



Article

Physiological and Quality Responses of Calendula (*Calendula officinalis* L.) to Co-application of Biochar composite and PGPR under Water Deficit Conditions

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Abstract: One of Egypt's biggest obstacles to sustainable agriculture is the lack of water. By assessing the effects of applying biochar composite (BC) which consists of biochar made from a mixture of wood (produced from woody ornamental nursery wastes) and biochar made from banana waste at a 2:1 (w/w) ratio either alone or in conjunction with tripartite microbial consortium comprising pure, active strains of *Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Bacillus polymyxa* as plant growth promoters (PGPR), this study investigated sustainable methods to reduce water stress in Calendula (*Calendula officinalis* L.). During the growing seasons of 2023/2024 and 2024/2025, two factorial field experiments with a split-plot design were carried out at the Medicinal Plant Farm, Faculty of Pharmacy, Cairo University, Giza, Egypt. Three irrigation schedules were applied to calendula plants: normal (14-day intervals; I1), moderate water deficit (21-day intervals; I2), and severe water deficit (28-day intervals; I3). The most effective regime, according to the results, was the 21-day irrigation interval, which saved 16% to 21% of irrigation water without affecting the calendula plants' vegetative development, flowering, or chemical analysis. Co-application of Biochar composite (BC) and Plant Growth-Promoting Rhizobacteria PGPR demonstrated the best biological synergy under this mild stress. When compared to the untreated control, this combined treatment greatly improved vegetative growth, flowering characteristics, total carotenoids and total flavonoids, total chlorophyll (a and b), total carbohydrates, and nutritional levels (N, P, and K). Additionally, it maintained relative water content (RWC%), accumulated proline and phenolic contents, and increased phytohormones, antioxidant enzymes, and water productivity all of which substantially mitigated yield losses caused by drought. The co-application of Biochar composite (BC) and PGPR treatment under moderate water shortage (21-day intervals) might be suggested as a sustainable agronomic strategy to enhance vegetative

growth, essential oil composition, and nutritional quality of *Calendula* plants based on the obtained results.

Keywords: *Calendula officinalis* L., Biochar composite, Plant Growth-Promoting, Rhizobacteria, drought stress.

1. Introduction

The most essential component for agricultural crops to grow and flourish is water. A lower supply of irrigation water than the demand is typically implied by the phrase "drought." Globally, osmotic stress has been identified as the most detrimental environmental stress factor (Marchin *et al.*, 2020). In many regions of the world, drought stress has been brought on by changing climatic circumstances (Seleiman *et al.*, 2021). Crop productivity has been negatively impacted by an increase in drought-prone areas. Two-thirds of the world's population is predicted to be impacted by water scarcity by 2050, which is predicted to pose major issues with plant development on fertile lands (AQUASTAT, 2023). Unfortunately, several nations have already measured and confirmed the existence of global warming in recent years. The European Environment Agency issued a warning in 2015 regarding the disturbance of agricultural cycles. Evapotranspiration rises with rising temperatures and little or no precipitation. Soil drying and salinization are caused by a drop in groundwater levels or water retention by soil particles (GIEWS, 2022). One important element that is thought to have a detrimental impact on plant growth and development and lower plant production is drought (Haghpanah *et al.*, 2024). Drought stress impairs photosynthesis, food absorption, transpiration, hormone production, and other metabolic processes in plants by disrupting enzyme pathways primarily involved in CO₂ fixation and adenosine triphosphate synthesis (Gupta *et al.*, 2020). One of the main problems with crop production is drought stress, which lowers cell turgor pressure and disrupts plant physiological and biochemical processes. They produce a lot of reactive oxygen species, which weaken the structure of organelles like chloroplasts. They break down thylakoids and induce membrane lipid peroxidation, which interferes with photosynthesis. Mineral absorption, assimilation, and cellular processes are all negatively impacted by water scarcity (Bhattacharya, 2021). Plants under drought stress have higher leaf temperatures but lower RWC, transpiration rate, and leaf water potential. On the other hand, under comparable stress conditions, leaves showed less osmotic adjustment and retained their capacity to relax their walls, which resulted in growth inhibition (Ferreira, 2019). The size of stomatal apertures decreased as a result of guard cells losing turgor pressure due to decreasing soil moisture brought on by drought stress. Reactive oxygen species (ROS) are overproduced during drought. Reactive oxygen species promote membrane lipid peroxidation by changing the metabolic and oxidative balance of plant cells. Excessive ROS buildup can cause photosynthetic pigments to break down, resulting in leaf senescence (Raja *et al.*, 2020).

There are 29 species in the genus *Calendula*, which belongs to the family Asteraceae and the order Asterales. The most popular of these is *Calendula officinalis* L., a herbaceous plant that is extensively grown for decorative, therapeutic, and cosmetic applications in many different nations. The Mediterranean region is home to this genus. The only species of the genus *Calendula* that is commonly used in therapeutic contexts worldwide is *Calendula officinalis* L. (Sharma and Kumari, 2021). This plant contains a wide variety of physiologically active secondary metabolites, as demonstrated by numerous phytochemical investigations. The leaves and flowers contain high concentrations of carotenoids, essential (volatile) oils, phenolic compounds, saponins, and triterpenoids. The flowers include terpenoids, flavonoids, coumarins, quinones, and essential compounds in addition to being a significant source of lutein. Along with terpenoids, flavonoids, coumarins, quinones, and vital amino acids, the blooms are also a major source of lutein. Calendic acid, a conjugated fatty acid that is frequently utilized in specific cosmetic formulations, is especially abundant in the seeds (Patil *et al.*, 2022). Comprehensive antibacterial, antiviral, anti-inflammatory, anticancer, and antioxidant qualities are displayed by *Calendula officinalis* L. Additionally, a variety of systemic illnesses, including issues with the central nervous system (CNS), have been addressed by both ancient and modern herbal medicines (Ak *et al.*, 2020). While the flowers are used as stimulants, antispasmodics, and emmenagogues, the leaves are said to be diaphoretic and expectorant. Its fresh juice was used to treat jaundice, constipation, and decreased menstrual flow, while the decoction of its petals was used as a

posset drink to treat smallpox and measles. Its florets are used in ointments to treat scars, wounds, herpes, ulcers, frostbite, skin damage, and blood purification. Its leaves are infused topically to treat varicose veins **(Preethi and Kuttan, 2021)**. A number of esteemed international herbal monographs and pharmacopoeias, such as the German Commission E, the European Scientific Co-operative on Phytotherapy (ESCOP), the British Herbal Pharmacopoeia (BHP), and the World Health Organization (WHO), have officially recognized and documented *C. officinalis* due to its profound anti-inflammatory and wound-healing properties. Modern preparations like Carophyllenic ointment, which is standardized for carotenoids obtained from flowers, show the therapeutic efficacy of *Calendula officinalis* L. **(Shahane et al., 2023)**. It is one of the main active ingredients in the homoeopathic formulation Traumeel, which is used to treat acute musculoskeletal injuries by reducing pain and oedema **(Cesnulevicius et al., 2020)**. Furthermore, in children with acute otitis media, naturopathic otic formulations as Otikon otic solution have shown therapeutic benefit in reducing otalgia **(Goldberg and Lin, 2021)**. *C. officinalis* extracts have strong antineoplastic action against a variety of tumour cell lines, including leukaemias, fibrosarcomas, melanomas, and carcinomas of the breast, cervix, prostate, pancreatic, and lung, according to in vitro tests **(Cruceriu et al., 2020)**. The antibacterial and antifungal properties of *C. officinalis* flower extracts provide compelling evidence for their therapeutic use in dermatology **(Taravat and Esmaeilzadeh, 2023)**. It is perfect for pre- and post-sun exposure creams because of its high saponin, gum, and mucilage content, which prevented secondary infections, reduced pruritus, erythema, dryness, and pain, and accelerated the resolution of scars **(Nicolaus et al., 2021)**. Through localised vasodilation, it improves skin tone **(Ghafourian et al., 2022)**.

The use of beneficial soil microorganisms as biological elicitors has become a crucial, environmentally friendly approach in the modern agricultural paradigm to mitigate the detrimental effects of drought stress on crop output **(Etesami and Jeong, 2021)**. The rhizosphere provides a home for sophisticated microbial populations that can form complicated mutualistic relationships with host plants, hence changing the plant-soil-microbe interaction **(Al-Askar et al., 2023)**. Plant Growth-Promoting Rhizobacteria (PGPR) are unique among them in that they can actively colonise root surfaces, maximise soil fertility, and induce systemic changes that control plant life in extreme moisture deficiencies. Drought-tolerant PGPR buffer the holobiont against environmental anomalies by inducing a multidimensional Induced Systemic Tolerance (IST) that balances direct nutritional supplementation with complex cellular and genetic reprogramming, as opposed to acting through isolated pathways **(Sukweenadhi et al., 2024)**. The systemic redesign of Root System Architecture (RSA) and hormonal signalling pathways is the main defence layer activated by PGPR under extreme water scarcity. By secreting important phytohormones like auxins, gibberellins (GAs), and cytokinins coupled with low-molecular-weight signalling chemicals like 2,4-diacetylphloroglucinol (DAPG), beneficial rhizobacteria actively re-engineer RSA **(Vacheron et al., 2021)**. A crucial biomarker for bacterial effectiveness under osmotic stress is microbial indole-3-acetic acid (IAA), which significantly increases the absorptive surface area for water and immobile nutrients by loosening root cell walls and promoting lateral root proliferation and root tip development **(Fukami et al., 2022)**. In order to reduce leaf transpiration without compromising photosynthetic efficiency, bacterial cytokinins simultaneously regulate long-distance root-to-shoot communication, regulating stomatal closure and metabolic activity **(Zahedi et al., 2024)**. Volatile organic compounds (VOCs) released by certain strains enhance this hormonal cross-talk by acting as airborne determinants that regulate the expression of regulatory stress proteins and functional plant genes in arid conditions **(Abulfaraj et al., 2025)**. In addition to structural changes, PGPR protects the host plant from drought-induced secondary oxidative and metabolic damage. In order to efficiently scavenge Reactive Oxygen Species (ROS) and stop membrane lipid peroxidation, inoculation proactively stimulates both enzymatic and non-enzymatic antioxidant defence systems **(Batool et al., 2020)**. A significant decrease in hydrogen peroxide (H₂O₂) level, malondialdehyde (MDA) accumulation, and electrolyte leakage is indicative of this antioxidant upregulation, which maintains the structural integrity of chloroplasts and stabilises the photosynthetic system **(Kasim et al., 2025)**. The microbial synthesis of multifunctional traits, such as the generation of iron-chelating siderophores, atmospheric nitrogen fixation, the solubilisation of inorganic minerals like rock phosphate, and the secretion of exopolysaccharides (EPS) that form a highly hydrated biofilm around the root cortex, mechanistically reinforces this internal physiological homeostasis **(Kour et al., 2021)**. For sustainable agriculture in dry areas, using tailored, drought-resistant PGPR consortia is a

long-term, synergistic biological strategy (Mishra *et al.*, 2024). Certain multi-strain combinations, usually anchored by *Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Bacillus polymyxa*, are among the most effective candidates isolated for generating water stress resistance through these synchronised processes. This particular consortium demonstrates a significant ability to maintain physiological homeostasis and crop yield under water-limiting conditions by acting as a tripartite biological shield that combines the atmospheric nitrogen fixation and antioxidant stimulation of *A. chroococcum*, the hyper-production of IAA and RSA modification of *A. lipoferum*, and the physical EPS moisture-retention barrier and phosphate solubilisation of *B. polymyxa* (He and Oh, 2023).

A finely textured, carbon-rich organic material produced by pyrolyzing biomass and organic leftovers from the forestry and agricultural industries is known as biochar (BC) (Chang-Chien *et al.*, 2025). This thermochemical breakdown takes place in anoxic or oxygen-limited environments, usually between 300 and 600 °C (Garzón-Camacho *et al.*, 2024). The synthesis and use of biochar, which serves as a stable, long-term terrestrial carbon sink while simultaneously reducing greenhouse gas emissions and increasing waste management efficiency, has attracted international attention in recent years as a crucial tool for mitigating climate change (Aduba *et al.*, 2025). Beyond its benefits for the environment, adding biochar to soil improves its mechanical, chemical, and physical characteristics. By lowering bulk density and raising general porosity, the use of biochar physically alters the architecture of the soil (Zhu, 2025). It optimises soil fertility and nutrient retention by increasing the soil's organic carbon content, cation exchange capacity (CEC), and adsorption capacity both chemically and biologically (Ghorbani, *et al.*, 2025). These changed characteristics encourage microbial dynamics and enzymatic activity, which makes the environment extremely conducive to the growth of beneficial soil microorganisms (Khalifa, 2025). Additionally, biochar is an efficient remediation agent that may remove toxins, steroids, and aquatic pollutants from soil and water matrices due to its strong affinity for binding organic molecules and heavy metals (Zhang *et al.*, 2025). Because of its ability to lessen the negative effects of moisture stress, biochar has become a feasible alternative for sustainable crop production in agricultural systems, especially in water-limited and drought-prone areas (Al-Garni, 2025). Biochar greatly enhances plant-available water by improving the soil's hydraulic qualities and water-holding capacity (Zhang and Smith, 2024). By reducing oxidative and osmotic stress, this increased moisture availability helps plants absorb water through their roots and encourage vegetative development (Li *et al.*, 2025). Biochar enrichment enhances photosynthetic efficiency, stomatal conductance, and overall water use efficiency (WUE) at the physiological level. By maximising electron transport pathways and increasing the activity of beneficial antioxidant enzymes within the crops, it also lessens damage caused by moisture stress (Rahman *et al.*, 2025). Therefore, applying biochar is a significant step toward protecting critical plant growth stages, from seed germination and seedling emergence to final crop yields and long-term agricultural sustainability, by improving fertiliser and water utilisation efficiency over extended periods (Ribeiro *et al.*, 2025).

Considering that drought is a significant issue in Egypt, it is necessary to assess the consequences of the water shortage. In this regard, the current study's goal was to ascertain the relationships between the treatment of *Calendula* (*Calendula officinalis* L.) under water stress using Plant Growth Promoters (PGPR) and biochar.

2. Materials and Methods

Site description and Experimental design

At the Medicinal Plant Farm, Faculty of Pharmacy, Cairo University, Giza, Egypt, two field tests were carried out in an open area in clay loam soil. throughout the seasons of 2023–2024 and 2024–2025, respectively. In order to investigate the effects of Plant Growth Promoters (PGPR) and in combination with biochar on *Calendula* under drought stress after 14 days (normal condition, I1), 21 days (moderate water deficit stress, I2), and 28 days (severe water deficit stress, I3), the experimental site is situated at 31° 12' E longitude, 30° 01' N latitude, and 22 m altitude above mean sea level.

The medicinal and aromatic plants research department in Dokki, Giza, graciously provided the calendula seeds, which were planted on September 21st/ 2023, and September 23rd/ 2024. After 40 days in both seasons, the seedlings were moved and planted in 2.0 × 2.5 m plots with three rows per plot. The intra-

row hill spacing in the same row was 30 cm, while the inter-row spacing was kept at 60 cm. One plant per hill was left after thinning (Maie, Mohsen and Ismail, 1916).

In the second week following the stage of transplanted seedlings, drought stress was administered. The treatments included 14 days (100%) of ET_c, 21 days (80%) of ET_c, and 28 days (60%) of ET_c from field capacity in the main plots (irrigation regimes depending on plant evapotranspiration). Sub-plots: biochar, combination of PGPR and biochar with mixture of PGPR bacteria, control (without any modifications), Thus, 12 interaction treatments were involved in the experiment. The Egyptian Ministry of Agriculture's prescribed dosages of nitrogen (N), phosphorus (P), and potassium (K) were applied to control plants.

Soil Hydro-Physical and Chemical Characteristics:

The physical, Hydrophysical and chemical characteristics of soil samples were determined at the laboratories of the Agricultural Research Center (ARC), Ministry of Agriculture, Giza, Egypt, and the Soil Physics Laboratory, Department of Soil and Water Sciences, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

For thorough physical and chemical characterization, soil samples were taken from the experimental location using a soil auger at a depth of 0–30 cm. After being air-dried and crushed, the samples were run through a 2-mm sieve. The methods outlined by (Klute, 1986) were used to determine the particle size distribution. The standard procedures described by (Page *et al.*, 1982) were used to analyze the chemical characteristics of the soil. According to (Klute, 1986) and (Blake *et al.*, 1986), Bulk density and soil-water constants, including field capacity and permanent wilting point, were determined at successive soil depths within the 0–60 cm soil profile. Tables (A) and (B) provide a summary of the experimental soil's baseline properties. Tables present the mean determined values for the 0–60 cm soil profile obtained before planting for each growing season.

Table (A). Soil physical and chemical properties at the experimental site

Season	Particle size distribution			Textural class	Chemical properties					
	Clay	Silt	Sand		O.M (%)	EC dSm ⁻¹ (1:5)	Available (ppm)			pH (1:2.5)
	%						N	P	K	
2023/2024	42.5	33.8	23.7	Clay loam	1.78	0.86	44.52	10.5	188.65	7.75
2024/2025					1.80	0.82	44.60	11.23	190.77	7.75

Table (B). Soil water constants and bulk density at the experimental site

Depths (cm)	Field capacity (%)	Permanent Wilting Point (%)	Available moisture (%)	Bulk density (g cm ⁻³)	Available moisture (mm/layer)	
First season 2022/2023						
0-15	35.4	18.6	16.8	1.18	29.7	59.0
15-30	33.6	17.7	16.3	1.20	29.3	
30-45	31.5	15.8	15.7	1.27	29.9	62.7
45-60	30.1	14.7	15.4	1.42	32.8	
Second season 2023/2024						
0-15	36.2	19.4	16.8	1.19	30.0	59.1
15-30	34.4	18.5	15.9	1.22	29.1	
30-45	32.1	16.4	15.7	1.30	30.6	65.0
45-60	31.1	15.7	15.4	1.49	34.4	

Preparation and Application of Biochar composite (BC) Treatment

The biochar composite (BC) was supplied by Waste Recycling, an Egyptian company. This was created by mixing biochar made from wood (produced from woody ornamental nursery wastes) and biochar made from banana waste at a 2:1 (w/w) ratio. This attempts to establish a synergistic balance: the biochar made from banana waste provides the soil with essential mineral nutrients, especially potassium, while the wood-derived biochar offers a highly porous structure and great long-term water retention capacity. Two weeks prior to the planting date, the activated biochar composite was added at a rate of three tonnes per feed and blended into the soil to a depth of five to ten cm. In order to support budding seedlings over extended irrigation intervals, this step attempted to build an instant water and nutrient store (**Leng *et al.*, 2021**). (Table C) summarises the biochar composite's baseline properties, which were established in accordance with (**International Biochar Initiative, 1915**).

Table (C). Physicochemical Properties of the Biochar Composite (BC) Used in the Study

Measured Parameter	1 st season	2 ^{ed} season
Weight of 1 m ³ (kg)	355	367
Organic Carbon (%)	57.42	53.37
Total N (%)	0.88	0.92
Total P (%)	0.52	0.62
Total K (%)	5.54	6.22
Fe (ppm)	122.04	162.34
Cu (ppm)	13.05	11.74
Zn (ppm)	27.3	38.0
Mn (ppm)	76.2	91.3
Ash content (%)	15.12	17.57
Specific surface area (m ² /g)	200	230
Water Holding Capacity (%)	142	163
Bulk Density (g/cm ³)	0.32	0.37
EC (ds/m)	2.45	3.10
pH	8.85	8.80

Preparation and Application of PGPR Treatment

A synergistic tripartite microbial consortium comprising pure, active strains of *Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Bacillus polymyxa* was kindly provided by the Microbiology Department, Soil, Water and Environment Research Institute (SWERI), Agriculture Research Center (ARC), Ministry of Agriculture, Giza, Egypt, to biologically inoculate calendula (*Calendula officinalis* L.) plants. To create a balanced microbial inoculum, the three bacterial strains were grown separately in their corresponding liquid broth media until they reached the logarithmic growth phase, guaranteeing a cell density of at least 108 CFU/mL. They were then combined in equal quantities (1:1:1 v/v/v). (**Bashan *et al.*, 2014**). (Table D) lists some of the features of the plant growth promoting (PGPR) that were utilized. Throughout the growth stages, three bacterial inoculations were carried out. Before being planted in the PGPR culture for the first time, calendula seedlings were soaked for half an hour. The second application was sprayed on the plants 35 days after transplanting, and the last application was sprayed when the flowers were budding. (**Fasciglione *et al.*, 2015**).

Table (D). Plant Growth-Promoting Characteristics of the Treatments Used in the Study

Strain	N ₂ -ase μmole C ₂ H ₄ /ml/hr	Phytohormons (μg/L culture)		
		Indole acetic acid	Gibberellins	Cytokinins
<i>Azotobacter chroococcum chroococcum</i>	285.7	72.5	105.8	156.0
<i>Azopirillum lipoferum</i>	66.34	5.8	85.6	327.0
<i>Bacillus polymyxa</i>	59.72	44.5	100.7	98.0

Recorded Data**1- Vegetative measurements**

Calendula (*Calendula officinalis* L.) plants were collected 120 days after transplantation in order to measure plant height (cm), number of branches per plant, fresh and dry herb weights (g/plant), and leaf area (cm²) of the median leaf of the main stem (measured after 90 days of transplanting).

2- Flowering parameters

Inflorescences of calendula were harvested immediately after the full opening throughout the flowering period then recorded, diameter of inflorescences (cm), Inflorescences count /plant and fresh and dry weights of inflorescences(g/plant). These parameters were recorded by collecting the inflorescences per plant were taken every 10 days started at (14th February - 28th May) when 80% of the flowers were opened. Then, Inflorescences dry yield/fed (Kg) was calculated mathematically.

3- Chemical analysis

In leaf samples, chlorophyll (a&b) (mg/gm fresh matter) was measured in accordance with (Nornai, 1982). The technique outlined by (A.O.A.C, 1990) was used to measure the total carotenoids and total flavonoids in air-dried ray flowers. Samples of leaves from both testing seasons were collected, dried at 70 °C for 72 hours, and finely powdered before the percentage of total carbs was calculated using the method outlined by (Herbert *et al.*, 1971). The modified micro Kjeildahl, as described by (Pregl, 1945) was used to calculate total nitrogen. Phosphorus was calculated using (King, 1951). Atomic absorption spectroscopy was used to measure potassium (Rawe, 1973). In accordance with (Bates, 1973) free proline was calculated. While R.W.C% was determined after 60 days from sowing, fresh mass (FM) and dry mass (DM) for shoots calendula were determined. Turgid weight (TM) was assessed. The RWC % of shoots were determined using the equation as described by (Sharp *et al.*, 1990) as follows:

$$\text{RWC \%} = (\text{FM} - \text{DM} / \text{TM} - \text{DM}) \times 100$$

4-Total Phenolic content and Antioxidant Enzymes

The total phenolic content was determined using the Folin-Ciocalteu colorimetric technique (Barka *et al.*, 2006). Gallic acids were used as the standard to test phenolics at 750 nm, and the results were represented as mM GA eq/g FW. Catalase (CAT) enzyme was measured using the method of (Aebi, 1983) for antioxidant enzyme assays (time). Super Oxide Dismutase (SOD) and Acerbate Peroxidase (APX) were measured in accordance with (Nakano and Asada, 1981). and (Donahue *et al.*, 1997), respectively.

5- Phytohormones in calendula plants.

After 60 days from sowing, 10 grams of fresh tissue per sample were homogenized with 70% (v/v) methanol and stirred overnight at 4°C to measure the phytohormone (IAA, GA3, and ABA). The resulting samples were filtered through a Millipore filter and then subjected to HPLC analysis in accordance with (Sümer and Palavan-Ünsal, 2004).

6- Dehydrogenase enzyme

The dehydrogenase activity was also determined according to (Skujins and Burns, 1976) at 60 days from sowing.

7- Applied irrigation water (AIW)

This treatment was carried out using a furrow surface irrigation technique. A flow meter installed in the main irrigation water pumping unit was used to measure the amount of applied irrigation water. The following formula is used to determine the orifice's discharge (**Schudson, 1978**).

$$Q = CA$$

Where: Q = discharge through orifice, (cm³/sec), C = coefficient of discharge (0.6 up to 0.8), A = cross-sectional area of the orifice (cm²), g = acceleration of gravity (981 cm/sec), h = head of water causing discharge through the orifice (cm).

8- Economic Water productivity

Water utilization efficiency (Ute) values were calculated according to (**Jensen, 1983**) as follow:

Ute (kg m⁻³) = fruits yield (kg fed⁻¹) /Applied irrigation water (m³ fed⁻¹). Applied irrigation water was recorded by a flowmeter installed in the main unit of irrigation water.

Statistical Analysis

According to (**Little and Hills, 1978**), statistical analysis was performed on the gathered data. The least significant difference test was used to separate the means at the 5% level (LSD 0.05).

Results

1- Vegetative growth characteristics:

(Table 1) shows the effects of biochar composite (BC), plant growth promoters (PGPR), and their interactions under irrigation intervals on the vegetative parameters of the Calendula plant throughout the two studied seasons.

Considering that the use of plant growth promoters (PGPR) and biochar composite (BC) treatments had a significant impact on the various vegetative growth traits of the calendula (*Calendula officinalis* L.) plant, whether separately or in combination. These traits include plant height (cm), number of branches per plant, medium leaf area of main stem, and fresh and dried plant weights (g). When compared to the untreated control plants, the administration of various treatments often encouraged vegetative development and produced notable improvements in the values of these traits. Plants treated with biochar composite (BC) plus plant growth promoters (PGPR) showed increases in the aforementioned attributes in both seasons, compared to those treated with PGPR only and BC solely, respectively. Similar enhancing effects on plant growth were noticed by (**Ajis *et al.*, 2022**), (**Shahrajabian and Sun, 2024**), (**Mahmoud and Agwah, 2024**) and (**El-Serafy and El-Sheshtawy, 2025**).

When irrigation intervals increased (i.e., from every 21 days to every 28 days) in both seasons, vegetative growth characteristics dramatically decreased (Table 1). When compared to irrigation every 14 days, irrigation every 28 days resulted in the largest decrease in vegetative features. (**Raza *et al.*, 2025**), (**Khalilzadeh *et al.*, 2020**) and (**Bistgani *et al.*, 2024**) achieved this outcome.

Significant effects on vegetative growth metrics were noted during both research seasons with regard to the interacting effects of biochar composite (BC) and PGPR inoculation under the different irrigation intervals (Table 1). When BC and PGPR inoculation were coupled throughout the applied irrigation intervals, these traits often increased significantly; plants treated with both agents (BC and PGPR) under a 14-day irrigation schedule showed the best results. In contrast, untreated plants declined sharply and reached their lowest levels under severe water stress conditions (irrigation every 28 days); however, the application of the combined treatment (BC and PGPR) under the same drought conditions (28 days interval) improved these vegetative growth parameters, an outcome seen in both seasons. Overall, the results in Table 1 demonstrated that when treated with both biochar composite (BC) and PGPR inoculation, plants under water stress (21 days interval) displayed vegetative growth values similar to those of plants under regular irrigation (14 days interval).

Table (1). Responses of plant vegetative parameters of calendula (*Calendula officinalis* L.) to co-application of biochar composite (BC) and PGPR under water deficit conditions during 2023/2024 and 2024/2025 seasons

Irrigation intervals	First season 2023/2024					Second season 2024/2025				
	Cont.	BC	PGPR	BC+ PGPR	Mean of Irr.	Cont.	BC	PGPR	BC+ PGPR	Mean of Irr.
Plant height (cm)										
14 days	44.19	56.45	61.79	66.63	57.27	46.67	58.73	65.42	71.45	60.57
21 days	42.70	48.15	59.12	64.52	53.62	44.07	50.88	61.83	68.73	56.38
28 days	40.19	46.05	51.80	54.27	48.08	42.97	48.14	53.75	56.44	50.33
Mean of BC&PGPR	42.36	50.22	57.57	61.81		44.57	52.58	60.33	65.54	
L.S.D.5% Irr	2.781					3.152				
L.S.D.5% BC x PGPR	3.524					4.745				
Interaction	2.126					3.032				
No. of branches/plant										
14 days	6.44	13.42	16.70	20.47	14.26	7.45	15.01	18.45	22.33	15.81
21 days	5.70	9.05	14.66	18.36	11.94	6.75	9.75	16.63	20.17	13.33
28 days	4.45	7.40	10.17	12.00	8.51	5.75	8.11	11.25	13.06	9.54
Mean of BC&PGPR	5.53	9.96	13.84	16.94		6.65	10.96	15.44	18.52	
L.S.D.5% Irr	0.982					1.221				
L.S.D.5% BC x PGPR	1.730					2.753				
Interaction	1.043					1.141				
Leaf area medium leaf (cm²)										
14 days	43.50	61.45	70.42	82.70	64.52	55.12	75.17	82.12	90.55	75.74
21 days	39.70	52.07	66.51	76.52	58.70	50.44	63.25	78.54	87.44	69.92
28 days	35.45	47.12	55.44	58.70	49.18	45.17	59.44	66.70	70.22	60.38
Mean of BC&PGPR	39.55	53.55	64.12	72.64		50.24	65.95	75.79	82.74	
L.S.D.5% Irr	3.573					4.147				
L.S.D.5% BC x PGPR	5.624					6.014				
Interaction	4.326					5.007				
Fresh weight of plant (gm)										
14 days	197.15	245.75	260.14	275.12	244.54	230.70	260.33	280.45	310.55	270.51
21 days	188.07	217.82	252.07	269.44	231.85	222.42	255.08	272.76	291.70	260.49
28 days	174.33	201.45	228.11	239.15	210.76	210.19	241.45	265.50	257.12	243.57
Mean of BC&PGPR	186.52	221.67	246.77	261.24		221.10	252.29	272.90	286.46	
L.S.D.5% Irr	7.152					8.244				
L.S.D.5% BC x PGPR	10.451					11.227				
Interaction	8.301					9.411				
Dry weight of plant (gm)										
14 days	30.32	37.61	39.77	42.02	37.43	35.36	39.80	42.82	47.33	41.33
21 days	28.96	33.42	38.56	41.17	35.53	34.11	39.01	41.67	44.51	39.83
28 days	26.90	30.97	34.97	36.62	32.37	32.28	36.97	40.58	39.32	37.29
Mean of BC&PGPR	28.73	34.00	37.77	39.94		33.92	38.59	41.69	43.72	
L.S.D.5% Irr	0.452					0.936				
L.S.D.5% BC x PGPR	1.110					1.534				
Interaction	0.877					1.004				

Biochar composite (BC) *Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Bacillus polymyxa* (PGPR) Irrigation intervals (Irr)

2- Flowering growth characteristics

(Table 2) shows the impact of biochar composite (BC), plant growth promoters (PGPR), and combinations of these two fertilizer types under irrigation intervals on Calendula plant flowering parameters throughout the two studied seasons.

The effects of biochar composite (BC) or/and plant growth promoters (PGPR) treatments in both seasons are shown by the results in Table 2. These treatments greatly enhanced the inflorescences count per plant, the width of inflorescences (cm), the fresh and dry weights of inflorescences per plant (g), and the dry weights of inflorescences per feed (Kg) as compared to the control. The best results were obtained by plants fertilized with biochar composite (BC) and inoculated with PGPR, followed by treatments treated with PGPR separately. Individual calendula plants treated with biochar composite (BC) displayed the least amount of blooming. The benefits of these therapies in line with (El-Serafy and El-Sheshtawy, 2025), (Singh *et al.*, 2024) and (Radhakrishnan *et al.*, 2024).

In comparison to plants that received irrigation every 14 days, the data in (Table 2) further shows that plants that received irrigation every 21 days had a significant impact on the inflorescence count/plant, diameter of inflorescences (cm), fresh and dry weights of inflorescences/plant (g), and dry weights of inflorescences/fed (Kg) in both seasons. Conversely, in both seasons, plants that were irrigated every 28 days had the lowest values for the blooming metrics examined. These findings concur with (Khalilzadeh *et al.*, 2020), (Bistgani *et al.*, 2024), (Ghaffari, *et al.*, 2025) and (Al-Ghzawi *et al.*, 2024).

Inflorescence count/plant, inflorescence diameter (cm), fresh and dry weights of inflorescences/plant (g), and dry weights of inflorescences/fed (Kg) were all considerably reduced by watering intervals of 21 and 28 days in both seasons, according to data in (Table 2). However, applying biochar composite (BC) or inoculating with PGPR at varying irrigation intervals greatly improves these blooming parameters. When compared to the control and individual treatments under the same irrigation interval, the combined treatment of biochar composite (BC) and PGPR inoculation under the long irrigation interval (28 days) often resulted in an increase in flowering parameters in the first and second seasons. In general, compared to plants exposed to severe water stress conditions (28 days), plants treated with the biochar composite (BC) and inoculated with PGPR under moderate water stress conditions (21 days) showed significant increases in the studied flowering characteristics.

3- Chemical analysis

A- In plant's flowers

The effects of biochar composite (BC), plant growth promoters (PGPR), and their interactions under irrigation intervals on the total carotenoids and total flavonoids content of calendula flowers over the two tested seasons are shown in (Fig. 1).

Regarding the effects of applying biochar composite (BC) or plant growth promoters (PGPR) on calendula flowers, the data in Figure 1 shows that all treatments enhanced the total carotenoids and total flavonoids content of calendula flowers during the two tested seasons. Plants that received the (BC) and were inoculated with PGPR showed the greatest gains, followed by those that were treated with PGPR alone. Conversely, the plants treated with BC had the lowest levels of total flavonoids and carotenoids. Similar boosting effects were observed in (Ajis *et al.*, 2022), (Shahrajabian and Sun, 2024) and (Mahmoud and Agwah, 2024).

Table (2). Responses of plant flowering parameters of calendula (*Calendula officinalis* L.) to co - application of biochar composite (BC) and PGPR under water deficit conditions during 2023/2024 and 2024/2025 seasons

Irrigation intervals	First season 2023/2024					Second season 2024/2025				
	Cont.	BC	PGPR	BC+ PGPR	Mean of Irr.	Cont.	BC	PGPR	BC+ PGPR	Mean of Irr.
Inflorescences count /plant										
14 days	9.33	22.40	27.33	35.50	23.64	13.25	28.02	34.40	40.15	28.96
21 days	7.05	13.70	25.45	31.75	19.49	11.14	18.10	30.16	37.70	24.28
28 days	6.70	11.50	16.40	19.75	13.59	9.45	16.70	21.45	25.50	18.28
Mean of BC&PGPR	7.69	15.87	23.06	29.00		11.28	20.94	28.67	34.45	
L.S.D.5% Irr	2.268					3.145				
L.S.D.5% BC x PGPR	3.426					4.017				
Interaction	2.985					3.512				
Diameter of inflorescences (cm)										
14 days	4.30	5.92	6.82	7.55	6.15	4.51	6.60	7.70	8.70	6.88
21 days	4.02	5.00	6.30	7.17	5.62	4.22	5.20	7.18	8.32	6.23
28 days	3.72	4.65	5.32	5.67	4.84	4.00	4.86	5.85	6.25	5.24
Mean of BC&PGPR	4.01	5.19	6.15	6.80		4.24	5.55	6.91	7.76	
L.S.D.5% Irr	0.355					0.420				
L.S.D.5% BC x PGPR	0.521					0.643				
Interaction	0.847					1.051				
Fresh weight of inflorescences/plant(g)										
14 days	56.32	81.37	93.60	113.83	86.28	62.30	87.76	98.64	116.05	91.19
21 days	51.73	63.92	88.07	98.60	75.58	57.81	70.51	93.65	110.83	83.20
28 days	49.46	60.88	70.38	76.63	64.34	52.74	66.42	75.40	80.37	68.73
Mean of BC&PGPR	52.50	68.72	84.02	96.35		57.62	74.90	89.23	102.42	
L.S.D.5% Irr	1.672					2.107				
L.S.D.5% BC x PGPR	2.645					3.841				
Interaction	2.111					3.205				
Dry weight of inflorescences/plant(g)										
14 days	8.45	12.21	14.04	17.08	12.95	9.35	13.16	14.80	17.41	13.68
21 days	7.76	9.59	13.21	14.79	11.34	8.67	10.58	14.05	16.63	12.48
28 days	7.42	9.13	10.56	11.50	9.65	7.91	9.96	11.31	12.06	10.31
Mean of BC&PGPR	7.88	10.31	12.60	14.46		8.64	11.23	13.39	15.37	
L.S.D.5% Irr	0.223					0.347				
L.S.D.5% BC x PGPR	0.416					0.563				
Interaction	0.538					0.605				
Dry weight of inflorescences/fed (kg)										
14 days	194.35	280.83	322.92	392.84	297.74	215.05	302.68	340.40	400.43	314.64
21 days	178.48	220.57	303.83	340.17	260.76	199.41	243.34	323.15	382.49	287.10
28 days	170.66	209.99	242.88	264.50	222.01	181.93	229.08	260.13	277.38	237.13
Mean of BC&PGPR	181.16	237.13	289.88	332.50		198.80	258.37	307.89	353.43	
L.S.D.5% Irr	5.263					6.745				
L.S.D.5% BC x PGPR	7.452					8.342				
Interaction	9.107					10.208				

Biochar composite (BC)
Irrigation intervals (Irr)

Azotobacter chroococcum, *Azospirillum lipoferum*, and *Bacillus polymyxa* (PGPR)

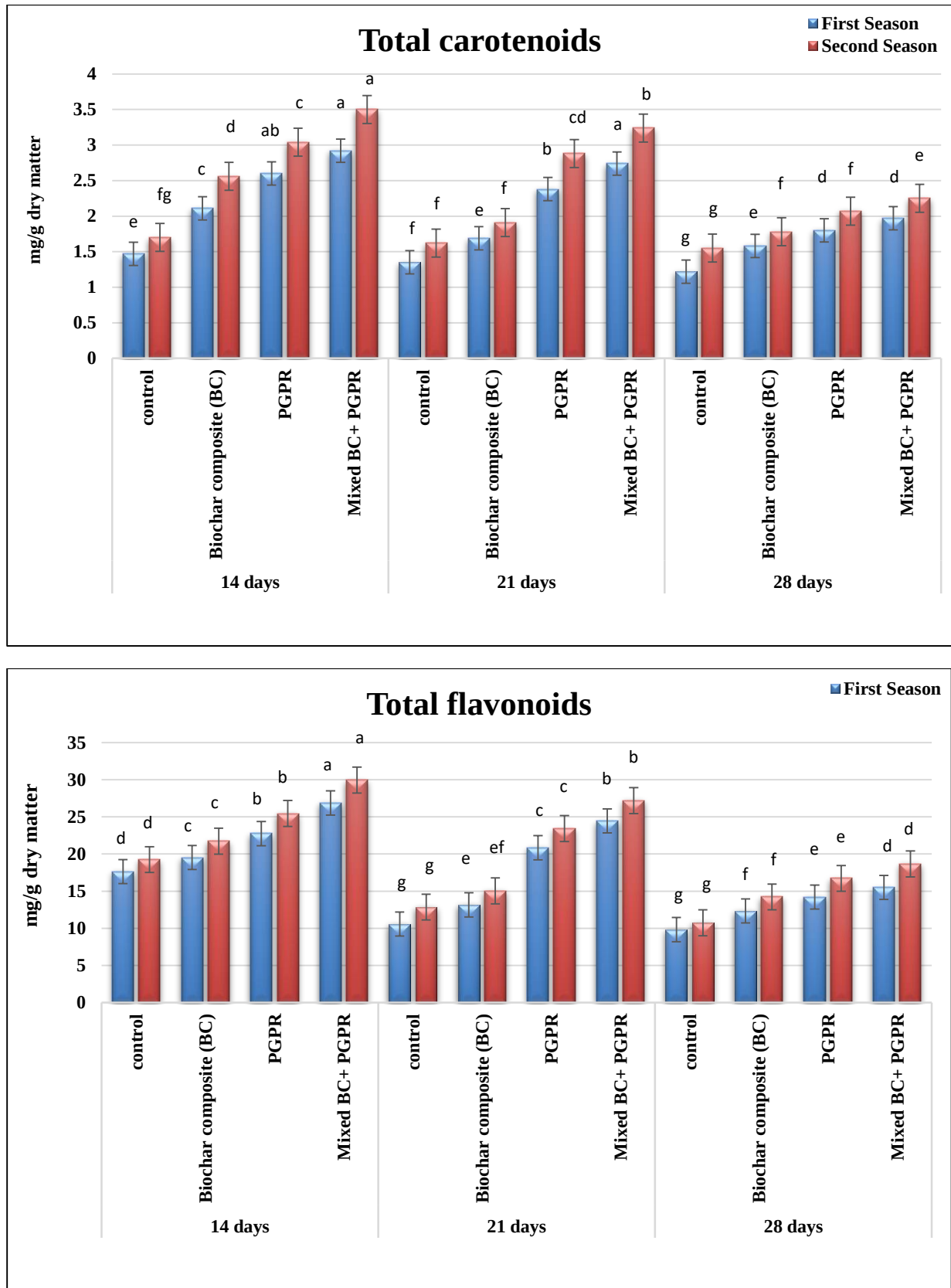


Fig. (1). Response total carotenoids and total flavonoids content in petals of *Calendula Officinalis* L. to co – application of biochar composite (BC) and PGPR under water deficit conditions during 2023/2024 and 2024/2025 seasons

Irrigation interval data were recorded in (Fig. 1). The data showed that both total carotenoids and total flavonoids gradually and significantly declined with increasing irrigation intervals (i.e., from every 14 days to every 21 days, and finally to every 28 days) in both seasons. When compared to plants that were irrigated every 14 days, those that were irrigated every 28 days had the lowest average values for the concentration of total flavonoids and total carotenoids. Similar improvements in chemical analysis were observed by (Raza *et al.*, 2025), (Ghaffari, *et al.*, 2025) and (Al-Ghzawi *et al.*, 2024).

The interaction between the application of biochar composite (BC) and PGPR under different watering intervals clearly had a substantial impact on the total carotenoids and total flavonoids of calendula flowers during both seasons (Fig. 1). The plants that received (BC) plus PGPR during a regular irrigation interval (14 days) had the highest content of total carotenoids and total flavonoids in calendula petals when compared to all other interaction treatments over both seasons. These plants were followed by plants that received the same treatment under irrigation (21 days), while the least effective treatment was using biochar composite (BC) alone under irrigation (28 days).

B- In Plant's leaves

The effects of biochar composite (BC), plant growth promoters (PGPR), and their interactions during irrigation intervals on the chemical analysis of calendula plants are shown in (Figs 2-7).

1- Total Chlorophyll (A&B) content, Total Carbohydrates and Mineral contents

It is clear from the data in (Figs. 2 and 3) that all treatments at the two tested seasons increased the chemical analysis of calendula plants expressed as total chlorophyll (A&B), total carbohydrates, nitrogen, phosphorus, and potassium when it came to the effects of biochar composite (BC) or/and Plant Growth Promoters (PGPR) treatments on the chemical analysis of calendula plants. The greatest values were displayed by plants treated with BC plus PGPR, followed by those treated with PGPR alone. In contrast, the least effective treatment was BC individually. Similar enhancing effects on chemical analysis were noticed by (Ajis *et al.*, 2022), (Shahrajabian and Sun, 2024) and (El-Serafy and El-Sheshtawy, 2025).

(Figs 2 and 3) contain information on irrigation intervals. The findings showed that when irrigation intervals rose (i.e., from every 14 days to every 21 days, and finally to every 28 days) in both seasons, total chlorophyll (a and b), total carbohydrates, nitrogen, phosphate, and potassium all gradually and dramatically reduced. When compared to plants that received irrigation every 28 days, plants that received regular irrigation every 14 days had the highest average values for the aforementioned chemical analysis. Similar improvements in chemical analysis were observed by (Raza *et al.*, 2025), (Bistgani *et al.*, 2024) and (Al-Ghzawi *et al.*, 2024).

The chemical analysis of calendula plants was significantly impacted by the combined treatments of BC and PGPR with varying watering intervals over the two tested seasons (Fig. 2 and 3). The interaction between BC and PGPR under a 14-day irrigation interval was the most effective in increasing the chemical analysis parameters seen in calendula plants when compared to the control group and all other reactive treatments. The identical treatment was then administered with a 21-day irrigation interval. The usage of the BC with PGPR reduced the impacts of drought and enhanced the measured chemical analysis results in comparison to the control plants, even though the calendula plants were subjected to drought stress with irrigation every 28 days.

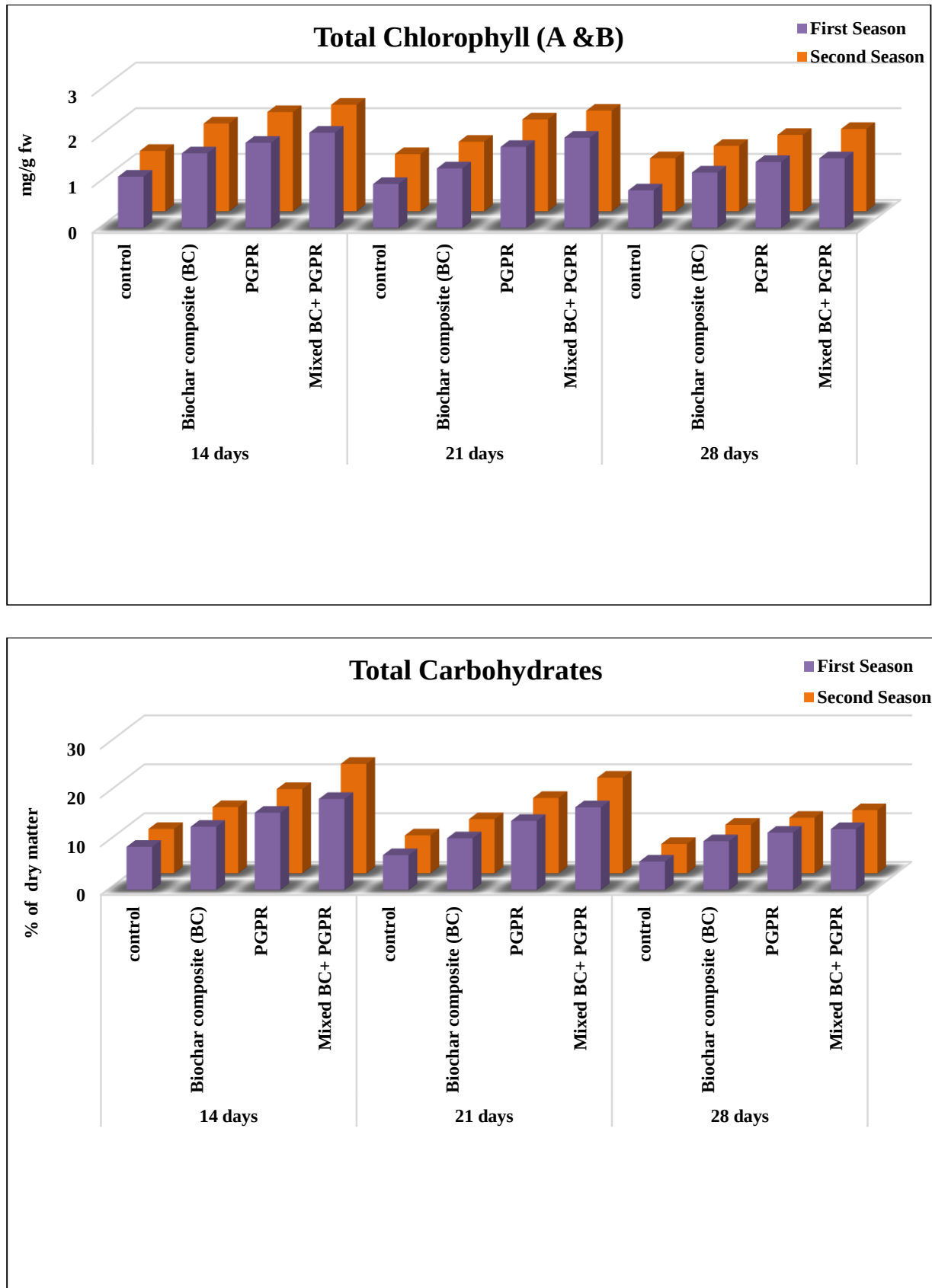


Fig. (2). Response total chlorophyll (A&B) content and total carbohydrates content in leaves of *Calendula Officinalis* L. to co – application of biochar composite (BC) and PGPR under water deficit conditions during 2023/2024 and 2024/2025 seasons

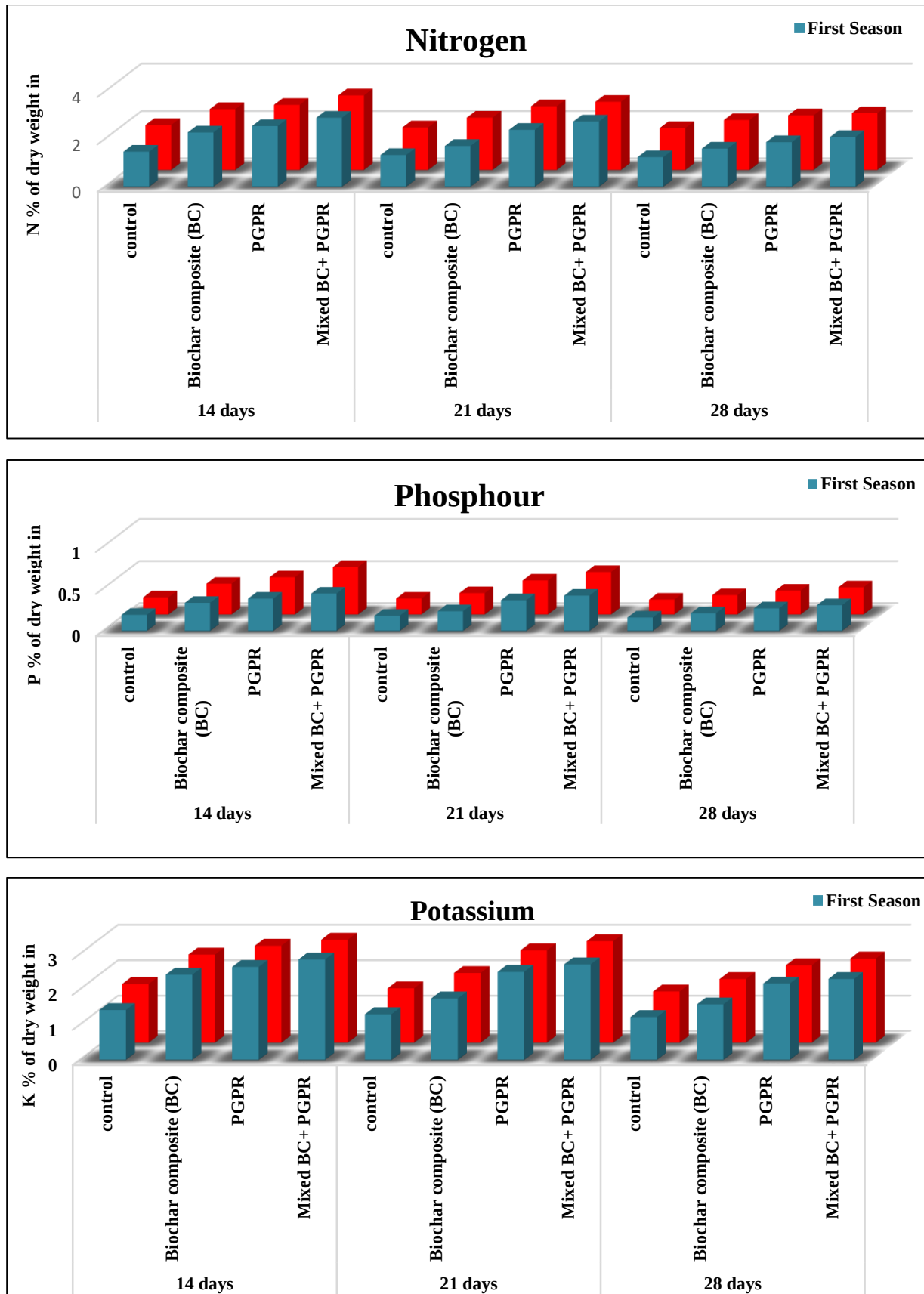


Fig. (3). Response the percentage of nitrogen, phosphorous and potassium in leaves of *Calendula Officinalis* L. to co – application of biochar composite (BC) and PGPR under water deficit conditions during 2023/2024 and 2024/2025 seasons

2- Osmotic parameter

Phenolic content, Leaf proline content and Relative water content (R.W.C. %)

Drought stress considerably changed the concentrations of phenolics, leaf proline, and relative water content (R.W.C.%), as seen in (Fig. 4). In both seasons, plants treated with either BC or PGPR had higher phenolic content, proline content, and relative water content (R.W.C%). The phenol content of plants treated with BC and PGPR was higher than that of untreated plants, but under drought-induced stress conditions, it decreased in both seasons, especially when irrigated every 28 days.

Untreated plants exhibited the highest proline accumulation under watering conditions of 21 or 28 days when compared to plants treated with BC and PGPR. Proline content increased noticeably in both BC and PGPR treatments. Plants treated with BC alone exhibited the highest proline accumulation when compared to plants grown in standard irrigation water (14 days), followed by plants treated with PGPR alone when irrigated every 28 days. Figure 4 shows the relative water content (R.W.C%) of plants treated with either BC, PGPR, or both. The (R.W.C%) decreased when there was water stress, particularly after 28 days of irrigation. On the other hand, using PGPR alone or in combination with BC increased the (R.W.C%) of plants throughout the same 28-day watering period and reduced the damage brought on by water stress. These findings support the conclusions drawn by (Çakmakçı *et al.*, 2023) and (Parmoon *et al.*, 2022).

3- Antioxidant Enzyme Activities of leaves

The results obtained in (Fig. 5) during the course of the two experimental seasons demonstrate that the use of biochar composite (BC) or/and plant growth promoters (PGPR) significantly boosted the ability of calendula leaves to scavenge free radicals produced by water stress when compared to the control group. Additionally, extended irrigation intervals (21 and 28 days) significantly increased the antioxidant enzyme activity in calendula leaves since there was less moisture available in both seasons. At irrigation intervals of 21 and 28 days, the activity of the enzymes catalase (CAT), acetyl peroxidase (APX), and superoxide dismutase (SOD) increased most in the leaves of untreated plants. Regardless of the irrigation interval, the application of PGPR alone or in conjunction with biochar composite (BC) significantly decreased the quantities of the enzymes catalase (CAT), acetyl peroxidase (APX), and superoxide dismutase (SOD) and lessened the harmful effects of oxidative stress. Inoculating the plants with biochar composite (BC) and PGPR resulted in a decrease in the activity of the previous antioxidant enzymes for both seasons, despite the fact that the plants were exposed to drought when irrigated every 28 days, which caused an increase in the activity of catalase, acetyl peroxidase, and superoxide dismutase enzymes, resulting in weak plant growth and a decrease in seed productivity and total carotenoids and total flavonoids content. These findings support the conclusions drawn by (Çakmakçı *et al.*, 2023) and (Parasar and Agarwala, 2025).

4- Phytohormones

According to the data shown in (Fig. 6), plants treated with biochar composite (BC) or/and plant growth promoters (PGPR) demonstrated an increase in measured phytohormones such as IAA, GA, and ABA in both seasons when compared to the control under varied watering intervals. Plants that received regular irrigation every 14 days performed the best under all treatments. Plants under water stress (irrigation every 21 or 28 days) exhibited a decrease in phytohormones across all treatments, in contrast to plants that were irrigated every 14 days. However, plants treated with BC and inoculated with PGPR showed a rise in these hormones during prolonged irrigation conditions (28 days), followed by plants treated with either BC or PGPR at the same irrigation interval.

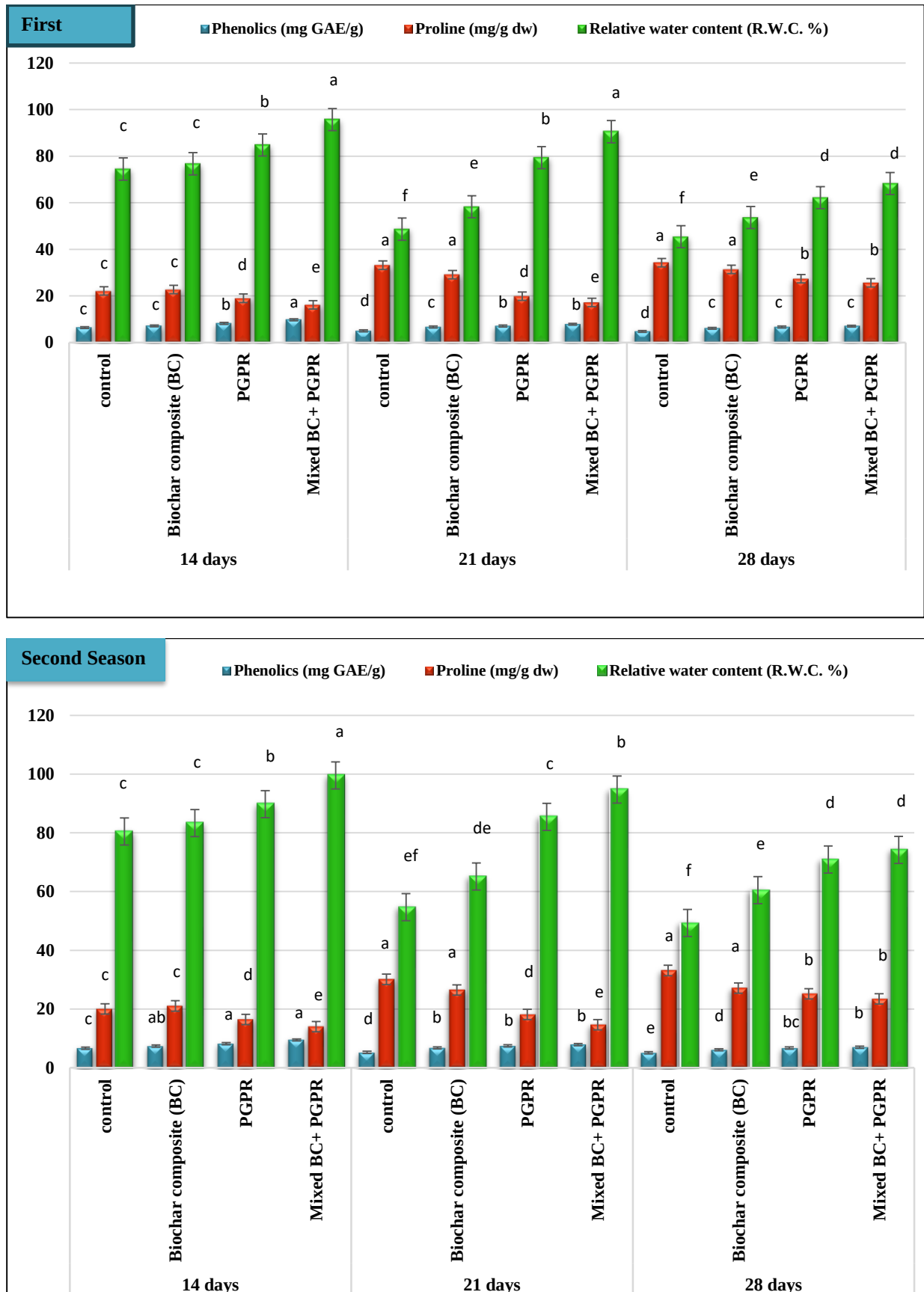


Fig. (4). Response the phenolic content, proline content and relative water content (R.W.C. %) in leaves of *Calendula Officinalis* L. to co – application of biochar composite (BC) and PGPR under water deficit conditions during 2023/2024 and 2024/2025 seasons

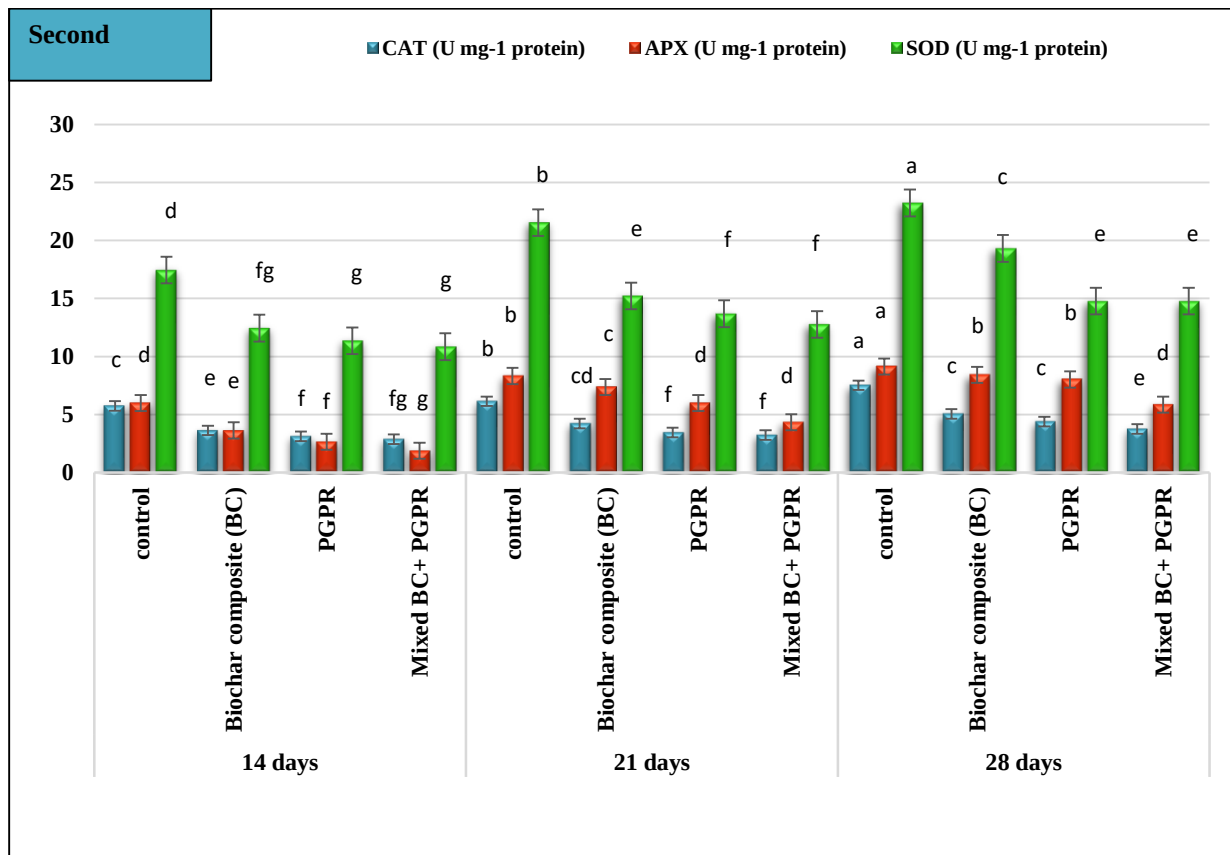
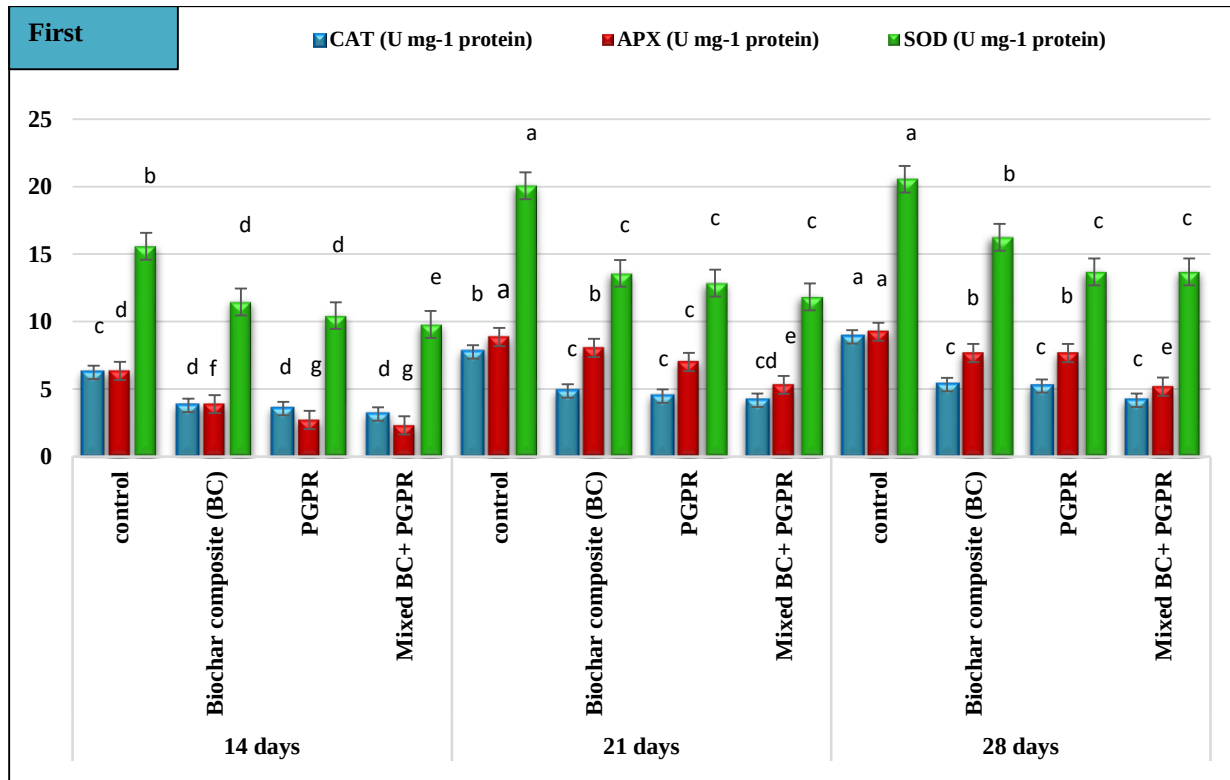


Fig. (5). Response the antioxidant enzymes in leaves of *Calendula Officinalis* L. to co – application of biochar composite (BC) and PGPR under water deficit conditions during 2023/2024 and 2024/2025 seasons

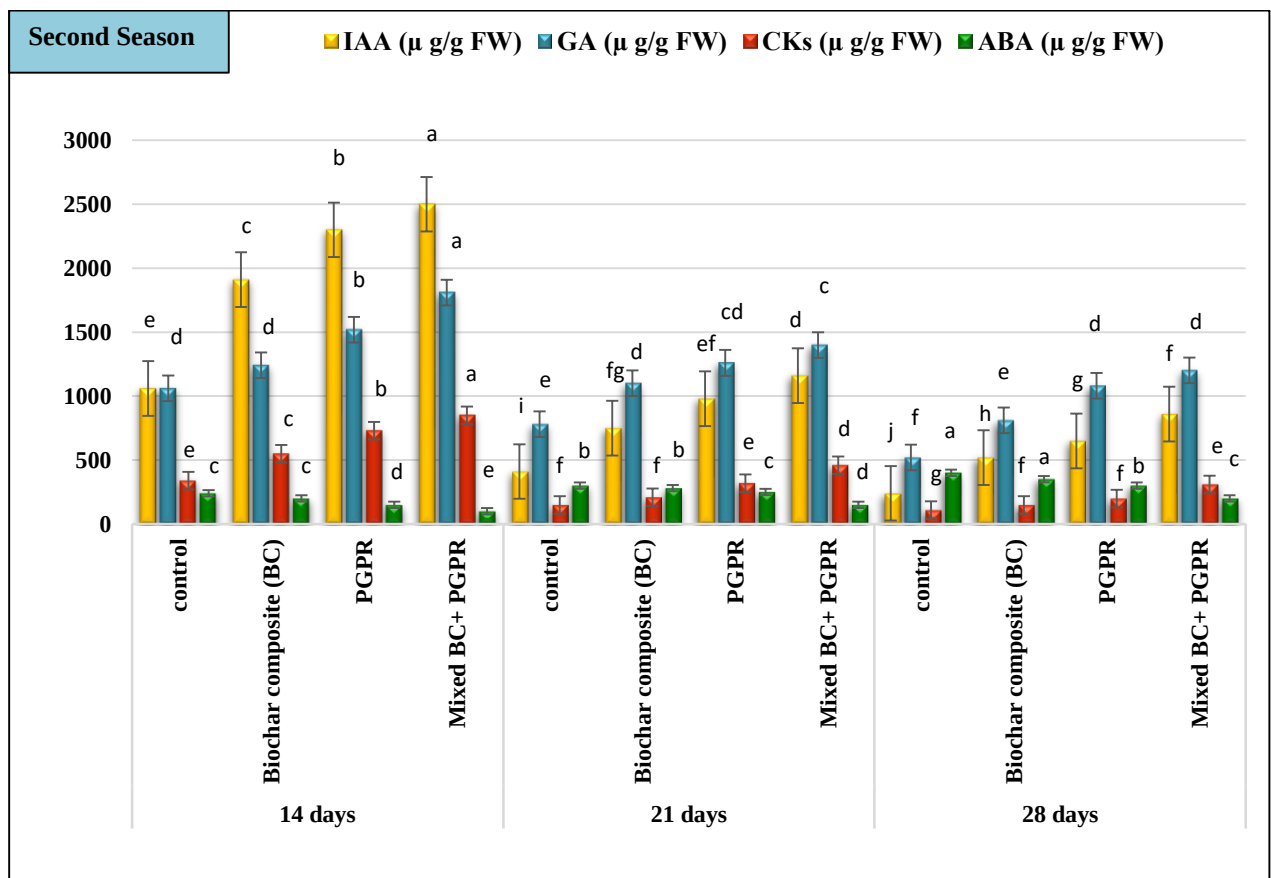
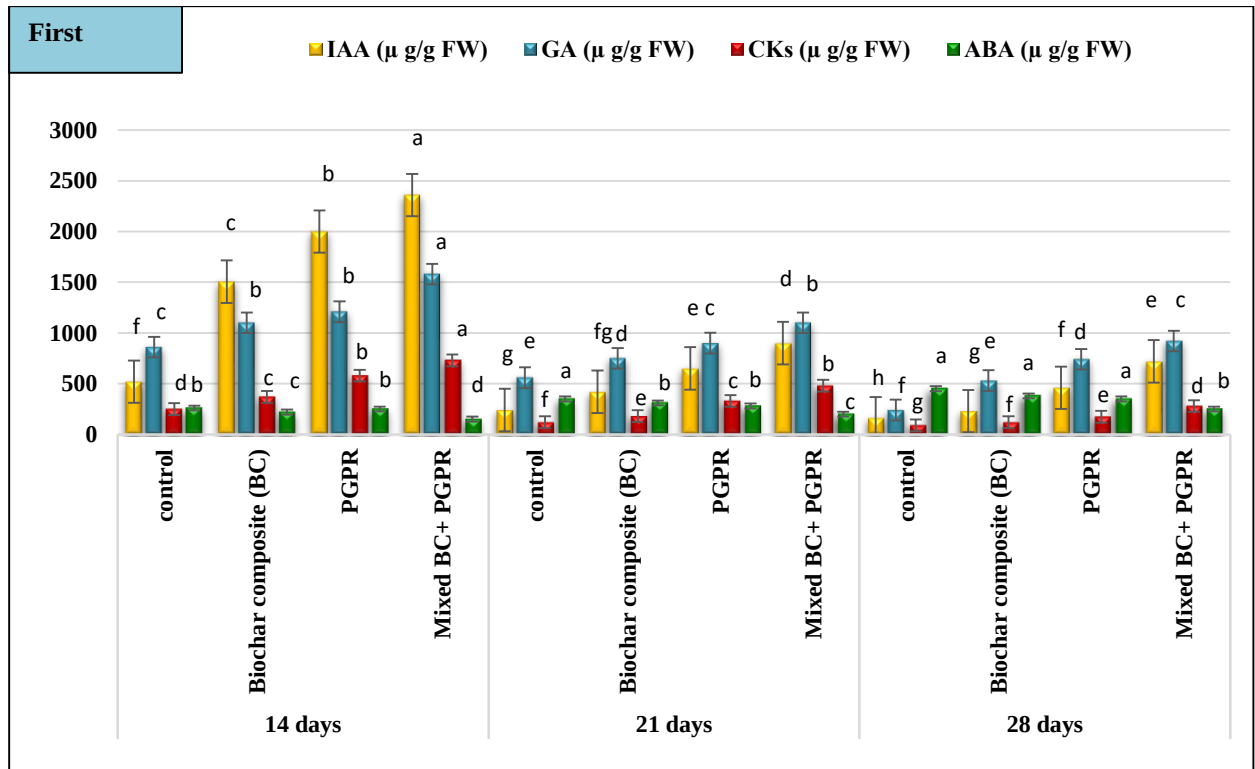


Fig. (6). Response the Phytohormone content in leaves of *Calendula Officinalis* L. to co – application of biochar composite (BC) and PGPR under water deficit conditions during 2023/2024 and 2024/2025 seasons

Abscissic acid (ABA) levels were also greater in calendula leaves under water stress (21 or 28 days) in both seasons compared to plants that were irrigated every 14 days. When compared to the control group during drought (21 or 28 days), plants treated with a combination of BC and infected with PGPR displayed a decrease in ABA content. Conversely, all treatments under water stress conditions (21 or 28 days) showed a decrease in cytok compared to plants treated with all treatments under 14 days irrigation settings. These findings support the conclusions drawn by (Al-Ghzawi *et al.*, 2024) and (Parasar and Agarwala, 2025).

C- In the Soil of calendula

1- Dehydrogenase activity (DHA)

The dehydrogenase enzyme (DHA) in the soil of calendula plants throughout the two studied seasons is affected by biochar composite (BC), plant growth promoters (PGPR), and their interactions during irrigation intervals (Fig. 7). The results show that all applied treatments significantly increased soil dehydrogenase (DH) activity during all irrigation intervals when compared to the untreated control. DH activity gradually decreased as the watering intervals were extended from 14 days to 21 and 28 days. During the 14-day irrigation period, all treatments had peak dehydrogenase (DH) activity; the synergistic treatment, which included BC and PGPR, produced the highest overall level of enzymatic activity. The control plants showed the least level of dehydrogenase (DH) activity among the various treatments using BC and PGPR, which were arranged in descending order. Extending the irrigation interval resulted in a proportionate decrease in DH activity across all examined treatments as compared to the 14-day interval. Despite the lack of moisture, the combination of BC and PGPR kept the highest activity levels, followed by PGPR, BC, and the control therapy. This demonstrates that combining these substances effectively decreased mild water stress while preserving high levels of microbial metabolic activity. These findings support the conclusions drawn by (Cun, 2025) and (Ferioun *et al.*, 2025).

2- Soil water relations

Different irrigation intervals (14, 21, and 28 days) have a strong and significant impact on both the monthly applied irrigation water amounts ($\text{m}^3/\text{fed}.$) and the overall seasonal water consumption over the growth seasons, according to the data in (Table 3).

The data showed that the decreased irrigation period (14 days) consistently recorded the highest total applied water, reaching 2380 and 2376 m^3/fed for the first and second seasons, respectively. Conversely, the total amount of water applied significantly decreased when the irrigation intervals were increased. The total applied water under the 21-day interval decreased to 1880 m^3/fed in the first season (a water savings of approximately 21.01%) and 1979 m^3/fed in the second season (a savings of 16.71%) when compared to the 14-day treatment. During a 28-day period, the lowest water use 1790 m^3/fed in the first season and 1883 m^3/fed in the second was noted.

The monthly distribution revealed the lowest water requirements of all treatments (between 162 and 160 m^3/fed), with a gradually increasing trend in water application starting in November. The primary causes of this are the early growth stages of the plants and low ambient temperatures. Then, as plants became older, their canopies grew, and the temperature rose, their water requirements progressively climbed, peaking in February and March. March had the greatest monthly water application (544 m^3/fed) throughout a 14-day period during the first season. The second season's peak slipped somewhat toward February (420 m^3/fed) but stayed high in March (565 m^3/fed). Important physiological stages including flowering and seed formation, as well as rising atmospheric evaporative requirements, coincide with this peak. The increase in total applied water under the shortened crop cycle is inextricably connected to the increasing frequency of irrigation episodes. Irrigation interval (14 days), as (Table 3) illustrates. On the other hand, by making the plants use up all of the moisture that is available from deeper soil layers, longer watering intervals (21 and 28 days) may increase Water Use Efficiency (WUE). These findings support the conclusions drawn by (Chen *et al.*, 2024) and (Zhang *et al.*, 2023).

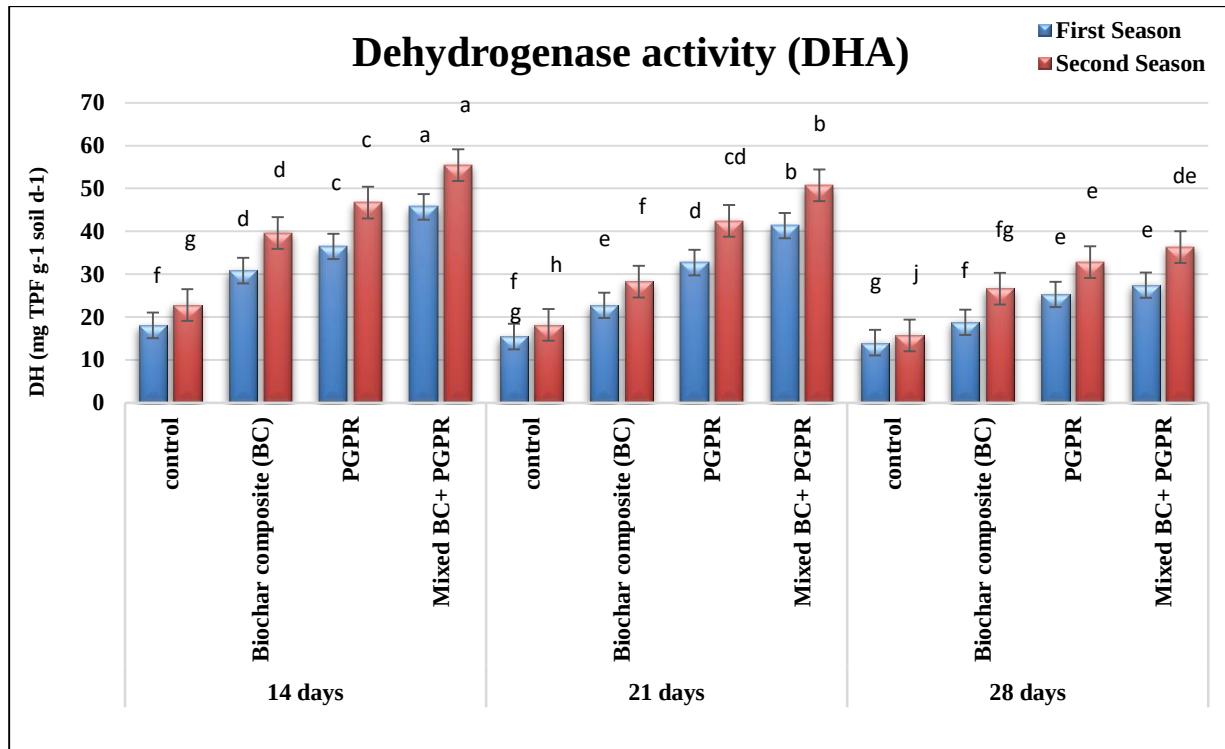


Fig. (7). Response the dehydrogenase activity in soil of *Calendula Officinalis* L. to co – application of biochar composite (BC) and PGPR under water deficit conditions during 2023/2024 and 2024/2025 seasons

3. The irrigation Water Productivity, (kg/m³)

The results presented in (Table 4) demonstrate that, in addition to the total seasonal water applied (m³/fed), irrigation at various intervals (14, 21, and 28 days) had a highly significant impact on the calendula crop's quantitative yield and irrigation water productivity (IWP) over the course of the two subsequent seasons. Extending the irrigation intervals from 14 days to 21 and 28 days resulted in a considerable decrease in the total volume of water applied per feddan (m³/fed) throughout both growing seasons.

In particular, water use decreased from 2380 to 1790 m³/fed in the first season and from 2376 to 1883 m³/fed in the second. When calendula (*Calendula officinalis* L.) plants were treated to progressive water shortage stress (by irrigating every 21 and 28 days), the inflorescences dry yield per feddan steadily dropped compared to the regular, well-irrigation treatment (14 days interval). The 14-day irrigation interval produced the highest production of inflorescences, which was 297.74 kg/fed. and 314.64 kg/fed. for the two consecutive seasons, respectively.

The maximum Irrigation Water Productivity (IWP) was considerably attained under the moderate irrigation interval (21 days), even if the 14-day irrigation interval yielded the highest absolute crop production. In the first and second seasons, it registered the highest values of 0.139 and 0.145 kg/m³, respectively. The intensive irrigation treatment (28 days) showed the lowest water consumption efficiency, while the 14-day interval came next. The rise in IWP throughout the course of the 21-day period indicates a satisfactory physiological equilibrium. At this intermediate interval, there was no discernible loss in crop output due to the large reduction in the total amount of water sprayed, demonstrating that plants under mild-to-moderate drought stress maximise gas exchange and maintain high photosynthetic water-use efficiency per m³ of water, in contrast to over-irrigation (14 days), which could otherwise lead to nutrient leaching or root zone hypoxia. The efficiency of the utilized. The lack of a discernible drop in yield production under water deficiency conditions (21 days intervals) indicates that a combination of PGPR actively maintained crop productivity through many growth-promoting mechanisms in the root zone. Furthermore, the addition of the wood-banana biochar composite (BC)

served as an essential buffer for the rhizosphere. This amendment effectively reduced the osmotic and oxidative stress brought on by the extended watering intervals by maximizing soil moisture retention and activating endogenous antioxidant defenses.

In this sense, changing irrigation can save water, as demonstrated by the data in Table 4. In the first season, extending the irrigation interval to 21 and 28 days instead of 14 days led to notable water savings of 500 m³/fed (21.01%) and 590 m³/fed (24.79%), respectively. Similar trends were seen in the second season, with water savings of 16.71% and 20.75% for intervals of 21 and 28 days, respectively. More importantly, the 21-day interval proved to be highly effective when these water savings were correlated with the characteristics of the calendula plant, such as vegetative growth, flowering, and total carotenoids content; it produced irrigation water savings of 16% to 21% while maintaining optimal biological synergy with treatments involving biochar composite (BC) and Plant growth promoters (PGPR) treatments that successfully protected the plants against yield loss caused by drought.

Table (3). Effect of irrigation treatments on the amounts of monthly applied irrigation water (m³/fed.) for the 2023/2024 and 2024/2025 growing seasons

Months	Irrigation intervals		
	(14 Day)	(21 Day)	(28 Day)
	2023/2024		
November	162	162	162
December	253	266	181
January	285	300	248
February	423	351	367
March	544	326	334
April	363	245	257
May	350	230	241
Total M ³ /Fed.	2380	1880	1790
	2024/2025		
	(14 Day)	(21 Day)	(28 Day)
	2024/2025		
November	160	160	160
December	228	216	192
January	346	232	248
February	420	300	332
March	565	434	400
April	332	320	285
May	325	317	266
Total m ³ /Fed.	2376	1979	1883

Table (4). The total water applied (m³/fed.), The yield (kg/fed.), The irrigation water Productivity, (kg/m³) under different treatments

Irrigation Interval	The total water applied, (m ³ /fed.)	The yield, kg/fed	The irrigation Water Productivity, kg/m ³	Water saved (m ³ /fed.)
	2023/2024			
(14 Days)	2380	297.74	0.125	-----
(21 Days)	1880	260.76	0.139	500
(28 Days)	1790	222.01	0.124	590
	2024/2025			
	2024/2025			
(14 Days)	2376	314.64	0.132	-----
(21 Days)	1979	287.10	0.145	397
(28 Days)	1883	237.13	0.126	493

4. Discussion

Due mostly to global warming, which exacerbates a number of abiotic stresses like drought, salinity, and soil acidity or alkalinity, climate change has become a major worldwide environmental problem during the past 20 years. Rising temperatures drastically reduce water availability and cause the gradual desiccation of soils and vegetation by speeding up air evaporation rates, which change the Earth's hydrological cycle. As a result, drought is now one of the most common and complicated abiotic hazards to sustainable agriculture, resulting in significant reductions in crop yields, food shortages, and a rise in forest fires (**Seleiman *et al.*, 2021**). Drought causes a plant's stomata to gradually close in order to reduce water loss, which at the same time interferes with cellular diffusion, root-to-shoot nutrient transfer, and water use efficiency. A series of detrimental morphological, physiological, biochemical, and molecular changes are set off by these disturbances. Drought stress essentially impairs cell division, elongation, and differentiation three fundamental biological processes for plant development which clinically shows up as limited root proliferation, reduced leaf area, and stunted stem extension. Moreover, photorespiration, cellular energy production, membrane integrity, pigment biosynthesis, lipid metabolism, and endogenous hormonal balance are all negatively impacted by the concurrent decrease in stomatal and mesophyll conductance, which severely limits carbon dioxide (CO₂) diffusion (**Haghpanah *et al.*, 2024**). Water deficits downregulate important photosynthetic proteins on a molecular and biochemical level, especially Rubisco activase and Rubisco large subunit-binding protein, which are essential for assembling the enzyme within chloroplasts and preserving the catalytic competence of the Rubisco active site, respectively. In the end, the decreased quantity of these proteins suppresses net CO₂ fixation by indirectly causing Rubisco breakdown. Drought stress also degrades chlorophyll-binding proteins and lowers overall chlorophyll levels by reducing the concentrations of chlorophyll (a and b) (**Amaral *et al.*, 2024**). By destroying pigment-protein complexes, changing the shape of chloroplasts, and disrupting thylakoid membranes and the electron transport chain, these multi-level disturbances seriously jeopardise the structural and functional integrity of the photosynthetic apparatus. Excessive carbohydrate buildup is encouraged by the imbalance in sugar and nutrient metabolism that results from stomatal regulation's limitation of carboxylation capacity. Reactive oxygen species (ROS) are overproduced as a result, causing severe oxidative stress that results in membrane lipid peroxidation, protein denaturation, and enzyme inactivation. In the end, the drastic drop in transpiration rates substantially hinders overall plant growth and productivity by suppressing xylem sap transfer, limiting vital enzymatic activities, and impairing symbiotic nitrogen fixation (**Raja *et al.*, 2020**).

Plant-growth-promoting rhizobacteria (PGPR) inoculation has become a very successful, economically feasible, and environmentally sustainable method of increasing agricultural productivity and reducing the negative effects of abiotic stresses, especially salinity and drought (**Etesami and Jeong, 2021**). By colonising the rhizosphere and forming a dynamic, mutualistic relationship with the host plant, these helpful soil microbes serve as biological elicitors that cause systemic tolerance (**Al-Askar *et al.*, 2023**). PGPR efficiently protects plants from environmental abnormalities by combining direct mechanisms like nutrient solubilisation and phytohormone modulation with indirect pathways like the control of stress-induced ethylene. This optimises plant growth, physiological homeostasis, and root system architecture (RSA) to guarantee survival and maintain yield under difficult field conditions (**Glick, 2020**).

The multifunctional, synergistic, and complementary mechanisms of the particular bacterial consortium consisting of *Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Bacillus polymyxa* are largely responsible for the remarkable improvement in plant biomass, RSA, and drought stress mitigation observed upon co-inoculation (**He and Oh, 2023**). The formation of strong roots and absorptive hairs greatly increases the soil surface area occupied by the root system, maximising water and nutrient uptake during moisture deficiency. This co-inoculation serves as an advanced bio-strategy that re-engineers the rhizosphere. Microbial secretion of low-molecular-weight signalling molecules, like 2,4-diacetylphloroglucinol (DAPG), and a highly integrated hormonal network are responsible for this architectural change (**Vacheron *et al.*, 2021**). The ability of these three strains to produce ACC

deaminase, which efficiently breaks down root-exuded 1-aminocyclopropane-1-carboxylic acid (ACC), suppressing the production of inhibitory "stress ethylene" and delaying tissue senescence, is a crucial aspect of this hormonal network (Vacheron *et al.*, 2021). In particular, the mitigation of ethylene feedback inhibition unlocks the physiological efficiency of indole-3-acetic acid (IAA), which is heavily generated by *Azospirillum lipoferum*. This enzymatic cleavage works in perfect harmony with other plant growth-promoting (PGP) features. This microbial IAA facilitates robust rhizosphere colonisation, encourages root elongation, and improves lateral branching by loosening cell walls, stimulating lateral root development, and increasing root exudation (Fukami *et al.*, 2022). Simultaneously, synchronised abscisic acid (ABA) adjustments fine-tune stomatal conductance, transpiration rates, and root hydraulic conductivity, while this intricate hormonal modulation indirectly optimises the plant's endogenous balance by reducing ACC levels and modifying the ethylene signalling pathway to prevent growth inhibition (Zahedi *et al.*, 2024). The consortium drives a powerful osmoregulatory adjustment that maintains cellular homeostasis and relative water content (RWC) in parallel with hormonal and enzymatic regulation. Proline biosynthesis genes are activated upon inoculation with these osmotolerant strains, and suitable solutes such as soluble sugars, total proteins, trehalose, and glycine betaine (GB) accumulate in both the bacterial cells and host tissues (Kasim *et al.*, 2025). *Azotobacter chroococcum* actively contributes to the creation of a greater water potential gradient within this metabolic framework, which maximises the use of biologically fixed nitrogen, essential nutrients, and antioxidants (Kour *et al.*, 2021) and prevents biological structures from drying out (Bhat *et al.*, 2023). While sugar and trehalose accumulation maintains cell turgor and regulates important physiological processes like development and senescence (Kasim *et al.*, 2025), accumulated GB acts as a molecular chaperone that stabilises membranes, protein quaternary structures, and enzyme folding under stress. Importantly, *Bacillus polymyxa* mechanically strengthens this internal physiological and biochemical resistance by secreting large amounts of exopolysaccharides (EPS) in addition to phosphate solubilisation (Shultana *et al.*, 2022). By forming a hydrophilic biofilm and a protective attachment zone between soil clay particles, bacteria, and the root system, these highly hydrated macromolecules which contain around 97% water in their polymer matrix restrict the influx of harmful ions. By promoting soil aggregation and forming a slow-drying moisture buffer that protects plant roots and helpful microorganisms from desiccation while also storing essential carbon sources, this EPS matrix physically modifies the microenvironment (Mishra *et al.*, 2024). Thus, a strong, tripartite biochemical and physical shield is established by the integration of ACC-deaminase mediated ethylene mitigation, IAA-driven root expansion by *A. lipoferum*, nutritional and metabolic osmotic adjustment supported by *A. chroococcum*, and the physical EPS-moisture barrier created by *B. polymyxa*, which explains the co-inoculated plants' superior drought tolerance and physiological homeostasis (Etesami and Jeong, 2021).

Agriculture faces previously unheard-of difficulties in maintaining crop output and maximizing dwindling water supplies in the face of escalating global climate change and growing arid regions. In order to achieve long-term agricultural resilience, it is now essential to develop creative, climate-smart agronomic solutions. Among these tactics, applying biochar (BC) to the soil has become a ground-breaking method that tackles soil deterioration, plant water scarcity, and ecological waste management all at once. Comprehensive changes in soil physicochemical and mechanical properties are brought about by the application of biochar (BC), which is characterized by decreased bulk density, decreased soil compaction, and an increase in overall porosity and soil architecture (Tanure *et al.*, 2019). Biochar is an effective water reservoir that improves soil moisture content and water retention in dry and semi-arid environments because of its extremely porous structure, which is rich in aromatic and heterocyclic functional groups (Hassan *et al.*, 2025). This structural change improves soil aeration and rhizosphere oxygen availability, which are essential for root development, while also optimizing pore space structure, which increases water penetration and drainage as demonstrated by saturated hydraulic conductivity in fine-textured soils (Heydari *et al.*, 2023). Additionally, adding biochar significantly increases the soil's cation exchange capacity (CEC), organic matter (OM), and organic carbon (OC) content while also changing the soil's pH and electrical conductivity (EC) (Ayaz *et al.*, 2021). These changes maximize nutrient availability and retention, allowing for a long-term, progressive release of vital macro- and micronutrients, including phosphorus (P), potassium (K), calcium (Ca), magnesium

(Mg), and nitrogen (N) through improved biological nitrogen fixation (**Gabhane *et al.*, 2020**). By creating an ideal micro-habitat that promotes microbial growth in the rhizosphere, the addition of biochar enhances soil biological dynamics at the biophysical and microbiological levels. Beneficial microorganisms, such as arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria, are encouraged to proliferate by this structural and nutritional support. These microbes directly improve plant nutrient intake, fortify root systems, and increase disease resistance (**Jabborova *et al.*, 2021**). Building a strong root system therefore improves the plant's ability to link soil particles, which greatly aids in efforts to conserve water and soil in drought-prone areas. Furthermore, plant shape and vegetative growth characteristics are dynamically regulated by the improved soil state and increased nutrient supply (**Khan *et al.*, 2021**). By reducing osmotic and oxidative stresses in plant tissues, biochar supplementation physiologically lessens the negative effects of drought and moisture stress (**Tanure *et al.*, 2019**). A crucial inorganic osmo-protectant biochar improves leaf relative water content (RWC) and restricts harmful (Na^+) uptake from saline or stressed soils by regulating root water absorption and raising cellular potassium (K^+) concentrations (**Hasnain *et al.*, 2023**). Cell growth and metabolic balance are maintained as a result. The degradation of photosynthetic pigments is directly prevented by this better water status; biochar increases the intake of magnesium (Mg^{2+}), a crucial element needed for chlorophyll production, which raises levels of total chlorophyll and carotenoid. Net photosynthetic rates are therefore increased (**Khan *et al.*, 2021**). Additionally, applying biochar causes important antioxidant enzymes to be profoundly upregulated, greatly increasing the activity of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) (**Rahman *et al.*, 2025**). This strong antioxidant ability efficiently scavenges reactive oxygen species (ROS), shielding cellular membranes and the photosynthetic system from oxidative damage caused by water stress (**Jabborova *et al.*, 2021**). In the end, increased water use efficiency (WUE), optimized biomass production, and sustained crop yields under challenging environmental conditions result from the synergistic improvement in soil-plant-water relations combined with extended nutrient availability (**Hasnain *et al.*, 2023**).

Conclusively, the combination of biochar composite (BC) and plant growth promoters (PGPR) under 21-day intervals produced the best results in terms of optimal water use and the maintenance of both the quality and quantity of the inflorescences dry yield/fed (Kg), even though all applied treatments improved the vegetative growth parameters, flowering parameters, and chemical analysis of calendula (*Calendula officinalis* L.) under irrigation intervals.

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