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CATHARANTHUS ROSEUS (L.) G. DON. MEDICINAL VALUE UNDER WATER-STRESS AND SALICYLIC ACID TREATMENT

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Abstract

Water stress is one of the main environmental factors that limit the productivity and medicinal quality of ornamental and medicinal plants. The present research evaluated the effect of long term irrigation intervals and salicylic acid (SA) application on growth, biochemical composition, antioxidant activity and antimicrobial potential of *Catharanthus roseus* (L.) G. Don. (Apocynaceae). Plants were watered at two intervals namely 2 days (2DWI) and 6 days (6DWI) along with foliar application of SA at different concentration (0, 0.01, 0.05 and 0.10 mM). The prolonged irrigation interval significantly reduced vegetative growth and flowering attributes such as leaf number, leaf area, plant dry weight, plant height and flower number per plant. On the other hand, drought stress led to increased total carbohydrates, proline accumulation, antioxidant activity and total phenolic content. Activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) were also improved under water-stress conditions. Foliar application of SA significantly alleviated the negative impact of drought stress and improved all growth parameters studied in the two irrigation regimes. The highest concentration of SA (0.10 mM) showed the most favourable improvements in vegetative growth, chlorophyll content and flower production. Furthermore, SA treatment increased antioxidant capacity by decreasing DPPH and β-carotene-linoleic acid IC50 values and increasing phenolic accumulation. Leaf extracts of *C. roseus* showed considerable antibacterial and antifungal activities, which were improved further with longer irrigation intervals and SA application. In general, the strongest antimicrobial activity was observed under 6DWI combined with 0.10 mM SA. The results indicate that controlled water stress enhanced the accumulation of bioactive compounds in *C. roseus*. Exogenous SA application could effectively alleviate the inhibition of growth and improve the physiological performance, antioxidant defence and medicinal value under drought stress. SA application could serve as a sustainable agronomic strategy for maintaining medicinal plant productivity and improving phytochemical quality under water-limited conditions, thereby supporting sustainable cultivation practices in arid and semi-arid regions.

Key words: *Catharanthus roseus*, Water stress, Antioxidants, Salicylic acid, Foodborne pathogens

Introduction

Ornamental plants contain a lot of medicinal and aromatic plants; they have attracted researchers worldwide as well as the industrial company's attention because of their pharmacological value and ability to survive under harsh climatic conditions. These dual purpose species, include *C. roseus* which produces terpenoid indole alkaloids such as vinblastine and vincristine; which are commonly used in traditional medicine and modern anticancer treatment (Kumar *et al*., 2021; Liu *et al*., 2021; Sharma *et al*., 2024). In addition to its known medical applications, *C. roseus* is commonly grown as decorative bedding plants due to its continuous flowering, adaptation to environmental conditions and high temperature and generally dry weather tolerance (Hassan *et al*., 2021; Salem *et al*., 2022).

C. roseus is of medicinal interest mainly due to the wide range of phytochemical molecules such as alkaloids, flavonoids, terpenoids and phenolic compounds with antibacterial, antimicrobial, antidiabetic and anticancer effects (Kumar *et al*., 2021; Sharma *et al*., 2024). Environmental stressors relatively can influence the synthesis, overall production and storage of different secondary metabolites by activating stress-responsive metabolic pathways (Liu *et al*., 2021; Ababaf *et al*., 2021). Controlled drought is therefore a viable strategy to increase the medicinal quality and phytochemical yield of medicinal herbs.

Drought stress is one of the most important abiotic stresses. It is one of the major environmental constraints on agricultural productivity on the planet. Water shortage causes osmotic imbalance and oxidative stress that are detrimental for growth, photosynthesis, membrane stability, nutritional intake and reproductive development (Farooq *et al*., 2019; Saleem *et al*., 2021). Drought stress might inhibit vegetative growth of medicinal plants like *C. roseus* but it could also increase biosynthesis of secondary metabolites and antioxidant compounds as a plant defence response (Ababaf *et al*., 2021; Hassan *et al*., 2021). Water deficit causes over-accumulation of reactive oxygen species (ROS) such as superoxide radicals, singlet oxygen, hydroxyl radicals and hydrogen peroxide that cause oxidative damages to proteins, nucleic acids, pigments and membrane lipids (Hasanuzzaman *et al*., 2020; Saleem *et al*., 2021). Plants protect themselves against oxidative stress by activating enzymatic antioxidant systems such as SOD, CAT, and APX and non-enzymatic antioxidants such as phenolics, flavonoids and proline (Hasanuzzaman *et al*., 2020; Sharma *et al*., 2023). Many recent studies have shown that in many cases drought stress drastically changes

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physiological and biochemical characters of *C. roseus*. Recent report indicated that drought stress may increase the antioxidant activities and stimulate the osmotic adjustment while reduced the growth and chlorophyll composition (Salem *et al*., 2022). Another report by Ababaf *et al*., (2021) found similar results and reported the accumulation of alkaloids. In the same topic, Al-Huqail and Ali, (2021) found that abiotic stress can significantly influence the morphophysiological traits and secondary metabolism. Salicylic acid (SA), as a phytohormone belonging to phenols may affects plant growth and flowers under different stress conditions. This phytohormone had been associated with the antioxidant drought tolerance mechanism as well as the osmotic adjustment, ion uptake, cell and memberane stability and expression of stress-responsive genes under abiotic stress conditions (Peng *et al*., 2021; Saleem *et al*., 2021). Recent investigations show a significant role as mediator of signalling for enhancing tolerance to drought, salinity, heavy metals and temperature stress through the control of antioxidant defense systems and maintenance of the redox equilibrium in cells (Rhaman *et al*., 2020; Sharma *et al*., 2023).

The external application of SA has been reported to enhance the drought tolerance of different plant species including medicinal species. Salem *et al*., (2022) showed that application of SA can improve the biomass production, leaf area and other phytochemical parameters like chlorophyll content as drought tolerance mechanism in *C. roseus* and that it can increase the alkaloid production (Hassan *et al*., 2021) and enhance the flowering traits, antioxidant capacity, chlorophylls and membrane and cellular stability of different ornamental plants such as petunia, chrysanthemum, marigold, basil and geranium under stressful environmental conditions (Elhindi *et al*., 2023; Yang *et al*., 2023). Besides its role in stress tolerance, SA has been suggested to be involved in enhanced biological production of secondary metabolites in medicinal plants. Liu *et al.,* (2021) showed that the syntheses of the alkaloids is associated with hormonal signalling in *C. roseus* plants and it may enhance the production of different phenolics and antioxidants in stressed medicinal plants (Rhaman *et al*., 2020; Sharma *et al*., 2023).

Unlike previous reports that mainly focused on either drought stress or salicylic acid application separately, the present study evaluated their combined influence on growth performance, antioxidant metabolism, and antimicrobial activity of *C. roseus* under prolonged irrigation intervals

Studies on the adaptation ability of medicinal plants to drought are increasing. However, there is dearth of information on the combined effects of extended irrigation intervals and SA administration on the growth traits, antioxidant metabolism and antibacterial activity of *C. roseus* under water-stress conditions. So the present study was done to investigate the effect of irrigation intervals and foliar application of salicylic acid on vegetative development, biochemical parameters, antioxidant defense systems and antibacterial potentialities of *C. roseus*.

Materials and Methods

Plant material: Uniform young plants (approximately 7 cm height) of *C. roseus* (L.) G. Don. were obtained from commercial nurseries in Riyadh, Saudi Arabia, spring 2023. The experiment was conducted in a controlled glasshouse at the University of King Saud. Plants were transplanted into 2.1 L pots containing a mixture of peat and perlite (3:1, w/w). Following growth establishment in 1 week, the plant were supplemented with a balanced fertilizer (Crystalon® 20:20:20 N:P:K) at 2 g L^{-1}. Plants were acclimatized for 3 weeks under controlled environmental conditions (15.1°C–27.5°C, 58%–67% relative humidity, and ~1000 µmol m^{-2} s^{-1} PAR at midday), consistent with the optimal greenhouse cultivation parameters for ornamental species. During this period, irrigation (38–50 mL per plant) was applied daily. After acclimatization, the plants underwent two irrigation regimes: watering every 2 days (2DWI) and every 6 days (6DWI) for six weeks to replicate well-watered and drought stress, respectively. SA solutions (degree of deacetylation > 95%; Sigma Aldrich, Germany) were applied on leaves at different concentration (0, 0.01, 0.05 and 0.10 mM) until run-off, two weeks prior to the stress imposition, following the previously described methods for biostimulant applications (Rojas-Pirela *et al*., 2024). The untreated plants were used as controls. The plants were arranged in a split-plot design employing a randomized complete block design (RCBD). The irrigation treatments allocated to main plots and SA concentrations were the subplots. Each treatment was replicated 5 times in each block of the 3 blocks.

Morphological and physiological measurements: Plants were harvested after 6 weeks of treatment. Growth parameters including plant height and number of leaves were measured. The leaf area was determined using digital image analysis with AutoCAD software. The total dry biomass was obtained after oven-drying the plant samples at 30°C to a constant weight. The total carbohydrate content was quantified in fresh leaves following DuBois *et al*., (1956). The mineral elements were determined from leaf sap extracts by inductively coupled plasma spectrophotometry (K^+ and Ca^{2+}). The proline composition in leaves was determined spectrophotometrically at 520 nm according to Bates *et al*., (1973). Antioxidants, chlorophyll, phenolics and enzyme activities Leaf samples were air-dried, ground to a fine powder and extracted in methanol (99%) for 24 h in the dark following the established protocol for phenolic compound extraction (Elansary *et al*., 2020). The antioxidant activity was determined using the DPPH radical scavenging experiment (Elansary *et al*., 2020) and the β-carotene–linoleic acid bleaching method (Elansary *et al*. 2020). The absorbance was measured at 517 and 470 nm. IC50 values were calculated from the inhibition curves. Butylated hydroxytoluene (BHT) was used as a positive control. Total phenolic content was quantified by the Folin–Ciocalteu method and expressed as gallic acid equivalents (mg GAE g^{-1} extract) (Singleton & Rossi, 1965).Chlorophyll content was determined spectrophotometrically as described by Moran & Porath (1980).

The activities of antioxidant enzymes such as CAT, APX and SOD and hydrogen peroxide (H2O2) content were determined by the standard spectrophotometric procedures (Nakano & Asada, 1981; Aebi, 1984).

Antimicrobial assays and medicinal properties: The antimicrobial activity of methanolic leaf extracts against selected gram-positive and gram-negative bacteria and fungal strains was evaluated. The selected bacteria for the analyses included *Listeria monocytogenes* (clinical isolate), *Bacillus cereus* (ATCC 14579), *Staphylococcus aureus* (ATCC 6538), *Micrococcus flavus* (ATCC 10240), *Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 35210). An economic group of fungi were tested in this experiment and included *Candida albicans* (ATCC 12066), *Penicillium ochrochloron* (ATCC 48663), *Aspergillus niger* (ATCC 6275), *A. ochraceus* (ATCC 12066), and A. flavus (ATCC 9643). Antibacterial and antifungal effects were tested using the microdilution method (Elansary *et al*., 2018). The minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibited the visible growth of the microorganisms, while the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were defined as the lowest concentrations that reduced the initial inoculum by 99.5%. Positive controls included streptomycin and ampicillin for antibacterial assays and fluconazole and ketoconazole for antifungal assays, whereas 5% dimethyl sulfoxide was used as a negative control. All experiments were conducted in triplicate and repeated twice.

Statistical analysis

Data are expressed as mean values ± standard deviation. One-way analysis of variance (ANOVA) was used for statistical analysis, and the least significant difference (LSD) test at $p<0.05$ was used to separate the means. We used SPSS (PASW Statistics, version 21) to do the analyses. The plants were arranged in a split-plot design employing a randomized complete block design (RCBD).

Results

Growth performance: The results presented in Table 1 demonstrate that both irrigation interval and salicylic acid (SA) application significantly affected vegetative growth and flowering characteristics of *C. roseus*.

Compared with the well-watered untreated plants (2DWI without SA), prolonged irrigation intervals (6DWI without SA) reduced leaf area, plant dry weight, and flower number by approximately 54.0%, 50.0%, and 33.1%, respectively, indicating the severe inhibitory effects of drought stress on vegetative growth and flowering of *Catharanthus roseus*. However, foliar application of salicylic acid substantially alleviated these reductions. Under normal irrigation conditions, treatment with 0.10 mM SA increased leaf number, leaf area, dry weight, plant height, and flower number by approximately 24.2%, 16.4%, 21.8%, 21.9%, and 17.5%, respectively, compared with untreated control plants.

Under prolonged irrigation intervals, 0.10 mM SA increased leaf number by approximately 36.3%, leaf area by 31.8%, dry weight by 35.9%, plant height by 34.4%, and flower number by 32.7% relative to untreated drought-stressed plants.

Leaf biochemical composition: The results presented in Table 2 demonstrate that both irrigation interval and salicylic acid (SA) application significantly affected leaf biochemical composition of *C. roseus*. Water deficit increased the accumulation of osmoprotective and metabolic compounds in the leaves. Total carbohydrates, potassium (K), calcium (Ca), and proline contents were generally higher under 6DWI than under 2DWI. Proline accumulation, a typical stress-response indicator, increased from 1.41 mg g^{-1} DW in the well-watered untreated plants to 1.54 mg g^{-1} DW under water stress.

SA treatment further enhanced the accumulation of these biochemical constituents. The highest values were consistently recorded with 0.10 mM SA under 6DWI, including 15.34% total carbohydrates, 30.5 mg g^{-1} DW K, 4.31 mg g^{-1} DW Ca, and 1.78 mg g^{-1} DW proline. These findings indicate that SA improved osmotic adjustment and nutrient retention under drought condition

Antioxidant activity and chlorophyll: Table 3 shows the antioxidant activity (DPPH and β-carotene assays), total phenolic content, and total chlorophyll content. Leaf extracts from drought-stressed plants exhibited enhanced antioxidant activity. Lower IC_{50} values in both DPPH and β-carotene-linoleic acid assays indicated stronger free radical scavenging activity under water stress and SA application. The strongest antioxidant activity was observed in plants treated with 0.10 mM SA under 6DWI, with DPPH and β-carotene IC50 values decreasing to 6.3 and 6.5 μg mL^{-1}, respectively.

Total phenolic content increased significantly under drought stress and with increasing SA concentration. The maximum phenolic content (14.5 mg GAE g^{-1}) was recorded under 6DWI with 0.10 mM SA, suggesting stimulation of secondary metabolite biosynthesis under stress conditions.

In contrast, total chlorophyll content declined under prolonged irrigation intervals, indicating drought-induced impairment of photosynthesis. Chlorophyll content decreased from 1.71 mg g^{-1} FW in control plants at 2DWI to 1.11 mg g^{-1} FW under untreated 6DWI plants. However, SA application partially restored chlorophyll levels, especially at 0.10 mM SA.

Antioxidant enzyme activities (SOD, CAT, and APX): The activities of the following key antioxidant enzymes: SOD, CAT, and APX. The activities of antioxidant enzymes increased in response to drought stress and SA treatment (Fig. 1). This enhancement reflects activation of the enzymatic antioxidant defense system to counteract oxidative damage caused by water deficit.

Hydrogen peroxide (H_2O_2) content also increased under prolonged irrigation intervals (Fig. 2), confirming the occurrence of oxidative stress. However, SA-treated plants showed moderated H_2O_2 accumulation compared with untreated stressed plants, indicating improved reactive oxygen species scavenging capacity.

Table 1. Irrigation intervals and SA effects on leaf number and area, dry weight, and plant height in *C. roseus.* Values are expressed as means (± sd).

Water interval	Treatment with SA (mM)	Leaf number (leaf plant^{-1})	Leaf area (cm^2 plant^{-1})	Plant dry weight (g plant^{-1})	Plant height (cm)	Number of flowers per plant
2DWI	0	17.8 ± 0.3c*	352.7 ± 11.1c	7.8 ± 0.1c	20.1 ± 0.1c	25.1 ± 0.2c
	0.01	19.0 ± 0.4b	381.6 ± 8.2b	8.7 ± 0.2b	22.8 ± 0.1b	26.8 ± 0.1b
	0.05	20.1 ± 0.2b	393.2 ± 12.1b	8.9 ± 0.1b	22.9 ± 0.2b	28.9 ± 0.3a
	0.10	22.1 ± 0.1a	410.5 ± 11.3a	9.5 ± 0.1a	24.5 ± 0.1a	29.5 ± 0.1a
6DWI	0	10.2 ± 0.1f	162.1 ± 8.7f	3.9 ± 0.1f	12.5 ± 0.1f	16.8 ± 0.1g
	0.01	12.2 ± 0.3e	182.2 ± 12.3e	4.7 ± 0.2e	15.1 ± 0.3e	18.6 ± 0.2f
	0.05	12.5 ± 0.1e	190.1 ± 7.6e	4.9 ± 0.2e	15.8 ± 0.1e	20.9 ± 0.1e
	0.10	13.9 ± 0.1d	213.7 ± 10.5d	5.3 ± 0.1d	16.8 ± 0.2d	22.3 ± 0.2d

*Means followed by different letters within columns are significantly different, based on the least significant difference test ($p \leq 0.05$)

Table 2. Irrigation intervals and SA effects on total carbohydrate, K, Ca, and proline composition in the leaves of *C. roseus leaves.* Values are means (± sd).

Water interval	Treatment with SA (mM)	Total carbohydrates (% DW)	K (mg g^{-1} DW)	Ca (mg g^{-1} DW)	Proline (mg g^{-1} DW)
2DWI	0	11.01 ± 0.1f	20.3 ± 0.3e	3.52 ± 0.03d	1.41 ± 0.01e
	0.01	12.81 ± 0.2d	24.6 ± 0.2c	3.85 ± 0.02b	1.58 ± 0.03c
	0.05	13.11 ± 0.1c	25.2 ± 0.1c	3.91 ± 0.05b	1.59 ± 0.05c
	0.10	14.45 ± 0.5b	27.3 ± 0.1b	4.17 ± 0.01a	1.65 ± 0.01b
6DWI	0	11.95 ± 0.3e	23.7 ± 0.3d	3.77 ± 0.02c	1.54 ± 0.01d
	0.01	13.55 ± 0.1c	27.1 ± 0.5b	3.94 ± 0.01b	1.63 ± 0.02b
	0.05	13.64 ± 0.3c	28.3 ± 0.1b	3.95 ± 0.03b	1.69 ± 0.01b
	0.10	15.34 ± 0.2a	30.5 ± 0.3a	4.31 ± 0.01a	1.78 ± 0.01a

*Means followed by different letters within columns are significantly different, based on the least significant difference test ($p \leq 0.05$)

Table 3. DPPH and β-Carotene-linoleic acid in leaf extracts, phenolic composition, and total chlorophyll of *C. roseus.* Values are means of triplicate determinations ±sd.

Water interval	Treatment with SA (mM)	Free radical scavenging activity of DPPH (IC_{50}, µg ml^{-1})	β-Carotene-linoleic acid assay (IC_{50}, µg ml^{-1})	Total phenolic content (mg GAE g^{-1})	Total chlorophyll content (mg g^{-1} FW)
2DWI	0	7.9 ± 0.05a	8.5 ± 0.05a	11.3 ± 0.3 c	1.71 ± 0.02c
	0.01	7.5 ± 0.03b	8.1 ± 0.03b	12.5 ± 0.1 b	1.93 ± 0.03b
	0.05	7.3 ± 0.01b	7.9 ± 0.01b	12.7 ± 0.1 b	1.98 ± 0.01b
	0.10	6.9 ± 0.03c	7.5 ± 0.03c	13.5 ± 0.2 b	2.21 ± 0.01a
6DWI	0	7.2 ± 0.02b	8.2 ± 0.01b	12.5 ± 0.2 b	1.11 ± 0.01f
	0.01	6.8 ± 0.02c	7.5 ± 0.03c	13.4 ± 0.3 a	1.25 ± 0.01e
	0.05	6.7 ± 0.03c	7.3 ± 0.02c	13.7 ± 0.1 a	1.29 ± 0.02e
	0.10	6.3 ± 0.01d	6.5 ± 0.01d	14.5 ± 0.1b	1.38 ± 0.01d

*Means followed by different letters within columns are significantly different based on the least significant difference test ($p \leq 0.05$)

Table 4. MIC and MBC of *C. roseus* leaf extracts (mg^{-1}mL) against bacteria.

Water interval	Treatment with SA (mM)	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus cereus*	*Micrococcus flavus*	*Pseudomonas aeruginosa*	*Listeria monocytogenes*
2DWI	0	0.21 ± 0.2	0.15 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.25 ± 0.01	0.22 ± 0.02
		0.58 ± 0.01	0.35 ± 0.01	0.18 ± 0.01	0.23 ± 0.01	0.56 ± 0.03	0.41 ± 0.03
	0.01	0.19 ± 0.01	0.14 ± 0.03	0.08 ± 0.01	0.09 ± 0.02	0.24 ± 0.02	0.21 ± 0.02
		0.54 ± 0.01	0.33 ± 0.01	0.17 ± 0.02	0.21 ± 0.01	0.55 ± 0.03	0.40 ± 0.04
	0.05	0.18 ± 0.01	0.13 ± 0.01	0.07 ± 0.00	0.08 ± 0.01	0.22 ± 0.02	0.20 ± 0.02
		0.47 ± 0.01	0.31 ± 0.01	0.16 ± 0.01	0.19 ± 0.01	0.53 ± 0.02	0.38 ± 0.01
	0.10	0.16 ± 0.01	0.11 ± 0.01	0.06 ± 0.01	0.07 ± 0.02	0.20 ± 0.01	0.19 ± 0.01
		0.45 ± 0.01	0.29 ± 0.01	0.14 ± 0.01	0.18 ± 0.01	0.50 ± 0.03	0.37 ± 0.031
6DWI	0	0.20 ± 0.01	0.14 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.24 ± 0.01	0.20 ± 0.02
		0.51 ± 0.01	0.33 ± 0.01	0.17 ± 0.01	0.22 ± 0.01	0.54 ± 0.04	0.39 ± 0.01
	0.01	0.18 ± 0.03	0.12 ± 0.07	0.07 ± 0.01	0.08 ± 0.02	0.22 ± 0.03	0.18 ± 0.01
		0.48 ± 0.01	0.30 ± 0.01	0.15 ± 0.02	0.20 ± 0.01	0.53 ± 0.01	0.37 ± 0.01
	0.05	0.16 ± 0.03	0.11 ± 0.07	0.06 ± 0.01	0.07 ± 0.02	0.21 ± 0.03	0.16 ± 0.01
		0.43 ± 0.01	0.28 ± 0.01	0.14 ± 0.01	0.18 ± 0.02	0.50 ± 0.01	0.35 ± 0.01
	0.10	0.15 ± 0.01	0.10 ± 0.03	0.06 ± 0.01	0.06 ± 0.01	0.19 ± 0.02	0.15 ± 0.01
		0.39 ± 0.01	0.27 ± 0.01	0.13 ± 0.02	0.16 ± 0.01	0.47 ± 0.05	0.32 ± 0.03
	Streptomycin	0.10 ± 0.01	0.22 ± 0.01	0.04 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.15 ± 0.01
		0.40 ± 0.01	0.43 ± 0.01	0.13 ± 0.01	0.19 ± 0.02	0.14 ± 0.01	0.31 ± 0.01
	Ampicillin	0.22 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.02	0.14 ± 0.01	0.15 ± 0.01
		0.41 ± 0.01	0.13 ± 0.01	0.16 ± 0.01	0.16± 0.01	0.21 ± 0.01	0.29 ± 0.01

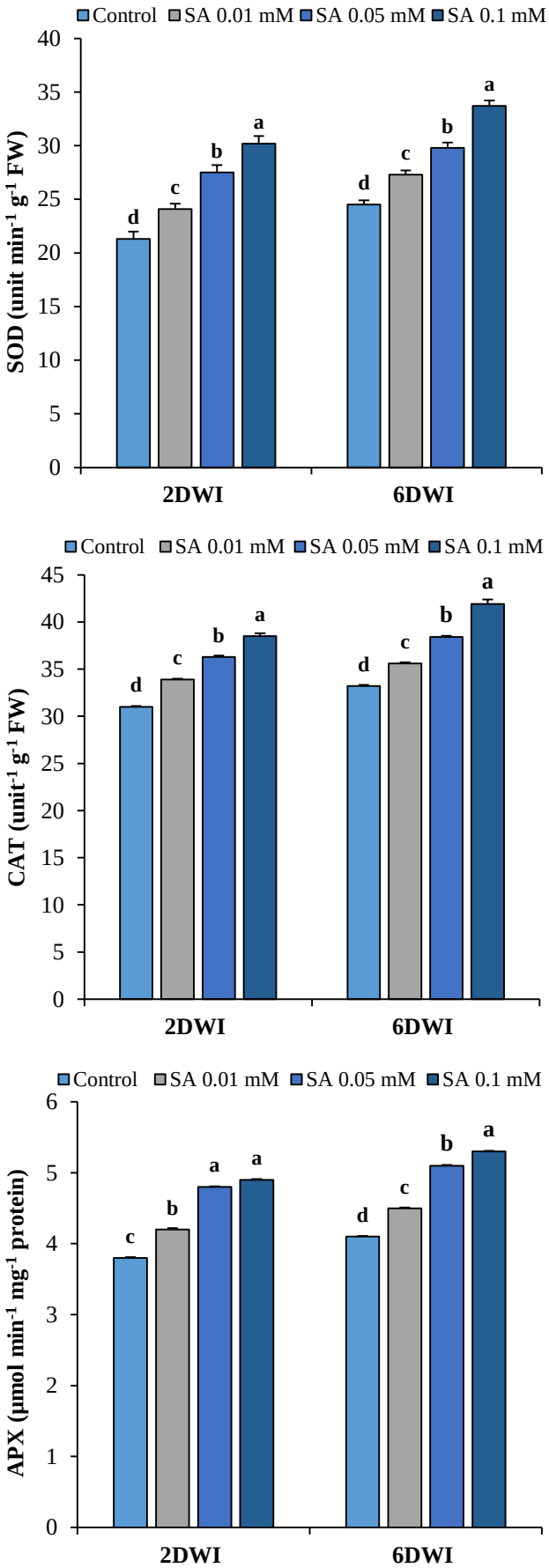

Fig. 1. SOD, CAT, and APX activities in *C. roseus* subjected to prolonged irrigation intervals and different SA concentrations. *Means followed by different letters within columns are significantly different based on the least significant difference test ($p \leq 0.05$).

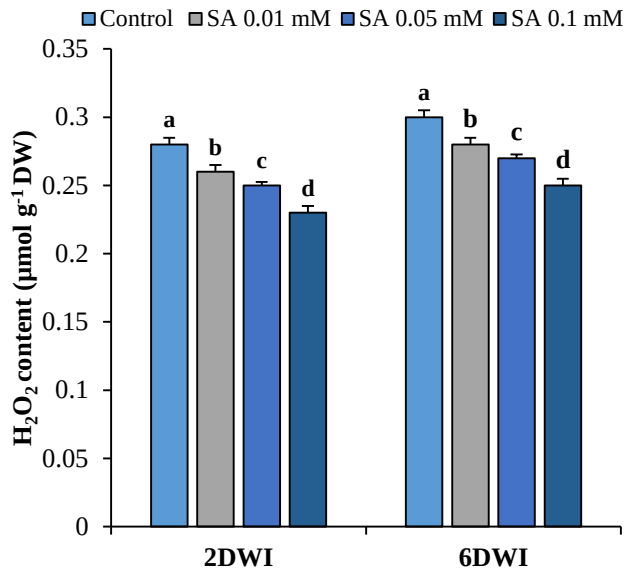

Fig. 2. H_2O_2 content in *C. roseus* subjected to prolonged irrigation intervals and different SA concentrations.
*Means followed by different letters within columns are significantly different based on the least significant difference test ($p \leq 0.05$).

Antibacterial and antifungal activities: The antibacterial activity of *C. roseus* extracts against several pathogenic bacteria is shown in Table 4. Leaf extracts of *C. roseus* demonstrated substantial antibacterial activity against both Gram-positive and Gram-negative bacteria, including *Escherichia coli, Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, and Listeria monocytogenes*.

The antimicrobial effectiveness increased under drought stress and with higher SA concentrations, as reflected by lower MIC and MBC values. The strongest antibacterial activity was generally observed at 6DWI combined with 0.10 mM SA. Among the tested bacteria, *Bacillus cereus* and *Micrococcus flavus* were the most sensitive organisms.

The antifungal activity of *C. roseus* extracts against several pathogenic bacteria is shown in Table 5. The extracts also showed strong antifungal activity against fungi including *Aspergillus niger, Aspergillus flavus,* and *Candida albicans*. Similar to the antibacterial results, drought stress and SA application enhanced antifungal potency. The lowest MIC and MFC values were observed under 6DWI with 0.10 mM SA treatment, indicating increased accumulation of antifungal bioactive compounds under stress conditions.

Quantitative comparison with standard antibiotics revealed that the antimicrobial efficacy of several SA-treated extracts approached that of streptomycin and ampicillin against certain tested bacterial strains. For example, the minimum inhibitory concentration (MIC) values of extracts obtained from plants treated with 0.10 mM SA under 6DWI conditions were substantially lower than untreated controls and, in some cases, differed by less than two-fold from the activity of standard antibiotics. Similarly, minimum bactericidal concentration (MBC) values showed considerable improvement following SA treatment, indicating enhanced bactericidal potential.

Table 5. MIC and MBC of *C. roseus* leaf extracts (mg^{-1}mL) against fungi.

Water interval	Treatment with SA (mM)	*Aspergillus niger* MIC MFC	*Aspergillus ochraceus* MIC MFC	*Aspergillus flavus* MIC MFC	*Penicillium ochrochloron* MIC MFC	*Candida albicans* MIC MFC
2DWI	0	0.18 ± 0.01 0.35 ± 0.01	0.16 ± 0.1 0.33 ± 0.01	0.14 ± 0.01 0.30 ± 0.01	0.23 ± 0.01 0.45 ± 0.03	0.15 ± 0.01 0.29 ± 0.03
	0.01	0.17 ± 0.01 0.33 ± 0.01	0.15 ± 0.1 0.32 ± 0.02	0.13 ± 0.01 0.29 ± 0.03	0.22 ± 0.01 0.43 ± 0.05	0.14 ± 0.01 0.28 ± 0.03
	0.05	0.15 ± 0.01 0.30 ± 0.01	0.14 ± 0.02 0.31 ± 0.01	0.12 ± 0.00 0.27 ± 0.01	0.21 ± 0.01 0.41 ± 0.03	0.13 ± 0.01 0.27 ± 0.03
	0.10	0.14 ± 0.01 0.29 ± 0.01	0.13 ± 0.02 0.27 ± 0.01	0.11 ± 0.01 0.25 ± 0.01	0.20 ± 0.02 0.39 ± 0.01	0.12 ± 0.01 0.26 ± 0.01
6DWI	0	0.16 ± 0.01 0.33 ± 0.01	0.15 ± 0.01 0.31 ± 0.01	0.13 ± 0.01 0.28 ± 0.01	0.22 ± 0.01 0.43 ± 0.01	0.14 ± 0.01 0.27 ± 0.01
	0.01	0.15 ± 0.01 0.30 ± 0.01	0.14 ± 0.02 0.29 ± 0.01	0.12 ± 0.01 0.26 ± 0.01	0.20 ± 0.01 0.41 ± 0.01	0.13 ± 0.01 0.25 ± 0.01
	0.05	0.13 ± 0.01 0.29 ± 0.01	0.12 ± 0.01 0.27 ± 0.01	0.11 ± 0.02 0.25 ± 0.01	0.18 ± 0.02 0.38 ± 0.03	0.12 ± 0.03 0.23 ± 0.01
	0.10	0.12 ± 0.01 0.27 ± 0.01	0.11 ± 0.03 0.24 ± 0.01	0.10 ± 0.01 0.24 ± 0.01	0.16 ± 0.01 0.35 ± 0.03	0.11 ± 0.02 0.21 ± 0.01
	FLZ	0.15 ± 0.01 0.30 ± 0.02	0.20 ± 0.01 0.33 ± 0.01	0.11 ± 0.01 0.24 ± 0.01	0.20 ± 0.01 0.36 ± 0.03	0.10 ± 0.01 0.21 ± 0.01
	KTZ	0.10 ± 0.01 0.21 ± 0.01	0.20 ± 0.01 0.40 ± 0.01	0.21 ± 0.01 0.43 ± 0.01	0.19 ± 0.01 0.41 ± 0.03	0.20 ± 0.01 0.40 ± 0.01

Discussion

Long irrigation intervals significantly reduced vegetative growth and flowering traits of *C. roseus* while the application of salicylic acid (SA) significantly ameliorated the adverse effects of drought (Table 1). Water stress significantly decreased leaf number, leaf area, dry biomass, plant height and number of flowers per plant.

Jaleel *et al*., (2007) reported similar responses of reduced biomass accumulation and growth performance of *C. roseus* under water deficit stress due to impaired cell expansion, reduced photosynthetic efficiency and altered osmotic balance.

The reduction of leaf area and biomass in the longer irrigation intervals may be attributed to stomatal closure and limited carbon assimilation in the water deficit conditions. It is well known that drought stress inhibits leaf initiation and expansion in order to reduce transpiration loss, but this adaptation reduces the photosynthetic surface area and biomass production as well. Salem *et al*., (2022) reported that water stress for *C. roseus* resulted in a significant reduction in shoot and root growth, relative water content and chlorophyll accumulation, which resulted in a decrease in plant productivity.

In our study, foliar application of SA significantly increased all growth parameters under normal and drought conditions and the positive effect increased with the increase of SA concentration where 0.10 mM SA showed highest values of leaf number, plant dry weight, plant height and flower number. Similar results were reported by Salem *et al*., (2022), they found increase in shoot weight, root weight, chlorophyll content and leaf area index under drought stress in *C. roseus*. They concluded that SA may has positive effects on plant growth and plays a major role as signalling molecule involved in the regulation of antioxidant metabolism, osmotic adjustment, nutrient uptake and photosynthetic activity. According to Neelam *et al*., (2014) SA significantly increased the dry weight, water content, chlorophyll concentration, soluble proteins and antioxidant enzyme activity in *C. roseus* under salinity stress. In addition, Idrees *et al*., (2013) reported that SA reduced heavy-metal-induced oxidative damage and improved vegetative growth and alkaloid biosynthesis in *C. roseus*.

The increase in the number of flowers after application of SA is particularly important because the flowering is highly sensitive to water deficit stress. In the present experiment, flower production was significantly reduced under drought stress, while the plants treated with SA maintained significantly higher floral development. Similar effects have been reported in ornamental crops where SA improved floral initiation, flower retention and ornamental quality under stress conditions. Antonić *et al*., (2020) showed that SA treatment under drought stress may increase drought tolerance and enhance the physiological performance of *Impatiens walleriana*, resulting in better ornamental growth. They concluded that the enhancement of flowering following SA application might be attributed to better partitioning of assimilates and hormonal regulation. SA has been associated with better endogenous balance of cytokinin and auxin, delayed senescence and increased availability of carbohydrates for reproductive development.

Matsoukas *et al*., (2012) reported the involvement of signalling molecules and hormonal regulators in floral induction pathways under stress conditions. The improved performance of the 0.10 mM SA treatment in the present study is consistent with previous reports indicating that moderate concentrations of SA stimulate plant metabolism, while very high concentrations may be inhibitory. Salem *et al*., (2022) found that higher bulk concentrations of SA were more effective in alleviating drought stress in *C. roseus*, while lower nano-SA concentrations produced similar results. Positive effects of SA on other medicinal and ornamental herbs under drought stress have also been reported.

SA treatment may enhance the antioxidant enzyme activities, alkaloid accumulation and biomass production in *C. roseus* under drought stress (Ababaf *et al*., 2021). Similarly, Sheikh *et al*., (2019) reported that SA treatment can enhance the anatomical development, vegetative growth and physiological performance of periwinkle plants treated with biotic and abiotic elicitors. Similar responses were reported in ornamental species such as petunia, chrysanthemum, marigold, basil, rosemary, and geranium, where SA improved drought tolerance by enhancing antioxidant defense systems and maintaining membrane stability. SA-induced stimulation of antioxidant enzymes reduces oxidative damage generated by reactive oxygen species during water deficit stress. The increase in plant dry weight and leaf production in the present experiment might also be attributed to improved osmotic adjustment and nutrient assimilation in SA-treated plants. Drought-stressed *C. roseus* accumulated higher levels of osmoprotectants and antioxidant compounds, thereby contributing to improved stress adaptation (Jaleel *et al*., 2007). The treatment of SA has also been associated with improved synthesis of secondary metabolites such as vinblastine and vincristine which are economically important alkaloids in *C. roseus*. SA enhanced alkaloid accumulation under both normal and drought conditions (Ababaf *et al*., 2021).

The enhanced antimicrobial activity observed in drought-stressed and salicylic acid-treated *Catharanthus roseus* plants may be associated with the increased accumulation of phenolic compounds and other secondary metabolites. Phenolics are well known for their antimicrobial properties because they disrupt microbial cell membranes, interfere with enzymatic activity, and inhibit pathogen growth. Drought stress and salicylic acid application are both reported to stimulate phenolic biosynthesis through activation of the phenylpropanoid pathway, thereby enhancing the biological activity of plant extracts (Hasanuzzaman *et al*., 2020; Sharma *et al*., 2023). Consequently, the stronger antimicrobial activity recorded under prolonged irrigation intervals combined with SA treatment is likely related to the elevated production of phenolics and antioxidant metabolites in *C. roseus*.

The present findings have important implications for the sustainable commercial cultivation and pharmaceutical utilization of *C. roseus* under water-limited environmental conditions. The ability of salicylic acid (SA) to alleviate drought-induced growth reduction while simultaneously enhancing antioxidant activity and antimicrobial potential suggests that SA application could be integrated into commercial production systems to improve both plant productivity and phytochemical quality.

From an agricultural perspective, the use of prolonged irrigation intervals combined with appropriate SA concentrations may contribute to water-saving cultivation strategies in arid and semi-arid regions where water availability is limited. Although drought stress reduced vegetative growth and flowering, SA-treated plants maintained acceptable growth performance and ornamental quality under reduced irrigation. Therefore, foliar application of SA could help growers reduce irrigation frequency while sustaining biomass production and flower yield, thereby improving water-use efficiency and reducing production costs.

In conclusion, the drought stress had generally negative effects on vegetative growth and flowering of *C. roseus*, while the exogenous application of SA efficiently mitigated the negative impacts. The improvement of growth and floral characteristics by SA may be associated with the improved photosynthetic performance, antioxidant metabolism, osmotic adjustment and hormonal balance. The highest concentration of SA (0.10 mM) was the most effective in improving growth and flowering under both irrigation conditions.

Conclusion

Results of the present study revealed that prolonged irrigation intervals significantly influenced the growth, physiological attributes, antioxidant metabolism and antimicrobial activity of *C. roseus*. Water stress caused reduction in vegetative growth and flowering performance as evidenced by decrease in leaf number, leaf area, plant biomass, plant height, chlorophyll content and flower production. However, drought stress induced accumulation of osmoprotectants, phenolic compounds, antioxidant enzymes and bioactive metabolites that enhanced the medicinal properties. Foliar application of salicylic acid effectively ameliorated the adverse effects of water deficit and significantly improved plant performance under normal and prolonged irrigation intervals. Maximum improvements in growth characteristics, chlorophyll content, antioxidant activity and antimicrobial potential were observed in plants treated with 0.10 mM SA among the tested treatments. SA treatment enhanced the activities of SOD, CAT and APX thus boosted antioxidant defense systems, and minimized the oxidative damage due to reactive oxygen species. Moreover, the leaf extracts of drought-stressed and SA-treated plants showed enhanced antibacterial and antifungal activities against different pathogenic microorganisms, suggesting that the combined treatment of water stress and SA application increased the accumulation of biologically active secondary metabolites. In conclusion, these results suggest that controlled drought stress in combination with exogenous SA application can be an effective agronomic strategy for improving drought tolerance, physiological performance and medicinal quality of *C. roseus*. These results provide useful insights for sustainable cultivation of medicinal and ornamental plants in water limited environments. Future studies should identify specific bioactive compounds associated with the enhanced antimicrobial activity observed under drought stress and SA treatment. Future studies should evaluate the effectiveness of SA under open-field and commercial cultivation conditions to verify whether the beneficial responses observed under controlled greenhouse conditions can be maintained under variable environmental stresses. Field-scale experiments involving different climatic regions, soil types, and irrigation systems would provide more realistic information regarding the practical applicability of SA for sustainable cultivation of *C. roseus* under water-limited environments.

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