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Growth and physiological responses of *Atriplex lentiformis* to variable levels of salinity

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Abstract
In the present paper, effect of salt stress on growth and physiology of *Atriplex lentiformis* was investigated. Plants were first germinated and transplanted to pots. Salt stress was given as Hoagland's solution spiked with50, 100, 200 and 300 mM NaCl. Leaf samples were harvested at 30, 60, and 90 days for determination of various parameters. Proline and sugars accumulation was also measured to determine the extent of tolerance of test plant to salinity. Low levels of salinity did not cause substantial inhibition of growth but higher concentrations induced a decline in root-shoot length and biomass. Chlorophyll content declined with increasing NaCl concentration, while proline and sugar content increased as compared to control. The results of present study indicate no significant effect of salinity on *A. lentiformis*. Improved to lerance to salt stress in *A. lentiformis* may be accomplished by decline in growth and photosynthetic activity and increased proline and sugar content. Based on these findings, the *Atriplex* can be suitable for revegetating moderately NaCl-contaminated soils.

Keywords: *Atriplex*, chlorophyll, halophyte, salt stress, salinity

1. Introduction
With the rapidly increasing population, scarcity of water and increasing salinization of agricultural lands is already threatening the food supply [1]. Plant exposure to abiotic stresses like as drought, high salinity, and extreme temperature leads to a major loss in crop productivity globally. Among the various abiotic stress factors, soil salinization is the biggest threat to inland agriculture. Salinity is one of the most widespread environmental threats to global crop production, especially in arid and semi-arid climates, where land degradation, water shortage and population growth are already a major concern [2].
Salinization transforms fertile and productive land to barren land, and often leads to loss of habitat and biodiversity. Salinity restricts plant growth by inducing physiological dysfunctions even at low salt concentrations [3].
Salt induces osmotic stress by limiting absorption of water from soil, and ionic stress resulting from high concentrations of potentially toxic salt ions within plant cells. Plants have evolved a variety of protective mechanisms to allow them to cope with these unfavourable environmental conditions for survival and growth, including the accumulation of ions and osmolytes such as proline [4]. The accumulation of these compounds prevents water loss and ion toxicity [5]. Extensive literature has shown that under environmental stress, plant cells synthesize various kinds of osmotically active solutes, such as minerals, carbohydrates, glycinebetaine, proline etc [6, 7].
The use of plants in the remediation of saline soils is an emerging lowcost and environment friendly approach in the reclamation of abandoned irrigated fields.[8] In this respect, the creation of highly productive fodder systems through the establishment of palatable halophytes has been shown to remediate saline/sodic soils as well as provide an income to resource poor farmers [9]. Halophytes are morphological, ecological and physiological well adapted to saline soils and are capable of producing high yields under such conditions.
Various species of halophytes grow naturally in salinized areas. The compartmentalisation and accumulation of ions in vacuole, presence of salt tolerancegenes, confer salt resistance to halophytes [10]. The re-vegetation of saline areas through halophytes is a form of pro-active phytoremediation [11].
The plant-based method of saline soil stabilization is of great importance, especially in developing countries like India where other techniques/methods are more expensive. In this perspective, the use of Na^+ and Cl^-accumulating plants for soil desalination is often recommended [12]. Flowers and Colmer, 2008 reported that halophytes are remarkable plants which have the ability to complete their life cycle in a substrate rich in high NaCl that are normally toxic to other species [13].
Atriplex species (saltbushes) are dominant in many arid and semi-arid regions of the world, particularly in areas with relatively high soil salinity. *Atriplex* spp. are among a group of halophytes that complete their life cycle even at high salinity levels and have the ability to uptake and sequester high micronutrients [14]. They have increased biomass production with salt increments in the growth medium ranging from 5 to 10 g l^{-1} NaCl [15]. *Atriplex* shrubs have adaptive features that enable them to tolerate adverse effects of salts internally, or excrete salt from cells and tissues [16].
In general, low salinity levels do not appear to have a deleterious effect on the growth of *Atriplex* spp. Several *Atriplex* species are valued as livestock forage especially in arid and salt affected areas also they have high protein, vitamins and minerals content [17]. This plant is often grown as fodder plant in arid/semi-arid areas because of its resistance to

drought and salt tolerance. Furthermore, *Atriplex* spp., such as *A. nummularia*, and *A. griffithii*have been shown to survive and grow under high saline environment [14].

Therefore, the aim of the present study was to elucidate the growth and tolerance of *Atriplex lentiformis* to salinity. In this research, growth parameters such as root length shoot length, total biomass, and chlorophyll content of *Atriplex lentiformis* was determined to elucidate the effects of salinity on the overall growth. Proline and sugars accumulationin test plant were also measured to determine their role in tolerance to salt stress.

2. Materials and methods

2.1. Experimental design

Seeds of *Atriplex lentiformis* were procured from Arid Forest Research Institute, Jodhpur, India. Seeds were sterilized in 0.1%$HgCl_2$and then allowed to germinate. After germination, three seedlings per pot were transplanted into4 kg garden soil. Watering was done on a two days basis to achieve soil water field capacity level. The seedlings were first allowed to grow for 6 weeks after which NaCl was added to pots at varying concentrations.Salt treatments with solutions of 0, 50, 100, 200 and 300 mM NaCl (NaCl dissolved in half strength Hoagland's solutions) were applied on plants. Watering was done to ensuring that the pots were maintained wet throughout the experiment.Pots were keptin a random block design. The whole experiment was conducted in green house for 4 months. Any symptoms of Na Cltoxicity exhibited by plants were noted during the entire experimental period. Ateach sampling dates, i.e., 30, 60, and 90 days after treatment (DAT), plants were harvested and taken for analysis. Plant growth parameters like root length, shoot length, biomass, and physiological parameters like chlorophyll, sugar content and proline accumulation were also determined.

2.2. Physico-chemical profiling of soil

Soil collected from the botanical garden of the college was tested for various parameters to obtain a clear profile. Soil pH was measured by a pH meter (SystronicsμpH system 361) with a water-soil ratio of 1:2.5. Electrical conductivity was measured by a conductivity meter (Systronics) by preparing a 1:2.5 soil suspension in water. Organic carbon was measured by modified Walkley-Black rapid dichromate oxidation method [18]. Where 10 ml 1N potassium dichromate ($K_2Cr_2O_7$) and 20 ml conc. H_2SO_4 were added to 5 g soil. After 30 min. 200ml distilled water was added along with 10 ml 85% phosphoric acid and 1 ml DPA indicator (Diphenylamine). This was titrated against N/2 ferrous ammonium sulphate. The value obtained was multiplied by the factor 1.724 to obtain organic matter content, as reported. Available potash was estimated by flame photometer (mediFlam), available nitrogen by micro-kjeldahl and available phosphate was also determined using digital spectrophotometer (systronics) [19].

Soil pH (1:2.5) was 7.11, EC in dS/m (1:2.5): 0.63, Organic carbon: 0.7%, available phosphorus: 21.7 kg/ha, available potash: 373kg/ha and available nitrogen 109.4 kg/ha.

2.3. Chlorophyll estimation

Chlorophyll content of each plant was estimated according to Arnon (1949) [20]. Fresh leaves 40 mg were soaked in 10 ml of 80% acetone and kept in a bottle covered with black carbon paper to prevent the entry of light and kept in a refrigerator for 4-5 days. The bottle was sealed to prevent evaporation of acetone. After 5 days, optical density was calculated by spectrophotometer at different wavelengths i.e. 630, 645, 652, 665 nm, respectively.

2.4. Determination of Proline content

Proline content in plants was estimated according to method given by Bates *et al.* (1973) [21]. Fresh leaves (500 mg) were taken and crushed with 10 ml of 3% sulphosalicylic acid. This was then centrifuged at 2000 rpm for 15-20 min. Two millilitres of supernatant was taken in a test tube and 2 ml each of ninhydrin and glacial acetic acid were added. The solution was boiled in water bath for 30 minutes and then transferred into an ice bath. After 30 min, 4 ml toluene was added and the test tube was shaken vigorously. The upper red chromophore (toluene layer) was taken and optical density was calculated at 520 nm. Toluene was used as control (reference blank).

2.5. Sugar estimation

Sugar estimation was done by using an throne reagent (Plummer, 1971) [22]. One gram of fresh plant material was crushed with 2 ml 80% ethanol and total volume was made to 10ml by adding distilled water. The content was centrifuged at 2000 rpm for 10-15 min and the supernatant was collected (for soluble sugars). To the residue, 10ml 1% $H_2SO_4$was added and centrifuged at 2000 rpm for 10-15 min. Then, 0.2 ml of the Supernatant was taken, and 0.8 ml distilled water and 9.0 ml anthrone was added. The solution was heated in water bath for 10 min and the percent of transmission at 620 nm was noted down. With the help of standard graph curve and formula, quantity of sugar was calculated. For control 1 ml distilled water + 9 ml anthrone was used.

3. Results & discussion

Values for chl 'a', chl'b' and total chlorophyllare depicted in fig.1.A progressive decline in chlorophyll content with the increasing NaCl concentration was observed at all the testing dates. Highest reduction in chlorophyll content was observed at 300m M NaCl. At 30 DAT, highest value of chlorophyll "a" was recorded in control i.e. 2.85 and the lowest value was recorded at 300m MNaCl i.e. 1.97.At 60 DAT, highest value was recorded in control i.e. 2.98 and lowest value at 300 mM NaCl i.e. 2.12. At 90 DAT also values decreased from control to high salt concentration. Highest value of 3.33 was recorded in control and lowest value of 2.94 at 300mMNaCl treatment. Total chlorophyll also decreased with increasing salt concentration. At 30 DAT, highest value of total chlorophyll was observed in control i.e. 3.88 and lowest value was recorded at 300mM NaCl treatment i.e. 2.73. At 60 DAT, highest value was observed in control i.e. 3.99 and lowest value was observed at 300mM NaCl treatment. At 90 DAT also similar trend was observed. Highest value was recorded in control i.e. 4.61 and lowest value was observed at 300mM NaCl treatment i.e. 3.86. However, not much reduction was observed at 90 DAT as compared to 30 DAT and 60 DAT. Results obtained are in agreement with many authors who reported declined chlorophyll content by increasing salt stress

[23]. Reduction in chlorophyll content is commonly observed phenomena as salinity increases and plants are subjected to salt stress. Gebauer and Ebert, 2003 also observed a decrease in chlorophyll a, chlorophyll b, and carotenoid content in *Tamarindus indica*under salt stress [24].

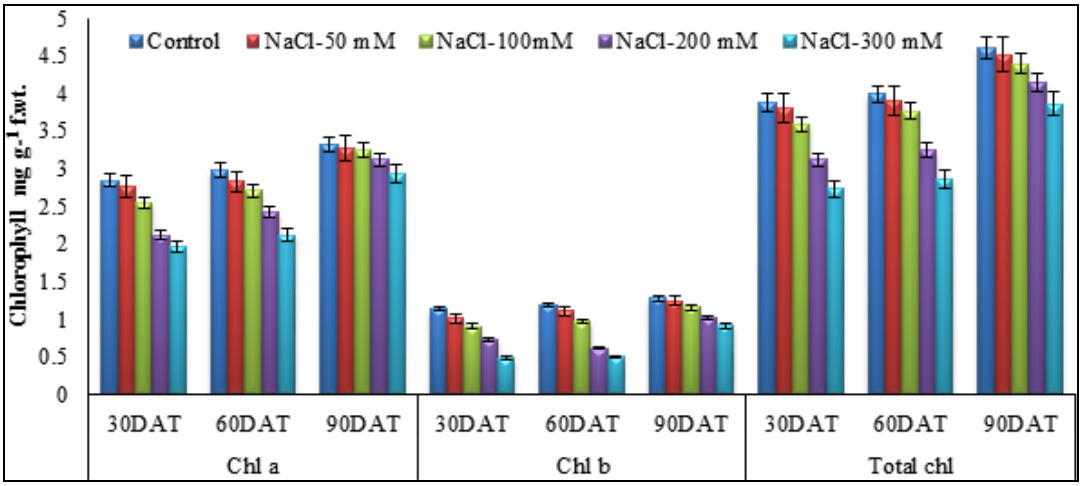

Fig 1: Chlorophyll content in *Atriplex lentiformis*

Chlorophyll is a key component for photosynthesis, which in turn, is a key metabolic event essential for growth and development of plants. However, photosynthesis is extremely sensitive to abiotic stresses such as salt, drought, frost and temperature [25]. According to Kao *et al.* 2006, salinity often leads to decrease in chlorophyll contents and photosynthesis rates. [26]Reduction in chlorophyll content under salinity stress is a commonly reported phenomenon because of its adverse effects on membrane stability [27]. Netondo *et al.* (2004) reported that 100 mM NaCl and higher salt concentration initiates chlorophyll reduction in plants [28].

Proline accumulation is a common metabolic response of higher plants to salinity [4]. Proline accumulation in leaves of many plant species grown in saline environments has been reported [29, 30].

Figure 2 depicts the data pertaining to proline content of the test plant i.e. *A. lentiformis*. It was observed that proline content of the leaves increased with increasing salt concentration. At 30 DAT, lowest value was recorded in control i.e. 0.17 and highest value was recorded at 300 mM NaCl i.e. 1.19. At 60 DAT also, values increased from control to 300 mM NaCl. Recorded value in control was 0.19 and 1.26 in 300 mM NaCl. Similar trend was observed at 90 DAT. Lowest value was recorded in control i.e. 0.18 and highest value of 1.45 was recorded in 300 mM NaCl treatment. Present investigation was in accordance with those of Bidai (2001) who reported proline synthesis and accumulation in leaves of *A. halimus* treated with NaCl+$CaCl_2$ and sea water [31]. Slama *et al.* (2007) also noticed an inclined in proline level in leaves of *Sesuvium portulacastrum* a perennial halophyte, stressed with 100 mM NaCl [32].

In salinity stress, osmolyte accumulations in cells contribute substantially to cytoplasmic osmotic adjustment [33]. Besides being an osmo-protectant, proline also plays an important role in detoxification of reactive oxygen species and act as an antioxidant [34]. Proline is a stable, less toxic and have resistant for oxidative damage in saline-stressed plants.

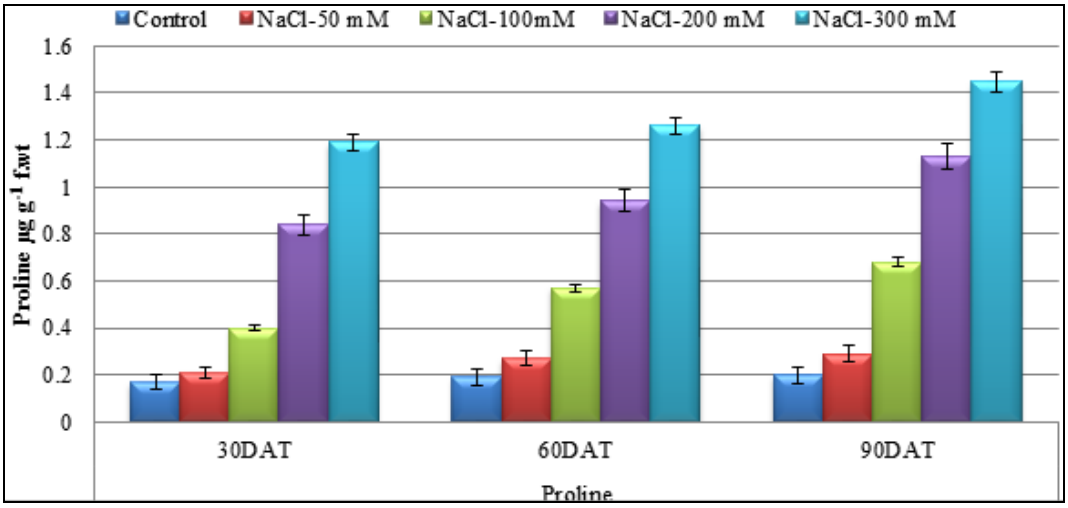

Fig 2: Proline content in *Atriplex lentiformis*

It is evident from fig. 3 that sugar content of *A. lentiformis* leaves was influenced by NaCl treatments. Application of NaCl increased sugar content in leaves with increasing salt concentration. At 30 DAT, all treatments show significant enhancement in sugar content with the increasing concentration of NaCl in comparison to control. Sugar content varied from 24.7(control) – 43.9 (300 mM NaCl) at 30 DAT to 26.2- 49.8 at 60 DAT. The picture was however not similar at 90 DAT. It was observed that at 90 DAT, the values were near to the control. At control the value was 31.3 and at 300 mM NaCl the value was 33.1. Similar values in control and at high salinity NaCl indicate the adaptability of the test plant to salt stress.

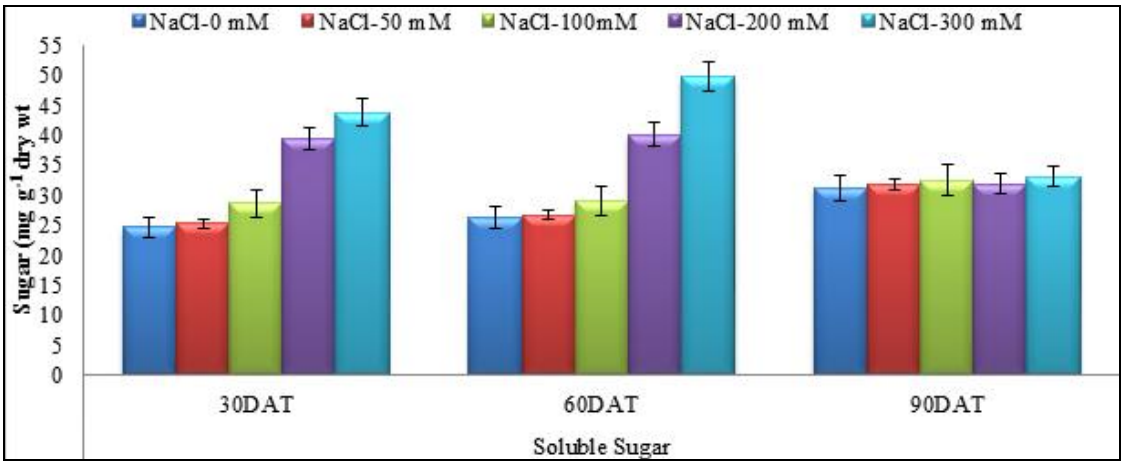

Fig 3: Sugar content in *Atriplex lentiformis*

Sugars are compatible solutes which accumulate in plant tissues when exposed to abiotic stresses, such as, drought, temperatures and salt stress. Sugar accumulation plays an important role in the plant defensive mechanisms of osmoregulation and energy preservation [35].

Carbohydrates represent the most important compounds so far as dry matter production and energy relations of cells concerned. Carbohydrate changes are of particular importance because of their direct relationship with such physiological processes as photosynthesis, translocation and respiration. Sugar content increases under lower levels of salinity [36] but in contrast to this Rathert (1983) observed that the sucrose and starch are the predominant carbohydrates affected by salinity, and soluble sugars are more sensitive to salt stress than starch. [37]Sugars in plants generally serve mainly as source of carbon and energy, osmotica, stress protectants and signal molecules. Accumulation of soluble sugars has been attributed as an important parameter of osmotic adjustment in the halophytes.

Studies have shown that carbohydrates like sucrose, glucose, and fructose have significant role in conferring adaptation to salt stress [38]. Carbohydrates are frequently associated with active osmotic adjustment and have long been known to increase in a wide range of plants grown under saline conditions [39].

Soluble carbohydrates and free amino acids have been mentioned as important compounds in osmoregulation in plants under water and salt stress [40]. Accumulation of these compatible solutes reduces osmotic potential in the cytoplasm and contributes to maintaining water homeostasis among several cellular compartments [41].

Root and shoot lengths of the *A. lentiformis*are presented in Table.1. Plant height reduced in the treatments supplemented with NaCl. Significantly reduction in the elongation of shoots and roots length was observed with the increasing salt concentration in soil. At 30 DAT, maximum root and shoot length was recorded in control i.e. 42.2 cm and 102.2 cm, respectively while the minimum root and shoot length i.e. 32.3 cm and 81.9 cm was recorded at 300 mM NaCl treatment. At 60 DAT also, maximum root and shoot length was recorded in control i.e. 68.7 cm and 128.8 cm, respectively while minimum root and shoot length i.e. 52.2 cm and 91.3 cm was recorded at 300 mM NaCl treatment. Similar trend was recorded at 90 DAT. Maximum roots and shoots length was recorded in control i.e. 76.2 cm and 161.6 cm respectively. Minimum root and shoot length was recorded at 300 mM NaCl with values 67.8 cm and 144.2 cm, respectively.

Table 1: Effect of NaCl treatments on root and shoot length of *Atriplex lentiformis*

Treatments	Root length (cm)			Shoot length (cm)		
	30DAT	60DAT	90DAT	30DAT	60DAT	90DAT
Control	42.2±1.8	68.7±1.3	76.2±2.8	102.2±3.6	128.8±2.9	161.6±3.6
NaCl-50 mM	41.3±2.1	67.8±2.7	75.2±1.4	100.7±1.7	126.9±1.6	160.7±1.8
NaCl-100mM	38.7±0.8	63.1±0.9	73.7±4.1	95.5±1.1	120.7±1.1	153.9±2.2
NaCl-200 mM	36.6±1.9	58.4±1.7	70.9±2.8	89.6±2.2	111.2±0.6	149.8±1.6
NaCl-300 mM	32.3±2.2	52.2±3.2	67.8±1.5	81.9±2.9	91.3±2.2	144.2±1.9

From the data recorded in the study, it is evident that shoots were more affected than roots. Similar results were also reported by a number of authors [42, 43]. According to Munns (1993), the first phase of the growth response results from the effect of salt outside the plant which results in reduced leaf growth and to a lesser extent root growth [44]. Number of authors in relation with growth characteristics had reported a significant decrease in shoot length of plants under the influence of salinity [45, 46].

Cheeseman, 1988 also reported that root growth is less sensitive compared to shoot growth under saline conditions therefore an increased root: shoot ratio is often observed when plants are subjected to saline conditions [47]. Inhibited shoot growth with continued root growth has been considered an adaptation in plant to salt and water stress [48]. Ramoliya *et al*. 2004 also reported that soil salinity suppresses shoot growth more than the root growth [49].

Data pertaining to biomass of test plant is depicted in fig. 4. It was observed in the study that with increasing salinity, there was reduction in the biomass. Highest values were recorded in control and lowest values were recorded at 300 mM NaCl treatment. At 30 DAT, highest value for biomass i.e.126.2 g was recorded in control and lowest value was recorded at 300 mM NaCl treatment i.e. 100.5 g. At 60 DAT, it was observed that there was increase in biomass with increasing salt concentration. Higher values at 100mM NaCl and 200 mM NaCl i.e. 158.3 g and 157.7 g respectively were recorded compared to the control value i.e. 154.4 g. However, at 300 mM NaCl, observed value of biomass (148.2 g) was less than control value of 154.4 g.

At 90 DAT also similar trend was observed. Higher values at 100 mM NaCl and 200 mM NaCl (227.5 g and 222.6 g, respectively) were recorded than the control value i.e. 221.4 g. At 300 mM NaCl, the observed value of biomass (219.8 g) was however less than control value of 221.4 g. It was observed in the study that biomass decreased with increasing salinity during initial growth period but once the plant adapts itself to salinity; its growth was not affected much, thus showing its high tolerance towards salt stress.

Plant growth is a major parameter used to assess the survival and adaptation of a plant species to environmental factors that decisively control biomass production [50].

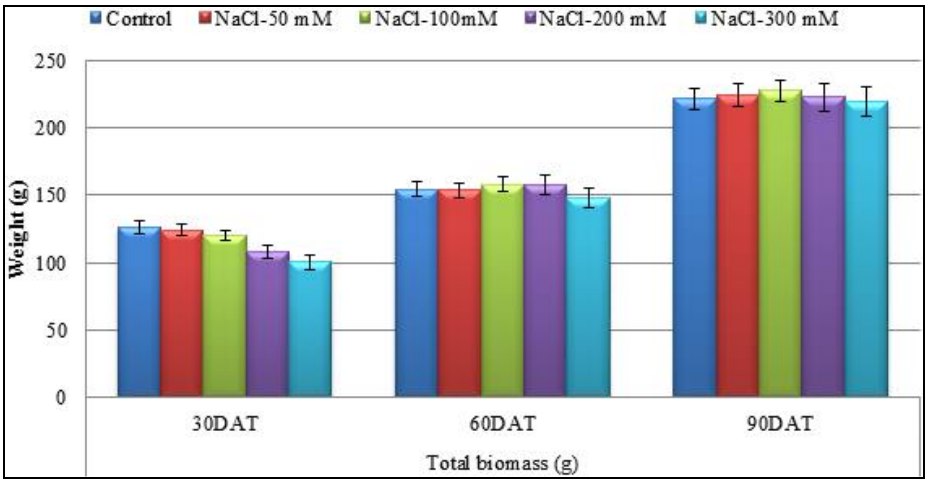

Fig 4: Biomass of *Atriplex lentiformis*

Halophytes are characterized in growing at highly saline soils and absorbing a high proportion of salts, especially Na^+ and Cl^- ions in their leaves. Among the halophytes the genus *Atriplex* has been extensively studied not only for its biomass productivities, but also for its fodder value and its ability to grow on degraded saline lands; improve soil conditions and its nutritional values [51].

Atriplex spp. are among a group of halophytes that complete their life cycle at high salinity levels and have the ability to accumulate high concentrations of micronutrients much greater than the required minimum [14]. *Atriplex* spp. has increased biomass production with salt increments in the growth medium ranging from 5 to 10 g l^{-1} NaCl [15]. A similar promotion of growth has also been reported for other halophytic species [52].

The *Atriplex* genus has several plant species that are capable to complete their life cycles under very stressful environmental conditions such as drought, high temperature and high salinity [14]. The growth and productivity of *Atriplex* under conditions of low and erratic rainfall are exceptional, and the adaptation of this species to high salinity makes its introduction very suitable [53]. Halophytes are characterized in growing at highly saline soils and absorbing a high proportion of salts, especially Na^+ and Cl^- ions in their leaves.

4. Conclusion

Our study shows that *A. lentiformis* is a moderate salt tolerant halophyte; it has the potential to complete its life cycle under high saline matrix. Its growth may be stimulated by the presence of salts in the growth medium. Salinity stress reduces chlorophyll content but not up to extent that it adversely affects the physiology of plant. Accumulation of proline and sugar under salt stress indicates the adaptability of the plant to saline conditions. Production of good biomass even at higher salinity indicates high tolerance of the plant to salt stress.The obtained results, in the context of this study; showed that the *A. lentiformis* could be promising in programs of rehabilitation of degraded pastoral zones and of the salty sites in arid and semi-arid regions.

5. Acknowledgment
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