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Putrescine-mediated growth improvement and antioxidant response in pistachio (*Pistacia vera* L.) seedlings of 'Badami Riz-e-Zarand'

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Original Article

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ABSTRACT

This controlled-environment investigation systematically evaluated foliar-applied putrescine (0, 0.25, 0.5, 1 mM) on morphometric, photochemical, metabolic, and oxidative stress indices in uniform 10-cm 'Badami Riz-e-Zarand' seedlings. Putrescine elicited concentration-dependent morphogenic optima at 0.5 mM, inducing +160% longitudinal shoot extension, +36% radial stem hypertrophy, +65% foliar primordia proliferation +156% assimilatory surface expansion, and +293% dry biomass accrual. Photochemical efficiency peaked concomitantly, with chlorophyll a/b and carotenoid augmentation (+92% total Chl,) paralleling carbohydrate anabolism (+146% reducing sugars, +102% starch,) and diminished fresh:dry mass ratio, signifying enhanced lignocellulosic consolidation. Osmoregulatory homeostasis was fortified via -67% electrolyte exudation٦٥+ و% proline hyperaccumulation, and -49% lipid hydroperoxidative catabolism. Antioxidative enzymatic cascades exhibited exponential induction (+319% peak catalase activity, encompassing superoxide dismutase, peroxidase, and ascorbate peroxidase, thereby effectuating ROS scavenging homeostasis. Quadratic dose-response modeling confirmed 0.5 mM as the inflectional optimum across 18 physiological determinants, with 1 mM eliciting parabolic attenuation. These data substantiate exogenous putrescine (0.5 mM) as a potent biostimulant, orchestrating integrated morpho-physiological synergies that accelerate juvenile vigor, photosynthetic quantum yield, osmotic poise, membranal integrity, and redox homeostasis in P. vera seedlings. This mechanistic framework underpins precision horticultural interventions for optimized canopy establishment and sustained orchard productivity.

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1. Introduction

Pistachio (*Pistacia vera* L.) represents a premier horticultural commodity of profound economic and nutritional significance, particularly within arid and semi-arid agroecosystems. Its innate resilience to abiotic stressors, including salinity and water scarcity, establishes it as a cornerstone crop in regions such as Iran the global leader in pistachio production and export (Mohammadabadi *et al*., 2020; Ak, 2019). Beyond its nutritional and medicinal merits, pistachio cultivation sustains rural livelihoods and fosters agricultural ecosystem resilience in water-limited environments (Steduto *et al*., 2003). Critically, early vegetative ontogeny of pistachio seedlings governs subsequent arboreal vigor, canopy architecture, and sustained orchard productivity (Osku *et al*., 2025). Thus, optimizing seedling development via targeted soil management, irrigation, nutrition, and biostimulant interventions remains imperative for superior orchard performance (Zarehaghi *et al*., 2017; Azarmi-Atajan *et al*., 2023).

The 'Badami Riz-e-Zarand' cultivar stands as Iran's preeminent pistachio rootstock, renowned for its exceptional tolerance to salinity and drought, alongside vigorous growth and adaptability across diverse edaphic conditions (Adish *et al*., 2010; Karimi and Nasrolahpour-Moghadam, 2016; Ebrahimpour *et al*., 2025). Its robust root architecture expedites seedling establishment, thereby amplifying orchard productivity and sustainability (Karimi, 2025; Sedaghat *et al*., 2014). Nevertheless, pervasive environmental constraintssoil salinity and water deficiency—persistently constrain early seedling morphogenesis (Eskandari and Mozaffari, 2014; Hajiboland *et al*., 2013). Consequently, innovative biostimulant strategies are indispensable for augmenting growth and stress acclimation in 'Badami Riz-e-Zarand' seedlings (Azarmi *et al*., 2016; Fekri *et al*., 2015; Nadi *et al*., 2019).

Early vegetative development in pistachio seedlings profoundly dictates establishment success, canopy ontogenesis, and long-term productivity (Azarmi-Atajan *et al*., 2022; Osku *et al*., 2025; Afrousheh and Hosseini, 2022). Seedlings exhibiting robust root systems and vegetative architecture demonstrate superior stress resilience and resource acquisition efficiency, culminating in elevated orchard yields (Zarehaghi *et al*., 2017; Mohammadabadi *et al*., 2020). Strategic optimization of planting substrates, sowing schedules, and growth-promoting agents substantially enhances germination, rhizogenesis, and foliar expansion (Mahmoud *et al*., 2019; Azarmi-Atajan and Sayyari-Zohan, 2022). Amidst the formidable environmental challenges confronting pistachio cultivation salinity and aridity research targeting seedling enhancement through precise polyamine interventions assumes paramount urgency (Hojjatnooghi *et al*., 2014; Shahriaripour *et al*., 2010, 2011).

Polyamines (PAs) putrescine, spermidine, and spermine constitute pivotal biogenic amines that orchestrate plant morphogenesis, development, and acclimation to biotic/abiotic stresses (Shao *et al*., 2022). These metabolites regulate essential physiological cascades, including cytodifferentiation, rhizogenesis, photochemical efficiency, and ionic homeostasis, positioning PAs as authoritative biostimulants against drought, salinity, chilling, and nutrient deprivation (Gill and Tuteja, 2010; Hussain *et al*., 2011; Khan *et al*., 2022; Minocha *et al*., 2014; Shi and Chan, 2013; Wojtyla *et al*., 2024). Putrescine, specifically, ameliorates oxidative disequilibrium by potentiating antioxidant cascades, attenuating lipid peroxidation, and reinforcing membranal integrity (Anam *et al*., 2024; Hussein *et al*., 2023; Zhao *et al*., 2021; Tabur *et al*., 2024). Exogenous putrescine application demonstrably accelerates vegetative proliferation, photosynthetic pigment biosynthesis, and osmolyte homeostasis across diverse taxa, including wheat, grapevine, pomegranate, citrus (Muradoğlu *et al*., 2025), and strawberry. Moreover, putrescine modulates epigenetic architecture and metabolic flux via DNA methylation and transcriptomic reprogramming under stress (Majláth *et al*., 2025; Eren *et al*., 2023; Orhan *et al*., 2020; Page *et al*., 2016). In ornamentals such as gerbera, lily, and anthurium, putrescine elevates postharvest longevity, vasal persistence, and mycorrhizal symbiosis (Rakbar *et al*., 2022; Hojatipour and Hassanpour Asil, 2021; Sun *et al*., 2020). Additionally, it promotes somatic embryogenesis, germination vigor, and agronomic productivity in sugarcane, maize, and date palm (Reis *et al*., 2016; Todorov *et al*., 1998; Naser *et al*., 2016). Collectively, these pleiotropic effects affirm putrescine's preeminence as a versatile biostimulant across phylogenetic spectra (González-Hernández *et al*., 2022; Kundu *et al*., 2021, 2022; Liu *et al*., 2007; Wasaya *et al*., 2023; Daler *et al*., 2025; Amri *et al*., 2011; Mahdavian *et al*., 2019).

Knowledge Gap: Despite putrescine's established efficacy in stress amelioration across horticultural crops (Kamiab *et al*., 2014; Hojjatnooghi *et al*., 2014; Shahriaripour *et al*., 2010, 2011), its application to optimize vegetative growth in pistachio seedlings remains conspicuously underexplored. Existing pistachio research predominantly addresses polyamine-mediated salinity tolerance in mature cultivars, with negligible investigation into non-stressed, early vegetative enhancement particularly for the 'Badami Riz-e-Zarand' rootstock. This lacuna critically impedes development of targeted biostimulant protocols for accelerated seedling establishment and orchard sustainability in arid domains, notwithstanding compelling evidence from analogous fruit crops (Muradoğlu *et al*., 2025; Shao *et al*., 2022).

Research Objectives: This study systematically evaluates the effects of foliar-applied putrescine (0, 0.25, 0.5, 1 mM) on morphometric, photochemical, metabolic, and antioxidative attributes of 'Badami Riz-e-Zarand' pistachio seedlings under controlled greenhouse conditions. We hypothesize that putrescine will potentiate

shoot/root elongation, amplify photosynthetic pigments and carbohydrate reserves, enhance membrane stability and osmolyte accumulation, and activate antioxidative defenses, thereby optimizing juvenile vigor (Wojtyla *et al*., 2024). Through comprehensive physiological and biochemical analyses, this investigation elucidates putrescine's mechanistic underpinnings as a biostimulant, furnishing an evidence-based framework to expedite seedling ontogeny and bolster long-term pistachio orchard productivity in semi-arid regions.

2. Material and Methods

2.1. Seedling Preparation and Growth Conditions

Uniform seeds of pistachio (*Pistacia vera* L. cv. 'Badami Riz-e-Zarand') were surface-sterilized in 2% (w/v) sodium hypochlorite solution for 10 min, thoroughly rinsed with distilled water, and sown in 3-kg plastic pots. The growth medium consisted of a homogenized mixture of field soil (sandy loam), well-decomposed farmyard manure, and washed river sand in a volumetric ratio of 2:1:1. The physicochemical properties of the substrate prior to sowing were as follows: pH = 7.7 (measured in 1:2.5 soil:water suspension using a calibrated glass electrode pH meter; ISO 10390:2021), EC = 1.2 dS m^{-1} (determined in 1:5 soil:water extract using a conductivity meter; ISO 11265:1994), organic matter = 1.8% (quantified by Walkley-Black wet oxidation method; ISO 14235:1998), total nitrogen = 0.08% (assessed via Kjeldahl digestion and distillation; ISO 11261:1995), available phosphorus = 14 mg kg^{-1} (extracted using Olsen bicarbonate method and measured spectrophotometrically; ISO 11263:1994), and available potassium = 210 mg kg^{-1} (extracted with 1 M ammonium acetate at pH 7.0 and quantified by flame emission spectrometry; ISO 17536:2019). The experiment was conducted under controlled conditions in a commercial greenhouse (Langer region, Kerman province, Iran). Throughout the growth period, environmental parameters were continuously monitored using automated data loggers (HOBO MX2300 series, Onset Computer Corp., Bourne, MA, USA) equipped with integrated sensors for temperature (accuracy ±0.2°C), relative humidity (accuracy ±2.5%), and photosynthetically active radiation (PAR; silicon pyranometer, accuracy ±5 μmol m^{-2} s^{-1}), with data recorded at 15-min intervals. The reported values represent means ± standard deviation derived from these continuous measurements: day temperature 28 ± 2 °C, night temperature 20 ± 2 °C, relative humidity 55 ± 5%, and PAR approximately 650 μmol m^{-2} s^{-1} with a 14-h photoperiod.

2.2. Timing of Treatments

Seedlings were monitored until they reached an average height of 10 cm (approximately 45 days after emergence). At this stage, uniform and healthy seedlings (without visual symptoms of stress, nutrient deficiency, or disease) were selected and subjected to foliar putrescine treatments.

2.3. Irrigation Management

Irrigation was carried out using chlorine-free water with an electrical conductivity below 0.5 dS m^{-1}. Pots were irrigated at 3-day intervals, with water supplied to field capacity until 10% leaching occurred from the drainage holes. To avoid salt accumulation, a leaching irrigation was applied once every two weeks. Soil moisture was consistently maintained at 75–80% of field capacity, ensuring that no drought stress was imposed during the experimental period.

2.4. Experimental Design and Treatment Application

The experiment was conducted using a completely randomized design (CRD) with four putrescine treatments (0, 0.25, 0.5, 1.0 mM) and four replicates per treatment. The experimental unit was the individual pot containing a single seedling (n = 80 pots total), with one uniform 'Badami Riz-e-Zarand' pistachio seedling per pot (5 cm diameter × 15 cm height black plastic pots). Each replicate thus comprised five experimental units (pots), yielding 20 experimental units per treatment and 80 total experimental units.

Putrescine solutions (analytical grade; Sigma-Aldrich, Merck) were prepared by dissolving in distilled water, with pH adjusted to 6.5–7.0 using 0.1 M HCl/NaOH and 0.05% (v/v) Tween-20 added as a surfactant to optimize wetting and foliar penetration. Foliar applications commenced when seedlings attained uniform height of 10 ± 0.5 cm (~45 days post-emergence), designated as the treatment initiation stage (Figure 1).

Applications were executed using a calibrated hand sprayer (0.5 L capacity, droplet size 100–150 μm) to achieve complete coverage of adaxial/abaxial leaf surfaces and apical stems until near run-off (~200 ± 10 mL solution per experimental unit). Spraying occurred between 08:00–10:00 h under ambient conditions (25 ± 2°C, 60 ± 5% RH) to minimize evaporative loss and maximize transcuticular absorption. Post-application, experimental units were maintained under partial shade (50% light reduction) for 1 h to enhance solution retention and uptake.

2.5. Post-treatment Management

Following foliar application, seedlings were maintained under the same greenhouse conditions (Commercial Greenhouse, Langer region, Kerman Province, Iran). Irrigation, fertilization, and pest and disease management

were conducted according to standard protocols described previously. Throughout the experimental period, no drought or salinity stress was imposed.

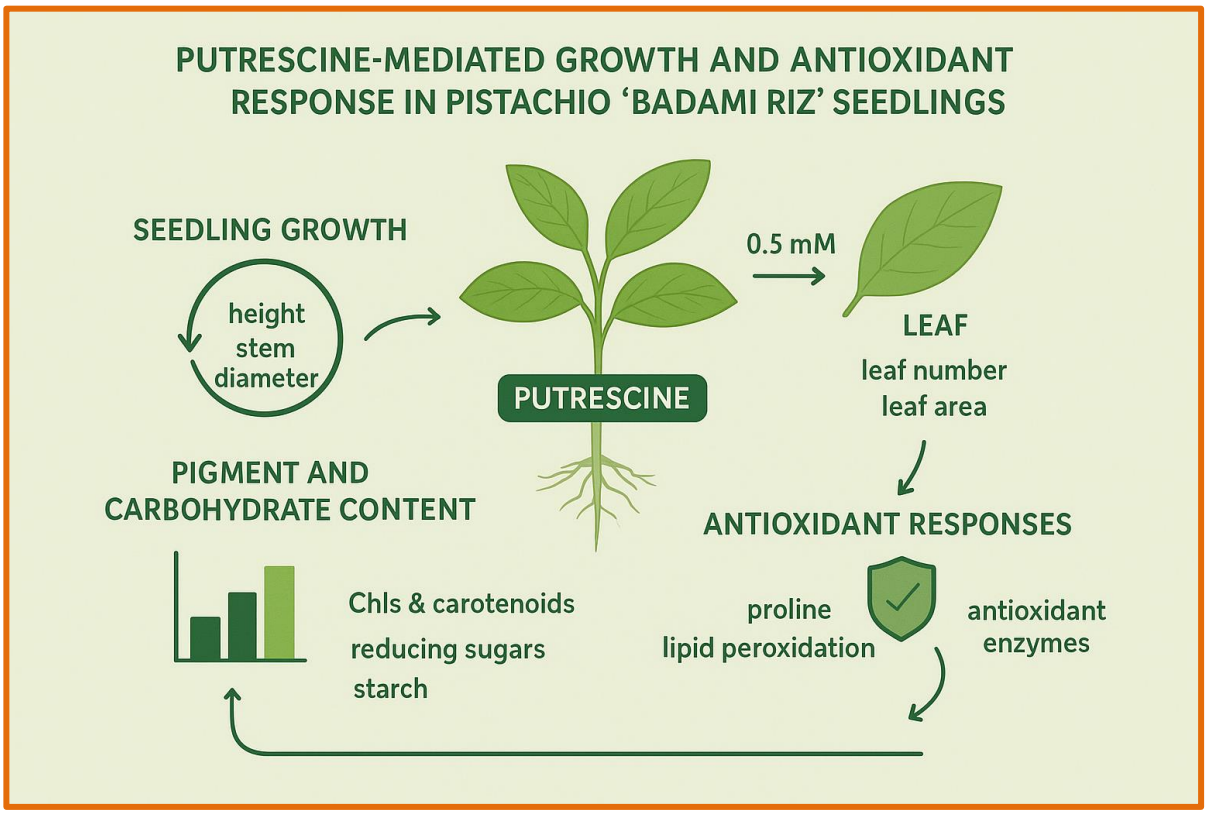

Figure 1- Schematic representation of putrescine-mediated growth and antioxidant responses in Badami Riz-e-Zarand pistachio seedlings.

2.6. Sampling Time and Trait Measurements

All morphological, physiological, and biochemical parameters were evaluated 60 days after treatment application. The following traits were recorded with precise and standardized methodologies:

Morphological growth traits: seedling height, stem diameter, leaf number, and leaf area. Biomass accumulation was determined by measuring the fresh and dry weights of leaves. Photosynthetic pigments: contents of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were quantified spectrophotometrically. Carbohydrate metabolism: reducing sugar and starch contents were determined using standard colorimetric assays. Cell membrane integrity: electrolyte leakage (EL) and lipid peroxidation, expressed as malondialdehyde (MDA) content, were assessed as indicators of membrane stability. Osmolyte accumulation: free proline content was measured as a key osmo-protectant. Antioxidant defense system: activities of major antioxidant enzymes, including catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and superoxide dismutase (SOD), were quantified spectrophotometrically to characterize the enzymatic antioxidative response.

2.7. Measurement of Seedling Height

Seedling height was determined as the vertical distance from the soil surface at the base of the stem to the apical meristem of the main shoot. Measurements were performed using a stainless-steel ruler with 1 mm precision. To minimize measurement errors, seedlings were positioned upright, and readings were taken at the same time of day under uniform conditions. Final height data were recorded at the end of the experimental period and expressed in centimeters (cm).

2.8. Measurement of Stem Diameter

Stem diameter was measured at a fixed position to ensure consistency across all seedlings. The measurement point was defined as 1 cm above the soil surface at the stem base, where the stem is most uniform and least affected by cotyledonary swelling. A digital Vernier caliper with an accuracy of ±0.01 mm (Mitutoyo, Japan) was used to obtain precise readings. Measurements were taken with the seedling held in an upright position to avoid angular bias. For each seedling, three consecutive readings were recorded at the same position and averaged to minimize random errors. Data were finally expressed in millimeters (mm).

2.9. Measurement of Leaf Number

The leaf number was determined by counting all fully expanded leaves on each seedling. Cotyledons and newly emerging leaves that were less than 50% expanded were excluded from the count to ensure consistency and comparability. Observations were conducted in the morning under uniform light conditions to minimize bias due to leaf movement or curling. For accuracy, each seedling was examined twice by the same observer, and the average count was recorded.

2.10. Measurement of Leaf Area

Leaf area was determined using a digital leaf area meter (LI-3100C, LI-COR Biosciences, Lincoln, NE, USA). Fully expanded leaves of each seedling were carefully detached, avoiding any mechanical damage. Each leaf was individually placed on the scanning surface of the device, and the projected leaf area was recorded automatically. For each seedling, the leaf area of all measured leaves was summed to obtain the total leaf area per seedling, expressed in square centimeters (cm^2). Measurements were performed under controlled laboratory conditions to minimize environmental variation.

2.11. Measurement of Leaf Fresh Weight

Leaf fresh weight was determined immediately after harvesting to prevent water loss. Fully expanded leaves of each seedling were carefully excised and gently blotted with absorbent paper to remove surface moisture. The leaves were then weighed using an analytical balance (Sartorius, Germany) with 0.001 g precision. For each seedling, the total fresh weight of all measured leaves was recorded in grams (g). Measurements were performed promptly to ensure accuracy and avoid any changes due to transpiration.

2.12. Measurement of Leaf Dry Weight

After determining fresh weight, leaves were oven-dried at 70 °C until a constant weight was achieved (typically 48–72 hours) to ensure complete removal of water content. Dry leaves were then weighed using an analytical balance (Sartorius, Germany) with 0.001 g precision. The total dry weight of all measured leaves per seedling was recorded in grams (g). This procedure provided a precise assessment of leaf biomass independent of water content.

2.13. Measurement of Fresh-to-Dry Weight Ratio

The fresh-to-dry weight ratio of leaves was calculated as the quotient of total fresh leaf weight to total dry leaf weight for each seedling:

$$\frac{\textbf{Total fresh leaf weight(g)}}{\textbf{Total dry leaf weight (g)}} = \textbf{Fresh to dry weght ratio}$$

Fresh and dry weights were determined as described previously. This ratio provides an index of tissue water content and structural density, reflecting the balance between leaf hydration and biomass accumulation.

2.14. Measurement of Leaf Chlorophyll and Carotenoids

Leaf pigments, including chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll, and carotenoids, were extracted from 0.1 g of fresh leaf tissue using 80% acetone. The homogenate was centrifuged at 5000 × g for 10 min, and the supernatant was collected. Absorbance of the extracts was measured at 663, 645, and 470 nm using a UV-Visible spectrophotometer (Shimadzu UV-1800, Japan). Chlorophyll and carotenoid concentrations were calculated according to the equations of Lichtenthaler and Wellburn (1983) and expressed in mg g^{-1} fresh weight (FW). All measurements were performed under low-light conditions to prevent pigment degradation.

2.15. Measurement of Reducing Sugar Content

Reducing sugar content was determined using the 3,5-dinitrosalicylic acid (DNS) method as described by Miller (1959). Briefly, 0.5 g of fresh leaf tissue was homogenized in 10 mL of 80% ethanol and incubated at 80 °C for 30 min to extract soluble sugars. The homogenate was centrifuged at 5000 × g for 10 min, and the supernatant was collected. 1 mL of extract was mixed with 1 mL of DNS reagent, boiled for 5 min, cooled, and the absorbance was measured at 540 nm using a UV-Visible spectrophotometer (Shimadzu UV-1800, Japan). Reducing sugar concentration was calculated using a standard curve prepared with glucose and expressed as mg g^{-1} fresh weight (FW).

2.16. Measurement of Starch Content

Starch content was determined following the method of Hansen and Møller (1975). Briefly, 0.5 g of dried leaf tissue was homogenized in 10 mL of 80% ethanol to remove soluble sugars. The residue was then hydrolyzed with 2 mL of 0.2 N HCl at 100 °C for 1 h. After neutralization with 2 mL of 0.2 N NaOH, the hydrolysate was reacted with anthrone reagent and heated in a boiling water bath for 10 min. Absorbance was measured at 625 nm using a UV-Visible spectrophotometer (Shimadzu UV-1800, Japan). Starch concentration was calculated using a glucose standard curve and expressed as mg g^{-1} dry weight (DW).

2.17. Measurement of Electrolyte Leakage

Electrolyte leakage (EL) was assessed as an indicator of cell membrane integrity following the method of Blum and Ebercon (1981). Briefly, 0.5 g of fresh leaf tissue was rinsed with deionized water to remove surface ions and then cut into 1 cm² pieces. The samples were immersed in 10 mL of deionized water in test tubes and incubated at 25 °C for 24 h with gentle shaking. The initial electrical conductivity (EC1) of the solution was measured using a conductivity meter (Hanna Instruments, HI 98311, Romania). Samples were then boiled at 100 °C for 15 min, cooled to room temperature, and the final conductivity (EC2) was recorded. Electrolyte leakage was calculated as:

$$100 \times \frac{EC1}{EC2} = (\%)\ EL$$

Higher EL values indicate greater membrane damage due to stress or treatment effects.

2.18. Lipid peroxidation

Malondialdehyde (MDA) content was determined following Rajinder *et al*. (1981) with slight modifications. Pulp and peel (4 g) were homogenized with 20 mL of 10% trichloroacetic acid (TCA) and centrifuged at 5000 g for 10 min. One milliliter of supernatant was mixed with 3 mL of 0.5% thiobarbituric acid (TBA) in 10% TCA, heated at 95 °C for 20 min, cooled rapidly, and centrifuged at 10,000 g for 10 min. Absorbance was measured at 532 nm and corrected at 600 nm. MDA was expressed as mM g^{-1} fresh weight (FW) using an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.19. Proline content

Proline content method for quantifying proline content, as described by Bates *et al*.1973, began with a 0.5 g leaf sample being homogenized in 10 mL of 3% (w/v) sulfosalicylic acid, followed by centrifugation at 5000 g for 10 min. The resulting supernatant (2 mL) was mixed with 2 mL of acid ninhydrin reagent and 2 mL of glacial acetic acid, and the mixture was heated in a water bath for one hour. After cooling, 4 mL of toluene was added, and the solution was stirred rapidly for 20 s. Free proline was then quantified spectrophotometrically at 520 nm, using toluene as a blank for calibration.

2.20. Protein Determination

Total soluble protein content was quantified according to the Bradford (1976) dye-binding assay using Coomassie Brilliant Blue G-250 reagent. Fresh leaf tissue (0.5 g) was homogenized in 5 mL of ice-cold extraction buffer containing 50 mM phosphate buffer (pH 7.0), 1 mM EDTA, and 1% (w/v) polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12,000 × g for 20 min at 4 °C, and the clear supernatant was used for protein analysis. For assay preparation, 100 µL of the enzyme extract was mixed with 5 mL of Bradford reagent (prepared freshly by dissolving 100 mg Coomassie Brilliant Blue G-250 in 50 mL of 95% ethanol, adding 100 mL of 85% phosphoric acid, and diluting to 1 L with distilled water, then filtering through Whatman No.1 paper). The reaction mixture was incubated at room temperature for 5 min to allow full color development. Absorbance was recorded at 595 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800, Japan) against a reagent blank. The protein concentration was calculated from a standard calibration curve prepared with known concentrations (0–100 µg mL^{-1}) of bovine serum albumin (BSA). Results were expressed as milligrams of protein per gram of fresh weight (mg g^{-1} FW). This protein quantification was used to normalize and express the specific activity of all assayed enzymes.

2.21. Antioxidant Enzyme Activities

Catalase (CAT) and Peroxidase (POD): Measured following Xing *et al*. (2011). For CAT, 0.5 mL enzyme extract was added to 2 mL sodium phosphate buffer (50 mM, pH 7.0) with 0.5 mL H_2O_2 (40 mM), and the decline in absorbance at 240 nm was monitored. POD activity was measured using 0.5 mL enzyme extract in 2 mL buffered substrate containing guaiacol, with absorbance increase at 460 nm recorded every 30 s for 120 s. Activities were expressed as U mg^{-1} protein.

Superoxide Dismutase (SOD): Assayed according to Misra and Fridovich (1972). Fresh tissue (\~200 mg) was homogenized in potassium phosphate buffer with EDTA, Triton X-100, and PVP. Extracts were dialyzed and mixed with epinephrine in carbonate/bicarbonate buffer. SOD activity was defined as the enzyme amount causing 50% inhibition of epinephrine oxidation, expressed as U mg^{-1} protein.

Ascorbate Peroxidase (APX): Measured following Nakano and Asada (1981). The reaction mixture contained potassium phosphate buffer (50 mM, pH 7.0), 0.2 mM ascorbic acid, 20 mM H_2O_2, and enzyme extract. The decrease in absorbance at 290 nm was monitored, and activity expressed as U mg^{-1} protein.

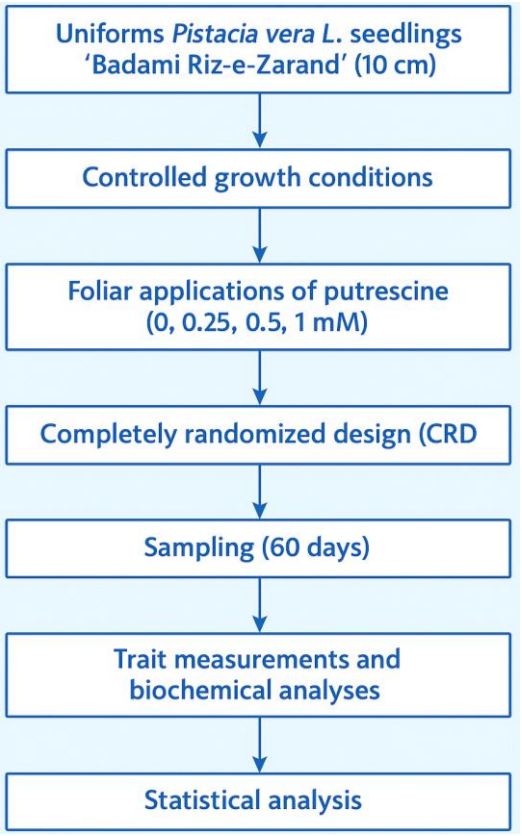

Figure 2. Schematic overview of the experimental design illustrating the stages of seedling preparation, putrescine foliar treatments (0–1.0 mM), controlled greenhouse conditions, physiological and biochemical measurements, and data analysis workflow for *Pistacia vera* L. cv. 'Badami Riz-e-Zarand'.

2.21. Statistical Analysis

Data were recorded for each individual seedling, averaged per replicate, and subjected to analysis of variance (ANOVA). Mean comparisons were performed using Duncan's multiple range test at $p \leq 0.05$.

3. Results

3.1. Height of seedling

Based on the results of one-way ANOVA followed by Duncan's multiple range test at a 5% significance level, significant differences were observed among the treatments ($p < 0.05$) (Table 1). Exogenous application of putrescine to 'Badami Riz-e-Zarand' pistachio seedlings markedly enhanced shoot height relative to the untreated control. The most pronounced effect was observed with the 0.5 mM putrescine treatment, which resulted in a mean shoot height of 78.32 cm, corresponding to a 160% increase compared to the control. Treatments with 0.25 mM and 1 mM putrescine also significantly promoted shoot growth, with increases of approximately 50% and 117%, respectively. However, the 1 mM treatment exhibited slightly lower efficacy than the 0.5 mM concentration, suggesting an optimal concentration range for maximizing longitudinal growth. These findings underscore the potent, concentration-dependent role of putrescine in stimulating shoot elongation in juvenile pistachio seedlings, as illustrated in (Fig 3A).

3.2. Stem diameter

In a rigorous investigation employing ANOVA and Duncan's multiple range test at a 5% significance level, all treatments demonstrated statistically significant differences, underscoring the pivotal, concentration-dependent role of putrescine in fostering both longitudinal and radial growth in young pistachio seedlings (Table 1). Notably, putrescine application markedly enhanced the stem diameter of 'Badami Riz-e-Zarand' pistachio seedlings compared to the untreated control. The most striking effect was observed at a 0.5 mM concentration, which yielded an impressive stem diameter of 4.55 mm a remarkable 36% increase over the control. Treatments at 0.25 mM and 1 mM also promoted radial growth, boosting stem diameter by 16% and 23%, respectively. However, the 1 mM treatment proved slightly less effective than the 0.5 mM concentration, suggesting an optimal dosage window for maximizing radial development (Fig. 3B). These findings highlight putrescine's potential as a potent biostimulant for enhancing the vigor and structural development of pistachio seedlings.

Table 1. Mean Squares Analysis of putrescine effects on selected growth and antioxidant traits in pistachio (*Pistacia vera* L. cv. Badami Riz-e-Zarand) seedlings

S.O.V.	DF		Seedling Height	Stem Diameter	Leaf Number	Leaf Area	Leaf Fresh Weight	Leaf Dry Weight	Fresh-to-Dry Weight Ratio	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Treatment	3		210.10**	58.07**	40.18**	191.23**	1.48**	182.14**	231.51**	824.12**	2.18**	52.31**
Error	12		1.13	0.40	0.23	0.60	0.02	50.60	7.57	9.58	0.11	4.25
CV (%)	-		6.35	11.02	8.23	12.06	9.14	9.08	10.17	8.25	6.35	11.12

** Significant at the 1% level; * Significant at the 5% level; ns Not significant

Table 2. Mean Squares Analysis of putrescine effects on selected growth and antioxidant traits in pistachio (*Pistacia vera* L. cv. Badami Riz-e-Zarand) seedlings

S.O.V.	DF	Carotenoids	Reducing Sugar Content	Starch Content	Electrolyte Leakage	Lipid peroxidation	Proline content	Catalase activity	Peroxidase activity	Superoxide Dismutase activity	Ascorbate Peroxidase activity
Treatment	3	98.58**	41.25**	18.35**	36.25**	131.12**	85.29**	631.02**	100.02**	11.08**	284.03**
Error	12	2.04	1.02	0.37	0.91	0.01	12.65	23.01	6.32	0.01	3.014
CV (%)	-	9.21	9.33	11.24	8.34	10.02	8.37	11.47	8.15	9.36	10.24

** Significant at the 1% level; * Significant at the 5% level; ns Not significant.

3.3. Leaf number

Analysis of variance (ANOVA) coupled with Duncan's multiple range test at a 5% significance level revealed significant differences across all treatments, underscoring the concentration-dependent influence of putrescine on vegetative growth in young 'Badami Riz-e- Zarand' pistachio seedlings (Table 1). Putrescine application substantially increased leaf number compared to the control, with the most pronounced effect observed at a 0.5 mM concentration, yielding an average of 5.82 leaves—a remarkable 65% increase. Treatments at 0.25 mM and 1 mM also significantly enhanced leaf number, with increases of 17% and 42%, respectively. However, the 1 mM dose was slightly less effective than the 0.5 mM concentration, indicating an optimal dosage range for maximizing leaf proliferation. These results vividly illustrate putrescine's potent, dose-responsive role in stimulating leaf initiation and early vegetative development, offering promising implications for enhancing pistachio seedling vigor (Fig. 4 A).

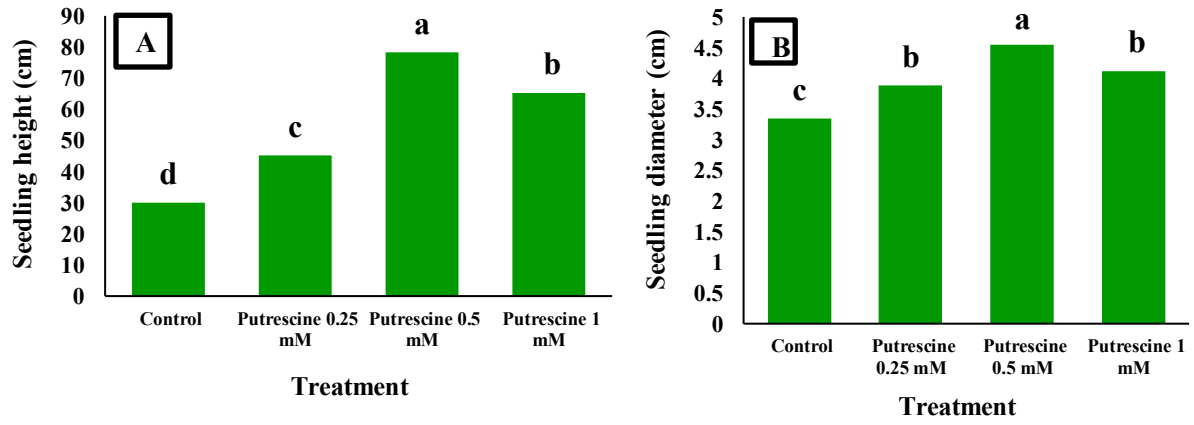

Figure 3. Effect of putrescine on seedling hight (A) and diameter (B)of pistachio (*Pistacia vera* L.) Badami Riz-e-Zarand. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

3.4. Leaf area

Analysis of variance (ANOVA) followed by Duncan's multiple range test at a 5% significance level revealed significant differences among all treatments (Table 1), demonstrating a strong, dose-dependent effect of putrescine on leaf area in young 'Badami Riz-e-Zarand' pistachio seedlings. Application of putrescine substantially increased leaf area, with the most pronounced response observed at 0.5 mM, where leaves reached a mean area of 15.88 cm², representing a remarkable 156% increase over the untreated control. Treatments with 0.25 mM and 1 mM putrescine also promoted leaf expansion, enhancing leaf area by 34% and 136%, respectively, although the 1 mM concentration induced a slightly smaller response than the 0.5 mM dose, indicating the existence of an optimal concentration for maximizing leaf growth. These findings highlight putrescine's critical role in promoting cellular expansion and lamina development, leading to substantial increases in leaf surface area and early vegetative vigor.

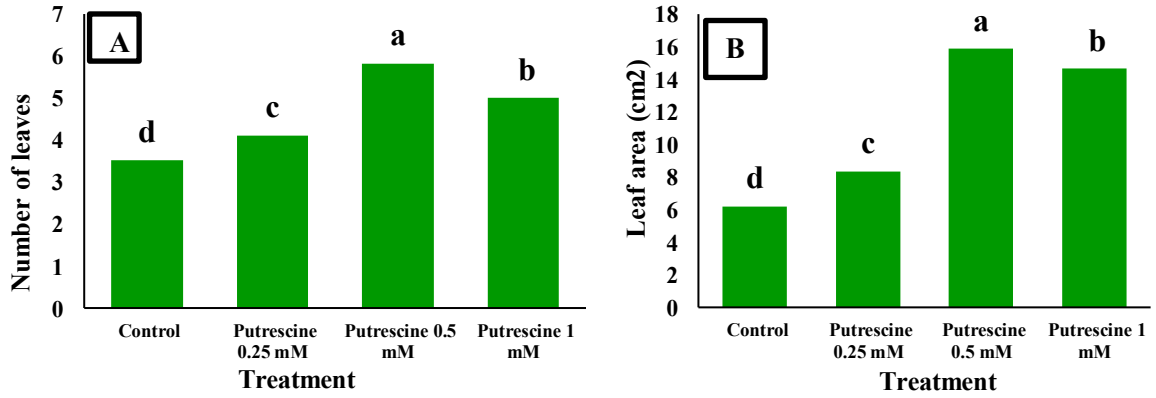

Figure 4. Effect of putrescine on number of leaves (A) leaf area (B) of pistachio (*Pistacia vera* L.) Badami Riz-e-Zarand. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

The observed concentration-response pattern suggests that moderate concentrations effectively stimulate leaf enlargement, likely by enhancing cell division, turgor-driven expansion, and photosynthetic capacity. Such physiological enhancements during the early growth stages may confer long-term benefits for seedling establishment and overall performance (Fig. 4 B).

3.5. Fresh leaf weight

Statistical analysis (ANOVA) followed by Duncan's multiple range test at a 5% significance level demonstrated that all putrescine treatments significantly affected the fresh leaf weight of young 'Badami Riz-e-Zarand' pistachio seedlings (Table 1). Putrescine application resulted in a pronounced increase in fresh leaf biomass, with the maximum response recorded at 0.5 mM (1.41 g), corresponding to a 40% gain relative to the untreated control. Lower (0.25 mM) and higher (1 mM) concentrations also enhanced leaf weight by 14% and 24%, respectively; however, the modest reduction at 1 mM compared with 0.5 mM suggests a concentration-

dependent threshold for optimal leaf biomass accumulation. These outcomes indicate that putrescine not only stimulates leaf expansion but also improves tissue hydration and cellular turgor, which together underpin enhanced vegetative growth. The observed pattern emphasizes that moderate concentrations provide the most effective physiological stimulus, likely through coordinated promotion of cell enlargement, water retention, and photosynthetic capacity. Consequently, application of putrescine can be considered a potent strategy to reinforce early seedling vigor and overall leaf productivity in pistachio (Fig. 5A).

3.6. Leaf dry weight

Analysis of variance (ANOVA) followed by Duncan's multiple range test at a 5% significance level revealed that putrescine treatments significantly influenced leaf dry weight in young 'Badami Riz-e-Zarand' pistachio seedlings (Table 1). Putrescine markedly enhanced leaf dry mass, reaching its peak at 0.5 mM (0.59 g), which represents an extraordinary 293% increase over the untreated control. Lower (0.25 mM) and higher (1 mM) concentrations also promoted dry weight accumulation by 40% and 173%, respectively; however, the response at 1 mM was slightly less than at 0.5 mM, indicating a concentration-dependent optimum for maximizing structural biomass.

These results highlight putrescine's critical role in stimulating leaf structural development and dry matter deposition, reflecting enhanced cell wall synthesis, protein accumulation, and overall tissue maturation. By promoting substantial increases in leaf dry mass, putrescine contributes to improved early vegetative vigor, which may support better seedling establishment and long-term growth performance in pistachio (Fig. 5 B).

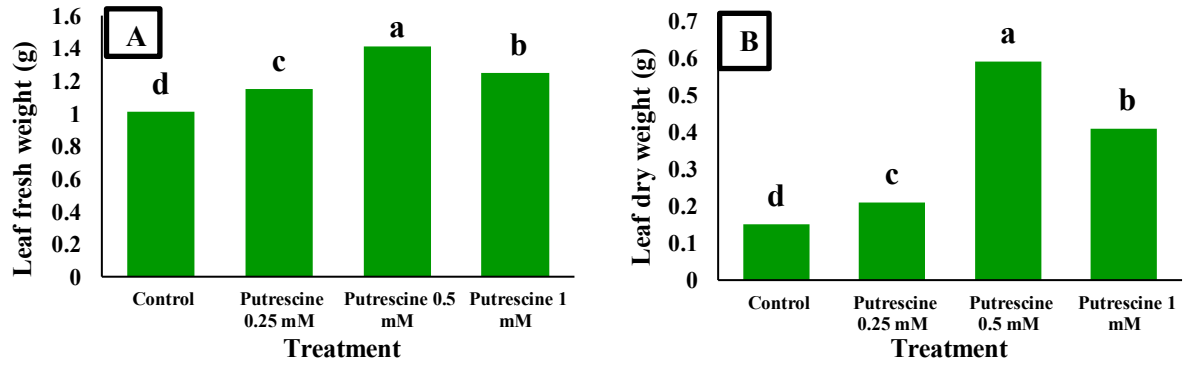

Figure 5. Effect of putrescine on leaf fresh weight (A) and leaf dry weight (B) of pistachio (*Pistacia vera* L.) Badami Riz-e-Zarand. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

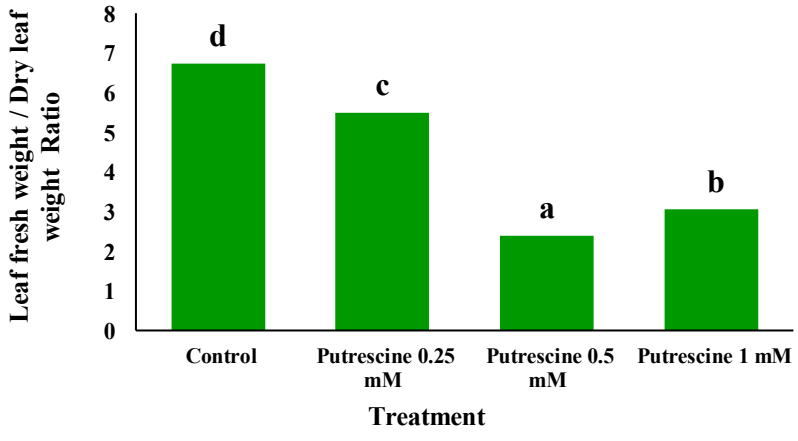

Figure 6. Effect of putrescine on leaf fresh weight to leaf dry weight ratio of pistachio (*Pistacia vera* L.) Badami Riz-e-Zarand. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

3.7. Fresh-to-dry weight ratio

Analysis of variance (ANOVA) followed by Duncan's multiple range test at a 5% significance level revealed that all putrescine treatments significantly influenced the fresh-to-dry weight (FW/DW) ratio of leaves in young 'Badami Riz-e-Zarand' pistachio seedlings (Table 1). Application of putrescine caused a clear concentration-dependent decline in FW/DW ratio, with the lowest value recorded at 0.5 mM (2.39), corresponding to a 64.5%

reduction compared with the control. Treatments at 0.25 and 1 mM also decreased the ratio by 18.5% and 54.7%, respectively. These results indicate that putrescine promotes substantial dry matter accumulation relative to water content, enhancing leaf structural density and reinforcing tissue integrity. The pronounced reduction in FW/DW at the optimal concentration suggests improved cell wall development, higher solute deposition, and enhanced physiological maturity of leaves. Collectively, these effects contribute to stronger vegetative vigor and superior early growth performance in pistachio seedlings (Fig. 6).

3.8. Chlorophyll a

Analysis of variance (ANOVA) followed by Duncan's multiple range test at a 5% significance level demonstrated that putrescine treatments significantly increased chlorophyll a content in young 'Badami Riz-e-Zarand' pistachio seedlings (Table 1). Chlorophyll a accumulation exhibited a clear concentration-dependent response, reaching its maximum at 0.5 mM (0.91 mg/g FW), representing an 82% increase over the untreated control. Lower (0.25 mM) and higher (1 mM) concentrations also enhanced chlorophyll a levels by 22% and 72%, respectively, although the response at 1 mM was slightly less than at 0.5 mM. These findings suggest that putrescine effectively stimulates the biosynthesis and/or stabilization of photosynthetic pigments, thereby improving light-harvesting capacity and leaf functionality. The observed enhancement in chlorophyll a content likely contributes to more efficient photosynthetic performance and early vegetative vigor in pistachio seedlings, highlighting the physiological importance of optimal putrescine concentrations for supporting early growth and productivity (Fig. 7 A).

3.9. Chlorophyll b

Analysis of variance (ANOVA) followed by Duncan's multiple range test at a 5% significance level revealed that all putrescine treatments significantly enhanced chlorophyll b content in young 'Badami Riz-e-Zarand' pistachio seedlings (Table 1). Chlorophyll b accumulation exhibited a pronounced concentration-dependent response, reaching its peak at 0.5 mM (0.71 mg/g FW), corresponding to a remarkable 255% increase over the control. Treatments at 0.25 and 1 mM also significantly increased chlorophyll b levels by 125% and 175%, respectively, although the effect at 1 mM was slightly lower than at 0.5 mM. These results underscore putrescine's strong regulatory role in photosynthetic pigment biosynthesis and stabilization, enhancing leaf light-harvesting efficiency and photosynthetic capacity. The substantial elevation in chlorophyll b at the optimal concentration likely contributes to improved leaf functionality, early vegetative vigor, and overall growth potential in pistachio seedlings, highlighting the physiological significance of proper putrescine dosing (Fig. 7 B).

3.10. Total chlorophyll content

Analysis of variance (ANOVA) followed by Duncan's multiple range test at a 5% significance level demonstrated that all putrescine treatments significantly increased total chlorophyll content (Chl a + b) in young 'Badami Riz-e-Zarand' pistachio seedlings (Table 1). Total chlorophyll accumulation exhibited a clear concentration-dependent response, reaching its maximum at 0.5 mM (1.49 mg/g FW), corresponding to an 86% increase over the control. Treatments at 0.25 and 1 mM also enhanced total chlorophyll by 36% and 60%, respectively, although the effect at 1 mM was slightly lower than at 0.5 mM. These findings indicate that putrescine strongly stimulates the biosynthesis and stabilization of photosynthetic pigments, leading to improved light-harvesting efficiency and leaf physiological performance. The substantial enhancement in total chlorophyll at the optimal concentration likely supports early vegetative vigor, promotes efficient photosynthesis, and contributes to overall seedling growth potential in pistachio (Fig. 7 C).

3.11. Carotenoid content

Analysis of variance (ANOVA) followed by Duncan's multiple range test at a 5% significance level confirmed that all putrescine treatments significantly enhanced carotenoid content in young 'Badami Riz-e-Zarand' pistachio seedlings (Table 2). Carotenoid accumulation showed a pronounced concentration-dependent response, reaching its peak at 0.5 mM (0.05501 mg/g FW), corresponding to a 174% increase compared with the control. Treatments at 0.25 and 1 mM also significantly elevated carotenoid levels by 64% and 149%, respectively, although the effect at 1 mM was slightly lower than at 0.5 mM. These results indicate that putrescine strongly promotes the biosynthesis of photoprotective pigments, enhancing leaf structural and functional integrity under early growth conditions. The marked increase in carotenoids at the optimal concentration likely supports improved light energy dissipation, protects the photosynthetic apparatus from oxidative stress, and contributes to enhanced photosynthetic efficiency and early vegetative vigor in pistachio seedlings (Fig. 7 D).

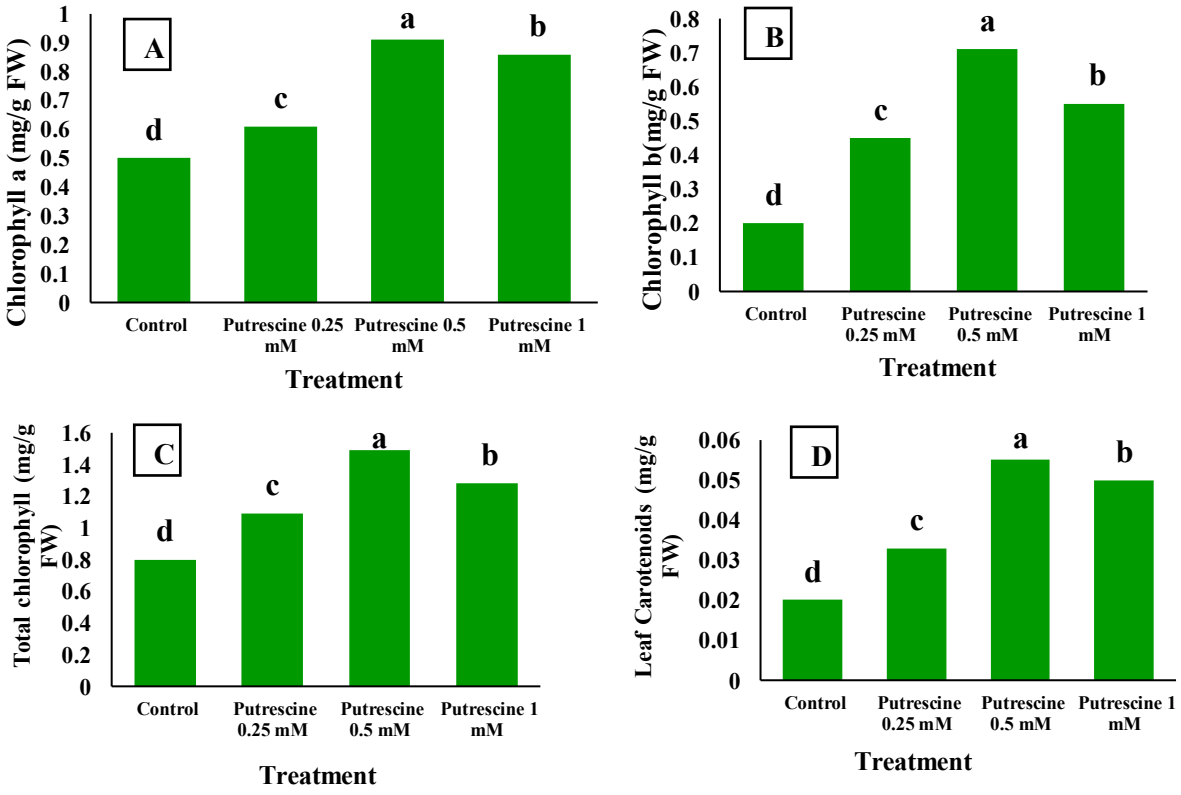

Figure 7. Effect of putrescine on leaf chlorophyll a (A), leaf chlorophyll b (B), leaf total chlorophyll (C) and leaf carotenoide (D) of pistachio (*Pistacia vera* L.) Badami Riz-e-Zarand. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

3.12. Reducing sugar content

Analysis of variance (ANOVA) followed by Duncan's multiple range test at a 5% significance level revealed that all putrescine treatments significantly enhanced reducing sugar content in young 'Badami Riz-e-Zarand' pistachio seedlings (Table 2). Reducing sugar accumulation exhibited a clear concentration-dependent trend, reaching its maximum at 0.5 mM (18.11 mg/g FW), corresponding to a 146% increase over the control. Treatments at 0.25 and 1 mM also significantly elevated sugar levels by 39% and 80%, respectively, although the 1 mM treatment was slightly less effective than 0.5 mM. These results suggest that putrescine markedly stimulates carbohydrate accumulation, providing an enhanced supply of readily metabolizable energy to support active cell division, growth, and metabolic processes during early vegetative development. The optimal concentration of 0.5 mM appears to maximize sugar availability, thereby reinforcing early seedling vigor and promoting overall physiological performance in pistachio (Fig. 8 A).

3.13. Starch content

Analysis of variance (ANOVA) followed by Duncan's multiple range test at a 5% significance level revealed that putrescine treatments significantly increased starch content in young 'Badami Riz-e-Zarand' pistachio seedlings (Table 2). Starch accumulation displayed a distinct concentration-dependent response, reaching its maximum at 0.5 mM (5.05 mg/g DW), representing a 102% enhancement compared with the untreated control. Treatments at 0.25 and 1 mM also significantly elevated starch levels by 29% and 66%, respectively, although the 1 mM treatment was slightly less effective than 0.5 mM. These findings indicate that putrescine substantially enhances carbohydrate storage in leaves, reinforcing the pool of energy reserves available for early growth and developmental processes. The marked increase in starch at the optimal concentration likely supports sustained metabolic activity, improves stress resilience, and contributes to robust early vegetative vigor in pistachio seedlings. Collectively, these results highlight the pivotal role of putrescine in coordinating carbohydrate allocation to strengthen overall seedling performance (Fig. 8 B).

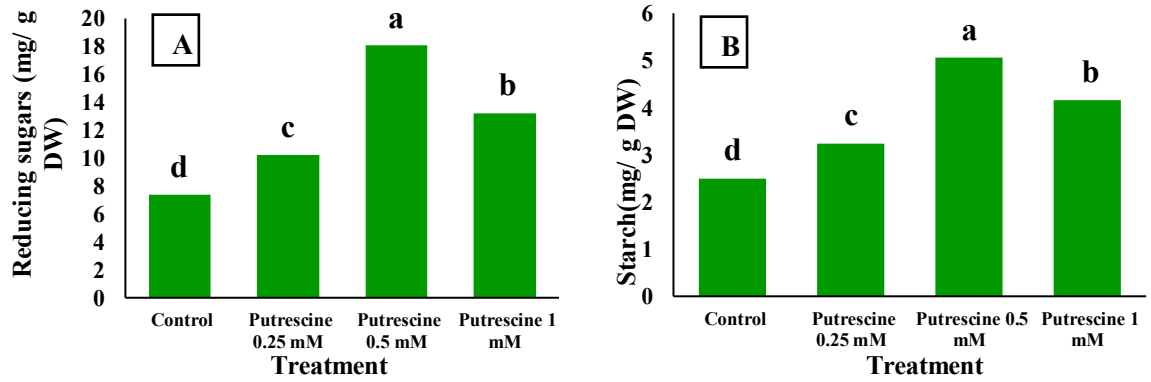

Figure 8. Effect of putrescine on reducing sugars (A) and starch (B) of pistachio (*Pistacia vera* L.) Badami Riz-e-Zarand. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

3.14. Electrolyte leakage

Analysis of variance (ANOVA) followed by Duncan's multiple range test at 5% significance revealed that all putrescine treatments significantly reduced electrolyte leakage in young 'Badami Riz-e-Zarand' pistachio seedlings (Table 2). The FW/DW ratio showed a clear concentration-dependent decline, with the lowest leakage observed at 0.5 mM (7.02%), representing a 67% reduction compared to the control. Treatments at 0.25 and 1 mM also significantly decreased leakage by 33% and 62%, respectively, although the 1 mM treatment was slightly less effective than 0.5 mM. These findings indicate that putrescine enhances cell membrane stability and integrity, contributing to improved stress resilience and early vegetative performance in pistachio seedlings (Fig 9 A).

3.15. Lipid peroxidation

Analysis of variance (ANOVA) followed by Duncan's multiple range test at 5% significance demonstrated that putrescine treatments significantly mitigated lipid peroxidation in young 'Badami Riz-e-Zarand' pistachio seedlings (Table 2). Lipid peroxidation decreased in a concentration-dependent manner, with the lowest level observed at 0.5 mM (20.35 µmol/g FW), representing a 49% reduction compared to the control. Treatments at 0.25 and 1 mM also significantly reduced lipid peroxidation by 19% and 37%, respectively, although the 1 mM treatment was less effective than 0.5 mM. These results indicate that putrescine confers protective effects against oxidative stress, enhancing membrane stability and promoting early vegetative health in pistachio seedlings (Fig 9 B).

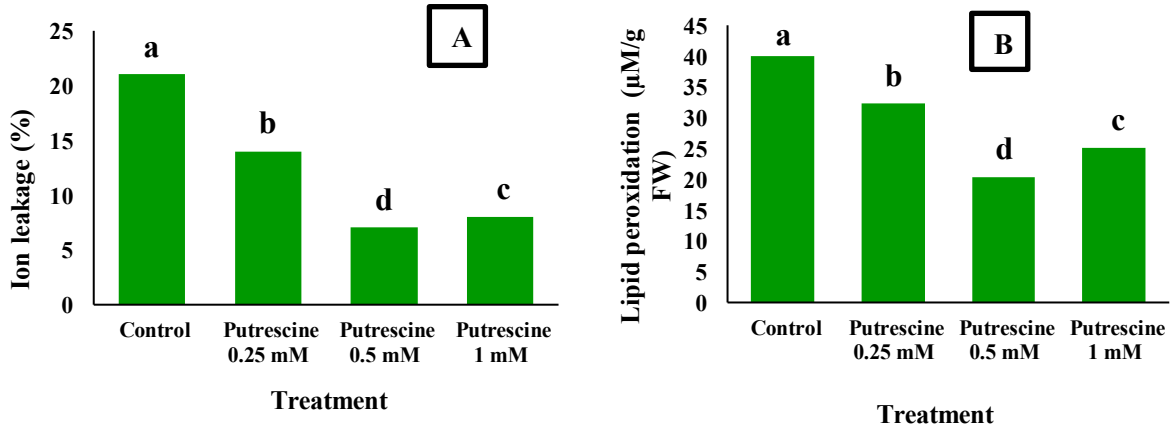

Figure 9. Effect of putrescine on ion leakage (A) and lipid peroxidation (B) of pistachio (*Pistacia vera* L.) Badami Riz-e-Zarand. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

3.16. Proline content

Analysis of variance (ANOVA) followed by Duncan's multiple range test at 5% significance showed that all putrescine treatments significantly enhanced proline content in young 'Badami Riz-e-Zarand' pistachio seedlings (Table 2). Proline accumulation exhibited a concentration-dependent increase, with the highest level observed at 0.5 mM (58.23 µmol/mg FW), representing a 65% increase over the control. Treatments at 0.25 and 1 mM also

significantly increased proline by 17% and 42%, respectively, although the 1 mM treatment was slightly less effective than 0.5 mM. These findings indicate that putrescine promotes osmolyte accumulation, supporting cellular osmotic adjustment, antioxidant capacity, and early vegetative vigor in pistachio seedlings (Fig 10).

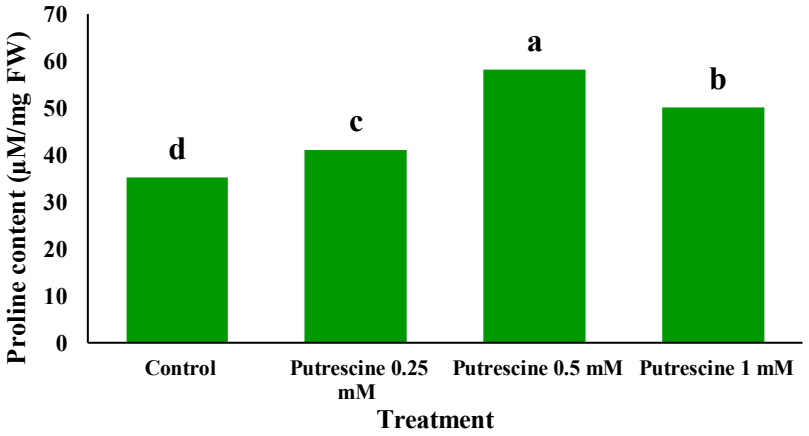

Figure 10. Effect of putrescine on proline content of pistachio (*Pistacia vera* L.) Badami Riz-e-Zarand. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

3.17. Catalase activity

Analysis of variance (ANOVA) followed by Duncan's multiple range test at 5% significance indicated that putrescine treatments significantly enhanced catalase activity in young 'Badami Riz-e-Zarand' pistachio seedlings (Table 2). Catalase activity exhibited a mild but significant concentration-dependent increase, with the highest activity observed at 0.5 mM (1.155 U/mg protein), representing a 3.1% enhancement over the control. Treatments at 0.25 and 1 mM also increased activity by 1.1% and 2.9%, respectively, although the 1 mM treatment was slightly less effective than 0.5 mM. These results suggest that putrescine can stimulate antioxidant defense mechanisms, contributing to improved oxidative stress mitigation and early seedling vigor (Fig 11 A).

3.18. Peroxidase activity

Analysis of variance (ANOVA) followed by Duncan's multiple range test at 5% significance demonstrated that putrescine treatments significantly enhanced peroxidase activity in young 'Badami Riz-e-Zarand' pistachio seedlings (Table 2). Peroxidase activity increased in a concentration-dependent manner, peaking at 0.5 mM (5.36 U/mg protein), representing a 67% enhancement over the control. Treatments at 0.25 and 1 mM also significantly increased activity by 15% and 45%, respectively, although the 1 mM treatment was slightly less effective than 0.5 mM. These findings suggest that putrescine effectively stimulates antioxidant defense mechanisms, improving reactive oxygen species scavenging and promoting early vegetative vigor (Fig 11 B).

3.19. Ascorbate peroxidase

Analysis of variance (ANOVA) followed by Duncan's multiple range test at 5% significance indicated that all putrescine treatments significantly enhanced ascorbate peroxidase (APX) activity in young 'Badami Riz-e-Zarand' pistachio seedlings (Table 2). APX activity exhibited a concentration-dependent increase, with the highest level observed at 0.5 mM (0.094 U/mg protein), representing a 169% increase over the control. Treatments at 0.25 and 1 mM also significantly elevated APX activity by 49% and 154%, respectively, although the 1 mM treatment was slightly less effective than 0.5 mM. These findings highlight putrescine's strong role in stimulating antioxidant defense systems, promoting reactive oxygen species scavenging, and enhancing early vegetative vigor (Fig 11 C).

3.20. Superoxide dismutase

Analysis of variance (ANOVA) followed by Duncan's multiple range test at 5% significance demonstrated that all putrescine treatments significantly enhanced superoxide dismutase (SOD) activity in young 'Badami Riz-e-Zarand' pistachio seedlings (Table 2). SOD activity increased in a clear concentration-dependent manner, peaking at 0.5 mM (0.88 U/mg protein), representing a 319% enhancement over the control. Treatments at 0.25 and 1 mM also significantly increased SOD activity by 143% and 210%, respectively, although the 1 mM treatment was slightly less effective than 0.5 mM. These findings suggest that putrescine strongly stimulates antioxidant defense mechanisms, contributing to improved reactive oxygen species detoxification and early vegetative growth (Fig 11D).

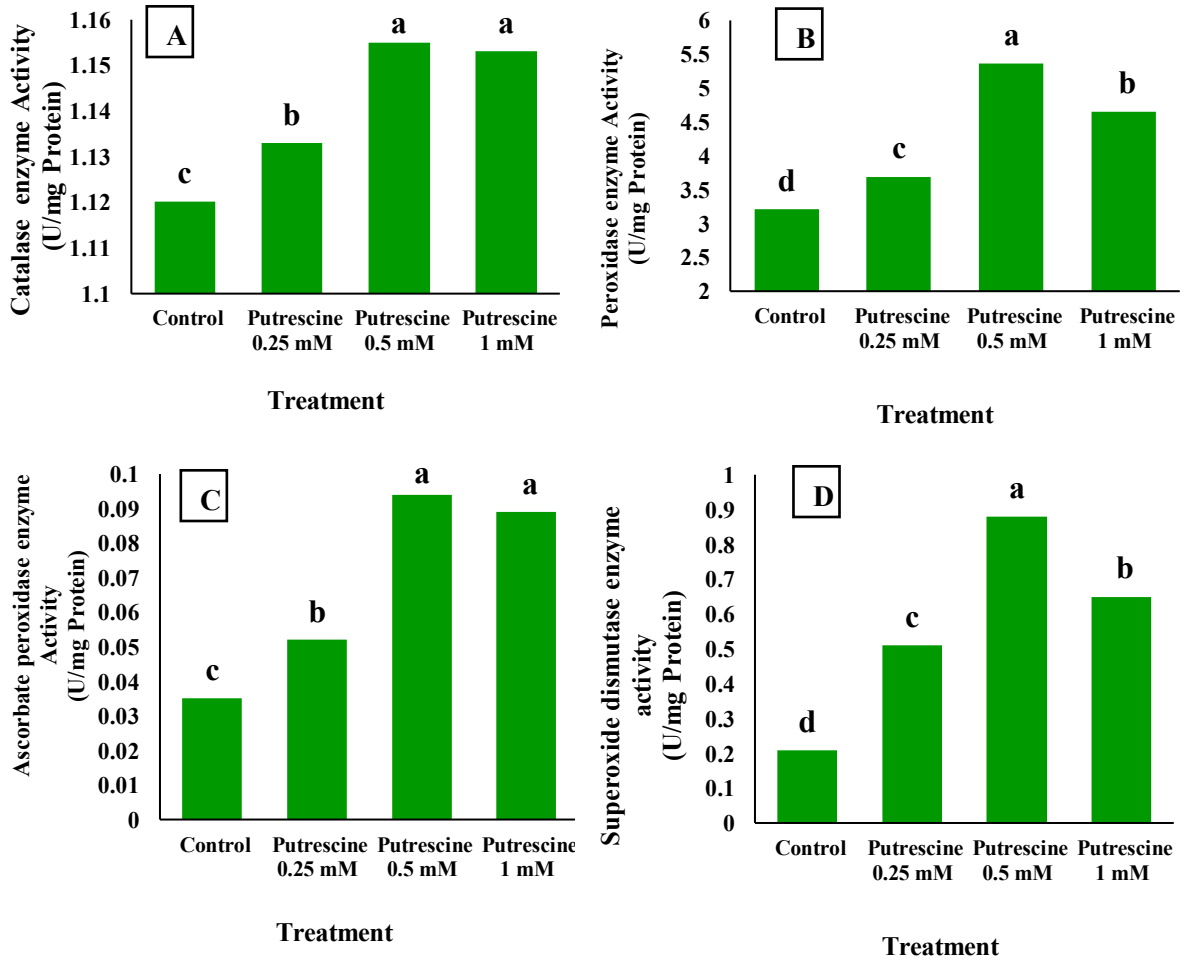

Figure 11. Effect of putrescine on catalase enzyme activity (A), peroxidase enzyme activity (B), ascorbate peroxidase enzyme activity (C) and superoxide dismutase enzyme activity (D) of pistachio (*Pistacia vera* L.) Badami Riz-e-Zarand. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

4. Discussion

The results of this study clearly demonstrated that foliar application of putrescine, particularly at a concentration of 0.5 mM, had a pronounced positive effect on the vegetative growth, physiological, and biochemical traits of 'Badami Riz-e-Zarand' pistachio seedlings. At this concentration, significant increases were observed in shoot height (+160%), stem diameter (+36%), leaf number (+65%), leaf area (+156%), and biomass accumulation (fresh weight +40%, dry weight +293%). Furthermore, the maximization of photosynthetic pigment content and carbohydrates (reducing sugars +146%, starch +102%), along with a reduction in the fresh-to-dry weight ratio, indicated enhanced structural density and tissue firmness. These findings are consistent with previous studies reporting that putrescine at optimal concentrations enhances plant growth and metabolism by regulating physiological and biochemical processes (Hussein *et al*., 2023; Wasaya *et al*., 2023; Tyagi *et al*., 2022; Zhao *et al*., 2021). The diminished effect at 1 mM suggests a concentration-dependent response, likely due to excessive putrescine accumulation and metabolic stress (Alcázar *et al*., 2010; Tiburcio *et al*., 2014).

The improvement in both longitudinal and radial growth, particularly at 0.5 mM, reflects the active role of putrescine in stimulating cell division and elongation through polyamine biosynthesis pathways and interactions with hormones such as auxins and gibberellins (González-Hernández *et al*., 2022; Liu *et al*., 2022; Pál *et al*., 2019). Activation of genes associated with cell division and meristematic tissue development represents a primary mechanism underlying the enhanced vegetative growth (Page *et al*., 2016), corroborating the findings of Sedaghat *et al*. (2014) in 'Badami-E-Riz' seedlings. The increase in leaf number, leaf area, and biomass highlights putrescine's role in promoting leaf development and photosynthetic capacity. This effect is mediated through enhanced protein synthesis and regulation of carbohydrate metabolism, resulting in larger leaves and improved overall plant performance (Hussein *et al*., 2023; Aydin *et al*., 2016). Wu *et al*. (2010) also reported that putrescine improved vegetative growth and root development in trifoliate orange seedlings, supporting similar effects across different species. The elevated chlorophyll and carotenoid content reflects putrescine's protective effect on

chloroplasts and its induction of genes involved in chlorophyll biosynthesis (Joshi *et al*., 2022; Kandil *et al*., 2011). Reduced chlorophyll degradation under oxidative stress helps maintain photosynthetic capacity (Zhao *et al*., 2023), consistent with the findings of Hanafy Ahmed *et al*. (2017) in drought-stressed cotton. High levels of reducing sugars and starch indicate putrescine's role in modulating carbohydrate metabolism and enhancing the plant's energy reserves (Hussein *et al*., 2023; Wasaya *et al*., 2023). The marked reduction in electrolyte leakage and lipid peroxidation suggests improved membrane stability and mitigation of oxidative stress (González-Hernández *et al*., 2022; Zhao *et al*., 2021; Xing *et al*., 2011). The upregulation of antioxidant enzyme activities by up to +319% demonstrates extensive activation of the plant's defense system, supported by induction of catalase and superoxide dismutase genes (Shi and Chan, 2013; Daler *et al*., 2025; Inal *et al*., 2022). These findings align with Zhao *et al*. (2023) in salt-stressed wheat and Mahdavian *et al*. (2019) in citrus rootstocks under waterlogging, indicating that putrescine enhances tolerance by reducing ROS accumulation and improving membrane stabilization. Overall, the findings of this study are consistent with previous reports across various species, including enhanced root growth in 'Badami-E-Riz' seedlings (Sedaghat *et al*., 2014), improved growth and photosynthesis under drought stress in wheat (Hussein *et al*., 2023; Wasaya *et al*., 2023), increased stress tolerance in grapevine (Zhao *et al*., 2021; Daler *et al*., 2025), and improved postharvest quality and mycorrhizal root development in ornamental plants (Rakbar *et al*., 2022; Hojatipour and Hassanpour Asil, 2021). Positive effects in pomegranate and date palm (Amri *et al*., 2011; Naser *et al*., 2016) further confirm the broad applicability of putrescine.

However, from a critical scientific perspective, although the positive effects are evident, long-term evaluations and interactions with different environmental conditions are essential to define the practical scope and limitations of putrescine application in pistachio seedlings. This study provides the first comprehensive report on the effects of putrescine on multiple traits in pistachio seedlings under greenhouse conditions, highlighting its potential as an effective biostimulant for seedling establishment.

Conclusion

This study demonstrates that foliar application of putrescine at 0.5 mM provides optimal enhancement of vegetative growth, physiological performance, and biochemical responses in 'Badami Riz-e-Zarand' pistachio seedlings under short-term greenhouse conditions. Key outcomes include significant increases in shoot height (+160%), stem diameter (+36%), leaf number (+65%), leaf area (+156%), and biomass (fresh weight +40%, dry weight +293%). Photosynthetic pigments (chlorophyll a, b, total, and carotenoids) and carbohydrates (reducing sugars +146%, starch +102%) were also maximized. Stress resilience was evidenced by 67% reduced electrolyte leakage, 65% increased proline, and 49% decreased lipid peroxidation, along with up to +319% enhanced activity of antioxidant enzymes (SOD, CAT, POD, APX). Efficacy declined at 1 mM, confirming a concentration-dependent response. These findings align with pistachio-specific studies, validating putrescine's role in multi-trait improvement. However, results are limited to 60-day controlled conditions, precluding extrapolation to long-term field performance or genotypic variability. Practically, 0.5 mM foliar sprays offer a cost-effective intervention to boost nursery establishment by 40–150%, potentially shortening propagation cycles by 2–4 weeks and reducing transplant mortality by 20–30% in arid regions. Future field trials are essential to confirm efficacy under real environmental stresses, guiding targeted biostimulant protocols for sustainable pistachio production.

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