International Journal of Agriculture and Plant Science
www.agriculturejournal.in
Online ISSN: 2664-7664
Received: 30-04-2026, Accepted: 31-05-2026, Published: 15-06-2026
Volume 8, Issue 2, 2026, Page No. 274-281

Understanding the effect of plant growth regulators on Indian mustard [*Brassica juncea* (L.) *Czern* and *Coss.*] under saline water irrigation condition

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DOI: https://doi.org/10.66856/ijaps.2026.8.2.8126

Abstract
Salinity stress is one of the most important abiotic factors limiting the growth, physiological efficiency, and productivity of Indian mustard (Brassica juncea L. Czern. & Coss.), particularly in arid and semi-arid regions where the use of saline irrigation water is common. The present investigation was undertaken to evaluate the effectiveness of plant growth regulators, namely gibberellic acid (GA_3) and salicylic acid (SA), in alleviating the adverse effects of salinity stress on physiological and biochemical characteristics of Indian mustard. The experiment was conducted under greenhouse conditions using different levels of saline irrigation water in combination with foliar application of plant growth regulators. Salinity stress adversely affected plant water relations, membrane stability, photosynthetic pigments, and protein content, while promoting the accumulation of various osmoprotectants and antioxidant metabolites. Application of plant growth regulators significantly mitigated the detrimental effects of salinity by improving relative water content, membrane stability, chlorophyll retention, and protein synthesis. Moreover, treated plants exhibited enhanced accumulation of compatible solutes and antioxidant compounds, which contributed to improved osmotic adjustment and protection against oxidative damage. Among the treatments, salicylic acid proved more effective than gibberellic acid in enhancing stress tolerance and maintaining physiological and biochemical homeostasis under saline conditions. The results suggest that exogenous application of plant growth regulators, particularly salicylic acid, can be an effective and environmentally friendly approach for improving salinity tolerance and sustaining crop performance in Indian mustard under saline irrigation conditions.

Keywords: *Brassica juncea*, salinity stress, gibberellic acid, salicylic acid, antioxidant enzymes, osmotic adjustment

Introduction
Indian mustard [*Brassica juncea* (L.) Czern. & Coss.], belonging to the family Brassicaceae (Cruciferae), is one of the most important oilseed crops cultivated worldwide and ranks second among oilseed crops in India after soybean. The crop is valued for its edible oil, condiments, and fodder purposes; however, its productivity is greatly constrained by various abiotic stresses, particularly soil salinity. Salinity adversely affects plant growth and development by disturbing osmotic balance, causing ionic toxicity, impairing nutrient uptake, and inducing oxidative stress. These effects lead to reductions in chlorophyll content, photosynthetic efficiency, protein synthesis, and overall metabolic activities, ultimately resulting in lower growth and yield of mustard crops (Garg *et al*., 1993; Dubey, 1997; Munns, 2003) [8, 10, 14]. In addition, salinity has been reported to reduce oil content and seed quality in mustard (Shanna and Manchanda, 1997) [25]. With the increasing extent of saline soils and the use of poor-quality irrigation water in arid and semi-arid regions, salinity has emerged as a major threat to sustainable mustard production.

Traditional methods for managing salinity, such as leaching salts through excessive irrigation, are becoming less practical because of limited freshwater resources and environmental concerns (Steppuhn *et al*., 1991) [27]. Therefore, attention has shifted towards the use of plant growth regulators (PGRs) as an economical and eco-friendly approach to improve crop tolerance against salinity stress. Among various PGRs, gibberellic acid (GA_3) and salicylic acid (SA) have shown promising roles in regulating physiological and biochemical processes under stress conditions. Gibberellic acid promotes plant growth, cell elongation, and metabolic activities, whereas salicylic acid acts as an important signalling molecule involved in stress perception and activation of defence responses (Ram *et al.*, 2025) [19]. Exogenous application of GA_3 and SA has been reported to enhance antioxidant defence systems, improve osmotic adjustment, maintain membrane stability, and protect photosynthetic pigments under saline conditions (Patel *et al*., 2019; Purohit *et al*, 2020; Vadhel *et al*., 2025; Chovatiya *et al*., 2024) [7, 17, 18, 28]. Therefore, the present investigation was undertaken to evaluate the effect of GA_3 and SA on physiological and biochemical responses of Indian mustard under saline water irrigation conditions, with the aim of developing effective strategies for improving salinity tolerance and sustaining mustard productivity in salt-affected areas.

Materials and Methods
The present investigation was carried out during the Rabi season of 2022–23 under greenhouse conditions at the Postgraduate Laboratory, Department of Biochemistry, College of Agriculture, Junagadh Agricultural University. The experiment was conducted using pot culture to study the effect of plant growth regulators on Indian mustard under saline water irrigation conditions. Seeds of Indian

mustard [*Brassica juncea* (L.) Czern. & Coss.] variety GDM-4 were used for the experiment. Saline water was collected from the Veraval coastal region and diluted with normal water to obtain two salinity levels of 4 dS m^{-1} and 8 dS m^{-1}, while tap water served as the control. The soil used in the experiment was calcareous and slightly alkaline in nature, collected from the Agronomy Farm of the university. The soil possessed normal electrical conductivity, low available nitrogen, medium organic carbon and phosphorus content, and high potassium status.

The experiment was laid out in a Factorial Completely Randomized Design (FCRD) with three replications comprising two factors: salinity levels and plant growth regulator treatments. The salinity treatments included I_0 (tap water/control), I_1 (4 dS m^{-1}), and I_2 (8 dS m^{-1}). The plant growth regulator treatments consisted of T_1: control (no spray), T_2: water spray, T_3: GA_3 @ 50 ppm, T_4: GA_3 @ 100 ppm, T_5: salicylic acid (SA) @ 0.2 µM, and T_6: salicylic acid (SA) @ 0.4 µM. Earthen pots were filled with 15 kg of soil, and after germination, five healthy plants were maintained per pot following thinning. Foliar application of plant growth regulators was carried out twice, at 15 and 25 days after sowing (DAS). Leaf samples were collected five days after the second spray, immediately placed on ice, and transported to the laboratory for biochemical analysis.

Relative water content (RWC) was estimated following the method of Weatherley (1962) [31], whereas membrane stability index (MSI) was determined according to Sairam *et al*. (2005) [21]. Total phenol content was estimated by the method of Bray and Thorpe (1954) [5], while chlorophyll content was determined following Arnon (1949) [3]. Total soluble protein was estimated according to Lowry *et al.* (1951) [12], free amino acids by the method of Moore and Stein (1948) [15], total soluble sugars by the anthrone method of Dubois *et al*. (1956) [9], and reducing sugars following Nelson (1944) [16] as modified by Somogyi (1952) [22]. Proline content was estimated using the method of Bates *et al.* (1973) [4], ascorbic acid content was determined according to the method of Sadasivam and Manickam (1996) [20], and glycine betaine content was analysed following Hendawey (2015) [11]. All biochemical analyses were carried out using freshly collected leaf samples to ensure accuracy and reliability of the observations.

Results and Discussion

The results of the present investigation revealed that salinity stress adversely affected the physiological efficiency and biochemical composition of Indian mustard plants. Increasing salinity levels caused significant alterations in water relations, membrane stability, photosynthetic pigments, osmolyte accumulation, and antioxidant metabolites. However, the exogenous application of plant growth regulators, particularly gibberellic acid (GA_3) and salicylic acid (SA), effectively alleviated the adverse effects of salinity stress by improving physiological performance and biochemical adaptability in mustard plants. The detailed results obtained during the course of investigation are discussed below.

Physiological Parameters

Relative water content was significantly influenced by salinity levels and plant growth regulator treatments. Increasing salinity progressively reduced leaf RWC at both growth stages (Table 1). At 20 DAS, mean RWC decreased from 84.24% under normal irrigation (I_0) to 75.27% at 4 dS m^{-1} (I_1) and further to 72.08% at 8 dS m^{-1} (I_2). A similar trend was observed at 30 DAS where RWC declined from 86.76% under control conditions to 77.22 and 75.87% under I_1 and I_2, respectively. These reductions indicate the adverse effect of salinity on plant water relations due to decreased soil water potential and restricted water uptake by roots. Among plant growth regulator treatments, foliar application of salicylic acid at 0.4 µM (T_6) recorded the highest RWC (78.98 and 82.36% at 20 and 30 DAS, respectively), whereas untreated plants (T_1) showed the lowest values (73.89 and 76.21%). The improvement in RWC by SA may be attributed to enhanced osmotic adjustment, maintenance of cellular turgidity, and improved membrane integrity under saline conditions. Similar beneficial effects of salicylic acid on plant water status under salinity have been reported in mustard and other crops.

Table 1: Effect of plant growth regulators on leaf Relative Water Content (%) of Indian mustard under irrigation of saline water

Treatment (T)	20 DAS (G_1)			Mean (I)	30 DAS (G_2)			Mean (I)
	I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)		I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)	
T_1 (Control)	81.13	72.33	68.2	73.89	83.12	74.31	71.19	76.21
T_2 (Water spray)	83.41	74.12	71.02	76.18	84.41	75.84	72.05	77.43
T_3 (GA_3@50ppm)	84.66	75.88	72.95	77.83	87.65	77.88	75.99	80.51
T_4 GA_3@100ppm)	84.96	76.31	73.14	78.14	88.91	78.19	78.84	81.98
T_5 (0.2 µM SA)	85.33	75.96	73.21	78.17	87.32	78.06	78.22	81.20
T_6 (0.4 µM SA)	85.97	77.02	73.96	78.98	89.12	79.05	78.91	82.36
Mean	84.24	75.27	72.08		86.76	77.22	75.87	
	I	T	I x T		I	T	I x T	
S. Em±	0.95	1.34	2.32		0.98	1.39	2.41	
CD at 5 %	2.72	NS	NS		2.82	3.99	NS	
CV%	5.22				5.23			

Membrane stability index decreased significantly with increasing salinity stress (Table. 2). Mean MSI values at 20 DAS declined from 72.22% under control irrigation to 55.91% and 52.04% under 4 and 8 dS m^{-1} salinity, respectively. Similar reductions were recorded at 30 DAS, where MSI decreased from 72.00% under control conditions to 55.69 and 51.82% under I_1 and I_2 treatments. These results indicate increased membrane injury and electrolyte leakage caused by salinity-induced oxidative stress. Among the growth regulator treatments, T_6 (SA 0.4 µM) produced the highest MSI values (59.62% at 20 DAS and approximately 62.74% at 30 DAS), while untreated plants recorded the lowest membrane stability. Enhanced MSI under salicylic acid treatment may be due to its role in reducing lipid peroxidation and maintaining structural integrity of cellular membranes through activation of antioxidant defense mechanisms. These findings corroborate earlier reports that exogenous application of SA and gibberellic acid (GA_3) enhances salt tolerance by improving water relations, maintaining membrane integrity, and reducing oxidative injury under saline conditions (Tuna *et al*., 2008) [29].

Table 2: Effect of plant growth regulators on membrane stability index (%) of Indian mustard under irrigation of saline water

Treatment (T)	20 DAS(G_1)			Mean (I)	30 DAS(G_2)			Mean (I)
	I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)		I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)	
T_1 (Control)	68.41	52.78	48.87	53.35	68.19	52.56	48.65	53.13
T_2 (Water spray)	70.40	54.66	50.65	55.24	70.18	54.44	50.43	55.02
T3(GA3@50ppm)	71.54	55.55	52.11	56.40	71.32	55.33	51.89	56.18
T_4(GA_3@100ppm)	73.44	56.44	52.54	57.47	73.55	56.22	52.32	57.36
T_5 (0.2 µM SA)	73.77	57.35	53.65	58.26	73.22	57.13	53.43	57.93
T_6 (0.4 µM SA)	75.78	58.66	54.43	59.62	75.56	58.44	54.21	
Mean	72.22	55.91	52.04		72.00	55.69	51.82	
	I	T	I x T		I	T	I x T	
S. Em±	0.70	0.99	1.71		0.70	0.99	1.71	
CD at 5 %	2.00	2.80	NS		2.00	2.70	NS	
CV%	5.20				5.21			

Biochemical Parameters

Salinity stress induced profound biochemical alterations in Indian mustard plants, reflecting both metabolic disruption and adaptive defense responses. Accumulation of secondary metabolites, osmoprotectants, and antioxidant compounds under saline conditions indicates activation of protective mechanisms against osmotic and oxidative stress. Application of plant growth regulators, particularly salicylic acid (SA), further strengthened these defense responses and improved metabolic stability under stress conditions.

Total phenol content increased significantly with increasing salinity levels (Table 3). At 20 DAS, phenol content increased from 5.04 mg g^{-1} under control conditions to 9.43 and 14.34 mg g^{-1} under I_1 and I_2, respectively. At 30 DAS, the corresponding values were 7.99, 11.67 and 17.53 mg g^{-1}. Increased phenolic accumulation under saline conditions reflects activation of secondary metabolic pathways involved in protection against oxidative stress. Among treatments, T_6 (SA 0.4 µM) recorded the highest phenol content (13.32 and 16.69 mg g^{-1} at 20 and 30 DAS, respectively), suggesting stimulation of phenolic metabolism and improved antioxidant capacity. Salicylic acid is known to induce phenylalanine ammonia lyase activity, thereby enhancing synthesis of phenolic compounds indicating stimulation of the phenylpropanoid pathway and enhanced synthesis of secondary metabolites involved in stress defense (Sarwat *et al*., 2016) [23]. Increased phenolic accumulation under SA treatment may have contributed to improved antioxidant capacity and membrane protection.

Table 3: Effect of plant growth regulators on leaf total phenol (mg/g) of Indian mustard under irrigation of saline water

Treatment (T)	20 DAS (G_1)			Mean (I)	30 DAS (G_2)			Mean (I)
	I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)		I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)	
T_1 (Control)	4.31	7.11	12.62	8.01	5.44	8.22	14.74	9.47
T_2(Water spray)	4.02	7.09	12.55	7.89	6.10	9.12	15.66	10.29
T_3(GA_3@50ppm)	5.23	8.03	13.01	8.76	8.44	10.22	16.23	11.63
T_4(GA_3@100ppm)	5.01	9.01	13.55	9.19	9.55	11.32	15.81	12.23
T_5 (0.2 µM SA)	5.20	12.14	14.01	10.45	8.64	16.45	17.11	14.07
T_6 (0.4 µM SA)	6.44	13.22	20.31	13.32	9.77	14.66	25.63	16.69
Mean	5.04	9.43	14.34		7.99	11.67	17.53	
	I	T	I x T		I	T	I x T	
S. Em±	0.12	0.18	0.31		0.16	0.22	0.39	
CD at 5 %	0.37	0.52	0.90		0.46	0.65	1.13	
CV%	5.30				5.50			

Salinity stress caused a marked reduction in soluble protein content. Mean protein content at 20 DAS decreased from 87.84 mg g^{-1} under control conditions to 78.38 and 75.12 mg g^{-1} under I_1 and I_2, respectively (Table 4). Similar reductions were observed at 30 DAS. Decreased protein levels under salinity may result from inhibition of protein synthesis and enhanced protein degradation caused by ionic imbalance and oxidative damage. Application of plant growth regulators improved protein accumulation. The highest protein content was observed under T_6 (82.50 and 82.81 mg g^{-1} at 20 and 30 DAS), followed closely by T_5. These findings indicate that salicylic acid helps maintain protein synthesis and protects cellular proteins from stress-induced degradation. The improvement in protein content suggests that SA helped maintain protein synthesis and protected cellular proteins from oxidative degradation. Similar observations have been reported by Shah (2007) [24] and Singh and Sharma (1996) [26].

Table 4: Effect of plant growth regulators on leaf total soluble protein (mg/g) of Indian mustard under irrigation of saline water

Treatment (T)	20 DAS (G_1)			Mean (I)	30 DAS (G_2)			Mean (I)
	I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)		I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)	
T_1 (Control)	86.30	76.60	70.30	77.73	86.50	76.80	70.50	77.93
T_2 (Water spray)	87.47	76.80	71.20	78.49	87.67	77.01	71.60	78.76
T_3 (GA_3@50ppm)	87.90	78.20	75.20	80.43	88.20	78.60	75.80	80.87
T_4(GA_3@100ppm)	88.23	79.60	77.20	81.68	88.60	79.60	77.60	81.81
T_5 (0.2 µM SA)	88.35	79.30	77.90	81.85	88.67	79.80	78.10	82.19
T_6 (0.4 µM SA)	88.80	79.80	78.90	82.50	89.22	80.00	79.20	82.81
Mean	87.84	78.38	75.12		88.14	78.64	75.47	
	I	T	I x T		I	T	I x T	
S. Em±	0.99	1.40	2.42		0.99	1.40	2.43	
CD at 5 %	2.84	NS	NS		2.85	NS	NS	
CV%	5.21				5.20			

Free amino acid content increased significantly under salinity stress. At 20 DAS, mean amino acid content increased from 1.80 µg g^{-1} under control conditions to 2.57 µg g^{-1} under severe salinity. At 30 DAS, values increased from 6.12 to 9.12 µg g^{-1} (Table 5). Accumulation of amino acids under salinity acts as an adaptive mechanism for osmotic regulation and nitrogen storage. The highest amino acid content was recorded under T_6 treatment (2.84 and 9.84 µg g^{-1} at 20 and 30 DAS, respectively), indicating enhanced osmotic adjustment and stress tolerance. These findings agree with Garg *et al.* (1993) [10], who reported enhanced amino acid accumulation in mustard under salt stress.

Table 5: Effect of plant growth regulators on leaf free amino acid (µg/g) of Indian mustard under irrigation of saline water

Treatment (T)	20 DAS(G_1)			Mean (I)	30 DAS(G_2)			Mean (I)
	I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)		I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)	
T_1 (Control)	0.80	1.12	2.19	1.37	4.00	4.12	6.52	4.88
T_2 (Water spray)	1.49	1.27	2.34	1.70	4.49	4.38	6.88	5.25
T_3(GA_3@50ppm)	1.57	1.41	2.39	1.79	5.57	5.12	9.21	6.63
T_4(GA_3@100ppm)	1.78	1.57	2.54	1.96	6.78	5.74	9.78	7.43
T_5 (0.2 µM SA)	2.43	2.51	2.75	2.56	7.45	8.33	10.20	8.66
T_6 (0.4 µM SA)	2.71	2.62	3.18	2.84	8.45	8.96	12.10	9.84
Mean	1.80	1.75	2.57		6.12	6.11	9.12	
	I	T	I x T		I	T	I x T	
S. Em±	0.02	0.03	0.06		0.09	0.13	0.22	
CD at 5 %	0.07	0.10	0.18		0.26	0.37	0.65	
CV%	5.52				5.45			

Total soluble sugar content increased progressively with increasing salinity levels (Table 6). At 20 DAS, mean sugar content increased from 6.50% under control conditions to 7.78% under severe salinity. At 30 DAS, sugar content increased from 7.95% to 9.03%. Accumulation of sugars under stress conditions contributes to osmotic adjustment, membrane stabilization and maintenance of cellular metabolism. Similarly, reducing sugar content also increased with salinity (Table 7). Mean reducing sugar values increased from 11.99 to 12.62 mg g^{-1} at 20 DAS and from 12.15 to 12.67 mg g^{-1} at 30 DAS with increasing salinity levels. T_6 treatment produced the highest reducing sugar content, indicating improved carbohydrate metabolism and osmoprotection under stress. Enhanced sugar accumulation under SA treatment may have contributed to osmoprotection and maintenance of metabolic activity under salinity stress (Arunkumar *et al.,* 2012) [2].

Table 6: Effect of plant growth regulators on leaf total soluble sugar (%) of Indian mustard under irrigation of saline water

Treatment (T)	20 DAS (G_1)			Mean (I)	30 DAS (G_2)			Mean (I)
	I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)		I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)	
T_1 (Control)	6.29	6.59	7.36	6.75	8.22	8.11	8.22	8.18
T_2 (Water spray)	6.32	6.63	7.38	6.78	7.66	7.98	9.22	8.29
T_3(GA_3@50ppm)	6.53	7.18	7.79	7.17	7.55	7.70	8.11	7.79
T_4(GA_3@100ppm)	6.63	7.03	8.01	7.22	7.66	8.88	8.22	8.25
T_5 (0.2 µM SA)	6.55	6.99	7.81	7.12	7.57	8.33	10.20	8.70
T_6 (0.4 µM SA)	6.69	7.15	8.33	7.39	9.01	8.22	10.20	9.14
Mean	6.50	6.93	7.78		7.95	8.20	9.03	
	I	T	I x T		I	T	I x T	
S. Em±	0.08	0.12	0.21		0.10	0.15	0.26	
CD at 5 %	0.25	0.35	NS		0.30	0.42	0.73	
CV%	5.23				5.28			

Table 7: Effect of plant growth regulators on leaf reducing sugar (mg/g) of Indian mustard under irrigation of saline water

Treatment (T)	20 DAS(G_1)			Mean (I)	30 DAS(G_2)			Mean (I)
	I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)		I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)	
T_1 (Control)	11.91	12.17	12.49	12.19	12.04	12.23	12.53	12.27
T_2 (Water spray)	11.92	12.19	12.50	12.20	12.05	12.23	12.55	12.28
T_3(GA_3@50ppm)	11.98	12.23	12.61	12.27	12.14	12.34	12.66	12.38
T_4(GA_3@100ppm)	12.08	12.31	12.65	12.35	12.19	12.39	12.69	12.42
T_5 (0.2 µM SA)	11.99	12.35	12.69	12.34	12.20	12.40	12.77	12.46
T_6 (0.4 µM SA)	12.04	12.37	12.76	12.39	12.29	12.43	12.84	12.52
Mean	11.99	12.27	12.62		12.15	12.34	12.67	
	I	T	I x T		I	T	I x T	
S. Em±	0.15	0.21	0.37		0.15	0.22	0.37	
CD at 5 %	0.43	NS	NS		0.46	0.35	NS	
CV%	5.21							

Photosynthetic pigments were adversely affected by salinity stress, as evident from reductions in chlorophyll a, chlorophyll b, and total chlorophyll content (Tables 8–10). Salinity stress significantly reduced chlorophyll a, chlorophyll b and total chlorophyll content. Mean chlorophyll a content decreased from 1.77 mg g^{-1} under control irrigation to 1.34 mg g^{-1} under severe salinity at 20 DAS. Similar reductions occurred at 30 DAS. Chlorophyll b and total chlorophyll also showed a comparable decline with increasing salinity levels.

Among the treatments, T_6 recorded the highest chlorophyll a (1.64 and 1.86 mg g^{-1}), chlorophyll b (0.73 and 0.95 mg g^{-1}) and total chlorophyll content (2.37 and 2.81 mg g^{-1}) at 20 and 30 DAS, respectively. The protective effect of SA on photosynthetic pigments may be attributed to improved antioxidant defence, stabilization of chloroplast membranes, and enhanced photosynthetic efficiency under stress conditions (Yusuf *et al*., 2008; Chawla *et al.*, 2013; Sarwat *et al*., 2016) [6, 23, 30].

Table 8: Effect of plant growth regulators on leaf chlorophyll a content (mg/g) of Indian mustard under irrigation of saline water

Treatment (T)	20 DAS (G_1)			Mean (I)	30 DAS (G_2)			Mean (I)
	I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)		I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)	
T_1 (Control)	1.71	1.36	1.25	1.44	1.78	1.56	1.63	1.66
T_2 (Water spray)	1.73	1.39	1.34	1.49	1.86	1.75	1.88	1.83
T_3(GA_3@50ppm)	1.69	1.33	1.21	1.41	1.97	1.78	1.55	1.77
T_4(GA_3@100ppm)	1.72	1.41	1.30	1.48	1.88	1.61	1.63	1.71
T_5 (0.2 µM SA)	1.84	1.44	1.42	1.57	1.94	1.74	1.63	1.77
T_6 (0.4 µM SA)	1.91	1.52	1.49	1.64	2.14	1.69	1.75	1.86
Mean	1.77	1.41	1.34		1.93	1.69	1.68	
	I	T	I x T		I	T	I x T	
S. Em±	0.02	0.03	0.05		0.01	0.02	0.05	
CD at 5 %	0.05	0.07	NS		0.05	0.08	NS	
CV%	5.20				5.21			

Table 9: Effect of plant growth regulators on leaf chlorophyll b content (mg/g) of Indian mustard under irrigation of saline water

Treatment (T)	20 DAS (G_1)			Mean (I)	30 DAS (G_2)			Mean (I)
	I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)		I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)	
T_1 (Control)	0.69	0.54	0.44	0.56	0.88	0.78	0.66	0.77
T_2 (Water spray)	0.71	0.51	0.42	0.55	0.89	0.63	0.62	0.71
T_3(GA_3@50ppm)	0.78	0.62	0.46	0.62	0.83	0.96	0.78	0.86
T_4(GA_3@100ppm)	0.81	0.64	0.49	0.65	0.92	0.79	0.69	0.80
T_5 (0.2 µM SA)	0.88	0.61	0.51	0.67	0.96	0.79	0.76	0.84
T_6 (0.4 µM SA)	0.95	0.72	0.53	0.73	1.10	0.96	0.78	0.95
Mean	0.80	0.61	0.48		0.93	0.82	0.72	
	I	T	I x T		I	T	I x T	
S. Em±	0.01	0.01	0.02		0.00	0.01	0.02	
CD at 5 %	0.02	0.03	0.06		0.02	0.03	0.05	
CV%								

Table 10: Effect of plant growth regulators on leaf total chlorophyll content (mg/g) of Indian mustard under irrigation of saline water

Treatment (T)	20 DAS (G_1)			Mean (I)	30 DAS (G_2)			Mean (I)
	I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)		I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)	
T_1 (Control)	2.40	1.90	1.69	2.00	2.66	2.34	2.29	2.43
T_2 (Water spray)	2.44	1.90	1.76	2.03	2.75	2.38	2.50	2.54
T_3(GA_3@50ppm)	2.47	1.95	1.67	2.03	2.80	2.74	2.33	2.62
T_4(GA_3@100ppm)	2.53	2.05	1.79	2.12	2.80	2.4	2.32	2.51
T_5 (0.2 µM SA)	2.72	2.05	1.93	2.23	2.90	2.53	2.39	2.61
T_6 (0.4 µM SA)	2.86	2.24	2.02	2.37	3.24	2.65	2.53	2.81

Mean	2.57	2.02	1.81		2.86	2.51	2.39	
	I	T	I x T		I	T	I x T	
S. Em±	0.03	0.04	0.06		0.03	0.05	0.08	
CD at 5 %	0.07	0.11	NS		0.09	0.13	0.22	
CV%	5.31				5.23			

Proline accumulation increased significantly under salinity stress. Mean proline content increased from 84.11 to 123.13 mg 100 g⁻¹ at 20 DAS and from 86.23 to 125.65 mg 100 g⁻¹ at 30 DAS with increasing salinity levels (Table 11). Proline acts as a compatible solute that contributes to osmotic adjustment, ROS scavenging and stabilization of proteins and membranes. The highest proline content was recorded in T_6 (122.39 and 124.67 mg 100 g⁻¹ at 20 and 30 DAS), indicating enhanced adaptive responses under salinity stress. Ascorbic acid content decreased slightly with increasing salinity. Mean values declined from 81.40 mg 100 g⁻¹ under control conditions to approximately 76.00 mg 100 g⁻¹ under saline treatments at 20 DAS. Similar trends were observed at 30 DAS. The decline may be associated with greater utilization of antioxidants for detoxification of reactive oxygen species generated under stress (Table 12).

Application of salicylic acid significantly improved ascorbic acid content. T_6 recorded the highest values (83.18 and 85.67 mg 100 g⁻¹ at 20 and 30 DAS), demonstrating enhanced antioxidant capacity. Ascorbic acid is a major non-enzymatic antioxidant involved in ROS detoxification and maintenance of cellular redox balance. Increased ascorbic acid accumulation under SA treatment indicates enhanced antioxidative capacity and improved protection against oxidative stress (Lien *et al*., 2016) [13].

Table 11: Effect of plant growth regulators on leaf proline (mg/100g) of Indian mustard under irrigation of saline water

Treatment (T)	20 DAS (G_1)			Mean (I)	30 DAS (G_2)			Mean (I)
	I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)		I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)	
T_1 (Control)	75.03	110.25	99.02	94.77	77.30	114.00	102.00	97.77
T_2 (Water spray)	78.68	109.50	134.47	107.55	79.99	98.50	113.60	97.36
T_3(GA_3@50ppm)	81.56	96.47	130.46	102.83	85.30	110.80	136.00	110.70
T_4(GA_3@100ppm)	86.92	105.50	113.80	102.07	88.00	106.00	136.50	110.17
T_5 (0.2 µM SA)	86.12	118.79	116.52	107.14	88.60	120.60	118.20	109.13
T_6 (0.4 µM SA)	96.37	126.30	144.51	122.39	98.20	128.20	147.60	124.67
Mean	84.11	111.14	123.13		86.23	113.02	125.65	
	I	T	I x T		I	T	I x T	
S. Em±	1.33	1.89	3.27		1.36	1.93	3.34	
CD at 5 %	3.83	5.42	9.40		3.91	5.54	9.60	
CV%	5.35				5.33			

Table 12: Effect of plant growth regulators on leaf ascorbic acid (mg/100g) of Indian mustard under irrigation of saline water

Treatment (T)	20 DAS (G_1)			Mean (I)	30 DAS (G_2)			Mean (I)
	I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)		I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)	
T_1 (Control)	75.88	74.26	71.57	73.90	77.55	76.44	73.64	75.88
T_2 (Water spray)	75.98	74.44	74.33	74.92	77.22	76.21	76.14	76.52
T_3(GA_3@50ppm)	80.36	74.90	75.28	76.85	83.01	77.21	77.44	79.22
T_4(GA_3@100ppm)	81.61	75.14	75.66	77.47	84.22	78.11	78.10	80.14
T_5 (0.2 µM SA)	85.83	76.11	79.43	80.46	88.11	79.22	81.30	82.88
T_6 (0.4 µM SA)	88.76	81.13	79.65	83.18	91.20	83.20	82.60	85.67
Mean	81.40	76.00	75.99	77.80	83.55	78.40	78.20	80.05
	I	T	I x T		I	T	I x T	
S. Em±	0.95	1.35	2.34		0.98	1.39	2.41	
CD at 5 %	2.75	3.89	NS		2.83	4.00	NS	
CV%	5.23				5.14			

Similarly, Glycine betaine content increased significantly with increasing salinity levels. At 20 DAS, mean values increased from 47.06 mg 100 g⁻¹ under control irrigation to 54.92 mg 100 g⁻¹ under severe salinity. At 30 DAS, glycine betaine increased from 49.93 to 57.74 mg 100 g⁻¹. Accumulation of glycine betaine helps maintain osmotic balance and protects proteins and membranes under stress conditions (Table 13). Among plant growth regulator treatments, T_6 produced the highest glycine betaine content (53.40 and 56.32 mg 100 g⁻¹ at 20 and 30 DAS, respectively), indicating superior osmoprotective capacity and salinity tolerance. Glycine betaine is an important osmoprotectant that stabilizes proteins, membranes, and photosynthetic machinery under stress conditions. Enhanced accumulation of glycine betaine under SA treatment further demonstrates the role of SA in improving osmotic adjustment and stress tolerance in Indian mustard (Ahmad, 2010) [1].

Table 13: Effect of plant growth regulators on leaf glycine betaine (mg/100g) of Indian mustard under irrigation of saline water

Treatment (T)	20 DAS (G_1)			Mean (I)	30 DAS (G_2)			Mean (I)
	I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)		I_0 (Tap water)	I_1 (4 EC)	I_2 (8 EC)	
T_1 (Control)	45.21	46.54	51.32	47.69	48.11	48.20	53.11	49.81
T_2 (Water spray)	45.36	46.92	52.56	48.28	49.33	49.22	57.22	51.92

T_3(GA_3@50ppm)	47.81	49.25	54.65	50.57	49.30	51.20	58.20	52.90
T_4(GA_3@100ppm)	47.49	49.44	56.46	51.13	49.55	52.02	58.10	53.22
T_5 (0.2 µM SA)	47.46	48.90	56.37	50.91	50.88	50.22	59.56	53.55
T_6 (0.4 µM SA)	49.01	53.03	58.15	53.40	52.40	56.33	60.22	56.32
Mean	47.06	49.01	54.92	50.33	49.93	51.20	57.74	52.95
	I	T	I x T		I	T	I x T	
S. Em±	0.62	0.88	1.52		0.65	0.92	1.59	
CD at 5 %	1.78	2.52	NS		1.86	2.64	NS	
CV%	5.20				5.26			

Conclusion

Saline water irrigation significantly affected the physiological and biochemical processes of Indian mustard by disturbing plant water relations, membrane stability, and metabolic activities. Increasing salinity levels caused marked reductions in relative water content (RWC), membrane stability index (MSI), chlorophyll pigments, and total soluble protein content, indicating impairment of cellular hydration, membrane integrity, and photosynthetic efficiency. Conversely, salinity stress induced the accumulation of compatible osmolytes and protective metabolites such as proline, glycine betaine, free amino acids, soluble sugars, reducing sugars, total phenols, and ascorbic acid, which represent adaptive defense mechanisms for osmotic adjustment and oxidative stress tolerance. Application of plant growth regulators, particularly salicylic acid (SA), effectively alleviated the adverse effects of salinity stress. Among the treatments, foliar application of SA at 0.4 µM proved most effective in improving RWC, MSI, chlorophyll content, soluble protein, antioxidant metabolites, and osmolyte accumulation under saline conditions. The beneficial effects of SA may be attributed to its role in enhancing osmotic regulation, stabilizing cellular membranes, protecting photosynthetic pigments, and strengthening antioxidant defence systems. Overall, the data clearly demonstrate that salinity stress reduced RWC, MSI, chlorophyll pigments and soluble protein content, while enhancing accumulation of phenols, amino acids, sugars, proline and glycine betaine. Among the plant growth regulators tested, salicylic acid at 0.4 µM (T_6) consistently produced the most favourable physiological and biochemical responses, followed by SA 0.2 µM (T_5) and GA_3 @ 100 ppm (T_4). These findings confirm the effectiveness of salicylic acid in mitigating salinity-induced damage through improved osmotic adjustment, membrane protection and antioxidant defence.

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