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Research paper

Drought Stress Mitigation through Antioxidant Defence Response in Sugar Beet Under Various Foliar Applications

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ABSTRACT

The rapid climate change is leading to serious changes in global weather patterns, influencing overall plant responses, where heat waves, cyclones, floods, and flash or long-term droughts become more frequent. Intensified evapotranspirative demand due to increased atmospheric temperature is leading to soil water depletion and drought. This has become a major concern in crop management. A two-year study was conducted during "winter-summer" season, evaluating the effect of three levels of irrigation and eight foliar treatments i.e., Nano silicon (N-Si) 1mM, Humic Acid (HA), Jasmonic Acid (JA), Salicylic Acid (SA), Boric Acid (B), Kaolin (KA), Chitosan (Chi) and control (water spray) on tropical sugar beet under Odisha condition. A significant impact of water scarcity was observed in the S_2 stressed plants, where Malondialdehyde (MDA) content increased from 5.05 to 13.8 nmol. g^{-1}. Confronted with the harmful effect of drought, sugar beet induces its defence mechanisms, when the stress intensity increased from moderate (7days) to severe (14days) for prolonged period. As a result, it induced increase in antioxidant enzyme activities in the leaves like CAT, SOD, APX, and POD were recorded 1.97, 205.29, 2.15, and 1.51 u.g^{-1}.min^{-1} respectively, under 14 days of severe stress cycles after 4th spray. Stress impact was also reflected in phenolics accumulation as well as proline. Foliar application of various treatments showed a significant ability to protect cell metabolism through up-regulating defence pathways against oxidative stress. Among all effective foliar treatments, SA, N-Si, Chitosan and JA exhibited as promising in alleviating prolonged drought stress in sugar beet.

Keywords: Climate Change, Drought, Antioxidant enzymes, Phenolics, MDA, Sugar beet

Climate change leads to the severity of drought by disturbing the rainfall patterns and invites a harsh situation during summer days with elevated temperatures mostly in tropical and sub-tropical regions. These climatic situations deplete soil water levels due to the excess moisture loss and reduced precipitation. Water scarcity creates a serious condition that severely affects plant growth and development by directly affecting cell membrane integrity, causing degradation of proteins, lipids and disturbs whole plant metabolism resulted ROS accumulation and oxidative stress damage (Ramadan *et al.,* 2025).

Sugar beet (*Beta vulgaris*. L) is a temperate biennial crop, though tropical varieties have been introduced, which symbolizes its importance. Tropical sugar beet is a moderately drought-tolerant plant has the ability to sustain up to 10 days of water withholding (Loho *et al.,* 2022). Plants develop natural defence mechanisms to survive under less water availability. Sugar beet activates antioxidant defence mechanisms by accumulating various osmolytes like proline scavenger enzymes such as CAT, SOD, POD and APX, along with phenolic compounds and flavonoids to neutralize reactive oxygen species (ROS) (Islam *et al.,* 2020). Under this crucial situation of climate change, it is required to improve drought tolerance in the plant through applying external supplements in the form of foliar sprays without disturbing soil health. During continuous stress, when plant's ability to overcome this stress is minimised, exogenous application of various components as growth regulators, minerals and anti-transpirants are emerged as an effective strategy to mitigate drought stress. JA and SA have been reported to increase antioxidant capacity and secondary metabolites accumulation under stressful conditions by regulating stomatal conductance, reducing water loss and preventing leaf senescence (Meier *et al.,* 2024). Chitosan serves as a natural biostimulant that mitigates the effect of harsh

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environmental conditions by sending signals to trigger cellular receptors and produce the secondary messengers and activating the transduction pathways. Kaolin is an anti-transpirant, forms a protective layer on leaf surface that helps plant to reduce water lose and providing mechanical support to the cells (Brito *et al.,* 2019). Foliar spray with Humic acid enhances the plant growth and tolerance under stress condition through modulating ROS signalling pathways (De Hita *et al.,* 2020). Besides these Boron, an essential micronutrient, has a role in plant defence under water scarcity by enhancing protective mechanisms in stressed plants (Naeem *et al.,* 2017). Supplementation with Silicon nanoparticles has become an effective method to mitigate the consequences of water stress, helps to reduce morphological and biochemical abnormalities in plants (Desoky *et al.,* 2025). To overcome the future water deficits caused by erratic climate patterns, fostering more research and application of foliar supplements to enhance tolerance, will be of utmost importance. It has been suggested that once sugar beet plants subjected to drought stress there is a possibility of developing tolerance through inducing stress memory in the plants (Leufen *et al.,* 2016). This may help in plant adaptation to certain extent but later resulted a severe yield loss under acute water depletion. In the view of these findings and as well as lack of studies on mitigation of drought stress effect in tropical sugar beet using foliar supplements, this research work was formulated with the objectives to improve drought tolerance in tropical sugar beet, through foliar treatments by enhancing defence mechanisms, which may reflect in sugar beet sustainability and production later.

MATERIALS AND METHODS

Experimental site

Two successful cultivation seasons during 2022-2023 and 2023-2024 were carried out in the research farm at the Institute of Agricultural Sciences (FAS), Binjhagiri, Odisha, with latitude 20°26' N, longitude 85°68' E, and 50.9 meters above sea level. During the experimental period the weather data recorded were the accumulated rainfall of 114.0mm and 65.4mm and the temperature range of 15.2°C- 37.5°C and 16-43.5 °C observed in 1st and 2nd year of study respectively.

Planting material and experimental details

Tropical sugar beet seeds of LS-6 variety were supplied by ICAR-IISR, Lucknow, and used as planting material for this experiment. A field experiment was conducted in December-May, provided with timely arrangements to avoid rain water. The experiment was laid out in a split- plot design with three main plots assigned for drought treatments such as normal irrigation for 3 days interval (NW), moderate drought stress by withholding irrigation for 7 days (S_1), severe drought stress by withholding irrigation for 14 days (S_2). The subplots contained 8 foliar sprays of Nano silicon (N-Si) 1mM, Humic Acid (HA) 300ppm, Jasmonic Acid (JA) 5µM, Salicylic Acid (SA) 100ppm, Boric Acid (B)1000ppm, Kaolin (KA) 5%, Chitosan (Chi) 200ppm and control (water spray) with three replications. Stress treatments were imposed after 50 DAS of normal growth and continued in cycles for two months with intermittent watering at recommended intervals, comprising 8 cycles of S_1 and 4 cycles of S_2. Along with stress treatments four sprays were applied in the interval of 15 days. First spray was given prior to stress induction as a preparatory measure for the plants. Samples were collected randomly from the subplots after 4th day of spray for morphological and biochemical analysis of 2nd, 3rd, and 4th foliar treatments before irrigating the field. Irrigation schedule was followed according to the stress plan and the parameters were analysed using fresh or dry leaf samples depending on the method used.

Antioxidant enzyme activity and Malondialdehyde (MDA) content in sugar beet leaves

Fresh healthy leaves were collected to extract the antioxidant enzymes using phosphate buffer. Catalase (CAT) activity was measured through the reaction between enzyme extract and H_2O_2 solution as mentioned by Beers & Sizer, (1952). Peroxidase (POD) activity was calculated as guaiacol peroxidase from the reaction between enzyme extract with guaiacol and H_2O_2 solutions according to Hammerschmidt *et al.,* (1982). Super Oxide Dismutase (SOD) activity was measured using NBT method as described by Dhindsa *et al.,* (1981). Ascorbate Peroxidase (APX) activity was calculated according to the Nakano & Asada, (1981) method. MDA was estimated using fresh leaf samples by following Cakmak & Horst, (1991) analytical procedure.

Non-enzymatic antioxidants and Osmolytes

Fresh leaf samples were used to determine total phenolic content (TPC) following catechol standard (Bray & Thrope, 1954). Tannin content was estimated from the dry leaf samples following Kavitha Chandran & Indera, (2016) procedure using Gallic acid standard. Dry leaf samples were used for the determination of Total flavonoid content (TFC) by following Zhishen *et al.,* (1999) procedure using quercetin standard. Proline amount in fresh leaves was measured using ninhydrin method (Bates *et al.,* 1973).

Statistical analysis

The average data of two seasons were analysed using SPSS v26 at probability level 0.05 ($P \leq 0.05$). The data including 3 levels of irrigation S (main factor), 8 foliar treatments T (sub factor), and 3 replications were subjected to homogeneity test before comparing the differences between means through ANOVA followed by LSD and Duncan tests. LSD test was expressed in this paper as a result of stage-wise analysis (after 2nd, 3rd, and 4th sprays separately). While Duncan test expressing the differences within the stress group NW, S_1, and S_2 separately after each spray in order to analyse the efficiency of treatments at each stress level.

RESULTS AND DISCUSSION

Effect of drought stress and foliar applications on proline content

Increasing irrigation intervals from 7 to 14 days and prolonged stress cycles led to a significant increment ($P \leq 0.05$) in proline content in sugar beet leaves in comparison to NW plants. The highest amount was recorded after 4th spray under S_2 in stress plants, with an increment of 305.81% against NW plants. A significant increase in proline content was observed with all foliar

Table 1: Interaction effect between various foliar treatments (T_1: Control, T_2: Nano Silicon, T_3: Humic Acid, T_4: Jasmonic Acid, T_5: Salicylic Acid, T_6: Boric Acid, T_7: Kaolin, T_8: Chitosan) and different drought stress levels on proline content in sugar beet leaves.

Foliar treatments	After 2nd spray			After 3rd spray			After 4th spray		
	Irrigation intervals								
	NW	S_1	S_2	NW	S_1	S_2	NW	S_1	S_2
T_1	1.23^{d}	1.88^{e}	2.44^{f}	2.10^{d}	3.17^{g}	5.97^{e}	2.41^{c}	4.87^{d}	9.78^{abc}
T_2	1.59^{c}	3.28^{b}	6.06^{de}	1.92^{e}	3.96^{e}	8.85^{cd}	2.10^{d}	3.99^{e}	8.95^{d}
T_3	1.65^{b}	2.91^{c}	6.25^{cd}	2.69^{a}	4.73^{b}	9.39^{ab}	2.90^{a}	4.78^{d}	9.45^{bc}
T_4	1.78^{a}	2.70^{d}	6.41^{c}	2.46^{b}	4.26^{d}	9.69^{ab}	2.69^{b}	5.19^{c}	9.82^{abc}
T_5	1.57^{c}	4.34^{a}	7.43^{a}	2.50^{b}	4.98^{a}	9.88^{a}	2.83^{a}	7.68^{a}	9.93^{a}
T_6	1.66^{b}	2.93^{c}	5.99^{e}	2.11^{d}	3.54^{f}	8.64^{d}	2.39^{c}	3.61^{f}	8.81^{d}
T_7	1.81^{a}	3.43^{b}	6.24^{cd}	2.51^{b}	4.01^{e}	9.32^{bc}	2.62^{b}	4.15^{e}	9.40^{c}
T_8	1.59^{c}	2.80^{cd}	6.86^{b}	2.32^{c}	4.51^{c}	9.75^{ab}	2.59^{b}	5.52^{b}	9.86^{ab}
Factors	S	T	SxT	S	T	SxT	S	T	SxT
LSD (P=0.05)	0.08	0.09	0.16	0.09	0.16	0.28	0.12	0.15	0.25

Statistical analysis represented as LSD (S): Least Significant Differences for stress, LSD(T): Least Significant Differences for Treatments, LSD(SxT): Least Significant Differences for interaction(n=3). Different letters indicated statistical significance($P \leq 0.05$) and referred to DMRT test.

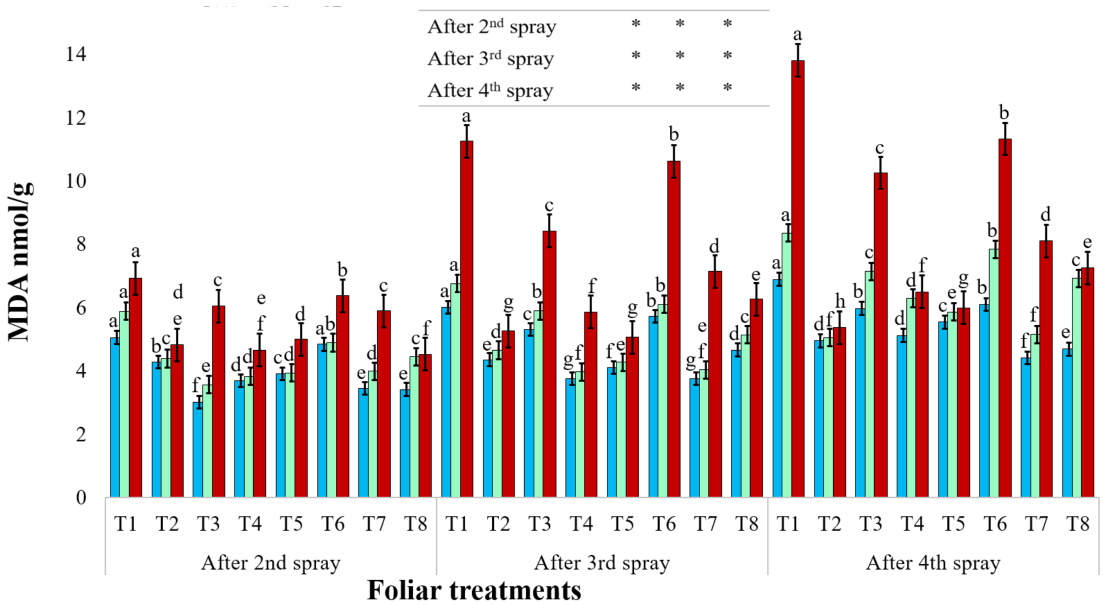

Fig. 1: Interaction effect between various foliar treatments (T_1: Control, T_2: Nano Silicon, T_3: Humic Acid, T_4: Jasmonic Acid, T_5: Salicylic Acid, T_6: Boric Acid, T_7: Kaolin, T_8: Chitosan) and different drought stress levels on MDA content in sugar beet leaves. Statistical analysis represents two years of average data. Error bars are expressed as means ± SE of three replicates. Different letters indicated statistical significance($P \leq 0.05$) and referred to DMRT test. * Referred to significance level at $P \leq 0.05$.

applications compared to stress control after second and third sprays. Treatments like N-Si, HA, B, and KA decreased the osmolyte after forth spray in S_1 as well as S_2, showing resistance under prolonged stress cycles, whereas the application of SA, Chi, and JA resulted in insignificant enhancement in proline content at $P \leq 0.05$ to alleviate stress effect, the value recorded as 9.93 $\mu g.g^{-1}$, 9.89 $\mu g.g^{-1}$ and 9.82 $\mu g.g^{-1}$ respectively after 4th spray under S_2 stress cycles compared to stress control 9.78 $\mu g.g^{-1}$ (Table 1).

Increasing proline content protects cell membrane, cellular enzymes and inhibits ROS in the plant cells under water shortage condition (Karimi *et al.,* 2023). Proline, an osmolyte that accumulates more when stress increases, was reported in tropical sugar beet (Loho *et al.,* 2025). Stress hormones JA and SA enhanced the proline accumulation in the sugar beet leaves by upregulating proline synthesis pathway performed as a defence mechanism under drought stress (Fugate *et al.,* 2018). According to Hidangmayum *et al.,* 2023, chitosan increases plant tolerance to stress through multiple mechanisms by activating the antioxidant defence system, which is evident in this research. Application of other treatments such as N-Si, B, KA,and HA balanced the stress effects in sugar beet leaves by reducing the need for proline accumulation which efficiently mitigated the impact of drought stress, showing their ability to overcome this stress (Zadegan *et al.,* 2023).

Effect of drought stress and foliar applications on MDA content

Imposing water stress caused significant lipid

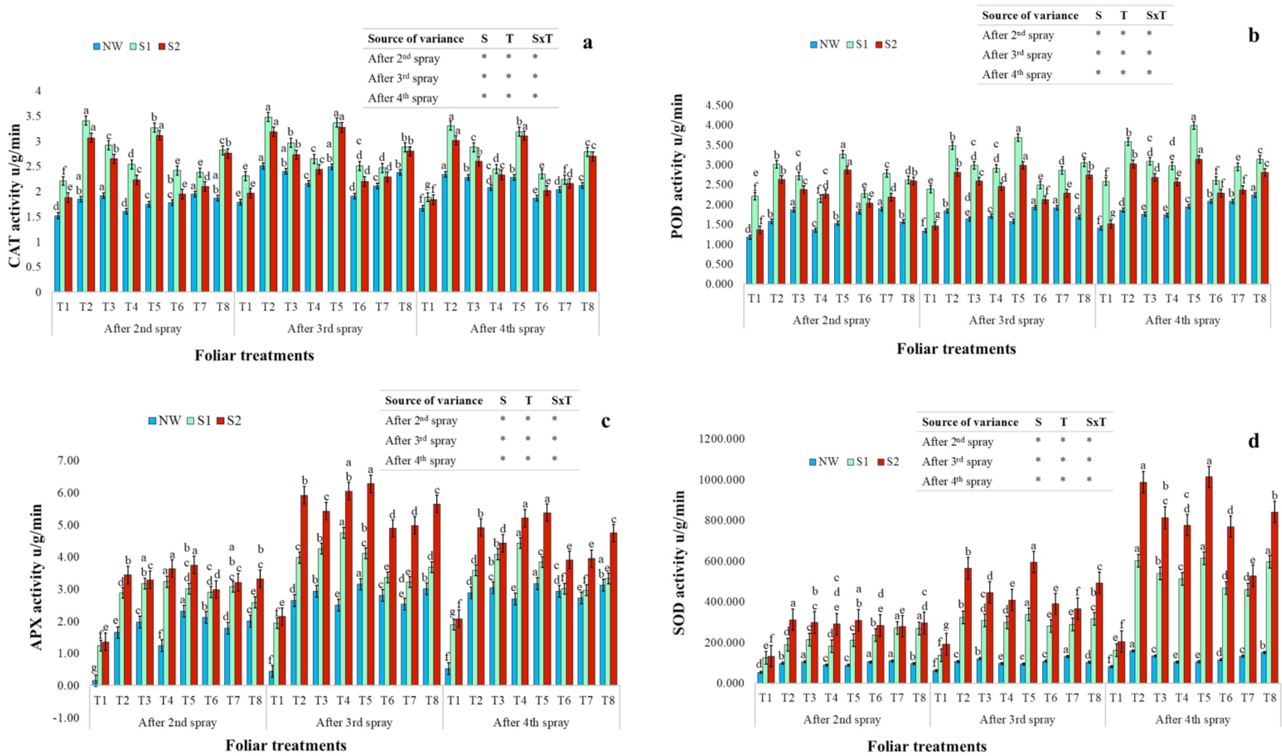

Fig. 2: Interaction effect between various foliar treatments (T_1: control, T_2: Nano Silicon, T_3: Humic Acid, T_4: Jasmonic Acid, T_5: Salicylic Acid, T_6: Boric Acid, T_7: Kaolin, T_8: Chitosan) and different drought stress levels on (a)CAT, (b)POD, (c)APX, (d)SOD activities in sugar beet leaves. Statistical analysis represents two years of average data. Error bars are expressed as means ± SE of three replicates. Different letters indicated statistical significance ($P \leq 0.05$) and referred to DMRT test. * Referred to significance level at $P \leq 0.05$.

peroxidation in sugar beet leaves, which was measured in terms of MDA amount. The prolongation of the drought period from 7 days to 14 days resulted in more accumulation of MDA, it was higher with the continuous drought condition and reached up to 100.29% over NW control after 2 months of stress cycles (S_2). Higher MDA level indicates greater cellular membrane damage and oxidative stress. Application of different sprays like N-Si, HA, JA, SA, B, KA, and Chi induced a reduction in MDA level in the sugar beet leaves under different stress durations ($P \leq 0.05$). N-Si (1mM) and SA (100ppm) marked as the most promising treatment with a decline in MDA content reached up to 61.09% and 56.59% less than stress control plants respectively under S_2 stress cycles after 4th spray (Fig. 1). MDA is an indicator for lipid damage in plant cells, increasing its accumulation under stress represents the level of cell injury in the leaves (Abdo *et al.,* 2024). According to Khan *et al.,* (2024), exogenous application of JA can reduce the production of ROS and prevent its toxicity on the cell membrane, N-Si can also reduce MDA accumulation by reducing stress signalling molecules through enhancing respiratory enzyme activity and resulted decreasing in oxidative stress (Alkahtani *et al.,* 2021). SA has been reported as an effective supplement to mitigate oxidative stress and reduce lipid peroxidation in plants (Mohi-Ud-Din *et al.,* 2021). Kaolin is also an anti-transpirant, showing its effective role in reducing MDA content by protecting the leaf surface and reducing evaporation (Faghihi *et al.,* 2025).

Effect of drought stress and foliar applications on antioxidant enzyme activity

Different behaviours were observed for antioxidant enzymes in response to stress severity. Drought stress increased antioxidant enzymes activity at $P \leq 0.05$ in S_1 and S_2 after every spray over normally irrigated (NW) plants. CAT and POD activity increased when the plants were subjected to 7 days of water withholding, while it reduced under 14 days irrigation interval compared to S_1 moderately stressed plants, showing acute stress effect. However, CAT increment was observed after 2nd spray to 3rd spray, showing highest accumulation of 2.31 u.g^{-1}.min^{-1} under S_1 and 1.97 u.g^{-1}.min^{-1} in S_2 after 3rd spray, tend to decline with the prolongation of drought exposing period after 4th spray (Fig. 2a). The prolongation of drought period up to two months stress cycles resulted continuous increase in POD activity which reached the peak of 2.58 u.g^{-1}.min^{-1}after 4th spray under S_1 and 1.51 u.g^{-1}.min^{-1} in S_2 compared with NW 1.41 u.g^{-1}min^{-1} (Fig. 2b). Significant rising in APX activity recorded from NW to S_1 and S_2 control non-treated plants ($P \leq 0.05$). Similar to CAT, the increment in APX activity after 3rd spray was less than after 4th spray for both S_1 and S_2, however, a continuous increase was recorded in case of NW. Maximum activity of APX in sugar beet leaves was recorded 2.15 u.g^{-1}.min^{-1} in S_2 after 3rd spray and declined to 2.08 u.g^{-1}.min^{-1} in S_2 control plants after 2 months of prolonged stress (Fig. 2c). SOD activity also showed a continuous increase from normal watering plants to moderate to

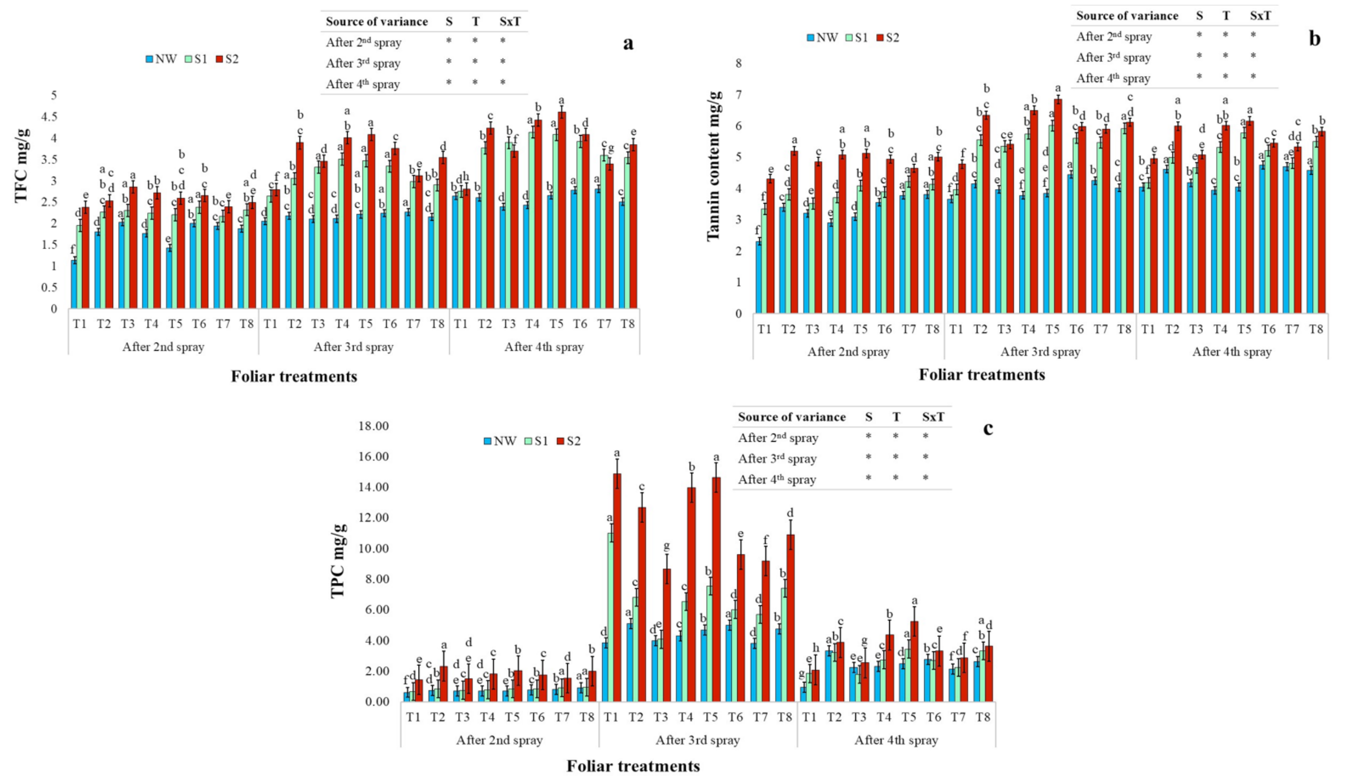

Fig. 3: Interaction effect between various foliar treatments (T_1: control, T_2: Nano Silicon, T_3: Humic Acid, T_4: Jasmonic Acid, T_5: Salicylic Acid, T_6: Boric Acid, T_7: Kaolin, T_8: Chitosan) and different drought stress levels on (a)TFC, (b)Tannin, (c)TPC in sugar beet leaves. Statistical analysis represents two years of average data. Error bars are expressed as means ± SE of three replicates. Different letters indicated statistical significance($P \leq 0.05$) and referred to DMRT test. * Referred to significance level at $P \leq 0.05$.

severe stress, this increment has been shown with the elongation of stress cycles till the end of stress imposition after 4th spray, where its activity was reached to 205.29 $u.g^{-1}.min^{-1}$ under 14 days of irrigation withholding (Fig. 2d). The enzyme activities were significantly enhanced by foliar supplementation under water deficit condition. Based on the comparison between the foliar treatments, CAT, POD, and SOD activities were increased under SA and N-Si application, where JA seemed as most promising in APX activity in S_1 cycle. Under S_2, after four cycles of stress till 4th spray, SA increased 69.02%, 107.95%, 158.65%, and 393.68% in CAT, POD, APX, and SOD activity, respectively against their non-treated stress controls (Fig. 2). Drought stress leads to the accumulation of reactive oxygen species (ROS) and disturbs plant cell functions (Soares *et al.,* 2019). Antioxidant enzymes play a crucial role in the scavenging process of ROS molecules. SOD is the first defence line against ROS injury, reduces the effect of superoxide anion, followed by other enzymes POD, CAT, and APX. APX increased rapidly until CAT and POD reached a certain level, then it declined when CAT is inhibited by severe stress, and in the same time POD increased in plant leaves to fulfil the lacking in H_2O_2 scavenging capacity (Wang *et al.,* 2019), which was clear and consistent in this study at prolonged stress after 4th spray.

Studies exhibited the effect of supplements in support of our results as SA and N-Si involved ROS detoxification by enhancing the expression of antioxidant enzyme genes and stimulating their activities (Khodadadi *et al.,* 2020). Application of JA at 5µM also showed its effectiveness in alleviating moderate drought stress by inducing scavenger enzymes activities (Ghaffari *et al.,* 2019).

Effect of drought stress and foliar applications on phenolic compounds

Increasing drought severity from 7 days to 14 days of irrigation withholding caused a significant increment at $P \leq 0.05$ in all phenolic components (TFC, TPC and Tannins) in sugar beet leaves throughout the imposed stress period. Flavonoid and tannin content continuously increased in both stress control plants under S_1 and S_2 of drought stress cycles. Under stress, after 4th spray, the values reached the highest accumulation of TFC was 2.75 $mg.g^{-1}$ in S_1 and 2.81$mg.g^{-1}$in S_2, where tannin increased 4.18 $mg.g^{-1}$in S_1 and 4.95 $mg.g^{-1}$in S_2 presented in Fig. 3. While TPC declined >80% after third to forth spray in stressed plants (Fig. 3). At an increase in stress cycles, foliar treatments enhanced TFC and tannin accumulation in the leaves, where TPC was reduced after 3rd spray under prolonged water scarcity. After 4th spray, SA, Chitosan, and JA were marked as the most effective foliar treatments enhancing the accumulation of these components under S_1 & S_2 stress in comparison to respective control plants. At initial stress of S_1, Boron and HA elevated TFC content, where Kaolin and Chitosan application enhanced TPC as well as tannin levels in sugar beet leaves. Under S_2 drought cycles after 4th spray, SA induced a significant increment up to 64.06%, 151.95%, and 24.44% in TFC, TPC, and tannin content respectively at P≤0.05 (Fig. 3). Under prolonged stress, phenolic levels reduced at the late stage of drought (Ghasemi *et al.,* 2023), which was observed after 4th spray. Phenolic compounds are an important part of the non-enzymatic scavenging system, phytohormones like SA and JA can increase the accumulation of these secondary metabolites by regulating their gene expression (Elsisi *et al.,* 2024). Flavonoids take place as co-substrate for peroxidases in H_2O_2 scavenging process (Namjoyan *et al.,* 2020), which explains its enhancement after 4th spray. As per results obtained, the degradation in TPC after 3rd foliar spray indicated effectiveness of these treatments acted differently in controlling ROS production (Alkahtani *et al.,* 2021). It is reported that tannins a type of polyphenol, play a crucial role in plant-environment interactions. The synthesis and accumulation of tannin in plants is regulated by defence related phytohormones (Iqbal & Poór, 2024), which was enhanced with SA and JA applications and its effect was showed consistent with the results explained in this investigation.

CONCLUSION

Climate change intensifies water shortage, resulted sever drought, which may be overcome by enhancing drought tolerance activities in plants by exogenous foliar treatments. To cope with certain adverse situations, plants induce several alternate physiological processes and modulate molecular mechanisms. Different irrigation intervals decreased the water availability to the plant, as a result, the sugar beet plant suffered from cellular membrane damage and oxidative stress by producing enzymatic and non-enzymatic antioxidants and accumulated osmolytes as proline, the effect was more acute under severe and prolonged drought cycles. Foliar treatments like N-Si, HA, JA, SA, B, KA, and Chi were surely beneficial to alleviate the drought impact in sugar beet. Among these, SA, JA, Chi and N-Si exhibited further enhancement in the internal defence mechanisms based on their type, degree and mode of action, ultimately helped the sugar beet plant to tolerate the harsh effects of water deficit stress.Depletion in water level is a major issue explaining yield loss caused due to climate change, somehow managed by using foliar applications, could be a effective strategy for the farmars to overcome certain harse situations of drought to oversee yield loss.

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