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Role of humic acid and salicylic acid on quality parameters and K/Na ratio of Groundnut (*Arachis hypogaea* L.) under salt and water stress

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Abstract
A field experiment was conducted during *kharif* 2015-16 to study the response of groundnut to humic acid and salicylic acid under salt and water stress conditions. Results revealed that application of saline water (4.0 dS m^{-1}) and water deficit irrigation (0.8 ETc) significantly decreased the quality parameters of groundnut such as oil, protein and shelling percent and due to saline water application there was increase in Na ion concentration in plant resulted in decreased K/Na ratio. Application of both humic acid and salicylic acid significantly increased the quality parameters and K/Na ratio in plant. The combined effect of saline water (4.0 dS m^{-1}) with humic acid (1500 ppm) application showed that humic acid alleviate the detrimental effect of saline water by an increasement in such quality parameters and K/Na ratio. The combined effect of humic acid and salicylic acid (HA+SA) was also found significant and recorded maximum protein content (26 %) and K/Na ratio (6.01). Application of salicylic acid (1.5 mM) also significantly increased the K/Na ratio in salt stressed conditioned and maintain the ionic balance in cell sap.

Keywords: ECiw, dS m^{-1}, Shelling, K/Na ratio

Introduction
Salt and water stress have been found to disrupt several physiological processes leading to reduction in quality parameters, ionic balance and yield. Salt stress induces ionic stress and osmotic stress in plant cells. A direct results of these primary effects is the enhanced accumulation of reactive oxygen species (ROS) that are harmful to plant cells at high concentration (Parida and Das, 2005) [11]. High concentrations of Na^+ in the soil also cause the disruption of intracellular ion homeostasis, membrane dysfunction and inhibition of metabolic activity, resulting in inhibition of growth and yield (Hasegawa *et al*., 2000) [6]. Yeilaghi *et al.* (2012) [17] examined the effects of salinity stress on seed oil content and fatty acid composition in various genotypes of safflower (*Carthamus tinctorius* L.).

The major functional groups of humic acids include carboxyl, phenolic hydroxyl, alcoholic hydroxyl, ketone etc. (Russo and Berlyn 1990) [15]. The mechanism of humic acid activity in promoting the plant growth is not completely known, but several explanations have been proposed by researchers such as increasing cell membrane permeability, oxygen uptake, respiration and photosynthesis, phosphate uptake, and root cell elongation (Vaughan 1974 and Russo and Berlyn 1990) [16, 15]. The malondialdehyde (MDA), lipid peroxidation and H_2O_2 contents reduce significantly after HA treatments (Kesba and El-Beltagi, 2012) [7].

Salicylic acid (SA) is a naturally occurring plant hormone, is an important signal molecule known to have diverse effects on biotic and abiotic stress tolerance. The positive effects of SA on groundnut plant have also been reported under drought stress (Rady *et al.* 2015) [12]. 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) [8]. El-Hak *et al.* (2012) [5] conducted a field experiment to study the beneficial effect of foliar spray of salicylic acid (200 ppm) on shelling per cent, protein content and yield characteristics of pea (*Pisum sativum L.*). The results showed the significant increase in shelling per cent and protein content in both the successive season of 2009/2010 and 2010/2011.

Materials and Methods

The experiment was conducted at Niche Area of Excellence, S.K. Rajasthan Agricultural University, Bikaner during *kharif* season of 2015. 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}. Groundnut plants variety HNG-10 were sown in open field at 15th june with 30 cm x 10 cm spacing. The experiment was carried out using 16 treatment combinations comprising two levels of saline water (control 0.25 dS $m^{-1}$and 4.0 dS m^{-1}), two level of potential evapotranspiration (1.0 PET and 0.8 PET) in main plots and two levels of humic acid (control and 1500 ppm) and two levels of salicylic acid (control and 1.5 mM) in sub plots. The experiment was laid out in a factorial split plot design and replicated four times. HA (1500 ppm) was applied in soil just after sowing along with fertigation. SA (1.5 mM) was applied twice as foliar application first at 30 DAS and second at 55 DAS.

Table 1: Composition of synthetic irrigation water.

EC (dS m^{-1})	Cations (me L^{-1})					Anions (me L^{-1})				
	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Total	Cl^{-}	CO_3^{2-}	HCO_3^{-}	SO_4^{2-}	Total
0.25	0.8	0.9	0.8	0.05	2.55	0.7	-	1.2	0.61	2.51
4	6.8	6.8	26.2	0.2	40.0	23.5	1.5	9.8	5.2	40.0

Irrigation level

For irrigation through drip, first irrigation (25 mm) was given immediately after sowing and subsequent irrigations were scheduled in alternate days as per treatment. The quantity of water was calculated as follows:

Irrigation water (mm) = PE × Kp × Kc

Where,
PE = Pan evaporation (mm)
Kp = Pan factor
Kc = Crop factor.

The lateral drip lines were laid on the soil surface as per treatment. Dripper to dripper spacing was 30 cm with 4 lit hr^{-1} discharge.

Quality parameters

Oil content

Oil content in kernel was determined by Soxhlet apparatus using petroleum ether (60-80^{0}C) as an extractant (A.O.A.C., 1960).

Protein content in kernel

Protein content (mg g^{-1} seed wt.) was determined by the method given by Lowry *et al.* (1951) [9].

Protein extraction

For extraction of protein content, 100 mg seeds was homogenized in 0.1 M NaCl. The homogenate was centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and final volume was made to 5 ml with the 0.1 M NaCl.

Protein quantification

1. An extract volume of 0.2 ml was taken in test tube and at the same time a series of test tubes 0.2, 0.4, 0.6, 0.8 & 1.0 ml of protein solutions were prepared by dissolving 10 mg of Bovin albumin serum protein in 100 ml of distilled water.
2. In each test tube the volume was made to 1.0 ml with distilled water. A tube with 1.0 ml of distilled water served as blank.

1.0 ml of the supernatant was taken and added 5 ml of alkaline copper reagent in separate test tubes, the mixture was kept at room temperature for 10 minutes. 0.5 ml of 1N folin phenol reagent was then added in each test tube. The mixture was incubated at room temperature for 30 minutes under dark and the absorbance of the blue colour was measured at 750 nm using spectroNic-20, zero absorbance was adjusted with the blank. The quantity of protein in the 100 mg of seeds was then calculated using the standard curve.

Reagent Preparation

Reagent A: 2 % Na_2CO_3 in 0.1 M NaOH
Reagent B: 0.5% copper sulphate ($CuSO_4.5H_2O$) in 1% potassium sodium tartrate.
Reagent C: Alkaline copper solution which was prepared by mixing reagent A and B in the ratio of 50:1 at the time of use.
Reagent D: The phenol reagent was diluted with glass distilled water in ratio of 1: 2.

$$\text{Protein (\%)} = \frac{\text{Protein (mg/g seed wt)}}{10}$$

Shelling Percentage

A composite sample of 100 gram was drawn from the bulk of the dry pods of each net plot randomly and shelled. The ratio of kernel to pod weight was worked out and expressed in percent.

$$\text{Shelling percentage} = \frac{\text{Kernel weight (g)}}{\text{Pod weight (g)}} \times 100$$

Result and Discussion

Quality parameters and ion contents

Application of saline water irrigation resulted in significant decrease in oil, protein and shelling percent (Table 2). K/Na ratio was also decreased significantly with saline water application (Table 3). The oil percent decreased significantly by 3.50 per cent, protein content by 3.59 per cent, shelling percent by 5.41 per cent and K/Na ratio by 68.36 per cent with 4.0 dS m^{-1} level of salinity of irrigation water, over control. These results are in harmony with Aydinşakir *et al.* (2015) [2] who found that salinity greatly affected the mineral nutrient composition of groundnut plants. Padole *et. al.* (1993) [10] also reported that protein content and oil content of groundnut decreased with saline conditions.

Due to Application of water deficit irrigation of 0.8 ET_c, oil content decreased significantly by 2.94 per cent and shelling by 1.80 per cent, over control. This might be due to moisture stress condition, which leads to low accumulation of photosynthates in kernel resulted in shrivelled kernels (Reddy *et al.*, 1978; Boote and Hammond, 1981) [14, 4].

The oil content increased significantly by 2.58 per cent, protein content by 2.92 per cent, shelling by 1.26 per cent and K/Na ratio by 132.21 per cent, with 1500 ppm level of humic acid application over control. This might be due to that humic substance might have various biochemical effects either at cell wall, membrane level or in the cytoplasm which in turn result in to enhanced photo-synthesis. These results corroborate the finding of Rajpar *et al.* (2011) [13] in mustard, El-Hak *et al.* (2012) [5] in pea and Bakry *et al*. (2014) [3] in flax.

Foilar application of Salicylic acid also significantly increased the oil content by 1.34 percent, protein content by 3.08 percent and K/Na ratio by 30.04 percent. Exogenous application of salicylic acid inhibit the Na^+ accumulation in plant cell, and stimulate the K^+, Ca^{2+} and Mg^{+2} uptake (Ahmed *at al.* 2009 for wheat) [1]. Alteration of mineral uptake by salicylic acid application may be one mechanism for the alleviation of salt stress.

Combined Effect - The combined effect of (saline water + irrigation levels), (saline water + humic acid) and (humic acid + salicylic acid) were found significant on this present investigation and showed in fig. 1,2,3, 4 and 5 respectively.

Table 4.2: Effect of saline water, irrigation level, humic acid and salicylic acid on quality parameters of groundnut plant.

Treatments	Quality parameters (%)		
	Oil	Protein	Shelling
Saline water (dS m^{-1})			
Control	41.94	26.17	61.54
4.0	40.47	25.23	58.21
S.Em.±	0.22	0.18	0.28
C.D. (P = 0.05)	0.70	0.59	0.88
Irrigation level (ETc)			
1.0 ETc	41.82	25.89	60.42
0.8 ETc	40.59	25.50	59.33
S.Em.±	0.22	0.18	0.28
C.D. (P = 0.05)	0.70	NS	0.88
Humic acid (ppm)			
Control	40.68	25.33	59.50
1500	41.73	26.07	60.25
S.Em.±	0.15	0.12	0.26
C.D. (P = 0.05)	0.42	0.35	0.75
Salicylic acid (mM)			
Control	40.93	25.31	59.71
1.5	41.48	26.09	60.04
S.Em.±	0.15	0.12	0.26
C.D. (P = 0.05)	0.42	0.35	NS

Table 4.3: Effect of saline water, irrigation level, humic acid and salicylic acid on Na^+, K^+ and K^+/Na^+ ratio of groundnut at flowering stage.

Treatments	Cations (%) Ratio		
	Na^+	K^+	K^+ / Na^+
Saline water (dS m^{-1})			
Control	0.22	1.26	5.72
4.0	0.52	0.94	1.81
S.Em.±	0.01	0.01	0.14
C.D. (P = 0.05)	0.03	0.03	0.32
Irrigation level (ETc)			
1.0 ETc	0.37	1.12	3.02
0.8 ETc	0.37	1.11	2.97
S.Em.±	0.01	0.01	0.14
C.D. (P = 0.05)	NS	NS	NS
Humic acid (ppm)			
Control	0.50	1.04	2.08
1500	0.24	1.16	4.83
S.Em.±	0.01	0.01	0.15
C.D. (P = 0.05)	0.02	0.02	0.3
Salicylic acid (mM)			
Control	0.41	1.08	2.63
1.5	0.33	1.13	3.42
S.Em.±	0.01	0.01	0.15
C.D. (P = 0.05)	0.02	0.02	0.3

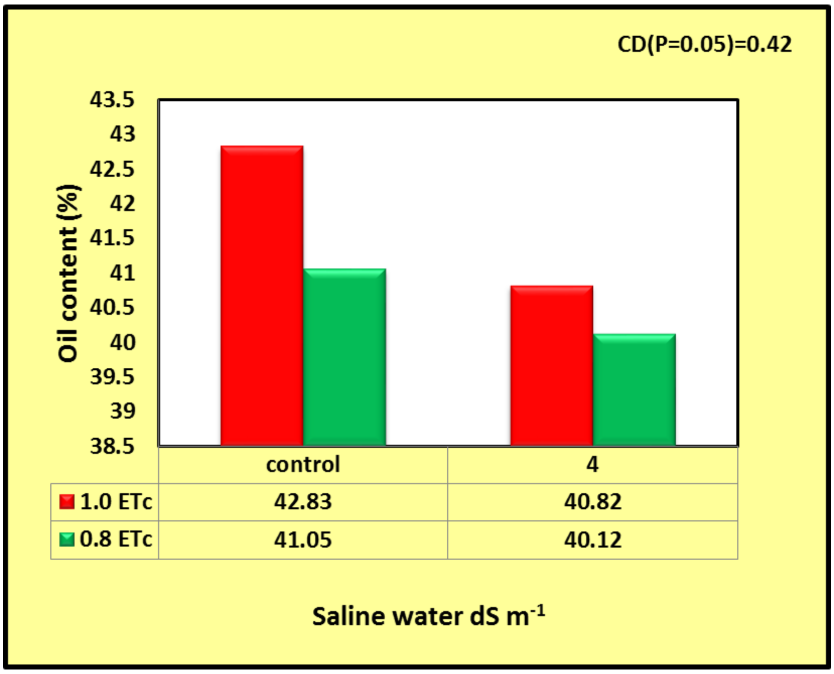

Fig 1: Combined effect of saline water and irrigation levels on oil content (%)

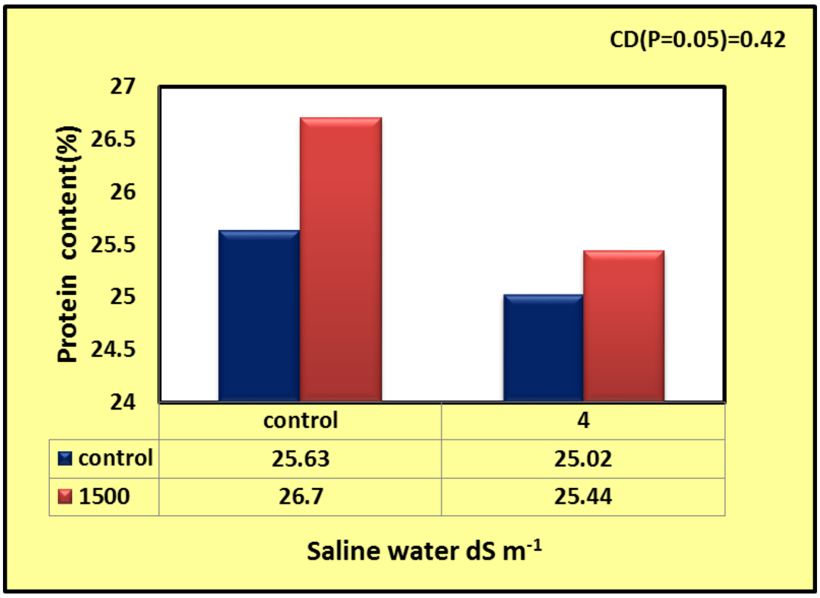

Fig 2: Combined effect of saline water and humic acid on protein content (%)

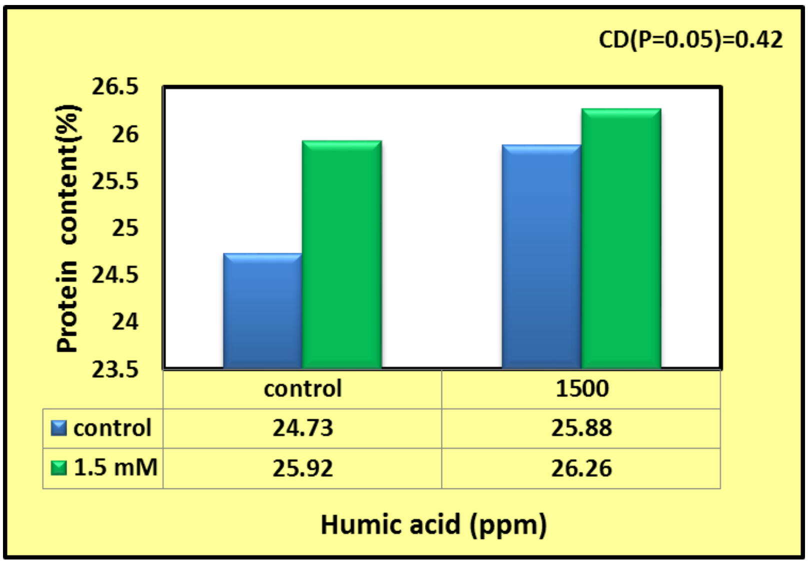

Fig 3: Combined effect of humic acid and salicylic acid on protein content (%)

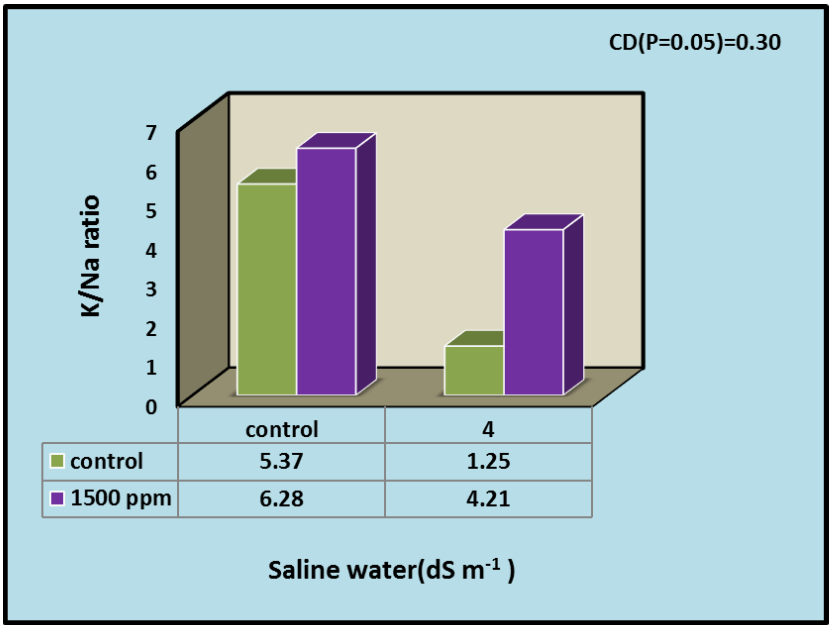

Fig 4: Combined effect of saline water and humic acid on K/Na ratio of groundnut at flowering.

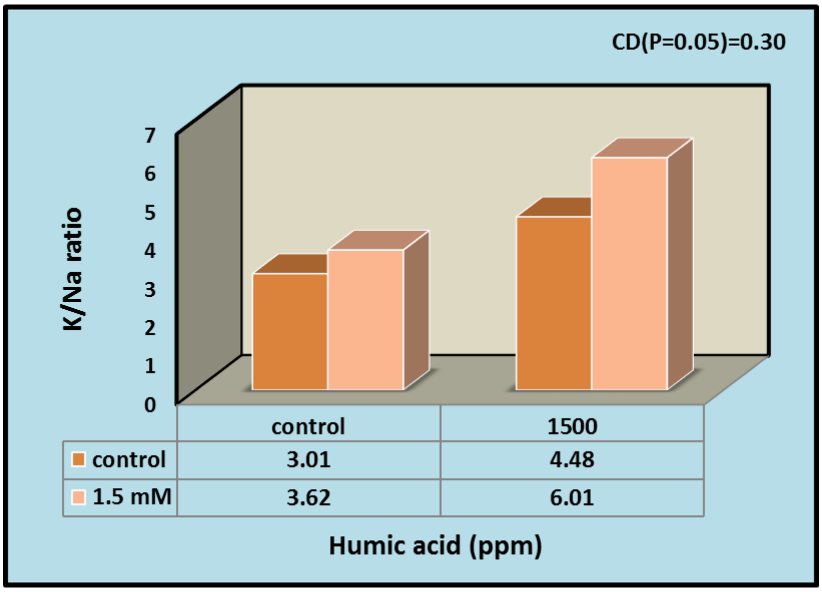

Fig 5: Combined effect of humic acid and salicylic acid on K/Na ratio of groundnut at flowering.

Conclusion

Based on the results of the present investigation, it may be concluded that the effect of salt (EC_{iw} 4.0 dS m-1) and water stress (0.8 ETc) on groundnut adversaly affected the K/Na ratio and quality parameters such as oil, protein and shelling percent. This adverse effect on groundnut can be effectively mitigated with the Combined treatment of soil application of humic acid (1500 ppm) with two spray of salicylic acid (1.5 mM), which alleviated the deleterious impacts of stress on ion content and quality parameters. Humic acid treatment can ameliorate the deleterious effects of salt and water stress by increasing antioxidant defence system and photosynthesis. Exogenous salicylic acid treatment (1.5mM) also ameliorate the negative effect of stress on the growth of groundnut.

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