

Evaluation of Oxidative Stress Tolerance, Growth Responses, Osmolites and Phytohormonal Estimation of the Selected Halophytes

Samiullah and Asghari Bano*

Department of Plant Sciences, Quaid-i-Azam University Islamabad.

*Corresponding author: asgharibano@yahoo.com

Phone No: + 92-05190643096

Abstract. The present study was aimed to determine physiological responses of the selected halophytes *Suaeda fruticosa* Forsk, *Atriplex leuococlada* Boiss, *Haloxylon salicornicum* (Moq.) and *Salicornica virginica* L. concomitant with the determination of soil characteristics collected from district Mardan. *Haloxylon salicornicum* present the highest concentration of K^+ , Ca^{2+} and Mg^{2+} ions in its leaves as compared to the other three selected halophytes, whereas Fe^{+2} , Cu^{+3} , Zn^{+2} and heavy metals like Co^{+3} and Ni^{+3} were higher in *Atriplex leuococlada*. The analysis of the physiological response mechanisms showed that *Atriplex leuococlada* possessed higher chlorophyll *a/b* ratio and protein content. Superoxide Dismutase (SOD), Peroxidase (POD) and Catalase (CAT) activities were found higher in the leaves of *Haloxylon salicornicum*. The production of abscisic acid (ABA) was significantly higher as compared to indole acetic acid (IAA) in all the selected plants. *Atriplex leuococlada* *Suaeda fruticosa* and *Salicornica virginica* possessed higher concentration of ABA. The *Salicornica virginica* and *Haloxylon salicornicum* appear to use sugar as osmolyte while proline was found significantly higher in *Suaeda fruticosa* and *Atriplex leuococlada*. Correlation present between K^+/Na^+ and proline content. Among the selected halophytes *Atriplex leuococlada* have higher K^+/Na^+ ratio as well as proline and protein contents.

Keywords: Abscisic acid, Proline, K^+/Na^+ ratio, Salinity, Halophytes

1. INTRODUCTION

Soil salinity is becoming a major problem due to various natural and man caused factors in almost all the regions of the world and especially in arid areas. Soil salinity is characterized by a high concentration of soluble salts in soil. Soils are considered saline when it's EC = 4dS/m or more [1]. The annual loss in the economy of Pakistan due to soil salinity has been estimated 300 US million dollars per year [2]. According to [3] adaptation of plants to soil salinity are of three various types: osmotic stress tolerance, Na^+ or Cl^- exclusion and high accumulation of Na^+ or Cl^- . The decrease in chlorophyll a, chlorophyll b, and carotenoid content in leaves under salinity has been reported by [4]. 5-aminolaevulinic acid (ALA) is the precursor of all tetrapyrroles converted to protochlorophyllides which in turn converted into chlorophyll when exposed to light. ALA is made from glutamate that was reported to decrease in salt stressed leaves [5;]. [6] Reported that proteins level decreased under salinity due to low uptake of nitrate ions. High proline content can be considered beneficial to stressed plants because a significant correlation between enhanced tolerance and proline accumulation in plants under saline condition has been reported [7]. Accumulation of selective ions ensures osmotic adjustments in plants, which occur through mass action, and it enables the plants to increase water retention and sodium exclusion [8].

To minimize the effects of oxidative stress, plant cells have evolved a complex antioxidant system, which is composed of glutathione, ascorbate and carotenoids as well as ROS-scavenging enzymes, such as: superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and

glutathione reductase (GR) [9]. ABA referred as a “stress phytohormone” because its level increases in response to various environmental stresses, mostly in drought condition [10]. [11] Reported that reduction in leaf growth under drought and salinity is due to a considerable accumulation of ABA.

The aim of the present study was compare the status of the salt stress response of four selected halophytes collected from Mardan district by their physiological and biochemical parameters.

2. MATERIALS AND METHODS

2.1. Plant samples collected

Four selected halophytes: *Suaeda fruticosa* Forsk, *Atriplex leucoclada* Boiss, *Haloxylon salicornicum* (Moq.), *Salicornia virginica* L. were collected by triplicate from different locations of Mardan district. Mardan is a district localized in the North-West Frontier Province of Pakistan, between an altitude of 400 to 1,700 masl, in 34° 05' to 34° 32' north latitudes and 71° 48' to 72° 25' east longitudes.

Three samples of the selected plant species at vegetative stage were collected with their rhizospheric soil, at a depth of 6 inches, the physicochemical analysis of the soil were done and the leaves of the plant samples were used to study the biochemical and physiological analysis.

2.2. Macro, micro and heavy metal analysis

Mineral or inorganic analysis of the bulk soil and leaves samples of selected plants were done, analyzing the macronutrients and micronutrients contents, and a heavy metals analysis were done, following the Ammonium Bicarbonate-DTPA method developed by [12] and by the Perchloric-acid digestion method for the leave samples.

2.3. Physiological and biochemical analysis of the selected halophytes

The analysis of the salt stress response of the selected halophytes was done taking the leaves of the plants. This foliar material was fractionated for the biochemical and physiological analysis as follows. The protein content was quantified according to the method of [13]. Sugar content was measure by the method of [14]. The chlorophylls and carotenoids content were determined by the method of [15]. The proline contents of leaves were also measured.

2.4. Determination of antioxidant enzymes

Fresh leaves (5g) were homogenized with 15ml of 0.05N phosphate buffer (PH 7.0) containing 10% poly vinyl poly pyrrolidore (PVPP) and 0.1 M Ethylene diamine tetra acetate (EDTA). Homogenate was centrifuged at 15,000 rpm for 15 min at 4°C. Supernatant was used for SOD and POD assay. SOD was determined by measuring inhibition of photochemical reduction of nitroblue tetrazolium (NBT) using method of [16]. POD was determined by the method of [17]. APOX and CAT activity was determined according to [18].

2.5. Extraction and purification of phytohormones

Extraction and purification of Abscisic acid and Indole acetic acid was made following the method of [19]. The samples were analyzed on HPLC (Agilent 1100) equipped with variable UV detector and C18 column (39×300 mm) [BondaPack Porasil C-18, 37/50 µm, Waters, Eschborn, BRD). Methanol and water in the ratio of 30:70 v/v were used as mobile phase @ of 1,500 µl min⁻¹ with a run time of 20 min sample⁻¹. The growth hormones were identified on the basis of retention time of phytohormone standards. IAA (indole-3-acetic acid) was studied at 280 nm wavelengths while ABA was analyzed at 254 nm respectively.

2.6. Statistical analysis

The data were analyzed statistically by an analysis of variance (Steel and Torrie) and the comparison between all the data obtained was made by Duncan's Multiple Range Test (DMRT).

3. RESULTS

3.1. Mineral nutrients and heavy metals as physiological saline response

Table 1 indicated that there was hyper accumulation of Na^+ , Ca^{++} , Mg^{++} , and K^+ ions in leaves of halophytes collected from saline areas of Mardan District. The distribution of the Na^+ content quantified in the plant species was: *Haloxylon salicornicum* > *Atriplex leucoclada* > *Salicornica verginica* > *Suaeda fruticosa*. P and NO_3^- were found in the range of 0.4-0.8 $\mu\text{g/g}$ in the soil.

The concentration of Ca^{+2} ions was higher in the leaves of *Haloxylon salicornicum* and *Salicornica verginica* as compared to *Atriplex leucoclada*. *Suaeda fruticosa* leaves have the minimum concentration of Ca^{+2} ions. The concentration of the K^+ ion founded in plant species was: *Haloxylon salicornicum* > *Salicornica verginica* > *Suaeda fruticosa* > *Atriplex leucoclada*. Higher concentration of Mg^{+2} ions was found in *Atriplex leucoclada* and *Haloxylon salicornicum* while least was found in *Salicornica verginica*. The Fe^{+2} , Cu^{+3} and Zn^{+2} ion concentration were higher in *Atriplex leucoclada*, followed by *Suaeda fruticosa* while *Salicornica verginica* showed minimum concentration of these three ions. Table 2 indicated that there was comparatively greater accumulation of Ni^{+3} , Li^{+1} , Pb^{+4} and Cd^{+2} ions in the leaves of selected halophytes as compared to that of soil. The concentration of Ni^{+3} ranged between 23 to 30 $\mu\text{g/g}$ in all plants. Similar accumulation of Li, Pb and Cd was found in all the four selected halophytes. *Atriplex leucoclada* had accumulated higher concentration of Fe^{+2} , Zn^{+2} and Cu^{+3} Co^{+3} and Ni^{+3} ions, while *Suaeda fruticosa* and *Salicornica verginica* had lower Co^{+3} accumulations. *Suaeda fruticosa* is the maximum accumulator of Pb^{+4} and Mn^{+2} . The results indicated that halophytes species differ in the accumulation of micronutrients.

3.2. Chlorophylls and carotenoid contents as a growth response to saline condition

Figure 1 indicates that plants growing in saline soils were significantly lower in chlorophyll's *a* and *b* contents, compared to other glycophytes; the chlorophyll *a* being significantly higher than chlorophyll *b*. Among the selected halophytes chlorophyll *a* content was found maximum in *Salicornica verginica* while it was minimum in *Atriplex leucoclada*; whereas chlorophyll *b* was found maximum in *Haloxylon salicornicum*. The chlorophyll *a/b* ratio was found higher in *Atriplex leucoclada* while total chlorophyll was found maximum in both *Haloxylon salicornicum* and *Salicornica verginica*. *Atriplex leucoclada*, *Haloxylon salicornicum* and *Salicornica verginica* had the higher carotenoid content among all species, the minimum amount of carotenoid was found in *Suaeda fruticosa*. (Figure 2)

3.3. Osmolite contents: (sugar, proline and protein)

Higher sugar content was found both in *Haloxylon salicornicum* and *Salicornica verginica*, while *Suaeda fruticosa* and *Atriplex leucoclada* have the minimum sugar content (Figure 3). Figure 4 shows the protein content and it was higher in *Atriplex leucoclada*, while *Haloxylon salicornicum* and *Salicornica verginica* has the lower but statistically similar protein content; minimum protein content was found in *Suaeda fruticosa*. The higher proline content was found in *Suaeda fruticosa* and *Atriplex leucoclada*, while *Haloxylon salicornicum* and *Salicornica verginica* has the lowest proline content.

3.4. Antioxidant enzymes in response to oxidative stress

Fig 5 indicated that POD activity was found higher in all the selected plants as compared to SOD. The POD contents did not differ significantly among the selected halophytes. However the SOD activity was significantly higher in *Haloxylon salicornicum*. Maximum APOX activity was found in *Atriplex leucoclada* which was at par with *Suaeda fruticosa* while *Salicornica verginica* and *Haloxylon salicornicum* showed least APOX activities (Fig 6). The CAT activity does not differ significantly among the four selected halophytes.

3.5. Phytohormones in saline response: Abscisic acid and Indole acetic acid contents

Figure 7 shows that both ABA and IAA content was found higher in *Haloxylon salicornicum*, while both *Salicornica verginica* and *Suaeda fruticosa* showed the lowest ABA and IAA content. Particularly, *Atriplex leucoclada* has the lower ABA content than *Haloxylon salicornicum* but higher than all other three compared halophytic species.

4. DISCUSSION

Salinity leads to the reduction of P, NO_3^- and Fe^{+2} ions in the soil due to the presence of high Na^+ and Cl^- ions concentration in the soil. [20] reported that salinity increase the uptake of Na^+ , Ca^{2+} , and Cl^- whereas the ratio of K^+/Na^+ decreases in saline condition. K^+/Na^+ ratio considered as an index of salt tolerance was higher in *Atriplex leuoclada* with a lower Ca^{2+} content but *Haloxylon salicornicum* which has the lowest K^+/Na^+ ratio has relatively higher Ca^{2+} than *Atriplex leuoclada*. Salinity caused significant decrease in chlorophyll's *a* and *b*, and carotenoid content in leaves [4]. According to [21] the decrease in chlorophyll *a* and *b* content is due to the increase in their degradation under saline condition. Higher amount of sugar was found in *Salicornia virginica* was supported that a strong correlation was reported between the sugar accumulation and the osmotic adjustment during abiotic stress condition [22]. [6] Reported that proteins level decreased under salinity due to low uptake of nitrate ions.

Proline content was found higher in *suaeda fruticosa* and *atriplex leuoclada* was according to [7] that significant correlation between enhanced tolerance and proline accumulation in plants under saline condition has occurred. Lower proline content but higher content of sugar in *salicornia virginica* and *haloxylon salicornicum* suggest that sugar can be used as osmolyte in these plants. [23] Demonstrated that salt stress leads to a decrease in SOD activity in salt-sensitive plants but to an increase in salt-tolerant one supported the present research work. The increase of catalase activity has been observed in *Calandula officinalis* and *Lycopersicon esculentum* plants under abiotic stress [24]. Abscisic acid plays a key role in plants under salinity condition [25]. *Atriplex leuoclada*, *Suaeda fruticosa* and *Salicornia virginica* showed higher concentration of ABA as compared to IAA. There is a strong interaction present between K^+/Na^+ ratio, ABA/IAA ratio and proline content. Among the selected halophytes *Atriplex leuoclada* have maximum ABA/IAA ratio as well as higher K^+/Na^+ ratio and higher proline and protein contents. While *Haloxylon salicornicum* and *Salicornia virginica* have lower K^+/Na^+ ratio, lower ABA/IAA ratio as well as lower proline and protein content.

5. CONCLUSION

K^+/Na^+ ratio, ABA and proline production interaction can be treated as a key character for salt tolerance. The various halophytes species also have ability to decontaminate soil from heavy metals. Of all the macro and essential micronutrient elements K^+ accumulation was lower whereas Ca^{+2} accumulations was higher than glycophytes.

6. REFERENCES

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Table: 1 Macro and micronutrient ($\mu\text{g/g}$) contents of rhizospheric soil and leaves of selected halophytes

| Metals | Na ⁺ | Ca ⁺² | K ⁺ | Mg ⁺² | Na ⁺ /K ⁺ | Fe ⁺² | Zn ⁺² | Cu ⁺³ |
|----------------------------|-----------------|------------------|----------------------|--------------------|---------------------------------|------------------|------------------|------------------|
| Soil | 14.8 +/-0.4 | 15 +/-0.1 | 0.08 +/- 0.002 | 3.6 +/- 0.07 | 185 +/-2.3 | 0.4 +/-0.01 | 0.07 +/-0.03 | 1.5+/- 0.05 |
| <i>S. fruticosa</i> | 2774 +/-14 | 15356 +/-24 | 9.1 +/-0.17 | 1708 +/-38 | 304 +/-2.6 | 635 +/-2.8 | 21 +/-0.04 | 215 +/-2 |
| <i>A.leuococlada</i> | 3565 +/-33 | 19582 +/-30 | 5.4 +/-0.20 | 4604 +/-49 | 659 +/-3.1 | 885 +/-3 | 26 +/-0.5 | 249 +/-1.2 |
| <i>H.salicornicu m</i> | 3854 +/-32 | 24613 +/-28 | 19.4 +/-0.5 | 6125 +/-49 | 199 +/-2.6 | 278 +/-4.0 | 24 +/-1 | 167 +/-1.1 |
| <i>S. verginica</i> | 3293 +/-6 | 26144 +/-40 | 12.4 +/-0.5 | 1239 +/-18 | 265 +/-1.7 | 375 +/-3 | 19 +/-0.5 | 207 +/-3.4 |

Table: 2 Heavy metal analysis ($\mu\text{g/g}$) of rhizospheric soil and leaves of selected halophytes

| Metals | Ni ⁺³ | Li ⁺¹ | Pb ⁺⁴ | Cd ⁺² | Cr ⁺³ | Co ⁺³ | Mn ⁺² |
|-----------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Soil | 0.05+/- 0.02 | 0.05+/- 0.03 | 1.3+/- 0.03 | 0.1+/- 0.002 | 0.05+/- 0.09 | 0.1+/- 0.02 | 0.5+/- 0.01 |
| <i>S. fruticosa</i> | 28+/- 1.7 | 9.2+/- 0.34 | 370+/- 5.2 | 26+/- 1.6 | 31+/- 0.8 | 10+/- 0.3 | 184+/- 2.9 |
| <i>A.leuococlada</i> | 30.5+/- 1.5 | 9.2+/- 0.1 | 318+/- 4.6 | 25+/- 0.5 | 26+/- 1.4 | 14+/- 0.5 | 152+/- 2.0 |
| <i>H.salicornicum</i> | 25.4+/- 0.6 | 9.3+/- 0.12 | 350+/- 2.02 | 26+/- 1.1 | 34+/- 1.7 | 13+/- 0.4 | 117+/- 1.7 |
| <i>S. verginica</i> | 23.4+/- 1.2 | 9.4+/- 0.08 | 346+/- 4.9 | 27+/- 1.4 | 49+/- 2.3 | 11+/- 1.0 | 143+/- 2.8 |

S.fruticosa:*Suaeda fruticosa*, *A.leuococlada*:*Atriplex leuococlada*,*H.salicornica*: *Haloxylon salicornicum*,*S.verginica*: *Salicornica verginica*. Leaves of selected halophytes along with rhizospheric soil (EC: 4.2dS/m; pH: 8.3-9.3) at a depth of 6 inches were collected with three replicates.+/- (standard error)

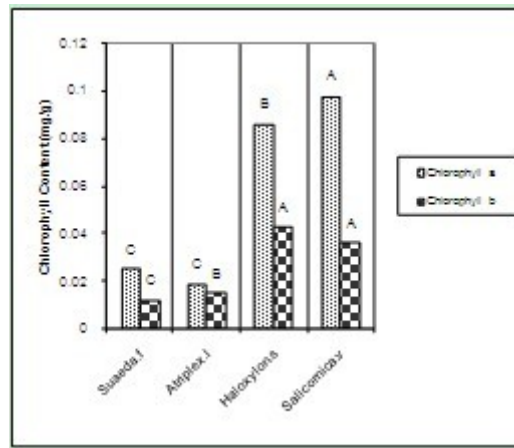


Fig: 1

Comparison of the Chlorophyll a and Chlorophyll b Content (mg/g) of Selected Halophytes of District Mardan. *Suaeda.f*: *Suaeda fruticosa*, *Atriplex.l*: *Atriplex leucoclada*, *Haloxyton.s*: *Haloxyton salicornicum*, *Salicornica.v*: *Salicornica virginica*. Leaves of selected halophytes were collected at vegetative stage. Three replicates were used.

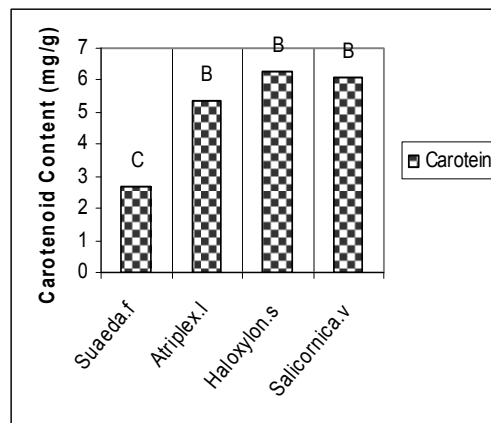


Fig: 2

Comparison of the Chlorophyll a, Chlorophyll b and Carotenoids Content (mg/g) of Selected Halophytes of District Mardan. *Suaeda.f*: *Suaeda fruticosa*, *Atriplex.l*: *Atriplex leucoclada*, *Haloxyton.s*: *Haloxyton salicornicum*, *Salicornica.v*: *Salicornica virginica*. Leaves of selected halophytes were collected at vegetative stage. Three replicates were used.

All bars which share same letters are non-significantly different at 5% level of significance.

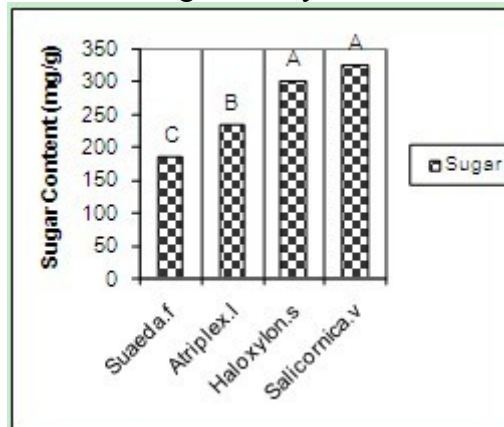


Fig: 3

Comparisons of Sugar Content (mg/g) of Selected Halophytes of District Mardan. *Suaeda.f*: *Suaeda fruticosa*, *Atriplex.l*: *Atriplex leucoclada*, *Haloxylon.s*: *Haloxylon salicornicum*, *Salicornica v*: *Salicornica virginica*. Leaves of selected halophytes were collected at vegetative stage. Three replicates were used.

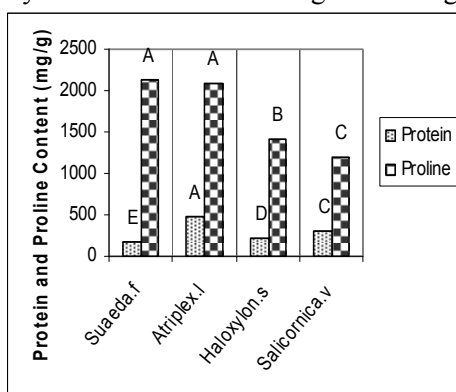


Fig: 4

Comparisons of Proteins (mg/g) and Proline Content (mg/g) of Selected Halophytes of District Mardan. *Suaeda.f*: *Suaeda fruticosa*, *Atriplex.l*: *Atriplex leucoclada*, *Haloxylon.s*: *Haloxylon salicornicum*, *Salicornica v*: *Salicornica virginica*. Leaves of selected halophytes were collected at vegetative stage. Three replicates were used.

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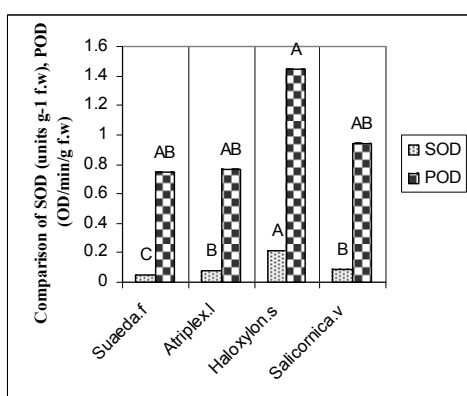


Fig: 5

Fig 1.1 Comparison of SOD (units g⁻¹ f.w) and POD (OD/min/g f.w), of the Selected Halophytes of District Mardan. *Suaeda.f*: *Suaeda fruticosa*, *Atriplex.l*: *Atriplex leucoclada*, *Haloxylon.s*: *Haloxylon salicornicum*, *Salicornica v*: *Salicornica virginica*. Leaves of selected halophytes collected at vegetative stage along with three replicates.

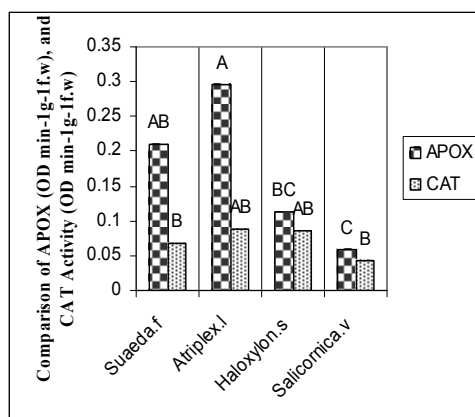


Fig: 6

Fig 1.2 APOX ($\text{OD min}^{-1}\text{g}^{-1}\text{f.w}$), and CAT Activity ($\text{OD min}^{-1}\text{g}^{-1}\text{f.w}$) of Selected Halophytes of District Mardan. *Suaeda.f: Suaeda fruticosa*, *Atriplex.l: Atriplex leucoclada*, *Haloxylon.s: Haloxylon salicornicum*, *Salicornica v: Salicornica verginica*. Leaves of selected halophytes collected at vegetative stage along with three replicates.

All bars which share same letters are non-significantly different at 5% level of significance.

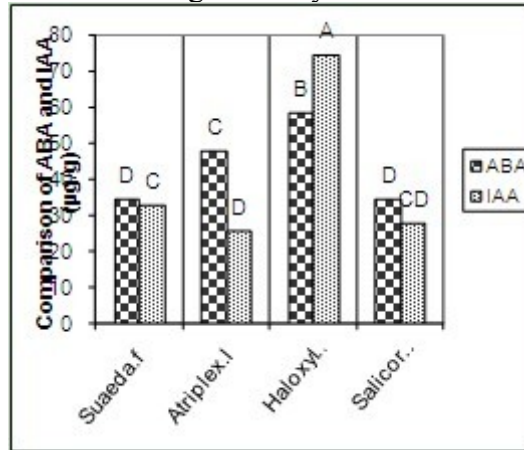


Fig: 7

Comparison of the ABA, IAA ($\mu\text{g/g}$) of Selected Halophytes of District Mardan. *Suaeda.f: Suaeda fruticosa*, *Atriplex.l: Atriplex leucoclada*, *Haloxylon.s: Haloxylon salicornicum*, *Salicornica v: Salicornica verginica*. Leaves of selected halophytes were collected at vegetative stage. Three replicates were used.

All bars which share same letters are non-significantly different at 5% level of significance