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IMPACT OF HUMIC ACID AND SALICYLIC ACID ON BIOCHEMICAL AND PHYSIOLOGICAL PARAMETERS OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) UNDER SALINITY STRESS

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ABSTRACT

A field experiment was conducted during *kharif* 2014-15 to study the response of tomato to different levels of humic acid and salicylic acid under salinity stress condition. Results revealed that the APX, SOD, GPX, CAT activities and electrolyte leakage increased while, RLWC decreased significantly with 4 dS m^{-1} and 8 dS m^{-1} level of salinity of irrigation water respectively, over control. Application of both HA and SA significantly increased the APX, SOD, GPX, CAT activities and RLWC while,electrolyte leakage decreased. The combined effect of saline water 0.25 dS m^{-1} (control) and 1500 ppm HA level recorded maximum APX (359.33 μmol^{-1} min^{-1} mg^{-1} FW), SOD (64.05 μmol^{-1} min^{-1} mg^{-1} FW), GPX (379.34 μmol^{-1} min^{-1} mg^{-1} FW), and CAT (40.99 μmol^{-1} min^{-1} mg^{-1} FW) activities. From the study it was concluded that combined treatment of soil application of HA (1500 ppm) with SA (1.5 mM) was found most effective, which alleviated the deleterious impacts of salinity stress on tomato plants.

KEY WORDS
Salinity
Humic acid
Salicylic acid
APX, SOD, GPX, CAT

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INTRODUCTION

Soil salinity is one of the major abiotic stresses that adversely affect plant productivity and quality (Zue, 2001). In India Majority of the underground waters contain high concentration of salts and their continuous use for irrigation adversely affects the crop production (Bali *et al.*, 2015). Salt stress has toxic effects on plants and lead to metabolic changes, like loss of chloroplast activity, decreased photosynthetic rate and increased photorespiration rate which then leads to an increased reactive oxygen species (ROS) production (Parida and Das, 2005). These ROS such as Superoxide (O_2^+), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), and single oxygen (O_2^-) causes oxidative stress on plants. High salt stress also disrupts homeostasis in water potential and in distribution. This disruption of homeostasis occurs at both the cellular and whole plant levels. Under physiological study state condition, there is balance between the production and scavenging of ROS (Skopelitis *et al.*, 2006). However, this homeostasis can be disturbed by a number of adverse environmental factors such as heat stress, metal stress, water stresses etc. Plants operate several mechanisms to counteract the adverse effects of salt. These mechanisms may be enhanced by the application of chemicals to the plants. One such mechanism is the activation of an antioxidant enzyme system, which may be influenced by the interaction of plant growth regulators and salt. Plants containing high activities of antioxidant enzymes have shown considerable resistance to the oxidative damage caused by ROS (Khan *et al.*, 2010). Plant growth regulators, such as HA and SAcan be used to promote growth and yield of plants under various stress conditions including salt stress.HAapplication in soil increasing cell membrane permeability, respiration and photosynthesis and root cell elongation (Russo and Berlyn, 1990).The malondialdehyde (MDA), lipid peroxidation and H_2O_2 contents reduce significantly after HA treatments (Kesbaand El-Beltagi, 2012). SA is a naturally occurring plant hormone, is an important signal molecule known to have diverse effects on biotic and abiotic stress tolerance. Low concentration of SA usually improves plant growth under salinity due to decreased concentrations of Na, Cl and H_2O_2 in plants, decreased electrolyte leakage, increased N and Ca contents and increased antioxidant enzyme activity (Khan *et al.*, 2010). The present investigations were, therefore, undertaken to evaluate the role of HA and SA in mitigating the adverse effect of saline water, biochemical and physiological parameters of tomato under different levels of saline water irrigation.

MATERIALS AND METHODS

The present investigation was carried out at College of Agriculture, S.K. Rajasthan Agricultural University Bikaner, during *kharif* season 2014-15. The soil of experimental site was sand in texture with pH_2 8.17, EC_2 0.43 dSm^{-1} and CEC 4.39

cmol (p^+) kg^{-1}. Tomato plants variety Pusa ruby was transplanted in open field during 1st week of august with 30 cm x 30 cm spacing.The experiment was carried out using 18 treatment combinations comprising three levels of saline water (control 0.25 dS m^{-1}, 4 dS $m^{-1}$and 8 dS m^{-1}). Three levels of HA (control, 750 ppm and 1500 ppm) and two levels of SA (control and 1.5mM) were tested. The treatment combinations were replicated three times in Factorial RBD and allocated randomly to different plots. All the three levels of saline water (0.25 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}) were applied in field after transplanting of tomato as per crop irrigation requirement. HA (750 ppm and 1500 ppm) were applied in soil just after transplanting along with fertigation. SA (1.5 mM) was applied twice as foliar application first at 30 DAT and second at 55 DAT.

Enzyme extraction

Fresh leaf sample at flowering stage (0.5g fresh wt.) was homogenized in ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM ethylene diamine tetra acetic acid (EDTA) and 1% polyvinyl polypyrrolidone (PVP). All operations for enzyme extraction were performed at 0 - 4°C.

Ascorbate peroxidase activity (APX) was measured by the method of Nakona and Asada (1987).Enzyme extract for superoxide dismutase activity (SOD) was estimated according to the method of Dhindhsa *et al.* (1981). For Glutathione peroxidase activity (GPX),the oxidation of Glutathione andCatalase activity (CAT) was measured as described by Chance and Maehly (1955). All enzyme activity was expressed as ($\mu mol^{-1} min^{-1} mg^{-1}$ FW).The relative leaf water content (RLWC) was estimating according to Slavik, 1974. For electrolyte leakage (EL)twenty five freshly cut leaf disc (0.5 cm diameter each) were floated on 15ml of deionized water with continuous shaking. The electrolyte content in the solution was measured immediately (C0) and after 3 h (C3) of incubation at room temperature using a conductimeter. Total electrolyte content was determined in the same way after boiling for 10 min. (TC). Results were expressed as per cent age of electrolyte leakage.

$$EL\ (\%) = \frac{100 \times (C3 - C0)}{TC}$$

RESULTS AND DISCUSSION

Biochemical parameters

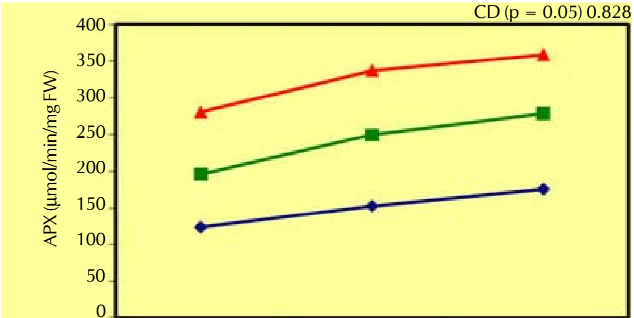
Salt stress is one of the major abiotic stress factors that affect almost every aspect of physiology and biochemistry of a plant. Saline water irrigation resulted in significant increase in APX, SOD, GPX and CAT (Table 1). The APX activity increased significantly by 23.20 and 35.41 per cent, SOD activity by 12.55 and 20.42 percent, GPX activity by 10.04 and 14.39 per cent per cent , CAT activity by 5.23 and 11.11 percent, with4 dS m^{-1} and 8 dS m^{-1} level of salinity of irrigation water respectively, over control. Plants protect themselves from oxidative damage due to ROS through both enzymatic and non-enzymatic defense mechanisms (Ardic *et al.,* 2009). The activities of these antioxidant enzymes were reported to increase under salt stresses cultivars generally show higher activity of these antioxidant enzymes (Ashraf and Ali, 2007; Fikret *et al.*, 2013)

HA application significantly increased the APX, SOD, GPX and CAT (Table 1).The APX activity increased significantly by 59.83 and 116.01 per cent , SOD activity by 76.69 and 139.85 per cent , GPX activity by 13.50 and 28.51 per cent and CAT activity by 48.35 and 113.89 per cent, with 750 ppm and 1500 ppm levels of HA application respectively, over control. The magnitude of increase in these enzymatic activities was much more as compared to application of saline water. ROS induced lipid peroxidation is one of the mechanisms responsible for cell damage. The malondialdehyde (MDA), lipid peroxidation and H_2O_2 contents reduce significantly after HA treatments (Kesbaand El-Beltagi, 2012), these finding suggest that application of HA probably not only improve the antioxidant defense enzymes system but also triggers the nonenzymatic antioxidants in plants (Zhanget *al.*, 2013). Application of SA resulted in significant increase in APX, SOD,

Table 1: Effect of saline water irrigation, HA and SA on biochemical and physiological parameters of tomato

Treatments	Biochemical parameters ($\mu mol^{-1} min^{-1} mg^{-1}$ FW)				Physiological parameters (%)	
	APX	SOD	GPX	CAT	RLWC	EL
Saline water (dS m^{-1})						
Control	200.36	39.67	295.25	26.20	79.23	19.96
4	246.84	44.65	324.92	27.57	76.42	28.99
8	271.31	47.77	337.74	29.11	70.62	36.98
S.Em. ±	1.66	0.41	2.65	0.27	0.84	0.28
C.D. (p = 0.05)	4.78	1.17	7.61	0.78	2.42	0.82
Humic acid (ppm)						
Control	151.00	25.57	280.30	17.93	71.91	31.00
750	241.34	45.18	317.38	26.60	75.74	28.76
1500	326.18	61.33	360.23	38.35	78.61	26.17
S.Em. ±	1.66	0.41	2.65	0.27	0.84	0.28
C.D. (p = 0.05)	4.78	1.17	7.61	0.78	2.42	0.82
Salicylic acid (mM)						
Control	208.92	40.25	300.53	25.31	74.22	29.79
1.5	270.09	47.81	338.07	29.94	76.62	27.50
S.Em. ±	1.36	0.33	2.16	0.22	0.69	0.23
C.D. (p = 0.05)	3.91	0.95	6.21	0.64	1.98	0.67

APX Ascorbate peroxidase activity; SOD Superoxide dismutase activity; GPX Glutathione peroxidase activity; CAT Catalase activity; RLWC Relative leaf water content; EL Electrolyte leakage



	Control	4	8
Control	124	153.07	175.92
750	195.87	249.45	278.69
1500	281.23	337.99	359.33

Saline water (dS m^{-1})

Figure 1: Combined effect of saline water irrigation and HA on APX (µmol^{-1} min^{-1} mg^{-1} FW)

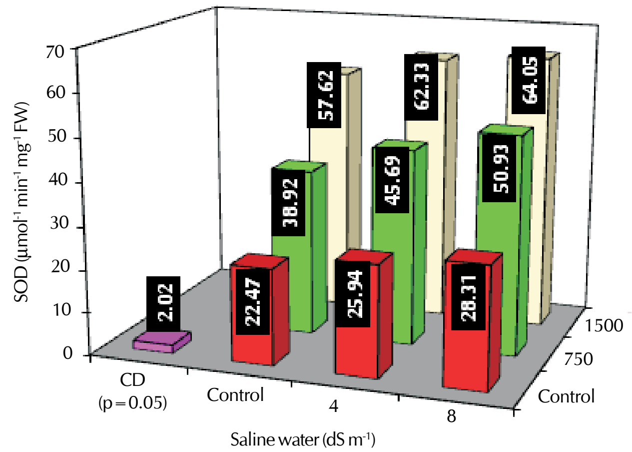


Figure 2: Combined effect of saline water irrigation and HA on SOD (µmol^{-1} min^{-1} mg^{-1} FW)

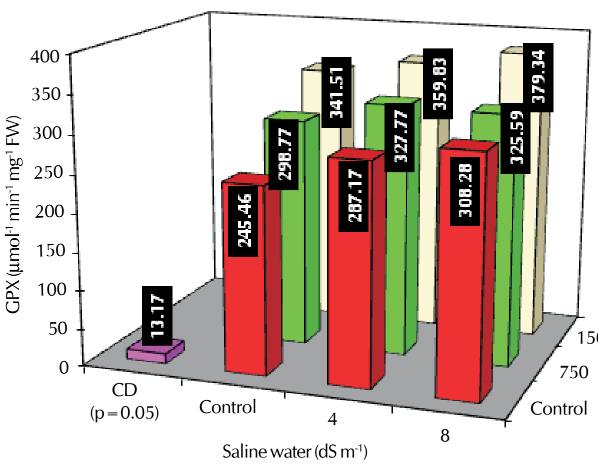


Figure 3: Combined effect of saline water irrigation and HA on GPX (µmol^{-1} min^{-1} mg^{-1} FW)

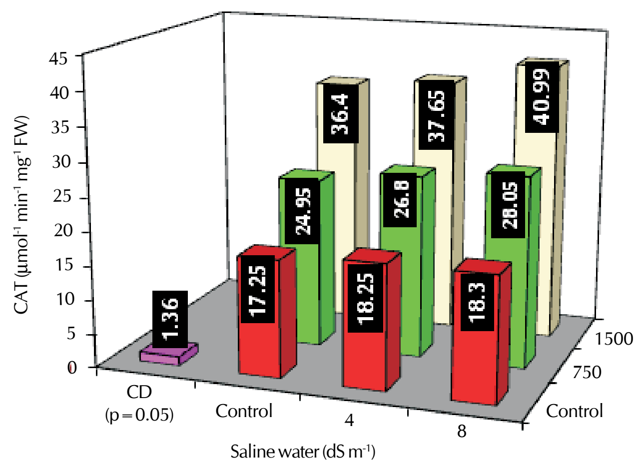


Figure 4: Combined effect of saline water irrigation and HA on CAT (µmol^{-1} min^{-1} mg^{-1} FW)

GPX and CAT (Table 1). The APX activity increased by 29.28 per cent,SOD activity by 18.78 per cent,GPX activity by 12.49 per cent,CAT activity by 18.30 per cent, with 1.5 mM SA application, over control. SA was found to enhance activities of antioxidant enzymes such as peroxidase, SOD, CAT, when sprayed exogenously to the salinity stressed plants (Szepsi *et al.*, 2008). The increase in the activity of antioxidant enzymes following SA application could be the indicator of build-up of a protective mechanism to reduce oxidative damage induced by salt stress(Khan *et al.,*2010). Few researchers also observed decrease in CAT activity by salicylic acid application under salt stress condition (Fahad and Bano, 2012).

The combined effect of saline water irrigation and HA application was found significant in enhancing the APX, SOD, GPX and CAT (fig. 1, 2, 3, 4). Significant maximum APX, SOD, GPX, CAT activities were recorded with the combined effect of saline water ECiw 8 dS m^{-1} and 1500 ppm HA level. This could probably by due to the self protective mechanism of plants under salt stress along with antioxidant properties of HA which led tosignificantly reduction in H_2O_2 contents by improving the contents of non-enzymatic antioxidants. As well as the antioxidant defense enzymes *viz:* APX, SOD, GPX and CAT(Zhang *et al.*, 2013).

Physiological parameters

Saline water irrigation resulted in significantly decreased the RLWC of leaves (Table 1). The RLWC decreased by 3.54 and 10.86 per cent with 4 dSm^{-1} and 8 dSm^{-1} level of salinity of irrigation water respectively, over control. This might be due to that higher salt concentration in nutrient solution increased the osmotic stress, which significantly reduced uptake of water, ultimately decreased the RLWC at higher salinity levels (Kirnak *et al.*, 2001). EL which is considered to be a promising indicator of stress-induced membrane damage, increased greatly with the increase in salinity level of irrigation water (Table 1).The EL increased by 45.24 and 85.27 per cent with 4 dSm^{-1} and 8 dSm^{-1} levels of saline water irrigation respectively, over control. High concentrations of Na or/ and decreased molar per cent ages of sterols and phospholipids (Wu *et al.,* 1998) with increasing salinity, might have caused membrane disorganization. The lipid peroxidation due to the accumulation of the ROS could be the principal cause of membrane disorganization (Sangtarashani *et al.*, 2013).

HA application significantly increased the RLWC of leaves (Table 1). RLWC increased by 5.32 and 9.31per cent with 750 ppm and 1500 ppm levels of HA application respectively, over control. Higher salt concentration in the soil solution causes osmotic stress, which significantly reduces uptake of water, under such conditions application of HA not only increases the water holding capacity of soil, but also maintain high soil water potential there by more water absorption by the root, thus increase in the RLWC of leaves (Boogar*et al*., 2014). Further application of HA also resulted in decreased EL (Table 1). The EL decreased by 7.22 and 15.58 per cent with 750 ppm and 1500 ppm levels of humic acid application respectively, over control. (David *et al*., 1994).HA improve plant physiological processes by enhancing the availability of major and minor nutrients as well as enhancing the vitamins, amino acids and ABA contents of the plants (Vanitha and Mohandass, 2014).

Salt stress induces water deficit and increases ionic and osmotic effects leading to oxidative stress and formation of ROS. In the present study, maximum oxidative stress was noted in terms of enhanced electrolyte leakage under salt stress, which was mitigated to some extent with the application of HA by modification of membrane fluidity and permeability (Aydin *et al*., 2012). SA significantly increased the RLWC of leaves (Table 1). The RLWC increased by 3.23 per cent with 1.5 mM SA application, over control. The RLWC, the water potential and osmotic potential of plants become more negative with increase in salinity. Foliar SA application might have increased the leaf diffusive resistance and lower transpiration rates, enhanced photosynthetic rate thereby increase in the RLWC of the leaf (Yildirim *et al*., 2008). Application of SA resulted in significant reduction in EL (Table 1). The EL decreased by 7.68 per cent over control with 1.5 mM SA. This could be due to reduction in the amount of ion leakage (measured as electrolytes) in salt stressed plant, indicating that SA application might have facilitated the maintenance of membrane functions (i.e., semi permeability) under stress conditions (Bayat *et al*., 2012).

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