

EFFECT OF GROWTH REGULATORS ON BIOCHEMICAL CONSTITUENTS AND PHYSIOLOGICAL PARAMETER IN MOTHBEAN (*Vigna aconitifolia* Jacq.) IRRIGATED WITH SALINE WATER

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ABSTRACT

Green house experiment was conducted to investigate the effect of growth regulator on physiological and biochemical changes in mothbean irrigated with saline water. The experimental design was factorial completely randomized design with two level of irrigation to induce salinity stress i.e. tap water (S₁) and 4 EC saline water (S₂) and different eight (T₁ to T₈) treatment combinations. The plants were sprayed with indol acetic acid, gibberellic acid, salicylic acid and their combination treatment after 15 and 25 DAS (Days after sowing) and samples was drawn after 20 and 30 DAS for the analysis. The observations were recorded for the parameters viz., relative water content, membrane stability index, leaf pH, total soluble sugar, reducing sugar, proline, glycine betaine, ascorbic acid. The results suggest that the collected samples of the mothbean that was irrigated with salt stress it shown decreased in the physiological parameters viz.; relative water content, membrane stability index but leaf pH showed reverse trend and it was increased in case of high salt concentration. The decreased in relative water contents in present experiment was low may be the effect of accumulation of osmolytes viz.; reducing sugar, total soluble sugar, proline and glycine betaine. Higher concentration of salt that increase total soluble sugar, reducing sugar, proline, glycine betaine and ascorbic acid content with increasing salt concentration. The leaf sample treated with growth regulators and their combination at different concentration that was increased physiological parameters viz.; relative water content, membrane stability index, escape leaf pH also regulate various plant metabolic process and modulate the production of varied osmolytes viz.; total soluble sugar, reducing sugar, proline, glycine betaine and ascorbic acid content This investigation has proved growth regulators (Indol acetic acid, Gibberellic acid, Salicylic acid) and their combination as a potential biomolecules in reducing the adverse effect of salt stress in plant. A growth regulator (Indol acetic acid, Gibberellic acid, Salicylic acid) has been showed to be beneficial for plant in growth and development under salinity stress.

Key words: *Vigna aconitifolia* Jacq., growth regulators, osmolyte, indol acetic acid, gibberellic acid, Salicylic acid, salinity stress, proline and glycine betaine.

INTRODUCTION

Mothbean (*Vigna aconitifolia* Jacq.) is a diploid species with $2n=2X=22$ chromosomes. Mothbean (*Vigna aconitifolia* Jacq.) is an important pulse crop of arid and semi-arid regions. Salinity can affect growth, dry matter accumulation and yield. It is well known that dry mass of plant is reduced in proportion to the increase in salinity. The reduction in growth of salinized plants may be related to salt induced disturbance of the plant water balance, and growth reduction under salinity stress include ionic imbalances, changes in nutrient and phytohormonal status, physiological processes, biochemical reactions, or a combination of such factors. (Kumar, 2000). An excess of soluble salts in the soil leads to osmotic stress, which results in specific ion toxicity and ionic imbalances (Munns, 2003) and the consequences of these can be plant demise (Rout and Shaw, 2001). This mechanism include, accumulation of different osmolites and phenolics compounds, induction of antioxidant and its related enzymatic system etc., (Vakharia *et al.*, 1997; Patel *et al.*, 2010; Kandoliya and Vakharia, 2013; Kandoliya and Vakharia, 2015 and Joshi *et al.*, 2018). Induced salt tolerance by exogenous application of various chemicals and hormones is a highly attractive approach to overcome the salinity threats (Trivedi *et al.*, 2018; Solanki *et al.*, 2018; Patel *et al.*, 2019a, Patel *et al.*, 2019b, Purohit *et al.*, 2020a and Purohit *et al.*, 2020b). Indole Acetic Acid (IAA) is one of the important plant growth regulators, which can manipulate a variety of growth and developmental phenomena in various crops. Growth regulators such as GA₃ acts as a mediator for acclimation of plants to leaf canopy, stimulates leaf area expansion and induces elongation and osmoregulation in internodes (Azuma *et al.*, 1997) in addition to increasing dry matter and biomass production (Gupta and Datta, 2001) and greatly enhancing the sink potential (Ouzounidou and Ilias, 2005). Salicylic acid also increases protein content in upper leaves in *Vicia faba* (Awastrii and Singh, 1999) and accumulation of phenolics content in groundnut leaves (Meena *et al.*, 2001). Negi and Prasad (2001) observed that in soybean leaves sprayed with lower concentration of salicylic acid increased the free amino acid content as compared to control, while reverse was true at higher concentration. Peroxidase activity was slightly increased with salicylic acid (Garg *et al.*,

1989). Thus, the green house experiment was conducted to investigate the effect of growth regulator on biochemical constituents and physiological parameter in mothbean (*Vigna aconitifolia* Jacq.) irrigated with saline water.

MATERIALS AND METHODS:

The green house experiment was conducted during *kharif* 2019-20 at Food testing Laboratory, Department of Biotechnology, Junagadh Agricultural University, Junagadh. Mothbean (*Vigna aconitifolia* Jacq.) seeds of variety *Gujarat Moth-1* were obtained from Main Pulse Research Station, Junagadh Agricultural University, Junagadh.

Treatments:

a) Salinity Level (2): Plant irrigated with saline water prepared by appropriate dilution of sea water [$S_1 \leq 2$ EC (Control, Tap Water), $S_2 \leq 4$ EC]

b) Growth regulators (8): T_1 - Control (without spray), T_2 - Sprayed with GA_3 @ 100 ppm, T_3 - Sprayed with SA @ 100 ppm, T_4 - Sprayed with IAA @ 500 ppm, T_5 - Sprayed with GA_3 @ 100 ppm + SA @ 100 ppm, T_6 - Sprayed with GA_3 @ 100 ppm + IAA @ 500 ppm, T_7 - Sprayed with SA @ 100 ppm + IAA @ 500 ppm, T_8 - Sprayed with GA_3 @ 100 ppm + SA @ 100 ppm + IAA @ 500 ppm.

c) Growth stage (2): D_1 -20 DAS, D_2 -30 DAS.

Spray of growth regulators with appropriate concentration was done at 15 and 25 days after the sowing (DAS). The sample was collected at 20 and 30 DAS respectively. Mothbean leaf were collected for various biochemical and physiological analysis at different stages (D_1 to D_2) after the spray of growth regulators (T_1 to T_8) from the pot irrigated with saline water having a different concentration (S_1 to S_2) and packed in plastic bag and brought to the laboratory under ice cold condition.

Biochemical parameters and physiological parameter assay

Total soluble sugar: Seedlings (0.1 gm) were extracted with 5 ml of 80% methanol and centrifuged at 3000 rpm for 10 minutes. Extraction was repeated 4 times with 80% methanol and supernatants were collected into 25 ml volumetric flasks. Final volume of the extract was made to 25 ml with 80% methanol. The extract (0.3 ml) was pipetted into separate test tubes and the tubes were placed in a boiling water bath to evaporate the methanol. One ml of Millipore water and 1 ml of 5% phenol was added in each test tube. Then 5 ml of sulphuric acid was added. The tubes were allowed to cool in ice-bath for 10-15 minutes. The intensity of colour was read at 490 nm on spectrophotometer. A standard curve was prepared using 10 mg glucose per 100 ml distilled water Dubois *et al.* (1956). The amount of total soluble sugar present in the sample was calculated by appropriate formula.

Reducing sugar: Reducing sugar was estimated by using Nelson and Somyogi method (Somogyi, 1952). In this method, Extraction was done same as total soluble sugar. Known aliquot (0.1 ml) was taken and volume made up to 1 ml by Adding distilled water. Then added 1 ml of alkaline copper reagent (Alkaline copper reagent: 4 gm copper sulphate, 24 gm sodium carbonate, 12 gm sodium potassium tartrate, 16 gm sodium bicarbonate, 180 gm anhydrous sodium sulphate were dissolved in 1000 ml of DW) to each tube. The tubes were placed in boiling water bath for 10 min. Then, added 1 ml Arsenomolybdate reagent (Arsenomolybdate reagent: 50 gm Ammonium molybdate, 42 ml concentrated sulphuric acid 6 gm disodium hydrogen arsenate were mix in 1000 ml DW) in each tube. Total volume made up 10 ml with DW. After 10 min, the absorbance was measured at 620 nm. A standard curve was prepared using 10 mg glucose per 100 ml distilled water.

Proline: Proline content was estimates by using method given by Bates *et al.* (1973). Leaf tissues weighed 0.1 gm and grinded in 5 ml of 3% sulphosalicylic acid. Aliquot from the sample extract (0.5-1.0 ml) and standard proline (0.1-0.6 ml from 0.05 mg/ml proline stock) were taken in a series of test tubes and the volume was made up to 1.0 ml with distilled water. Then 2 ml glacial acetic acid and 2 ml Ninhydrin reagent (1.25 gm Ninhydrine + 30 ml glacial acetic acid + 8 ml 6 M phosphoric acid in 12 ml distilled water) were added. Then tubes were kept in boiling water bath for 1 hrs. The tubes were cooled in running water at room temperature. After that, 4 ml toluene was added. The absorbance was recorded from toluene phase at 520 nm in spectrophotometer. The free proline was calculated as stated below and expressed as $mg.g^{-1}$

Glycine betaine: Glycine-betaine was done from fresh leaves per the method of Hendawey (2015). Finely ground leaf material (0.5 g) was mechanically shaken with 20 ml of distilled water for 16 hrs. at 25°C. The samples were then filtered and the filtrate was stored in freezer until analysis. Thawed extracts were diluted 1:1 with 2 N sulphuric acid. Aliquot (0.5 ml) was measured into test tube and cooled in ice for 1 hour 0.2 ml of cold potassium iodide- iodine reagent [Iodine (15.7 g) and potassium iodide (20 g) were dissolved in 100 ml of water and kept in fridge at 4°C] was added and the mixture was gently mixed with vortex mixture. The samples were stored at 0 to 4°C for 16 hr. After the expiration of the period samples were transferred to centrifuge tubes and then centrifuged at 10,000 g for 15 min at 0°C. The supernatant was carefully aspirated with 1 ml micropipette. The periodite crystals were dissolved in 9 ml of 1, 2-dichloroethane. Vigorous vortex mixing was done to effect complete solubility in developing solvent. After 2.0-2.5 hrs. an absorbance was measured at 365 nm. Reference standards of glycine-betaine (50-200 μgml^{-1}) were prepared in 2 N sulphuric acid and the amount of glycine betaine present in the sample was calculated by appropriate formula.

Ascorbic Acid: Ascorbic acid was estimated by Dinitrophenyl Hydrazine (DNPH) method of Sadasivam and Manickam (1992). Leaves (0.5 gm) of all treatments were extracted with 5 ml of 6% TCA (Trichloro Acetic Acid) using mortar and pestle. Homogenized material was centrifuged or filtered and the supernatant was collected. Known aliquot (0.1 ml) was taken and

volume made up to 1 ml by adding distilled water. 2 ml DNPH reagent (2% DNPH in 9 N H₂SO₄) was added in each tube. Then add 2 drop of 10% Thiourea. Kept in boiling water bath at 80°C for 15 min. Finally 5ml 80% H₂SO₄ was added and cooled. The intensity of colour was read at 540 nm on spectrophotometer.

Relative water content (RWC): Known weight (gm) of fresh leaf of mothbean were taken and transferred in a petri dish, and to this 25 ml distilled water was added and kept for four hour. Then the leaves were taken out, dried by blotting paper and weighed (Turgid weight). The leaf was kept in oven at 84°C for 5 hrs and weighted until constant weight was obtained. After this RWC were estimated as per formula and expressed as per cent relative water content (Weatherley, 1962).

$$\text{Relative Water Content (\%)} = \frac{\text{Fresh weight (g.)} - \text{Dry weight (g.)}}{\text{Turgid weight (g.)} - \text{Dry weight (g.)}} \times 100$$

Membrane stability index (MSI): Known weights (gm) of fresh leaf of mothbean were taken in test tubes and 10ml of distilled water was added in each. Test tubes were kept at 40°C in a water bath for 30 min and electrical conductivity (EC) of the sample was measured (C₁) using a conductivity meter. Than after test tubes were incubated at 100°C in the boiling water bath for 15 min and their electrical conductivity were measured (C₂). MSI was calculated using the given formula by (Sairam *et al.* 1997).

$$\text{Membrane Stability Index (MSI)} = 1 - (C_1/C_2) \times 100$$

Leaf pH: Few leaves were taken to measure, roll them into a tight ball and squeeze out of a few drops of sap using a press. Generally, the mature leaves of the plants give the most accurate leaf pH. The plant tissue can be measured by using the pH meter. (Johannes *et al.* 2011).

RESULTS AND DISCUSSION

4.2.4 Total soluble sugar

The data on total soluble sugar (%) analyzed from leaf tissue of mothbean collected from plants treated at 15 DAS and 25 DAS with different concentration of growth regulators and their combination (T₁ to T₈) grown in a pot irrigated with tap water (S₁) and saline water (S₂) 4 EC at two different stages G₁ (20 DAS) and G₂ (30 DAS) are depicted in Fig. 1 and 2.

Among the salinity level, treatment S₂ irrigated with saline water 4 EC showed highest amount (2.44 %) of total soluble sugar while the pot irrigated with tap water (S₁) showed lowest value (2.32 %) for total soluble sugar (Fig. 1 A).

Among the different stages, mean value of total soluble sugar non significantly varied between 2.38 % and 2.39 % (Fig. 1 B). The content was increased from 20 DAS (2.38 %) to 30 DAS (2.39 %).

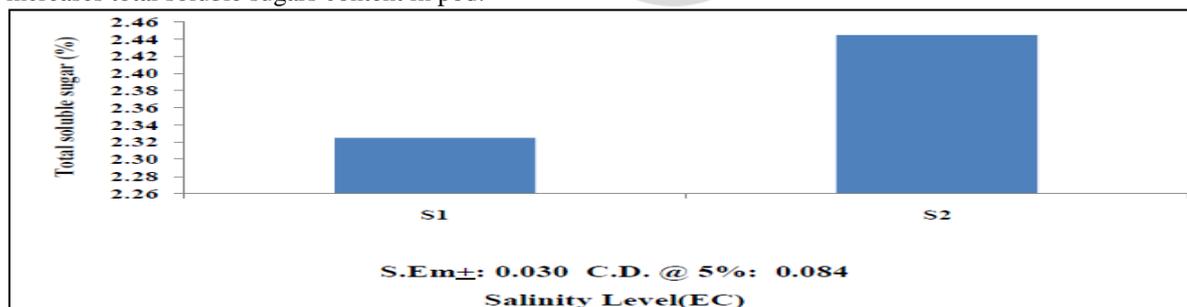
Imposition of spray treatment of growth regulators and their combination found statistical significant (Fig. 1C). The tissues obtain from mothbean pots treated with T₈ [GA₃ @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm] revealed higher amount of mean total soluble sugar (2.61 %) and the mean lowest content was noted for the tissues received from T₁ (2.24 %).

Interaction effect of S X T for total soluble sugar was revealed significant differences in leaf tissue of mothbean (Fig. 2 A). The highest value (2.69 %) of total soluble sugar content was observed for the S₁T₈ i.e. in plant irrigated with tap water combine with GA₃ @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm treatment. The lowest value (2.19 %) of total soluble sugar content was observed in plant irrigated with saline water under control condition (S₂T₁).

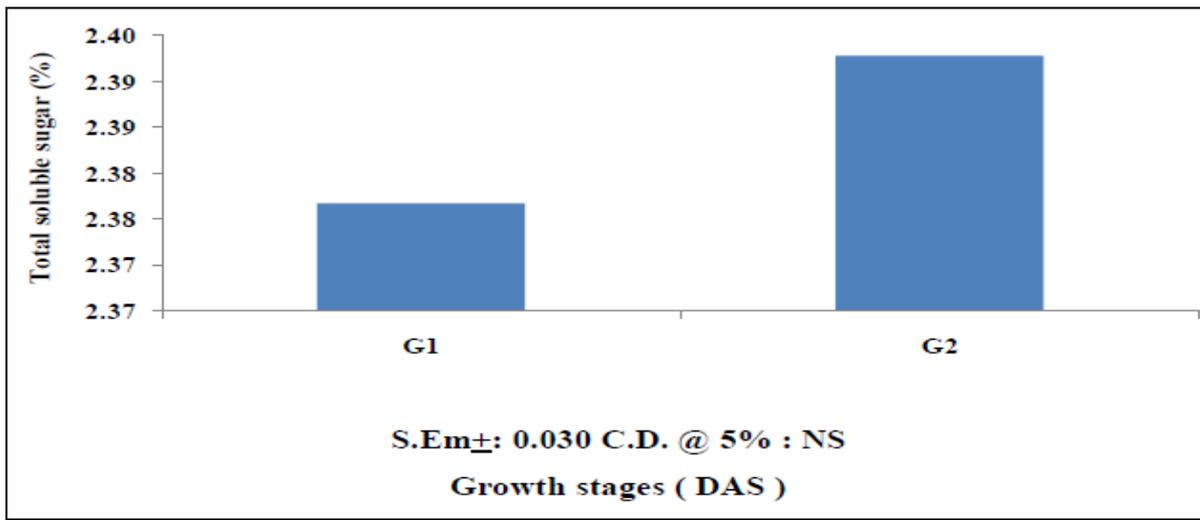
Interaction effect of G X T for total soluble sugar content was revealed significant differences in leaf tissue of mothbean (Fig. 2 B). The highest value (2.66 %) of total soluble sugar content was observed in G₁T₈ i.e. in plant treated with GA₃ @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm after at 20 DAS. The lowest value (2.20 %) of total soluble sugar content was observed for G₂T₁ i.e. plant was control condition after 30 DAS.

Interaction effect of S X G for total soluble sugar content was revealed significant differences in leaf tissue of mothbean (Fig. 2 C). The highest value (2.50 %) of total soluble sugar content was observed in S₂G₂ i.e. in plant irrigated with saline water after 30 DAS. The lowest value of total soluble sugar content was observed for S₁G₂ (2.29 %) i.e. in plant irrigated with tap water after 30 DAS.

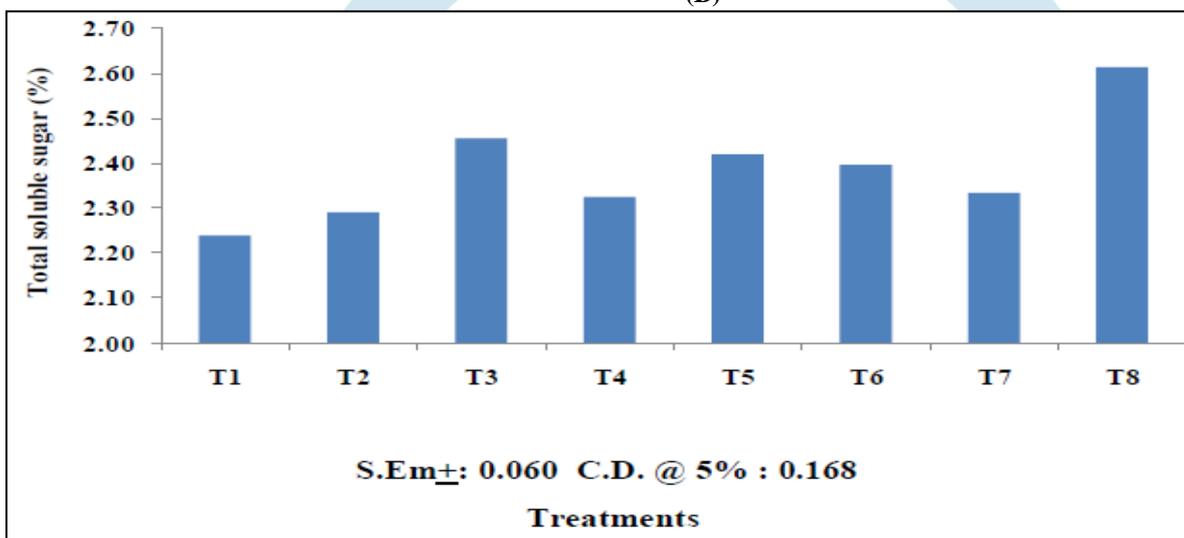
These results were in agreement with Li *et al.* (2010) reported that GA₃ significantly improved seed germination characteristics under drought stress. Compared with no-GA₃ priming treatment, seeds with 300 mg·l⁻¹ GA₃ showed a significantly increased soluble sugars (47.2% increase) in rapeseed (*Brassica napus* L.). Kandoliya *et al.* (2015) reported that abiotic stress treatment increases total soluble sugars content in pod.



(A)

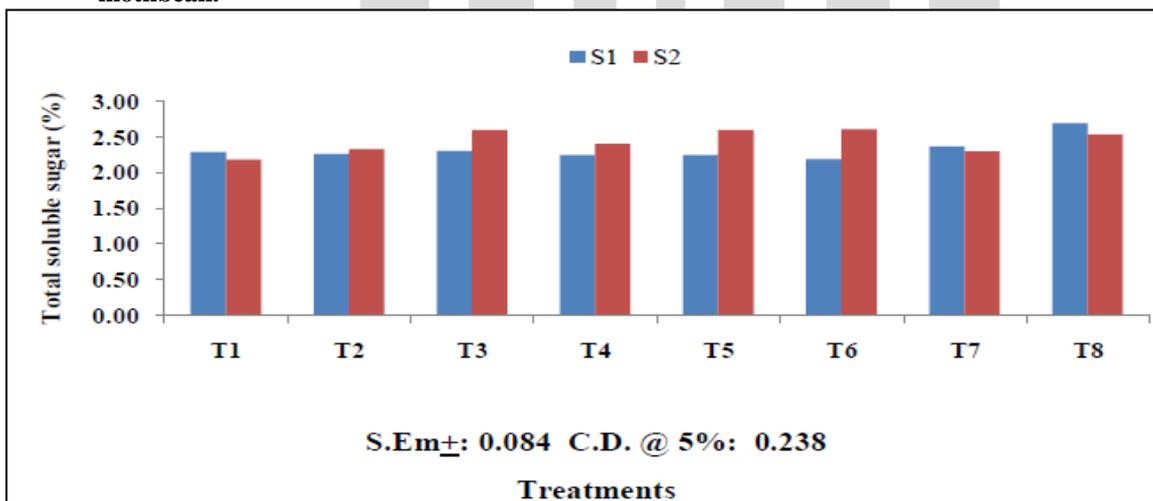


(B)



(C)

Fig.1: Mean effect of [A] salinity (S), [B] growth stages (G), [C] treatments (T) on total soluble sugar (%) in leaf tissue of mothbean.



(A)

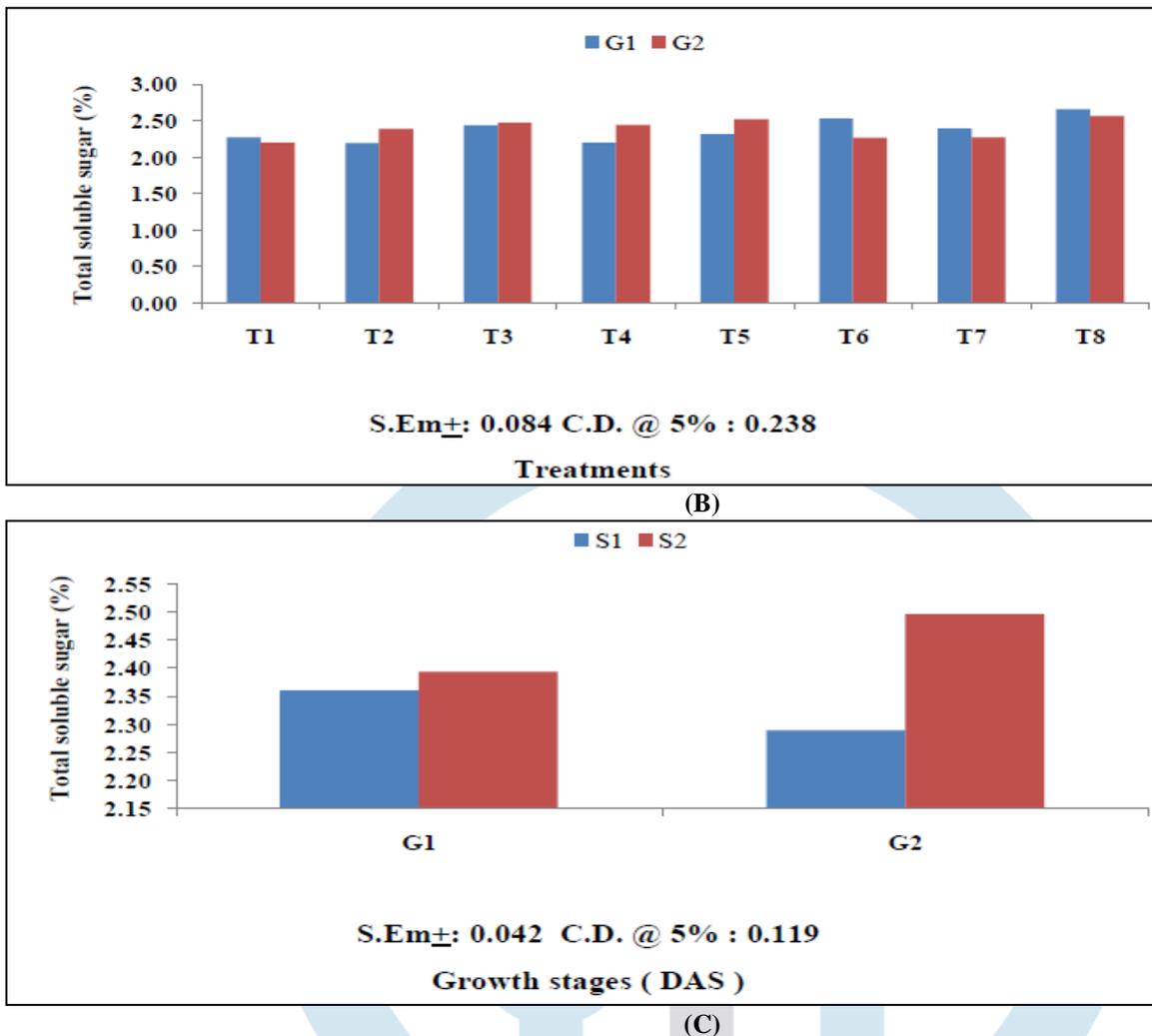


Fig. 2: Interaction effect of [A] Salinity (S) X Treatments (T), [B]Growth Stages (G) X Treatment (T), [C]Salinity (S) XGrowth Stages (G) on total soluble sugar (%) in leaf tissue of mothbean.

4.2.5 Reducing sugar

The data on reducing sugar (%) analyzed from leaf tissue of mothbean collected from plants treated at 15 DAS and 25 DAS with different concentration of growth regulators and their combination (T₁ to T₈) grown in a pot irrigated with tap water (S₁) and saline water (S₂) 4 EC at two different stages G₁ (20 DAS) and G₂ (30 DAS) are depicted in Fig. 3 and 4.

Among the salinity level, treatment S₂ saline water 4 EC irrigated with tap water showed highest amount of reducing sugar (2.86 %) while the pot irrigated with tap water (S₁) showed lowest value for reducing sugar (2.24 %) (Fig. 3 A).

Among the different stages, mean value of reducing sugar significantly varied between 2.75 % and 2.36 % (Fig. 3 B). The content was increased from 20 DAS (2.75 %) to 30 DAS (2.36 %).

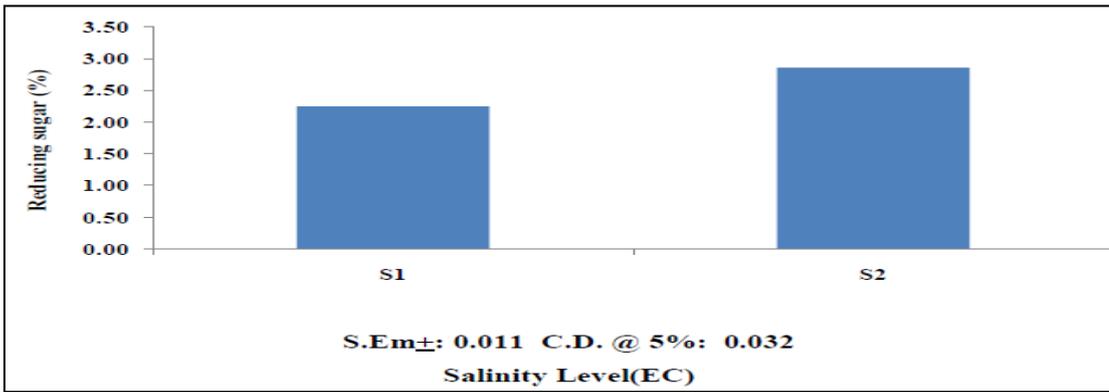
Application of spray treatment including growth regulators and their combination found statistical significant (Fig. 3 C). The tissues obtain from mothbean pots treated with T₈ [GA₃ @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm] revealed higher amount (3.74 %) of mean reducing sugar. The mean lowest content was noted for the tissues received from T₁ (1.49 %).

Interaction effect of S X T for reducing sugar was revealed significant differences in leaf tissue of mothbean (Fig. 4 A). The highest value (4.20 %) of reducing sugar content was observed for the S₂T₈ i.e. in plant irrigated with saline water combine with GA₃ @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm treatment. The lowest value (1.13 %) of reducing sugar content was observed in plant irrigated with tap water under control condition (S₁T₁).

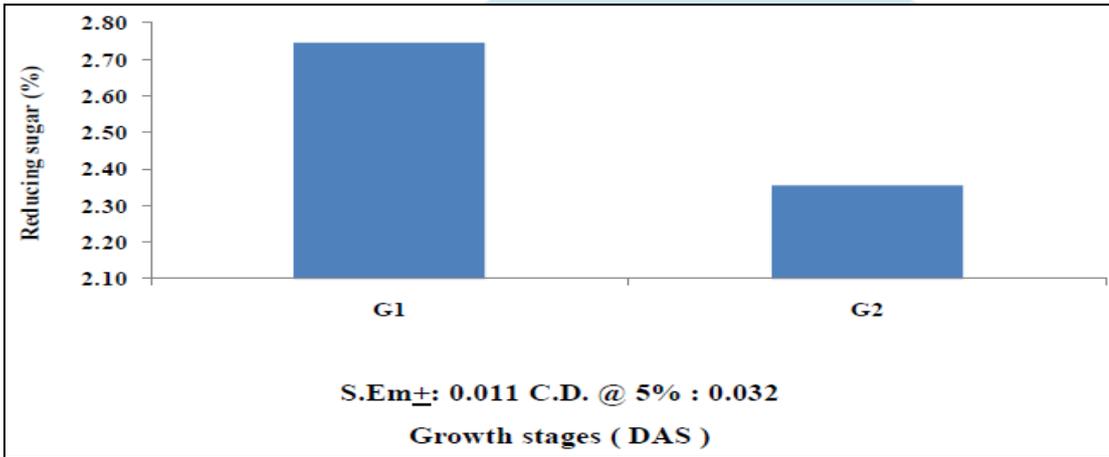
Interaction effect of G X T for reducing sugar content was revealed significant differences in leaf tissue of mothbean (Fig. 4 B). The highest value (3.87 %) of reducing sugar content was observed in G₁T₈ i.e. in plant treated GA₃ @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm after 20 DAS. The lowest value (1.40 %) of reducing sugar content was observed for G₂T₁ i.e. plant was control condition after 30 DAS.

Interaction effect of S X G for reducing sugar content was revealed significant differences in leaf tissue of mothbean (Fig. 4 C). The highest value (3.10 %) of reducing sugar content was observed in S₂G₁ i.e. in plant irrigated with saline water (4 EC) after 20 DAS. The lowest value of reducing sugar content was observed for S₁G₂ (2.09 %) i.e. in plant irrigated with tap water after 20 DAS.

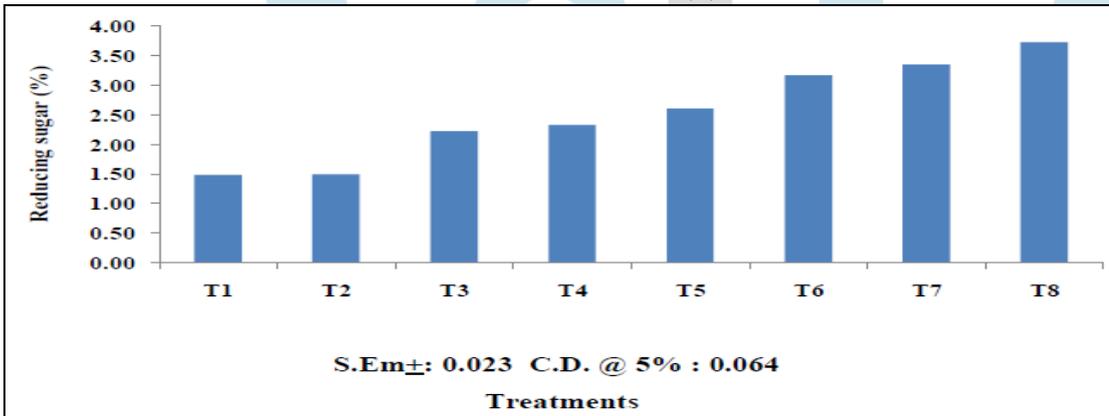
These results were in agreement with Mini *et al.* (2015) reported that sodium chloride solutions exposure to salt stress which increased sugar content. Anaya *et al.* (2015) studied the effects of salt stress and seeds soaked in SA on physiological and biochemical parameters of *Vicia faba* L. Salinity increases the concentration of soluble sugar while seeds soaked in SA reduce their concentration/activity.



(A)

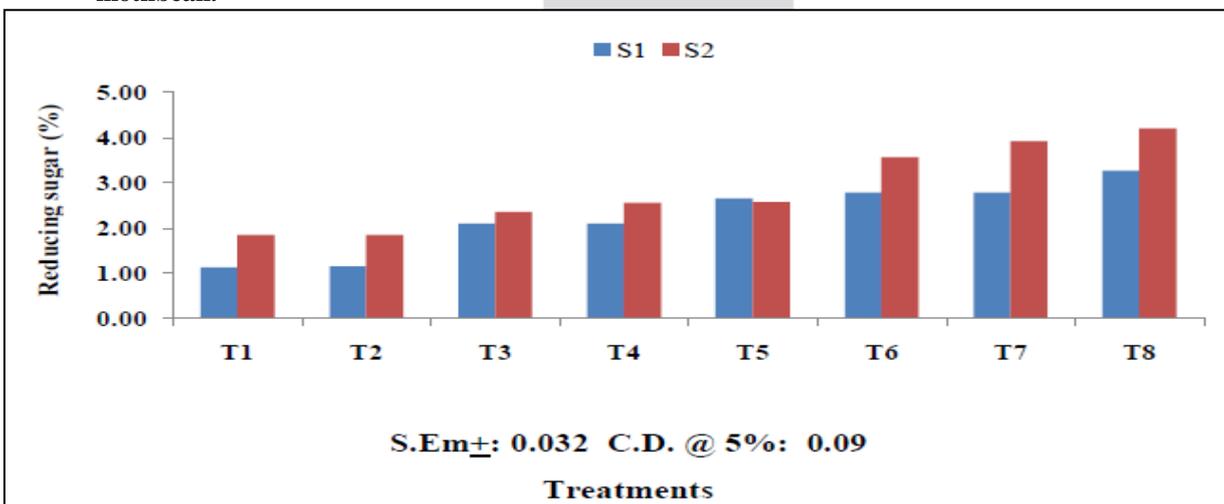


(B)



(C)

Fig.3: Mean effect of [A] salinity (S), [B] growth stages (G), [C] treatments (T) on reducing sugar (%) in leaf tissue of mothbean.



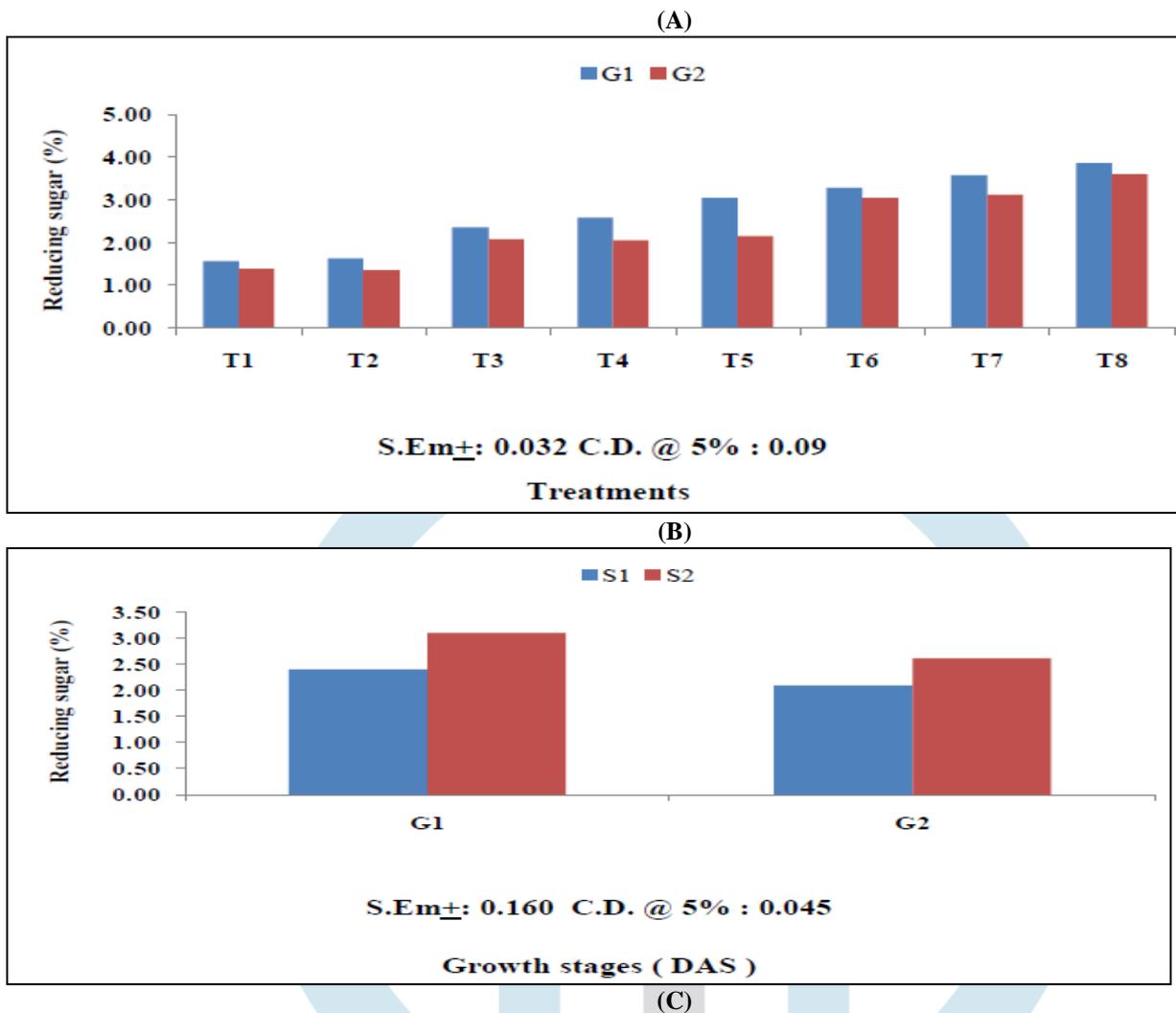


Fig.4: Interaction effect of [A] Salinity (S) X Treatments (T), [B] Growth Stages (G) X Treatment (T), [C] Salinity (S) X Growth Stages (G) on reducing sugar (%) in leaf tissue of mothbean.

4.2.8 Proline

The data on proline (mg.g^{-1}) analysed from leaf tissues of mothbean collected from plants treated at 15 DAS and 25 DAS with different concentration of growth regulators and their combination (T_1 to T_8) grown in a pot irrigated with tap water (S_1) and saline water (S_2) 4 EC at two different stages G_1 (20 DAS) and G_2 (30 DAS) are depicted in Fig. 5 and 6.

Among the salinity level, treatment S_2 irrigated with saline water showed highest amount of proline (4.97 mg.g^{-1}) while the treatment S_1 irrigated with tap water showed lowest value for proline (3.66 mg.g^{-1}) (Fig. 5 A).

Among the different stages, mean value of proline significantly varied between 3.69 mg.g^{-1} and 4.94 mg.g^{-1} (Fig. 5 B). The content was increased from 20 DAS (3.69 mg.g^{-1}) to 30 DAS (4.94 mg.g^{-1}).

Spray treatment of growth regulators and their treatment combination found statistical significant (Fig. 5 C). The tissues obtain from mothbean pots treated with T_8 [GA_3 @ 100 ppm + SA @ 100 ppm + IAA @ 500 ppm] revealed higher amount (5.10 mg.g^{-1}) of mean proline and which was followed by T_7 [SA @ 100 ppm + IAA @ 500 ppm (4.94 mg.g^{-1})] and T_6 [GA_3 @ 100 ppm + IAA @ 500 ppm (4.60 mg.g^{-1})] irrespective of salinity level and growth stages. The mean lowest content was noted for the tissues received from T_1 (3.51 mg.g^{-1}).

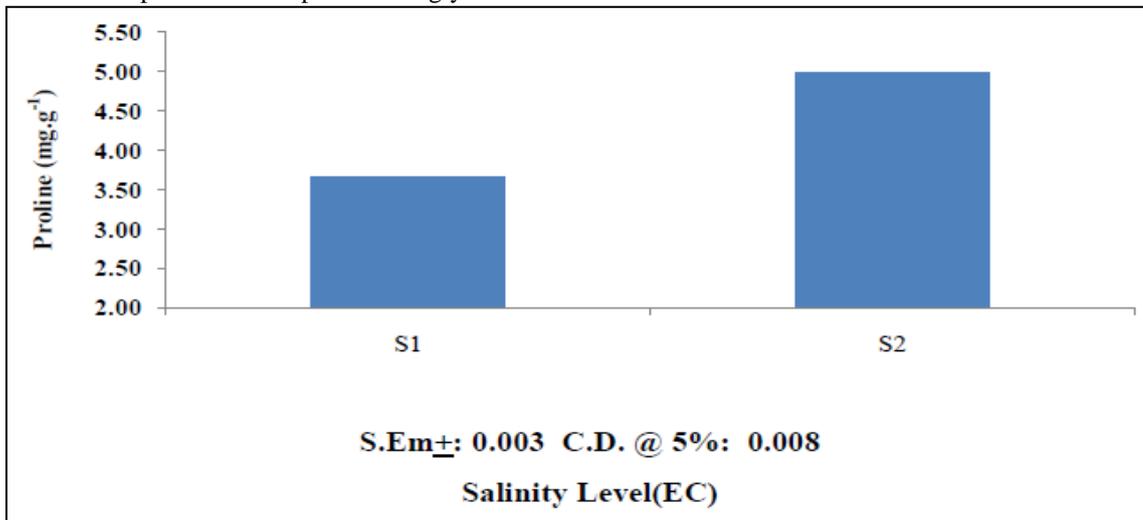
Interaction effect of S X T for proline was revealed significant differences in leaf tissue of mothbean (Fig. 6 A). The highest value (5.83 mg.g^{-1}) of proline content was observed for the S_2T_8 i.e. in plant irrigated with saline water combine with GA_3 @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm treatment. The lowest value (2.91 mg.g^{-1}) of proline content was observed in plant irrigated with tap water under control condition (S_1T_1).

Interaction effect of G X T for proline content was revealed significant differences in leaf tissue of mothbean (Fig. 6 B). The highest value (5.86 mg.g^{-1}) of proline content was observed in G_2T_8 i.e. in plant treated with GA_3 @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm after at 30 DAS. The lowest value (2.99 mg.g^{-1}) of proline content was observed for G_1T_1 i.e. plant was control condition after 20 DAS.

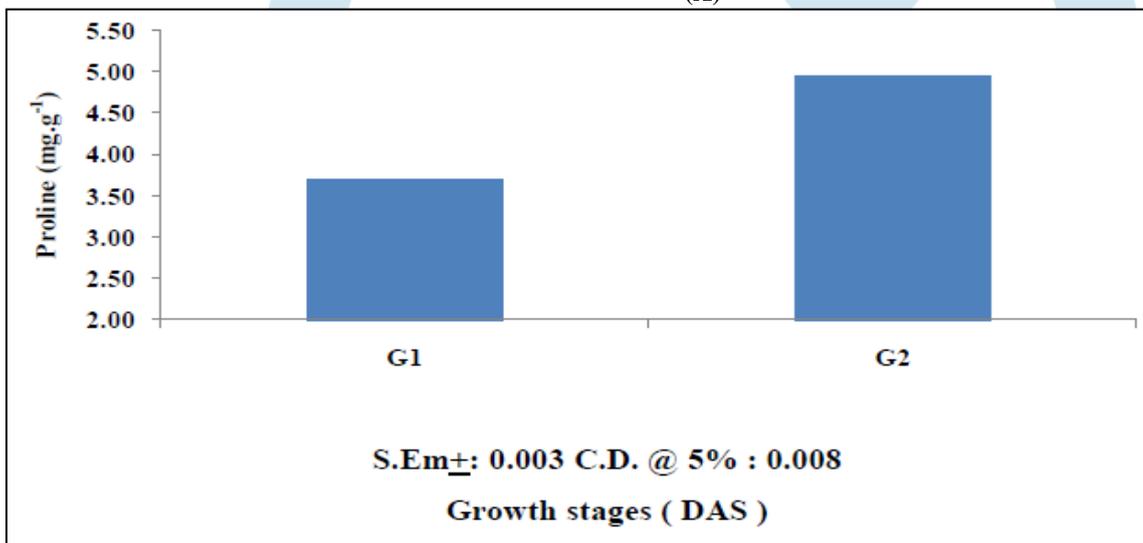
Interaction of S X G effect for proline content was concerned; it showed significant differences (Fig. 6 C). The highest value (5.36 mg.g^{-1}) of proline content was observed in S_2G_2 i.e. in plant irrigated with saline water 4 EC after 30 DAS. The lowest value (2.79 mg.g^{-1}) of proline content was observed for S_1G_2 i.e. in plant irrigated with tap water after 20 DAS.

These results were in agreement with Saha *et al.*, (2010) suggested that mungbean plants can be acclimatized to lethal levels of salinity by pre-treatment with sublethal levels of NaCl by increased growth and photosynthetic pigments of the seedlings,

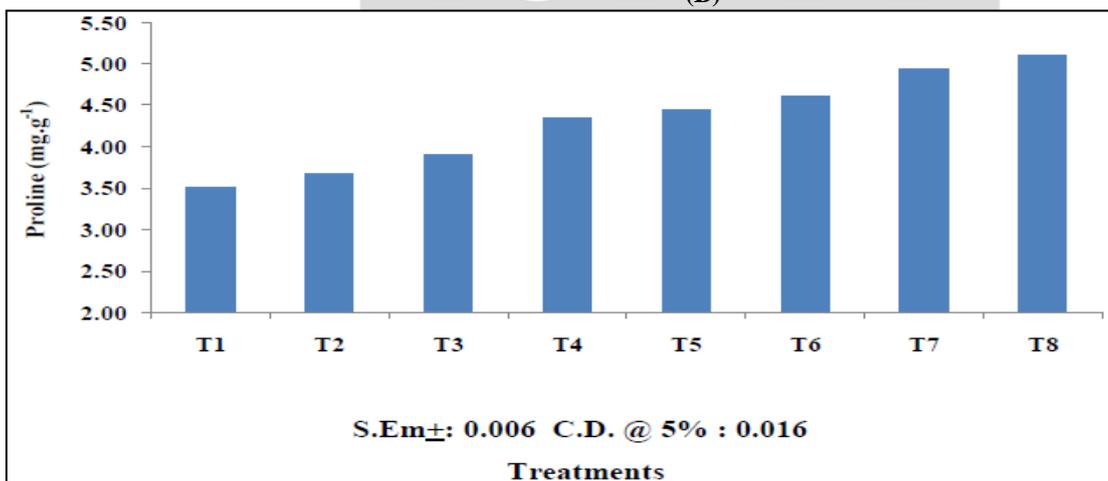
increasing accumulation of osmolytes like proline content. Bengu (2012) reported that salinity increased the proline content but the application of salicylic and gibberellic acids, was found to alleviate the adverse effects of salinity stress on the parameters also Solanki *et al.*, (2018) observed that biochemical parameter like proline and glycine betain was increased with higher concentration of salt stress. Salicylic acid can regulate various plant metabolic process and modulate the production of varied biochemical parameter like proline and glycine betain.



(A)

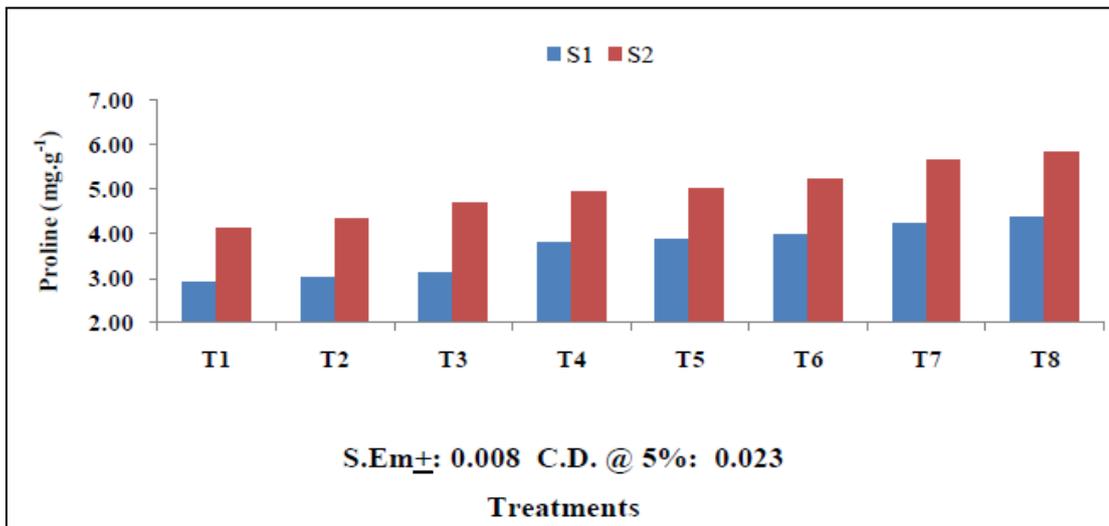


(B)

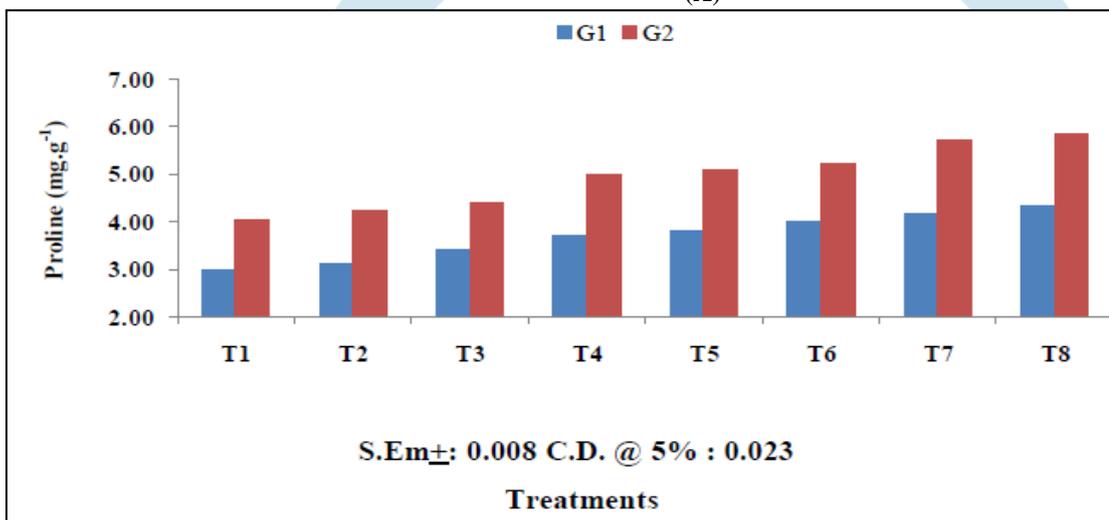


(C)

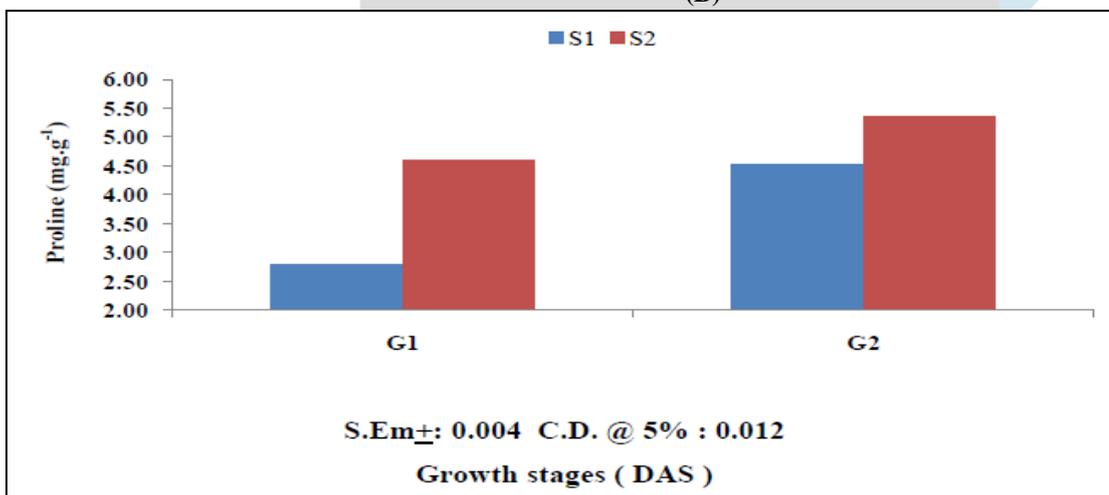
Fig.5: Mean effect of [A] salinity (S), [B] growth stages (G), [C] treatments (T) on proline (mg.g⁻¹) in leaf tissue of mothbean.



(A)



(B)



(C)

Fig.6: Interaction effect of [A] Salinity (S) X Treatments (T), [B] Growth Stages (G) X Treatment (T), [C] Salinity (S) X Growth Stages (G) on proline (mg.g⁻¹) in leaf tissue of mothbean.

4.2.9 Glycine betaine

The data on glycine betaine (mg.g⁻¹) analysed from leaf tissues of mothbean collected from plants treated at 15 DAS and 25 DAS with different concentration of growth regulators and their combination (T₁ to T₈) grown in a pot irrigated with tap water (S₁) and saline water (S₂) 4 EC at two different stages G₁ (20 DAS) and G₂ (30 DAS) are depicted in Fig. 7 and 8.

Among the salinity level, treatment S₂ irrigated with tap water showed highest amount of glycine betaine (4.38 mg.g⁻¹) while the pot irrigated with tap water showed lowest value for glycine betaine (3.07 mg.g⁻¹) (Fig. 7 A).

Among the different stages, mean value of glycine betaine significantly varied between 2.88 mg.g⁻¹ and 4.56 mg.g⁻¹ (Fig. 7 B). The content was increased from 20 DAS (2.88 mg.g⁻¹) to 30 DAS (4.56 mg.g⁻¹).

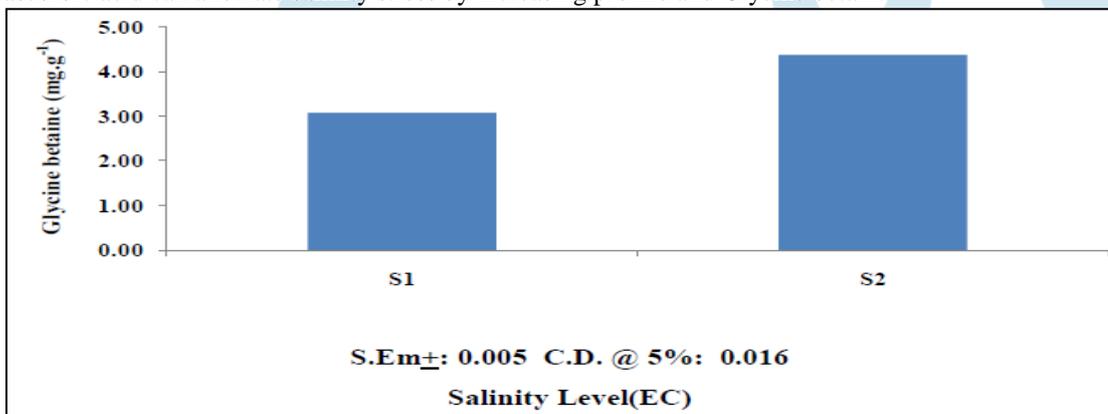
Treatment of growth regulators and their combination found statistical significant (Fig. 7 C). The tissues obtain from mothbean pots treated with T₈ [GA₃ @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm] revealed higher amount (4.77 mg.g⁻¹) of mean glycine betaine and which was followed by T₇ [SA @ 100 ppm + IAA @ 500 ppm (4.61 mg.g⁻¹)] and T₆ [GA₃ @ 100 ppm + IAA @ 500 ppm (4.15 mg.g⁻¹)] irrespective of salinity level and growth stages. The mean lowest (2.54 mg.g⁻¹) content was noted for the tissues received from T₁.

Interaction effect of S X T for glycine betaine was revealed significant differences in leaf tissue of mothbean (Fig. 8 A). The highest value (5.35 mg.g⁻¹) of glycine betaine content was observed for the S₂T₈ i.e. in plant irrigated with saline water combine with GA₃ @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm treatment. The lowest value (1.87 mg.g⁻¹) of glycine betaine content was observed in plant irrigated with tap water under control condition (S₁T₁).

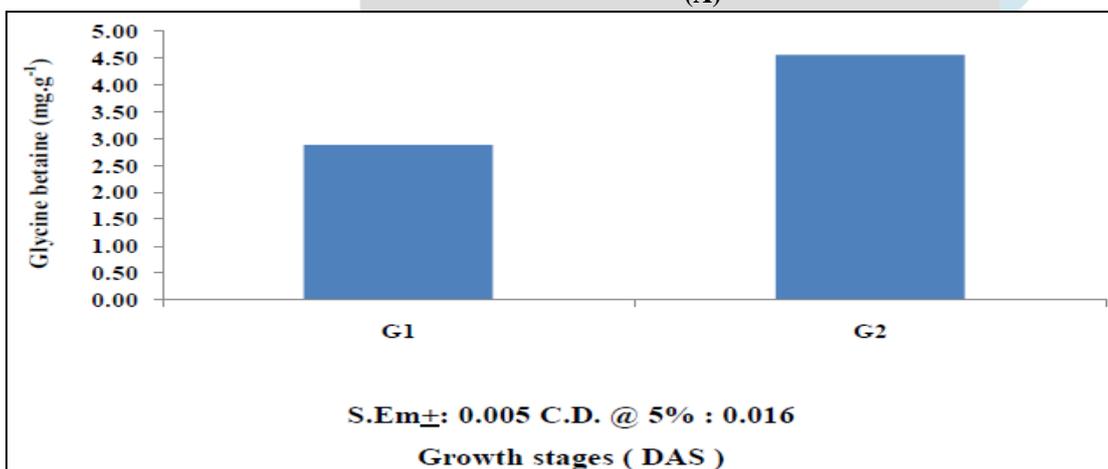
Interaction effect of G X T for glycine betaine content was revealed significant differences in leaf tissue of mothbean (Fig. 8 B). The highest value (5.85 mg.g⁻¹) of glycine betaine content was observed in G₂T₈ i.e. in plant treated with GA₃ @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm after 30 DAS. The lowest value (1.93 mg.g⁻¹) of glycine betaine content was observed for G₁T₁ i.e. plant was control condition after 20 DAS.

Interaction effect of S X G for glycine betaine content was revealed significant differences in leaf tissue of mothbean (Fig. 8 C). The highest value (5.34 mg.g⁻¹) of glycine betaine content was observed in S₂G₂ i.e. in plant irrigated with saline water (4 EC) after 30 DAS. The lowest value (2.35 mg.g⁻¹) of glycine betaine content was observed for S₁G₁ i.e. in plant irrigated with tap water after 20 DAS.

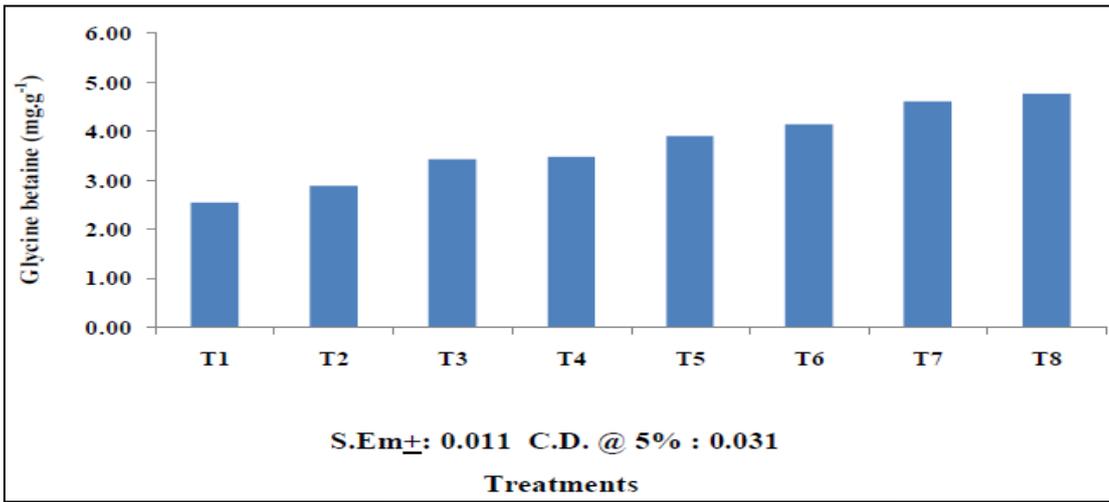
These results were in agreement with Jaleel *et al.* (2009) studied the effects of NaCl stressed on blackgram (*Vigna mungo* (L) Hepper) plants and reported that salinity treatment decreased the protein content and increased the glycine betaine (GB) in black gram compared with the control. Abbasil and faghani (2015) reported the role of salicylic acid and ascorbic acid in the alleviation of salinity stress in wheat (*Triticum aestivum* L.). Results showed that 300 mM (NaCl/1.8 CaCl₂) seed pre-treatment by 2.5% ascorbic acid can alleviate salinity stress by increasing proline and Glycine betaine.



(A)

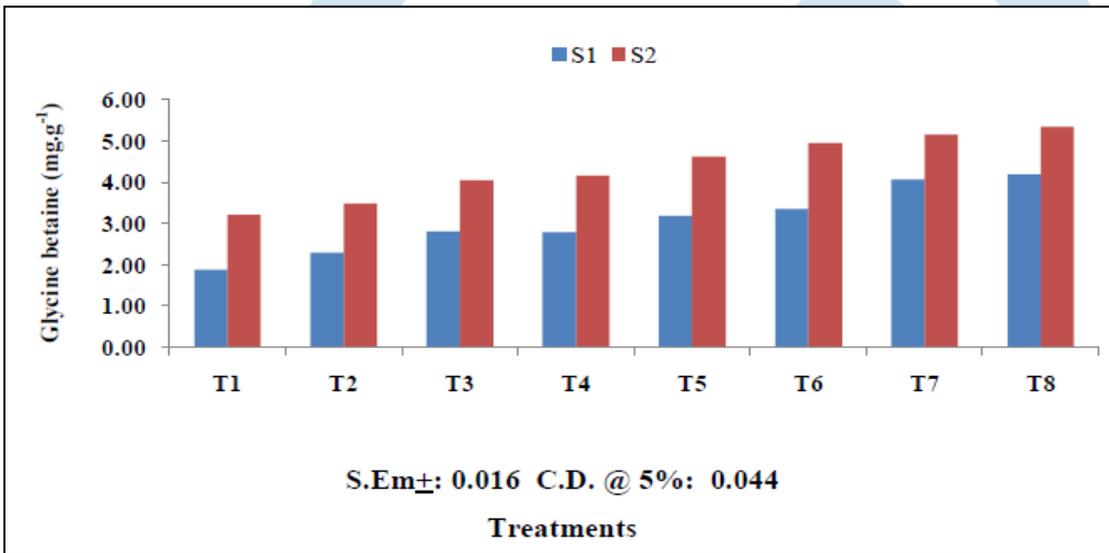


(B)

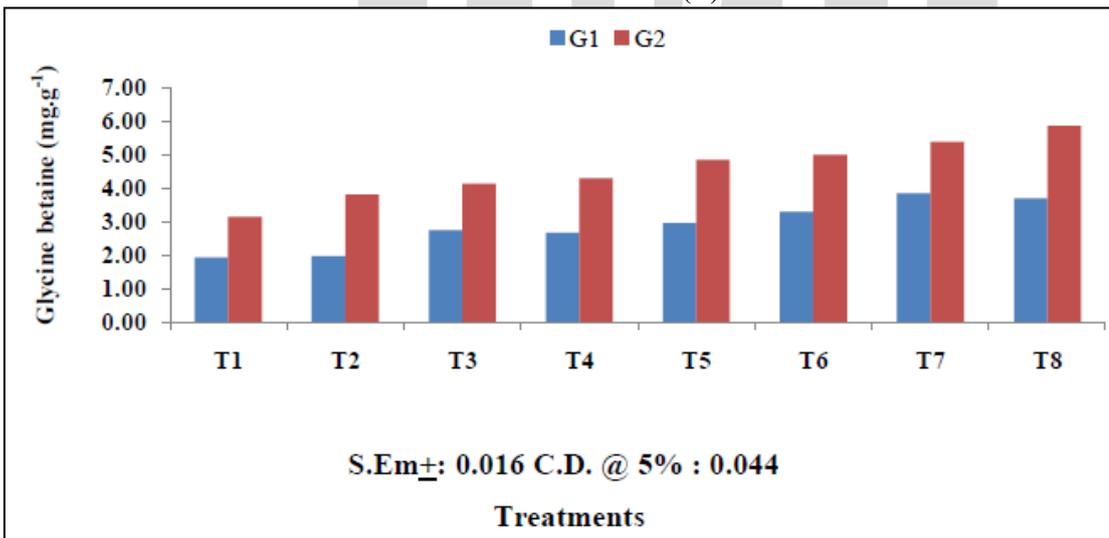


(C)

Fig.7: Mean effect of [A] salinity (S), [B] growth stages (G), [C] treatments (T) on glycine betaine (mg.g⁻¹) in leaf tissue of mothbean.



(A)



(B)

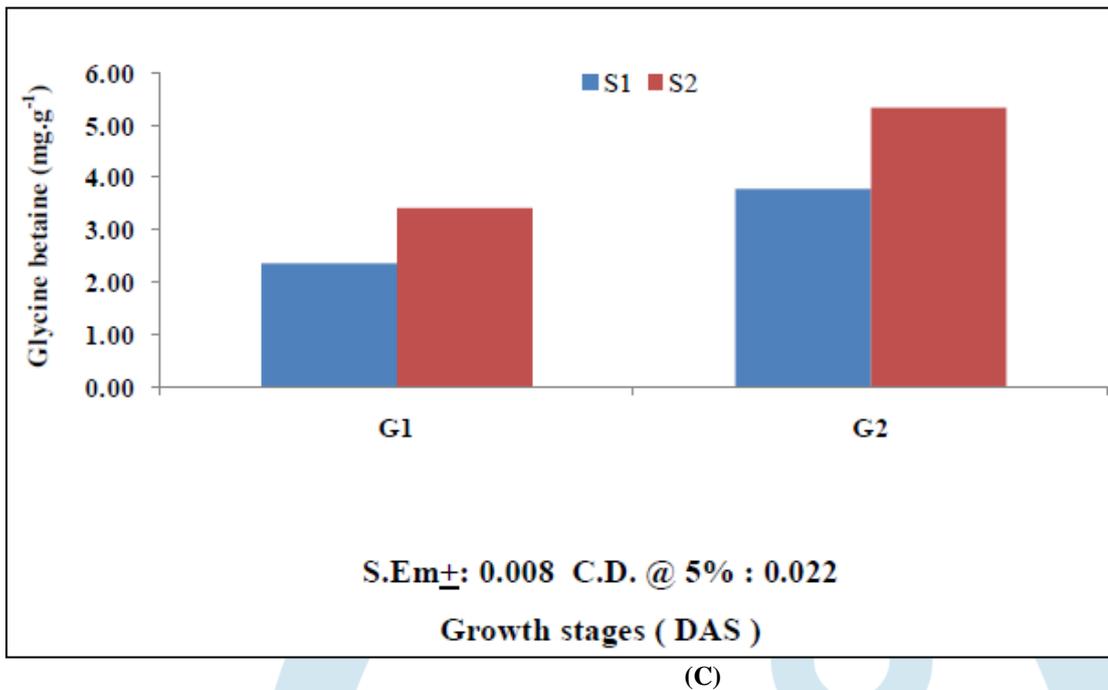


Fig.8: Interaction effect of [A] Salinity (S) X Treatments (T), [B]Growth Stages (G) X Treatment (T), [C]Salinity (S) X Growth Stages (G) on glycinebetain (mg.g⁻¹) in leaf tissue of mothbean.

4.2.10 Ascorbic acid

The data on ascorbic acid (mg.g⁻¹) analyzed from leaf tissues of mothbean collected from plants treated at 15 DAS and 25 DAS with different concentration of growth regulators and their combination (T₁ to T₈) grown in a pot irrigated with tap water (S₁) and saline water (S₂) 4 EC at two different stages G₁ (20 DAS) and G₂ (30 DAS) are depicted in Fig. 9 and 10.

Among the salinity level, treatment S₂ irrigated with tap water showed highest amount of ascorbic acid (7.58 mg.g⁻¹) while the pot irrigated with tap water showed lowest value for ascorbic acid (6.67 mg.g⁻¹) (Fig. 9 A).

Among the different stages, mean value of ascorbic acid significantly varied between 6.15 mg.g⁻¹ and 8.10 mg.g⁻¹ (Fig. 9 B). The content was increased from 20 DAS (6.15 mg.g⁻¹) to 30 DAS (8.10 mg.g⁻¹).

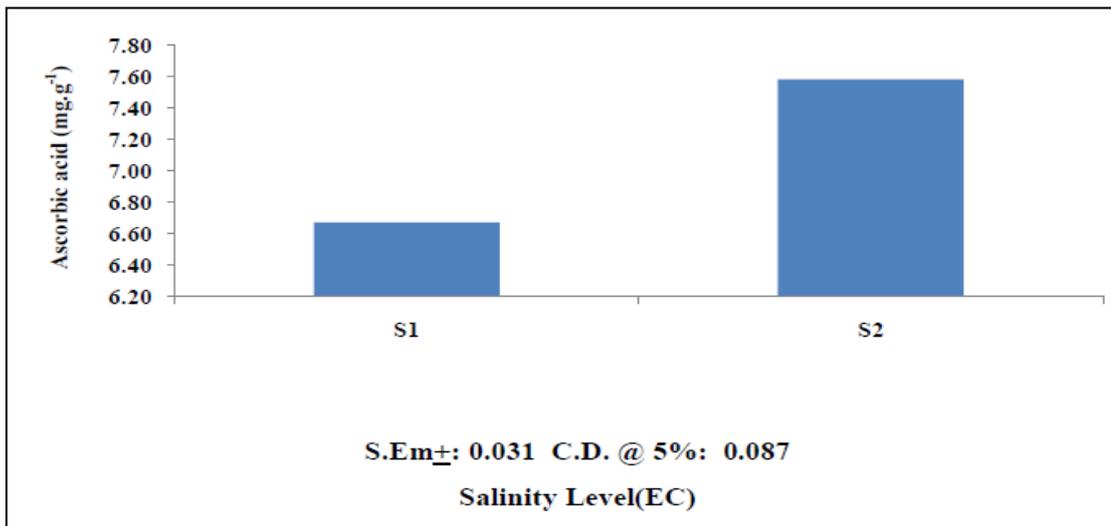
Treatment of growth regulators and their combination found statistical significant (Fig. 9 C). The tissues obtain from mothbean pots treated with T₈ [GA₃ @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm] revealed higher amount (8.41 mg.g⁻¹) of mean ascorbic acid and which was followed by T₇ [SA @ 100 ppm + IAA @ 500 ppm (8.18 mg.g⁻¹)] and T₆ [GA₃ @ 100 ppm + IAA @ 500 ppm (7.81 mg.g⁻¹)] irrespective of salinity level and growth stages. The mean lowest (5.76 mg.g⁻¹) content was noted for the tissues received from T₁.

Interaction effect of S X T for ascorbic acid was revealed significant differences in leaf tissue of mothbean (Fig. 10 A). The highest value (8.86 mg.g⁻¹) of ascorbic acid content was observed for the S₂T₈ i.e. in plant irrigated with saline water combine with GA₃ @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm treatment. The lowest value (5.25 mg.g⁻¹) of ascorbic acid content was observed in plant irrigated with tap water under control condition (S₁T₁).

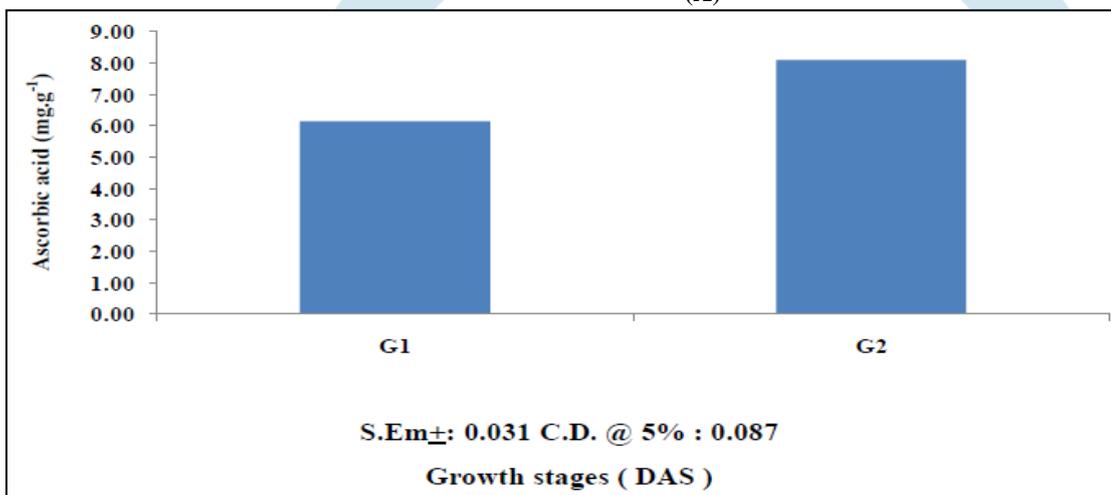
Interaction effect of G X T for ascorbic acid content was revealed significant differences in leaf tissue of mothbean (Fig. 10 B). The highest value (9.41 mg.g⁻¹) of ascorbic acid content was observed in G₂T₈ i.e. in plant treated with GA₃ @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm after at 30 DAS. The lowest value (4.76 mg.g⁻¹) of ascorbic acid content was observed for G₁T₁ i.e. plant was control condition after 20 DAS.

Interaction effect of S X G for ascorbic acid content was revealed nonsignificant differences in leaf tissue of mothbean (Fig. 10 C). The highest value (8.60 mg.g⁻¹) of ascorbic acid content was observed in S₂G₂ i.e. in plant irrigated with saline water (4 EC) after 30 DAS. The lowest value (5.74 mg.g⁻¹) of ascorbic acid content was observed for S₁G₁ i.e. in plant irrigated with tap water after 20 DAS.

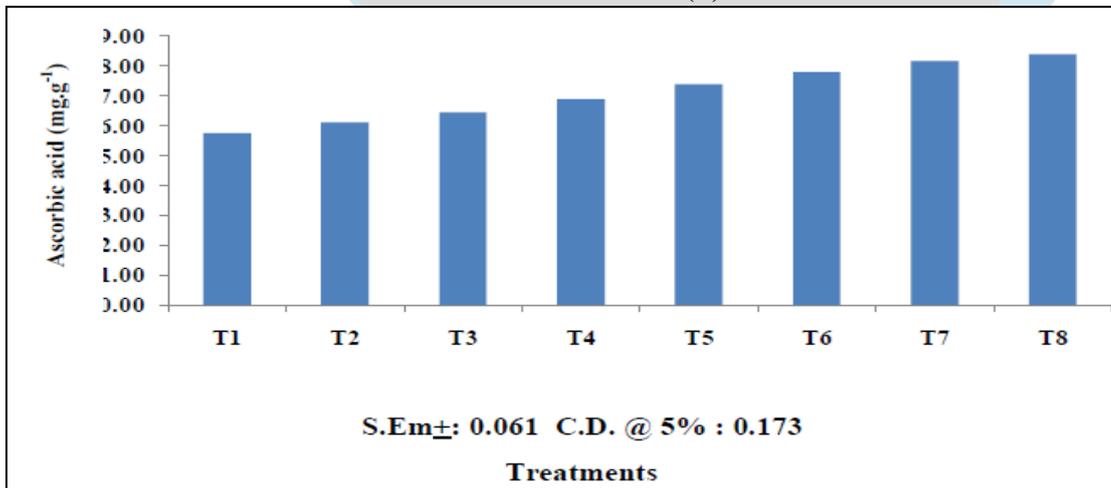
These results were in agreement with Saeed (2016) who reported that exogenous application of IAA increased the activities of ascorbic acid in the salt stress in rice.



(A)

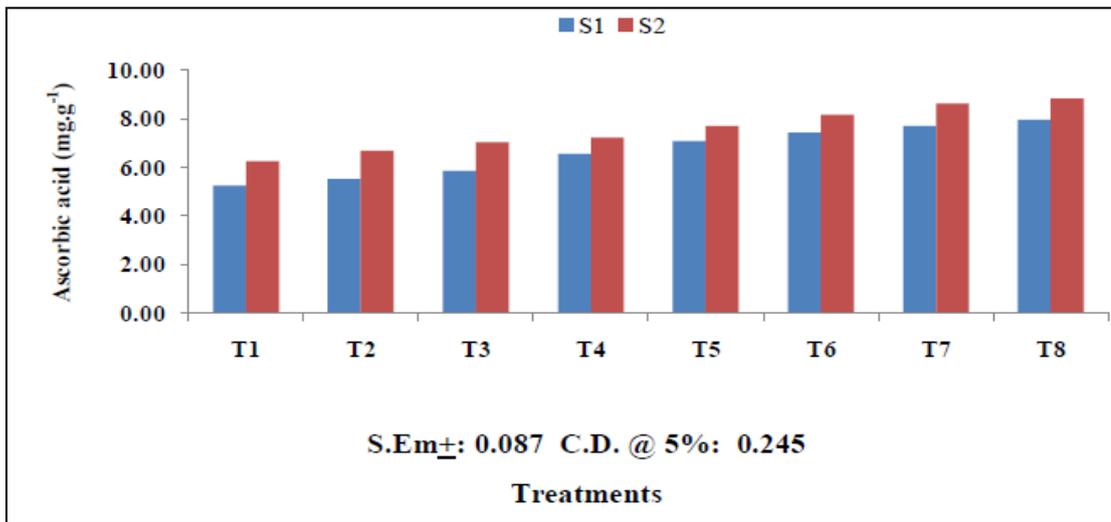


(B)

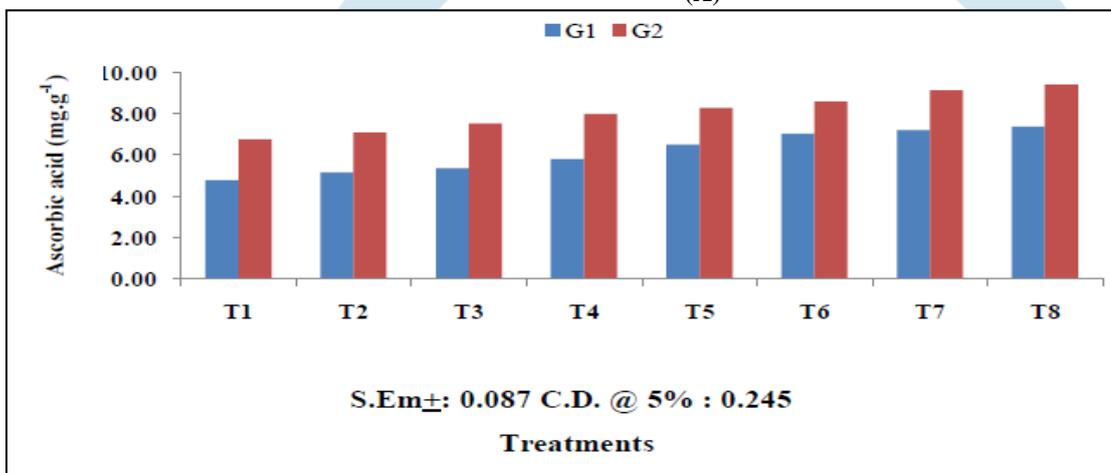


(C)

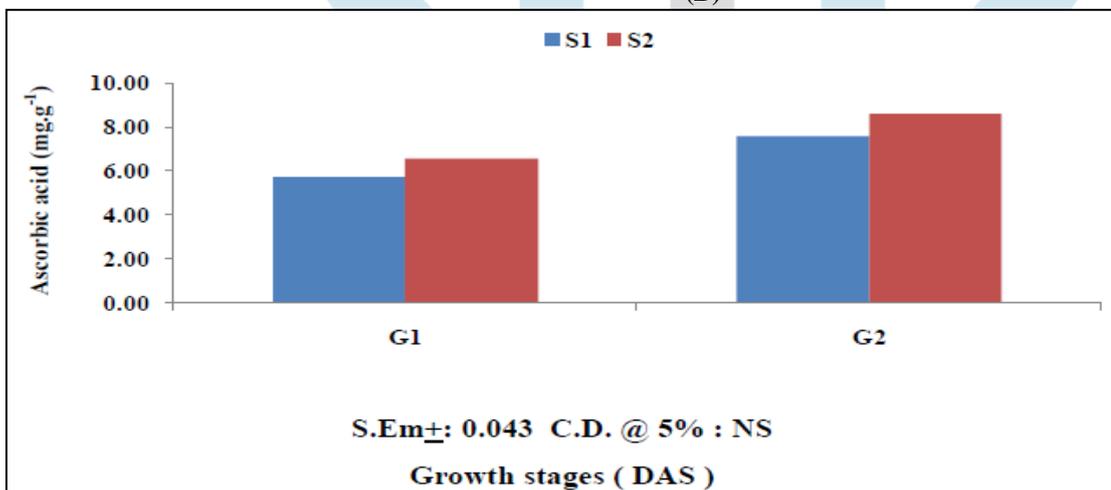
Fig.9: Mean effect of [A] salinity (S), [B] growth stages (G), [C] treatments (T) on ascorbic acid (mg.g⁻¹) in leaf tissue of mothbean.



(A)



(B)



(C)

Fig.10: Interaction effect of [A] Salinity (S) X Treatments (T), [B] Growth Stages (G) X Treatment (T), [C] Salinity (S) X Growth Stages (G) on ascorbic acid (mg.g⁻¹) in leaf tissue of mothbean.

Relative Water Content (RWC)

The data on relative water content (%) analyzed from leaf tissue of mothbean collected from plants treated with different concentrations of growth regulators (T₁ to T₈) grown in a pot irrigated with two different concentration of saline water concentration (S₁ and S₂) at two different stages (G₁ and G₂) are depicted in Fig. 11 and 12.

Among the salinity level, treatment S₁ irrigated with tap water showed highest amount of relative water content (85.66 %) while the S₂ pot irrigated with saline water of 4 EC showed lowest amount of relative water content (84.06 %) (Fig. 11 A).

Among the different stages, mean value of relative water content significantly varied between 87.36 % and 82.36 % (Fig. 11 B). The content was decreased with increased in crop growth from 20 DAS (87.36 %) to 30 DAS (82.36 %).

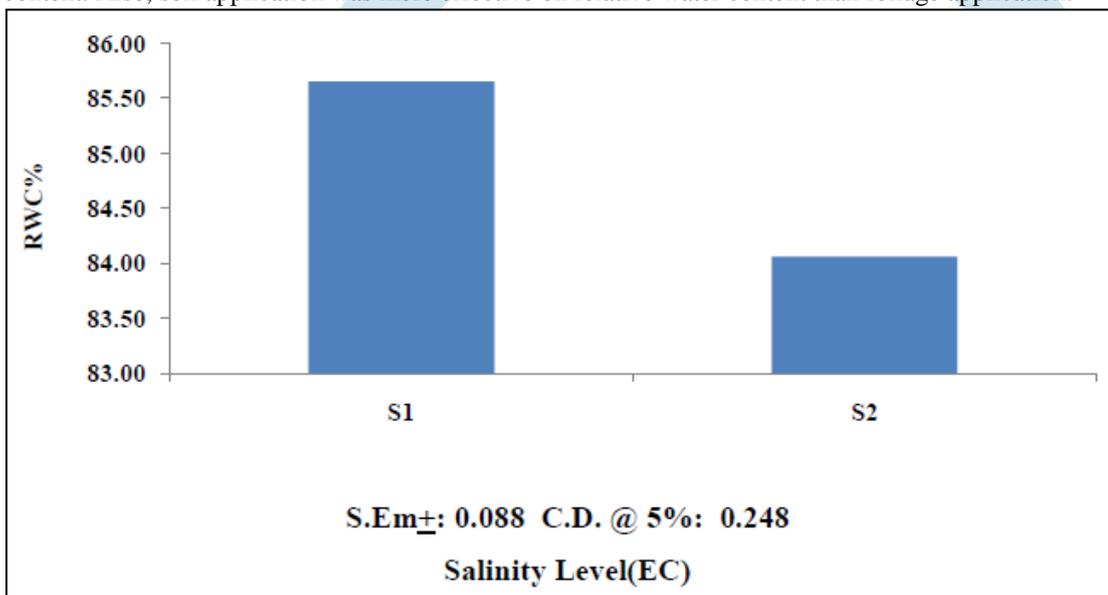
Imposition of different treatments of growth regulators resulted significant difference for the relative water content (Fig. 11 C). The tissues obtain from mothbean pots treated with T₈ [GA₃ @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm] revealed higher amount of mean relative water content (86.25 %) and which was followed by T₇ [SA @ 100 ppm + IAA @ 500 ppm (86.03 %)] and T₆ [GA₃ @ 100 ppm + IAA @ 500 ppm (86.03 %)] irrespective of salinity level and growth stages. The mean lowest content was noted for the tissues received from T₁ (83.16 %).

Interaction effect of S X T for relative water content revealed nonsignificant differences in leaf tissue of mothbean (Fig. 12 A). The highest value (87.35 %) of relative water content was observed for the S₁T₈ i.e. in plant irrigated with tap water and treated with GA₃ @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm treatment. The lowest value (82.17 %) of relative water content was observed in plant irrigated with saline water 4 EC under control condition (S₂T₁).

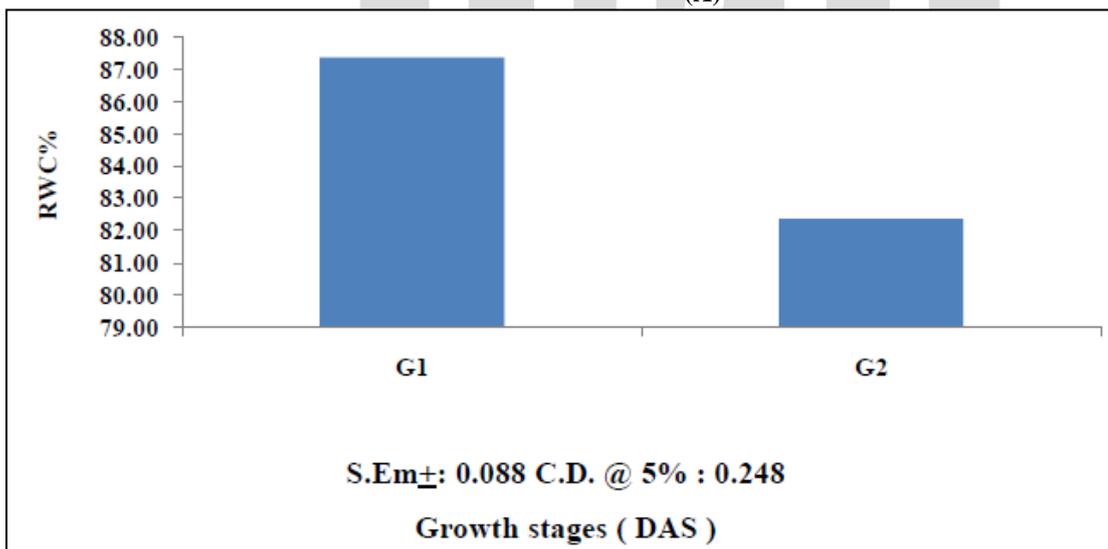
Interaction effect of G X T for relative water content showed nonsignificant differences for relative water content in mothbean (Fig. 12 B). The highest value (88.83 %) of relative water content was observed in G₁T₈ i.e. in plant treated GA₃ @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm after at 20 DAS. The lowest value (80.55 %) of relative water content was observed for G₂T₁ i.e. plant was control condition after 30 DAS.

Interaction effect of S X G for relative water content was revealed significant differences in leaf tissue of mothbean (Fig. 12 C). The highest value of relative water content was observed in S₁G₁ i.e. in plant irrigated with tap water (control condition) after 20 DAS (87.63 %). The lowest value of relative water content was observed for S₂G₂ (81.04 %).

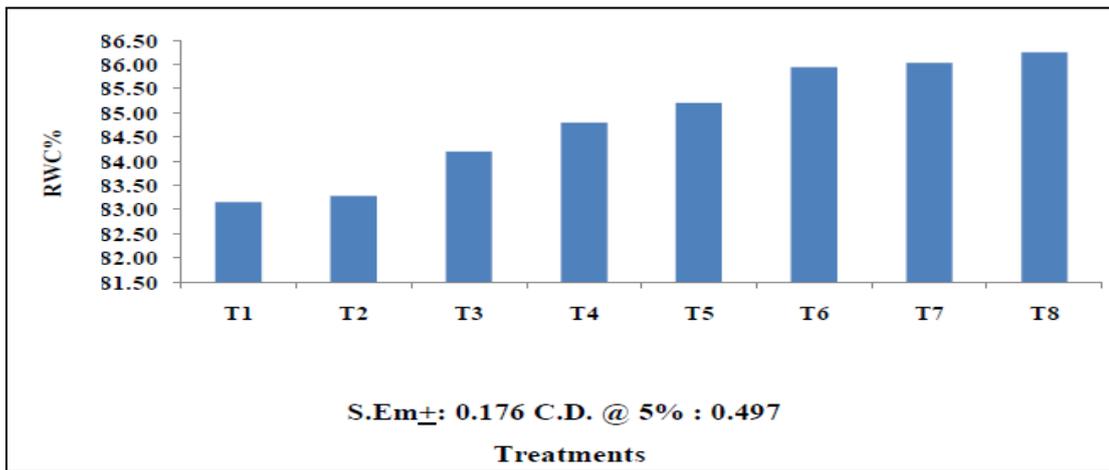
These results were in agreement with Imami *et al.* (2011) also observed that the salicylic acid application increase relative water content. Also, soil application was more effective on relative water content than foliage application.



(A)

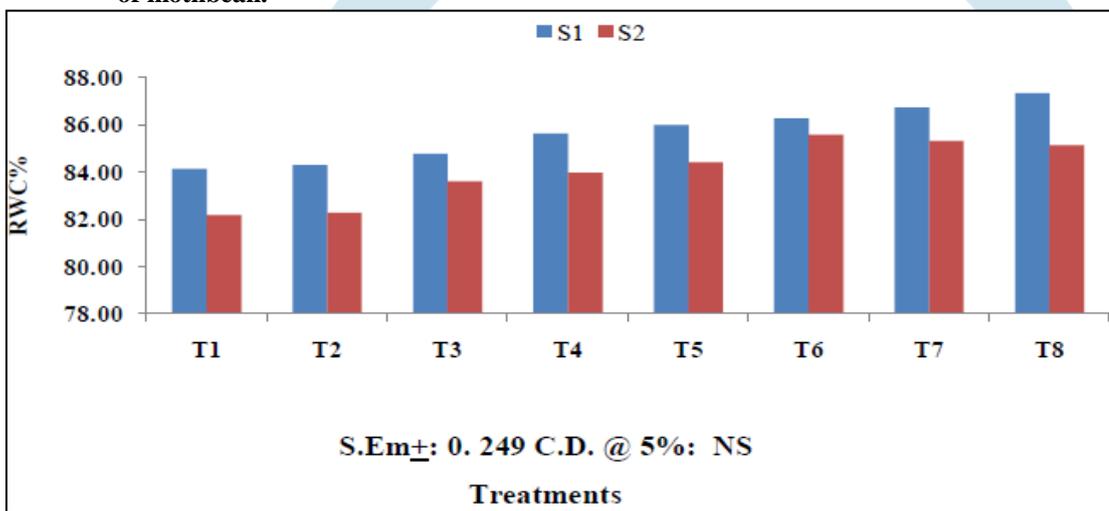


(B)

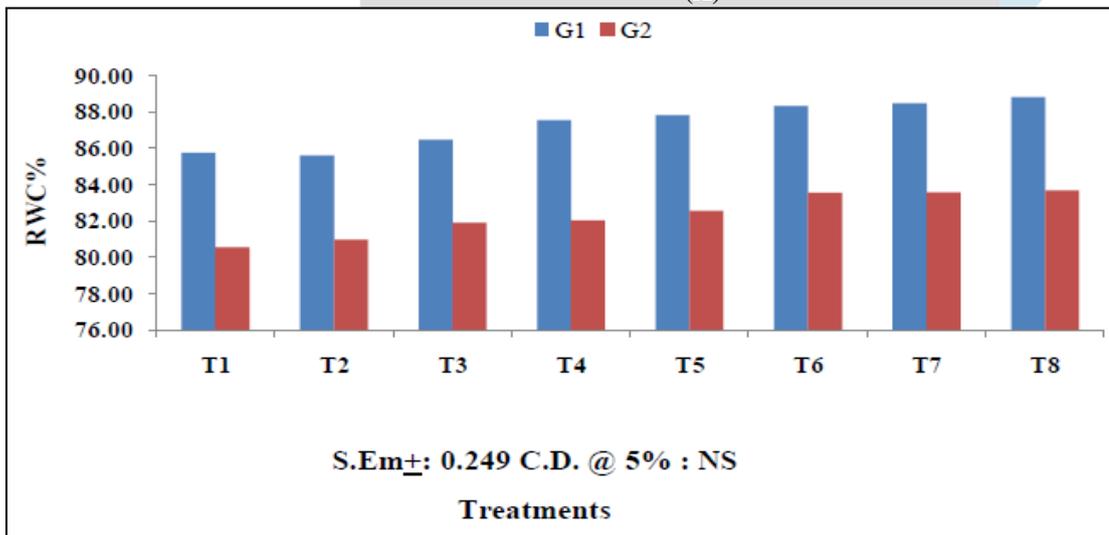


(C)

Fig.11: Mean effect of [A] salinity (S), [B] growth stages (G), [C] treatments (T) on relative water content (%) in leaf tissue of mothbean.



(A)



(B)

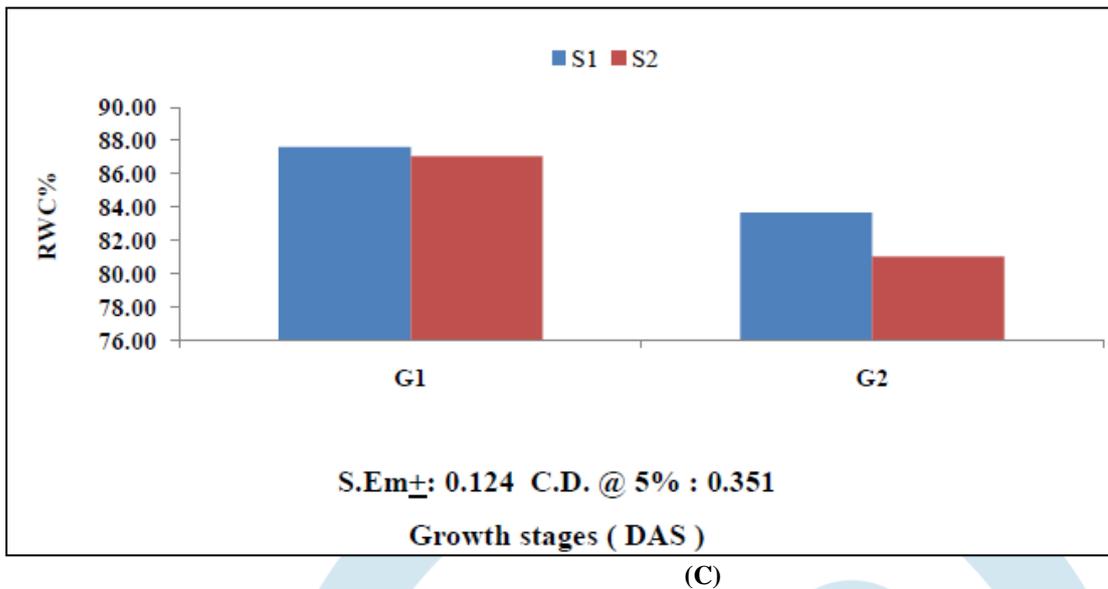


Fig.12: Interaction effect of [A] Salinity (S) X Treatments (T), [B] Growth Stages (G) X Treatment (T), [C] Salinity (S) X Growth Stages (G) on relative water content (%) in leaf tissue of mothbean.

4.1.2 Membrane stability index (MSI)

The data on membrane stability index (%) analyzed from leaf tissue of mothbean collected from plants treated with different concentrations of growth regulators and their combination (T_1 to T_8) grown in a pot irrigated with two different concentration of saline water concentration (S_1 and S_2) at two different stages (G_1 and G_2) are depicted in Fig. 13 and 14.

Among the salinity level, S_1 irrigated with tap water showed highest value for membrane stability index (64.23 %) while the S_2 irrigated with saline water showed declined value for membrane stability index (51.07 %) (Fig 13 A).

Among the different stages, mean value of membrane stability index significantly varied between 57.40 % and 57.89 % (Fig. 13 B). The content was increased with increased in crop growth from 20 DAS (57.40 %) to 30 DAS (57.89 %).

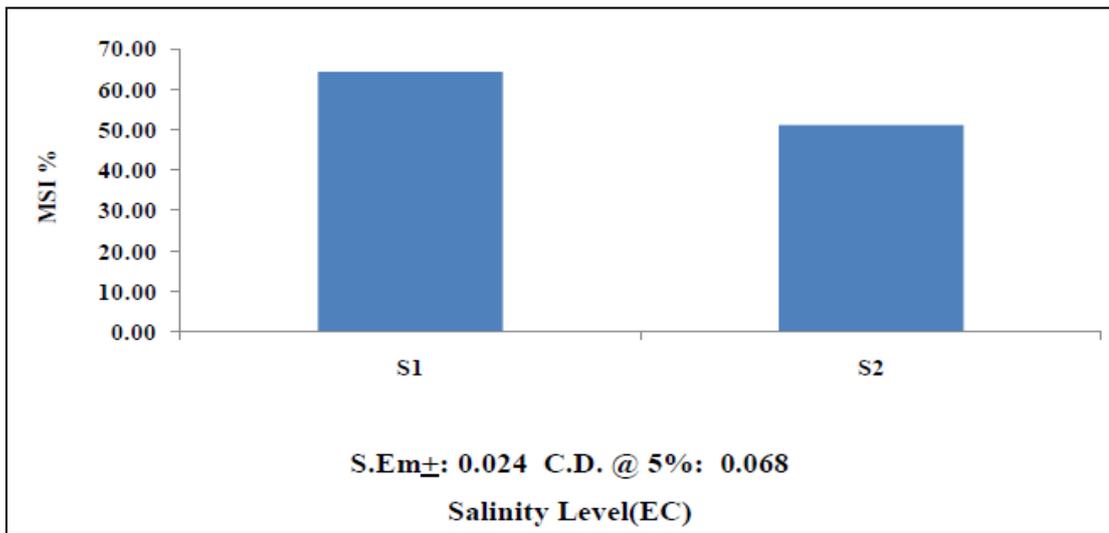
Imposition of different treatments of growth regulators resulted significant difference for the membrane stability index (Fig. 13 C). The tissues obtain from mothbean pots treated with T_8 [GA_3 @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm] revealed higher amount of mean membrane stability index (62.04 %) and which was followed by T_7 [SA @ 100 ppm + IAA @ 500 ppm (61.03 %)] irrespective of salinity level and growth stages. The mean lowest content was noted for the tissues received from T_1 (52.85 %).

Interaction effect of S X T for membrane stability index revealed significant differences in leaf tissue of mothbean (Fig. 14 A). The highest value (68.57 %) of membrane stability index was observed for the S_1T_8 i.e. in plant irrigated with tap water and treated with GA_3 @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm treatment. The lowest value (46.45 %) of membrane stability index was observed in plant irrigated with saline water 4 EC under control condition (S_2T_1).

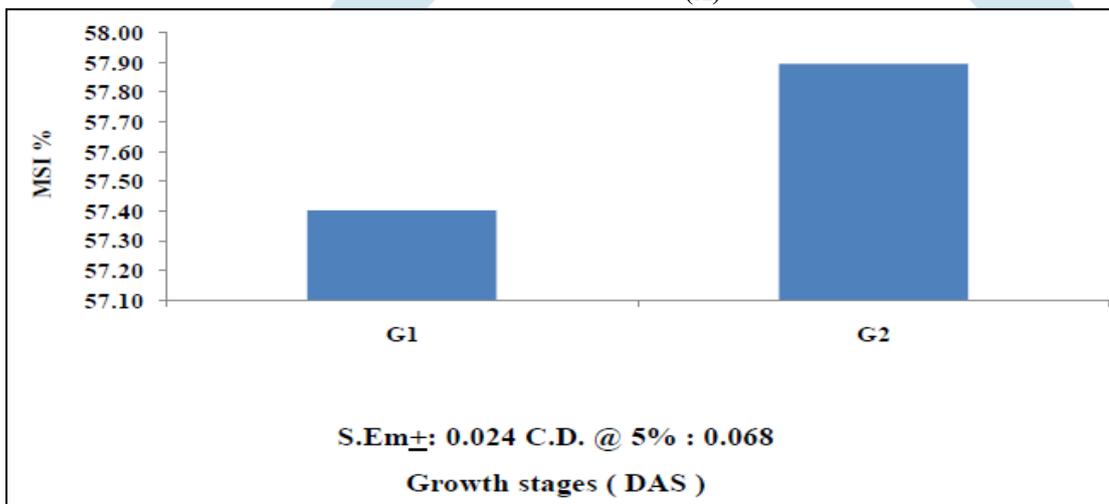
Interaction effect of G X T for membrane stability index showed significant differences for membrane stability index in mothbean (Fig. 14 B). The highest value (63.66 %) of membrane stability index was observed in G_2T_8 i.e. in plant treated with saline water (4 EC) and GA_3 @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm after at 30 DAS. The lowest value (52.45%) of membrane stability index was observed for G_2T_1 i.e. plant was control condition after 30 DAS.

Interaction effect of S X G for membrane stability index was revealed significant differences in leaf tissue of mothbean (Fig. 14 C). The highest value (65.06 %) of membrane stability index was observed in S_1G_2 i.e. in plant irrigated with tap water (control condition) after 30 DAS. The lowest value (50.72 %) of membrane stability index was observed for S_2G_2 i.e. in plant irrigated with saline water 4 EC after 30 DAS.

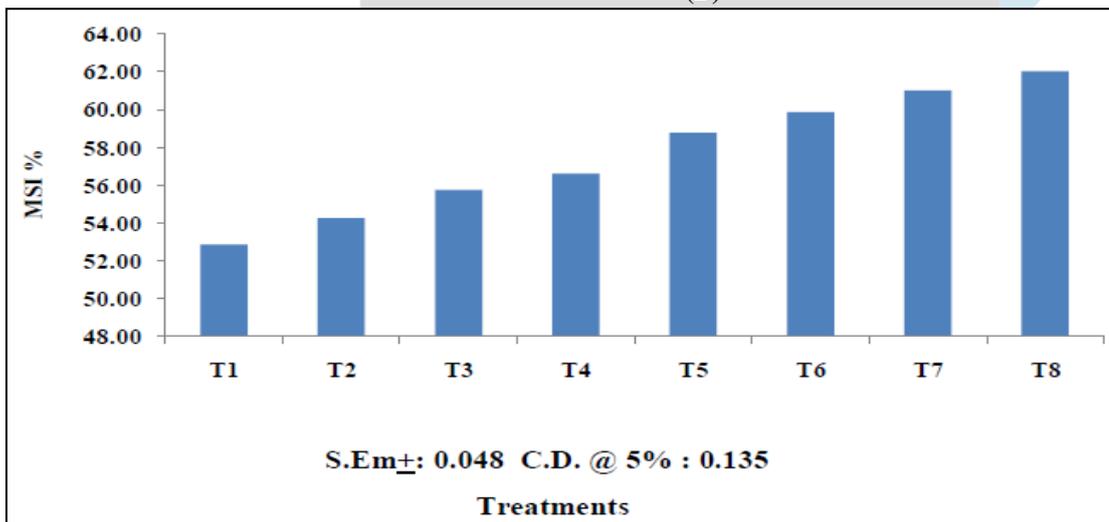
These results were in agreement with Muhammad *et al.* (2012) who reported that the membrane stability index decreases with the increase in salinity levels in sugarbeet also Abdulaziz *et al.* (2014) who reported that membrane stability index (MSI) and tissue water content (TWC) were negatively affected with increasing concentration of NaCl.



(A)

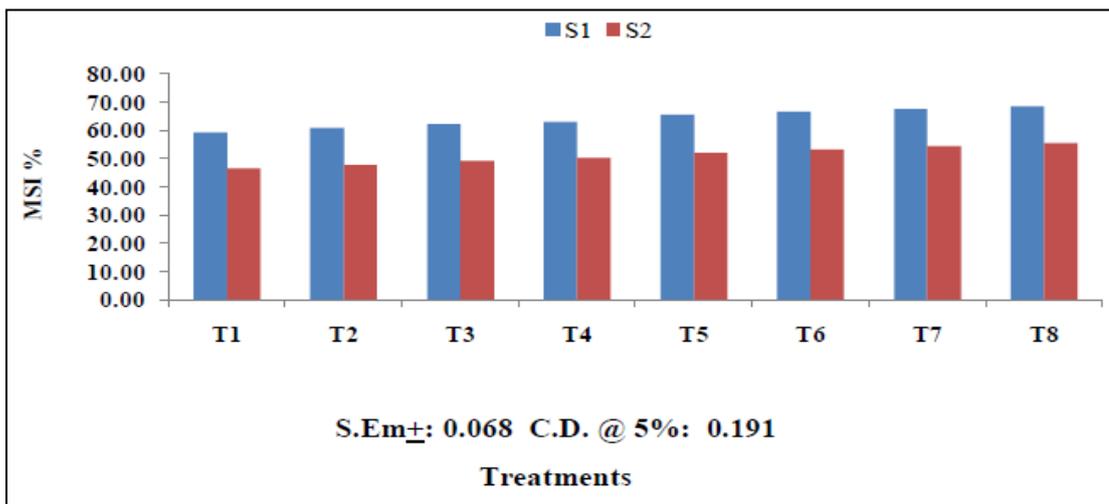


(B)

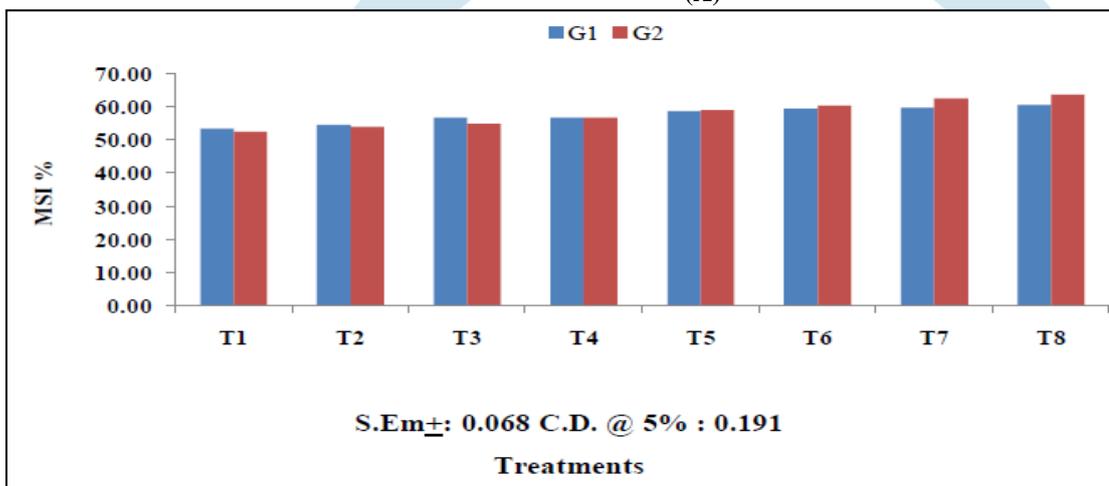


(C)

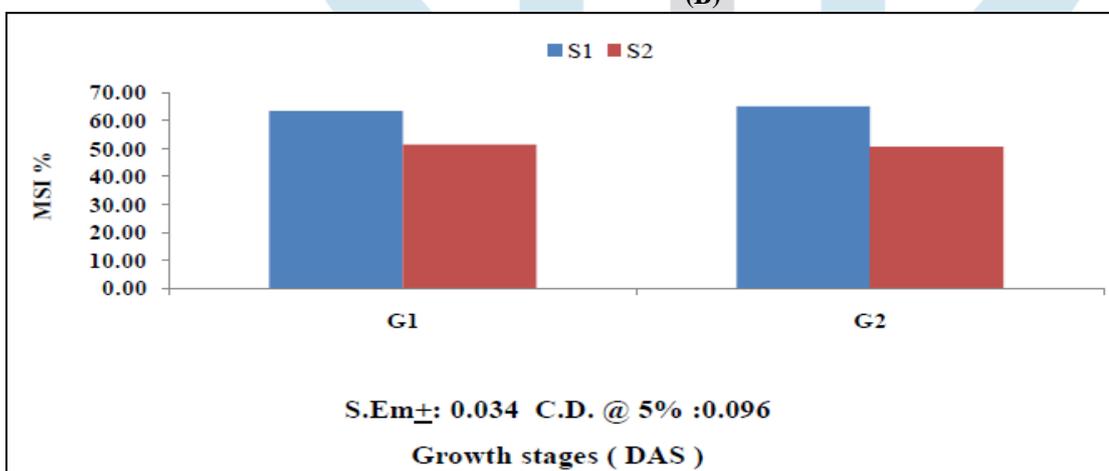
Fig.13: Mean effect of [A] salinity (S), [B] growth stages (G), [C] treatments (T) on membrane stability index (%) in leaf tissue of mothbean.



(A)



(B)



(C)

Fig.14: Interaction effect of [A] Salinity (S) X Treatments (T), [B]Growth Stages (G) X Treatment (T), [C]Salinity (S) X Growth Stages (G) on membrane stability index (%) in leaf tissue of mothbean.

4.1.4 Leaf pH

Leaf pH was analyzed from leaf tissue of mothbean collected from plants treated with different concentrations of growth regulators and their combination (T₁ to T₈) grown in a pot irrigated with different concentration of saline water (S₁ and S₂) are depicted in Fig. 15 and 16.

Among the salinity level, S₂ irrigated with saline water 4 EC showed higher amount of leaf pH (6.37) while the pot S₁ irrigated with tap water showed lower value for leaf pH (6.36) (Fig. 15 A).

Among the different stages, mean value of leaf pH significantly varied between 6.06 and 6.67 (Fig. 15 B). The content was increased from 20 DAS (6.06) and 30 DAS (6.67).

Application of spray treatment of growth regulators and their combination found statically nonsignificant for (Fig. 15 C). The tissues obtain from mothbean pots treated with T₈ [GA₃ @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm] revealed higher amount

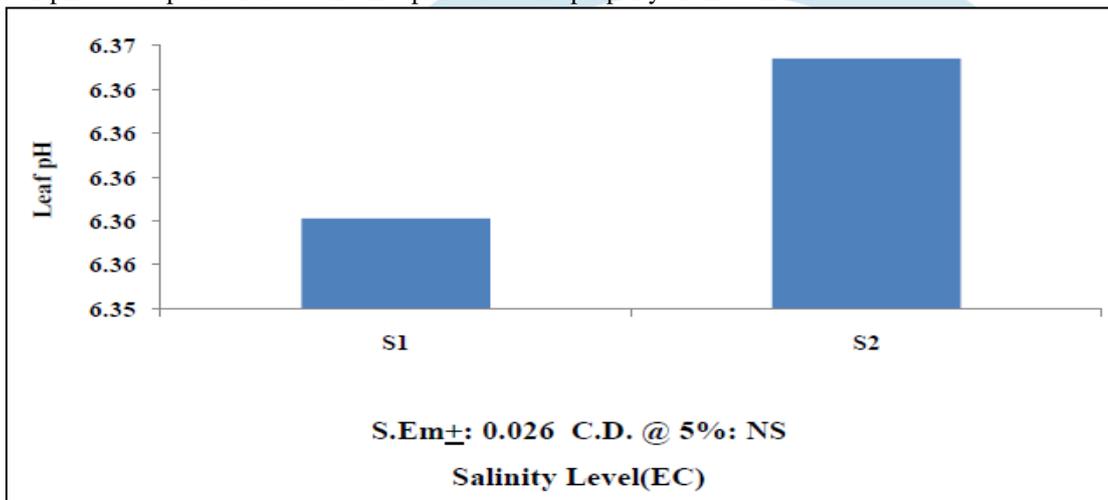
of mean leaf pH (6.54) and which was followed by T₇ [SA @ 100 ppm + IAA @ 500 ppm (6.43)] and T₆ [GA₃ @ 100 ppm + IAA @ 500 ppm (6.44)] irrespective of salinity level and growth stages. The mean lowest content was noted for the tissues received from T₁ (6.18).

Interaction effect of S X T for leaf pH was revealed nonsignificant differences in leaf tissue of mothbean (Fig. 16 A). The highest value (6.56) of leaf pH was observed for the S₂T₈ i.e. in plant irrigated with saline water 4 EC with GA₃ @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm treatment spray. The lowest value (6.17) of leaf pH was observed in plant irrigated with saline water 4 EC in control condition (S₂T₁).

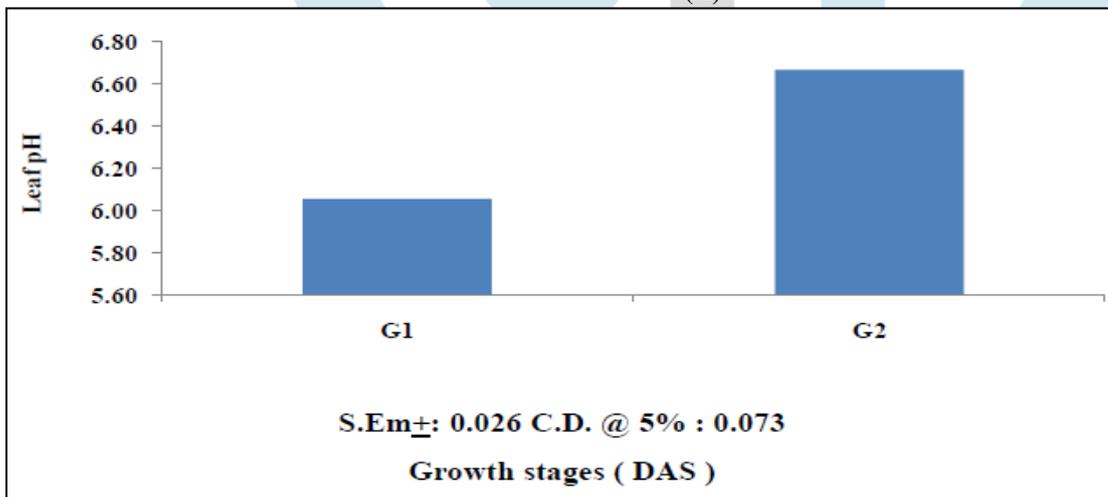
Interaction effect of G X T for leaf pH was revealed significant differences in mothbean (Fig. 16 B). However, the highest value (6.85) of leaf pH was observed for G₂T₈ i.e. in plant treated with [GA₃ @ 100 ppm + SA @ 100 ppm+ IAA @ 500 ppm] after at 30 DAS. The lowest value (5.92) of leaf pH was observed for G₁T₁ i.e. the plant under control condition after 20 DAS.

Interaction effect of S X G for leaf pH was revealed significant differences in leaf tissue of mothbean (Fig. 16 C). The lowest value (6.02) of leaf pH was observed in S₂G₁ i.e. in plant irrigated with saline water 4 EC after 20 DAS. The highest value (6.71) of leaf pH was observed for S₂G₂ i.e. in plant irrigated with saline water 4 EC after 20 DAS.

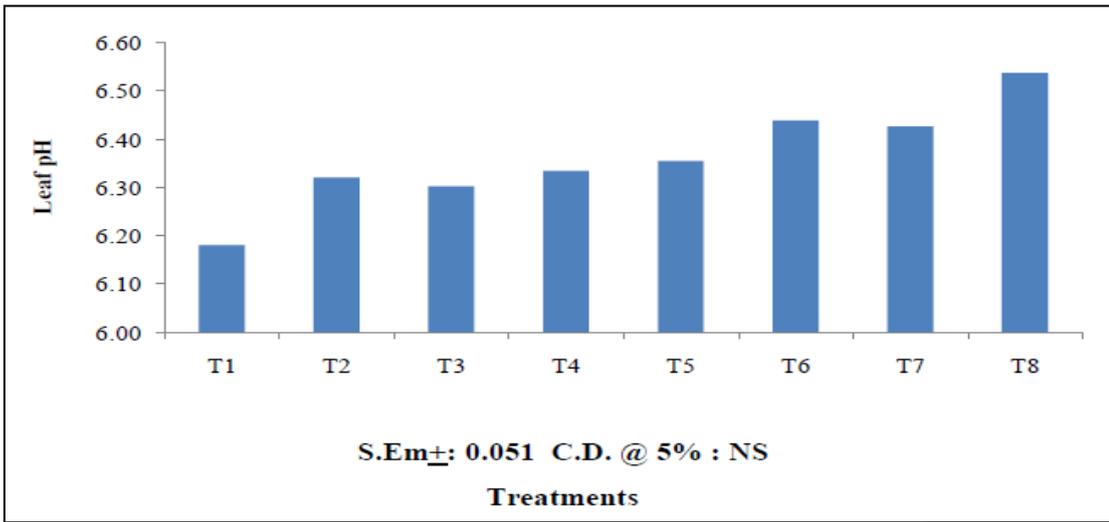
These results were in agreement with Johannes *et al.* (2011) observed variation in leaf pH due to salt stress and reported that the leaf pH was a species related and independent to the property of the soil.



(A)

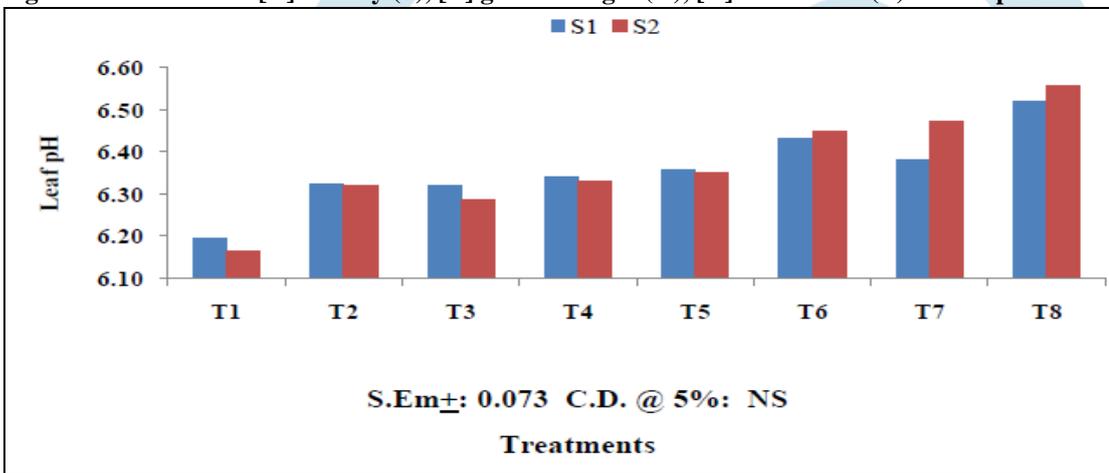


(B)

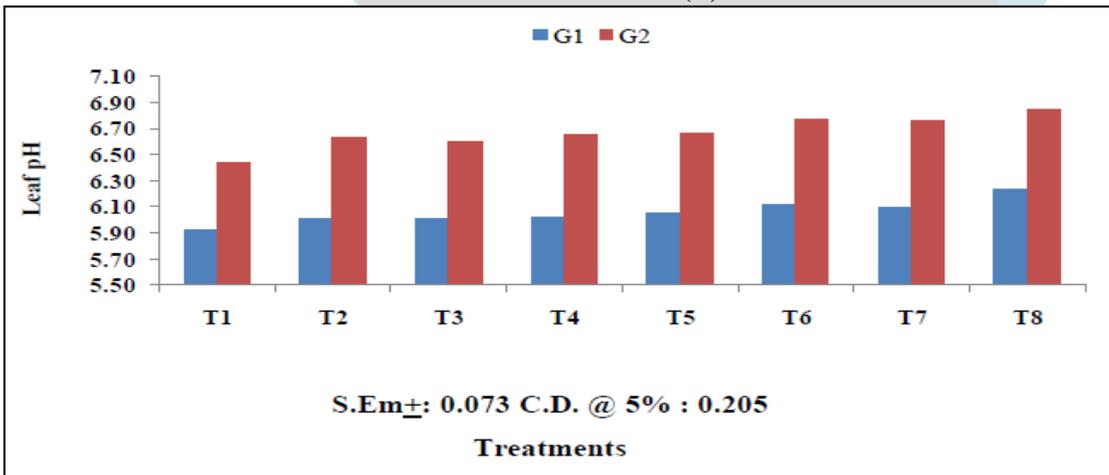


(C)

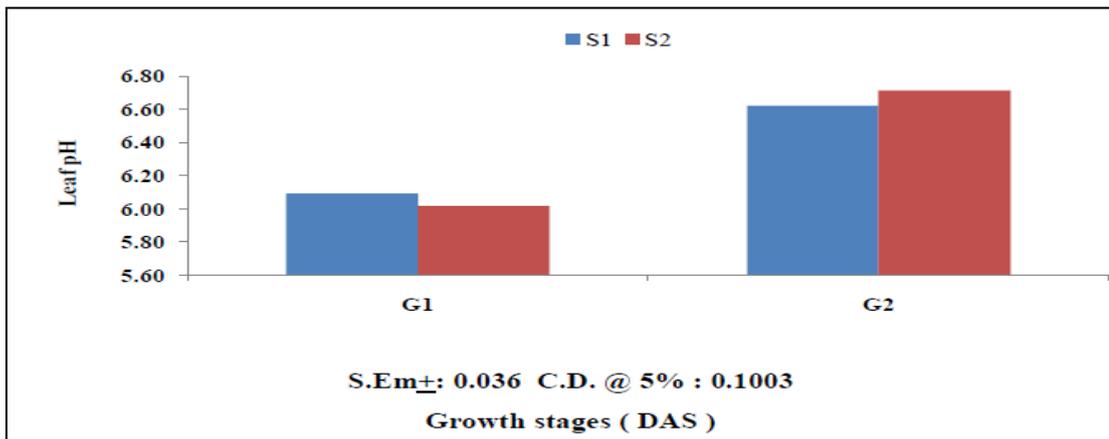
Fig.15: Mean effect of [A] salinity (S), [B] growth stages (G), [C] treatments (T) on leaf pH in leaf tissue of mothbean.



(A)



(B)



(C)

Fig.16: Interaction effect of [A] Salinity (S) X Treatments (T), [B] Growth Stages (G) X Treatment (T), [C] Salinity (S) X Growth Stages (G) on leaf pH in leaf tissue of mothbean.

CONCLUSION

The results suggest that the biochemical constituents and physiological parameter were affected due to salinity stress in mothbean. The collected samples of the mothbean that was irrigated with salt stress it shown decreased in the physiological parameters viz.; relative water content, membrane stability index, but leaf pH showed reverse trend and it was increased in case of high salt concentration. Higher concentration of salt that increase biochemical constituents viz.; total soluble sugar, reducing sugar, proline, glycine betaine and ascorbic acid content with increasing salt concentration. The leaf sample treated with growth regulators and their combination at different concentration that was increased physiological parameters viz.; relative water content, membrane stability index, escape leaf pH. Growth regulators and their combination can regulate various plant metabolic process and modulate the production of varied osmolytes viz.; total soluble sugar, reducing sugar, proline, glycine betaine and ascorbic acid content. This investigation has proved growth regulators (Indol acetic acid, Gibberellic acid, Salicylic acid) and their combination as a potential biomolecules in reducing the adverse effect of salt stress in plant. A growth regulator (Indol acetic acid, Gibberellic acid, Salicylic acid) has been showed to be beneficial for plant in growth and development under salinity stress.

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