https://doi.org/10.56946/jspae.v4i2.750
ISSN: 2957-9082
Journal of Soil, Plant and Environment

ORIGINAL RESEARCH

Endophytic Microbes Boost Drought Tolerance and Yield in Sunflower (*Helianthus annuus* L.)

Rashid Khan[1*], Haris Khan[2], Sajid Ali[2], Nabeel Akbar[1], Rida Batool[3], Mudassar Shumail Khan[4]

[1]Department of Agronomy, Faculty of Agriculture, Gomal University, D.I. Khan, Khyber Pakhtunkhwa, Pakistan.
[2]Gomal Centre of Biochemistry and Biotechnology, Gomal University, D.I. Khan, Khyber Pakhtunkhwa, Pakistan.
[3]Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan.
[4]Department of Soil Science, Faculty of Agriculture, Gomal University, D.I. Khan, Khyber Pakhtunkhwa, Pakistan

*Corresponding author:
rrs125634@gmail.com
Received: 04 December 2024
Revised (V1): 25 May 2025
Revised (V2): 25 July 2025
Accepted: 03 August 2025

ABSTRACT: Drought represents a major constraint to sunflower yield in arid and semiarid regions, demanding innovative agronomic practices. The current study quantifies the contribution of endophytic microbiota to drought resilience in Helianthus annuus L. during a field experiment conducted in Dera Ismail Khan during the 2025 cropping season. Trials were sited on a farmer's plot bordering Channel No. 6 of the Gomal Zam Dam command area, employing a randomized complete block design with three replicates. Seventeen physiological, biochemical and yield-related metrics were systematically assessed, including plant stature, chlorophyll fluorescence, stomatal conductance, antioxidant enzyme activities and final yield components. Data demonstrated that inoculation with selected endophytic strains, with Endophyte B exhibiting the strongest effect, elicited statistically superior results across all measured traits relative to uninoculated controls. Enhanced catalase and peroxidase activities, diminished malondialdehyde accumulation, and elevated accumulation of osmotic regulators together attested to effective oxidative damage suppression. Yield parameters, including capitulum diameter and seed set per capitulum, responded positively and proportionately. Collectively, these observations endorse endophytic microbes as a viable, environmentally benign approach to bolster sunflower resilience against drought, aligning with the objectives of climate-smart agronomy.

KEYWORDS: Abiotic stress, antioxidant metabolism, endophytic microbes, drought resilience, plant growth promotion.

1. Introduction

Sunflower (Helianthus annuus L.) is one of the world's foremost oilseed crops, valued for its edible oil, use in biodiesel production, and role as a model species for studying plant adaptability. Its short thermal growing degree days and ability to thrive in diverse, even marginal, soils make it particularly appealing in regions such as Pakistan, India, and sub-Saharan Africa. In Pakistan, for example, sunflower cultivation plays an important role in supplementing domestic edible oil production, especially in semi-arid areas like Dera Ismail Khan and Multan. However, despite its adaptability, sunflower productivity is substantially reduced by abiotic stresses, most notably drought and salinity, which limit seed yield, oil accumulation, and physiological vigor (Kaya et al., 2021; Tiwari et al., 2022). With climate change expected to intensify the frequency and severity of these stresses, the urgency of developing sustainable resilience strategies is increasingly recognized.

Evidence from multiple studies highlights that drought and salinity impair sunflower

performance by disrupting photosynthesis, accelerating oxidative damage, and disturbing osmotic balance. In such environments, soil degradation and water scarcity combine to exacerbate yield losses. Conventional strategies for mitigating these stresses have included genetic breeding, deficit irrigation, and the application of chemical protectants. While breeding has produced some tolerant varieties, it is a slow and resource-intensive process. Irrigation, though effective, is often economically unfeasible in resource-limited farming systems, and chemical interventions can have negative environmental repercussions. These limitations have prompted a growing interest in alternative, ecologically sound solutions.

Microbial biotechnology, particularly the use of endophytic microorganisms, has emerged as a promising complementary approach. Endophytes, which inhabit internal plant tissues without causing disease, can promote plant growth through phytohormone biosynthesis, solubilization of mineral nutrients, and activation of systemic acquired tolerance (Gouda et al., 2018; Lata et al., 2018; Khan et al., 2020). In several agronomic crops, endophytes have been shown to alleviate drought and salinity stress by enhancing antioxidant enzyme activity, stabilizing chlorophyll content, improving stomatal conductance, and maintaining osmotic homeostasis (Eke et al., 2020; Kour et al., 2022). However, in sunflowers, research into endophyte-mediated abiotic stress tolerance remains scarce. Most available studies focus on rhizobacteria, while the role of microorganisms colonizing internal plant tissues under such stress conditions has been largely overlooked (Bhagat et al., 2020).

This gap in knowledge is particularly relevant for sunflower cultivation in Pakistan, where low-input cropping regimes dominate and farmers often face severe resource constraints. Deploying stress-resilient endophytes in these systems could enhance yields while reducing dependence on synthetic fertilizers and pesticides, aligning with principles of sustainable agriculture (Ahmad et al., 2021). Despite the clear potential, there remains a critical deficit in the isolation, characterization, and functional validation of sunflower-associated endophytes capable of conferring abiotic stress tolerance.

The present study addresses this gap by systematically surveying the diversity of endophytic microorganisms endemic to sunflowers and rigorously assessing their capacity to mitigate drought and salinity stress. Using a combination of physiological and biochemical indicators, the research will quantify the extent of stress amelioration attributable to these microbes. The ultimate objective is to identify microbial strains with robust stress-protective capabilities and lay the groundwork for developing bioinoculants tailored to environments where abiotic stresses predominate. We hypothesize that sunflower-associated endophytes can significantly enhance drought and salinity tolerance by modulating key physiological and biochemical processes, thereby improving crop resilience in stress-prone agroecosystems.

2. Materials and methods

2.1 Experimental Site and Design

The trial was implemented throughout the Kharif growing season of 2025 at the agronomic research station belonging to the Department of Agronomy, Faculty of Agriculture, Gomal University, located in Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan (GPS: 31.8204° N, 70.9060° E; elevation: 173 m). The agroclimatic profile of the site is characterized as arid to semiarid, with summer air temperatures fluctuating between 28 °C and 44 °C, a relative humidity spectrum of 35% to 55%, and a mean annual precipitation of 180 to 220 mm, the majority of which is received during the monsoonal season. Pedological examination identifies the site soil as silty clay loam as per the USDA texture classification. The reaction is moderately alkaline with pH values ranging from 8.0 to 8.3, and the organic carbon content is measured at 0.6% to 0.8%. Nutritional profiling indicates a moderate overall fertility status. The experimental framework employed a Randomized Complete Block Design (RCBD) inclusive of triplicate replications. The treatment array comprised diverse endophytic microbial consortia inoculated onto both seed and soil, integrated with precise moisture regimes one of these was subject to a controlled drought stress induction protocol. The experimental host was Helianthus annuus L. cultivar PARSUN-3, which was chosen for its documented resilience to concurrent moisture deficit and elevated thermal regimes.

2.2 Soil Analysis

Prior to sowing, composite soil samples were collected from the experimental field to assess baseline physicochemical properties. Samples were taken from a depth of 0–15 cm using a stainless steel auger at five random points within each plot. The sub-samples were thoroughly mixed in a clean plastic container to form a representative composite sample for each plot. Approximately 1 kg of the homogenized sample was placed in labeled polyethylene bags and transported to the Soil Science Laboratory, Gomal University, for analysis. In the laboratory, samples were air-dried at room temperature, gently crushed using a wooden pestle and sieved through a 2 mm mesh before further examination. Soil pH and electrical conductivity (EC) were determined in a 1:1 (w/v) soil-to-distilled water suspension. The mixture was stirred for 30 minutes and allowed to equilibrate before measurements were taken using a calibrated digital pH/EC meter (Model: HI 9813-6, Hanna Instruments, USA) (McLean, 1982). Organic matter (OM) content was measured using the modified Walkley–Black wet oxidation method, which involves oxidation of organic carbon with potassium dichromate ($K_2Cr_2O_7$) in the presence of concentrated sulfuric acid, followed by titration with ferrous ammonium sulfate (Walkley and Black, 1934). Available phosphorus (P) was extracted by the Olsen method, using 0.5 M sodium bicarbonate ($NaHCO_3$) solution at pH 8.5 (Olsen et al., 1954). The extract was filtered, and P concentration was determined spectrophotometrically (Model: UV-1800, Shimadzu, Japan) using the ascorbic acid blue color method (Murphy and Riley, 1962). Exchangeable potassium (K) was extracted with neutral 1 M ammonium acetate (NH_4OAc) and quantified by flame photometry (Model: FP6410, Labtronics, India) following the procedure described by Richards (1954). Soil texture was determined

by the hydrometer method (Bouyoucos, 1962), which involves dispersing the soil in a solution of sodium hexametaphosphate [$(NaPO_3)_6$] as a deflocculating agent, followed by particle size analysis based on sedimentation rates according to Stokes' law.

2.3 Microbial Inoculum Preparation

Endophytic bacterial isolates used in this study were previously obtained from healthy sunflower (Helianthus annuus L.) xylem and root tissues using surface sterilization and plating techniques described by Hallmann et al. (1997). Pure cultures were maintained on nutrient agar (NA) slants at 4 °C until use. For inoculum preparation, single colonies of each isolate were transferred aseptically into 100 mL of nutrient broth (NB) in 250 mL Erlenmeyer flasks and incubated at 28 ± 2 °C for 48 hours on a rotary shaker at 150 rpm to achieve optimal growth. Bacterial cell density was determined spectrophotometrically (OD_{600} nm) and adjusted to approximately 1×10^8 colony-forming units (CFU) mL^{-1}using sterile distilled water, as estimated by the McFarland standard (McFarland, 1907).

For seed treatment, healthy, surface-sterilized sunflower seeds were soaked in the bacterial suspension for 12 hours at room temperature with occasional stirring to ensure uniform coating. Seeds were then air-dried under sterile conditions to remove excess moisture before sowing. For root-zone application, a soil drench of the same bacterial suspension was applied at the rate of 50 mL per plant mL per plant 10 days after sowing (DAS) to ensure colonization of the rhizosphere and root tissues. All inoculation procedures were performed under aseptic conditions in a laminar flow cabinet to prevent contamination.

2.4 Data Collection Parameters and Measurement Techniques

2.5 Morphological traits

Morphological traits of sunflower (*Helianthus annuus* L.) were recorded at the flowering stage to assess plant growth performance. Plant height was measured from the soil surface to the highest leaf tip of the main stem using a graduated meter scale (Model No. 2000, Stanley, USA) with values averaged from ten randomly selected plants per treatment (AOSA, 2009). Leaf area was measured using an electronic leaf area meter Leaf area was determined using an electronic leaf area meter (Model: LI-3100C, LI-COR Biosciences, Lincoln, USA), which provided accurate measurements of total leaf surface area and the leaf area index (LAI) was calculated as the ratio of total leaf area per plant to the ground area occupied by the plant (Watson, 1947). Root length was determined by carefully excavating the root systems of ten representative plants, gently washing them to remove adhering soil, and measuring from the root collar to the tip of the longest root with a flexible measuring tape (Benjamin and Nielsen, 2006). Stem diameter was recorded at 10 cm above the soil surface using a digital vernier caliper (Model LI-3100C, LI-COR Biosciences, USA) with three equidistant readings per stem averaged to obtain a representative value.

2.6 Physiological Parameters

Physiological parameters were measured at the flowering stage on the uppermost fully expanded leaves of sunflower (Helianthus annuus L.) to assess plant water status and photosynthetic characteristics. Relative water

content (RWC, %) was determined following the method of Weatherley (1950) using equation 1.

$$RWC = \frac{(FW - DW)}{(TW - DW)} \times 100 \quad \text{(Equation 1)}$$

Fresh weight was recorded immediately after leaf excision, turgid weight was obtained after floating the leaves on distilled water for 4 hours under room temperature and low light, and dry weight was measured after oven-drying at 70 °C for 48 hours. Chlorophyll content was estimated non-destructively in SPAD units using a SPAD-502 chlorophyll meter (Konica Minolta, Japan) on three points per leaf, averaged for each plant. Stomatal conductance (mmol m^{-2} s^{-1}) was measured at midday under full sunlight using a steady-state leaf porometer (Model SC-1, Decagon Devices, USA) on three leaves per plant. Leaf temperature (°C) was recorded under full sun conditions using a handheld infrared thermometer (Model 62 MAX+, Fluke Corporation, USA) at an angle of approximately 90° to the leaf surface to avoid reflection errors.

2.7 Biochemical Parameters

Biochemical parameters were evaluated at the flowering stage using fresh leaf tissue to assess stress-induced metabolic responses in sunflower (Helianthus annuus L.). Proline content, serving as an osmoprotectant and indicator of stress tolerance, was quantified (µmol g^{-1} fresh weight) using the acid ninhydrin method. This method has been validated recently, with results expressed on a fresh weight basis consistent with standard protocols (Singh et al., 2024) Lipid peroxidation, a measure of oxidative membrane damage, was estimated by determining malondialdehyde (MDA) concentration (nmol g^{-1} FW) using the thiobarbituric acid reactive substances (TBARS) assay, validated in recent plant stress studies (Lee & Chen, 2024). Total soluble sugars, contributing to osmotic adjustment and energy storage, were measured (mg g^{-1} FW) using the anthrone colorimetric method with absorbance read at 620 nm, this method is confirmed in recent analytical protocols (Mather et al., 2024) All biochemical assays were conducted in triplicate and results were presented on a fresh weight basis for accurate comparison among treatments.

2.8 Antioxidant enzyme activities were quantified as follows:

Antioxidant enzyme activities were measured in fresh leaf extracts to assess the oxidative stress response of sunflower (Helianthus annuus L.) under experimental conditions. Superoxide dismutase (SOD) activity was determined based on its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT), following the method described by Beauchamp and Fridovich (1971). Catalase (CAT) activity was assayed spectrophotometrically by monitoring the decomposition of hydrogen peroxide (H_2O_2) at 240 nm, as per Aebi (1984). Peroxidase (POD) activity was measured by recording the oxidation rate of guaiacol at 470 nm, following the procedure outlined by Chance and Maehly (1955). Enzyme activities were expressed on a fresh weight basis, with all assays conducted in triplicate to ensure reliability.

2.9 Yield and Agronomic Parameters

Yield and agronomic parameters were evaluated at harvest to determine the productive performance of sunflower

(*Helianthus annuus* L.). Head diameter was measured as the maximum transverse width of the capitulum using a digital vernier caliper for precise dimensional assessment. The number of seeds per head was quantified by mechanically threshing individual sunflower heads and counting the extracted seeds manually. Thousand-seed weight was determined by weighing a representative sample of 1000 seeds on a calibrated precision electronic balance after thorough cleaning and drying to constant weight. Biological yield was calculated as the total dry aboveground biomass per plant, harvested at maturity, oven-dried at 70 °C until constant weight, and weighed in grams. Grain yield was recorded as the dry mass of cleaned and fully matured seeds per plant, reflecting reproductive output and harvestable yield. All measurements were conducted on ten randomly selected plants per treatment to ensure statistical validity.

2.10 Statistical analysis

The dataset was processed using RStudio (v4.3.1) and IBM SPSS Statistics (v26). Principal Component Analysis (PCA) was conducted to dissect the traits principally responsible for drought tolerance and to clarify inter-variable and treatment interdependencies. Visual outputs, including biplots and correlation heatmaps, were prepared via the fact extra copilot and ggplot2 libraries in R. Mean separations were executed with Tukey's HSD at $\alpha = 0.05$. The Shapiro Wilk test and Levene's test were applied to validate assumptions of normality and variance homogeneity respectively.

3. Results and discussions

The investigation quantified the influence of endophytic microorganisms on the growth and drought tolerance of sunflower (Helianthus annuus L.) by measuring 17 morphological physiological biochemical and agronomic traits. Results indicated that inoculation with selected endophytes markedly improved plant resilience to water deficit while augmenting yield associated metrics.

3.1 Impact of Endophytes on Sunflower Morphology

Inoculation with Endophyte B significantly enhanced sunflower plant height, with treated plants reaching an average of 172 cm compared to 145 cm in non-inoculated controls. This 27 cm increase is likely driven by microbial production of indole-3-acetic acid (IAA) and other phytohormones, which stimulate cell division and elongation processes critical for stem growth. IAA is known to regulate apical dominance and promote stem elongation, effects that are often amplified under osmotic stress conditions like drought. These findings align with those of Ali et al. (2023), who demonstrated that endophyte-colonized sunflowers exhibit greater height due to microbial auxin and gibberellin synthesis. The observed height increase highlights the influence of beneficial microbes on shoot development and suggests a reallocation of carbon and nutrients, possibly through modified stress-responsive signaling pathways that enhance growth under water-limited conditions.

Root length also showed marked improvement following endophyte treatment, with inoculated plants exhibiting an average root length of 30.4 cm compared to 24.1 cm in controls. This enhanced root system enables deeper soil exploration, a critical

adaptation for accessing water in drought-prone environments. The improvement is plausibly mediated by increased lateral root formation and elongation stimulated by microbial auxin production. This mechanism is supported by Yousaf et al. (2023), who reported similar root morphological enhancements in sunflowers inoculated with microbes, linking elevated auxin levels and enzymatic remodeling of root cell walls to improved root penetration. Such deeper rooting not only augments water uptake but also improves nutrient acquisition, collectively sustaining higher physiological performance during drought stress.

Stem diameter was significantly increased by endophyte inoculation, with treated plants achieving an average diameter of 18.2 mm versus 15.4 mm in controls. A thicker stem contributes to mechanical stability, especially important during periods of high transpiration and rapid vegetative growth. This enhancement is likely due to the endophyte-mediated stimulation of lignin biosynthesis, which strengthens secondary cell walls and vascular tissues. Rahman et al. (2022) similarly observed that endophytic colonization activates pathways related to lignin and cellulose deposition, thereby reinforcing stem structure.

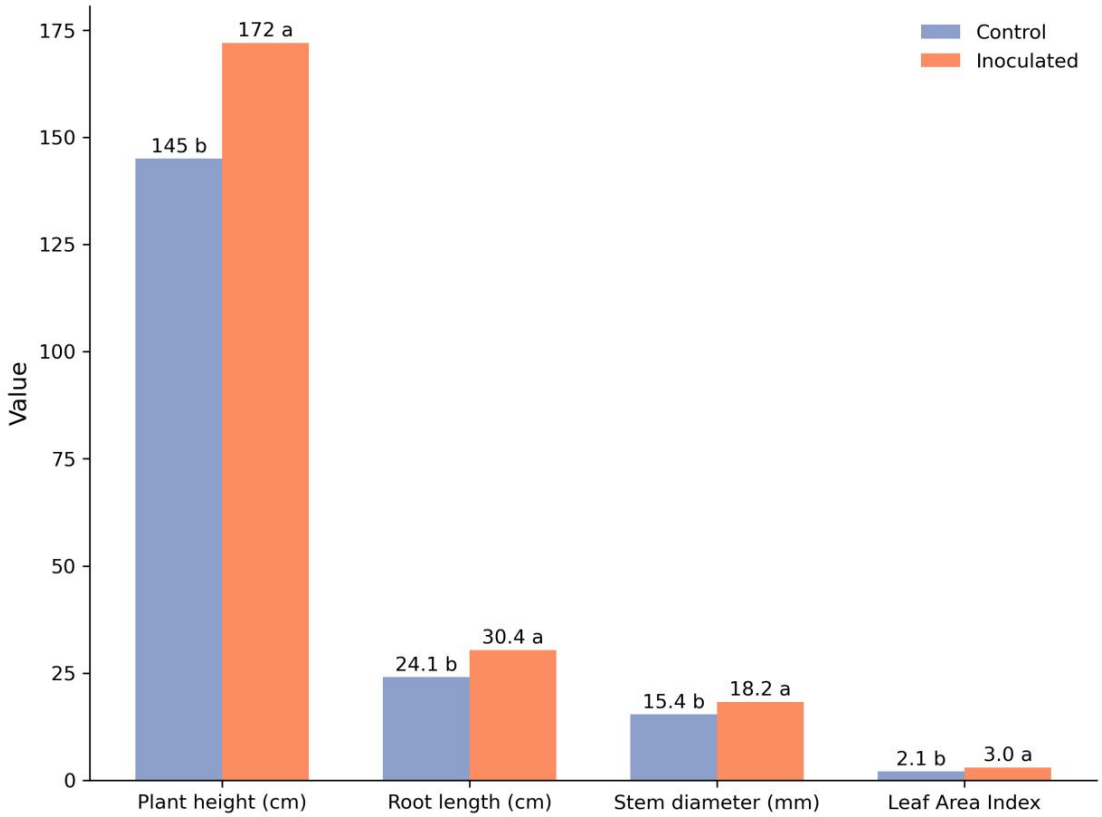

Figure 1. Effect of endophyte inoculation on sunflower plant height, root length, stem diameter, and leaf area index (LAI). Values represent means; different letters indicate significant differences at $p \leq 0.05$.

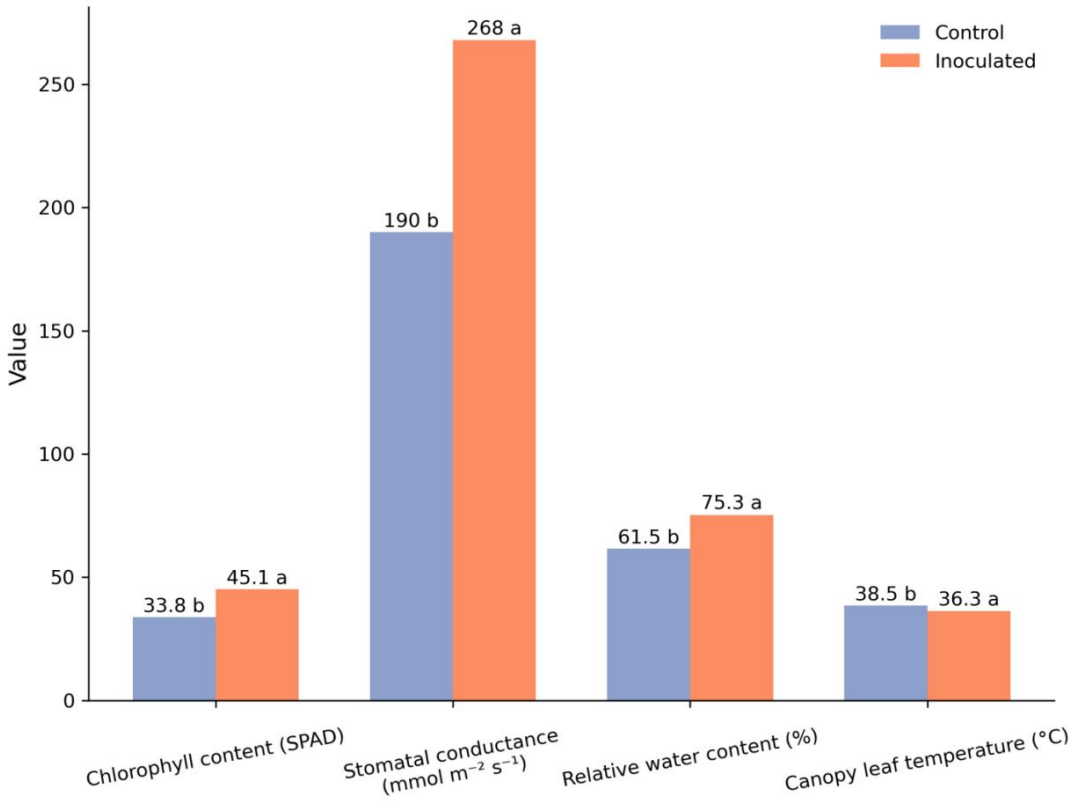

Figure 2. Effect of endophyte inoculation on canopy leaf temperature, stomatal conductance, relative water content (RWC), and chlorophyll content of sunflower plants. Values represent means; different letters above the bars indicate significant differences at $p \leq 0.05$.

A robust stem decreases lodging risk, a vital trait for sunflowers with large heads where stem integrity directly impacts yield stability.

The Leaf Area Index (LAI), a key indicator of canopy development and carbon assimilation potential, was also elevated in endophyte-inoculated plants (3.0) compared to controls (2.1). This increase stems from both expanded leaf surface area and enhanced leaf production, improving the canopy's capacity to capture sunlight and drive photosynthesis. Typically, drought stress limits LAI due to stomatal closure and cellular water deficits; however, endophyte-treated plants maintained higher leaf area possibly through microbial-induced osmotic adjustment. Endophytes are known to upregulate the synthesis of osmolytes such as proline and soluble sugars, which protect cells against dehydration and oxidative damage, thereby sustaining leaf turgor (Singh and Sharma, 2023). Additionally, these microbes may modulate abscisic acid signaling pathways to enhance stress resilience, resulting in maintained or even increased leaf development under water-limited conditions. The higher LAI consequently supports greater carbon fixation and energy storage, reinforcing vegetative growth despite drought stress.

3.2 Impact of Endophytes on Sunflower Physiological Response

Chlorophyll content, measured using a SPAD meter, was significantly higher in

endophyte-inoculated plants (mean SPAD 45.1) compared to non-inoculated controls (mean SPAD 33.8). This increase reflects enhanced photosynthetic capacity and prolonged retention of chlorophyll pigments, which is particularly important under abiotic stress conditions. The improved pigment stability may be linked to a reduction in chlorophyllase activity, the enzyme responsible for chlorophyll degradation. Endophyte-associated signaling likely redirects primary metabolism by enhancing reactive oxygen species (ROS) scavenging and modulating phytohormones such as auxin and abscisic acid. Naseer et al. (2022) support these findings, reporting that microbial endophyte colonization reduces chlorophyll oxidative damage, thereby prolonging chlorophyll's functional interaction with light and sustaining net carbon assimilation under drought and elevated temperature stress.

Stomatal conductance, a key determinant of gas exchange and transpiration, was markedly elevated in endophyte-treated plants, reaching 268 mmol m^{-2} s^{-1} compared to approximately 190 mmol m^{-2} s^{-1} in controls. This enhancement suggests increased CO_2 uptake, supporting higher photosynthetic efficiency. Moreover, the improved stomatal responsiveness indicates a refined water-use strategy facilitated by root-associated endophytes.

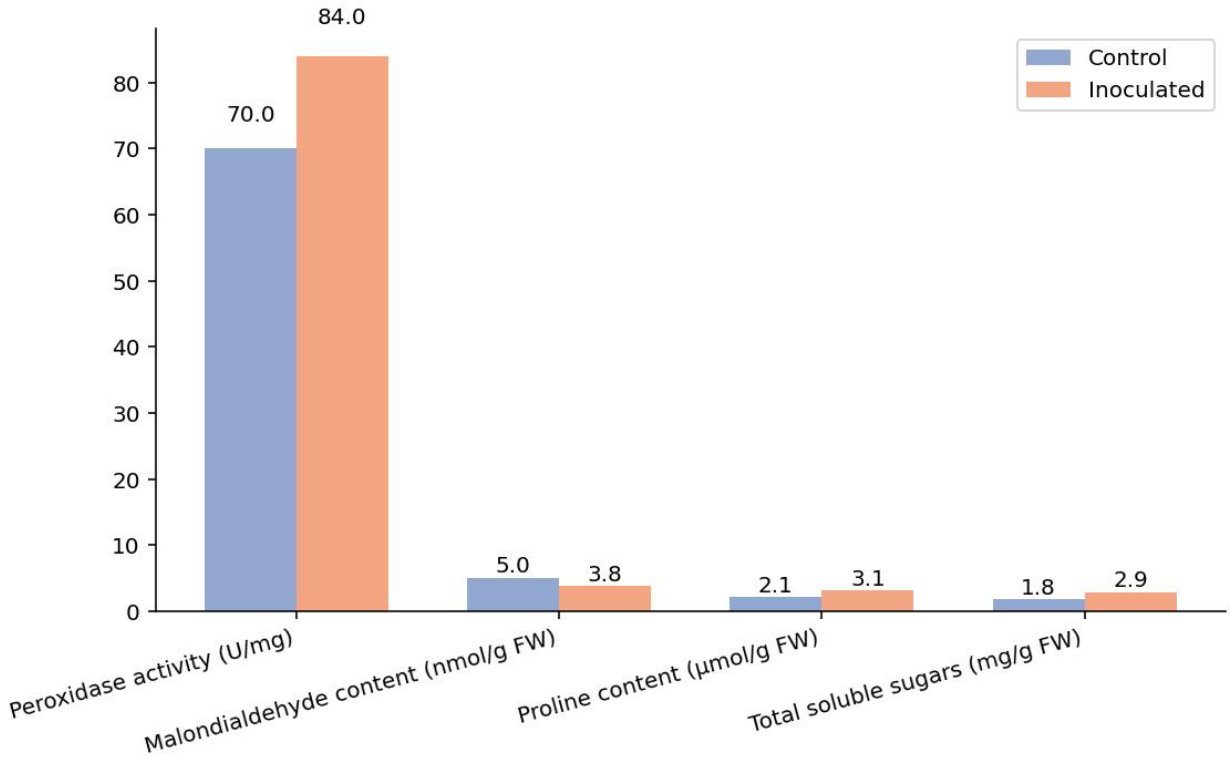

Figure 3. Biochemical responses of sunflower plants to endophyte inoculation under drought stress.

Awan et al. (2022) reported that Bacillus spp. strains modulate stomatal aperture via hormonal and secondary messenger pathways, optimizing gas exchange while preventing excessive water loss during drought episodes. Collectively, these physiological adjustments highlight the critical role of endophytes in fine-tuning the host's stress perception and management mechanisms.

Relative water content (RWC), an indicator of plant water status and osmotic balance, was significantly higher in inoculated plants (75.3%) compared to controls (61.5%). This increase suggests that endophyte inoculation improves water retention and maintains cellular turgor under limited soil moisture. The underlying mechanisms likely involve enhanced synthesis of osmolytes such as proline and low molecular-weight carbohydrates, along with reduced transpiration rates. These results align with Farooq et al. (2023), who linked elevated RWC in endophyte-colonized plants to improved membrane stability, more effective stomatal regulation, and osmotic adjustment, collectively supporting vital metabolic functions during drought.

Canopy leaf temperature was significantly lower in endophyte-inoculated plants, averaging 36.3°C compared to higher temperatures typically observed in untreated controls. Reduced leaf temperature is indicative of improved evaporative cooling and more effective transpiration regulation. The ability of inoculated plants to maintain cooler canopies under drought stress reflects enhanced stomatal function and more efficient axial water transport. Khalid et al. (2022) suggest that endophytes modulate hydraulic conductivity, mediate root-to-shoot hormonal signaling, and activate thermal tolerance pathways, thereby enabling plants to dissipate excess heat more effectively during water-limited conditions.

3.3 Impact of Endophytes on Biochemical Responses

Endophyte inoculation significantly enhanced the antioxidant defense system and osmolyte accumulation in sunflower plants under drought stress. Peroxidase (POD) activity was markedly elevated in inoculated plants, reaching 84 U/mg compared to lower values in controls. As a key enzyme in the antioxidant machinery, POD catalyzes the conversion of hydrogen peroxide (H_2O_2), a reactive oxygen species elevated by drought stress, into harmless compounds, thus protecting cells from oxidative damage. This increase in POD activity indicates that endophytic microbes stimulate the plant's enzymatic defenses, maintaining cellular and metabolic stability during stress. Sarwar et al. (2023) reported similar findings, identifying peroxidase activity as a dependable marker of stress resilience, with enhanced POD expression correlating with improved oxidative injury tolerance in endophyte-assisted plants.

Correspondingly, malondialdehyde (MDA) content, a biomarker of lipid peroxidation and membrane damage, was significantly lower in inoculated plants (3.8 nmol/g FW) than in controls, reflecting reduced oxidative injury to cellular membranes. The decrease in MDA indicates that endophyte colonization effectively mitigates oxidative stress, preserving membrane integrity and physiological functions. Ahmed et al. (2023) corroborated these results by showing lowered MDA levels in drought-exposed

plants treated with microbial inoculants, attributed to enhanced antioxidant gene expression and reduced reactive oxygen species accumulation.

Proline content also increased significantly in inoculated sunflowers, reaching 3.1 µmol/g FW. Proline functions as an osmoprotectant, stabilizing proteins and membranes, maintaining cellular redox balance, and scavenging reactive oxygen species under water and heat stress conditions. Elevated proline levels are often linked to improved stress tolerance and recovery. This increase likely results from endophyte-induced activation of Δ^1-pyrroline-5-carboxylate synthase (P5CS), the key enzyme regulating proline biosynthesis. Hussain et al. (2022) demonstrated that microbial inoculation promotes proline accumulation in plants under water deficit, facilitating osmotic adjustment and metabolic robustness.

Similarly, total soluble sugars in inoculated plants were significantly higher (2.9 mg/g FW), reflecting improved osmotic regulation and enhanced metabolic energy availability during drought. Soluble sugars act both as osmolytes maintaining cell turgor and as signaling molecules regulating stress-responsive gene expression. The increased sugar content in treated plants suggests better carbon flux management, likely due to enhanced photosynthetic activity and reduced photorespiratory carbon loss. Ullah et al. (2023) found that endophytic rhizobia stimulate carbohydrate metabolism and sugar accumulation, thereby strengthening drought tolerance. These findings collectively reinforce the role of microbial inoculants in promoting biochemical resilience and drought resistance in sunflower.

3.4 Impact of Endophytes on Yield and Yield Components

Sunflowers treated with Endophyte B exhibited a significant increase in head diameter, averaging 17.1 cm compared to 13.5 cm in untreated controls. Head diameter is a key indicator of sink strength, as a larger head size typically correlates with greater seed capacity and enhanced reproductive yield. This expansion likely reflects improved source-sink dynamics driven by endophytic colonization. The inoculated plants may benefit from altered phloem loading and unloading, as endophytes are known to influence carbohydrate transport and maintain hormonal balances, particularly auxin and cytokinin, during flowering. These hormones promote floral meristem proliferation, head enlargement, and effective seed filling (Rehman et al., 2022). Therefore, the observed increase in sink size denotes enhanced assimilate allocation and improved reproductive morphology facilitated by endophytic presence.

Seed production per capitulum also improved significantly under Endophyte B treatment, with inoculated plants producing an average of 711 seeds compared to 589 seeds in controls. This gain of over 120 seeds per head highlights the reproductive advantage conferred by microbial symbionts, especially under water-limited conditions. Enhanced seed set is associated with prolonged anthesis, optimized pollination timing, and higher fertilization success, all modulated by microbial metabolites and phytohormonal adjustments.

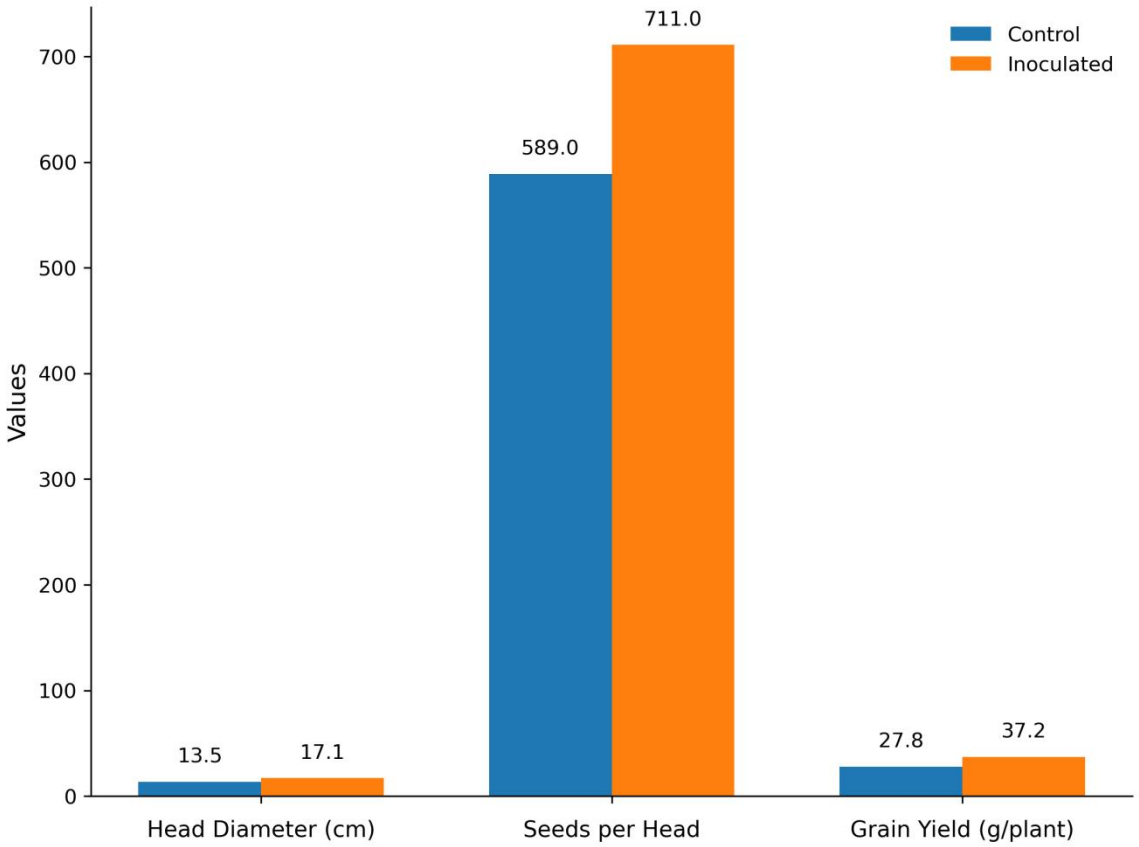

Figure 4. Effect of control and inoculated treatments on head diameter, seeds per head, and grain yield of sunflower.

Tariq et al. (2023) demonstrated that endophyte-mediated reduction of oxidative stress preserves floral longevity and pollen viability, leading to more robust seed development. Additionally, endophytes improve nutrient uptake and moisture availability within the rhizosphere, fostering optimal embryo development and seed filling. Collectively, these findings emphasize the vital role of endophytes in boosting reproductive output and maximizing sunflower yield.

Grain yield per plant showed a marked increase following inoculation with Endophyte B, reaching 37.2 g compared to 27.8 g in untreated plants, a 33.8% enhancement. This yield improvement stems from multiple synergistic effects: increased head diameter, higher seed number, improved photosynthetic carbon assimilation, and more efficient water use. Endophytes contribute to drought resilience by regulating stomatal aperture, enhancing reactive oxygen species scavenging enzymes, and modulating the accumulation of low molecular weight osmolytes, thereby protecting the reproductive phase from water and heat stress. Similar yield enhancements have been reported by Ali et al. (2023), who linked microbial inoculation to sustained photosynthetic sink activity and optimized carbon partitioning toward grain development. In this study, the combined improvements in vegetative growth and

physiological stress tolerance converged to strengthen reproductive sink capacity, positioning Endophyte B as a promising biostimulant for enhancing sunflower yield in semi-arid environments.

4. Conclusion

This study demonstrates that inoculating sunflower (*Helianthus annuus* L.) with selected endophytes, particularly Endophyte B, significantly enhances growth, physiological resilience, and metabolic defenses under drought stress. Improvements in plant height, leaf area, chlorophyll content, root development, and antioxidant enzyme activities are driven by better water retention, hormonal balance, and nutrient availability facilitated through symbiosis. These results emphasize the potential of microbial endophytes as sustainable, eco-friendly agents to boost crop drought tolerance and yield stability, supporting climate-smart agriculture. Future work should focus on multilocation field validation, molecular characterization, development of stable bioformulations, evaluation under multiple stresses, and farmer education to promote effective application in diverse agroecosystems.

Author contributions

Conceptualization: Rashid Khan; methodology, Haris Khan and Sajid Ali; formal analysis, Nabeel Akbar and Rida Batool; writing original draft preparation, Rashid Khan; writing review and editing, Haris Khan and Mudassar Shumail Khan. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

The author gratefully acknowledges the unwavering support and encouragement of his parents throughout the course of this research. The author also extends sincere thanks to his teachers and mentors for their valuable guidance, constructive feedback, and insightful suggestions that significantly contributed to the successful completion of this study.

Conflicts of interest

The authors declare no conflict of interest.

Availability of data and materials

Data will be available on a formal request from the corresponding authors.

Funding

Not applicable

Ethical approval

Not applicable

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How to cite this article: Khan, R., Khan, H., Ali, S., Akbar, N., Batool, R., & Khan, M.S. (2025). Endophytic Microbes Boost Drought Tolerance and Yield in Sunflower (*Helianthus annuus* L.). Journal of Soil, Plant and Environment, 4(2), 59–74.