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Research Article

Willow Bark Extracts Ameliorate the Vegetative and Flowering Attributes of Tuberose CV. Single AT Calcareous Soil of High pH

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Abstract

The study's goals are to assess different weeping willow bark extract concentrations, ideal application times, and how these factors combine to improve tuberose growth and flower quality. The growth and quality production of plants is negatively affected by several biotic and abiotic stresses including high potential of hydrogen (pH). Plants biostimulants are environmentally and eco-friendly which may involve in promoting sustainable production. Willow is not only a source of salicylic acid (SA) but also known as a natural and local source of bioactive, inexpensive and self-achieved plant biostimulants. An experiment on the effect of weeping willow bark extracts and their time of application on the growth and flower production of tuberose was carried out at the Ornamental Horticulture Nursery, Department of Horticulture, The University of Agriculture Peshawar, Pakistan in 2022. The Soil texture of the experimental site was silt loam with high pH (8.1). The experimental design was a Complete Block Design, arranged in a split plot. There were two factors: Factor A (main-plot) application time i.e. 20, 30, 40 days after plant emergence and Factor B (sub-plot) willow bark extracts i.e. 0, 2, 4, 6 and 8%. The experimental findings revealed that WBE and their application time significantly improved all the studied parameters in tuberose. Interaction between *Willow Bark Extracts* **(**WBE) and time of application was found significant for leaf area and floret fresh weight. Maximum number of leaves, days to last floret opening, plant height, chlorophyll content, floret spike, days of vase life and least days to spike emergence were observed with application of 8% of WBE, it was statistically similar with application of WBE at 6 and 4%. Maximum leave plant^{-1}, chlorophyll content; extended vase life and early spike emergence were noticed in plants sprayed 40 days after emergence. Maximum days to last floret opening, florets spike^{-1}; tallest plants and least days to first floret opening were recorded in plants of tuberose treated 40 days after emergence that were statistically similar to the findings observed when plants were sprayed 30 days after emergence. It is concluded that foliar application of WBE at 4% proved best and economical for better growth and flower production with superior quality in tuberose. While, application of WBE, 40 days after plant emergence was found most effective for most of the studied attributes in Tuberose.

Keywords

Tuberose, Weeping Willow Bark, Plant-biostimulant, Production, Sustainable Crop Production

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1. Introduction

Facing the problem of overusing chemical compounds in agriculture, such as plant protection products and growth regulators there exists a growing demand for their biological replacements, but that provide the same quantity and quality of crops. One of the groups of such substances is biostimulants. Scientists are interested in plant biostimulants which have been identified as natural and one of the most-concerned agronomic factors during the last few decades [1]. They may include both, chemical compounds and/or microorganisms which enhance plant metabolism when applied to plants or rhizosphere. One of the most important features of biostimulants is their multi-task activity. The metabolic mechanisms may differ strongly but appear independently of each other: from enhancing nutrient uptake and protection against oxidative stress to improving reproduction attributes [2, 3]. According to their composition, biostimulants (*Moringa leaf* extract, Kelp extracts, *Aloe vera* extracts, etc.) can be supplied in low concentration on plant [4].

One of the well-known biostimulants is weeping willow (*Salix babylonica* L.) bark extracts (WBE). Willows (*Salix spp*.), belong to the family Salicaceae. The trees are well known to flourish in marshy areas throughout the world. The weeping willow tree is known as a medicinal plant used in various drugs (aspirin) [5]. Besides medicinal advantages, weeping willow tree provides agricultural benefits as well. It also helps in stabilizing erosion on river banks etc.

The extracts of weeping willow are rich in several important nutrients such as nitrogen, phosphorus, potassium, calcium, boron, manganese, iron, zinc and magnesium. Willow also extract contains growth regulators or phytohormones such as salicylic acid, auxin IBA (Indole-3-butyric acid), salicylate and phenolics that may be utilized as growth promoters [6, 7]. It can be found several important secondary metabolites in willow bark extracts which are involved in plant metabolism. These metabolites include derivatives of salicin, flavonoids, tannins and polyphenols. Due to its complex composition, it enhances tolerance (against biotic & abiotic stresses) through various morphological and physiological as well as biochemical mechanisms [8].

The extracts have been used on various crops for their growth and development improvement. Due to the presence of antioxidant compounds, WBE exhibits strong anti-stress activity in plants. [7] revealed that WBE protected plants against salinity stress. The extract is also used as a pesticide to control *Botrytis cinerea* and *Penicillium expansum* [9, 10], The European Union for pesticide legislation recognized the WBE for application in agriculture for its fungicidal properties [6, 10]. The stimulating role of WBE in vegetative plant propagation, e.g. in adventitious rooting and in vitro propagation [11] also results from salicylic acid and phenolic concentration. Besides its antifungal and hormonal influence, WBE enhances calcium (Ca), magnesium (Mg) and potassium (K) uptake and increases micronutrient concentration [1]. It is found to enhance the growth, development and quality of flowers in a wide range of crops [12].

The extracts improved the leaf protein concentration and decreased the negative effects of salinity throughout the growth period. Weeping willow leaves, as well as barks extracts, can be used for seed treatment to enhance seedling growth and establishment under stress conditions [7]. The bark extract was found to be more effective than the willow leaf extract [13]. SA as a phenolic compound is known for its anti-stress activity and has a key role in enhancing resistance and regulating the physiological processes in various plants [14-16] studied the effect of SA on various crops.

Tuberose belongs to the family Asparagaceae and is one of the important ornamental tuberous plants. It originates from Mexico [17]. Tuberose is grown in the United States of America (USA), Italy, France, South Africa, and North Carolina on a large scale [18]. It is most commonly used for garden decoration and cut flowers as well as for its fragrant essential oil [19]. It has great significance and commercial value in the floriculture trade because of its extended vase life and fragrance [20]. It thrives best in sunny locations and produces inflorescences of high quality. Tuberose requires well-drained soil for good production and it produces inferior-quality spikes in water logged soils. A soil with good fertility having loam texture and pH ranging from 6.5 to 7.5 is considered best for it. In Pakistan, especially at Peshawar the soil pH is high and most of the micro-nutrients are not available causing plant stresses and resulting in poor performance of tuberose [21].

The weeping willow bark extract may play an important role in plant growth in Peshawar conditions, especially on alkaline soil. Little research has been done on the influence of weeping willow bark extract on the growth and production of various ornamental plants. Thereby, keeping in view the above-mentioned problems, the commercial importance of tuberose and the possible potential effects of weeping willow bark extract, an experimental trial was performed with the objectives of evaluating various concentrations of weeping willow bark extract, proper time of application as well as their interaction for better growth and quality flower production in tuberose.

2. Materials and Methods

2.1. Experimental Site

An experimental trial was carried out at the Ornamental Horticulture Nursery, The University of Agriculture Peshawar Pakistan during the year 2022.

2.2. Experimental Design

The two-factorial-experiment was established in the Randomized Complete Block Design (RCBD) and the Factors were arranged according to the split plot method. There were three replications of each combination (03). Factor A was spray time (S) of WBE: 20, 30 and 40 days after plant emergence and Factor B included five concentrations of WBE (assigned to sub plot), 0; 2; 4; 6 and 8%.

2.3. Preparation of Field, Collection of Bulbs, Planting of Tuberose Bulbs and Management Practices

The soil was ploughed and well-prepared for the tuberose bulb plantation. Soil samples from a depth of 0-30 cm were taken in three replications before the experiment to analyze soil properties. They are presented in Table 1. Old bulbs of tuberose were harvested from the field at the Ornamental Horticulture Nursery, the University of Agriculture Peshawar, Pakistan on the 10^{th} of March. The grading was carried out and bulbs of uniform size (3.5 cm / 50 g) were selected. The experiment was established on the 15^{th} of March by planting bulbs at a distance of 20 cm x 30 cm and a depth of 7cm. The field was irrigated immediately after planting of bulbs and then subsequently irrigated when needed, usually at 6-8 days intervals. Weeding and hoeing were carried out uniformly.

***Table 1**. Results of the analysis of the soil used in this investigation.*

Sand (%)	Silt (%)	Clay (%)	Electrical conductivity (EC) (dSm^{-1})	Ph	Nitrogen %	Phosphorus ($mg.kg^{-1}$)	Potassium ($mg.kg^{-1}$)	Calcium ($mg.kg^{-1}$)
15.0	60.0	25.0	0.35	8.1	0.06	4.10	130	114

2.4. WBE Preparation

For the preparation of WBE, fresh willow bark was collected from young shoots of the weeping willow tree (*Salix babylonica*) from Wersak Road Peshawar. To obtain 2% of WBE, 10 g of willow bark was chopped into approximately 1 cm pieces. Then they were placed in distilled water and the total volume was adjusted to 500 mL (milliliter) by adding distilled water. The mixture was kept at 90 ℃ for 30 minutes and constantly stirred at 400 rpm. The solution was filtered using a cheese cloth. The solution was cooled at room temperature and was ready to use (Mutlu-Durak and Kutman 2021. Likewise, the 4, 6 and 8% solutions were made.

C_1 = 0 (Distilled water)
C_2 = 2% (10g/500ml)
C_3 = 4% (20g/500ml)
C_4 = 6% (30g/500ml)
C_5 = 8% (40g/500ml)

2.5. Time of WBE Application

WBE was applied in the morning time, at 8 a.m.

2.6. Studied Attributes

Data were collected on the number of Leaves, Leaf area (using leaf area meter LICOR LI-3000CAP, made in United States), days to spike emergence, days to first floret opening, days to last floret opening. Plant height data were taken from five plants of all treatments by measuring the plant from surface of soil (plant base) to the inflorescence tip. Finally average data was calculated. Greenery index were recorded on the leaves of five plants using SPAD (Soil Plant Analysis Development meter) of the model SPAD-502 plus made in Japan and then mean value was worked out. Fresh florets were taken from the spikes of 5 plants (for all treatments) and were weighed with the SHIMADZU electrical balance made in Japan and then the average was determined. The cut flowers of tuberose (40 cm) were harvested on 70% from the plant for each treatment early in the morning and placed in the vase containing distilled water at the Horticulture Lab. (temperature of 32.7 ℃ and 52% relative humidity). The means were recorded from the date of placement till the senescence of the spike.

2.7. Statistical Procedure

Data were analyzed through the software Statistics 8.1 for generating ANOVA. In case of significant findings, the means were separated using LSD test [22].

3. Results

There were several major changes in plant growth parameters experienced with the use of plant extract. The number of leaves per plant was significantly affected by willow bark extract (WBE) with the greatest number of leaves in plants treated with WBE at 8 and 6% and the least number after application of distilled water. Application time also affected this trait of plants, with an increased number in plants treated 40 days after the emergence of bulbs. The interaction between WBE and times of application was not significant (Table 2).

However, interaction depicted the substantial effect of WBE on leaf area in plants sprayed with 8%, 40 days after emergence. As a result, the rise of value in this plant attribute is up to 37% more than control treatment. Though, statistically similar findings were also proven with 4 and 6% WBE, while the smallest leaf area in plants sprayed with distilled water (0% of WBE) at the same stage of application (Table 2; Figure 1) was observed. Chlorophyll (SPAD) in leaves was significantly affected by WBE concentrations and different intervals of foliar application, interaction was non-significant. The highest SPAD was noted in plants which received 8% WBE with 15.48% increase compared to water-treated plants. Their effect was not far different from WBE at 4 and 6% concentration. (Table 2). It could be seen that a significant increase in plant height (by 15.74%) was seen in plots receiving 8% of WBE but its effect was statistically similar to the WBE applied at 4 and 6% concentrations, whereas control plants produced the lowest shoots. Application of WBE 40 days after plant emergence was considerably higher for height than the rest of treatments (application time) (Table 2).

Table 2. *Effect of WBE and their foliar spray on number of leaves (NL), leaf area (LA), chlorophyll content (CHC) and plant height (PH) at different stages in tuberose.*

Factors (treatments)	**Studied attributes**			
Spray time [days after plants emergence] (S)	NL	LA	CHC	PH
20	19.95b	557.35B	65.24b	93.82b
30	20.15b	571.54B	65.57b	98.51ab
40	21.35a	648.50A	70.82a	100.46a
LSD	0.96	47.08	4.49	4.71
CV [%]	4.64	4.73	6.6	4.76
Significance	*	**	*	*
Willow Bark Extracts (WBE) [%]				
Distilled water	17.96C	439.53D	60.63C	87.37B
2	19.55BC	532.97C	65.30B	92.89B
4	21.18AB	624.01B	69.07A	101.15A
6	21.59A	666.41AB	69.29A	102.89A
8	22.14A	699.39A	71.74A	103.70A
LSD	1.65	45.97	3.76	7.55
CV [%]	6.1	5.89	4.25	5.87
Significance	**	**	**	**
Interaction S × WBE	NS	**Figure 1	NS	NS

NS: Non-significant; *: Significant at $P \leq 0.05$; **: Significant at $P \leq 0.01$, LSD: least significant difference, CV: coefficient of variation
Means followed by different letters are significantly different from each other at $P \leq 0.05$ (Lower case) and $P \leq 0.01$(Upper case).

In our experiment, it was found that foliar application of WBE and timing did positively affect plant traits. Noticeable changes in plant head appearance were observed after the WBE application. The analysis of data confirmed the acceleration of spike emergence with an increase in extract concentration up to 8%. Spray time also influenced time taken by spikes to emerge. An improvement was shown when foliar treatment of WBE was done 40 days after plants emergence (Table 3). Changes in flower characters with delay in days of first floret opening and sharp increase in days to last floret opening were then recorded after 8% WBE treatment (Table 3). On the other hand, spray time, e.g. application of WBE 40 days showed a sink in days to first floret opening. At the same time, days to the last floret opening went up. Interaction remained non-significant for both parameters (Table 3). WBE and spray time strongly improved the number of florets per spike while interaction did not., the most productive florets were proved after 8% extract application, but they were also approximately the same with that of 6 and 4%. Florets grew significantly to a maximum when we applied WBE 40 days after plant emergence (Table 3). Florets fresh weight as a part of the one-year investigation was more prolific by application of WBE at 8%, while water-treated plants produced the least (Table 3). The fresh floret climbed noticeably with foliar treatment of WBE 40 days after plant emergence, whilst a decline was observed for plants sprayed 20 days after emergence (Table 3). For this parameter, interaction was also found significant with considerable fresh weight at 8% of WBE treatment 40 days after plants emergence (Figure 2). The vase life (days) of tuberose was significantly affected by various concentrations of WBE and foliar application time. A soar of vase life was observed when sprayed the plants with 8, 4 and 6% WBE (Table 3). However the use of distilled water resulted in the shortest vase life (Table 3). Moreover, sprays times also affected this plant attribute. Plants treated 40 days after emergence demonstrated a leap of vase life (Table 3).

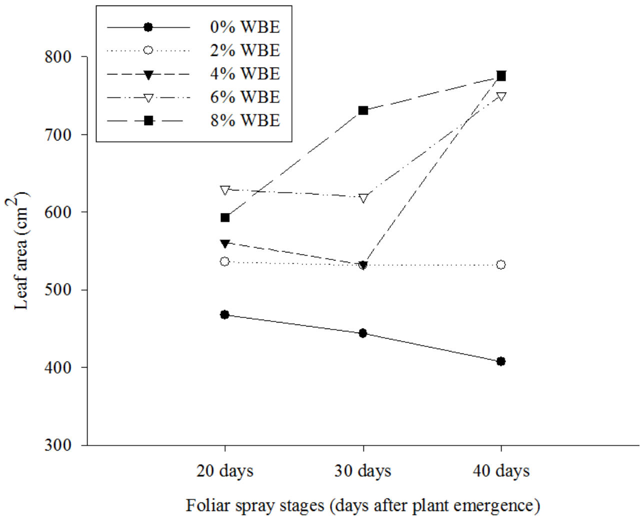

***Figure 1**. Interaction effect of WBE and their foliar spray on leaf area (cm^2) at different stages in tuberose.*

***Table 3**. Effect of WBE and their foliar spray on days to spike emergence (DSE), days to first floret opening (DFFO), days to last floret opening (DLFO), number of florets spike (NFS), floret fresh weight (FFW) and vase life (VL) at different stages in tuberose.*

Factors (treatments)	**Studied attributes**					
Spray time (S)	DSE	DFFO	DLFO	NFS	FFW	VL
20	54.54a	71.37A	80.66b	30.15b	31.86b	6.80C
30	53.48a	70.19AB	82.06ab	32.15ab	33.76b	8.73B
40	50.70b	69.21B	85.26a	34.08a	37.46a	11.20A
LSD	2.35	1.91	3.58	2.85	3.21	1.74
CV [%]	4.39	1.62	4.28	8.77	9.24	11.61
Significance	*	**	*	*	*	**
Willow Bark Extracts (WBE) [%]						
Distilled water	57.28A	72.14A	81.33BC	25.25C	24.28D	6.27C
2	54.77AB	71.70AB	80.27C	28.73B	29.87C	7.38C
4	50.82BC	68.81BC	84.55AB	34.66A	38.83B	10.90A
6	52.84BC	71.14AB	81.72ABC	34.92A	36.84B	9.33B
8	48.80C	67.51C	85.44A	37.07A	41.98A	10.66AB
LSD	4.13	2.89	4.04	2.73	2.72	1.53
CV [%]	5.93	3.13	3.7	6.46	6.02	13.07
Significance	**	**	**	**	**	**
Interaction S ×WBE	NS	NS	NS	NS	**Figure 2	NS

NS: Non-significant; *: Significant at $P \leq 0.05$; **: Significant at $P \leq 0.01$.
Means followed by different letters are significantly different from each other at $P \leq 0.05$ (Lower case) and $P \leq 0.01$(Upper case).

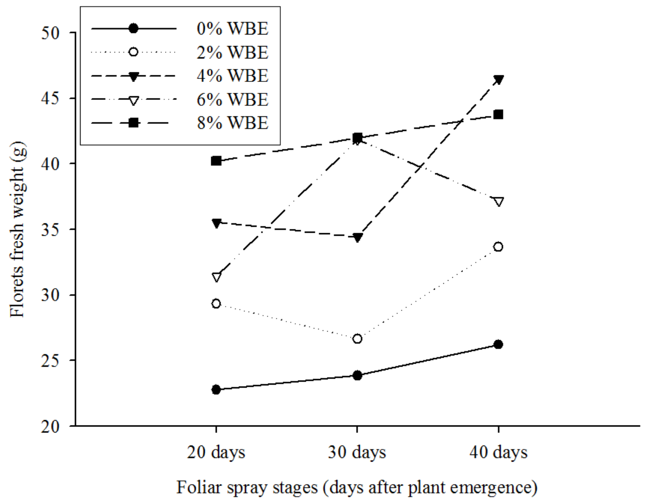

Figure 2. *Interaction effect of WBE and the time of foliar spray on floret fresh weight spike^{-1} (g) in tuberose.*

4. Discussion

The Plants were assigned into subplots at calcareous soil of high pH (8.1) and five different concentrations of WBE were applied as bio-stimulant to them according to three defined application times, this to improve their growth and development. In terms of physiology and biochemistry and the light of several types of research, willow bark extract (WBE) might be a source of various macro and micronutrients (N: nitrogen, P: phosphorus, K, Ca, B: boron, Mn: manganese, Fe: iron, Zn: zinc and Mg). It could also contain the phytohormones such as salicylic acid, auxin, salicylates and Phenolic compounds. Moreover, the extract could be rich in polyphenols and salicin [6, 7]. Its numerous compositions demonstrate that it may have a beneficial effect on various ornamental plant species.

Considerable increase in leaf development (number of leave and leaf area) after application of WBE in higher concentrations can be attributed to the synergetic effect of salicylic acid (SA), Auxin, mineral nutrients and other phenolic compounds found in the willow bark. WBE is an important botanical extract which is referred to natural bio stimulant and has been shown to have a positive influence on various plant attributes including the number and area of leaves. It may be involved in cell division, cell elongation and cell expansion due to its content of Auxin. The study of [23] demonstrated the significant influence of willow bark extract on *Lactuca sativa* plants with an increase in its vegetative development.

Foliar application of WBE had resulted in the highest content of chlorophyll in tuberose. Auxin contained in the bark may play a role in the division and elongation of plant cells. SA on the other hand can promote the enzyme's activities, control plant growth and development, and enhance photosynthesis as well as biochemical & physiological responses. Investigation of [24] found that willow bark extract increased the chlorophyll content of wheat seedlings. Applying willow bark extract to maize plants led to an increase in chlorophyll content [25].

The application of WBE decreased days to spike emergence in tuberose. Its foliar spray resulted in the least days to spike emergence as compare to control (Table 2). It could induce the early emergence of spike and controlled various physiological responses of plants. It was found that willow bark extract significantly reduced the time to spike emergence in wheat plants [26]. Another study revealed that applying willow bark extract to tomato plants led to earlier flowering and fruiting compared to untreated plants [27].

The use of plant biostimulants, such as WBE had a significantly impact on plant height in tuberose. Botanical extract's application ameliorate various plants attributes including plant growth and development, root development, plant resistance to biotic and abiotic stresses and callus formation [28]. It is also well known as a stimulating agent of seeds. Seed treatment with biostimulant (WBE) influenced significantly and improved the seedlings of maize [7]. Substantial positive growth of shoot length was noted when applied willow extract to maize seeds with good germination [29]. Applying willow bark extract to potted chrysanthemum plants led to an increase in plant height compared to untreated plants [30]. Willow bark extract also increased the height of potted cucumber plants [31].

The foliar spray of WBE resulted in the least days to flowering and regulated various physiological responses of the plant. The result showed that early flowering was obtained in plants when sprayed with WBE (Table 3) associated with improvement of fragrance. WBE may enhance photosynthesis and carbohydrate in leaves perhaps because of this reason the early florets opening took place. WBE consist of several essential mineral nutrient and PGR (plant growth regulator) such as salicylic acid and auxin (IBA) known to induce early growth and inflorescence. The extract stimulates earlier root initiation in cuttings of lavender and chrysanthemum [11].

The foliar application of WBE significantly improved the flower quality and prolonged flowering (Table 3). These results may be due to the role of WBE, as a natural plant hormone, which interacts as a prominent substance and improves the tolerance of plants against biotic stresses. The extract increases flower longevity because it can inhibit ethylene biosynthesis. It is proved that the mixture of SA and sucrose improved flower opening in ornamental orchid species [32].

The foliar application of WBE significantly affected florets per spike. The outcomes demonstrated that a large florets number was observed in plants that received treatment of WBE as compare to control treatment (Table 3). This result might be due to the high photosynthetic rate in leaves, the tallest spikes produced caused after the application of biostimulant treatment. Due to its many components of bioactive substances, willow extracts are involved in flower formation and development [33].

Foliar application of biostimulants such as WBE highly improves several reproductive growth parameters which consequently increase the economic value of ornamental crops. WBE application increased the number of leaves, plants height, leaf chlorophyll content (Table 2) and strong spikes as a result healthy plants with high florets fresh weight were produced. It influenced the physiological processes of plant growth and development. The greatest head weight of *Lactuca sativa* was observed with the application of willow extract [23]. WBE positively influenced and enhanced the plant biomass, flower size that might increased fresh weight of florets. The use of plants extract caused an increase in head weight of *Lactuca sativa* [23].

Application of WBE also enhanced the vase life of tuberose. WBE contains a phenolic compound that can slow down the activity of ACC (aminocyclopropane-1-carboxylic acid) oxidase which is known as the ethylene pre-cursor because of its SA content. Hence it reduces ethylene production and prolongs the cut flower life, like it was found in gerbera. The influence of SA on the post-harvest life of the flowers can be due to its property in enhancing the plants systemic resistance against pathogens that may block xylem vessels. The SA Pre-harvest spray improved the quality of crop and vase life in rose flower production [34, 35] stated that the application of SA on tuberose had a significant impact on the post-harvest life of cut flowers. [36] also found that SA extended post-harvest life of cut flowers in *Gerbera jamesonii*.

5. Conclusion and Recommendations

Our study agreed that botanical extract (Willow bark) may play a crucial role in ameliorating the growth, development, and quality flower production of tuberose. The application of WBE at 8% was proved more prolific than the rest of the treatments. Most of the studied parameters were greater at this concentration. However, the effect was similar to that of 6 and 4% for most of the parameters. Among different foliar application times, WBE when applied 40 days after plants emergence resulted in a greatest value of all the plant attributes. Based on the result, it can be concluded that application of WBE at the range of 4-8% and their application 40 days after plant emergence could be recommended for quality flower production of tuberose.

Abbreviations

SA	Salicylic Acid
WBE	Willow Bark Extracts
IBA	Indole-3-Butyric Acid
Ca	Calcium
Mg	Magnesium
K	Potassium
USA	United States of America
pH	Potential of Hydrogen
RCBD	Randomized Complete Block Design
EC	Electrical Conductivity
SPAD	Soil Plant Analysis Development Meter
mL	Milliliter
NL	Number of Leaves
LA	Leaf Area
CHC	Chlorophyll Content
PH	Plant Height
NS	Non-Significant
LSD	Least Significant Difference
CV	Coefficient of Variation
DSE	Days to Spike Emergence
DFFO	Days to First Floret Opening
DLFO	Days to Last Floret Opening
NFS	Number of Florets Spike
FFW	Floret Fresh Weight
VL	Vase Life
N	Nitrogen
P	Phosphorus
B	Boron
Mn	Manganese
Fe	Iron
Zn	Zinc
PGR	Plant Growth Regulator
ACC	Aminocyclopropane-1-Carboxylic Acid

Author Contributions

SOLEVO Martin designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript, managed the analyses of the study, 'managed the literature searches. Masood Ahmad and Abdul Mateen Khattak supervised the work. All authors read and approved the final manuscript.

Data Availability Statement

The corresponding author will provide as per the request the data presented in this study.

Conflicts of Interest

The authors declare no conflicts of interest.

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