1M2	58.33[c]	14.35[de]	9.68[c]	154.03[c]	72.00[c]	29.00[c]
I2M2	53.50[de]	12.33[f]	8.65[d]	125.00[g]	59.33[f]	23.86[e]
I3M2	49.83[f]	10.00[g]	7.60[e]	93.00[l]	44.76[i]	18.34[i]
I4M2	47.36[g]	8.30[hi]	6.65[fg]	73.33[n]	35.30[k]	14.50[k]
I1M3	61.50[b]	17.00[b]	10.66[b]	165.60[b]	78.00[b]	31.16[b]
I2M3	57.50[c]	14.66[d]	9.33[cd]	145.00[d]	68.66[d]	28.24[cd]
I3M3	52.83[e]	12.66[f]	7.65[e]	113.00[h]	54.50[g]	22.75[fg]
I4M3	47.16[g]	10.33[g]	7.00[ef]	99.00[k]	47.50[h]	19.50[h]
I1M4	65.16[a]	18.00[a]	12.64[a]	186.66[a]	89.00[a]	35.50[a]
I2M4	61.50[b]	15.66[c]	10.66[b]	154.10[c]	73.00[c]	30.33[b]
I3M4	57.50[c]	13.66[e]	9.65[c]	155.00[c]	62.53[e]	25.95[e]
I4M4	52.50[e]	12.66[f]	7.60[d]	108.00[i]	52.66[g]	21.91[g]
LSD	1.64	0.95	0.99	2.12	1.87	1.12
Mean	52.64	12.44	8.59	120.79	57.11	23.65

I1, I2, I3, and I4: irrigation at 100%, 80%, 60%, and 40% field capacity (FC), respectively. M1, M2, M3, and M4: 0, 50, 100, and 200 µM melatonin, respectively. Means with the same letter(s) within column are not significantly different at $p < 0.05$ using the LSD test

contrast to the control group with 100% FC, without the application of MT treatment. In contrast, various levels of MT demonstrated a significant reduction in the detrimental impact caused by the decrease in irrigation water from 100 to 80% FC, along with an increase in total chlorophyll content compared to the control group, particularly noticeable at a concentration of 100 µM of MT.

Hydrogen peroxide and malondialdehyde

Compared with the control treatment of 100% FC, drought stress treatments (40, 60, and 80% FC) without a foliar spray of MT significantly enhanced the ROS (H_2O_2) content in leaves (Fig. 3A). In contrast, MT treatment under drought stress significantly decreased the ROS (H_2O_2) content in the leaves, especially at 200 µmol compared with 100% FC. Therefore, it is shown in Fig. 3B that the reduction of irrigation water rates from 100 to 80% FC significantly increases the content of malondialdehyde (MDA) content in the leaves of *S. rechingeri* plants. Regarding the interaction effects between drought stress treatments and MT foliar application, it could be seen that at 40% FC treatment along with MT treatment, there was a significant increase in MDA production relative to the control. However, an increase in MT concentration reduced MDA production in *S. rechingeri* plants.

Phytochemical markers

Total phenol content

In plants subjected to drought stress, the total phenol content was significantly higher. However, the application of MT increased their production (Fig. 4A). The highest values (28.10 mg GAE/g DW) of TPC were achieved by 200 µM, and the lowest values (11.20 mg GAE/g DW) were recorded with 100% FC in the absence of MT. The content of total phenol increased by 150.89% with the application of MT under drought stress.

Rosmarinic acid

S. rechingeri is rich in phenolic compounds, mainly rosmarinic acid (RA) (Fig. 5). The content of RA is presented in Fig. 4B. The highest content (7.43 mg/g DW) of RA was observed in 40% FC and 100 µM MT treatments. In 100% FC, the lowest RA content (2.10 mg/g DW) was found in the absence of MT application.

EOs content and yield

The findings indicated that the level of EOs content increased as the drought stress level and MT concentration rose. The plants treated with 200 µM MT and 40% FC exhibited the highest EOs content (3.63%), whereas those treated with 100% FC without MT showed the

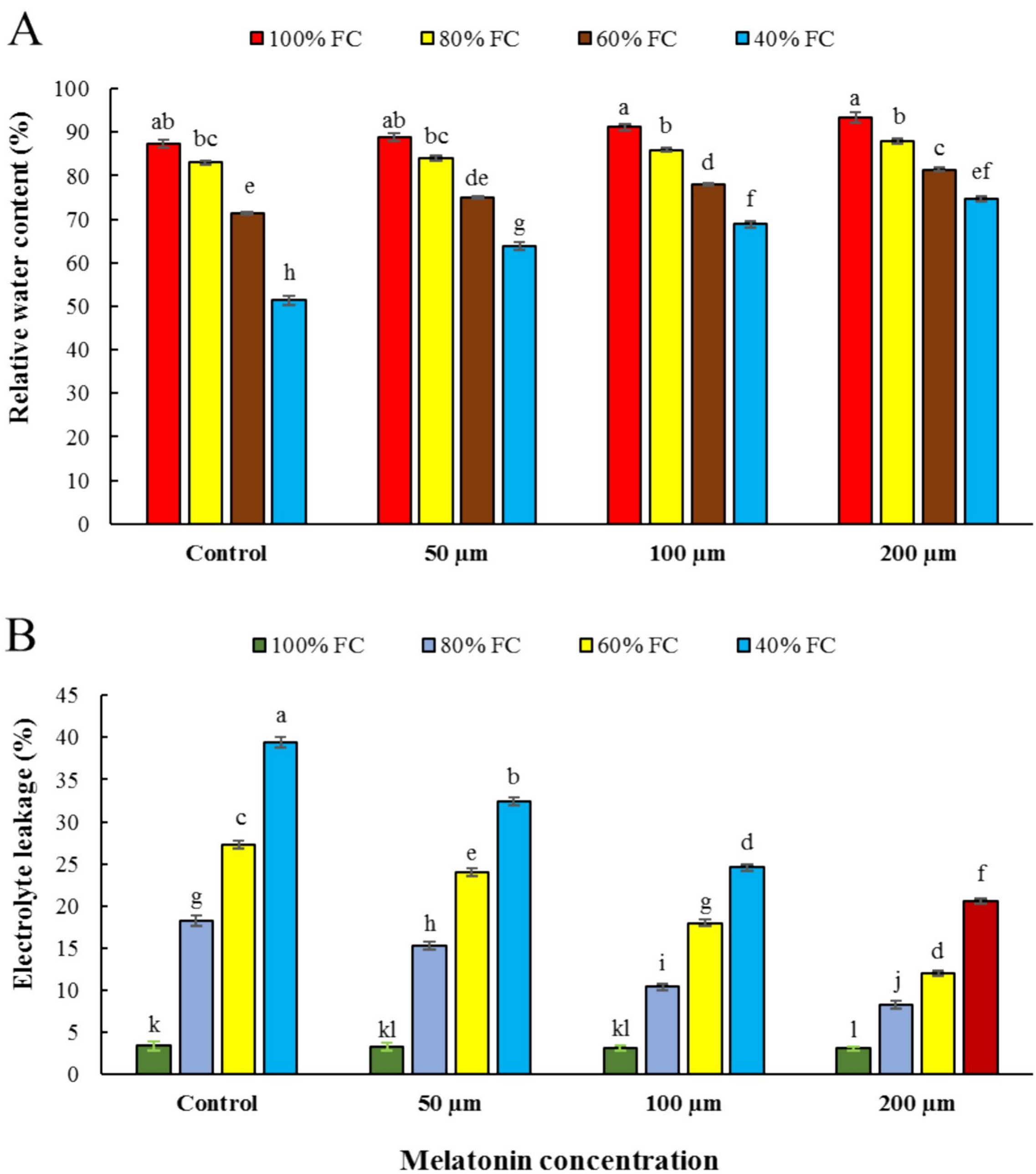


Fig. 1 Effect of melatonin treatment on relative water content (**A**) and electrolyte leakage (**B**) of *S. rechingeri* plants under drought stress. The mean comparisons were performed using the LSD test at $p \leq 0.05$ significant level. Data are presented as means ± SE ($n = 3$). Means followed by the same letter(s) are not significantly different

lowest content (2.12%) (Fig. 6A). The EOs yield is influenced by both leaf yield and EOs content. The plants exposed to 100% FC and 200 µM MT produced the highest EOs yield (0.96 g/plant), while those treated with 40% FC and 50 µM MT had the lowest yield (0.44 g/plant) (Fig. 6B).

Carvacrol in the EO

Carvacrol is the main component of the EOs of *S. rechingeri* (Fig. 7). The maximum content of carvacrol (95.66%) was observed in 40% FC and 200 µM MT. The minimum content of carvacrol (81%) was measured in 100% FC without MT treatment (Fig. 8A). At the maximum drought level, MT application increased the carvacrol percentage by approximately 18.09%, compared

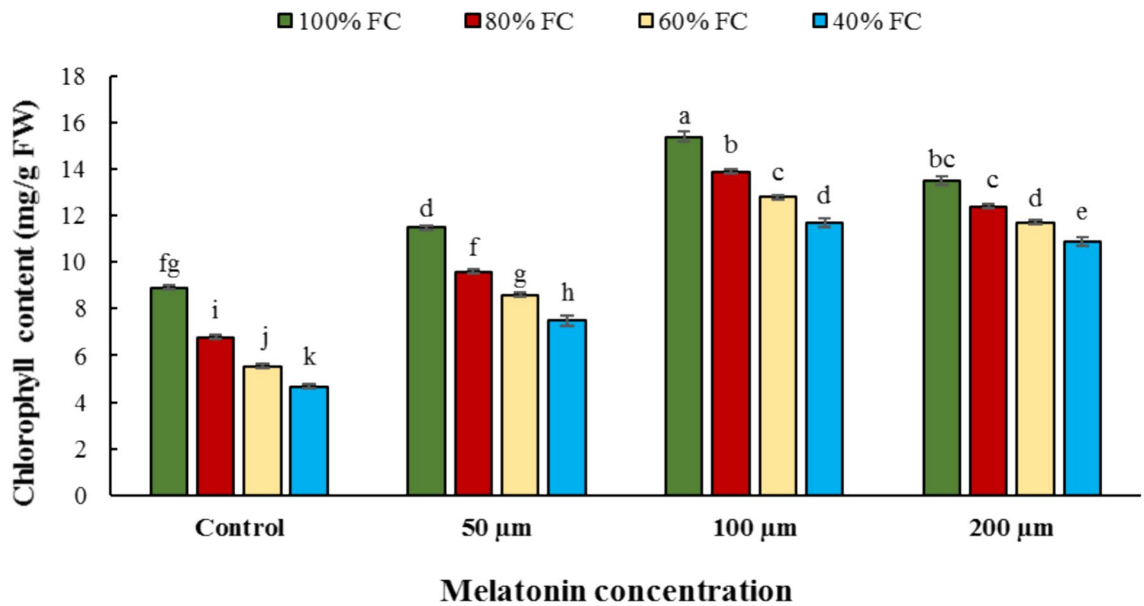


Fig. 2 Effect of melatonin treatment on chlorophyll content of *S. rechingeri* plants under drought stress. The mean comparisons were performed using the LSD test at $p \leq 0.05$ significant level. Data are presented as means ± SE ($n = 3$). Means followed by the same letter(s) are not significantly different

to the control. By increasing MT concentrations and drought stress, the results indicated a higher level of carvacrol.

Antioxidant activity

The FRAP assay was employed to examine the antioxidant activity capacity. The results in Fig. 8B indicate a noteworthy enhancement in antioxidant activity capacity under drought stress compared to the control plants. Various levels of exogenous MT were found to augment the antioxidant activity capacity under drought-stress conditions. The most substantial increase in antioxidant activity capacity was observed in plants treated with 40% FC and 200 µM MT. In comparison, the lowest value was recorded in plants subjected to 100% FC without MT.

Correlation analysis

The results for the correlation between agro-morphological, physio-biochemical, and phytochemical traits (Fig. 9) revealed that the total phenol content had positive and significant correlations with the carvacrol ($r = 0.94$), EOs content ($r = 0.81$) and antioxidant activity ($r = 0.93$). The traits of total phenol content ($r = 0.94$), antioxidant activity ($r = 0.81$), rosmarinic acid ($r = 0.95$), and EOs content ($r = 0.91$) showed a positive and significant correlation with carvacrol content. Also, a positive and significant correlation was observed between rosmarinic acid and total phenol content ($r = 0.96$), carvacrol content ($r = 0.95$), EOs content ($r = 0.85$), and antioxidant activity ($r = 0.87$). Drug weight as an important economic trait had a positive and significant correlation with plant height ($r = 0.94$), leaf length ($r = 0.98$), leaf width ($r = 0.96$), fresh weight ($r = 0.99$), and dry weight ($r = 0.99$) and had a negative and significant correlation with the relative water content. The final function of each medicinal plant in terms of the desired metabolites is obtained from the function of the medicinal organ of the plant. Because the secondary metabolites (including carvacrol and rosmarinic acid) of *S. rechinjeri* are mainly obtained from drug weight, therefore, any factor that causes increasing the drug weight in this plant can be effective in the production of more metabolites. Therefore, these attributes can be considered by plant breeders.

Discussion

Drought stress represents an important abiotic factor impacting plant development and biomass yield by constraining various physiological, biochemical, and molecular pathways such as photosynthesis, protein metabolism, and lipid biosynthesis, among others [35]. Due to the lack of water around the world, determining the exact amount of water needed to produce products seems necessary and necessary to avoid possible water losses [36]. In this study, the effect of MT was evaluated based on agro-morphological, biochemical, and phytochemical traits (especially the content of carvacrol in EOs and rosmarinic acid in extract) of *S. rechingeri* under drought stress. The findings indicated a notable

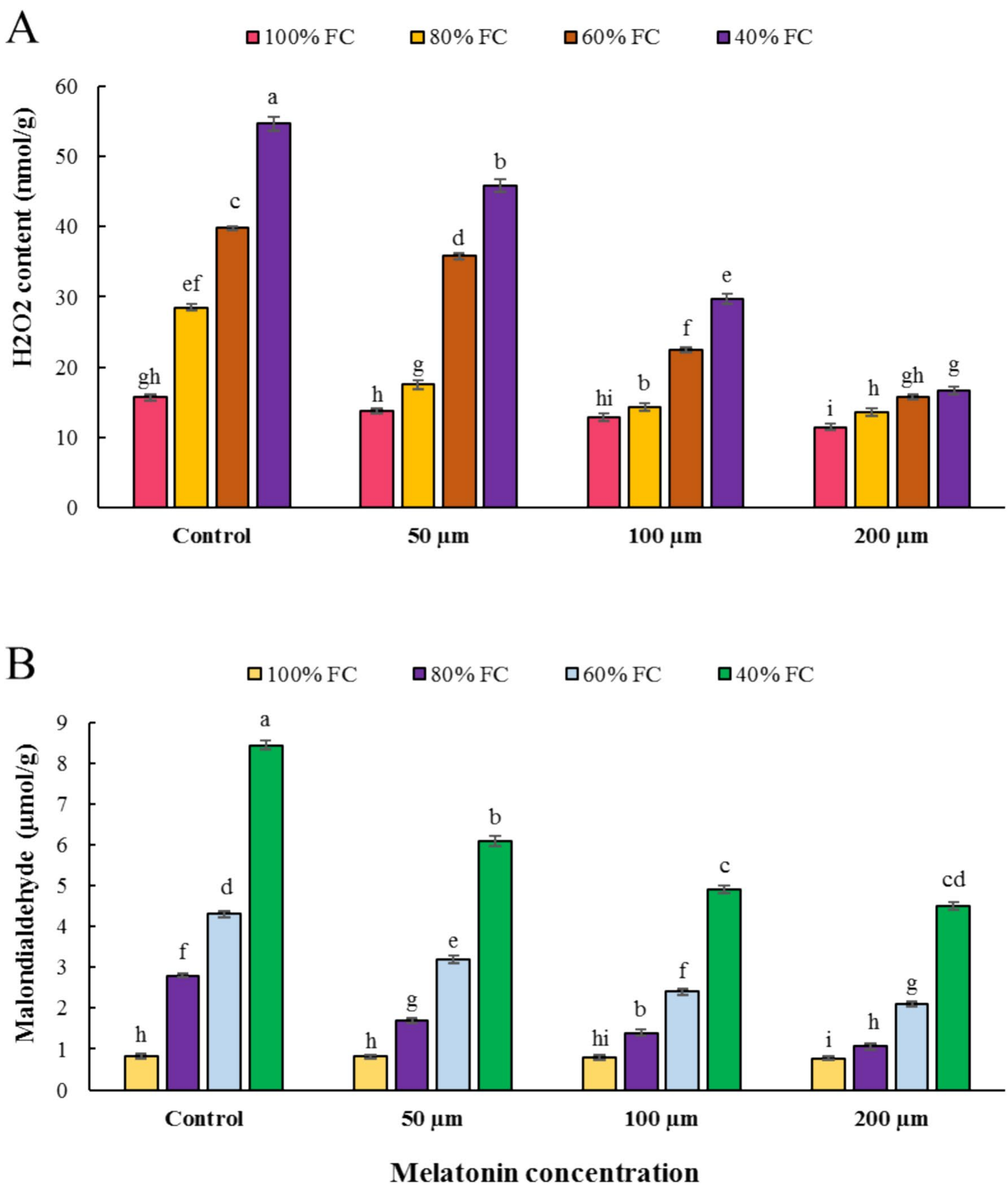


Fig. 3 Effect of melatonin treatment on H_2O_2 content (**A**) and malondialdehyde (**B**) of *S. rechingeri* plants under drought stress. The mean comparisons were performed using the LSD test at $p \leq 0.05$ significant level. Data are presented as means ± SE ($n = 3$). Means followed by the same letter(s) are not significantly different

deterioration in all plant growth parameters of drought-treated plants compared with the control group. This outcome could potentially be attributed to the decline in photosynthesis, heightened evapotranspiration, reduced cell turgidity, restricted CO_2 assimilation caused by stomatal closure, and ultimately, hindered cell division during periods of drought [37]. The introduction of exogenous MT demonstrated a positive impact on plant growth under drought stress, accompanied by an elevation in MT levels (Fig. 10). Particularly noteworthy was the significant enhancement in plant height, leaf length, leaf width, fresh weight, dry weight, and drug weight

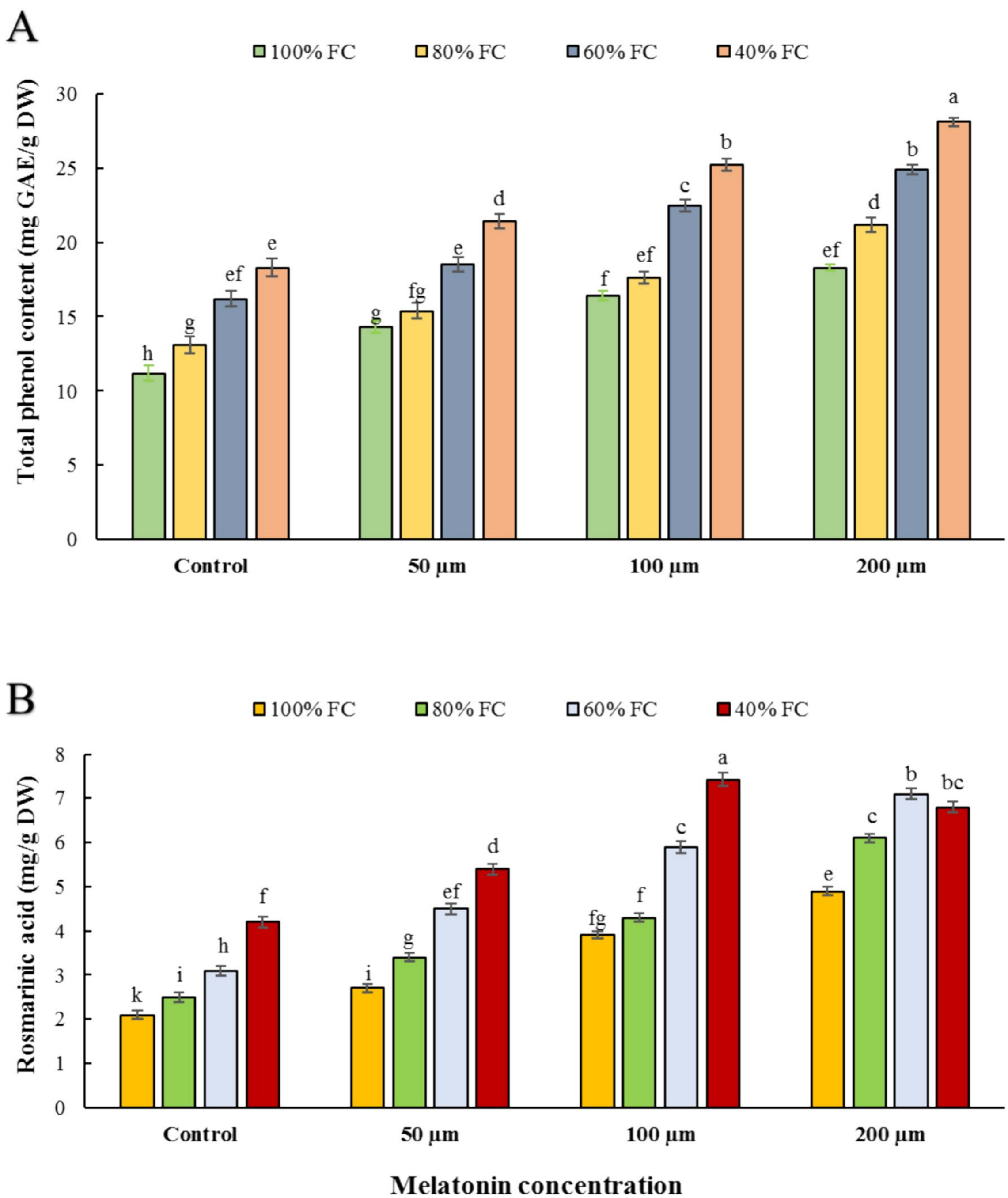


Fig. 4 Effect of melatonin treatment on total phenol content (**A**) and rosmarinic acid (**B**) of *S. rechingeri* plants under drought stress. The mean comparisons were performed using the LSD test at $p \leq 0.05$ significant level. Data are presented as means ± SE ($n = 3$).Means followed by the same letter (s) are not significantly different

observed in the 200 μM MT treatment group, when compared to plants subjected solely to drought conditions. In stressful conditions, MT as a strong antioxidant helps plants against oxidative stress caused by ROS and improves growth traits by increasing the content of chlorophyll and increasing the efficiency of photosynthesis [38]. Furthermore, MT enhances plants' defense mechanisms against environmental stresses, pathogens, and pests, thereby decreasing plants' susceptibility to both biotic and abiotic stresses [39]. In the current study, the reduction in RWC due to drought stress may be attributed to the inhibition of water movement from roots to

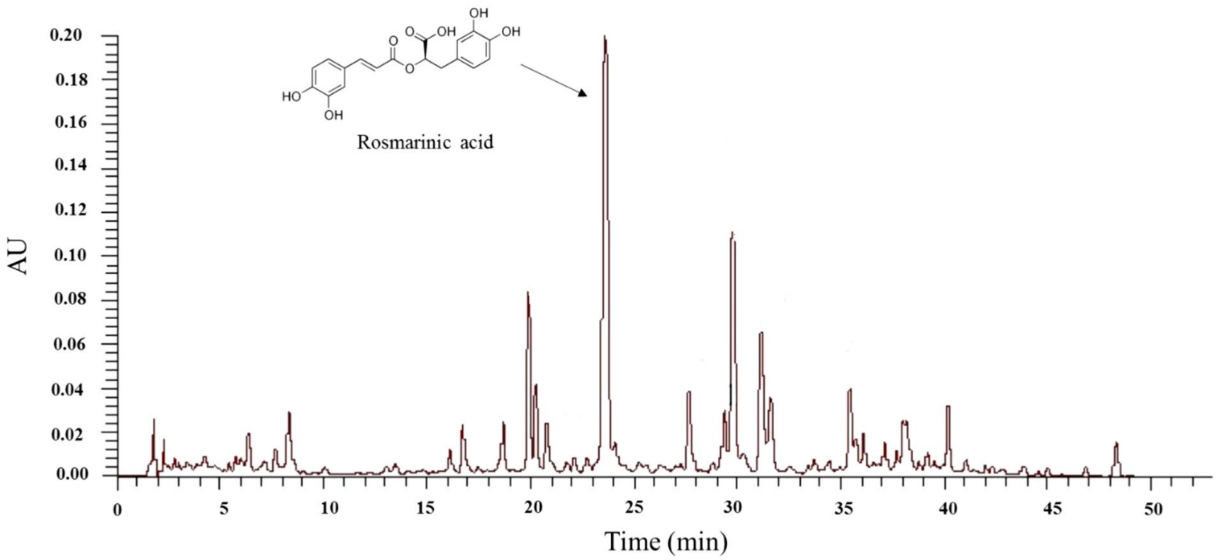


Fig. 5 The HPLC–PDA chromatogram of *S. rechingeri* extract. under treatment of 100 µM MT and 40% FC

shoots, mediated by factors such as mesophyll cell turgidity, low leaf water potential, increased leaf thickness, or reduced soil moisture [37–41]. In their study, Cui et al. [41] suggested that the application of exogenous MT could potentially mitigate the effects of drought stress on plants by regulating water balance and cell turgor. The assessment of EL was conducted to gauge the impact of drought stress on membrane permeability, showing an elevation in EL toward the intercellular spaces under drought conditions. Conversely, plants treated with 200 µM MT exhibited reduced EL levels compared to the untreated control under drought stress conditions. Drought stress-induced lipid peroxidation, triggered by the generation of ROS, resulted in membrane permeability, leading to increased EL levels [42]. The severity and duration of stress directly correlated with membrane damage, while the application of MT alleviated the negative effects of drought stress on *S. rechingeri*. Elevated levels of H_2O_2 were found to promote lipid peroxidation, causing membrane impairment and subsequent electrolyte leakage, as reported by Cui et al. [41]. The vulnerability of chlorophyll to water scarcity is noteworthy, with specific plant species experiencing a decrease in chlorophyll levels in response to drought-induced stress [43, 44]. In the present investigation, a reduction in chlorophyll content was observed under conditions of drought stress. Our observations were substantiated by the research outcomes of Campos et al. [45] and Sharma et al. [46], which proposed that the application of MT on stressed plants resulted in an enhancement of photosynthetic pigment, thereby retarding chlorophyll degradation and leaf chlorosis. Previous studies examining the impact of MT on chloroplast functions have consistently demonstrated its beneficial effects under various abiotic stress conditions [37, 45–49]. The levels of H_2O_2 and MDA exhibited a significant increase in response to the drought stress treatments, according to the findings of the present study. It was observed that the application of MT resulted in a decrease in these levels, indicating the potential of MT to function as an antioxidant in mitigating the damage caused by the accumulation of ROS. The generation of superoxide anions in plant cells under drought-stress conditions is believed to be modulated by MT, either through augmentation of the scavenging process or regulation of superoxide anion production, as suggested by previous studies [50–52]. Furthermore, MT has been shown to enhance the efficiency of H_2O_2 scavenging in plants subjected to drought stress, as reported in various research works [50, 53]. The proficient scavenging of ROS mediated by MT in plants experiencing drought stress conditions serves to protect the integrity of plant cell walls. This protective mechanism is further evidenced by the reduced MDA content levels and diminished electrolyte leakage observed in plants treated with MT under conditions of water deficit [47, 54, 55]. Prolonged exposure to stress and heightened production of ROS in plant organisms may trigger detrimental pathways, such as lipid peroxidation. The quantification of MDA is widely acknowledged as a reliable metric for assessing lipid peroxidation levels [56]. Within the context of the current investigation, MDA content exhibited an escalation in response to drought-induced stress due to lipid peroxidation and impairment of the plasma membrane. Nonetheless, the application of MT demonstrated

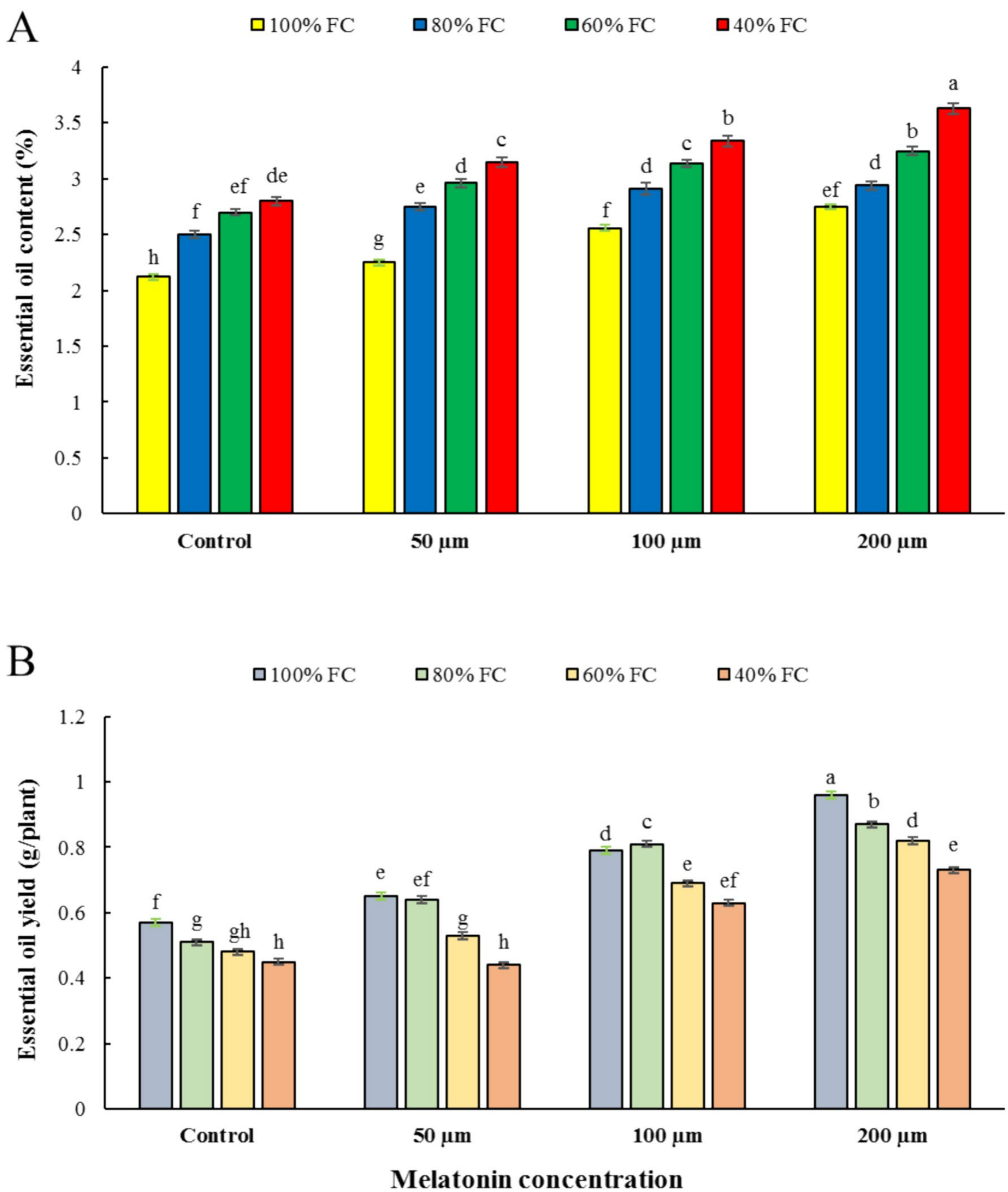


Fig. 6 Effect of melatonin treatment on essential oil content (**A**) and yield (**B**) of *S. rechingeri* plants under drought stress. The mean comparisons were performed using the LSD test at $p \leq 0.05$ significant level. Data are presented as means ± SE ($n = 3$). Means followed by the same letter(s) are not significantly different

a significant mitigation of the harmful impacts associated with drought stress.

Phenolic compounds, such as phenols, represent the secondary metabolites typically synthesized under abiotic stresses [57]. These compounds are crucial in mitigating oxidative damage by detoxifying ROS [35, 37, 57]. The antioxidant activity of phenolic compounds involves the deactivation of lipid ROS or the prevention of hydroperoxide decomposition into ROS [46]. Consequently, it is plausible that the upregulation of phenolic compound production contributed to ROS scavenging in *S. rechingeri* plants under drought conditions. Our study

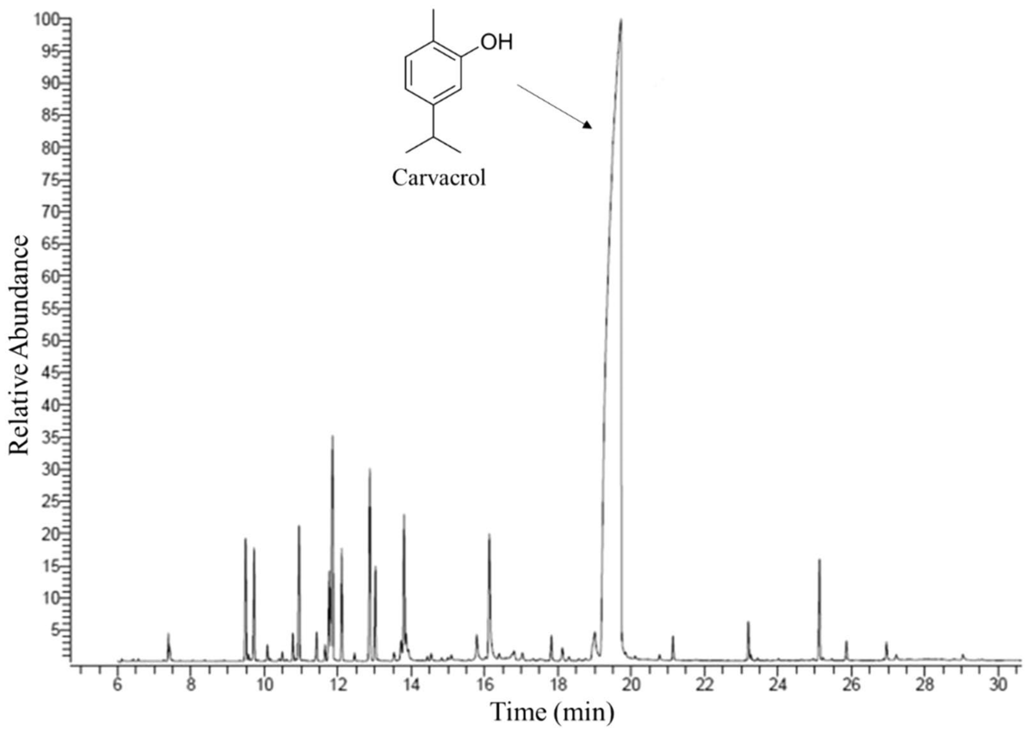


Fig. 7 The GC–MS chromatogram of *S. rechingeri* essential oil under treatment of 200 µM MT and 40% FC

revealed a more excellent total phenol content in plants subjected to drought stress compared to control plants. These findings align with those of Caser et al. [35], who similarly noted an increased total phenol content in plants treated with drought compared to controls. Furthermore, the application of exogenous MT served as a signaling agent in plants experiencing drought stress, leading to a significant increase in synthesizing these secondary metabolites to improve plant resistance against oxidative stress [49].

The extract of *S. rechingeri* is rich in phenolic compounds, particularly rosmarinic acid (RA) [22]. The concentration of RA was influenced by many factors, including species, cultivar, stage of growth, and environmental circumstances [58]. In our study, drought stress notably amplified the RA content. Moreover, the application of MT substantially increased the RA content and heightened the antioxidant capability in the leaves of *S. rechingeri*. In addition, the highest amount of rosmarinic acid was observed in *Ocimum basilicum* L. under drought stress [59] and in *Dracocephalum kotschyi* Boiss. under salt stress [60] at the highest concentration of MT. MT as an abiotic elicitor, induces the production of ROS, regulates defense responses, increases the activity of antioxidant enzymes, and increases the accumulation of phenolic compounds through stimulation of molecular signal transduction and regulation of gene expression which leads to plant immune response. This compound triggers the expression of key genes that lead to the production of Phenylalanine ammonia-lyase (PAL). PAL is an important enzyme in the phenylpropanoid pathway and increases the accumulation of phenolic compounds including RA in plants [60]. These results indicate that MT altered the composition of phenolic compounds in plants exposed to drought, favoring those with superior antioxidant properties. Consequently, these compounds could effectively counteract ROS, leading to a reduction in electrolyte leakage (EL) levels and hydrogen peroxide (H_2O_2) content in the leaves of plants treated with MT compared with those subjected to drought alone. To investigate the beneficial impact of foliar MT application on enhancing drought stress tolerance, the assessment of antioxidant activity serves as a crucial parameter for evaluating ROS scavenger. The FRAP assay indicated a rise in activity under conditions of drought stress alone and when MT was applied in conjunction with drought, compared to plants that were not subjected to stress. Our findings aligned with those of Cui et al. [41] and Ye et al. [61], indicating that the external application of MT contributed to an increase in stress tolerance by enhancing the overall antioxidant capabilities of the plant. Furthermore, we noted a positive correlation between the levels of phenolic

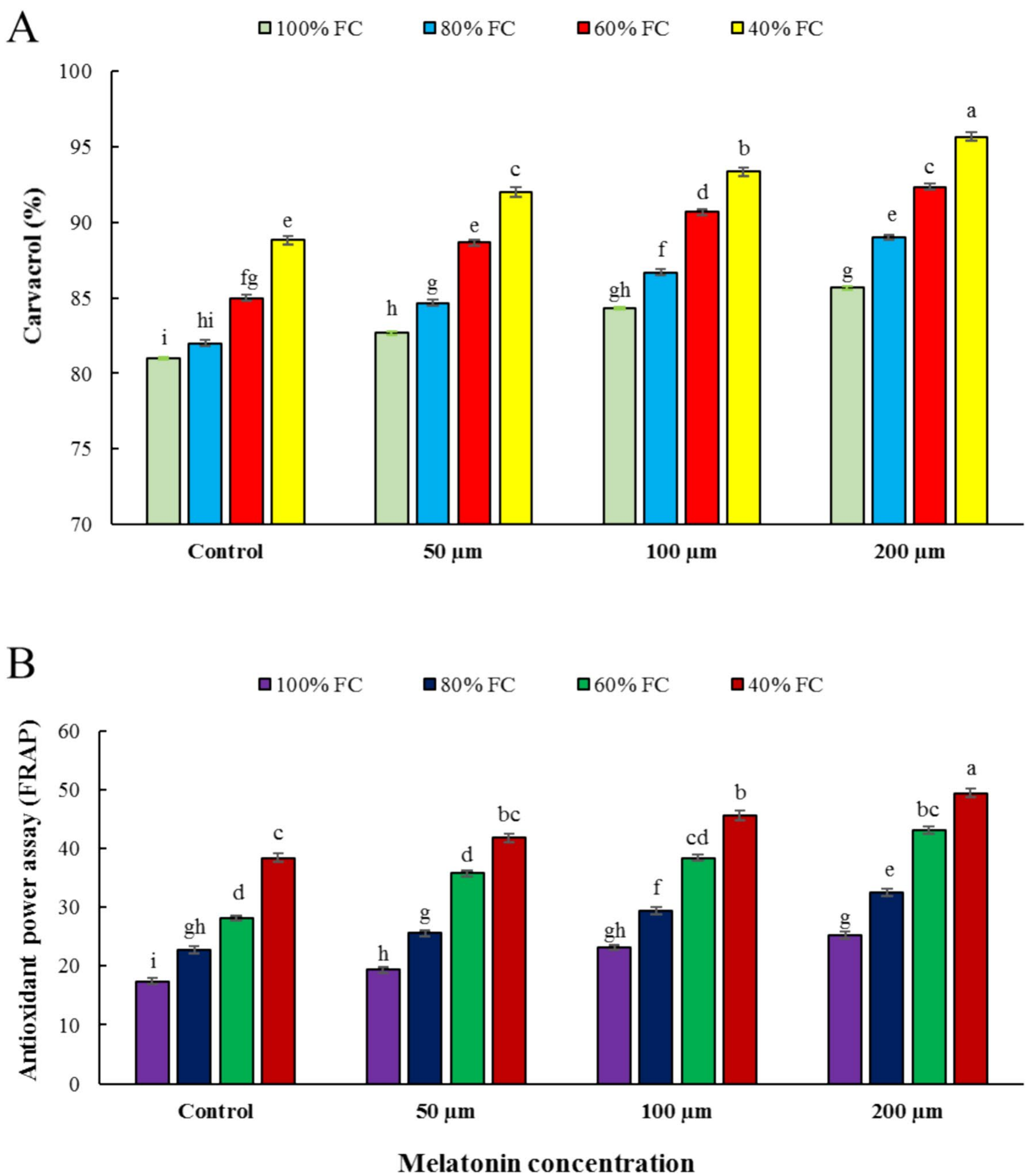


Fig. 8 Effect of melatonin treatment on carvacrol (**A**) and antioxidant power assay (B) of *S. rechingeri* plants under drought stress. The mean comparisons were performed using the LSD method at $p \leq 0.05$ significant level. Data are presented as means ± SE ($n = 3$). Means followed by the same letter(s) are not significantly different

compounds and antioxidant activity. These outcomes suggest that the predominant portion of the antioxidant activity observed in *S. rechingeri* stems from the phenolic compounds. The antioxidative potential is a product of synergistic or antagonistic influences arising from the interactions between various polyphenolic compositions with one another and with other constituents of the food matrix or organism. The augmentation of antioxidant capacity facilitated by MT is not only linked to the heightened levels of endogenous MT and polyphenolic constituents but also their intricate reactions [62]. The content of EOs and their constituent compounds in different plants is affected by environmental and genetic factors [63]. An increase in

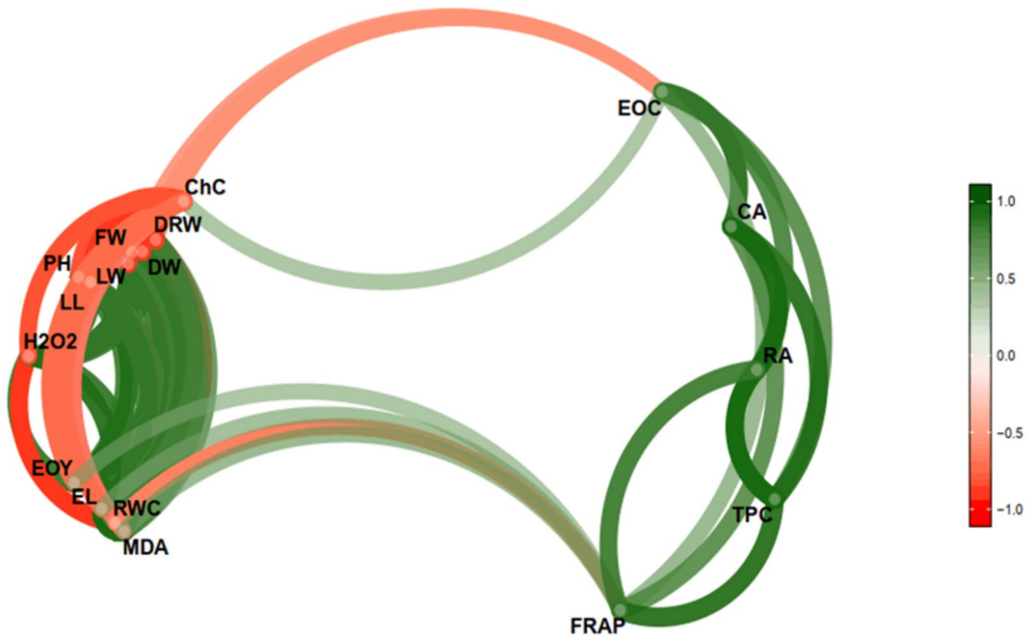


Fig. 9 Network correlation representation of morphological, biochemical and phytochemical traits of *S. rechingeri* under melatonin and drought treatments. TPC: Total phenol content; CA: Carvacrol; RA: Rosmarinic acid; EOC: Essential oil content; FRAP: Antioxidant power assay; EL: Electrolyte leakage; MDA: Malondialdehyde; EOY: Essential oil yield; H_2O_2: Hydrogen peroxide; DW: Dry weight; DRW: Drug weight; FW: Fresh weight; LL: Leaf length; LW: Leaf weight; PH: Plant height; ChC: Chlorophyll content; RWC: Relative water content

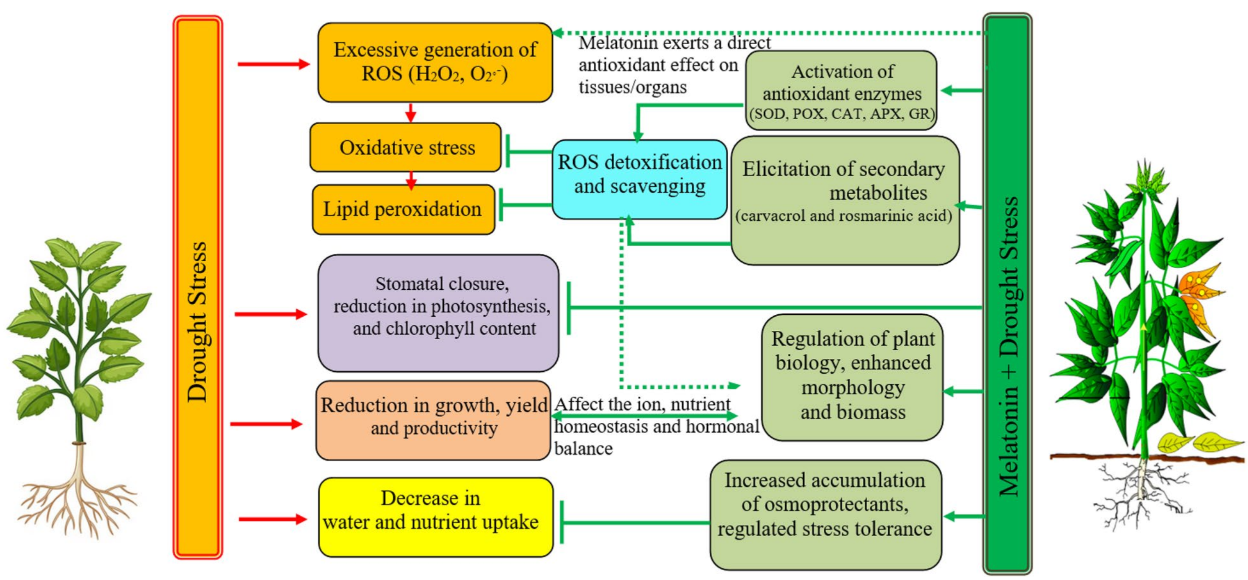


Fig. 10 The multiple mechanisms of melatonin-induced alleviation of drought sress responses in plants based on the present study's findings and related literature [66]. The negative impacts of drought stress are mainly due to the over-accumulation of reactive oxygen species [(ROS) including superoxide (O_2), hydroxy radical (OH), hydrogen peroxide (H_2O_2), and alkoxy radical (RO)], and the inhibition of cellular processes like photosynthesis, and catabolic of chlorophyll, resulting in decreased growth and metabolism. The disturbance of cellular redox regulation caused by drought stress induces further ROS production. This process is followed by damage to DNA, proteins, and lipids via oxidative burst. Several mechanisms behind the exogenous melatonin-mediated alleviation of drought stress can be discussed: (i) regulates the activation of enzymatic and non-enzymatic (*i.e.*, secondary metabolites such as carvacrol and rosmarinic acid) antioxidants, (ii) enhances the accumulation of osmoprotectants through the maintenance of organic compounds such as amino acids, which further affect the retention of water in the leaf tissue. Moreover, exogenously applied melatonin may also act directly as an antioxidant agent against ROS and lipid peroxidation, ensuring the survival of plants under drought stress conditions

the EOs content in different Lamiaceae species under drought stress has been reported [64]. The external application of MT has increased the content of EOs of bitter orange [65], rosemary [66], and *Salvia* species [67] in stressful conditions.

Conclusion

According to our findings, the administration of MT may serve as an effective strategy to enhance the growth of *S. rechingeri* plants under drought stress. Specifically, the application of MT at a concentration of 200 μM was found to enhance various morphological growth parameters, including plant height, leaf length, leaf width, fresh weight, dry weight, and drug weight in situations of limited water availability. The application of MT led to improved physiological indicators such as RWC and chlorophyll content during water stress. Furthermore, the levels of EL, MDA, and H_2O_2 generally decreased following MT treatment. The phytochemical traits analysis revealed that under water stress conditions, the production of secondary metabolites, including TPC, RA, EOs content, EOs yield, and carvacrol, exhibited an increase. Furthermore, the application of MT treatment further enhanced these traits, specifically in drought stress. Our investigation demonstrated that MT mitigates the impact of drought stress predominantly by stimulating the antioxidant defense mechanism in *S. rechingeri* plants. Further research efforts exploring the complexities of this method and focusing on clarifying the effects of externally administered MT on changes in particular molecular and biochemical pathways (including the carvacrol biosynthesis pathway) will provide new perspectives on the direct and indirect mechanisms of MT 's effectiveness in plant systems.

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Author contributions
YD: conceptualization, methodology, investigation, formal analysis, writing—original draft. GE: supervision, conceptualization, methodology, data curation, writing—original draft. MBA: conceptualization, methodology, data curation, validation, writing—review and editing. MM: investigation, formal analysis, validation, writing. M.G: software, formal analysis, writing—review and editing.

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Data availability
All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Declarations

Ethics approval and consent to participate
This manuscript is an original research and has not been published or submitted in other journals. This study does not involve any human or animal testing.

Consent for publication
All authors listed have read the complete manuscript and have approved submission of the paper.

Competing interests
The authors declare no competing interests.

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