

Research Article

Effective Microorganisms Improve Growth, Nutrients Uptake, Normalized Difference Vegetation Index, Photosystem II, and Essential Oil While Reducing Canopy Temperature in Water-Stressed Salvia sclarea Plants

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Clary sage (*Salvia sclarea* L.), a member of the Lamiaceae family of aromatic plants, is used as a flavoring in the food, fragrance, and cosmetic industries. Egypt's food and pharmaceutical industries require more natural resources, thus new crops have been introduced to meet the demand. In addition, various environmental conditions, such as water stress, influence aromatic plant growth and essential oil output. The microorganisms included in biofertilizers that have enzymes that solubilize minerals include plant growth-promoting rhizobacteria (PGPRs). Therefore, a field experiment was carried out to test how irrigation management, i.e., 100% of reference evapotranspiration (ETo) (full irrigation) or 50% ETo (water stress), can affect herbal yield, essential oil, and physiological aspects of clary sage, as well as how to improve clary sage plants utilizing biofertilizer in the field in Egypt's Mediterranean climate zone. The main processes through which PGPRs aid clary sage plants in coping with water stress include increased macro and micronutrient concentrations (P, N, K, Ca, Mg, Zn, B, Mn, Cu, and Fe), dehydrogenase activity, essential oil, and physiological and growth traits of photosystem II (Fv/Fm), chlorophyll content (SPAD-value), plant's health (normalized difference vegetation index, NDVI), shoot dry weight, and leaf area in clary plants. Application of different PGPRs reduced canopy temperature (CT), thus improving clary sage plants either under 100% ETo or 50% ETo. Consequently, the usage of PGPRs is appropriate for alleviating environmental stresses experienced by clary sage plants and has potential use in maintaining productivity in water stress and may thus be regarded as an important component of sustainable agricultural practices.

1. Introduction

Many useful medications are derived from medicinal plants, which are employed in many countries for various therapeutic processes [1]. These herbs are still used to treat common maladies by 60–80% of the world's population [2]. The Lamiaceae (Labiatae) plant family is one of the most significant dicotyledon families, with more than 240 genera. This family is highly valued for its aesthetic appeal and medicinal benefits since it has exterior glandular structures that produce volatile oil [3].

The Lamiaceae family includes the fragrant herb known as clary sage (*Salvia sclarea* L.). It serves as a flavoring additive in the food, fragrance, and cosmetics industries [4, 5]. Clary sage essential oil is utilized in the fragrance, soft drink, and liquor industries [6]. Setzer [7] found that the oil has medical use in aromatherapy for its anxiolytic, as well as digestive properties [8]. An herb that is both therapeutic and aromatic, clary sage is being researched as a potential heavy metal phytoremediation plant. It is a member of the class of zinc (Zn) accumulators [9]. Egypt's food and pharmaceutical industries require more natural resources; thus, new crops have been introduced to meet the demand.

Various environmental conditions, such as water stress, influence aromatic plant growth and essential oil output [10]. The essential oils extracted from aromatic plants change composition due to water stress. One of the most limiting constraints for plant growth and productivity in many agricultural areas across the world is drought stress or a lack of water [11-14]. Drought stress causes water potentials in the root zone to become sufficiently negative, which reduces water availability and prevents plant growth and development [11]. Stomatal closure, a decrease in the rate of photosynthesis, and plant growth are only a few examples of the physiological and metabolic changes brought on by drought stress [15]. Also, drought stress causes morphophysiological reactions in plants such as leaf area decrease, shoot growth, root growth enhancement, stomata closure, growth rate reduction, rapid antioxidant and soluble chemical accumulation, and enzyme activation [16]. It was reported that irrigation of Dracocephalum moldavica at 40% of field capacity (severe drought stress) reduced plant height, leaf area, internode length, shoot yield, and essential oil yield when compared to 100% or 60% of field capacity [17]. Burnett et al. [18] reported that Salvia splendens' stem and root lengths were decreased by water stress. In several therapeutic plants, for example, Achillea millefolium and Salvia officinalis, increased drought stress levels resulted in lower plant height and shoot weight [19].

One of the most crucial aspects of agronomic management is providing soil fertility through the application of balanced nutrition and the delivery of essential nutrients to generate the highest possible yield and quality of medicinal goods [20]. However, in recent decades, the use of chemical inputs in farming has resulted in a decline in soil fertility [21]. Nowadays, it is well accepted that using biofertilizers instead of chemical fertilizers can improve soil fertility and result in the production of sustainable agricultural products. The microorganisms that are included in biofertilizers that have enzymes that solubilize minerals include plant growthpromoting rhizobacteria (PGPRs) [22], allowing them to transform nonabsorbent minerals into absorbable nutrients in biological processes [23]. Plant growth is significantly influenced by a variety of growth-promoting bacteria, including Azospirillum, Azotobacter, Bacillus, and Pseudomonas [24]. Several studies have shown the major influence of plant growth-promoting bacteria on plant growth and development [25-28]. Nadjafi et al. [29] found that applying biofertilizers to Thymus vulgaris L. and Salvia officinalis L. maintained P and N availability while also boosting vegetative characteristics including plant height and fresh and dry weight. In addition, during the two growing seasons, Yasari et al. [30] found that in the first and second harvests, biofertilizers with Azospirillum and Azotobacter increased plant height and the fresh and dry weight of the plant's aerial parts. Azospirillum and Azotobacter were found to have a substantial impact on basil plant growth and plant height in

a different study [31]. Growth-promoting bacteria influence crop yield quantitatively and qualitatively by fixing nitrogen, enhancing K and P solubility, raising the biological availability of soil nutrients, reducing pathogenicity, and generating hormones that control plant growth [32]. Inoculation with PGPRs enhanced leaf chlorophyll content, leaf area, biomass, and other features [33]. Azotobacter and Pseudomonas treatment significantly raised chlorophyll and total chlorophyll concentrations in soybean compared to control [34]. One of the most important processes by which these bacteria achieve their goals is growth stimulation by PGPRs, which takes place through the synthesis of plant growth regulator hormones, e.g., indole acetic acid (IAA) [35], ethylene [36], cytokinin [37], and gibberellic acid [38]. Vinca roots were injected with various strains of growthstimulating bacteria, including B. megaterium, P. fluorescens, Azospirillum lipoferum, and Azotobacter chroococcum, to increase chlorophyll concentration and K, P, and N uptake [39], and they came to the conclusion that PGPRs could boost growth, nutrient absorption, and productivity. The number of leaves and branches on Coleus vettiveroides increased significantly as a result of the use of biofertilizers [40].

Remote sensing methods are frequently employed to assess the biophysical condition of vegetation, particularly water stress brought on by a lack of water in the soil. Crop water status has been closely tracked via remote sensing of canopy temperature. The basis of this method is the physical relationship between transpiration rate and crop canopy temperature. Due to a lack of water in the crop root zone, the canopy temperature rises as transpiration (the primary cooling process) declines, and vice versa [41]. Nondestructive infrared thermometry is a technique for measuring canopy temperature that can accurately evaluate crop water status [42]. Given the correlation between CT and grain production as well as stress tolerance indices like the stress tolerance index and mean productivity, CT may be an effective tool for selecting drought resistance in plant breeding and predicting water status [42]. The cooler CT that resulted from increased stomatal conductance and more transpiration was associated with an increase in wheat yield [43]. The GreenSeeker is a built-in optical sensor that analyses the normalized differential vegetation index (NDVI) using red and near-infrared (NIR) light produced by light-emitting diodes (LEDs) [44]. The normalized difference vegetation index (NDVI) is the most popular vegetation index that measures how green the vegetation is [45]. Based on correlations between NDVI and a drought index with a meteorological basis, it was discovered that NDVI is a good indication of vegetation response to drought [46].

Research on the effects of drought stress on crops is a major area of interest, especially concerning medicinal and aromatic plants in dry areas, to increase yield and essential oil production. To investigate how PGPRs affect Salvia's capacity for photosynthetic activity and uptake of nutrients, an experiment was conducted, taking into account the role of medicinal herbs and biological fertilizers in the creation of the product's quantitative and qualitative yield. Therefore, this study aimed to investigate how drought stress affected yield, leaf nutrient concentration, and essential oil content in clary sage plants. Moreover, we tested how deficit irrigation including water stress affects clary sage in terms of herbal yield, essential oil yield, and physiological features, as well as how to improve clary sage plants by utilizing biofertilizer in the field in Egypt's Mediterranean climate zone.

2. Materials and Methods

2.1. Experimental Procedures. At the Agricultural Experiments and Research Station, Faculty of Agriculture, Cairo University, Giza, Egypt (30°01'38"N, 31°11'35"E), a field experiment was conducted during the 2019-20 growing season. Figure 1 illustrates the average temperature and relative humidity at the experimental location for the 2019-20 season.

Clary sage (*Salvia sclarea* L.) seeds were introduced by the Jelitto Seed Company, Germany. Uniformly sized and colored seeds were cleaned in distilled water, sterilized in a 10% sodium hypochlorite solution for approximately 15 minutes, and then dried by air.

The seeds were planted on December 26th, 2019, in plastic pots $(23 \times 18 \text{ cm})$ with a soil mixture of 1:1 sand to clay. On February 26, 2020, the uniform seedlings were planted in the open field. Each experimental plot was 5.5 m by 4.5 m, with a 50 cm distance between lines of clary sage (planting density of 14500 plants ha⁻¹). A combination driller that permitted the simultaneous application of fertilizer and seeds was employed, and the trial field was plowed before planting. Physical and chemical analysis at the experimental site, which is characterized as loamy-clay soil, was done before sowing. Coarse sand (%) was 1.6, fine sand (%) 33.1, silt (%) 40.2, clay (%) 25.1, pH 7.3, organic matter 1.85%, available N 7.3 mg/kg, available P 6.4 mg/kg, and available K 160 mg/kg.

Before transplanting, during the soil preparation for culture, phosphorus (P) fertilizer at $32 \text{ kg}\cdot\text{P}\cdot\text{ha}^{-1}$ superphosphate (15.5% P₂O₅) was added as a single dose. Two identical doses of potassium sulfate (48% K₂O) were applied two separate times, the first before transplanting and the second one a month later, at a rate of 95 kg·K·ha⁻¹. According to the Ministry of Agriculture and Land Reclamation in Egypt, all other cultural practices were followed.

A split-plot design in a randomized complete block design with three replicates was used in the experiment. Irrigation was assigned as the main plot. Each main plot was split into five subplots (split-plots), four of which received an inoculation of Bacillus subtilis, Pseudomonas fluorescens, Cyanobacteria, or Trichoderma, while the fifth subplot acted as a control that was not given any treatment. Two irrigation regimes were used in the main plots, viz., (1) 100% of reference evapotranspiration (ETo) throughout the growing season as a well-watered treatment (IR100) and (2) 50% of ETo throughout the season as a water-stressed treatment (IR50). Consequently, the experiment included ten treatments that combined two factors (irrigation regimes and nitrogen-fixing bacteria biofertilizers), viz., T1, 100% ETo without nitrogen-fixing bacteria; T2, 100% ETo + B. subtilis; T3, 100% ETo + Ps. flu; T4, 100% ETo + Cyanobacteria; T5,

100% ETo + *Trichoderma*; T6, 50% ETo without nitrogenfixing bacteria; T7, 50% ETo + *B. subtilis*; T8, 50% ETo + *Ps. flu*; T9, 50% ETo + *Cyanobacteria*; and T10, 50% ETo + *Trichoderma*.

For bacterial inoculants, Bacillus subtilis and Pseudomonas fluorescens were kindly supplied by the Microbiology Department, Soil, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Ministry for Agriculture and Land Reclamation, Giza. Bacillus subtilis was grown in a nutrient broth medium [47] to reach 10⁸ cfu/ml, while *Pseudomonas fluorescens* was grown in Kings medium [48] to reach 10⁸ cfu/ml. Cyanobacteria inoculum is composed of a mixture of Anabaena fertilissima, Nostoc muscorum, and Spirulina platensis. Anabaena fertilissima and Nostoc muscorum strains were grown and propagated on BG11medium [49], while Spirulina platensis was grown on Zarrouk medium [50]. The strains were kindly supplied by the Department of Agricultural Microbiology, ARC, Giza, Egypt. The efficient nitrogen-fixing two Cyanobacteria strains were Anabaena fertilissima and Nostoc muscorum. Trichoderma harzianum was obtained from the Plant Pathology Research Institute, ARC, Giza, for fungal inoculants. Trichoderma harzianum was refreshed from spore suspensions in flasks containing 50 ml of yeast extract dextrose broth [51].

The difference between reference evapotranspiration demand (ETo) and rainfall served as the foundation for the two irrigation (IR) regimes. The following method used to calculate crop evapotranspiration (ETc) or irrigation levels under standard conditions:

$$ETc = ETo \times Kc,$$
 (1)

where ETc is crop evapotranspiration (mm/day); ETo is reference crop evapotranspiration (mm/day); and Kc is the crop coefficient.

Using the Penman–Monteith equation, ETo was determined [52]. Values of reference crop evapotranspiration (ETo) and irrigation (IR) water amounts in each irrigation treatment throughout the experimental period of 2019/20 are shown in Table 1. A gravimetric method was used to calculate the percentage of soil moisture content.

Data from a weather station installed in the facilities at the Agricultural Research Center, Giza, Egypt, were used to calculate ETo. The plants were completely irrigated without experiencing any water stress for the course of the experiment, as indicated by the 100% ETo. Only 50% of the irrigation volume used in the 100% ETo irrigation was administered to the treatment with 50% ETo. After the uniform seedlings were transplanted into the open field on February 26, 2020, these watering levels were used until the end of the growing season for the experimental period. The same day was used to irrigate every water treatment. Along with that, rainfall totals were recorded during the trial. The irrigation water's chemical properties include EC 0.41 dS/m, SAR 2.8%, and pH 7.35, and it was taken from an irrigation canal close to the experimental region. A flow meter connected to the irrigation pump was used to measure the irrigation water's volume.



FIGURE 1: The data of average temperature, and relative humidity at the experimental site in 2019/20 season.

TABLE 1: Values of reference crop evapotranspiration (ETo), and irrigation (IR) water amount in each water treatment throughout the experimental period of 2019/20.

Voor	Month	Dave	No. of	ETo (m	n ³ ∙ha ⁻¹)	IR (m	³ ·ha ^{−1})
I cai	Monui	Days	days	100%	50%	100%	50%
2020	February	26-28	3	88.5	44.2	98.4	49.2
2020	March	1 - 10	10	293.9	146.9	326.9	163.5
2020	March	11-20	10	348.0	174.0	387.2	193.6
2020	March	21-31	11	434.6	217.3	483.5	241.7
2020	April	1 - 10	10	419.4	209.7	503.3	251.6
2020	April	11-20	10	471.4	235.7	565.7	282.9
2020	April	21-30	10	535.7	267.9	642.9	321.4
2020	May	1 - 10	10	570.0	285.0	698.3	349.1
2020	May	11-20	10	684.2	342.1	838.1	419.0
2020	May	21-31	11	849.4	424.7	1040.5	520.3
2020	June	1 - 10	10	716.2	358.1	886.3	443.1
2020	June	11-20	10	662.2	331.1	819.4	409.7
2020	June	21-25	5	350.2	175.1	433.4	216.7
	Total		120	6423.6	3211.8	7723.8	3861.9

2.2. Measurements

2.2.1. Measurements of Plant Growth. To evaluate the growth parameters in terms of total leaf area per plant and shoot dry weight per plant, ten plants were randomly selected from each plot (replication) 120 days after transplanting. Using a leaf area meter, the total leaf area of each plant was determined (LiCOR 3100; Licor, Lincoln, NB, USA). After 48 hours of oven drying at 70°C, the estimated dry weight of each shoot was determined.

2.2.2. Normalized Difference Vegetation Index (NDVI). The normalized difference vegetation index (NDVI) was determined with a field-portable sensor (GreenSeeker® Handheld Crop Sensor; Trimble Navigation Limited, Westminster, CO, USA). At 120 days following transplanting, measurements were obtained per plot.

2.2.3. Measurements of Chlorophyll Fluorescence (Fv/Fm). A portable Handy PEA fluorometer was used to measure the fluorescence of leaf chlorophyll in five plants from each treatment (Hansatech Instruments Ltd., Kings Lynn, U.K.). At 120 days after transplantation, measurements were obtained.

2.2.4. Measurements of Chlorophyll Content (SPAD Value). A SPAD-502Plus meter was used to record SPAD values (Konica Minolta, Inc., Japan). The SPAD-502Plus meter was used to measure leaf absorbance in the red and near-infrared bands to assess chlorophyll content. At 120 days after transplantation, twelve independent SPAD measurements were taken per plot.

2.2.5. Measurements of Canopy Temperature (CT). Canopy temperature (CT) was measured 120 days after transplanting using a near-infrared temperature sensor, the Fluke Thermal Imager Ti32 (Fluke Corporation, Everett, WA, USA). Two measurements per plot were made in total.

2.2.6. Measurements of Concentrations of Macronutrients and Micro-nutrients. In an electric oven set at 70°C, the shoot samples were dried to a consistent weight before being ground. Concentrations of macronutrients, viz., potassium (K^+) , nitrogen (N), phosphorus (P), magnesium (Mg^{2+}) , calcium (Ca^{2+}), and micronutrients, viz., zinc (Zn^{2+}), boron (B), iron (Fe³⁺), manganese (Mn²⁺), and copper (Cu²⁺), were measured in the dried shoots at 120 days after transplanting old plants. Total N was measured via the micro-Kjeldahl technique. Using stannous chloride-ammonium molybdate as a colorimetric reagent, phosphorus was measured [50], after its sodium bicarbonate extraction [53]. Using a flame photometer, K⁺ was determined (ELE Flame Photometer, Leighton Buzzard, UK). Atomic absorption spectrophotometry was used to determine the concentrations of Mg²⁺, Ca²⁺, Fe³⁺, Zn²⁺, Mn²⁺, and Cu²⁺ [54]. N, P, K⁺, Ca²⁺, and Mg^{2+} were expressed as grams per kilogram, g·kg⁻¹, while Fe³⁺, Mn^{2+} , Zn^{2+} , and Cu^{2+} were expressed as mg·kg⁻¹.

2.2.7. Oil Yield Measurement. To prevent mechanical damage, plants were hand-picked 10 cm above the ground and then weighed right away to determine their fresh weight (FW). To determine the essential oil content expressed in mL·kg⁻¹ DW, four randomized subsamples of 125 g of vegetal material (a total of 500 g) per plot were distilled after being dried for two days in the shadows with air circulation. The distillation was done utilizing a vapor dragging system and a Clevenger-type distiller. The distillation time for each sample was set to 1.5 hours. The remaining material from each plant was packaged in clean paper bags, labeled, and oven-dried for 48 hours at 65°C to calculate the associated dry weight (DW) [55].

2.2.8. Total Phenol Measurement. At 120 days, plant samples were taken, and the total phenol concentration in plant leaves was measured [56]. In 10 ml of 80% methanol, 1 g of tissue was homogenized and stirred for 15 min at 70°C. Following the addition of 1 ml of methanolic extract, the mixture was incubated at 25°C for 3 minutes with 5 ml of distilled water, 2501 of Folin reagent (1N), 1 ml of saturated Na₂Co₃ solution, and 1 ml of distilled water. The produced blue color's absorbance was measured at 725 nm. Using a standard curve produced by a Folin reaction with a phenol solution and represented in g/GH/g DW dry tissue, the total phenol concentration was calculated.

2.2.9. Dehydrogenase (DA) Measurements. Plant samples were obtained and dehydrogenase activity (DA) was measured at 120 days. The activity of dehydrogenase was measured using triphenyl tetrazolium chloride (TTC) as an artificial electron receptor [57].

2.3. Statistical Analysis. After using the Levene test to check for homogeneity of error variance [58] and using the Shapiro–Wilk test for normality of distribution [59], a split-plot design was used to perform the analysis of variance on all the data [60]. Tukey's HSD (honestly significant difference) test was used to analyze statistically significant differences between means at $p \le 0.05$. GenStat 19th Edition was used for performing the statistical analysis and principal component analysis (VSN International Ltd., Hemel Hempstead, UK).

3. Results

3.1. Plant Nutrients under Water Stress. Irrigation at 50% of reference crop evapotranspiration (ETo) compared to full irrigation with 100% ETo resulted in a significant decrease in the concentration of N, P, K, Ca, Mg, Zn, Mn, Fe, and Cu, while there was a significant increase in B in clary plants (Table 2). Inoculation of clary plants with *Bacillus subtilis* or *Pseudomonas fluorescens* or *Cyanobacteria* or *Trichoderma* resulted in a significant increase in all mentioned nutrients compared to untreated control plants (Table 2). It is worth

mentioning that inoculation of clary plants with *Trichoderma* gave the highest concentration of N, P, K, Ca, Mg, Zn, Mn, Fe, and Cu in clary plants compared to all other treatments. Incorporation of *Trichoderma* resulted in an increase in N, P, K, Ca, Mg, Zn, Mn, Fe, and Cu by 22.0, 16.0, 29.1, 17.0, 12.6, 6.2, 6.9, 27.8, and 18.8%, respectively, compared to untreated control plants. All of the aforementioned traits showed substantial interaction effects, and it was noticed that application of all nitrogen-fixing bacteria (NFB) caused improved concentration of nutrients either under full irrigation or under water stress. Luckily, integration of only *Trichoderma* nitrogen-fixing bacteria under 50% ETo caused a substantial increase in N, K, B, Mn, and Fe, and this increase was 14.5, 11.6, 19.9, 3.1, and 5.1%, respectively (Table 2).

3.2. Oil, Total Phenol, and Dehydrogenase Activity under Water Stress. Irrigation at 50% ETo compared to full irrigation with 100% ETo significantly increased oil (%) and total phenol while significantly decreased dehydrogenase activity (Table 3). Inoculation of clary plants with Bacillus subtilis or Pseudomonas fluorescens or Cyanobacteria or Trichoderma resulted in a significant increase in all abovementioned traits compared to untreated control plants (Table 3). For example, inoculation of clary plants with Trichoderma compared to control resulted in an increase in oil, total phenol, and dehydrogenase activity by 24.6, 21.8, and 13.6%, respectively. All of the aforementioned traits showed substantial interaction effects, and it was noted that application of all NFB improved oil, total phenol, and dehydrogenase activity either under full irrigation or under water stress. Fortunately, the incorporation of only Trichoderma nitrogen-fixing bacteria under 50% ETo resulted in a significant increase in oil, total phenol, and dehydrogenase activity by 21.6, 28.7, and 29.9%, respectively (Table 3).

3.3. Physiological Response under Water Stress. Canopy temperature (CT) of clary sage plants at 120 days after transplanting under full irrigation with 100% ETo (A) and water-stressed at 50% of ETo (B) using full visible, full infrared, and blending levels between full infrared and full visible is shown in Figure 2. Irrigation at 50% ETo compared to full irrigation with 100% ETo significantly reduced Fv/Fm, chlorophyll content and NDVI, while significantly increasing the canopy temperature of clary plants inoculated with nitrogen-fixing bacteria (NFB) (Table 4; Figure 2). Inoculation of clary plants with Bacillus subtilis or Pseudomonas fluorescens or Cyanobacteria or Trichoderma grown under two irrigation treatments significantly increased Fv/Fm, chlorophyll content, and NDVI, while significantly decreasing canopy temperature (Table 4). For instance, inoculation of clary plants with Trichoderma compared to control resulted in an increase in Fv/Fm, chlorophyll content, and NDVI by 19.3, 20.5, and 18.1%, respectively, while decreasing canopy temperature by 8.9%. All of the aforementioned traits showed substantial interaction effects, and it was noticed that application of all NFB

Treatment	Ν	Р	K (g·kg ⁻¹)	Ca	Mg	В	Zn	$\frac{Mn}{(mg \cdot kg^{-1})}$	Fe	Cu
Irrigation (IR)										
IR100	16.3 ^{†a}	6.30^{a}	17.8 ^a	20.9^{a}	3.27 ^a	2.34^{b}	62.1 ^a	99.6 ^a	200^{a}	5.13 ^a
IR50	15.2 ^b	5.25 ^b	15.7 ^b	16.1 ^b	2.84 ^b	2.47 ^a	56.7 ^b	95.4 ^b	151 ^b	4.25 ^b
NFB										
Control	13.8 ^d	5.35 ^d	14.2 ^c	17.2 ^d	2.83 ^c	2.32 ^b	57.6 ^c	94.1 ^d	145 ^b	4.29 ^d
B. subtilis	15.9 ^c	5.64 ^c	16.4 ^b	17.9 ^d	2.98 ^b	2.47^{a}	58.2 ^c	96.5 ^c	180^{a}	4.70^{b}
Ps. flu	16.0 ^{bc}	5.86 ^b	17.3 ^{ab}	18.0 ^c	3.12 ^a	2.48^{a}	59.6 ^b	97.6 ^{bc}	184^{a}	4.55 ^c
Cyanobacteria	16.3 ^b	5.80 ^{bc}	17.5 ^{ab}	19.3 ^b	3.18 ^a	2.47^{a}	60.4^{ab}	98.9 ^b	185 ^a	4.80^{b}
Trichoderma	16.9 ^a	6.21 ^a	18.4^{a}	20.1 ^a	3.18 ^a	2.27 ^b	61.2 ^a	100.6 ^a	185 ^a	5.10 ^a
IR×NFB										
T1	14.3 ^d	5.76 ^{cd}	15.3 ^c	19.3 ^b	2.93 ^{cd}	2.02 ^d	60.9 ^{bc}	96.0 ^{de}	151 ^{bcd}	4.55 ^c
T2	16.4^{b}	5.93 ^c	17.1 ^{bc}	19.9 ^b	3.13 ^{bc}	2.63 ^a	61.7 ^{ab}	98.9 ^{bc}	216 ^a	5.15 ^b
Т3	16.6 ^b	6.46 ^b	18.4^{ab}	19.9 ^b	3.33 ^{ab}	2.42 ^{bc}	62.4 ^{ab}	100.1^{ab}	213 ^a	5.05 ^b
T4	16.9 ^{ab}	6.36 ^b	18.5 ^{ab}	22.2 ^a	3.43 ^a	2.52^{ab}	62.3 ^{ab}	100.8^{ab}	210 ^a	5.25 ^b
T5	17.3 ^a	6.97 ^a	19.7 ^a	23.2 ^a	3.54 ^a	2.12 ^d	63.0 ^a	102.2 ^a	212 ^a	5.66 ^a
Т6	13.3 ^e	4.95^{f}	13.2 ^d	15.2 ^d	2.73 ^d	2.63 ^a	54.3 ^f	92.1 ^f	139 ^d	4.04 ^e
T7	15.3 ^c	5.35 ^e	15.7 ^c	16.0 ^{cd}	2.83 ^d	2.32 ^c	54.6 ^f	94.1 ^{ef}	143 ^{cd}	4.24 ^{de}
Т8	15.4 ^c	5.25 ^{ef}	16.1 ^c	16.0 ^{cd}	$2.90^{\rm d}$	2.53 ^{ab}	56.7 ^e	95.0 ^{de}	154 ^{bc}	4.05 ^e
Т9	15.6 ^c	5.25 ^{ef}	16.5 ^{bc}	16.4 ^c	2.93 ^{cd}	2.43 ^{bc}	58.4 ^{de}	96.9 ^{cd}	160 ^b	4.34 ^{cd}
T10	16.4 ^c	5.45 ^{de}	17.1 ^{bc}	17.1 ^c	2.83 ^d	2.42 ^{bc}	59.3 ^{cd}	98.9 ^{bc}	159 ^b	4.55 ^c

TABLE 2: Effects of nitrogen-fixing bacteria (NFB) on concentrations of macronutrients (N, P, K, Ca, and Mg) and micro-nutrients (B, Zn, Mn, and Fe, Cu) of clary (*Salvia sclarea*) plants grown under two irrigation (IR) treatments.

[†]Mean values within the same column for each trait with the same lowercase letter are not significantly different according to Tukey's honestly significant difference (HSD) test at $p \le 0.05$. Measurements were done at 120 days after transplanting. IR100, 100% of reference crop evapotranspiration (ETo); IR50, 50% of reference crop evapotranspiration (ETo); T1, 100% ETo without nitrogen-fixing bacteria; T2, 100% ETo + B. subtilis; T3, 100% ETo + *Ps. flu*; T4, 100% ETo + *Cyanobacteria*; T5, 100% ETo + *Trichoderma*; T6, 50% ETo without nitrogen-fixing bacteria; T7, 50% ETo + *B. subtilis*; T8, 50% ETo + *Ps. flu*; T9, 50% ETo + *Cyanobacteria*; T10, 50% ETo + *Trichoderma*.

TABLE 3: Effects of nitrogen-fixing bacteria (NFB) on oil (%), total phenol (μ g/GH/g DW), and dehydrogenase activity (DA) (μ g Tpf/g dry soil/day) of clary (*Salvia sclarea*) plants grown under two irrigation (IR) treatments.

Treatment	Oil (%)	Total phenol (μ g/GH/g DW)	DA (µg tpf/g dry soil/day)
Irrigation (IR)			
IR100	1.01^{+b}	93.2 ^b	177 ^a
IR50	1.11 ^a	115.4 ^a	123 ^b
NFB			
Control	0.89 ^c	94.4 ^d	132 ^d
B. subtilis	1.02 ^b	99.1 ^{cd}	160 ^{ab}
Ps. flu	1.09 ^{ab}	104.1 ^{bc}	166 ^a
Cyanobacteria	1.21 ^a	108.9 ^b	143 ^c
Trichoderma	1.11^{ab}	114.9 ^a	149 ^{bc}
IR × NFB			
T1	0.85^{d}	87.0 ^g	163 ^c
T2	$0.92^{\rm cd}$	90.6 ^{fg}	189 ^{ab}
T3	1.01 ^{a-d}	93.2 ^{efg}	193 ^a
T4	$1.20^{\rm a}$	96.0 ^{efg}	171 ^{bc}
T5	$1.09^{ m abc}$	99.0 ^{def}	169 ^c
Т6	0.93 ^{bcd}	101.7 ^{de}	100^{f}
T7	1.12 ^{abc}	107.7 ^{cd}	130 ^{de}
Т8	1.16 ^a	115.0 ^{bc}	140^{d}
Т9	1.22 ^a	121.7 ^{ab}	114 ^{ef}
T10	1.13 ^{ab}	130.8 ^a	130 ^{de}

[†]Mean values within the same column for each trait with the same lowercase letter are not significantly different according to Tukey's honestly significant difference (HSD) test at $p \le 0.05$. Measurements were done at 120 days after transplanting. IR100, 100% of reference crop evapotranspiration (ETo); IR50, 50% of reference crop evapotranspiration (ETo); T1, 100% ETo without nitrogen-fixing bacteria; T2, 100% ETo + B. subtilis; T3, 100% ETo + Ps. flu; T4, 100% ETo + Cyanobacteria; T5, 100% ETo + Trichoderma; T6, 50% ETo without nitrogen-fixing bacteria; T7, 50% ETo + B. subtilis; T8, 50% ETo + Ps. flu; T9, 50% ETo + Cyanobacteria; T10, 50% ETo + Trichoderma.



FIGURE 2: Canopy temperature (CT) of clary sage plants at 120 days after transplanting under full irrigation with 100% of reference crop evapotranspiration (ETo) (A) and water stressed at 50% of ETo (B) using full visible, full infrared, and blending level between full infrared and full visible.

either under full irrigation or water stress improved all mentioned traits as it increased Fv/Fm, chlorophyll content, and NDVI, while decreasing canopy temperature. Luckily, 50% ETo combined with the application of *Trichoderma* nitrogen-fixing bacteria compared to full irrigation with 100% ETo significantly increased Fv/Fm, chlorophyll content, and NDVI by 5.2, 4.4, and 6.7%, respectively. Moreover, 50% ETo combined with the application of

TABLE 4: Effects of nitrogen-fixing bacteria (NFB) on the quantum efficiency of photosystem II (PSII) in dark- and light-adapted conditions (Fv/Fm), chlorophyll content (SPAD-value), plant's health (normalized difference vegetation index, NDVI), and canopy temperature (CT) expressed in Celsius degrees (°C), of clary (*Salvia sclarea*) plants grown under two irrigation (IR) treatments.

Treatment	Fv/Fm	SPAD	NDVI	СТ
Irrigation (IR)				
IR100	0.779^{+a}	32.9 ^a	0.64 ^a	37.9 ^b
IR50	0.703 ^b	28.7 ^b	0.58^{b}	49.5 ^a
NFB				
Control	0.661 ^e	27.5 ^e	0.55 ^e	46.1 ^a
B. subtilis	0.735 ^d	29.9 ^d	0.60^{d}	43.9 ^b
Ps. flu	0.750 ^c	31.1 ^c	0.61 ^c	43.3 ^{bc}
Cyanobacteria	0.769 ^b	32.4 ^b	0.63 ^b	42.9 ^{cd}
Trichoderma	0.789 ^a	33.2 ^a	0.65 ^a	42.3 ^d
IR×NFB				
T1	0.711 ^g	29.3 ^{fg}	0.58^{f}	40.2^{d}
T2	0.764 ^d	31.8 ^d	0.63 ^{cd}	38.1 ^e
T3	0.784 ^c	33.2 ^c	0.64 ^c	37.5 ^{ef}
T4	0.804^{b}	34.5 ^b	0.66^{b}	37.1 ^{ef}
T5	0.830 ^a	35.7 ^a	0.68^{a}	36.6 ^f
T6	0.611^{h}	25.8 ⁱ	0.52 ^g	52.0 ^a
T7	0.706 ^g	27.9 ^h	0.57^{f}	49.7 ^b
T8	0.715 ^g	28.9 ^{gh}	0.58^{f}	49.2 ^{bc}
Т9	0.734^{f}	30.4^{ef}	0.60^{e}	48.7 ^{bc}
T10	0.748^{e}	30.6 ^e	0.62^{d}	48.1c

[†]Mean values within the same column for each trait with the same lowercase letter are not significantly different according to Tukey's honestly significant difference (HSD) test at $p \le 0.05$. Measurements were done at 120 days after transplanting. IR100, 100% of reference crop evapotranspiration (ETo); IR50, 50% of reference crop evapotranspiration (ETo); T1, 100% ETo without nitrogen-fixing bacteria; T2, 100% ETo + B. subtilis; T3, 100% ETo + *Ps. flu*; T4, 100% ETo + *Cyanobacteria*; T5, 100% ETo + *B. subtilis*; T8, 50% ETo + *Ps. flu*; T9, 50% ETo + *Cyanobacteria*; T10, 50% ETo + *Trichoderma*.

Trichoderma decreased canopy temperature by 8.2% compared to 50% ETo without using NFB (Table 4).

3.4. Growth and Yield Response under Water Stress. Irrigation at 50% ETo compared to full irrigation with 100% ETo significantly reduced leaf area and shoot dry weight of clary plants inoculated with nitrogen-fixing bacteria (NFB) (Table 5). Inoculation of clary plants with Bacillus subtilis or Pseudomonas fluorescens or Cyanobacteria or Trichoderma grew under two irrigation treatments significantly increased leaf area and shoot dry weight (Table 5). For example, inoculation of clary plants with Trichoderma compared to control increased leaf area and shoot dry weight by 69.1 and 62.2%, respectively. All of the aforementioned traits showed substantial interaction effects, and it was noticed that application of all NFB either under full irrigation or water stress improved all mentioned traits as it increased leaf area and shoot dry weight. Luckily, 50% ETo combined with the application of Trichoderma nitrogen-fixing bacteria compared to full irrigation with 100% ETo significantly increased leaf area and shoot dry weight by 23.1 and 17.8%, respectively (Table 5).

TABLE 5: Effects of nitrogen-fixing bacteria (NFB) on leaf area per plant (LA) in dm^2 and shoot dry weight per plant (g) of clary (*Salvia sclarea*) plants grown under two irrigation (IR) treatments.

Treatment	LA (dm ²)	SDW (g)
Irrigation (IR)		
IR100	147 ^a	741 ^a
IR50	114 ^b	548 ^b
NFB		
Control	95 ^e	490 ^e
B. subtilis	117 ^d	570 ^d
Ps. flu	130 ^c	613 ^c
Cyanobacteria	149 ^b	756 ^b
Trichoderma	161 ^a	794 ^a
IR×NFB		
T1	119 ^e	566 ^c
T2	130 ^d	662 ^b
Т3	144 ^c	675 ^b
T4	165 ^b	881 ^a
T5	176 ^a	921 ^a
T6	72 ^g	413 ^e
T7	$104^{\rm f}$	478 ^d
Т8	116 ^e	550 ^c
Т9	134 ^d	632 ^b
T10	146 ^c	667 ^b

[†]Mean values within the same column for each trait with the same lowercase letter are not significantly different according to Tukey's honestly significant difference (HSD) test at $p \le 0.05$. Measurements were done at 120 days after transplanting. IR100, 100% of reference crop evapotranspiration (ETo); IR50, 50% of reference crop evapotranspiration (ETo); T1, 100% ETo without nitrogen-fixing bacteria; T2, 100% ETo + B. subtilis; T3, 100% ETo + *Ps. flu*; T4, 100% ETo + *Cyanobacteria*; T5, 100% ETo + *B. subtilis*; T8, 50% ETo + *Ps. flu*; T9, 50% ETo + *Cyanobacteria*; T10, 50% ETo + *Trichoderma*.

3.5. Correlation Matrix. Table 6 shows Pearson's correlation coefficients for all examined variables of clary sage plants cultivated under two water management treatments and five biofertilizer treatments. Yield (shoot dry weight) and the majority of the examined traits, including N, P, K, Ca, Mg, Zn, Mn, Fe, Cu, Fv/Fm, SPAD, NDVI, and LA, were found to be strongly correlated. In addition, there was a negative correlation between yield and B, TP, and CT, with the notable exception of CT, where this correlation was only highly significant ($p \le 0.01$). Although it was positive, the association between yield (shot dry weight) and oil or DA was not statistically significant.

3.6. Principal Component Analysis (PCA) of Clary Plants under Irrigation Treatments. Figure 3 illustrates a principal component analysis (PCA) biplot for the first two principal component (PC) scores, PC1 vs. PC2, concerning the categorization of 19 clary plant variables measured under irrigation treatments and biofertilizer treatments. Treatments are presented by green crosses (x) and traits by blue pluses (+). The figure identifies which treatments performed the best in each treatment and in each mega environment. The sectors divide the biplot into sectors by drawing lines from the origin. Mega environments encircle features that belong to the same sector with an

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$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	0.874* 0.987** 0.845** 0.879** -0.271 0.859** 0.962** 0.840** 0.876** 0.338 -0.159 0.738* 1 0.925** 0.970** 0.911** 0.946** -0.227 0.901** 0.980** 0.885** 0.934** 0.264 -0.255 0.734* 0.972** 1 0.886** 0.983** 0.870** 0.894** -0.213 0.882** 0.989** 0.884** -0.917** 0.302 -0.164 0.724* 0.989** 0.987** 1 -0.920** -0.749* -0.950** -0.883** 0.306 -0.939** -0.884** -0.917** 0.255 0.691* -0.927** 0.987** 1 0.821** 0.960** 0.816** 0.828** -0.308 0.846** 0.964** 0.750* 0.832** 0.409 -0.024 0.601 0.967** 0.957** 0.972** -0.702** 1 0.821** 0.960** 0.816** 0.828** -0.308 0.846** 0.964** 0.750* 0.832** 0.409 -0.024 0.601 0.967** 0.957** 0.972** -0.766** 0.960** 0.861** 0.923** 0.893** 0.229 0.841** 0.947** 0.805** 0.894** 0.348 -0.157 0.592 0.927** 0.968** 0.955** -0.766** 0.960** inficant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; B, boron; Zn, zinc; Mn in; Cu, copper; Oil, oil%; TP, total phenol; DA, dehydrogenase activity; Fv/Fm, chlorophyll fluorescence; SPAD, chlorophyll content; NDVI, normalized difference vegetation index; CT, canopi		0.815^{**}	0.690^{*}	0.799^{**}	0.763^{*}	-0.184	0.844^{**}	0.721^{*}	0.848^{**}	0.788^{**}	-0.265	-0.664^{*}	1					
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	0.925** 0.970** 0.911** 0.946** -0.227 0.901** 0.980** 0.895** 0.934** 0.264 -0.255 0.734* 0.972** 1 0.886** 0.983** 0.870** 0.894** -0.213 0.882** 0.989** 0.874** 0.914** 0.302 -0.164 0.724* 0.989** 0.987** 1 -0.920** -0.749* -0.950** -0.883** 0.306 -0.939** -0.822** -0.884** -0.917** 0.255 0.691* -0.927** -0.797** -0.849** -0.809** 1 0.821** 0.960** 0.816** 0.828** -0.308 0.846** 0.964** 0.750* 0.832** 0.409 -0.024 0.601 0.967** 0.957** 0.972** -0.722** 1 0.821** 0.960** 0.816** 0.828** -0.229 0.841** 0.947** 0.965** 0.894** 0.348 -0.157 0.592 0.957** 0.968** 0.955** -0.766** 0.960** nifcant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; B, boron; Zn, zinc; Mn in; Cu, copper; Oil, oil%; TP, total phenol; DA, dehydrogenase activity; Fv/Fm, chlorophyll huorescence; SPAD, chlorophyll content; NDVI, normalized difference vegetation index; CT, canopi		0.874^{**}	0.987^{**}	0.845^{**}	$0.879^{* *}$	-0.271	0.859^{**}	0.962^{**}	0.840^{**}	0.876^{**}	0.338	-0.159	0.738^{*}	1				
0.886* 0.983** 0.870** 0.894** -0.213 0.882** 0.989** 0.874** 0.914** 0.302 -0.164 0.724* 0.989** 0.987** 1 -0.920** -0.749* -0.950** -0.883** 0.306 -0.939** -0.822** -0.884** -0.917** 0.225 0.691* -0.927** -0.797** -0.849** -0.809** 1 0.821** 0.960** 0.816** 0.828** -0.308 0.846** 0.964** 0.750* 0.832** 0.409 -0.024 0.601 0.967** 0.957** 0.972** -0.722* 1 0.861** 0.923** 0.893** 0.299** -0.229 0.841** 0.947** 0.805** 0.894** 0.348 -0.157 0.592 0.927** 0.968** 0.955** -0.766** 0.960** 30.661** 0.967** 0.893** 0.809** -0.229 0.841** 0.947** 0.805** 0.894** 0.348 -0.157 0.592 0.927** 0.968** 0.955** -0.766** 0.960** 30.661** 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; B, boron; Zn, zinc; Mn	0.886* 0.983** 0.870** 0.894** -0.213 0.882** 0.989** 0.874** 0.914** 0.302 -0.164 0.724* 0.989** 0.987** 1 -0.920** -0.749* -0.950** -0.883** 0.306 -0.939** -0.822** -0.884** -0.917** 0.225 0.691* -0.927** -0.797** -0.849** -0.809** 1 0.821** 0.960** 0.816** 0.828** -0.308 0.846** 0.964** 0.750* 0.832** 0.409 -0.024 0.601 0.967** 0.957** 0.972** -0.756** 0.960** 0.861** 0.923** 0.893** 0.899** -0.229 0.841** 0.947** 0.805** 0.894** 0.348 -0.157 0.592 0.927** 0.968** 0.955** -0.766** 0.960** nificant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; B, boron; Zn, zinc; Mn in; Cu, copper; Oil, oil%; TP, total phenol; DA, dehydrogenase activity; FV/Fm, chlorophyll fluorescence; SPAD, chlorophyll content; NDVI, normalized difference vegetation index; CT, carop		0.925^{**}	0.970^{**}	0.911^{**}	0.946^{**}	-0.227	0.901^{**}	0.980^{**}	0.895^{**}	0.934^{**}	0.264	-0.255	0.734^{*}	0.972^{**}	1			
$ \begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	-0.920** -0.749* -0.950** -0.883** 0.306 -0.939** -0.822** -0.884** -0.917** 0.225 0.691* -0.927** -0.797** -0.849** -0.809** 1 0.821** 0.960** 0.816** 0.828** -0.308 0.846** 0.964** 0.750* 0.832** 0.409 -0.024 0.601 0.967** 0.957** 0.972** -0.722* 1 0.861** 0.923** 0.893** 0.899** -0.229 0.841** 0.947** 0.805** 0.894** 0.348 -0.157 0.592 0.927** 0.968** 0.955** -0.766** 0.960** prificant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; B, boron; Zn, zinc; Mn in; Cu, copper; Oil, oil%; TP, total phenol; DA, dehydrogenase activity; FV/Fm, chlorophyll fluorescence; SPAD, chlorophyll content; NDVI, normalized difference vegetation index; CT, carop		$0.886^{* *}$	0.983^{**}	0.870^{**}	0.894^{**}	-0.213	0.882^{**}	0.989^{**}	0.874^{**}	0.914^{**}	0.302	-0.164	0.724^{*}	0.989^{**}	0.987^{**}	1		
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0.861** 0.923** 0.893** 0.899** -0.229 0.841** 0.947** 0.805** 0.894** 0.348 -0.157 0.592 0.927** 0.968** 0.955** -0.766** 0.960** gifticant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; B, boron; Zn, zinc; Mr	0.861** 0.923** 0.893** 0.899** -0.229 0.841** 0.947** 0.805** 0.894** 0.348 -0.157 0.592 0.927** 0.968** 0.955** -0.766** 0.960** printicant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; B, boron; Zn, zinc; Mr on; Cu, copper; Oil, oil%; TP, total phenol; DA, dehydrogenase activity; Fv/Fm, chlorophyll fluorescence; SPAD, chlorophyll content; NDVI, normalized difference vegetation index; CT, canop		0.821^{**}	0.960^{**}	0.816^{**}	0.828^{**}	-0.308	0.846^{**}	0.964^{**}	0.750^{*}	0.832^{**}	0.409	-0.024	0.601	0.967^{**}	0.957^{**}	0.972^{**}	-0.722^{*}	1
printicant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; B, boron; Zn, zinc; Mn	inificant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; B, boron; Zn, zinc; Mn on; Cu, copper; Oil, oil%; TP, total phenol; DA, dehydrogenase activity; FV/Fm, chlorophyll fluorescence; SPAD, chlorophyll content; NDVI, normalized difference vegetation index; CT, canop		0.861^{**}	0.923^{**}	0.893^{**}	0.899^{**}	-0.229	0.841^{**}	0.947^{**}	0.805^{**}	0.894^{**}	0.348	-0.157	0.592	0.927^{**}	0.968^{**}	0.955^{**}	-0.766^{**}	0.960^{**}
	on; Cu, copper; Oil, oil%; TP, total phenol; DA, dehydrogenase activity; Fv/Fm, chlorophyll fluorescence; SPAD, chlorophyll content; NDVI, normalized difference vegetation index; CT, canopy	60	nificant at 1	the 0.05 lev	el (2-tailed)). **Correlat	ion is sign	nificant at t	he 0.01 leve	l (2-tailed).	. N, nitroge	n; P, phos	sphorus; K,	potassium	; Ca, calciur	n; Mg, mag	gnesium; B,	boron; Zn,	zinc; Mn



FIGURE 3: A principal component analysis (PCA) biplot for the first two principal component (PC) scores, PC1 vs. PC2, related to the classification of 19 variables of clary plants measured under irrigation treatments. Treatments are in green colour, and traits are in blue colour. T1, 100% ETo without nitrogen-fixing bacteria; T2, 100% ETo + B subtilis; T3, 100% ETo + Ps. flu; T4, 100% ETo + Cyanobacteria; T5, 100% ETo + Trichoderma; T6, 50% ETo without nitrogen-fixing bacteria; T7, 50% ETo + B. subtilis; T8, 50% ETo + Ps. flu; T9, 50% ETo + Cyanobacteria; T10, 50% ETo + Trichoderma. N, nitrogen; P phosphorus; K potassium; Ca, calcium; Mg, magnesium; B boron; Zn, zinc; Mn, manganese, Fe, iron; Cu, copper; Oil, oil%; TP, total phenol; DA, dehydrogenase activity; Fv/Fm, chlorophyll fluorescence; SPAD, chlorophyll content; NDVI, normalized difference vegetation index; CT, canopy temperature; Fv/Fm, chlorophyll fluorescence; SPAD, chlorophyll content; NDVI, normalized difference vegetation index; LA, leaf area per plant in dm²; SDW, shoot dry weight per plant in g.

ellipse. By joining the farthest treatments to create a polygon that encloses every treatment, a convex hull has been created. The projection quality from the initial N-dimensional table is shown by the eigenvalues. In this case, the first eigenvalue, 14.295, accounts for 75.2% of the overall variability. The first two factors allow us to represent 89.3% of the initial variability of the data. The first three factors allow us to represent 95.1% of the initial variability of the data. In this biplot, there are four megaenvironments. The first mega environment included one trait (CT), the second included B, and the third included two traits (TP and oil). The last mega environment included all other studied traits. Treatments that exist in the same sector as a specific attribute (environment) typically perform best for that trait (environment). For example, treatments T5, T4, T3, and T2 relocated in the same sector as a trait (environment) (included P, K, N, Mg, Ca, Mn, Fe, Zn, Cu, Fv/Fm, SPAD, NDVI, DA, LA, and SDW). Therefore, we expect these treatments to produce the best plant performance in this particular trait (environment).

4. Discussion

Leaf macronutrients and microconcentration in our experiment were decreased due to drought stress, except for B, which increased (Table 2). Our findings are consistent with those who found a considerable decrease in N, P, K, and Mg [55]. The decrease in leaf N can be attributed to a decrease in the activity of leaf nitrate reductase (NR), which is linked to photosynthetic activity and the availability of C skeletons, both of which are reduced when there is a water shortage [61]. A rise in leaf N content in other crops during drought conditions has been seen in numerous research studies, for example, Leymus chinensis [62] and Pinus halepensis [63]. This increase in leaf nitrogen concentration has been previously shown to be caused by nitrogen translocation to leaves to produce a special protein that helps plants fight off the impacts of water shortages [64]. Drought stress in our experiment led to a drop in the concentration of P in the leaves. Mild water stress caused a considerable drop in leaf P content in Ocimum gratissimum plants [65]. Our study's results are in line with previous research that found a drop in leaf K content when Thymus daenensis plants were grown in dry conditions with 20, 50, or 80% soil water depletion [66]. Other Lamiaceae species, such as Ocimum basilicum and Ocimum americanum, showed a drop in leaf K content when grown in a water-stressed environment [67]. Our results were in opposition to those who reported a rise in leaf K concentration in Salvia sclarea plants cultivated in drought conditions at 70% reference evapotranspiration [68]. The drop in leaf K concentration under drought stress conditions could be explained by the fact that water shortage impacts stomatal control, resulting in decreased photosynthetic capacity and the intake of K ions to maintain and regulate turgidity and stomatal control, as indicated [69].

In this study, the application of NSB and phosphatesolubilizing bacteria (PSB) had a positive impact on plant growth, chlorophyll content, and nutrient uptake. Recent studies have demonstrated that microorganisms that promote growth can increase plant growth by (1) lessening the amount of ethylene in developing plant roots, where ACC deaminase is produced [70]; (2) producing ethylene and other plant growth regulators [36], indole acetic acid [35], cytokinin [37], and gibberellic acid [71]; (3) nitrogen stabilization by living organisms [72]; (4) synthesis of siderophores, chitinases, antibiotics, fluorescent pigments, and cyanide that have antagonistic effects on phytopathogenic microorganisms [73]; and (5) solubilization of mineral phosphates and other nutrients [74].

PGPRs boost photosynthesis and plant growth by improving physiological features (increased chlorophyll concentration). According to a study, *Azotobacter* and *Pseudomonas* significantly increased the chlorophyll content and total chlorophyll content of soybean leaves when compared to the control (which did not get fertilizer), which is consistent with the results of our study [34]. In addition, the study in [75] found that PGPRs enhanced chlorophyll concentration, root, and stem growth. Moreover, branches numbers were enhanced as a result of the PGPR application [30]. It is worth mentioning that the changes in nutrient elements are related to photosynthetic parameters/pigments (for example, SPAD value and Fv/Fm). In our study, we reported a positive, strong association between either SPAD or Fv/Fm with all studied nutrients (K, N, P, Mg, Ca, Mn, Zn, Fe, and Cu) except for B (Table 6).

The basil plant grew taller after being fertilized with biofertilizers containing Azospirillum and Azotobacter [31], which is consistent with our findings. The usage of Azospirillum, Azotobacter, and Pseudomonas increased the plant height of Anethum graveolens L., which could be related to increased nutrient uptake efficiency, photosynthetic pigments, and leaf area [76]. Bacteria that promote plant development by increasing nutrient intake and chlorophyll content are known as growth-stimulating bacteria. PGPRs improve plant access to high-energy nutrients including N, P, and K, as well as their absorption and translocation into the leaves [77], resulting in increased chlorophyll synthesis and photosynthesis. Numerous studies have demonstrated how PGPRs can increase the levels of N, P, and K in plant tissues [78, 79], which is consistent with the findings of this study. Furthermore, three species of PGPRs (Azospirillum, Pseudomonas, and Azotobacter) caused the plant to absorb the most N, P, and K [80]. N and P play a crucial role in increasing the quantity and surface area of leaves by controlling the process of cell division and the production of chlorophyll as a growth factor [81]. As stated by Glick [82], PGPRs have a favorable and incremental influence on growth indicators, including leaf area and plant biomass. In comparison to the control treatment, the application of Azotobacter and Azospirillum produced an incremental in plant height and the number of Salvia leaves [83]. Furthermore, Azotobacter increases yield, dry weight, total N content, and plant growth [84].

The factors that can cause the canopy temperature to deviate from the air temperature include plant water availability, air temperature and humidity, solar radiation, wind speed, and the consequent canopy microclimate [85]. A potent tool for measuring and monitoring water stress is the use of infrared thermometry and canopy temperature. A quick, affordable, and effective method to assess water stress in medicinal plants is canopy temperature as measured by infrared thermometry. According to reports, in typical weather conditions, wheat genotypes with lower canopy temperatures have better physiological and metabolic properties than those with higher canopy temperatures [86]. The results from this study revealed a similar phenomenon in clary sage plants with different canopy temperatures (Table 4). Using Baccharis crispa Spreng, plants exposed to higher levels of water stress (25 and 50% ETo) displayed hotter leaves than control plants (100, 125, and 150 percent ETo) [87]. Some features of climatic extremes and variability that agricultural production is subjected to can be tempered by irrigation [88]. In our study, 100% ETo reduced CT, consequently improving crop performance. In addition, the application of different nitrogen-fixing bacteria (NFB) reduced CT, thus improving clary sage plants either under 100% ETo or even under water stress (50% ETo). In our study, a highly significant correlation between CT and yield

has been reported, i.e., r = -0.766 (p < 0.01). Because it was shown that treatments with less ETo resulted in leaves with higher temperatures, infrared thermometry can be used as an alternative for the quick and correct management of irrigation in medicinal plants (Table 4; Figure 2).

A multispectral, two-band, optical reflectance sensor called GreenSeeker measures the normalized difference between two infrared wavelengths, 656 nm and 774 nm. The following measurements can be made quickly with a GreenSeeker instrument: vegetative green biomass, canopy photosynthetic capacity, green area index, leaf area index, biomass, and nutrient concentration. On its LCD display panel, the sensor shows the observed value as an NDVI reading (which ranges from 0.00 to 0.99). The amount of light that is detected serves as a direct gauge of the crop's health; the greater the reading, the healthier the plant. In an ideal situation, an earlier study suggested utilizing NDVI as a selection criterion to increase grain yield in wheat [89]. The output of GreenSeeker can be utilized to forecast yield, biomass accumulation, growth rate, soil coverage, early vigor, and abiotic stress detection [90]. Linear regressions were found to be the most straightforward modification to fit the associations between NDVI and dry aboveground biomass for wheat genotypes [91]. We found a highly substantial correlation coefficient in our study, r = 0.955between NDVI and yield (shoot dry weight) (Table 4; Table 6). In linear regression between NDVI and yield, R-squared (R^2) was 0.912 (p < 0.01). In conclusion, the GreenSeekerTM portable active sensor spectroradiometer proved to be a helpful tool for NDVI prediction.

Water stress results in an over-reduced state that affects secondary chemical compounds and the concentration of essential oils [92]. In our study, plants of Salvia sclarea responded favorably to dryness by producing more essential oils (Table 3). According to earlier research, there are discrepancies between our findings and those of other publications because of variations in the severity and duration of the drought, the physiological status of the plant, plant species, and even cultivars of the same species, for example, considering the findings of other reports which are in direct conflict with our findings [68]. Similarly, some researchers have documented a decline in the content of essential oil in certain Lamiaceae species, such as Salvia officinalis [93]. Due to the stress brought on by the lack of water, a loss in leaf area under drought conditions may be associated with an increase in essential oil content and a higher density of oil glands.

Water-stressed plants lose turgor, which inhibits growth and cell development in the aerial region of the plant, particularly in the stems and leaves [94]. Dry weight decreased significantly as a result of drought stress, according to the findings of our investigation (Table 5). Our experiment's dry biomass reduction was consistent with other Lamiaceae species' results obtained by other researchers. Water stress, for example, has been linked to a decrease in dry weight in *Salvia* spp. *Salvia* spp. plants were demonstrated to have decreased dry weight when exposed to moderate drought stress (MD) and severe drought stress (SD) (50 and 40% of field water capacity, respectively) [55, 95, 96]. On the other hand, the study in [93] found that *Salvia officinalis* plants cultivated in a water-stressed environment had a lower dry weight.

5. Conclusions

Irrigation of clary sage plants at 50% of reference crop evapotranspiration (ETo) caused significant decreases in concentrations of K, N, P, Mg, Ca, Mn, Zn, Fe, and Cu, dehydrogenase activity, photosystem II (Fv/Fm), chlorophyll content (SPAD-Value), plant's health (NDVI), leaf area, and shoot dry weight, while causing significant increases in oil percent and total phenol. Meanwhile, once clary sage plants were inoculated with Bacillus subtilis, Pseudomonas fluorescens, Cyanobacteria, or Trichoderma, all of the above attributes, as well as oil and total phenol, increased significantly compared to untreated control plants. Especially inoculation of clary sage plants with Trichoderma gave the highest concentration of K, N, P, Mg, Ca, Mn, Zn, Fe, Cu, oil, total phenol, dehydrogenase activity, Fv/Fm, chlorophyll, NDVI, leaf area, and shoot dry weight, while inoculation decreased canopy temperature in clary plants compared to all other treatments. Clary sage plants performed better under 100% ETo or 50% ETo when various PGPRs were applied to minimize canopy temperature (CT). This might be a result of sufficient soil moisture, which is important in arid regions and depends on or is boosted by proper irrigation. According to the study's findings, PGPB inoculation has a positive impact on plant performance regardless of irrigation level, but it has a much better potential to increase plant productivity than other types of inoculation.

Data Availability

The authors state that all data generated or analyzed during this study are included in this article. The full data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Naayem M. Elgam contributed to investigation and original draft preparation. Adel B. Salama contributed to conceptualization, funding acquisition, investigation and original draft preparation. Heba Sh. Shehata also contributed to conceptualization, investigation, review, and editing. Magdi T. Abdelhamid contributed to conceptualization, investigation, funding acquisition, provision of resources, supervision, data curation, software provision, visualization, validation, review, and editing.

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