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Effect of Soluble Silica on the Plant Growth, Leaves Chlorophyll Content and Bulb Quality of the Garlic (*Allium sativum* L.) against Drought Stress

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ABSTRACT

Background: Garlic is an aromatic spice with nutritional and medicinal values. Climatic variations such as drought, adversely affect Garlic's morpho-physiological, biochemical attributes and diminish its bulb quality. Soluble silica is a plant-available form of silicon (Si). Silica is beneficial for plant's healthy growth especially against stress conditions. The present study was aimed to evaluate the effect of soluble silica on the garlic plant growth, leaves chlorophyll content and bulb quality against drought stress.

Methods: Garlic was cultivated during the *Rabi* season (2019-2020) in the experimental field Lohar pipliya (Dewas), India. Randomized experimental blocks consisted of six treatments as follows: T_1- Control (well-watered), T_2- Drought stress (water supply reduced by about 50%), T_3- Drought + a basal dose of NPK fertilizers (BDF) in soil, T_4, T_5, T_6- Drought + BDF + foliar sprays of 7.5, 10 and 12.5 ml L^{-1} soluble silica respectively. Silica sprays were applied seven-time at a regular interval of 15-days. After 120-days of sowing, plant growth in terms of plants height and number of leaves $plant^{-1}$ were measured. 120-days leaves chlorophyll, MDA and proline content were estimated. Garlic bulb's quality in terms of fresh-dry weight and diameter were recorded at the final harvest.

Result: Results revealed that drought significantly reduced Garlic's plant height, bulb quality (weight and diameter), chlorophyll (Chl-*a* and Chl-*b*) and increased MDA, proline content in contrast to the well-watered garlic. Fertilizers (BDF) feebly improved garlic bulb quality. However, fertilizers (BDF) supplemented with soluble silica sprays (7.5 ml L^{-1}<12.5 ml L^{-1}<10 ml L^{-1}) significantly increased plant height, bulb weight and diameter, leaves chlorophyll, proline and reduced MDA content over the drought-stressed Garlic. Fertilizers (BDF) supplemented with Soluble silica maintained plant water content, reduced oxidative stress and increased leaves chlorophyll content. These resulted in good quality garlic bulbs in terms of weight and diameter despite the drought stress.

Key words: Chlorophyll, Drought stress, Garlic bulb quality, MDA, Proline, Soluble silica.

INTRODUCTION

Garlic (*Allium sativum* L., Family Alliaceae) is the second-largest bulbous crop that originated 5000 years ago in Central Asia (Benke *et al*., 2020). India (5.3%) is the second principal producer of Garlic after China (79.8%) and exports it in the fresh-dehydrated form to other countries such a Bahrain, Germany and Japan (Lawande *et al*., 2009). Garlic is widely used as a spice in flavoring food because of the distinctive aroma of organosulphur compounds like allicin (diallyl thiosulphate) (Bhatia *et al*., 2020). Garlic has various health benefits; it is used as a natural antibiotic for the common cold, fever, bronchitis and lowers serum cholesterol and triglycerides in cardiovascular diseases (Reyes *et al*., 2018, Morales-Gonzalez *et al*., 2020, Vijaya *et al*., 2020).

Garlic production solely depends on the irrigation water supply because of its shallow roots and water-sensitive nature. Adverse climatic variations such as drought severely affect garlic growth, quality and yield in drylands (Sattar *et al*., 2020). Water scarcity inhibits seed germination and seedling growth (Maksimovic *et al*., 2021), causes cellular dehydration, stomatal closure and imbalance in reducing equivalents in plants (Hussain *et al*., 2019). These trigger synthesis of reactive oxygen species (ROS) in cellular organelle like mitochondria, chloroplast, peroxisome. ROS damage membrane lipid, protein, nucleic acid and negatively affect plant growth and development (Chung *et al*., 2020, Hasanuzzaman *et al*., 2020).

Routinely used agriculture practices and fertilizers were not enough for the plants to cope with the climatic adversities and require additional growth nutrients. Silicon (Si) is a quasi-essential (Zargar *et al*., 2019), an anti-stress agent against abiotic and biotic stresses (Malhotra and Kapoor, 2019). Although Si is abundant in soil, plants are not able to utilise its complex form (Zargar *et al*., 2019). Soluble silica (Agribooster™) a new water-soluble form of silicon, that can be utilised directly by plants and exhibits numerous beneficial effects on plant growth, quality and yield against climatic

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adversities. Previous studies suggested that soluble silica mitigated heavy metal (Pb) toxicity on *Vigna radiata* (Goyal *et al*., 2017), *Triticum aestivum* (Sharma *et al*., 2018). Si mitigated salt stress in Soybean (Chung *et al*., 2020), drought stress in Maize (Parveen *et al*., 2019), sudden environmental stress (Rangwala *et al*., 2018) and elevated temperature in Barley (Hussain *et al*., 2019). Polysilicate ($SiO_2.nH_2O$) impregnates plant tissues like sclerenchyma, epidermis, vascular bundle to provide strength and rigidity against external stress (Mandlik *et al*., 2020). It maintained turgor pressure, nutrient accumulation in Canola against the drought stress (Bukhari *et al*., 2020). Si balanced the water status of plants by apoplastic role (Thorne *et al*., 2020), improved chlorophyll pigments and antioxidants (SOD, CAT and POD) activity in maize seedlings (Sattar *et al*., 2020).

Our study explored the possibilities of the use of soluble silica foliar sprays (a plant-available form of silicon) along with routinely used fertilizers (BDF) to reduce the adverse effect of drought on Garlic's plant growth, 120-days leaves chlorophyll (Chl-*a* and Chl-*b*), MDA, proline content and bulb quality.

MATERIALS AND METHODS

Experimental field and plant material

A field experiments on the garlic (*Allium sativum L.*) crop of variety-Amleta were carried out during *Rabi* season (28 October 2019 to 21 March 2020) at the agricultural research field, Lohar pipliya village, Dewas (MP), India (22° 54′ 42″N longitude and 75° 59′31″E latitude). Growth and Biochemical parameters of 120-days Garlic leaves and harvested matured bulbs were recorded at the Department of Biochemistry, Govt. Holkar Science College, Indore (M.P).

The soil characteristics before treatments were: pH 7. 98, E.C 0.92 d Sm^{-1}, Organic carbon 0.48%, N, P, K, are 196, 11.2 and 480 kg ha^{-1} respectively. There was no rainfall and field temperature was varied from 22°C to 34°C during the complete growth period.

Experimental design

The experimental setup was designed as randomized complete blocks consisted of six treatments, each having a 2×1.8 m^2 block area (Fig 3a). Garlic bulbs were carefully separated into individual cloves and sown in the field so that the distal end was upward and covered with soil.

Tt. no.	After 15-days of sowing, Garlic plants were treated with six treatments as follows:
T_1	Control (well-watered).
T_2	Drought stress (water supply reduced by about 50% as compared to control).
T_3	Drought stress + a basal dose of N, P, K fertilizers in soil (BDF).
T_4	Drought stress + BDF + foliar spray of 7.5 ml L^{-1} soluble silica fertilizer.
T_5	Drought stress + BDF + foliar spray of 10 ml L^{-1} soluble silica fertilizer.
T_6	Drought stress + BDF + foliar spray of 12.5 ml L^{-1}soluble silica fertilizer.

Here, soluble silica fertilizer (Agribooster™) was used as a plant-available form (PAF) of silicon (Si). It consisted of 13.1 % SiO_2. Silica was sprayed seven-time in a regular interval of 15-days from sowing up-to-the Garlic bulb maturity. Weeds were removed manually. After 120-days of sowing (DAS), Garlic leaves from each treatment were sampled for biochemical assay and at final harvest matured Garlic bulbs were collected for quality assay (Fig 3c and 3d).

Plant growth (height and number of leaves plant^{-1})

120-days (DAS) Garlic plant height (cm) and the number of leaves were recorded in triplicates (Fig 3b) (Wu *et al*., 2015).

Bulb quality (weight and diameter)

After harvest, Garlic bulbs and pseudo-stem diameter (cm), were measured through a vernier calliper. Its fresh and dry weight (gm) was measured using an electronic balance in triplicates (Mann, 1952).

Chlorophyll content

Chl-*a and* Chl-*b* content of 120-days Garlic leaves (200 mg) were extracted in 80% alcohol and estimated using Lichtenthaler and Welburn (1983) equation:

Chl-*a* ($\mu g\ mL^{-1}$) = 12.21 (A_{663}) - 2.81 (A_{646}) and Chl-*b* ($\mu g\ mL^{-1}$) = 20.13 (A_{646}) - 5.03 (A_{663}).

Malondialdehyde (MDA) estimation

MDA level of 120-Days Garlic leaves (100 mg) were estimated using: $A = \varepsilon cl$,

where

A = Absorbance at 532 nm.

ε = Extinction coefficient (155 $mM^{-1}cm^{-1}$).

l = length of cell (1cm).

C = Concentration (Heath and Packer, 1968).

Proline estimation

Proline content of 120-Days Garlic leaves (500 mg) were determined using a standard curve and estimated on a fresh weight basis of sample: µmoles of Proline/g tissue = µg proline/ ml × toluene (ml) × 5/ g sample 115.5 (Bates *et al*., 1973).

RESULTS AND DISCUSSION

Chlorophyll content in garlic leaves

As shown in Table 1 and Fig 1, Chl-*a* and Chl-*b* content in Garlic leaves were reduced under drought stress (T_2) compared to the well-watered control (T_1). Here, reduced fresh weight, increased MDA and proline content in Garlic leaves (T_2) (Table 1) indicates that water scarcity has induced oxidative stress in plants. Abid *et al*.,(2018) revealed that stomatal closure in drought stressed-wheat leaves, reduces carbon assimilation, cause an imbalance between excited electrons and their use in light reaction to trigger overgeneration of reactive oxygen species (ROS). Chlorophyll content in Garlic leaves might have reduced due to oxidative damage of chloroplast thylakoid membrane and inhibition of chlorophyll biosynthesis enzymes by ROS. Our suggestions were in favor of other findings that drought reduced chlorophyll content in maize hybrid varieties Xida-319 and Xida-889 (Hussain *et al*., 2019) and Wheat (Pozo *et al*., 2020). Routinely used fertilizers (BDF) (T_3) insignificantly improved Chl-*a* and Chl-*b* content in Garlic leaves as compared with drought Garlic leaves (T_2). Supplementation of silica sprays to fertilizers (BDF) significantly increased leaves Chl-*a* and Chl-*b* content (T_4, T_5, T_6) in order 7.5 ml L^{-1} <12.5 ml L^{-1} <10.0 ml L^{-1} over the Garlic grown under drought without any treatments (T_2). Previous studies of Ma, (2012), Sapre and Vakharia, (2016), Greger *et al*., (2018), Alamri *et al*., (2020) **s**uggested that silicon deposits at the leaf bundle sheath and form the silica cuticle layer (epidermis) to reduce the transpirational water loss in plants. In our study, reduced MDA level and highly significantly increased proline (osmoprotectant) content in garlic treated with BDF+silica sprays (T_4, T_5, T_6) as compared to drought garlic (T_2) shows that silica has balanced water content in plants and might have prevented Chloroplast thylakoid membrane from oxidative damage. Our study is in favour with Dehghanipoodeh *et al., (*2018) that Potassium silicate (K_2SiO_3) (10 mmol L^{-1}) maintained the water content of the Strawberry plants. Sun *et al*., (2021) also showed that 15 g L^{-1} of water-soluble silicon (Si-50G, Si-60G) has improved proline, sugar and protein (osmolytes) content to prevent membrane damage and increased chlorophyll content in maize seedlings.

Growth attributes of garlic plants

In our study, the Garlic plant's growth was assessed based on height and the number of leaves on the plants. As shown in Table 2 and Fig 2, drought (T_2) reduced garlic plant height significantly and the number of leaves plant^{-1} insignificantly over the well-watered plant (T_1). It is evident that plants absorb growth nutrients from soil in a water-soluble form. Here, garlic plant's height were reduced might be due to insufficient availability of water-soluble nutrients from soil. Secondly, reduced light-capturing pigments (Chl) possibly affected plants photosynthetic activity and diminished garlic plant height in drought stress. The basal dose of NPK fertilizers (T_3) has slightly improved plant growth against drought stress (T_2). However, BDF+silica sprays ($T_{4,5,6}$) have

Table 1: Showing the effect of soluble silica on chlorophyll (Chl-*a*, Chl-*b* and Chl-*a*/*b*), MDA and proline content of garlic leaves against drought stress.

Type	Conc. of Silica (ml L^{-1})	Chl *a* (mg/g)	Chl *b* (mg/g)	Chl *a*/*b* (mg/g)	MDA (mM/g)	Proline (µmoles/g)
Control	0	13.182±0.15	6.364±0.20	2.073±0.09	0.00299±0.0001	14.66±0.57
		a, b**, c*	a, b*, c*	a, b*, c*	a, b*, c*	a, b*, c*
Drought	0	11.728±0.42	5.976±0.07	1.926±0.01	0.00347±0.00003	16.33±0.58
		a**, b, c^{ns}	a*, b, c^{ns}	a*, b, c^{ns}	a**, b, c^{ns}	a*, b, c^{ns}
Drought + BDF	0	11.839±0.22	6.146±0.09	1.922±0.04	0.00324±0.0001	17.33±1.15
		a**, b^{ns}, c	a^{ns}, b^{ns}, c	a^{ns}, b^{ns}, c	a*, b^{ns}, c	a*, b^{ns}, c
Drought +	7.5 ml L^{-1}	12.512±0.42	6.134±0.06	2.040±0.002	0.00317±0.0001	20.33±1.15
BDF +		a*, b*, c^{ns}	a^{ns}, b*, c^{ns}	a^{ns}, b*, c^{ns}	a^{ns}, b*, c*	a**, b**, c*
silica	10.0 ml L^{-1}	12.544±0.42	6.154±0.06	2.049±0.01	0.00299±0.0002	20.66±0.57
		a*, b*, c*	a^{ns}, b*, c*	a^{ns}, b*, c*	a^{ns}, b*, c**	a***, b***, c*
	12.5 ml L^{-1}	12.648±0.42	6.171±0.03a	2.048±0.01	0.00311±0.0001	19.66±0.57
		a*, b*, c*	^{ns}, b*, c*	a^{ns}, b*, c*	a^{ns}, b*, c*	a***, b**, c*

^{ns} indicates not significant, * indicates significant, ** indicates highly significant *** indicates extremely significant. a-shows the p-value in contrast to control, b- shows the p-value, in contrast to, drought stress, c-shows the p-value in contrast to fertilzer.

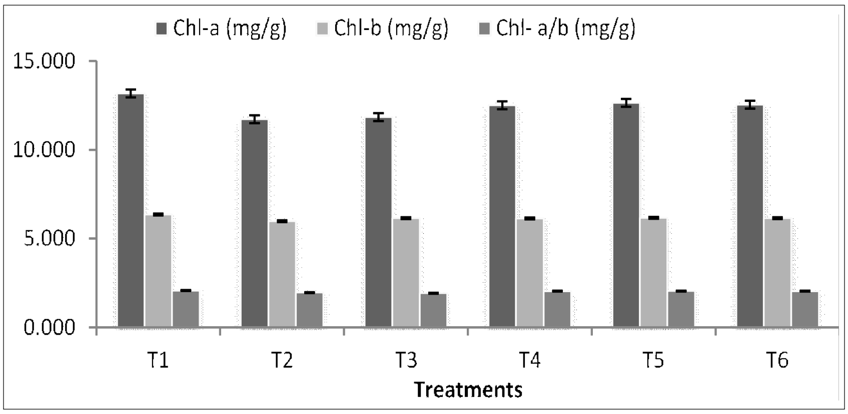

Fig 1: Effect of soluble silica on Chl-*a*, Chl- *b*, Chl- *a*/*b* content in garlic leaves against drought stress.

Table 2: Showing the effect of Soluble silica on growth and bulb quality of garlic against drought stress.

Type	Conc. of silica (ml L^{-1})	Height of garlic plant (cm)	No. of leaves $plant^{-1}$	Pseudo-stem diameter (cm)	Garlic bulb diameter (cm)	Fresh weight of Garlic bulb (gm)	Dry weight of Garlic bulb (gm)
Control	0	75.333±3.785 a, b**, c*	7.000±1.000 a, b^{ns}, c^{ns}	0.567±0.1528 a, b^{ns} ,c^{ns}	3.867±0.2887 a, b*,c^{ns}	38.42 ±1.3 a, b**, c	20.113±1.3805 a, b*, c^{ns}
Drought	0	52.833±5.107 a**, b, c^{ns}	6.333±0.577 a^{ns}, b, c^{ns}	0.500±0.100 a^{ns}, b*** c^{ns}	2.900±0.500 a*, b, c^{ns}	28.22±1.4 a**, b, c^{ns}	15.40±0.624 a*, b, c*
Drought + BDF	0	58.000±5.408 a*, b^{ns}, c	6.667±0.577 a^{ns}, b^{ns}, c	0.567±0.115 a^{ns}, b^{ns} ,c	3.600±0.300 a^{ns}, b^{ns}, c	31.23±1.7 a*, b^{ns}, c	17.423±4.245 a^{ns}, b*, c
Drought + BDF + Silica	7.5 ml L^{-1}	65.67±4537 a^{ns}, b**, c*	7.333±1.155 a^{ns}, b*, c*	0.567±0.058 a*, b**, c**	4.100±0.100 a**, b**,c**	33.55±1.5 a*, b^{ns}, c*	22.707±1.308 a**, b**, c**
	10.0 ml L^{-1}	74.333±4.509 a^{ns}, b**, c*	8.0±1.00 a^{ns}, b*, c**	0.607±0.058 a*, b**,c**	4.500±0.100 a**, b**,c**	40.727±0.595 a*, b***, c**	32.043±1.257 a***, b**, c**
	12.5 ml L^{-1}	73.00±1.328 a^{ns}, b**, c**	8.0±1.00 a^{ns}, b*, c**	0.633±0.208 a*, b**, c**	4.467±0.153 a**, b**,c**	36.977±2.9 a^{ns}, b*, c*	27.09 ±1.257 a***, b**, c**

^{ns} indicates not significant, * indicates significant, ** indicates highly significant. a-shows the p-value in contrast to control, b-shows the p-value, in contrast to, drought stress, c-shows the p-value in contrast to fertilizer.

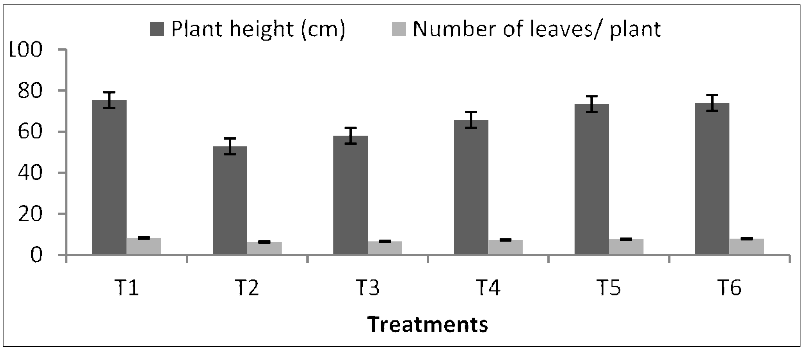

Fig 2: Effect of soluble silica on the height of the Garlic plants under drought stress.

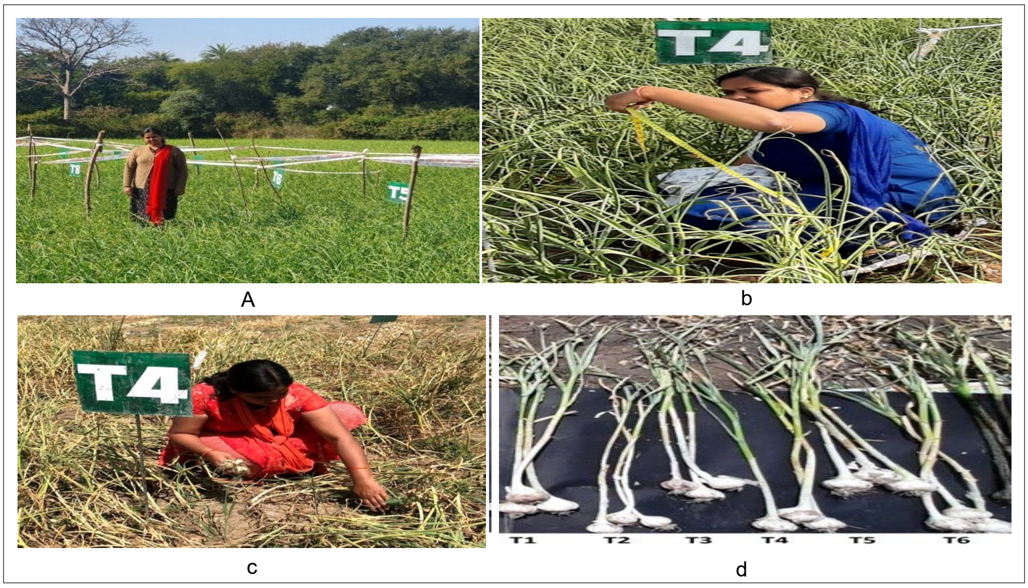

Fig 3 a)**:** Showing randomised blocks for garlic; b) Showing garlic plant height measurement;
c) Garlic bulb collection from research field; d) Comparison between Garlic grown in different treatments.

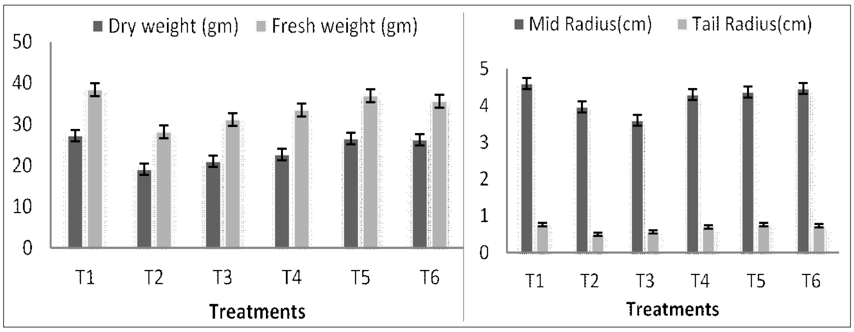

Fig 4: Effect of soluble silica on the fresh-dry weight and diameter of the Garlic bulbs against drought stress.

significantly increased plant height over the drought plants(T_2) in order, 7.5 ml L^{-1} <12.5 ml L^{-1} <10 ml L^{-1}. Our study is in agreement with Kowalska *et al*., (2020) that foliar sprays of orthosilicic acid (Si) (24%) increased plant height and number and weight of ear in spring wheat. Si increased plant growth by balancing water and nutrient content (Rizwan *et al*., 2015). Similarly, 1000 kg ha^{-1} treatment of potassium silicate (K_2SiO_3) increased the pH of the soil and P, Ca, Zn, S, Mo, Mn and Cu nutrients available to the plants (Greger *et al*., 2018). 250 kg ha^{-1} dose of Si (Diatomaceous earth, Calcium silicates, Bagasse ash) with a basal supply of fertilizer increased plant growth by enhancing nitrogen and potassium intake (Gade *et al.,* 2019), (Minden *et al*., 2020). Various Osmolytes such as proline, antioxidants and secondary metabolites helps plants to combate drought stress (Kusvuran and Dasgan, 2017). Silicon enhanced proline and antioxidants in wheat to reduce oxidative stress in term of H_2O_2 level (Sapre and Vakharia, 2016). Similarly in our study, silica prevented plants lodging, increased proline content, reduced MDA level that might have increased photosynthetic food synthesis and uptake of essential nutrients from the soil for plants vegetative growth.

Garlic bulb quality

Garlic bulb quality is the main criteria for the farmers to earn a profitable income. A perusal of our data presented in Table 2 and Fig 4, revealed that drought (T_2) considerably reduced the fresh-dry weight and diameter of the Garlic bulb over the control (T_1). As per Omar and Wabel (2010), the Garlic bulb is mainly constituted by water (65%), sugar, protein and fiber. Water deficiency and reduced leaves chlorophyll content might have declined photosynthetic food storage and affected bulb weight and diameter negatively. However, foliage applied silica sprays (7.5, 10, 12.5 ml L^{-1}) + NPK fertilizers ($T_{4,}T_{5,}T_6$) in the soil improved bulb diameter, fresh-dry weight in contrast to the bulbs grown under limited water supply (T_2). This is in favor with Bhangare and Shinde (2020) that foliarly applied silicon has improved onion bulb diameter. Dorairaj *et al*.,(2020) showed that silicon increased uprightness in rice plants and thus due to more photosynthetic activity weight of the panicle and 100 rice grains was increased. Foliage applied Si protected Soybean from lodging by lignin deposition and increased photosynthetic rate in soybean leaves. Magd *et al*., (2013) reported that bulb weight depends on the quantity of organic food such as sugar and protein transferred from leaves to the bulb. Hussaina *et al*., (2021) showed that foliage applied Si protected Soybean from lodging by lignin deposition and enhanced photosynthetic rate in Soybean leaves. Our studies show that silica mitigated water stress and increased chlorophyll content in Garlic plants (T_4, T_5, T_6) as compared to drought plants. Sufficient water availability and increased chlorophyll content in silica treated leaves might have increased photosynthetic food synthesis and translocation and accumulation of synthesised food to bulb increased bulb weight and diameter compared with the drought bulbs.

CONCLUSION

Drought (water scarcity) induced oxidative stress and reduced chlorophyll (light-harvesting pigments) in Garlic leaves. These might have declined photosynthetic food storage and negatively affected Garlic plant height, bulb fresh-dry weight and diameter. Routinely used fertilizers insignificantly improved bulb quality. Supplementation of fertilizers (BDF) with silica foliar sprays significantly relieved Garlic plants from oxidative stress by retaining water content and increasing proline (osmoprotectant) concentration. Probably silica also increased uptake of essential growth nutrients of soil and NPK fertilizers (BDF). Thus, the interactive effect of soluble silica foliar sprays and NPK fertilizer (BDF) in soil made it possible to obtain good quality Garlic bulbs in terms of weight and diameter despite the drought. Our studies suggested that soluble silica can be integrated with routine agriculture practices to protect plants from drought stress.

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Conflict of interest: None.

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