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ARTICLE

Biochar-Loaded *Purpureocillium lilacinum* Formulation Enhances Soil Water Retention, Drought Tolerance, and Essential Oil Productivity of Spearmint (*Mentha spicata* L.)

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ABSTRACT: Water deficit severely constrains the productivity and essential oil yield of medicinal and aromatic crops grown in sandy soils. This study assessed whether a biochar-loaded *Purpureocillium lilacinum* formulation could improve soil properties, drought tolerance, growth, mineral nutrition, soil biological activity, and essential oil production of spearmint (*Mentha spicata* L.) under full and deficit irrigation. A two-season field experiment was conducted in 2024 and 2025 using a split-plot design arranged in a randomized complete block with three replicates. Irrigation regimes, including 100% and 75% of crop evapotranspiration (ETc), were assigned to main plots, whereas bioformulation treatments, including untreated control, biochar alone, free *P. lilacinum*, and biochar-loaded *P. lilacinum*, were assigned to subplots. The formulation maintained high viability during storage, with fungal counts declining from 8.30×10^7 to 5.10×10^7 colony-forming units (CFU) g^{-1} after 60 days, while pH and electrical conductivity remained relatively stable. Deficit irrigation reduced soil organic matter, water-holding capacity, available macronutrients, plant growth, chlorophyll content, relative water content, leaf nutrient concentrations, essential oil yield, and dehydrogenase activity, while increasing proline accumulation. Conversely, bioformulation treatments improved most measured traits, with biochar-loaded *P. lilacinum* producing the strongest response. This treatment enhanced soil water retention and nutrient availability, reduced bulk density, improved biomass accumulation and physiological status, and maintained high essential oil yield under both irrigation regimes. Overall, biochar-loaded *P. lilacinum* represents a promising bioformulation for improving spearmint productivity, drought resilience, soil biological activity, and essential oil performance in sandy soils under deficit irrigation.

KEYWORDS: Biochar; *Purpureocillium lilacinum*; spearmint; deficit irrigation; sandy soil; essential oil; drought tolerance; soil dehydrogenase activity

1 Introduction

Water deficit is a major constraint limiting the growth, productivity, and metabolic performance of medicinal and aromatic plants, particularly in arid and semi-arid production systems [1,2]. Reduced soil water availability restricts nutrient uptake, stomatal conductance, photosynthetic activity, cell expansion, and biomass accumulation, thereby affecting both herb yield and the biosynthesis of economically important secondary metabolites [3,4]. In aromatic crops, drought may also alter essential oil yield and composition, although the magnitude and direction of these changes depend on stress intensity, plant species, developmental stage, and crop management practices [5,6]. Accordingly, sustainable approaches that improve soil water retention and support plant physiological performance under deficit irrigation are increasingly needed for the production of high-value aromatic crops in water-limited environments [7,8].

Spearmint (*Mentha spicata* L.) is an economically important medicinal and aromatic plant cultivated primarily for its essential oil, which is widely used in food, pharmaceutical, cosmetic, and flavoring industries [9,10]. Its commercial value is closely related to essential oil yield and chemical composition, particularly the relative abundance of major monoterpenes such as carvone, limonene, and 1,8-cineole, as well as other oxygenated compounds [11,12]. However, spearmint productivity and essential oil traits are highly sensitive to environmental conditions, especially water availability. Previous studies on *M. spicata* and other Lamiaceae species have shown that water deficit and salinity can alter chlorophyll content, oxidative stress responses, proline accumulation, biomass production, and essential oil characteristics [13,14]. Improving drought resilience in spearmint while maintaining essential oil productivity and quality is therefore an important agronomic and biochemical objective.

Biochar has attracted considerable attention as a sustainable soil amendment due to its porous structure, high surface area, relative stability, and capacity to improve soil physical and chemical properties [15,16]. In coarse-textured soils, biochar can enhance water-holding capacity, reduce bulk density, improve nutrient retention, increase cation-exchange capacity, and decrease nutrient leaching [17,18]. Recent reviews and meta-analyses further indicate that biochar can improve soil fertility and crop performance, although its effects vary according to feedstock type, pyrolysis conditions, soil texture, application rate, and environmental context [19,20]. Under drought-prone conditions, these properties may help conserve water in the root zone, sustain nutrient availability, and support plant physiological activity during deficit irrigation [21].

In addition to its direct effects on soil properties, biochar can serve as a carrier material for microbial inoculants [22]. Its porous matrix, surface functional groups, moisture-retention capacity, and stable carbon framework can provide protective microhabitats for beneficial microorganisms, thereby improving their survival, adhesion, establishment, and functional persistence in the rhizosphere [22,23]. Biochar-based microbial inoculants are therefore increasingly viewed as integrated tools that combine soil conditioning with biological plant-growth promotion and stress mitigation [24].

Purpureocillium lilacinum, formerly known as *Paecilomyces lilacinus*, is a beneficial soil fungus widely recognized for its biological control activity, particularly against plant-parasitic nematodes [25,26]. Beyond its biocontrol function, available evidence suggests that *P. lilacinum* can contribute to plant growth by improving root performance, photosynthetic pigments, biomass accumulation, and rhizosphere functionality [25,27]. However, the practical effectiveness of fungal inoculants under field or semi-field conditions is often constrained by poor survival, limited persistence, and reduced adaptation to unfavorable soil environments. Suitable carrier-based formulations are therefore required to improve the stability and field performance of beneficial fungal inoculants.

Recent studies also reflect growing interest in biologically based crop-support strategies, including microbial culture filtrates, field evaluation under drought-prone environments, and endophytic bacterial

and fungal isolates under stress-associated conditions [28–30]. However, such studies are largely disease- or host-specific and do not directly address the use of biochar-mediated delivery systems for improving drought tolerance and essential oil productivity in aromatic crops. Combining biochar with *P. lilacinum* may therefore provide a dual-function bioformulation in which biochar serves as both a soil conditioner and a protective carrier for the fungus. This approach may enhance fungal viability, support rhizosphere establishment, improve soil water retention and biological activity, and ultimately strengthen plant performance under water-limited conditions.

Despite the potential of biochar-based microbial delivery systems, limited information is available on the use of biochar-loaded *Purpureocillium lilacinum* formulations for improving drought tolerance and essential oil productivity in aromatic plants. The purpose of this study was to determine whether biochar-mediated delivery of *P. lilacinum* could improve soil water retention, plant drought tolerance, growth performance, physiological status, mineral nutrition, soil biological activity, and essential oil productivity of spearmint (*Mentha spicata* L.) under full and deficit irrigation. The study also aimed to assess whether the biochar-loaded fungal formulation provides greater functional benefits than biochar alone or free *P. lilacinum* inoculation under water-limited sandy-soil conditions.

2 Materials and Methods

2.1 Experimental Site and Soil Characterization

The field experiment was carried out during two consecutive summer seasons, 2024 and 2025, at the Ismailia Agricultural Research Station, Ismailia Governorate, Egypt (30°35′41.9″ N, 32°16′45.8″ E). Before transplanting, surface soil samples were collected from the 0–30 cm layer and analyzed for selected physical and chemical properties using standard soil analytical procedures [31].

2.2 Biochar Preparation and Characterization

Biochar was produced from rice straw residues by slow pyrolysis at 500°C for 2 h under oxygen-limited conditions, following the procedure described by Lee et al. [32]. After pyrolysis, the material was ground and passed through a 0.5-mm sieve to obtain a uniform particle size. The prepared biochar was used both as a soil amendment and as a carrier material for *Purpureocillium lilacinum*. Biochar was incorporated into the soil before transplanting at a rate of 7.35 m^3 $feddan^{-1}$. Its main chemical characteristics are shown in Table 1.

2.3 Fungal Isolate and Preparation of Purpureocillium lilacinum Inoculum

The isolate was tentatively identified as *Purpureocillium lilacinum* based on cultural and microscopic characteristics. Molecular identification using ITS-rDNA sequencing was not performed for the exact working isolate used in this study; therefore, no GenBank accession number is available. In addition, the isolate has not been deposited in a public culture collection such as ATCC, CGMCC, or CBS. The fungal culture was maintained in the culture collection of the Microbiology Department, Soil, Water and Environment Research Institute, Agricultural Research Center, Egypt, and was used as a morphology-based working isolate. Accordingly, species-level identification should be interpreted with caution, and the absence of molecular confirmation and public strain-deposit accession number is acknowledged as a limitation of this study.

The isolate was tentatively identified as *Purpureocillium lilacinum* based on cultural and microscopic characteristics. This morphological identification was based on colony appearance on potato dextrose agar (PDA), including colony color, growth pattern, texture, and sporulation, as well as microscopic

examination of conidiophores, phialides, conidial chains, and conidial morphology using a light microscope according to standard taxonomic criteria for Purpureocillium/Paecilomyces-like fungi. However, molecular confirmation, such as ITS-rDNA sequencing, was not performed for the exact working isolate used in the present experiment; therefore, species-level identification should be interpreted as morphology-based and represents a limitation of this study. Colony morphology was examined on potato dextrose agar (PDA), including colony color, growth pattern, texture, and sporulation. Microscopic identification was performed by examining conidiophores, phialides, conidial chains, and conidial morphology using a light microscope, according to standard taxonomic criteria for Purpureocillium/Paecilomyces-like fungi.

Molecular identification was not performed for the exact working isolate used in the present experiment; therefore, no GenBank accession number is reported for this isolate. Future work will include ITS rDNA-based molecular confirmation and sequence deposition to support strain-level identification.

Before use, the fungal culture was maintained on potato dextrose agar (PDA) slants at 4 ± 1°C and periodically subcultured to preserve its viability. For inoculum preparation, the isolate was cultured on PDA plates and incubated at 25 ± 2°C for 7–10 days until abundant conidial production was achieved. Conidia were harvested by flooding the culture surface with sterile distilled water containing 0.05% (v/v) Tween 80 and gently scraping the colony surface with a sterile glass rod. The resulting suspension was filtered through two layers of sterile cheesecloth to remove mycelial fragments. The conidial concentration was determined using a hemocytometer and adjusted to 1×10^7 conidia mL^{-1} for preparation of the free fungal inoculum and the biochar-loaded formulation. The use of *P. lilacinum* as a beneficial fungal inoculant was based on its documented biocontrol and plant-growth-promoting potential [25,26].

Conidial viability was evaluated using a germination test [33]. Briefly, an aliquot of the conidial suspension was spread onto PDA plates and incubated at 25 ± 2°C for 18–24 h. At least 100 conidia were examined microscopically. A conidium was considered germinated when the germ tube length was equal to or greater than the conidial diameter. Conidial viability was calculated as the percentage of germinated conidia relative to the total number of conidia examined. Only conidial suspensions with viability greater than 90% were used for preparation of the experimental treatments.

Table 1: Selected chemical properties and available nutrient contents of the biochar used in the experiment.

Property	Value
Total carbon (C; %)	82.48
Total nitrogen (N; %)	0.70
Hydrogen (H; %)	1.35
Sulfur (S; %)	ND
pH (1:2.5 biochar:water suspension)	7.73
Electrical conductivity, EC (1:5 biochar:water extract; dS m^{-1})	0.85
Available nitrogen (N; mg kg^{-1})	46.7
Available phosphorus (P; mg kg^{-1})	35.0
Available potassium (K; mg kg^{-1})	3705

ND: not detected.

2.4 Preparation of Biochar-Loaded Purpureocillium Lilacinum Formulation

Biochar was sterilized at the Egyptian Atomic Energy Authority before being used as a carrier material for *Purpureocillium lilacinum*. The sterilized biochar was moistened with the prepared conidial suspension

until it reached approximately 50–60% of its water-holding capacity. It was then mixed thoroughly under aseptic conditions to ensure uniform distribution of the fungal inoculum.

The inoculated biochar was incubated at 25 ± 2°C for 10–14 days to allow fungal colonization of the biochar particles. After incubation, the biochar-loaded fungal formulation was air-dried under aseptic shaded conditions and stored in sterile containers at 4°C until field application. Fungal viability in the final formulation was determined by serial dilution plating on PDA, and the viable fungal population was expressed as colony-forming units per gram of formulation (CFU g^{-1}), The representative appearance and packaged form of the final biochar-loaded *Purpureocillium lilacinum* formulation are shown in Fig. 1.

Figure 1: Representative appearance of the final biochar-loaded *Purpureocillium lilacinum* formulation. The formulation consisted of sterilized biochar particles colonized by *Purpureocillium lilacinum* and packed in sterile, sealed containers for refrigerated storage until field application. The magnified panel illustrates fungal colonization of the biochar particles, while the lower panels show the approximate particle size and final packaged formulation.

2.5 Irrigation Management

Irrigation treatments were scheduled according to crop evapotranspiration (ETc), calculated using the FAO-56 approach as follows:

$$ETc = ETo \times Kc$$

where ETo is the reference evapotranspiration (mm day^{-1}), calculated from daily climatic data obtained from the nearest meteorological station using the FAO Penman–Monteith equation, and Kc is the crop coefficient corresponding to the developmental stage of spearmint [34]. For calculation purposes, Kc values of 0.50, 0.75, 1.05, and 0.85 were used for the initial, crop-development, mid-season, and late-season stages, respectively.

Two irrigation regimes were imposed: full irrigation at 100% ETc and deficit irrigation at 75% ETc. All plots received equal irrigation during the first 14 days after transplanting to ensure successful crop establishment. Thereafter, irrigation treatments were initiated and maintained throughout the experimental period. Irrigation water was delivered through a drip irrigation system consisting of lateral lines spaced 0.70 m apart, and emitters spaced 0.30 m apart, with a nominal discharge rate of 4.0 L h^{-1}.

Irrigation was applied every 2–3 days, depending on prevailing weather conditions. The amount of water applied at each irrigation event was calculated based on ETc, plot area, and an assumed irrigation-system efficiency of 90%. The seasonal irrigation water applied was approximately 5200 m^3 ha^{-1} under the 100% ETc regime and 3900 m^3 ha^{-1} under the 75% ETc regime. Irrigation duration was adjusted separately for each irrigation treatment, while irrigation frequency remained similar. The applied water volume was monitored using flow meters installed on the irrigation lines.

2.6 Plant Material and Growth Conditions

Uniform and healthy spearmint (*Mentha spicata* L.) seedlings were obtained from a certified commercial nursery located in Giza Governorate, Egypt. At transplanting, seedlings were approximately 6–8 weeks old, 12–15 cm in height, and had 4–6 fully developed leaves. Plants showing visible symptoms of nutrient deficiency, pest infestation, or mechanical damage were excluded.

Transplanting was carried out during the first week of March in both growing seasons. Seedlings were transplanted at 30 cm between plants and 45 cm between rows under drip irrigation. Each experimental subplot measured approximately 3.6 m^2 and consisted of four rows, each 2.0 m long, with approximately 24 plants per subplot. The outer plants and terminal plants of each row were considered border plants, whereas plants from the central rows were used for measurements and sampling.

All plots received uniform agronomic management throughout both seasons. Phosphorus fertilizer was incorporated into the soil before transplanting at a rate equivalent to approximately 30–45 kg P_2O_5 ha^{-1}. Nitrogen fertilizer was applied at a total rate of approximately 90–120 kg N ha^{-1} in three equal doses: after establishment, after the first cut, and during subsequent vegetative regrowth. Potassium fertilizer was applied at approximately 60–90 kg K_2O ha^{-1} in two or three split applications. Fertilizers were supplied through the irrigation system or applied adjacent to the plant rows according to local production practices.

Weeds were removed manually whenever necessary. Pest and disease incidence was monitored regularly, and no pesticides were applied unless infestation exceeded the locally accepted economic threshold. All management practices were applied uniformly to all treatments. No additional microbial inoculants, biofertilizers, or soil amendments were applied except those specified in the experimental treatments.

Plants were harvested at approximately 50% flowering. Two cuts were obtained during each growing season. The first cut was conducted approximately 90–105 days after transplanting, and the second cut was performed approximately 60–75 days after the first.

2.7 Experimental Framework

The experiment was arranged as a split-plot design within a randomized complete block structure with three replicates. Irrigation regime was assigned to the main plots and consisted of two levels: 100% ETc and 75% ETc. Four soil bioformulation treatments were randomly assigned to the subplots within each main plot: untreated control, biochar alone, free *P. lilacinum* inoculation, and biochar-loaded *P. lilacinum* formulation.

Accordingly, the experiment included eight treatment combinations, resulting from the combination of two irrigation regimes and four bioformulation treatments. Each treatment combination was replicated three times, giving a total of 24 experimental subplots. This arrangement allowed assessment of the main

effects of irrigation regime and bioformulation treatment, as well as their interaction, on soil water retention, soil physicochemical properties, plant growth, drought-related physiological responses, herb yield, essential oil percentage, essential oil yield, and essential oil composition of spearmint.

2.8 Formulation Measurements

The biochar-loaded *P. lilacinum* formulation was evaluated for fungal viability, storage stability, moisture content, pH, and electrical conductivity (EC). These measurements were selected because the physicochemical properties of biochar carriers can influence microbial attachment, survival, and storage stability [35]. All measurements were performed in triplicate.

Fungal viability was determined immediately after formulation preparation and after 30 and 60 days of storage at 4°C. For each determination, 1.0 g of the formulation was aseptically transferred into 9.0 mL of sterile distilled water containing 0.05% (v/v) Tween 80. The suspension was vortexed thoroughly for 2–3 min to detach fungal propagules from the biochar particles. Serial tenfold dilutions were prepared, and 0.1 mL aliquots from the appropriate dilutions were spread onto PDA plates following the standard serial-dilution and viable plate-count procedure [36]. Plates were incubated at 25 ± 2°C for 3–5 days, after which colonies showing the characteristic morphology of *P. lilacinum* were counted. Plates containing approximately 30–300 colonies were used for enumeration.

The viable fungal population was expressed as CFU g^{-1} of formulation and calculated as follows:

$$\text{CFU } g^{-1} = \text{number of colonies} \times \text{dilution factor} \times 10$$

where the factor 10 accounts for the 0.1 mL plated volume. Storage stability was assessed from changes in the viable fungal population during the 60-day storage period and expressed as CFU g^{-1} and as the percentage of the initial viable population remaining at each sampling time.

For pH determination, the formulation was mixed with distilled water at a 1:2.5 (w/v) ratio, shaken for 30 min, and allowed to equilibrate before measurement using a calibrated pH meter. Electrical conductivity was determined in a separate 1:5 (w/v) formulation-to-water extract using a calibrated EC meter, following general analytical procedures described for biochar materials [37]. The pH and EC meters were calibrated using appropriate standard buffer and conductivity solutions before analysis.

Moisture content was determined gravimetrically by drying approximately 5.0 g of formulation at 105°C until constant weight, following the general procedure described in ASTM D1762-84 [38]. Moisture content was calculated on a wet-weight basis as follows:

$$\text{Moisture content (\%)} = [(\text{fresh weight} - \text{oven-dry weight})/\text{fresh weight}] \times 100.$$

2.9 Soil Measurements

Soil samples were collected from the root zone after harvest during both growing seasons to evaluate the effects of irrigation regime and bioformulation treatments on soil physicochemical properties and microbial activity. Samples were air-dried, gently crushed, passed through a 2-mm sieve, and analyzed for organic matter (OM), water-holding capacity (WHC), bulk density, and available N, P, and K according to standard soil analysis procedures [31]. Soil organic matter was determined using the standard oxidation method. Available nitrogen, phosphorus, and potassium were determined using standard analytical procedures commonly applied for soil fertility assessment. Water-holding capacity was determined gravimetrically, whereas bulk density was measured using the core method [31].

Soil dehydrogenase activity was determined as an indicator of microbial activity using the colorimetric reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF), and results were expressed as μg TPF g^{-1} soil h^{-1} [39].

2.10 Plant Growth, Physiological Traits, Essential Oil, and Leaf Nutrient Analysis

Plant growth and physiological measurements were recorded during each growing season. Plant height was measured from the soil surface to the terminal growing point, and the number of branches per plant was counted. Fresh biomass was recorded immediately after harvest, whereas dry biomass was determined after oven-drying plant samples at 70°C until constant weight. Chlorophyll content was estimated on fully expanded leaves using a SPAD chlorophyll meter [40]. Relative water content (RWC) was determined using fresh, turgid, and dry leaf weights [41]. Proline content was determined as a drought-stress indicator using the colorimetric method described by Bates et al. [42].

Essential oil was extracted from fresh spearmint aerial parts by hydrodistillation using a Clevenger-type apparatus [43]. Essential oil percentage was calculated on a fresh-weight basis, and essential oil yield was calculated relative to fresh herb yield. The chemical composition of the essential oil was analyzed using gas chromatography–mass spectrometry (GC–MS). The GC–MS analysis was performed using an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass selective detector and equipped with an HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. One microliter of diluted essential oil was injected in split mode, with the injector temperature set at 250°C. The oven temperature was programmed from 60°C to 240°C at 3°C min^{-1} and then held at 240°C for 10 min. The mass spectrometer was operated in electron-impact ionization mode at 70 eV, and mass spectra were recorded over the m/z 50–550 range. Volatile compounds were identified by comparing their mass spectra and retention behavior with the NIST mass spectral library and published mass spectral data [44]. The main identified constituents included carvone, limonene, 1,8-cineole, trans-carveol, cis-dihydrocarvone, β-caryophyllene, and germacrene D.

For leaf nutrient analysis, leaf samples were collected, washed with distilled water, oven-dried at 70°C until constant weight, ground, and digested for macroelement determination. The concentrations of P, K, Ca, and Mg in leaf tissues were determined using ICP-OES, whereas nitrogen was determined by the Kjeldahl method when applicable [45].

2.11 Statistical Analysis

Data from each growing season were analyzed separately using analysis of variance appropriate for a split-plot randomized complete block design with three blocks. Irrigation regime was considered the main-plot factor, whereas bioformulation treatment was considered the subplot factor. The statistical model evaluated the effects of irrigation regime, bioformulation treatment, and their interaction using the appropriate whole-plot and subplot error terms. When significant effects were detected, irrigation regime × bioformulation treatment combination means were separated using Tukey's honestly significant difference test at $p \leq 0.05$. Data are presented as means ± standard deviation (n = 3).

3 Results

3.1 Initial Soil Characteristics

The initial soil analysis showed that the experimental soil was sandy in texture, with low organic matter content and relatively low available macronutrient levels (Table 2). These baseline soil characteristics were used to describe the initial field condition before treatment application.

Table 2: Some physical and chemical properties of the experimental soil.

Category	Soil Characteristic	Value
Particle-size distribution	Coarse sand (%)	50.4
	Fine sand (%)	40.4
	Silt (%)	3.20
	Clay (%)	6.00
	Texture class	Sandy
Chemical properties	$CaCO_3$ (%)	1.40
	pH (1:2.5 soil:water suspension)	7.92
	EC, saturated paste extract (dS m^{-1})	0.37
	Organic matter (%)	0.32
Soluble cations	Ca^{2+} (meq L^{-1})	0.94
	Mg^{2+} (meq L^{-1})	0.89
	Na^{+} (meq L^{-1})	1.45
	K^{+} (meq L^{-1})	0.45
Soluble anions	CO_3^{2-} (meq L^{-1})	ND
	HCO_3^{-} (meq L^{-1})	1.42
	Cl^{-} (meq L^{-1})	1.02
	SO_4^{2-} (meq L^{-1})	1.29
Available nutrients	Nitrogen (N; mg kg^{-1})	40.0
	Phosphorus (P; mg kg^{-1})	15.0
	Potassium (K; mg kg^{-1})	67.0

ND: not detected.

3.2 Shelf-Life Stability of Biochar-Loaded Purpureocillium lilacinum Formulation

Before formulation preparation, the conidial suspension showed viability greater than 90% and was therefore used to prepare the free fungal inoculum and the biochar-loaded formulation. The biochar-loaded *Purpureocillium lilacinum* formulation maintained relatively high fungal viability during storage, although viable counts declined gradually over time (Table 3). The viable population decreased from 8.30×10^7 CFU g^{-1} at preparation to 6.75×10^7 CFU g^{-1} after 30 days and 5.10×10^7 CFU g^{-1} after 60 days. Similarly, $\log_{10}$ CFU values declined from 7.92 at day 0 to 7.83 and 7.71 after 30 and 60 days, respectively. Moisture content decreased from 28.6% at preparation to 26.9% after 30 days and 24.8% after 60 days. By contrast, pH and EC showed only minor changes during the storage period, ranging from 6.82 to 6.74 and from 0.91 to 0.95 dS m^{-1}, respectively.

Table 3: Shelf-life stability of the biochar-loaded *Purpureocillium lilacinum* formulation during storage.

Storage Time	Viable Count (CFU g^{-1} Formulation)	Log_{10} CFU g^{-1}	Moisture Content (%)	pH	EC (dS m^{-1})
Day 0	$(8.30 \pm 0.42) \times 10^{7a}$	7.92 ± 0.02^{a}	28.6 ± 0.9^{a}	6.82 ± 0.05^{a}	0.91 ± 0.03^{a}
Day 30	$(6.75 \pm 0.35) \times 10^{7b}$	7.83 ± 0.02^{b}	26.9 ± 0.8^{b}	6.78 ± 0.04^{a}	0.93 ± 0.04^{a}
Day 60	$(5.10 \pm 0.31) \times 10^{7c}$	7.71 ± 0.03^{c}	24.8 ± 0.7^{c}	6.74 ± 0.06^{a}	0.95 ± 0.03^{a}

Values are presented as means ± standard deviation (n = 3). Within each column, means followed by different lowercase letters are significantly different among storage times at $p \leq 0.05$ according to Tukey's HSD test. CFU: colony-forming units; EC: electrical conductivity.

3.3 Effects on Soil Organic Matter, Water-Holding Capacity, Bulk Density, and Available Macronutrients

Table 4 presents the effects of irrigation regime and bioformulation treatments on soil organic matter, water-holding capacity, and bulk density during the two growing seasons. In both seasons, deficit irrigation at 75% ETc recorded lower soil organic matter and water-holding capacity, and higher bulk density, than full irrigation at 100% ETc. Across irrigation regimes, biochar only, free *Purpureocillium lilacinum*, and biochar-loaded *Purpureocillium lilacinum* recorded higher soil organic matter and water-holding capacity and lower bulk density than the untreated control.

Table 4: Effects of irrigation regimes and bioformulation treatments on soil organic matter, water-holding capacity, and bulk density during two growing seasons. Data were analyzed using split-plot analysis of variance, and means were separated using Tukey's HSD test at $p \leq 0.05$.

Bioformulation Treatment	First Season			Second Season		
	100% ETc	75% ETc	Treatment Mean	100% ETc	75% ETc	Treatment Mean
Organic matter (%)						
Control	0.48 ± 0.02^{f}	0.43 ± 0.02^{g}	0.46^{D}	0.51 ± 0.02^{f}	0.46 ± 0.02^{g}	0.49^{D}
Biochar only	0.72 ± 0.03^{c}	0.66 ± 0.03^{d}	0.69^{B}	0.78 ± 0.03^{c}	0.72 ± 0.03^{d}	0.75^{B}
Free *Purpureocillium lilacinum*	0.61 ± 0.03^{d}	0.55 ± 0.02^{e}	0.58^{C}	0.66 ± 0.03^{d}	0.60 ± 0.03^{e}	0.63^{C}
Biochar-loaded *Purpureocillium lilacinum*	0.88 ± 0.04^{a}	0.79 ± 0.03^{b}	0.84^{A}	0.96 ± 0.04^{a}	0.87 ± 0.04^{b}	0.92^{A}
Irrigation mean	0.67^{I}	0.61^{II}		0.73^{I}	0.66^{II}	
Water-holding capacity (%)						
Control	18.6 ± 0.7^{f}	15.9 ± 0.6^{g}	17.25^{D}	19.2 ± 0.8^{f}	16.7 ± 0.7^{g}	17.95^{D}
Biochar only	25.8 ± 1.0^{c}	23.4 ± 0.9^{d}	24.60^{B}	27.1 ± 1.1^{c}	24.6 ± 1.0^{d}	25.85^{B}
Free *Purpureocillium lilacinum*	21.7 ± 0.9^{d}	19.8 ± 0.8^{e}	20.75^{C}	22.8 ± 0.9^{d}	20.9 ± 0.8^{e}	21.85^{C}
Biochar-loaded *Purpureocillium lilacinum*	31.4 ± 1.2^{a}	28.6 ± 1.1^{b}	30.00^{A}	33.0 ± 1.3^{a}	30.1 ± 1.2^{b}	31.55^{A}
Irrigation mean	24.38^{I}	21.93^{II}		25.53^{I}	23.08^{II}	
Bulk density (g cm^{-3})						
Control	1.58 ± 0.04^{a}	1.62 ± 0.04^{a}	1.60^{A}	1.56 ± 0.03^{a}	1.60 ± 0.04^{a}	1.58^{A}
Biochar only	1.45 ± 0.03^{c}	1.48 ± 0.03^{bc}	1.47^{C}	1.42 ± 0.03^{c}	1.45 ± 0.03^{bc}	1.44^{C}
Free *Purpureocillium lilacinum*	1.52 ± 0.03^{b}	1.55 ± 0.04^{ab}	1.54^{B}	1.49 ± 0.03^{b}	1.52 ± 0.03^{ab}	1.51^{B}
Biochar-loaded *Purpureocillium lilacinum*	1.36 ± 0.02^{e}	1.40 ± 0.03^{d}	1.38^{D}	1.32 ± 0.02^{e}	1.36 ± 0.02^{d}	1.34^{D}
Irrigation mean	1.48^{II}	1.51^{I}		1.45^{II}	1.48^{I}	

Values are presented as means ± standard deviation (n = 3). Within each parameter and growing season, different lowercase superscript letters indicate significant differences among irrigation regime × bioformulation treatment combinations; different uppercase superscript letters indicate significant differences among bioformulation-treatment means; and different superscript Roman numerals indicate significant differences between irrigation means, according to split-plot analysis of variance followed by Tukey's HSD test at $p \leq 0.05$. ETc: crop evapotranspiration.

The biochar-loaded *Purpureocillium lilacinum* formulation recorded the highest soil organic matter and water-holding capacity and the lowest bulk density under both irrigation regimes and in both seasons. Under 100% ETc, this treatment recorded organic matter values of 0.88 and 0.96% in the first and second seasons, respectively, compared with 0.48 and 0.51% in the untreated control. Water-holding capacity reached 31.4 and 33.0%, whereas bulk density decreased to 1.36 and 1.32 g cm^{-3} in the first and second

seasons, respectively. Under 75% ETc, the same treatment recorded organic matter values of 0.79 and 0.87%, water-holding capacity values of 28.6 and 30.1%, and bulk density values of 1.40 and 1.36 g cm^{-3} in the first and second seasons, respectively.

Table 5 presents the effects of irrigation regime and bioformulation treatments on soil available nitrogen, phosphorus, and potassium during the two growing seasons. In both seasons, deficit irrigation at 75% ETc recorded lower available N, P, and K than full irrigation at 100% ETc. Across irrigation regimes, biochar only, free *Purpureocillium lilacinum*, and biochar-loaded *Purpureocillium lilacinum* recorded higher available N, P, and K than the untreated control.

Table 5: Effects of irrigation regimes and bioformulation treatments on available nitrogen, phosphorus, and potassium in soil during two growing seasons. Data were analyzed using split-plot analysis of variance, and means were separated using Tukey's HSD test at $p \leq 0.05$.

Bioformulation Treatment	First Season			Second Season		
	100% ETc	75% ETc	Treatment Mean	100% ETc	75% ETc	Treatment Mean
Available Nitrogen (mg kg^{-1})						
Control	42.6 ± 1.7^{f}	37.8 ± 1.5^{g}	40.20^{D}	44.1 ± 1.8^{f}	39.4 ± 1.6^{g}	41.75^{D}
Biochar only	56.8 ± 2.1^{c}	50.2 ± 1.9^{d}	53.50^{B}	60.3 ± 2.3^{c}	53.7 ± 2.0^{d}	57.00^{B}
Free *Purpureocillium lilacinum*	52.4 ± 2.0^{d}	46.5 ± 1.8^{e}	49.45^{C}	55.6 ± 2.1^{d}	49.6 ± 1.9^{e}	52.60^{C}
Biochar-loaded *Purpureocillium lilacinum*	68.9 ± 2.5^{a}	61.7 ± 2.3^{b}	65.30^{A}	73.2 ± 2.7^{a}	66.4 ± 2.5^{b}	69.80^{A}
Irrigation mean	55.18^{I}	49.05^{II}		58.30^{I}	52.28^{II}	
Available phosphorus (mg kg^{-1})						
Control	5.2 ± 0.3^{f}	4.6 ± 0.2^{g}	4.90^{D}	5.5 ± 0.3^{f}	4.9 ± 0.3^{g}	5.20^{D}
Biochar only	7.4 ± 0.4^{c}	6.5 ± 0.3^{d}	6.95^{B}	7.9 ± 0.4^{c}	7.0 ± 0.3^{d}	7.45^{B}
Free *Purpureocillium lilacinum*	6.8 ± 0.3^{d}	5.9 ± 0.3^{e}	6.35^{C}	7.2 ± 0.4^{d}	6.3 ± 0.3^{e}	6.75^{C}
Biochar-loaded *Purpureocillium lilacinum*	9.1 ± 0.4^{a}	8.0 ± 0.4^{b}	8.55^{A}	9.8 ± 0.5^{a}	8.7 ± 0.4^{b}	9.25^{A}
Irrigation mean	7.13^{I}	6.25^{II}		7.60^{I}	6.73^{II}	
Available potassium (mg kg^{-1})						
Control	158 ± 6^{f}	145 ± 5^{g}	151.50^{D}	164 ± 6^{f}	151 ± 6^{g}	157.50^{D}
Biochar only	198 ± 7^{c}	181 ± 7^{d}	189.50^{B}	207 ± 8^{c}	190 ± 7^{d}	198.50^{B}
Free *Purpureocillium lilacinum*	186 ± 7^{d}	170 ± 6^{e}	178.00^{C}	194 ± 7^{d}	178 ± 6^{e}	186.00^{C}
Biochar-loaded *Purpureocillium lilacinum*	232 ± 8^{a}	214 ± 8^{b}	223.00^{A}	244 ± 9^{a}	226 ± 8^{b}	235.00^{A}
Irrigation mean	193.50^{I}	177.50^{II}		202.25^{I}	186.25^{II}	

Values are presented as means ± standard deviation (n = 3). Within each nutrient and growing season, different lowercase superscript letters indicate significant differences among irrigation regime × bioformulation treatment combinations; different uppercase superscript letters indicate significant differences among bioformulation-treatment means; and different superscript Roman numerals indicate significant differences between irrigation means, according to split-plot analysis of variance followed by Tukey's HSD test at $p \leq 0.05$. ETc: crop evapotranspiration.

The biochar-loaded *Purpureocillium lilacinum* formulation recorded the highest available N, P, and K values under both irrigation regimes and in both seasons. Under 100% ETc, available N reached 68.9 and 73.2 mg kg^{-1} in the first and second seasons, respectively, compared with 42.6 and 44.1 mg kg^{-1} in the untreated control. Available P reached 9.1 and 9.8 mg kg^{-1}, whereas available K reached 232 and 244 mg kg^{-1} in the first and second seasons, respectively. Under 75% ETc, the biochar-loaded *Purpureocillium lilacinum*

formulation recorded available N values of 61.7 and 66.4 mg kg^{-1}, available *p* values of 8.0 and 8.7 mg kg^{-1}, and available K values of 214 and 226 mg kg^{-1} in the first and second seasons, respectively.

The results presented in Fig. 2 show that irrigation regime and bioformulation treatments affected vegetative growth and biomass accumulation in spearmint during both growing seasons. Deficit irrigation at 75% ETc recorded lower plant height, branch number, fresh biomass, and dry biomass than full irrigation at 100% ETc. Across both irrigation regimes, biochar only, free *Purpureocillium lilacinum*, and biochar-loaded *Purpureocillium lilacinum* recorded higher values for all growth traits than the untreated control.

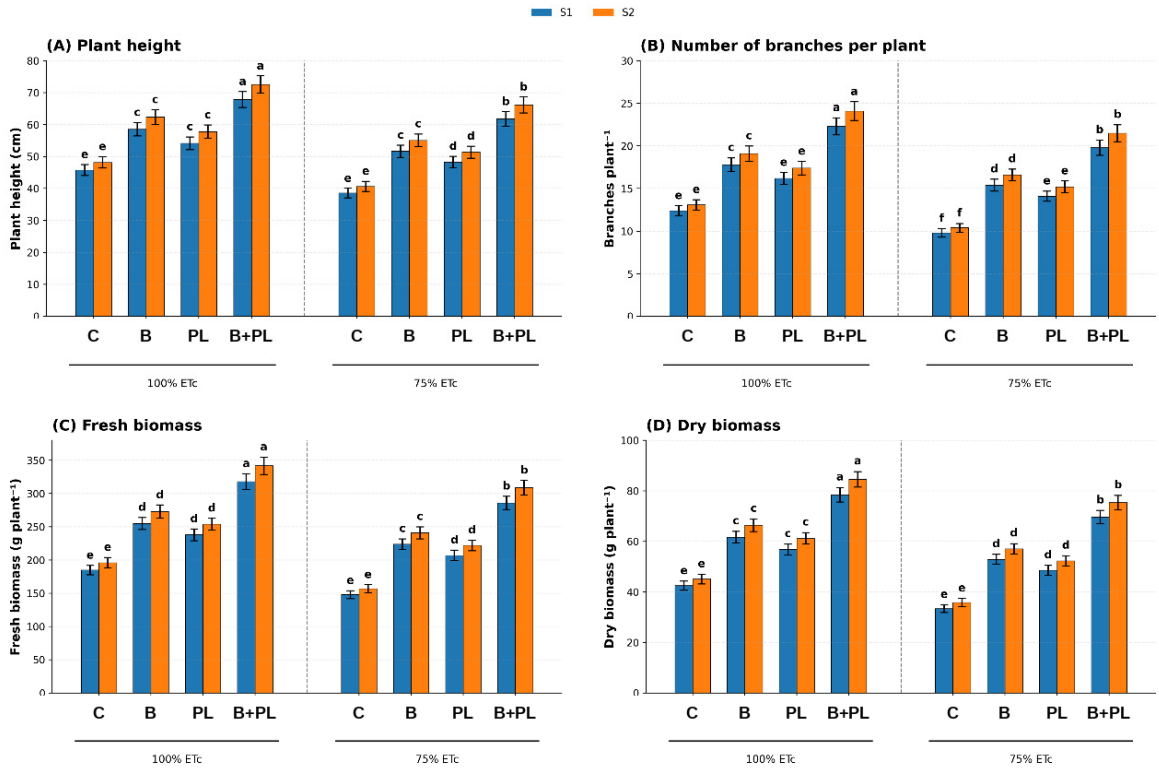

Figure 2: Effects of irrigation regimes and bioformulation treatments on plant height (**A**), number of branches per plant (**B**), fresh biomass (**C**), and dry biomass (**D**) of spearmint (*Mentha spicata* L.) During the first (S1) and second (S2) growing seasons. Bars represent means ± standard deviation (n = 3). Within each growing season, different lowercase letters indicate significant differences among irrigation regime × bioformulation treatment combinations according to split-plot analysis of variance followed by Tukey's HSD test at $p \leq 0.05$. ETc: crop evapotranspiration, C: control, B: biochar only, P: free *Purpureocillium lilacinum*, B+PL: biochar-loaded *Purpureocillium lilacinum*.

The biochar-loaded *Purpureocillium lilacinum* formulation recorded the highest plant height, number of branches, fresh biomass, and dry biomass under both irrigation regimes and in both seasons. Under 75% ETc, this treatment maintained higher growth and biomass values than the untreated control, biochar only, and free *Purpureocillium lilacinum* treatments. Growth and biomass values were generally higher in the second season than in the first season across most treatment combinations.

The results presented in Fig. 3 show that irrigation regime and bioformulation treatments affected SPAD chlorophyll value, relative water content (RWC), and proline content in spearmint during both growing seasons. Deficit irrigation at 75% ETc recorded lower SPAD and RWC values and higher proline content than full irrigation at 100% ETc. Across both irrigation regimes, biochar only, free *Purpureocillium*

lilacinum, and biochar-loaded *Purpureocillium lilacinum* recorded higher SPAD and RWC values than the untreated control.

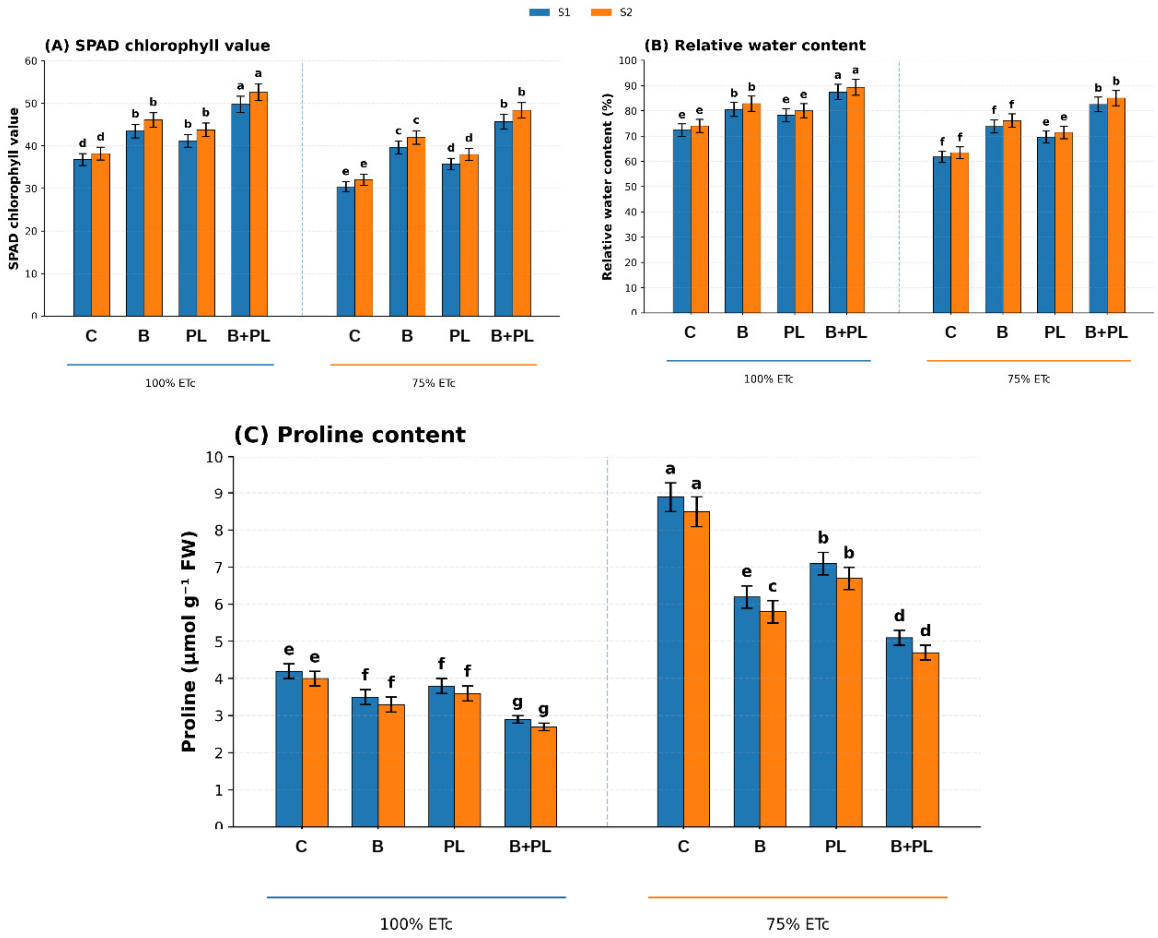

Figure 3: Effects of irrigation regimes and bioformulation treatments on SPAD chlorophyll value (**A**), relative water content (**B**), and proline content (**C**) of spearmint (*Mentha spicata* L.) During the first (S1) and second (S2) growing seasons. Bars represent means ± standard deviation (n = 3). Within each growing season, different lowercase letters indicate significant differences among irrigation regime × bioformulation treatment combinations according to split-plot analysis of variance followed by Tukey's HSD test at $p \leq 0.05$. ETc: crop evapotranspiration; FW: fresh weight. C: control, B: biochar only, P: free *Purpureocillium lilacinum*, B+PL: biochar-loaded *Purpureocillium lilacinum*.

The biochar-loaded *Purpureocillium lilacinum* formulation recorded the highest SPAD and RWC values under both irrigation regimes and in both seasons. Under 75% ETc, this treatment also recorded lower proline content than the untreated control and the individual biochar-only or free *Purpureocillium lilacinum* treatments. Physiological trait values were generally higher in the second season than in the first season across most treatment combinations, whereas proline accumulation was highest in the untreated control under 75% ETc.

The results presented in Fig. 4 show that irrigation regime and bioformulation treatments affected leaf macroelement concentrations of spearmint during both growing seasons. Plants grown under full irrigation at 100% ETc recorded higher concentrations of N, P, K, Ca, and Mg than those grown under deficit irrigation at 75% ETc. The untreated control recorded the lowest leaf macroelement concentrations under both irrigation regimes and in both seasons.

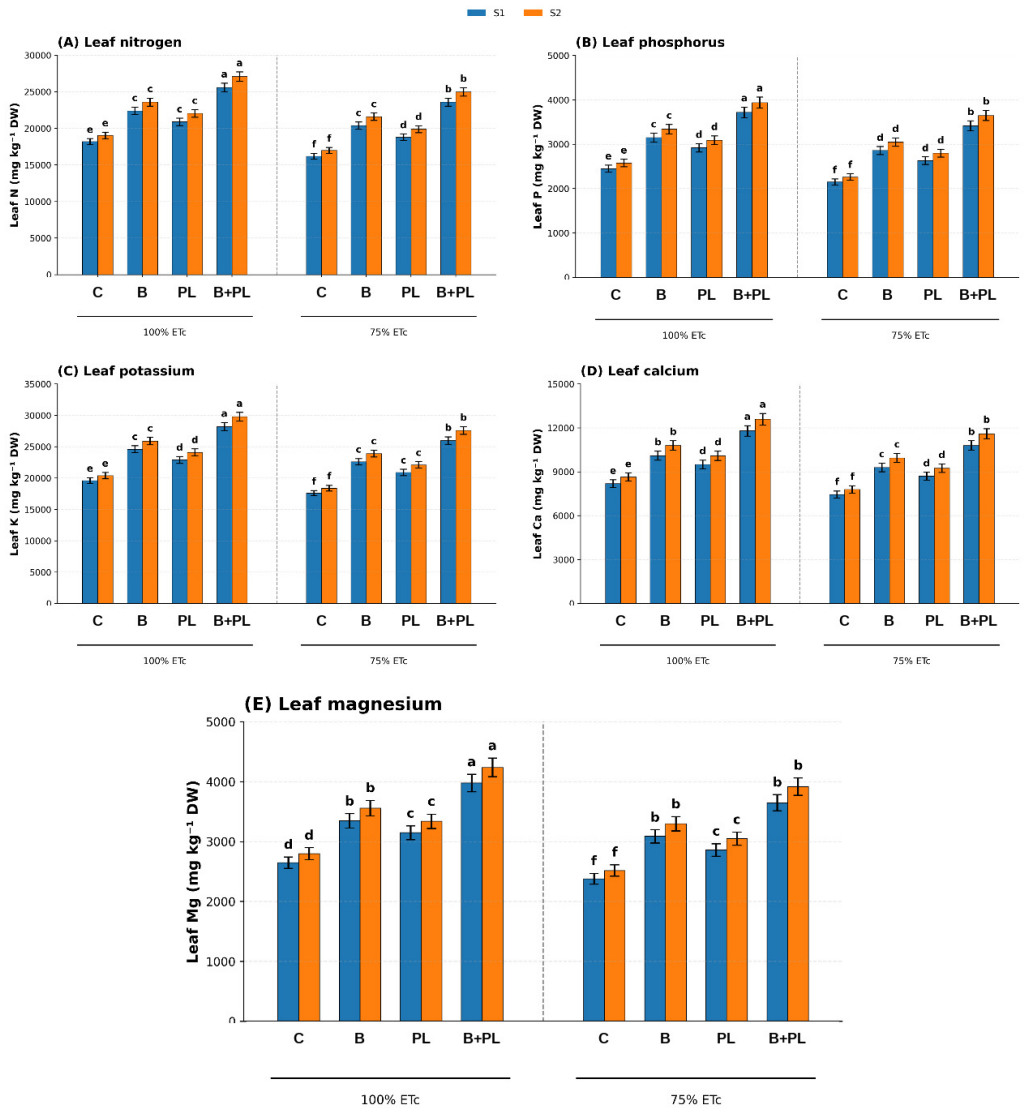

Figure 4: Effects of irrigation regimes and bioformulation treatments on leaf nitrogen (**A**), phosphorus (**B**), potassium (**C**), calcium (**D**), and magnesium (**E**) concentrations in spearmint (*Mentha spicata* L.) During the first (S1) and second (S2) growing seasons. Bars represent means ± standard deviation (n = 3). Within each growing season, different lowercase letters indicate significant differences among irrigation regime × bioformulation treatment combinations according to split-plot analysis of variance followed by Tukey's HSD test at $p \leq 0.05$. ETc: crop evapotranspiration; DW: dry weight. C: control, B: biochar only, P: free *Purpureocillium lilacinum*, B+PL: biochar-loaded *Purpureocillium lilacinum*.

Across irrigation regimes, biochar only, free *Purpureocillium lilacinum*, and biochar-loaded *Purpureocillium lilacinum* recorded higher leaf N, P, K, Ca, and Mg concentrations than the untreated control. The biochar-loaded *Purpureocillium lilacinum* formulation recorded the highest concentrations of all measured macroelements under both irrigation regimes and in both seasons. Under 75% ETc, this treatment maintained higher leaf macroelement concentrations than the untreated control, biochar only, and free *Purpureocillium lilacinum* treatments.

The results presented in Fig. 5 demonstrate that irrigation regimes and bioformulation treatments markedly influenced the percentage and yield of essential oil and soil dehydrogenase activity in spearmint

during both growing seasons. In general, deficit irrigation at 75% ETc reduced essential oil yield and soil dehydrogenase activity compared with full irrigation at 100% ETc. This response can be explained by the negative effects of water limitation on plant biomass accumulation, root activity, nutrient uptake, and soil microbial processes. Since essential oil yield depends not only on oil percentage but also on total fresh herb biomass, any reduction in vegetative growth under deficit irrigation is expected to reduce the final oil yield per plant.

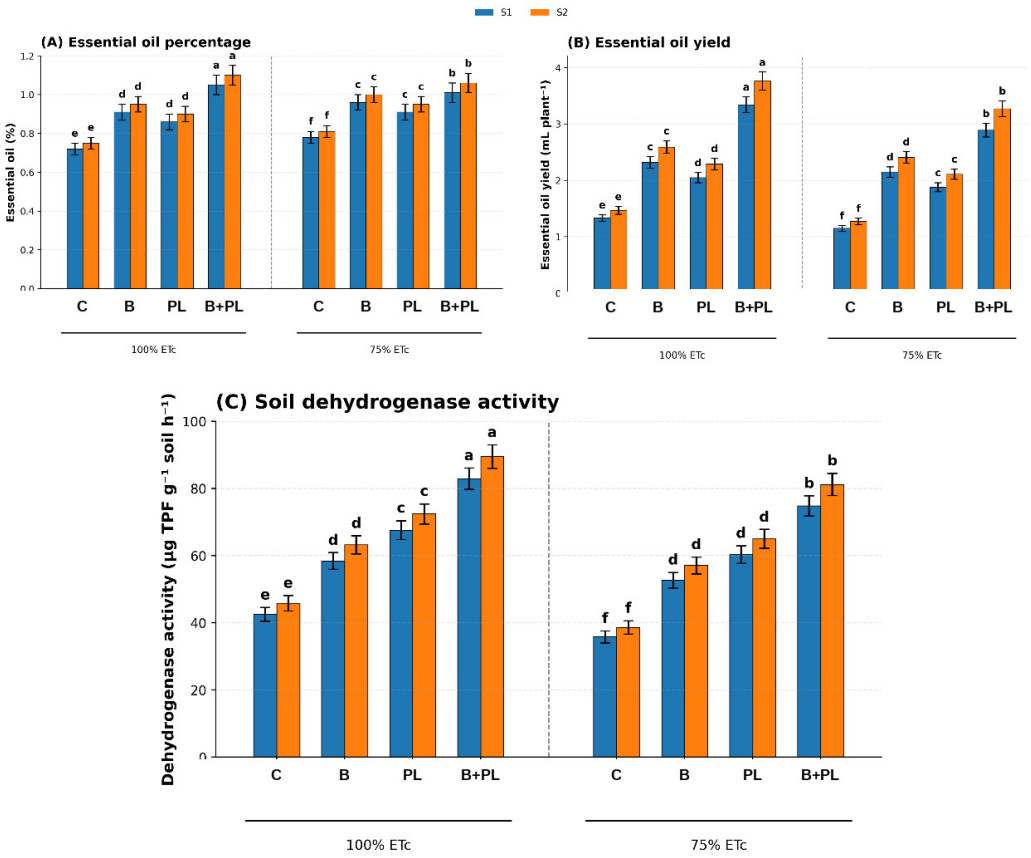

Figure 5: Effects of irrigation regimes and bioformulation treatments on essential oil percentage (**A**), essential oil yield (**B**), and soil dehydrogenase activity (**C**) of spearmint (*Mentha spicata* L.) During the first (S1) and second (S2) growing seasons. Bars represent means ± standard deviation (n = 3). Within each growing season, different lowercase letters indicate significant differences among irrigation regime × bioformulation treatment combinations according to split-plot analysis of variance followed by Tukey's HSD test at $p \leq 0.05$. ETc: crop evapotranspiration; TPF: triphenyl formazan. C: control, B: biochar only, P: free *Purpureocillium lilacinum*, B+PL: biochar-loaded *Purpureocillium lilacinum*.

Interestingly, the essential oil percentage showed a slightly different response from the essential oil yield. Under deficit irrigation, oil percentage was not always reduced and, in some cases, appeared slightly higher than the corresponding control under full irrigation. This may reflect a common response in aromatic plants, in which moderate water stress can stimulate secondary metabolism and increase the relative accumulation of certain volatile compounds. However, this increase in oil percentage does not necessarily mean higher productivity, because the reduction in fresh biomass under water stress can offset the increase in oil concentration. Therefore, essential oil yield is a more practical indicator of commercial

productivity than oil percentage alone. Across both irrigation regimes, biochar only, free *Purpureocillium lilacinum*, and biochar-loaded *P. lilacinum* increased essential oil percentage and yield compared with the untreated control. Biochar alone enhanced oil productivity, most likely by improving soil water-holding capacity, reducing nutrient loss, and maintaining better root-zone conditions. These improvements may have supported higher photosynthetic activity and biomass production, which are essential for oil biosynthesis and accumulation. In addition, improved potassium and calcium availability under biochar treatments may have contributed to better physiological activity and secondary metabolite formation.

Free *P. lilacinum* inoculation also increased essential oil traits compared with the control, indicating the fungus's beneficial role in improving plant performance. This effect may be related to enhanced rhizosphere activity, nutrient mobilization, and improved plant physiological status. However, the performance of free fungal inoculation was generally lower than that of the biochar-loaded formulation, particularly under deficit irrigation. This suggests that applying the fungus without a protective carrier may reduce its survival, persistence, and functional efficiency in sandy soil, especially when water availability is limited. The biochar-loaded *P. lilacinum* formulation recorded the highest essential oil percentage and essential oil yield under both irrigation regimes and in both seasons. This confirms the complementary interaction between biochar and the fungal inoculant. Biochar likely acted as a protective carrier for *P. lilacinum*, enhancing fungal survival and establishment in the rhizosphere while improving soil moisture retention and nutrient availability. Meanwhile, the fungal component may have contributed to better root activity, nutrient uptake, and physiological performance. Together, these effects supported greater biomass production and enhanced oil biosynthesis. Soil dehydrogenase activity followed a similar trend, with the highest values recorded under biochar-loaded *P. lilacinum*. Dehydrogenase activity is considered an indicator of overall microbial oxidative activity in soil; therefore, its increase suggests improved microbial functioning and biological activity in the rhizosphere. The reduction in dehydrogenase activity under deficit irrigation indicates that water limitation suppressed microbial processes, whereas the biochar-loaded formulation helped maintain higher biological activity even under 75% ETc. This is particularly important in sandy soils, where microbial activity is often limited by low organic matter and poor moisture retention. The slight improvement observed in the second season may be attributed to cumulative effects of biochar persistence, improved soil structure, enhanced water retention, and better fungal establishment over time. Overall, the results indicate that biochar-loaded *P. lilacinum* was the most effective treatment for improving both the biological quality of the soil and spearmint's essential oil productivity. The treatment was especially valuable under deficit irrigation, where it reduced the negative impact of water stress and maintained relatively high oil yield and microbial activity.

Figs. 6 and 7 show the GC–MS chromatograms of spearmint essential oil during the first season in both S1–C1 and S1–C2, clearly demonstrating that the irrigation regime and bioformulation treatments influenced the volatile profile of the extracted oil. The chromatographic pattern was characterized by the appearance of several major volatile constituents typically associated with spearmint essential oil, particularly carvone and limonene, which represented the most dominant peaks. Other detected compounds, including α-pinene, β-pinene, β-myrcene, 1,8-cineole, trans-carveol, cis-dihydrocarvone, β-caryophyllene, and germacrene D, appeared as secondary constituents contributing to the overall essential oil profile. In both S1–C1 and S1–C2, the untreated control under 75% ETc showed the lowest peak intensities, indicating that deficit irrigation without amendment reduced the accumulation of the main volatile constituents. This reduction may be attributed to the negative effect of water limitation on plant growth, nutrient uptake, photosynthetic activity, and secondary metabolite biosynthesis. The 100% ETc control showed relatively higher peak responses than the 75% ETc control, confirming the positive role of adequate irrigation in supporting

essential oil formation. The application of biochar-loaded *Purpureocillium lilacinum* markedly enhanced the GC–MS profile under both irrigation regimes. Under 100% ETc, this treatment produced the strongest chromatographic response, with clear increases in the major peaks, especially carvone and limonene. This indicates that the combined biochar–fungal formulation improved the physiological and nutritional status of spearmint, thereby enhancing the biosynthesis and accumulation of major monoterpenes. Biochar likely improved soil water retention, nutrient availability, and root-zone conditions, while *P. lilacinum* contributed to rhizosphere activity and nutrient mobilization. Under 75% ETc, biochar-loaded *P. lilacinum* maintained a stronger and more balanced chromatographic profile than the untreated deficit-irrigated control. This suggests that the formulation helped mitigate the adverse effects of water deficit on essential oil composition. Although deficit irrigation reduced the overall peak intensities compared with full irrigation, the biochar-loaded treatment preserved the main volatile constituents and supported higher relative abundance of key compounds. Overall, comparisons of S1–C1 and S1–C2 revealed that the qualitative composition of spearmint essential oil remained broadly stable, while the relative intensities of major peaks varied with irrigation level and bioformulation treatment. The biochar-loaded *P. lilacinum* formulation was the most effective treatment in enhancing the chromatographic profile of spearmint essential oil, particularly through increasing the abundance of carvone, limonene, and related monoterpenes. These results support the role of biochar-based fungal bioformulation as an effective strategy for improving essential oil quality and maintaining volatile compound production under deficit irrigation conditions.

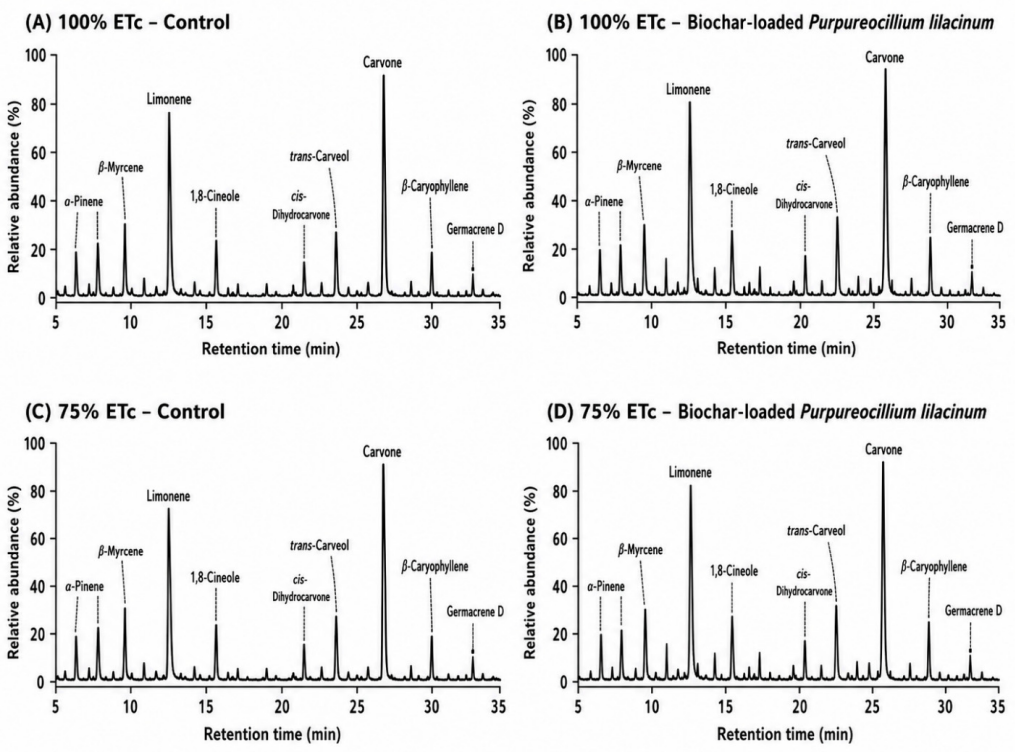

Figure 6: Representative GC–MS chromatograms of spearmint (*Mentha spicata* L.) Essential oil obtained during the first cut of the first growing season (S1–C1) under 100% ETc with the untreated control (**A**), 100% ETc with the biochar-loaded *Purpureocillium lilacinum* formulation (**B**), 75% ETc with the untreated control (**C**), and 75% ETc with the biochar-loaded *Purpureocillium lilacinum* formulation (**D**). The major identified volatile compounds and their retention times are indicated above the corresponding peaks. ETc: crop evapotranspiration; GC–MS: gas chromatography–mass spectrometry.

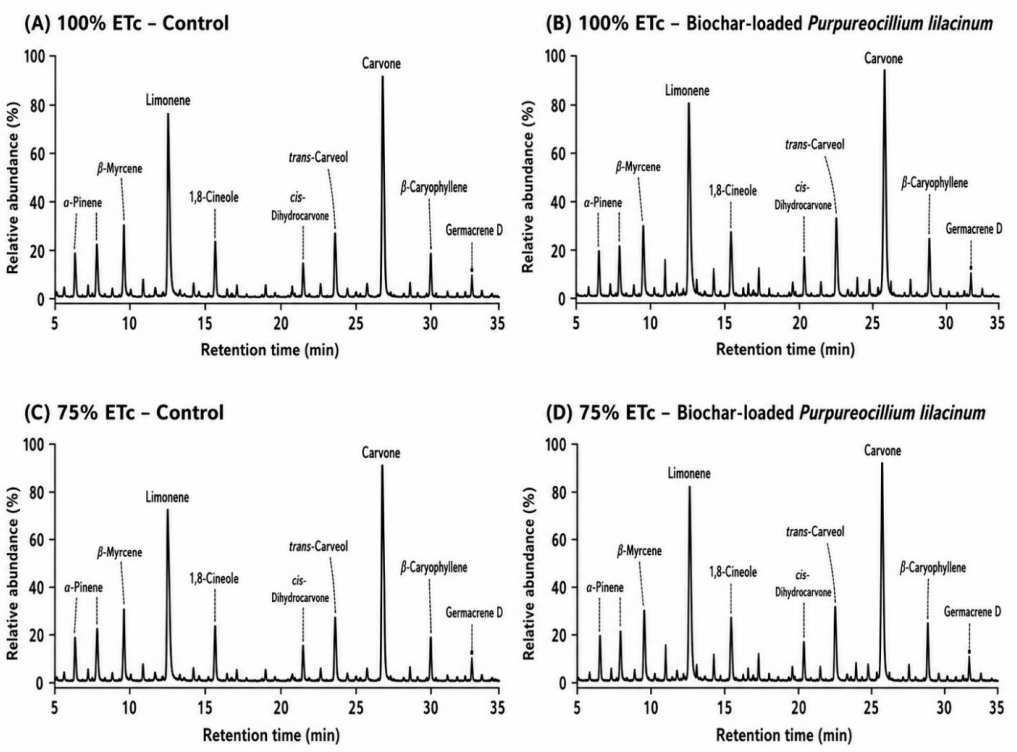

Figure 7: Representative GC–MS chromatograms of spearmint (*Mentha spicata* L.) Essential oil obtained during the second cut of the first growing season (S1–C2) under 100% ETc with the untreated control (**A**), 100% ETc with the biochar-loaded *Purpureocillium lilacinum* formulation (**B**), 75% ETc with the untreated control (**C**), and 75% ETc with the biochar-loaded *Purpureocillium lilacinum* formulation (**D**). The major identified volatile compounds and their retention times are indicated above the corresponding peaks. ETc: crop evapotranspiration; GC–MS: gas chromatography–mass spectrometry.

4 Discussion

The present study showed that the biochar-loaded *Purpureocillium lilacinum* formulation was more effective than either biochar alone or free fungal inoculation in improving soil quality, plant growth, physiological performance, mineral nutrition, essential oil productivity, and soil biological activity of spearmint under both full and deficit irrigation. This response suggests that the combined formulation acted through complementary soil-conditioning and biological mechanisms. Biochar improved the physical and chemical conditions of the sandy soil, whereas P. lilacinum likely enhanced rhizosphere biological activity and supported plant growth. This integrated effect was particularly relevant under 75% ETc, where a restricted water supply can reduce nutrient mobility, microbial activity, root functioning, and biomass accumulation. The result is also consistent with the broader interest in biologically based crop-support strategies under stressful field conditions [28–30].

From a physiological perspective, the improved performance under deficit irrigation appears to result from the combined contribution of both formulation components rather than from a single mechanism. Biochar probably played the primary structural role by improving soil water retention and nutrient conservation in sandy soil, whereas *Purpureocillium lilacinum* may have contributed biologically by supporting rhizosphere activity, root functioning, and nutrient acquisition. Because root architecture and phytohormone production were not directly measured, any direct hormonal stimulation by the fungus remains a hypothesis that requires further verification.

The improvements in soil organic matter, water-holding capacity, and bulk density under biochar-containing treatments are consistent with biochar's known capacity to modify the physical structure of coarse-textured soils. Because biochar particles generally possess high porosity, low bulk density, and large surface area, their incorporation can improve pore distribution, increase water retention, and reduce rapid drainage [46,47]. These effects are especially important in sandy soils, where low clay and organic matter contents limit water storage and nutrient retention. Previous reviews and experimental studies have shown that biochar can mitigate drought effects by improving soil structure, enhancing water retention, reducing bulk density, and increasing nutrient availability to plants [48–50]. In the present study, the biochar-loaded *P. lilacinum* treatment produced the greatest water-holding capacity and the lowest bulk density, indicating that the combined formulation improved the root-zone environment more efficiently than either component alone [51].

The increase in available N, P, and K under biochar-loaded *P. lilacinum* may be attributed to improved nutrient retention, reduced leaching, and enhanced biological nutrient cycling. Biochar can retain nutrient ions on its surface and within its porous matrix, whereas microbial inoculants may support nutrient mobilization through rhizosphere activity. The fungal component may also have contributed indirectly by improving root activity and nutrient acquisition. Previous studies on biological control fungi, including *Paecilomyces*/*Purpureocillium lilacinum*, have reported improvements in plant growth and rhizosphere performance, supporting the view that this fungus can function not only as a biocontrol agent but also as a plant-growth-supporting microorganism [52,53]. Therefore, loading *P. lilacinum* onto biochar may have enhanced fungal survival and persistence, allowing stronger effects on nutrient availability and plant performance.

The vegetative growth response of spearmint was consistent with the observed improvements in soil physical properties and nutrient availability. Deficit irrigation reduced plant height, branch number, fresh biomass, and dry biomass, reflecting the adverse effects of water limitation on cell expansion, photosynthetic activity, nutrient uptake, and assimilate accumulation. However, plants treated with biochar-loaded *P. lilacinum* maintained markedly higher growth under 75% ETc than untreated plants. This indicates that the formulation partially compensated for reduced irrigation by improving water and nutrient supply in the root zone. Similar findings have been reported in biochar-based studies under abiotic stress, where improved soil moisture conservation, nutrient retention, and photosynthetic performance were associated with better crop growth [46,48,54,55].

The physiological responses further confirmed the formulation's stress-mitigating role. Deficit irrigation reduced SPAD chlorophyll values and relative water content, while increasing proline accumulation. Reduced chlorophyll content and RWC indicate impaired leaf hydration and photosynthetic capacity, whereas increased proline reflects osmotic adjustment under water stress. The biochar-loaded *P. lilacinum* treatment increased SPAD and RWC while reducing proline accumulation compared with the deficit-irrigated control. This lower proline accumulation should not be interpreted as a weaker stress response; rather, it suggests that treated plants experienced lower stress intensity because of improved soil water retention, nutrient availability, and rhizosphere functioning. By maintaining better hydration and nutritional status, the formulation likely reduced the need for excessive osmolyte accumulation. This interpretation agrees with previous reports showing that biochar amendments can improve plant water relations and physiological performance under drought and combined abiotic stress conditions [48,54,55].

Leaf macroelement concentrations followed the same general response pattern. The highest concentrations of N, P, K, Ca, and Mg were recorded under biochar-loaded *P. lilacinum*, particularly under full irrigation, while the same treatment also maintained relatively high nutrient concentrations under deficit

irrigation. These findings indicate improved nutrient uptake and translocation to leaves. Improved K and Ca nutrition may be especially relevant under water stress, because K is involved in stomatal regulation and osmotic adjustment, whereas Ca contributes to membrane stability and stress signaling. Thus, improved mineral nutrition may partly explain the superior growth and physiological performance observed under the biochar-loaded fungal treatment.

The essential oil results highlight the distinction between essential oil percentage and essential oil yield. Deficit irrigation slightly increased oil percentage in some treatments, possibly due to stress-induced stimulation of secondary metabolism. However, essential oil yield per plant decreased under deficit irrigation because oil yield is strongly dependent on biomass production. Therefore, essential oil yield is a more relevant indicator of commercial productivity than oil percentage alone. The highest oil yield was obtained with biochar-loaded *P. lilacinum*, indicating that the formulation improved both biomass accumulation and oil productivity. Spearmint oil is commonly characterized by oxygenated monoterpenes and monoterpene hydrocarbons, particularly carvone and limonene [9,56–59]. Previous GC/MS studies have shown that carvone and limonene are the dominant constituents of Mentha spicata essential oil, although their proportions vary with genotype, environment, harvest stage, and extraction conditions [9,56–59]. The higher essential oil yield observed under the biochar-loaded treatment may therefore reflect improved physiological and nutritional conditions that supported biomass production and monoterpene biosynthesis.

Soil dehydrogenase activity was also maximized by the biochar-loaded *Purpureocillium lilacinum* formulation. Because dehydrogenase activity is widely used as an indicator of microbial oxidative activity, its increase suggests enhanced soil biological functioning. Biochar can provide protected microsites and carbon-rich surfaces that support microbial colonization, whereas fungal inoculation can directly contribute to rhizosphere biological activity. In addition, the porous structure and adsorption capacity of biochar may have temporarily retained nutrients and microbially derived metabolites within the root zone, thereby creating a more favorable microhabitat for microbial activity and enzyme functioning. However, nutrient sequestration and adsorption of fungal metabolites were not directly measured in the present study; therefore, this mechanism should be interpreted cautiously. The combination of biochar and *Purpureocillium lilacinum*, therefore, likely created a more favorable biological environment for nutrient cycling and plant performance. Previous studies have also reported positive effects of biochar on soil moisture retention, microbial respiration, and biological activity in sandy and sandy-loam soils [50,57,58,60,61]. In addition, recent work on fungal- and biologically based approaches supports the broader potential of beneficial fungi as components of integrated plant-support and plant-protection strategies, although their effects depend strongly on host species, stress type, and application context [62].

Although the storage stability of the biochar-loaded formulation was evaluated *in vitro* for 60 days, the persistence of Purpureocillium lilacinum in the rhizosphere at the end of the field seasons was not quantified as CFU g^{-1} soil. Therefore, the proposed improvement in fungal persistence under field conditions should be considered an inferred mechanism rather than a directly measured response.

The present findings are also consistent with recent reports on the use of biostimulant-based approaches to improve the performance of Mentha species under water-limited conditions. D'Agostino et al. [63] reported that gallic acid enhanced yield and essential oil quality of Mentha spicata under both deficit- and well-watered conditions, supporting the value of phytostimulants for improving aromatic crop performance under variable water supply. Similarly, Torabi Giglou et al. [64] showed that the combined application of Kitoplus® growth stimulant and chitosan-coated iron oxide nanoparticles improved physiological performance and essential oil biosynthesis in peppermint under drought stress. Compared with these foliar or nanoparticle-based approaches, the present study provides a soil-applied microbial bioformulation

strategy, in which biochar functions as both a soil conditioner and a carrier for *Purpureocillium lilacinum*. This comparison indicates that different biostimulant strategies can support growth, physiological stability, and essential oil productivity in Mentha species under deficit irrigation, although their mechanisms of action differ according to the applied material and delivery system.

Overall, the findings indicate that biochar-loaded *P. lilacinum* is a promising bioformulation for improving spearmint productivity, drought tolerance, soil biological activity, and essential oil performance in sandy soils under deficit irrigation. Its advantage over biochar alone and free fungal inoculation suggests that biochar-based delivery may improve the functional efficiency of beneficial fungal inoculants under water-limited conditions. Nevertheless, further studies should verify fungal persistence in the rhizosphere, characterize root colonization, and quantify changes in essential oil composition in greater detail to clarify the mechanisms underlying the observed responses.

5 Conclusion

The present study demonstrated that biochar-loaded *Purpureocillium lilacinum* is an effective bioformulation for improving spearmint productivity, drought tolerance, essential oil yield, and soil biological activity in sandy soil under deficit irrigation. Deficit irrigation at 75% ETc reduced soil organic matter, water-holding capacity, available macronutrients, vegetative growth, physiological performance, leaf mineral nutrition, essential oil yield, and dehydrogenase activity, while increasing proline accumulation. However, biochar, free *Purpureocillium lilacinum*, and especially biochar-loaded *Purpureocillium lilacinum*, mitigated these adverse effects. Among the tested treatments, the biochar-loaded *Purpureocillium lilacinum* formulation consistently produced the greatest improvements across both growing seasons. Under 75% ETc, this treatment increased soil water-holding capacity from 15.9 to 28.6% in the first season and from 16.7 to 30.1% in the second season, corresponding to an approximately 1.8-fold increase compared with the untreated control. It also increased available nitrogen from 37.8 to 61.7 mg kg^{-1} in the first season and from 39.4 to 66.4 mg kg^{-1} in the second season, corresponding to approximately a 1.6–1.7-fold increase over the untreated control. These improvements were accompanied by increases in plant biomass, chlorophyll status, relative water content, leaf macroelement concentrations, essential oil yield, and soil dehydrogenase activity, while bulk density and stress-associated proline accumulation decreased. Overall, the findings suggest that biochar can serve as both a soil amendment and an efficient carrier for *Purpureocillium lilacinum*, thereby improving the functional performance of this beneficial fungus under water-limited sandy-soil conditions. Further studies should evaluate long-term field performance, rhizosphere persistence, strain-level molecular confirmation, essential oil composition, and economic feasibility under commercial production systems.

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