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Article

Optimizing Tuberose Production Using Mycorrhiza and Biostimulants to Enhance Drought Tolerance

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Abstract: Water deficit can significantly limit the sustainable production of plants, resulting in reduced growth, development, and flowering. Previous studies have shown that the use of biostimulants improves plant stability and promotes growth under low irrigation conditions. The study aims to evaluate the effects of biostimulators on the growth, development and flowering of tuberose (*Polianthes tuberosa* L.) under water deficit conditions. The experiment was conducted using a completely randomized design with three replications in pots and eight treatments: four irrigation regimes (100%, 80%, 60%, and 40%), and four biostimulant treatments (foliar application of seaweed extract at concentrations of 500, 1000, and 2000 ppm, humic acid at concentrations of 150, 300, and 600 ppm, inoculation of the bed with mycorrhiza, and a control treatment without biostimulators). The length, diameter, fresh and dry weight of the flower pedicel, the number of leaves per plant, the fresh and dry weight of the leaves, stem and root of each plant, as well as the content of photosynthetic pigments, phenol, proline, carbohydrates and flavonoids were measured. The results of this study showed that the growth and development of tuberoses were positively affected by different irrigation levels and biostimulants. The highest morphological characteristics were observed in plants irrigated at 100% field capacity. In contrast, the irrigation regime treatment of 40% of the field capacity combined with 600 mg/liter of humic acid had the most detrimental effect on plant growth indicators. Under stress conditions, the plant also exhibited increased levels of proline, carbohydrates, and flavonoids, which are commonly used as indicators of stress tolerance.

Keywords: water deficit; biostimulant; plant growth; tuberose; drought tolerance; irrigation regimes

1. Introduction

Tuberose (*Polianthes tuberose* L.) is a bulbous plant originating from Mexico [1]. It is highly valued for its fragrant, long-lasting flowers, which are widely used as a popular scent of cut flower industry [2]. Additionally, tuberose is widely cultivated for its essential oils, which are highly prized in the perfume industry, and is also a popular ornamental plant, blooming the spring and early autumn [1]. Its unique fragrance and versatility have made tuberose a sought-after crop for both commercial and horticultural purposes. Water deficit is a significant factor that affects the growth and postharvest quality of cut flowers [3,4]. Previous studies have demonstrated that water limitation or water stress can have a profound impact on the quantity and quality of cut flower yields, as well as their ornamental value [5,6]. Specifically, water deficit can negatively affect the flowering process in many plant species, including tuberose, by reducing the formation of new flowers and altering the overall flowering pattern [7,8].

To alleviate the negative impacts of water deficit on floricultural products, various compounds such as plant hormones, biostimulant s, and chemical nutrients have been used. Among these, biostimulants are a promising agroecological practice that involves the utilization of bioactive compounds from ethno-medicinal plants [9]. These compounds are gaining popularity for their environmentally friendly and cost-effective production methods. However, despite their potential benefits, there is a lack of information on the natural biostimulant s that can be extracted from plants

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[9]. Further research is needed to explore the efficacy of these biostimulant s in mitigating the negative impacts of water deficit on floricultural products.

Seaweed extract is a potent biostimulant that can be used to alleviate the effects of water stress on plant growth and productivity [10,11]. By increasing the concentration of chlorophyll in plant leaves and amylase enzyme in plant organs, seaweed extract enables the breakdown of unusable sugars in the plant, thereby promoting plant growth and development [12]. The application of seaweed extract has been shown to have numerous benefits for plant growth and productivity, including increased plant height, leaf number, and root growth, as well as accelerated flowering time, increased fruit formation, and delayed leaf senescence [11,13,14]. Moreover, seaweed extract has been found to improve plant resistance to environmental stresses such as drought, salinity, and temperature, making it a valuable tool for promoting plant growth and productivity in challenging environmental conditions. Another biofertilizer that has gained attention in recent years is the endophytic arbuscular mycorrhizal fungi (AMF), which forms a symbiotic relationship with plants to enhance their growth and stress tolerance [16]. AMF interacts with plants by increasing the central carbon metabolism flux among the TCA cycle, GABA shunt, and glyoxylic pathway [16]. This interaction not only promotes plant growth but also reduce in oxidative damage in stress conditions [17] and alters the diversity and structure of root and soil microbial communities in plant bed cultures [18,19]. Furthermore, in drought conditions, the symbiosis between plants and mycorrhiza can stimulate growth by generating phytohormones and providing essential nutrients [20]. This highlights the potential of AMF as a biofertilizer to improve plant growth and productivity, particularly under stressful environmental conditions.

We hypothesized that water deficit plays a significant role in affecting the quantity and quality of indices of bulbs, stems, and cut flowers of tuberosa, and that Biostimulants can mitigate the negative effects of water deficit on this species. However, there is a lack of information on the responses of tuberosa to biostimulators such as Alaria extract and mycorrhiza symbiosis under water deficit conditions. Therefore, this study was conducted to investigate the effects of biostimulators on morphological and physiological changes in tuberose plants grown under water deficit conditions. This study aims to provide a theoretical basis for understanding the responses of tuberose cut flowers to water deficit, which could be applied to optimize growth and maximize cut flowers yield.

2. Material and Methods

2.1. Experimental Design

To evaluate the effect of biostimulators on the quality and quantity of tuberose under water deficit, a pot experiment was conducted in the research greenhouse of Agricultural Faculty, University of Zabol. A mixture of garden soil, sand, and completely decayed manure (2:1:1) was used as bulb bed culture, as determined by our pilot works. The experiment was designed as a factorial arrangement with a completely randomized design, consisting of three with replications. The factors included four levels of water deficit: WD0 (100% Field Capacity; FC), WD1 (80% FC), WD2 (60% FC), and WD3 (40% FC), and four biostimulants treatments: (1) foliar application of seaweed extract at concentrations of 500, 1000, and 2000 ppm, (2) humic acid at concentrations of 150, 300, and 600 ppm, (3) inoculation of the bed with mycorrhiza, and (4) a control treatment without biostimulators.

2.2. Estimation of Morphological Indices

At the end of the experiment, various morphological and growth parameters were measured to evaluate the effects of biostimulators on tuberose plants under water deficit conditions. The measured parameters included: number of leaves and flowering stems per plant, leaf area, diameter of the flowering stem, length of the flowering spike, number of flower buds in the spike, and diameter of the spikes. Additionally, fresh and dry weights of the flowering stems and spikes were also measured to assess the overall biomass production of the plants.

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2.3. Estimation of Physiological Indices

2.3.1. Photosynthetic Pigments

Accurately weighted 0.5g of fresh plant leaf sample was taken and homogenized in a mortar with liquid nitrogen. Then, 20 ml of 80% acetone solvent was added to the homogenized sample mixture. The mixture was centrifuged at 6000 rpm for 10 minutes. The supernatant was separated and analyzed for Chlorophyll-a, Chlorophyll-b, and carotenoids content using a spectrophotometer. The spectral absorbance for Chlorophyll-a, Chlorophyll-b, and carotenoids was calculated using the following equation (Eq. 1) [21].

$$\text{Chlorophyll a} = 15.65\,A666 - 7.340\,A653$$
$$\text{Chlorophyll b} = 27.05\,A653 - 11.21\,A666$$
$$\text{Total Chlorophyll} = \text{Chl. a} + \text{Chl. b}$$
$$\text{Carotenoids} = 1000(A470) - 2.27(\text{mg chl. a}) - 81.4(\text{mg chl. b})/227$$

2.3.2. Total Phenol Content

Total phenol contents were estimated using the Folin Ciocalteu reagent method (McDonald et al., 2001) [22]. A dilute extract of the sample (0.5 ml of 1:10 g/ml) or gallic acid used as a standard was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml, 1M). The mixture was allowed to stand for 10 minutes, and the absorbance was measured by colorimetry at 765 nm. standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/l solutions of gallic acid in methanol: water (50:50, v/v). Total phenol contents were expressed in terms of gallic acid equivalent (mg/g of dry mass), with gallic acid used as the reference compound.

2.3.3. Carbohydrate

The phenol-sulphuric acid method was used to estimate the carbohydrates content of the samples [23]. A 0.1g powder of each accession was mixed with 5mL of 2.5 N-HCl and heated in a water bath for 3 hours. The mixture was then neutralized by adding sodium carbonate and the volume was increased to 100 mL. Glucose was used as the standard for carbohydrate estimation. Each experimental sample (standard and maize accessions) was mixed with 1 ml of 5% phenol solution and 5ml of 96% sulphuric acid solution and incubated at 30°C for 20 minutes. After incubation, the absorbance was measured at 490 nm and linear regression equation (Eq. 2) was used to estimate carbohydrate content of the selected maize accessions.

$$X = \frac{(Y - 0.2981)}{0.0237\ (\text{Eq. 2})}$$

2.3.4. Proline Estimation

Proline content in the explant tissues was extracted and analyzed according to the method of [24]. Samples (100 mg) were homogenized in aqueous sulfosalicylic acid (3% w/v; 10 ml) and stored at room temperature for 48 h and the mixture was then centrifuged at 10,000 rpm for 10 minutes. Subsequently, 2 ml of supernatant was reacted with an equal volume of each ninhydrin reagent (5 g ninhydrin in 120 ml of glacial acetic acid and 48 ml distilled water, and 32 ml H_3PO_4) and glacial acetic acid. The reaction mixture was then placed in a bain-marie at 70 °C for 1 h and cooled in an ice bath. The reaction mixture was vigorously mixed with 5 ml toluene by stirrer for 10-15 seconds, allowing the tubes to stand at least for 20 minutes in darkness at room temperature for toluene and aqueous phase separation. The toluene phase was carefully poured into test tubes, and the absorbance was measured at 520 nm using a spectrophotometer. The concentration of proline was calculated from a standard curve using the following equation: (µg proline in extract/115.5)/g sample = µmol/g FW.

2.4. Data Analysis

Data were analyzed by using SAS 9.1 software. A generalized liner model was employed for variance analysis of the data, and means were compared using the Duncan test at a significance level of $P < 0.05$

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3. Results

Variance analysis revealed that the morphological and physiological indices of tuberose were significantly influenced by the factors of different water conditions and biostimulants in the present study.

3.1. Morphological Indices

3.1.1. Growth Characteristics

The results of the variance analysis (Table 1) indicated that tuberose growth characteristics were significantly affected by water deficit treatments and Biostimulant treatments, as well as their interaction. Specifically, the results showed that water deficit treatments significantly impacted leaf number, leaf fresh and dry weight, and root fresh and dry weight. Biostimulant treatments also significantly affected all growth characteristics, except root dry weight. Furthermore, the interaction between water deficit and biostimulators had a significant impact on leaf fresh and dry weight, and root fresh weight.

Table 1. Results of variance analysis of experimental factors on tuberose growth characteristics. .

Treatment	df	Leaf number	Leaf fresh weight	Leaf dry weight	Root fresh weight	Root dry weight
Water deficit treatments	7	12.399**	573.417**	58.643**	3.169**	0.258**
Biostimulant treatments	3	58.558**	48.188**	3.881**	2.230**	0.097^{ns}
Water deficit + Biostimulant	21	3.470^{ns}	16.188**	2.896**	0.915**	0.093^{ns}
Error	64	2.76	1.184	0.143	0.34	0.06

3.1.2. Bulb and Bulb Leaf Characteristics

Both bulb fresh and dry weight, as well as bulb leaf number, were significantly influenced by the factors of water deficit and biostimulant treatments (Table 2). However, neither water deficit nor biostimulant treatments had a significant effect on bulb leaf fresh weight. The results also showed a significant effect of the interaction between the two factors on bulb fresh and dry weight, bulb leaf number, and bulb leaf fresh weight (Table 2). Moreover, the results revealed that biostimulators and their interaction with water deficit had no significant effects on bulb leaf dry weight, whereas water deficit treatments significantly impacted this characteristic of bulb leaf.

Table 2. Results of variance analysis of experimental factors on tuberose bulb and bulb leaf characteristics.

Treatment	df	Bulb fresh weight	Bulb dry weight	Bulb leaf fresh weight	Bulb leaf dry weight	Bulb leaf number
Water deficit treatments	7	167.276**	7.578**	0.223^{ns}	0.122**	10.527**
Biostimulant treatments	3	63.527**	3.278**	0.442^{ns}	0.007^{ns}	4.333**
Water deficit + Biostimulant	21	16.323**	0.891**	0.766**	0.026^{ns}	1.4**
Error	64	6.741	0.49	0.296	0.017	0.468

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3.1.3. Flower Characteristics

The results showed that both water deficit and biostimulators treatments, as well as their interaction, had a significant impact on the flower characteristics of tuberose, including flower stem length, spike diameter, flower stem fresh weight, and dry weight (Table 3).

Table 3. Results of variance analysis of experimental factors on tuberose flower characteristics.

Treatment	df	Flower stem length	flower diameter	spike fresh weight	spike dry weight
Water deficit treatments	7	1827.14**	232.09**	103.34**	1.40**
Biostimulant treatments	3	109.42**	15.42**	19.65**	0.53**
Water deficit + Biostimulant	21	8.42**	0.72**	0.50**	0.01**
Error	64	1.51	0.40	0.17	0.005

3.2. Physiological Indices

3.2.1. Photosynthesis Pigments

The results of the variance analysis showed that the photosynthesis pigments of tuberose were significantly affected by the separate effects of both water deficit and bio-stimulator factors (Table 4). However, the interaction between water deficit and biostimulators had no significant effect on the assessed photosynthesis pigments, including chlorophyll a, b, and total chlorophyll, as well as carotenoid.

Table 4. Results of variance analysis of experimental factors on tuberose photosynthesis pigments.

Treatment	df	Chl a	Chl b	Total Chl	Carotenoid
Water deficit treatments	7	0.721*	1.707**	4.574**	0.307^{ns}
Biostimulant treatments	3	2.730**	0.82**	4.223**	9.884**
Water deficit + Biostimulant	21	0.192^{ns}	0.225^{ns}	0.344^{ns}	0.302^{ns}
Error	64	0.205	0.193	0.365	0.313

3.2.2. Secondary Metabolites

The results showed that the secondary metabolites of tuberose were significantly affected by biostimulators treatments and their interaction with water deficit. However, the separate effects of water deficit were significant on carbohydrate and proline but had no significant effects on phenol and flavonoid (Table 5).

Table 5. Results of variance analysis of experimental factors on tuberose secondary metabolites.

Treatment	df	phenol	flavonoid	carbohydrate	proline
Water deficit treatments	7	0.055^{ns}	0.007^{ns}	0.233**	0.048**
Biostimulant treatments	3	0.209**	0.061**	0.145**	0.000**
Water deficit + Biostimulant	21	0.068**	0.015**	0.022**	0.000**
Error	64	0.045	0.008	0.000	0.000

3.3. Results of Mean Comparison

Mean compression were presented for indices that were significant under the interaction of both water deficit and Biostimulant factors. The results showed that bio stimulators treatments had different significant effects on alleviating the negative effects of water deficit on tuberose characteristics.

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3.3.1. Morphological Indices

The mean comparisons showed that biostimulators treatments could enhance the morphological indices of tuberose under water deficit conditions (Table 6). The highest amounts of leaf fresh and dry weight, root fresh weight, bulb fresh and dry weight, bulb leaf number, and bulb leaf fresh weight were obtained under 100% FC irrigation with the application of mycorrhiza and 2000 ppm of seaweed extract treatments. Moreover, under other water conditions, there were significant differences between the effects of treatments. For example, in the 80% FC irrigation, the application of 150 and 300 ppm of acid humic, respectively, resulted in higher fresh and dry weight of leaves. However, the mean comparisons for underground characteristics showed no significant difference between effects of some biostimulant treatments on root fresh weight, bulb fresh and dry weight, bulb leaf number, and bulb leaf fresh weight compared to the control condition under 100% and 80% FC irrigation (Table 6). In contrast, under 60% and 40% FC irrigation, the treatment with seaweed extract (2000 ppm) had a greater effect on increasing leaf fresh and dry weight compared to other biostimulator treatments. Additionally, under these irrigation regimes, the application of Biostimulant treatments could increase the fresh and dry weights of underground characteristics compared to the control treatment.

Table 6. Mean comparison of morphological indices of tuberose under different water deficit conditions and biostimulant treatments.

		Growth and bulb characteristics						
Water deficit	**Biostimulant treatments**	**Leaf fresh weight (g)**	**Leaf dry weight (g)**	**Root fresh weight (g)**	**Bulb fresh weight (g)**	**Bulb dry weight (g)**	**Bulb let number**	**Bulb let fresh weight (g)**
100% FC	SE 500 ppm	81.83^{bc}	9.12^{b}	3.50^{b-e}	19.38^{b-e}	3.83^{a-d}	5.00^{a-d}	3.86^{bc}
	SE 1000 ppm	80.55^{cd}	8.54^{bc}	3.48^{b-e}	22.03^{abc}	3.77^{a-d}	5.66^{ab}	3.87^{bc}
	SE 2000 ppm	85.24^{b}	11.03^{a}	5.60^{a}	29.23^{a}	5.00^{a}	6.33^{a}	4.63^{b}
	AH 150 ppm	75.89^{efg}	7.26^{def}	4.52^{ab}	21.06^{a-d}	4.32^{abc}	5.00^{a-d}	4.21^{bc}
	AH 300 ppm	78.36^{cde}	8.07^{bcd}	3.66^{b-e}	18.18^{b-e}	3.93^{abc}	5.33^{abc}	3.40^{c}
	AH 600 ppm	77.38d^{ef}	7.35^{cde}	4.00^{a-e}	16.30^{b-e}	3.28^{a-e}	4.00^{b-e}	3.80^{bc}
	M	88.81^{a}	11.79^{a}	3.65^{b-e}	14.35^{cde}	2.63^{b-e}	3.00^{d-e}	6.17^{a}
	C	74.06^{fgh}	6.43^{e-j}	5.15^{abc}	23.04^{ab}	4.49^{ab}	5.66^{ab}	4.06^{bc}
80% FC	SE 500 ppm	72.16^{hi}	6.08^{f-k}	3.33^{b-e}	16.16^{b-e}	3.14^{a-e}	4.00^{b-e}	4.04^{bc}
	SE 1000 ppm	72.20^{hi}	5.37^{h-k}	4.26^{abc}	18.34^{b-e}	3.61^{a-d}	4.33^{a-d}	3.95^{bc}
	SE 2000 ppm	72.43^{ghi}	5.57^{ijk}	4.60^{ab}	20.28^{bcd}	3.97^{abc}	4.66^{a-d}	4.34^{bc}
	AH 150 ppm	73.92^{fg}	6.82^{e-h}	4.05^{abc}	15.71^{b-e}	2.85^{a-e}	4.00^{b-e}	4.00^{bc}
	AH 300 ppm	72.96^{fgh}	7.09^{d-g}	3.58^{bcd}	14.49^{cde}	3.09^{a-e}	3.33^{cde}	4.40^{bc}
	AH 600 ppm	71.09^{ij}	5.08^{k}	3.14^{de}	15.76^{b-e}	3.22^{a-e}	3.66^{b-e}	4.35^{bc}
	M	77.01^{def}	6.77^{e-i}	3.66^{bcd}	14.53^{cde}	3.05^{a-e}	3.33^{cde}	4.39^{bc}
	C	72.01^{hi}	5.99^{g-k}	5.22^{ab}	21.01^{a-d}	4.20^{abc}	5.33^{abc}	3.94^{bc}
60% FC	SE 500 ppm	70.99^{ij}	5.12^{k}	3.24^{de}	15.79^{b-e}	3.00^{a-e}	3.66b-e	4.35^{bc}
	SE 1000 ppm	72.15^{hi}	5.85^{h-k}	4.26^{abc}	14.85^{b-e}	3.14^{a-e}	3.33cde	4.50^{b}
	SE 2000 ppm	72.44^{gh}	8.87^{bc}	3.35^{cde}	14.82^{b-e}	3.05^{a-e}	3.66^{b-e}	4.08^{bc}
	AH 150 ppm	69.27^{ijk}	5.06^{k}	3.45^{cd}	14.97^{b-e}	3.08^{a-e}	3.66^{b-e}	4.11^{bc}
	AH 300 ppm	70.42^{ij}	5.50^{jk}	2.88^{de}	13.14^{de}	2.17^{cde}	3.00^{de}	4.37^{bc}
	AH 600 ppm	70.55^{ij}	5.88^{h-k}	3.77^{bcd}	14.60^{cde}	2.86^{a-e}	3.66^{b-e}	4.03^{bc}
	M	71.20^{hij}	6.05^{g-k}	3.29^{cde}	13.97^{cde}	2.23^{b-e}	3.33^{cde}	3.95^{bc}

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40% FC	C	69.85^{ijk}	5.94^{g-k}	3.32^{cde}	14.95^{b-e}	2.85^{a-e}	3.33^{cde}	4.49^{b}
	SE 500 ppm	70.44^{ij}	5.06^{k}	3.83^{bc}	14.79^{b-e}	2.37^{b-e}	4.00^{b-e}	3.73^{c}
	SE 1000 ppm	70.42^{ij}	5.08^{k}	4.14^{abc}	20.04^{bcd}	4.23^{abc}	5.66^{ab}	3.52^{c}
	SE 2000 ppm	72.16^{hi}	5.52^{jk}	3.60^{cd}	15.12^{b-e}	2.73^{de}	3.66^{b-e}	4.09^{bc}
	AH 150 ppm	68.82^{lmn}	5.08^{k}	4.14^{abc}	18.35^{b-e}	3.74^{a-d}	4.66^{a-d}	3.92^{bc}
	AH 300 ppm	69.29^{k-n}	5.03^{k}	3.15^{de}	13.97^{cde}	2.23cde	4.33^{cde}	4.21^{b}
	AH 600 ppm	66.53^{n}	5.00^{k}	2.38^{e}	11.07^{e}	1.15^{e}	2.00^{e}	4.36^{bc}
	M	68.79^{lmn}	5.43^{jk}	2.90^{d-e}	11.56^{e}	1.63^{de}	3.00^{de}	3.90^{bc}
	C	66.94^{mn}	5.36^{jk}	3.71^{b-e}	15.93^{b-e}	3.19^{a-e}	4.00^{b-e}	2.24^{d}

FC; field capacity, SE; seaweed extract, AH; acid humic, M; mycorrhiza, C; control.

The indices of flower stem and spike were significantly influenced by water deficit and biostimulant s. The application of biostimulant treatments significantly alleviated the negative effects of water deficit on the flowering indices of tuberose. The mean comparisons showed that the highest flower stem length was obtained with the application of 2000 ppm of seaweed extract under 100% and 80% FC irrigation condition (Table 7). Moreover, all biostimulant treatments significantly increased flower diameter under the irrigation regimes compared to the control treatment. Additionally, in 60% and 80% FC irrigation, the highest fresh and dry weight of spike was obtained with the application of 2000 ppm of seaweed extract. Furthermore, under water deficit conditions in 60% and 40% FC irrigation, the io-stimulant treatments enhanced the flowering characteristics of tuberose compared to the control condition, with the treatment of 2000 ppm of seaweed extract having the more significant effects on reducing the effects of water deficit.

Table 7. Mean comparison of flowering indices under water deficit and biostimulators treatments.

Water deficit	**Biostimulant treatments**	**Flowering characteristics**			
		Flower stem length (cm)	**Flower diameter (mm)**	**Spike fresh weight (g)**	**Spike dry weight (g)**
100% FC	SE 500 ppm	61.68^{def}	11.55^{bcd}	12.20^{abc}	1.46^{c-f}
	SE 1000 ppm	64.96^{bcd}	17.08^{ab}	12.28^{abc}	1.77^{ab}
	SE 2000 ppm	68.84^{ab}	17.28^{ab}	13.17^{a}	1.98^{a}
	AH 150 ppm	62.31^{de}	16.01^{ab}	10.62^{def}	1.23^{f-j}
	AH 300 ppm	63.60^{cde}	16.78^{ab}	11.25^{cde}	1.42^{d-g}
	AH 600 ppm	63.70^{cde}	18.02^{a}	11.74^{bcd}	1.64^{bcd}
	M	64.50^{cd}	17.99^{a}	11.36^{b-e}	1.42^{d-g}
	C	57.98^{fgh}	13.82^{cde}	7.93^{ijk}	1.13^{hi}
80% FC	SE 500 ppm	62.41^{de}	16.84^{ab}	11.04^{cde}	1.34^{f-i}
	SE 1000 ppm	67.10^{abc}	17.10^{ab}	11.94^{bcd}	1.70^{bc}
	SE 2000 ppm	69.45^{a}	17.01^{ab}	12.68^{ab}	1.86^{ab}
	AH 150 ppm	59.94^{efg}	15.61^{bc}	10.06^{e-h}	1.23^{f-j}
	AH 300 ppm	60.00^{efg}	17.05^{ab}	10.71^{def}	1.45^{c-g}
	AH 600 ppm	57.95^{fgh}	17.37^{ab}	11.05^{cde}	1.60^{b-e}
	M	57.78^{fghi}	17.23^{ab}	10.19^{efg}	1.39^{d-h}
	C	56.07^{ghi}	13.37^{efg}	7.28^{jkl}	1.13^{h-l}
60% FC	SE 500 ppm	50.81^{kl}	12.34^{e-j}	8.99^{ghi}	1.31^{f-i}
	SE 1000 ppm	53.90^{ijk}	12.59^{e-i}	9.64^{fgh}	1.33^{f-i}
	SE 2000 ppm	55.08^{hij}	13.50^{def}	9.58^{fgh}	1.46^{c-f}

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	AH 150 ppm	50.47^{kl}	12.68^{e-i}	8.12^{ij}	1.15^{h-k}
	AH 300 ppm	51.15^{jkl}	13.32^{e-h}	8.72^{hi}	1.19^{g-k}
	AH 600 ppm	51.17^{jkl}	13.34^{efg}	8.80hi	1.37^{e-h}
	M	49.99^{klm}	13.82^{cde}	8.08^{ij}	1.22^{f-j}
	C	45.96^{no}	11.21^{i-m}	6.22^{lm}	0.88^{lmn}
	SE 500 ppm	43.09^{op}	9.20^{mn}	10.62^{def}	1.04^{j-m}
	SE 1000 ppm	46.35^{mno}	10.30^{j-n}	7.66^{ijk}	1.10^{i-m}
	SE 2000 ppm	49.33^{lmn}	11.26^{h-m}	7.93^{ijk}	1.23^{f-j}
40% FC	AH 150 ppm	43.07^{op}	9.33l^{mn}	6.04^{lm}	0.86^{mn}
	AH 300 ppm	44.95^{op}	9.71^{k-n}	6.71^{kl}	0.94^{k-n}
	AH 600 ppm	46.43^{mno}	11.31^{g-l}	6.89^{jkl}	1.01^{j-m}
	M	44.13^{op}	11.51^{f-k}	6.84^{jkl}	0.96^{klm}
	C	41.92^{p}	8.31^{n}	5.03^{m}	0.68^{n}

FC; field capacity, SE; seaweed extract, AH; acid humic, M; mycorrhiza, C; control.

3.3.2. Physiological Indices

The results showed a significant difference among the effects of water deficit levels on the concentrations of phenol, flavonoid, carbohydrate, and proline in leaves of tuberose. Moreover, the application of biostimulant s can alleviate the negative effects of water deficit on the secondary metabolites of tuberose. Consequently, biostimulant treatments led to an increase in the concentrations of phenol, flavonoid, carbohydrate, and proline content in the leaves of tuberose. The treatments of 1000 and 2000 ppm of seaweed extract and 300 and 600 ppm of acid humic had a more positive effect on increasing the total phenol compound (Table 8). A higher flavonoid concentration was obtained under 40% FC irrigation and with the treatments of 150 and 300 ppm of acid humic. The highest concentration of total carbohydrate was obtained through mycorrhiza symbiosis with the tuberose plant under 40% FC water deficit (Table 8). However, there was no significant effect among biostimulant treatments and the control condition under 40% FC irrigation on proline concentration (Table 8).

Table 8. Mean comparison of Physiological indices under water deficit and biostimulators treatments.

Water deficit	Biostimulant treatments	Phenol	Flavonoid	Carbohydrate	Proline
	SE 500 ppm	0.416^{efj}	0.047^{fg}	0.20^{op}	0.503^{m}
	SE 1000 ppm	0.525^{cde}	0.084^{ef}	0.19^{p}	0.504^{m}
	SE 2000 ppm	0.439^{efj}	0.034^{fg}	0.19^{p}	0.510^{m}
	AH 150 ppm	0.130^{hi}	0.16^{e}	0.31^{lm}	0.514^{klm}
100% FC	AH 300 ppm	0.359^{fj}	0.21^{d}	0.37^{ij}	0.512^{lm}
	AH 600 ppm	0.473^{def}	0.15^{e}	0.39^{ghi}	0.511^{m}
	M	0.489^{def}	0.20^{d}	0.40^{fgh}	0.506^{m}
	C	0.689^{bc}	0.24^{cd}	0.33^{kl}	0.523^{jkl}
	SE 500 ppm	0.847^{a}	0.092^{ef}	0.22^{o}	0.528^{j}
	SE 1000 ppm	0.710^{b}	0.11^{ef}	0.21^{op}	0.535^{ij}
80% FC	SE 2000 ppm	0.495^{de}	0.095^{ef}	0.21^{op}	0.546^{hi}
	AH 150 ppm	0.211^{jh}i	0.082^{ef}	0.33^{kl}	0.535^{ij}
	AH 300 ppm	0.454d^{ef}	0.10^{ef}	0.34^{jk}	0.53^{0j}

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	AH 600 ppm	0.643^{cd}	0.15^{e}	0.38^{hi}	0.534^{ij}
	M	0.629^{cd}	0.27^{c}	0.44^{e}	0.525^{ijk}
	C	0.684^{bc}	0.34^{ab}	0.43^{ef}	0.555^{gh}
	SE 500 ppm	0.593^{cd}	0.064^{efg}	0.31^{l}	0.573^{def}
	SE 1000 ppm	0.843^{a}	0.082^{ef}	0.28^{mn}	0.580^{cd}
	SE 2000 ppm	0.768^{ab}	0.095^{ef}	0.27^{n}	0.568^{def}
60% FC	AH 150 ppm	0.451^{def}	0.25^{c}	0.39^{ghi}	0.578^{cde}
	AH 300 ppm	0.465^{def}	0.26^{c}	0.40^{fgh}	0.570^{def}
	AH 600 ppm	0.665^{bcd}	0.23^{cd}	0.45^{e}	0.567^{efg}
	M	0.756^{ab}	0.24^{cd}	0.68^{b}	0.564^{fg}
	C	0.678^{bc}	0.22^{d}	0.50^{d}	0.587^{c}
	SE 500 ppm	0.614^{cd}	0.10^{ef}	0.70^{b}	0.612^{ab}
	SE 1000 ppm	0.916^{a}	0.06^{bcd}	0.41^{fg}	0.611^{ab}
	SE 2000 ppm	0.834^{a}	0.10^{ef}	0.30^{lm}	0.611^{ab}
	AH 150 ppm	0.567^{cde}	0.33^{ab}	0.39^{ghi}	0.617^{ab}
40% FC	AH 300 ppm	0.761^{ab}	0.36^{a}	0.41^{fg}	0.616^{ab}
	AH 600 ppm	0.776^{ab}	0.18^{de}	0.43^{ef}	0.613^{ab}
	M	0.721^{b}	0.16^{e}	0.84^{a}	0.605^{b}
	C	0.469^{def}	0.10^{ef}	0.62^{c}	0.622^{a}

FC; field capacity, SE; seaweed extract, AH; acid humic, M; mycorrhiza, C; control.

4. Discussion

Drought stress poses a significant challenge to agricultural plant production, adversely impacting the growth and development of various plant species [11]. The results of the current study showed that the morphological and physiological indices of Tuberose were significantly affected by water deficit (Tables 1-5). The morphological indices were significantly reduced under various levels of water stress (Tables 6 and 7). These findings are consistent with previous studies, which reported that water deficit stress limits stem growth and development of cut flowers [25–27]. Similarly, another study found that the drought stress led to a reduction in the leaf area, stem length, and fresh weight of Zinnia elegans [28]. The effects of water deficit on morphological indices of cut flower stems can have significant implications for their marketability, as reduced quality traits can result in lower profits for floriculture trading. However, the application of biostimulant s in the current study showed that these compounds can have positive effects on enhance growth parameters and mitigating the negative impacts of water deficit on Tuberose (Table 6 and 7).

In this respect, Zulfiqar et al. [29] reported that biostimulant s can serve as an effective tool in mitigating the adverse effects of abiotic stresses, including drought, salinity, heavy metals, and extreme temperatures, which limit plant production. These compounds act by altering gene expression, metabolism, and phytohormone production, as well as encouraging the accumulation of compatible solutes and antioxidants, ultimately leading to enhanced plant growth under stress conditions [29,30]. The findings of the current study demonstrate that the use of seaweed extract can significantly mitigate the adverse impacts of water deficit on the development of Tuberose. Previous studies have shown significant effects of seaweed extract on alleviating the negative effects of drought stress on Pot marigold (*Calendula officinalis* L.) [31], Chicory (*Cichorium intybus* L.) [32], Suggarcane (*Saccharum spp. hybrids*) [33,34]. Mycorrhiza and acid humic are others bio stimulants that have played significant roles in alleviating the adverse effects of water deficit on the growth of Tuberose. Mycorrhiza, with its strategic associations between plant roots and soil-borne symbiotic fungi, plays a pivotal role in enhancing the drought tolerance of existing plants [35]. Another study showed that incubating the African marigold (*Tagetes erecta*) plant with mycorrhizal fungus stimulated all growth indices compared to non-treated plants [36].

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In addition, the present study revealed substantial impacts of water deficit and bio-treatments on photosynthetic pigments independently (Table 4), while the interacted effect of these treatments was found to be not significant. Previous studies have reported negative effects of water deficit on the quality of ornamental plants, particularly leading to lower relative chlorophyll content in Brompton stock (*Matthiola incana* L.) [37], Carnation (*Dianthus caryophyllus* L.). The negative effects of water deficit on chlorophyll can be related to the role of chloroplasts as a source of reactive oxygen species (ROS) products, as the increased demand for respiration in mitochondria under water deficit results in the high production rates of this substance [38,39]. Moreover, the analysis of physiological indices in this study revealed a significant impact of water deficit levels on the accumulation of phenol and flavonoid compounds. These compounds exhibited increased levels under mild water deficit conditions, while their levels decreased under severe water deficit at 60 and 40% field capacity (Table 8). Furthermore, there was a notable increase in carbohydrate and proline levels with the severity of water deficit (Table 8). In this regard, a pervious study on rose flowers showed significant effects of drought on yield and quality of this ornamental plant. The physiological study of roses revealed a significant reduction in carbohydrate accumulation, while showing an increase in the levels of total phenols, total flavonoids, total anthocyanin, and volatile compounds compared to normal growth conditions [40].

5. Conclusions

This study demonstrated that the application of biostimulant s to alleviate water deficit irrigation can be effectively implemented in pot production of tuberose in greenhouse condition as an irrigation water conservation strategy while maintaining yields. The results of this research will be invaluable for advising flower producers on how to minimize agricultural water consumption. Furthermore, this study highlights the effectiveness of biostimulant s in sustaining tuberose growth and flowering responses across different water deficit irrigation strategies. The use of biostimulants is an effective method for managing horticultural products and making decisions regarding agricultural water management. On the other hand, it is essential to identify the differences in how biostimulant s alleviate water stress effects on different species in order to successfully apply deficit irrigation as a water preservation plan. Recognizing the right biostimulants enables practitioners to efficiently and effectively implement deficit irrigation. According to these findings, the use of seaweed extract (AE) under water deficit conditions at 60% field capacity ensure the conservation of water resources and minimize any adverse effects of water deficit on species growth and productivity.

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