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Effect of Exogenous Application of Salicylic Acid on the Vegetative and Reproductive Growth Parameters of *Cyamopsistetragonoloba* L. (Guar) under Sea-Salt Stress

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ABSTRACT

Salinity is one of the major challenging problems of low crop yield in the present age. To cope with this challenging problem, effect of exogenous application of salicylic acid (SA) was carried out using *Cyamopsis tetragonoloba* L. (guar) as experimental plant. In currents project, we studied the role of SA in the form of foliar spray in a concentration of 0.5mMfor the sea salt stress of 0, 2.5 dS/m^{-1} and 5 dS/m^{-1} concentration. Different vegetative growth parameters such as root and shoot length, number of leaves, leaf area, fresh and dry biomass, total chlorophyll, proteins, carbohydrates and K^+/Na^+ ratio were recorded and compared for salt treated and controlled plants. Reproductive growth parameters like number and weight of pods, and number and weight of seeds were also recorded. The results conclude that salt stress cause significant reduction in the vegetative as well as reproductive growth of *Cyamopsis tetragonoloba* L. while application of SA enhances all these growth parameters. It has been also realized that SA having role in the overcoming of deleterious effects of high salt content as well as stimulate growth related phenomena. This work suggests exogenous use of SA, for crops grown in salt affected areas, for the better production and yield to fulfill the need of food requirements in the future.
KEYWORDS: Salinity, Salicylic acid, chlorophyll, protein, carbohydrates, biomass.

INTRODUCTION

High salt stress is one of the worse problems facing by the agricultural land of today's world that in turn limits plant growth and yield [1]. Excess of salts in a field reduces capacity of soil water potential and penetration, physiological draught and sometimes cause plants death as well [2]. Among other causes of soil salinity, NaCl is considered to be the major reason [3]. Even more adverse conditions occur at molecular, biochemical and physiological levels of plants in arid and semiarid saline areas of the globe, that limit crop productivity by many folds [4, 5, 6]. High salts content of a soil, reduce assimilation potential as well as overall growth of a plant. Accumulation of sodium (Na^+) and chloride (Cl^-)ions in a field alter osmotic potential and availability of water [7]. Excess in sodium and chloride ions concentration in an agricultural field also compete with other essential elements for absorption such as Nitrogen, phosphorus, potassium, calcium and magnesium [8, 9].

Salicylic acid (SA) is a phenolic compound and a plant stress hormone. It is an endogenous plant growth regulator and take part in many physiological events of a plant. Key roles of SA for a plant include induction of flowering, facilitation and regulation of ions absorption by plants root, stomatal movement and thermogenesis activation in lily (*Arum*) plant [10]. During abiotic stresses like draught, heat and salinity, it functions as signaling molecule and induces immune responses in plants against these abiotic stresses [10, 11].Regulation of many physiological activities of plants like nutrients absorption by plant roots and their transportation in xylem tissues, permeability of cell membrane for various materials, rate of photosynthesis, growth and somatal movement may be carried out by the exogenous application of SA [12, 13, 14].Study on SA shows that it has important role against heat, ozone, UV-B rays, high osmotic potential stress and heavy metals [15, 16].Research on the SA also confirmed that it has role for *Zea mays* (maize), *Hordeum vulgare* (barley), *Phaseolus vulgaris* (bean), *Helianthus annuus* (sunflower), *Lens culinaris* (lentil) and *Triticumaestivum* (wheat) against salinity [17, 18].

Cyamopsis tetragonoloba L. (guar) is an annual bushy herb grows up to three meters and belongs to family leguminoceae. Leaves are compounds with three leaflets (trifoliate) and reach up to 10 cm in length. Flowers are usually white or red colored. Fruit is a pod which is yellowish-green, straight and hairy. Pod is 12 cm in length which constitute 5-12 bean shaped hard seeds. Root is tap root and deep, reaches deepest part of the soil to absorb maximum amount of water [19, 20, 21]. The plant loves arid and semiarid hot climatic conditions with low rain fall

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but can also grow in sub-humid climates of moderate rain fall. It is drought-tolerant plant which grows at elevation of 1000 meters from the sea level. The plant show active growth only in rainy season while remain inactive during hot and dry season of the year. It grows in an area of having mean annual rain fall 250 to 1000 mm but shows best growth and seeds formation in an area having less than800 mm of mean annual rain fall. High humidity and rain fall has negative effects on the rate of fertilization, development of pod, quality and quantity of seeds. In humid climatic conditions and high rain fall areas, the plant shows more leafy growth, suitable as fodder crop for cattle and green manure. The plant shows a wide range of adaptability to soil conditions such as alkaline, saline or less fertile soil. Medium textured, sandy loam alluvial and fertile soil is the best soil for its growth. This plant does not grow in highly saline black soil [19, 20, 21, 22].The reason for the present work was to determine the effect of exogenous application of SA on growth, important biochemical compounds, ionic composition and yield of guar plants grown under saline conditions.

MATERIALS AND METHODS

Plant material and growth conditions: *Cyamopsis tetragonoloba* L. (Guar) seeds were provided by the International Center for Biosaline Agriculture, Dubai. In this experiment 24 clay pots were taken, forming two sets, each set having 12 pots. The experiment was designed as follows:

I - Control and salt treatments: The first set was subjected to 5 dS/m^{-1}, 2.5 dS/m^{-1} and 0 dS/m^{-1} sea-salt irrigation.

II - Salt treatment and salicylic acid foliar application: The second set was subjected to 5 dS/m^{-1}, 2.5 dS/m^{-1} and 0 dS/m^{-1} sea-salt irrigation and at the age of two weeks, 0.5mM SA was applied in the form of foliar spray on guar seedlings and then sprayed again at 45 days of age.

All pots have small pore at the base for draining. For each treatment, there were three replicates while there were total of 24 clay pots in two sets. Three replicas were, 5 dS/m^{-1}, 2.5 dS/m^{-1} and 0 dS/m^{-1}(control) sea-salt concentrations. Three Kg sandy loam, thoroughly washed with distilled water, was filled in each clay pot. Then Hoagland's solution was applied to each pot with full saturation. Surface sterilization of seeds was carried out with 0.1% $HgCl_2$ for 60 seconds followed by rinsing with distilled water for three times. Five uniform size seeds were grown in each pot. Each clay pot was provided with 150 ml of tape water on daily basis. At three leaf stages, all but only one seedling were removed from the pots. Completely randomized design (CRD) method was applied to all pots in the Department of Botany, University of Karachi, Karachi (UKK). Sea salt was provided in the form of solution (1.5 L) with gradual increase, two times a week. Root and shoot lengths, number of branches, leaves and pods per plant and fresh and, dry biomass of all the plants were carried out and recorded. For biochemical analysis, leaves were collected at best growth period.

Chlorophyll concentration: The protocol of Maclachlam and Zalik was used to study chlorophyll content of fresh leaves [23].

Carbohydrate Content: Total carbohydrate contents were determined using Yemm and Willis protocol with the help of Anthron reagent [24].

Total Protein contents: Bradford, 1976 protocol was applied for the extraction and quantification of total proteins [25].

Mineral Estimation of Vegetative Parts: For the extraction and quantification of sodium and potassium ions, 0.5 grams of shade dried root, stem and leaves were taken in ash solution in 50 ml of de-ionized water. Cations concentration was studied with the help of PFP1 flame photometer.

Experimental design: Completely randomized design (CRD) method with three different salt concentrations and replicates was used.

Statistical analysis: Duncan's multiple range test (DMRT) ($P < 0.05$) was applied on the DATA for the analysis of variance (ANOVA) using software SPSS version 21.0.

RESULTS

Plant Growth:

Results from present study showed that plants treated with sea-salt exhibited significant decrease ($P<0.001$) in shoot and root lengths, leaves, leaf area, biomass (fresh and dry), pods (number and weight) and seeds (numbers and weight) as compared to non-treated plants (Figures 1-6). It has also been observed that sea-salt treated plants showed significant decrease ($P<0.001$) in the number of pods, seeds and biomass per plant as compared to non-treated plants (Figures 7-9). It has been observed in the present data that exogenous application of SA exhibited significant ($P<0.01$) increases in most of the above mentioned parameters.

Chlorophyll: In present investigation excess of salts cause significant ($P<0.001$) decrease in chlorophyll content (Figures 10-13). Foliar application of salicylic acid causes non-significant pigment increase in guar plant at high salt stress.

Total Carbohydrates: Total carbohydrates shown in the figure 14 of sea-salt treated plants reveal significant increase ($P<0.001$) as compared to untreated plants. Application of SA on the leaves showed non-significant improvement in total carbohydrates in the control as well as salt treated plants.

Total Proteins: Figure 15 describes a clear decrease ($P<0.001$) in total proteins of sea-salt treated *Cyamopsis tetragonoloba* L. as compared to untreated plants. In the present attempt, it is clear that foliar application of SA non-significantly enhances level of proteins during NaCl stress.

Ionic Composition of Different Plant Parts: Figure 16, 17 and 18 indicates significant increase ($P<0.001$) in the amount of Na^+ ions while significant decrease ($P<0.001$) in the K^+ ions in salt treated plants as compared to control plants. Present work showed that exogenous application of SA significantly ($P<0.05$) improves K^+/Na^+ ratio in the salt treated as well as controlled plants.

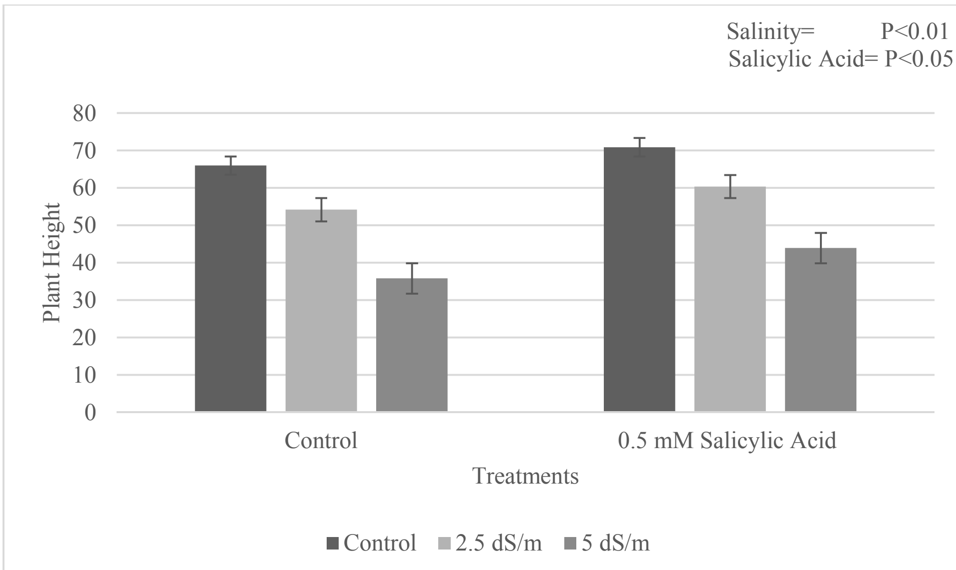

Figure 1. Effect of exogenous application of SA on the shoot length (cms) of *Cyamopsis tetragonoloba* L. grown in seasalt stress

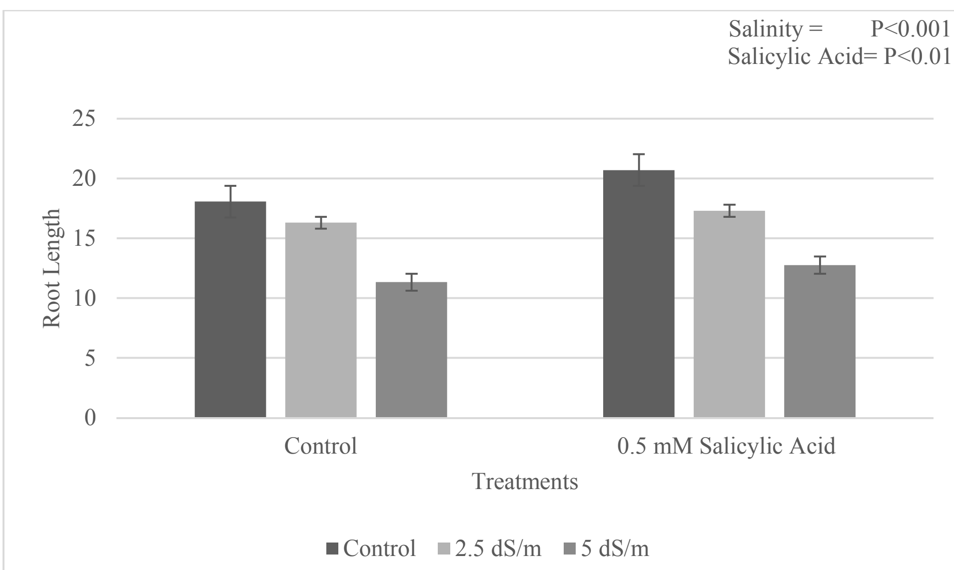

Figure 2. Effect of exogenous application of SA on the root length (cms) of *Cyamopsis tetragonoloba* L. grown in seasalt stress

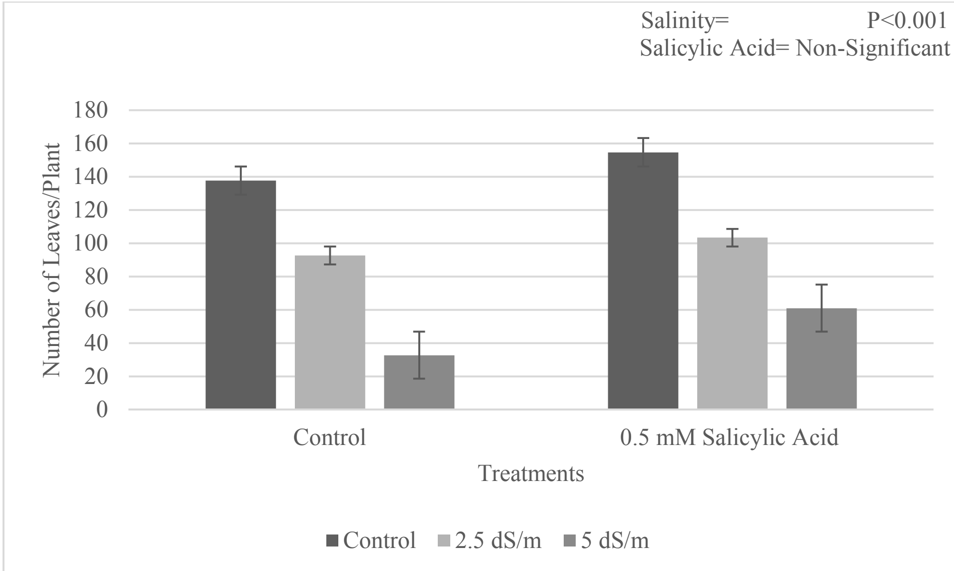

Figure 3. Effect of exogenous application of SA on the number of leaves/plant of *Cyamopsis tetragonoloba* L. grown in seasalt stress

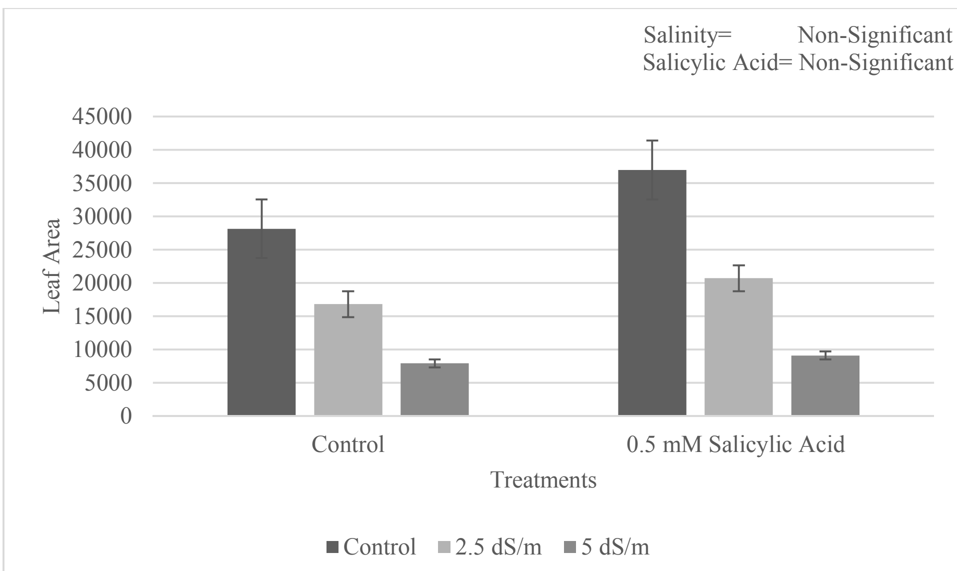

Figure 4. Effect of exogenous application of SA on the leaf area (mm^2) of *Cyamopsis tetragonoloba* L. grown in seasalt stress

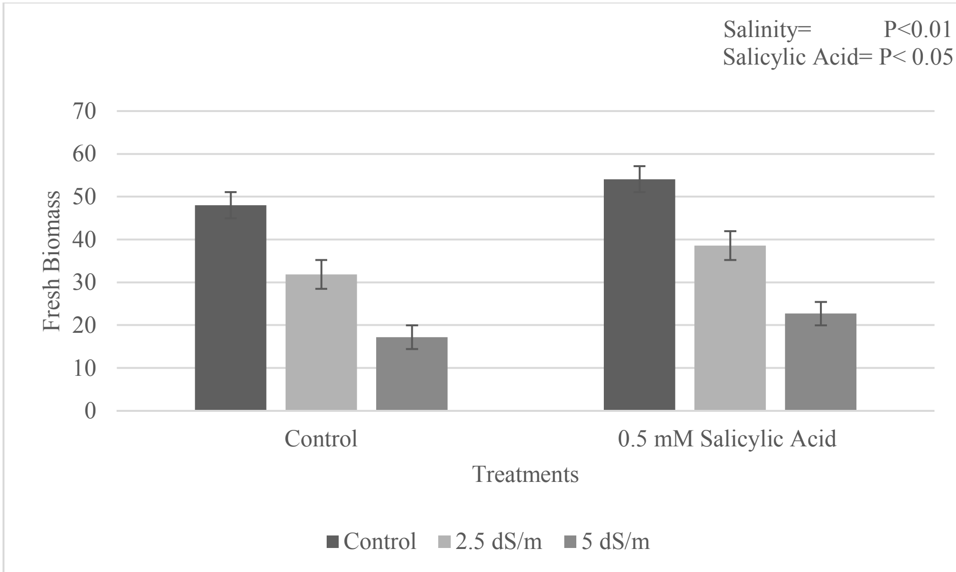

Figure 5.Effect of exogenous application of SA on the fresh biomass (gms) of *Cyamopsis tetragonoloba* L. grown in seasalt stress

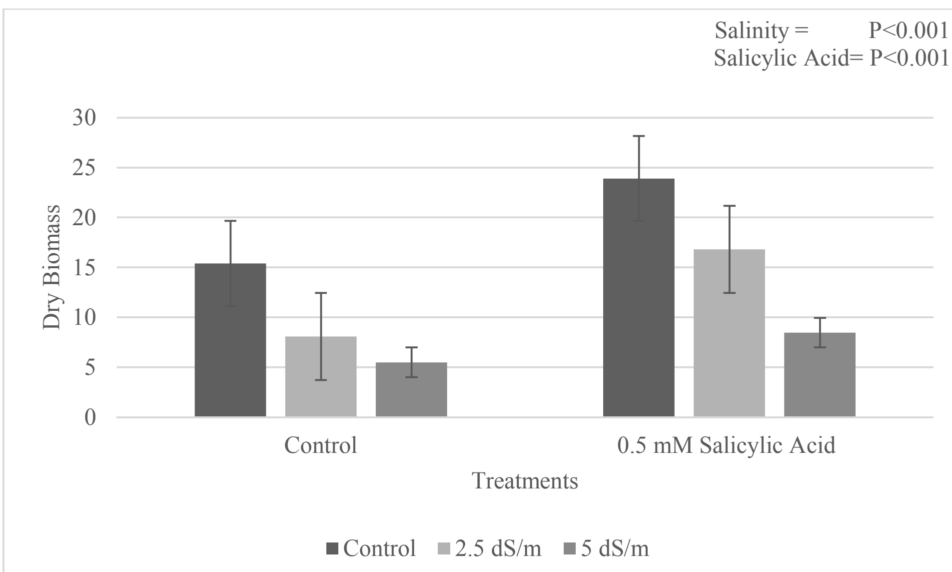

Figure 6. Effect of exogenous application of SA on the dry biomass (gms) of *Cyamopsis tetragonoloba* L. grown in seasalt stress

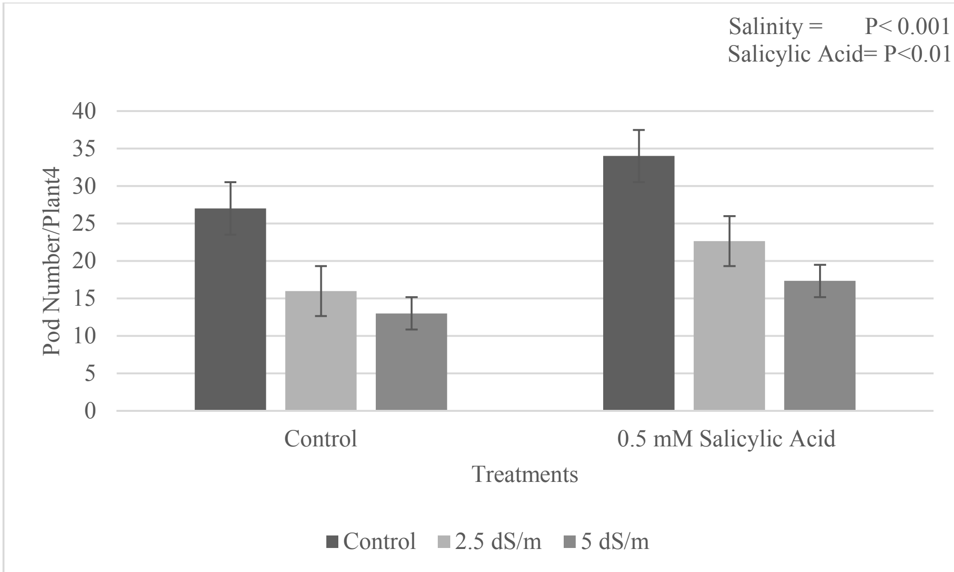

Figure 7. Effect of exogenous application of SA on the pod number/plant of *Cyamopsis tetragonoloba* L. grown in seasalt stress

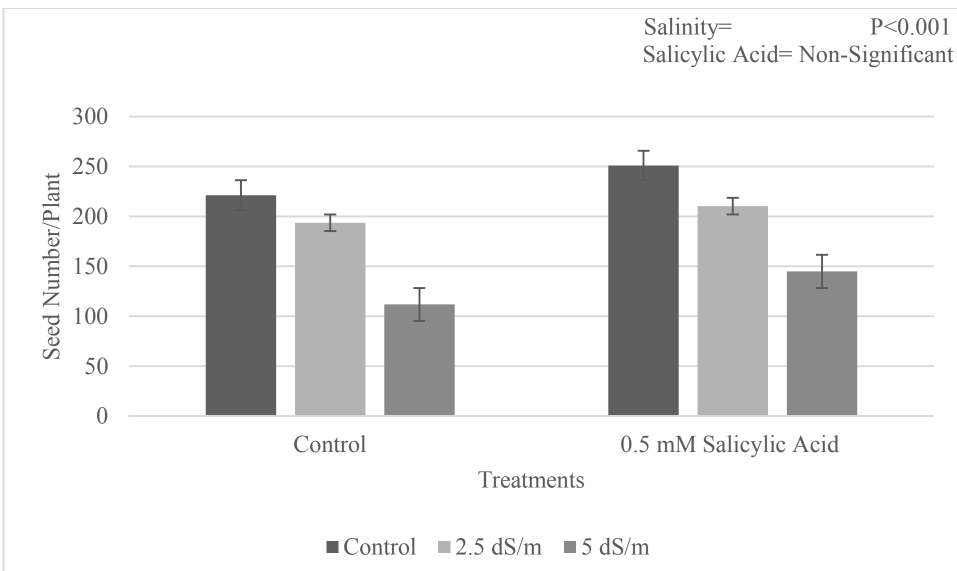

Figure 8.Effect of exogenous application of SA on the seed number/plant of *Cyamopsis tetragonoloba* L. grown in seasalt stress

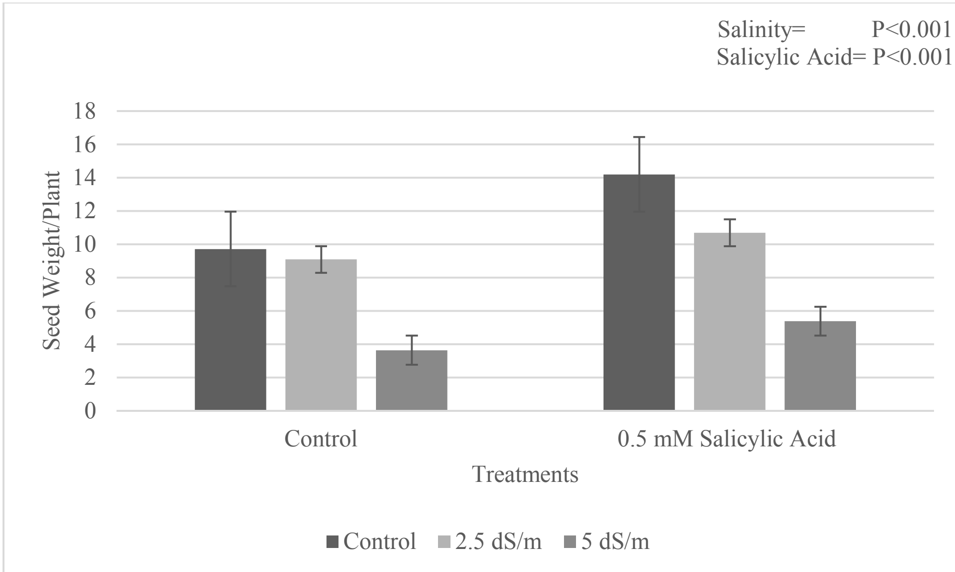

Figure 9. Effect of exogenous application of SA on the seed weight/plant of *Cyamopsis tetragonoloba* L. grown in seasalt stress

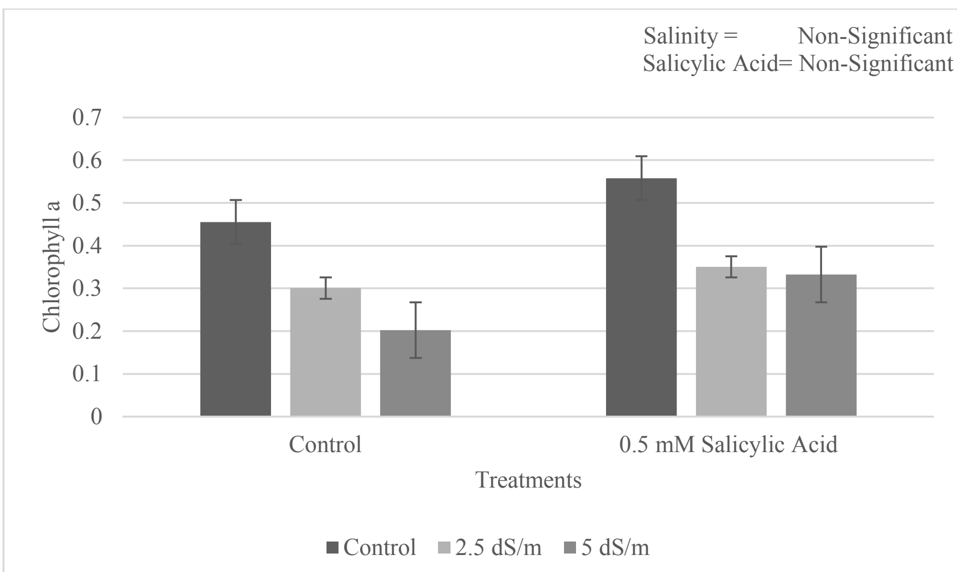

Figure 10. Effect of exogenous application of SA on chlorophyll a (mg/gm fresh wt) of *Cyamopsis tetragonoloba* L. grown in seasalt stress

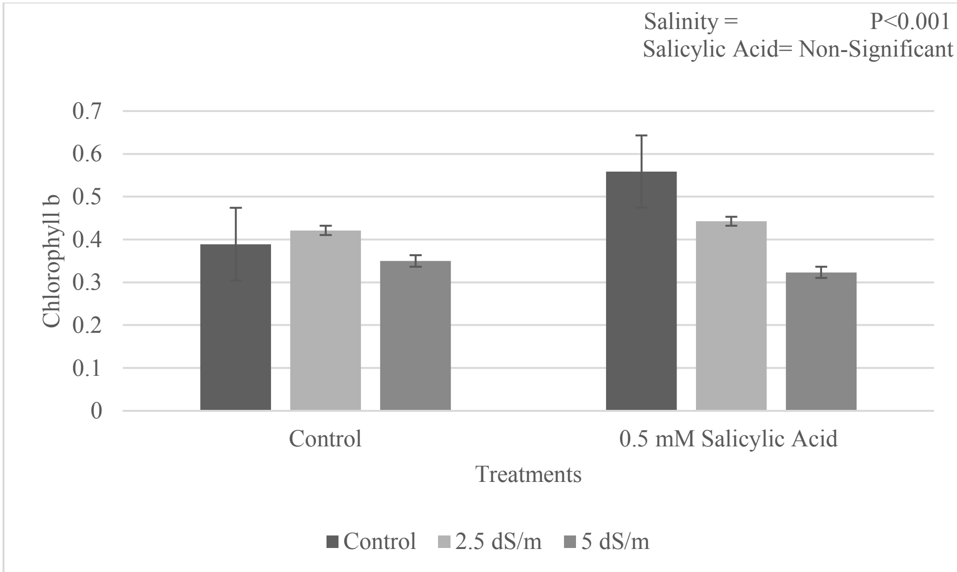

Figure 11. Effect of exogenous application of SA on chlorophyll b (mg/gm fresh wt) of *Cyamopsis tetragonoloba* L. grown in seasalt stress.

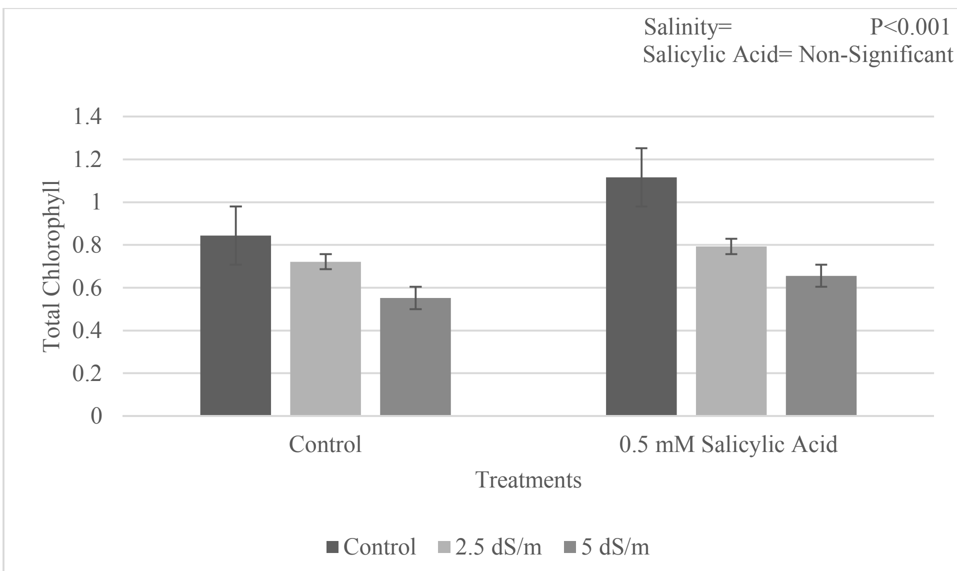

Figure 12. Effect of exogenous application of SA on total chlorophyll of *Cyamopsis tetragonoloba* L. grown in seasalt stress

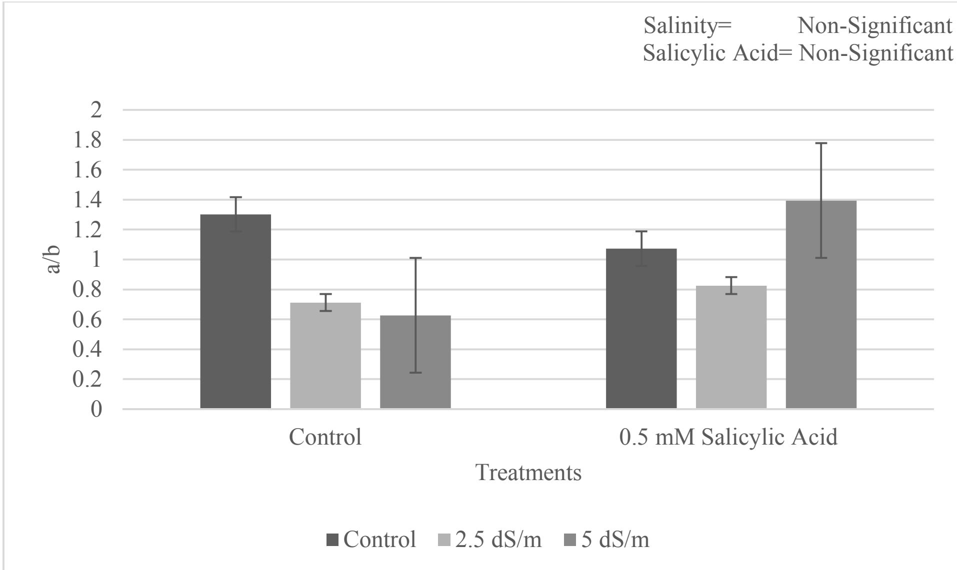

Figure 13.Effect of exogenous application of SA on chlorophyll a/b ratio of *Cyamopsis tetragonoloba* L. grown in seasalt stress

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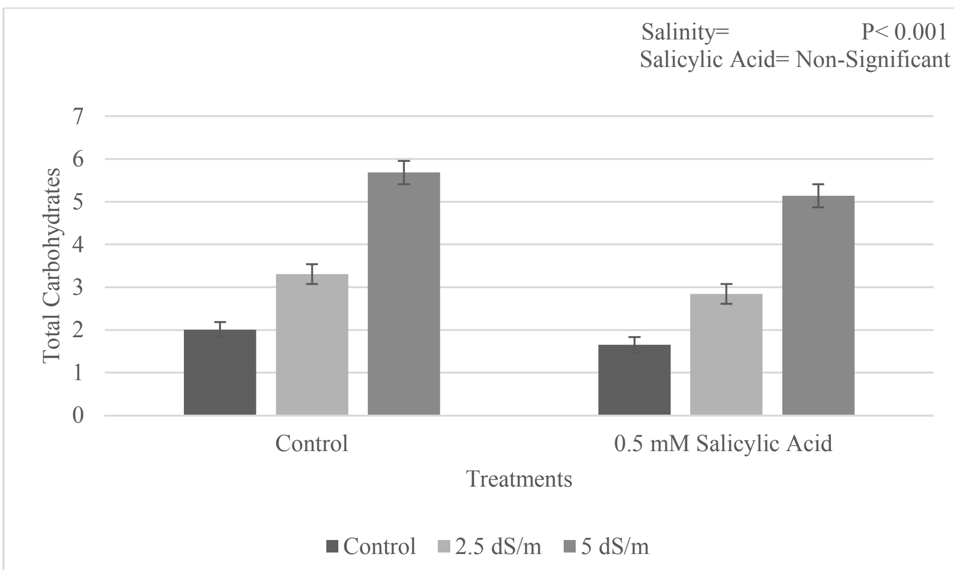

Figure 14. Effect of exogenous application of SA on total carbohydrates (mg/gm fresh wt) of *Cyamopsis tetragonoloba* L. grown in seasalt stress

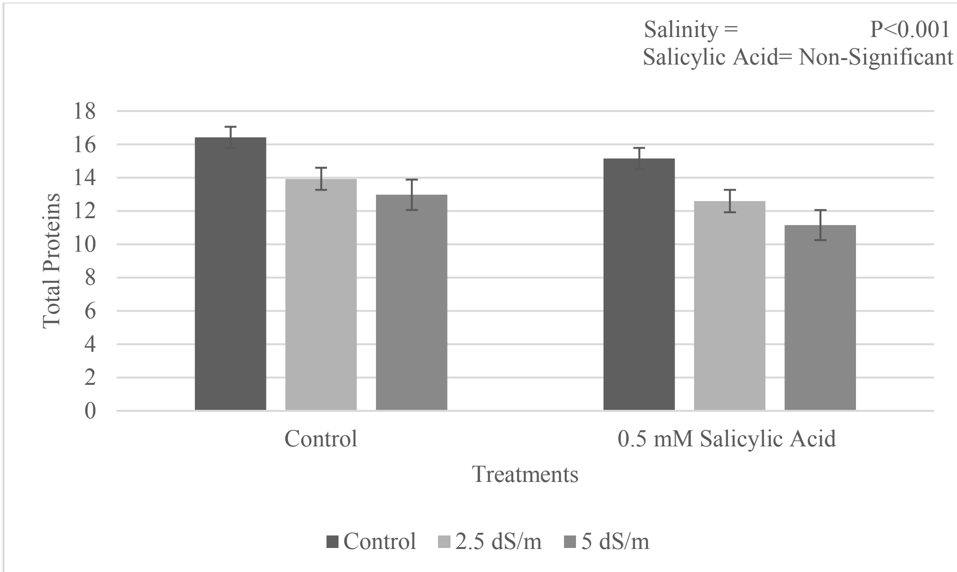

Figure 15. Effect of exogenous application of SA on total proteins (mg/gm fresh wt) of *Cyamopsis tetragonoloba* L. grown in seasalt stress.

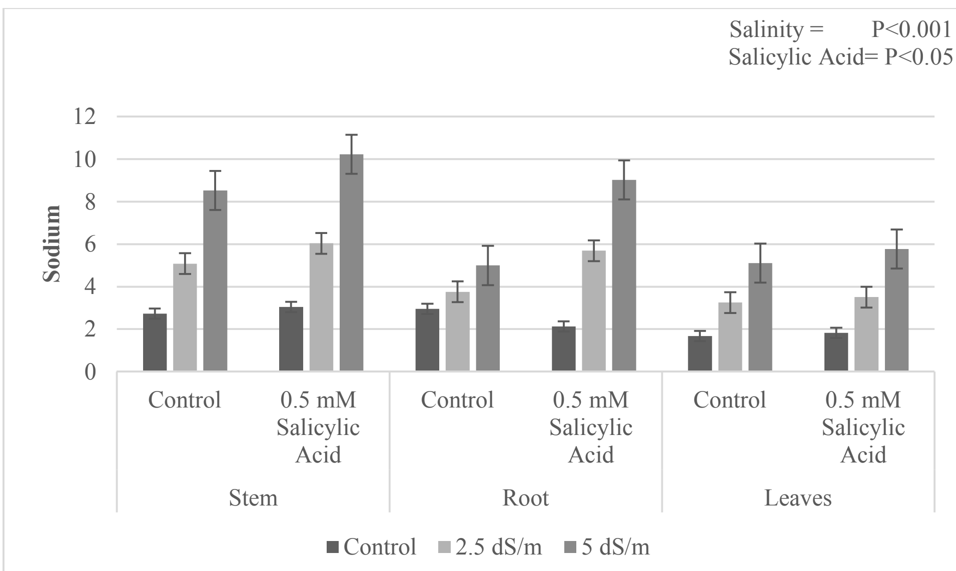

Figure 16. Effect of exogenous application of SA on Na^+ ions concentration in different parts (stem, root, leaves) of *Cyamopsis tetragonoloba* L. grown in seasalt stress

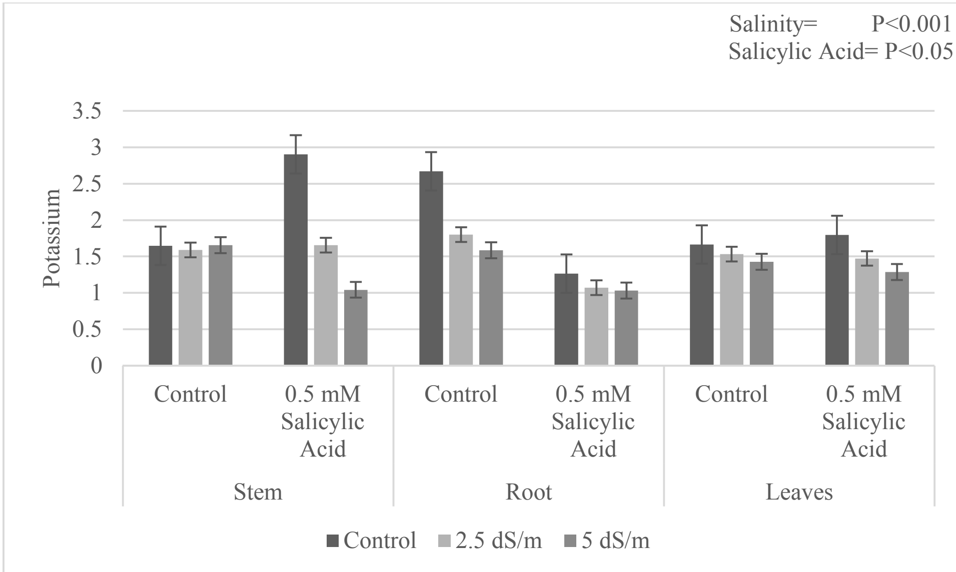

Figure 17. Effect of exogenous application of SA on K^+ ions concentration in different parts (stem, root, leaves) of *Cyamopsis tetragonoloba* L. grown in seasalt stress

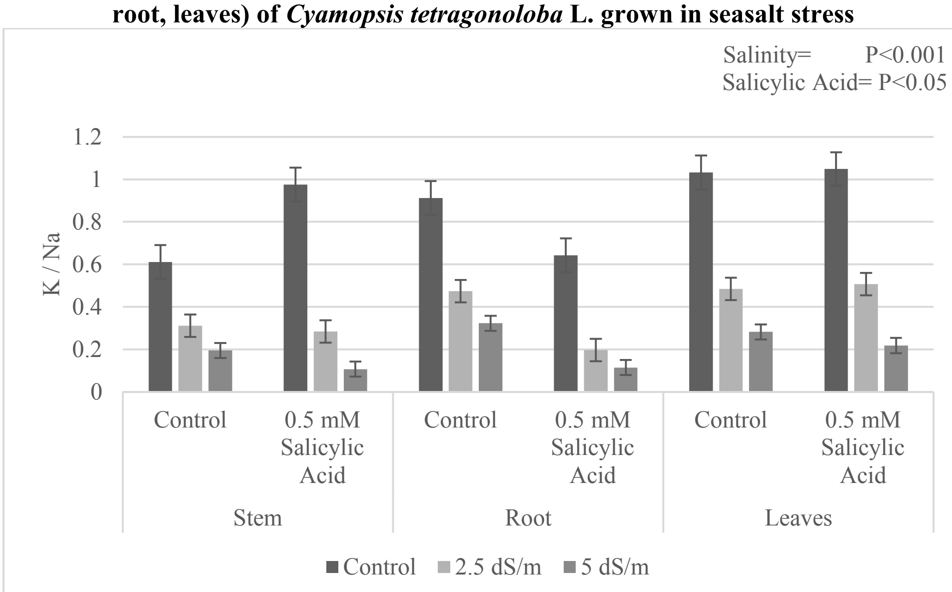

Figure 18.Effect of exogenous application of SA on K+/Na+ ions ratio in different parts (stem, root, leaves) of *Cyamopsis tetragonoloba* L. grown in seasalt stress

DISCUSSION

Plant Growth:

Salt stress has adverse effects on different parameters related to plant growth. Findings were noted in a study on *Vigna aconitifolia* L. (moth bean) [26], on *Raphanus sativus* L. (radish) [27, 28], on *Vigna unguiculata* L. (cowpea) [29] and on *Vigna mungo* L. [30]. They investigated that increased concentrations of NaCl develop a decline in the overall growth of the plants. Similar type of results were also shown by Welfare [31] studying *Cirerarietinum* L. while [32] confirmed it for the leaves of *Phaseolus acutifolius* L. (teprary bean), *Vigna unguiculata* L. (cowpea), and *Phaseolus filiformis* L. (wild bean). Reduction in the number of leaves was reported by these investigators during NaCl stress as compared to control plants. Reduction in the area of leaves of *Vigna aconitifolia* L. also occurs at enhanced concentration of NaCl [26]. A significant decrease in leaf area of *Beta vulgaris* L. (sugar beet) in response to salt stress using different concentrations (zero, 50, 100, 150 mmol) of sodium chloride, was also recorded [27, 28]. Study on *Avena sativa* L. (oat) [33] and *Fragaria ananssa* L. also showed similar results that exposure of these plants to NaCl salinity cause reduction in the leaf area [34].During salinity, all metabolic processes, biochemical as well as physiological activities, of plants are disturbed which leads into suppress growth. This has been confirmed by increasing concentration of salts and hence decreasing in biomass [35, 36]. Decrease in the leaf area and leaves number, may be the cause of reduction in biomass quantity [37, 38].

Significant enhancement has been shown by the plants in all growth parameters in controlled condition as compared to salt treated plants. Using *Triticum aestivum* L. (wheat), Hamid et al., found similar results [39]. Under high saline conditions, *Helianthus annuuas* L. (sunflower) showed noticeable increase in the growth by applying 200 mg L-1 of SA [40].During drought, it has been reported that foliar application of SA at the rate of 100 mg L-1 for *Helianthus annuuas* L., show significant increase in plant growth and biomass [41]. Exogenous application of SA overcome the negative effects of NaCl on fresh and dry mass for *Hordium vulgare* (barley) [17], *Lycopersicon esculentum* L. (tomato) [42, 43], *Zea mays* L. (maize) [18, 44] and *Cucumis sativus* L. (cucumber) [45]. This increase in the growth and biomass of a salt treated plant may be the result of SA application which enhance uptake of minerals by the root and photosynthetic rate [6, 43, 46,]. A single factor could not be considered for the reduction in plant growth and biomass during saline environment because many and different biochemical, physical and physiological factors may be involved at different stages of plant growth. High Na^+ absorption by the roots and their subsequent distribution in various parts of plants, especially in the leaves, cause leaf death, reduced rate of assimilation and reduced transportation of total photosythates to actively growing parts of a plant [4].

There is a differential competition between vegetative parts and developing panicles for the supply of carbohydrates in the salt affected plants which cause sterility and hence decrease in the yield [47]. High salts content results in the death of pollen grains which reduces seeds development, is considered the major cause of low yield by some workers [48].It is clear from the present data that exogenous application of SA increases plant biomass, pods number, and seeds number and biomass. For the bean plants under salt stress, flowering and early pods forming stages are the most important and sensitive because reduction of pods number occurs at these stages during salt stress. Application of SA also enhances IAA synthesis which in turn cause plant growth by increasing number of nodes and inter nodes so, pods number are increased. Foliar application of 0.5 mM of SA show increased number of pods and seeds in green beans. SA enhances rate of photosynthesis by increasing the formation of photosynthetic pigments and leaf area index. Other advantageous effects of SA are, reduction in the toxicity and stress of Na^+ and Cl^- ions, increase in the efficiency of water use and increase in the rate of photosynthesis. It also improves formation and transportation of assimilates to the newly forming seeds which is the most sensitive stage of plant life cycle [49].

Chlorophyll:

Photosynthesis is the first and most important physiological process in plants affected by high salt contents. Decrease in the chlorophyll contents of a salt affected plant is because of the enhanced activity of chlorophyllase enzyme, which produces N_2 by degrading chlorophyll molecules for the synthesis of glutamic acid, a precursor of proline and other amino acids. More proline has been detected in plants during salt stress [50]. Another possible reason of chlorosis, in salt affected plants, is the interference of salt ions in the biosynthesis of chlorophyll, by reducing the activity of enzymes responsible for the formation of chlorophyll molecules [51]. Chlorosis has been studied in many crop plants during salt stress [7]. High Na^+ and Cl^- ions are very toxic for the plants and cause degradation of chlorophyll molecules, destruction of chloroplast proteins as well as loosening of chlorophyll binding proteins [52].

SA helps in the lowering of negative effects of reactive oxygen species (ROS) on chlorophyll by activating antioxidant systems [53]. Increase in the chlorophyll contents in the leaves of *Phaseolus vulgaris* L. has been recorded by applying SA during high salinity [54]. Biosynthesis of chlorophyll molecules and mobilization of internal tissue nitrate is activated by the presence of SA and may be the cause of high chlorophyll contents during salt stress [55]. In *Zea mays* L., exogenous application of SA causes stimulation of ribulose-1, 5-biphosphate carboxylase (Rubisco) which in turn enhance its photosynthetic capacity [18]. It has been also reported that application of SA lowers the activity of ACC synthase, responsible for the production of ethylene hormone [56]while ethylene is responsible for the degradation of chlorophyll molecules [57].

Total Carbohydrates:

Total carbohydrates mostly showed increase in plant under salt stress condition. In a similar type of study done by El-Tayeb on *Avena sativa* L. high soluble sugars were detected in the root and buds during NaCl stress [17]. *Prosopis alba* L. also showed high soluble carbohydrates in their roots during salt stress [58]. Storage of soluble glucose during salt stress is possible by its reduction or its conversion to starch [59]. The high amount of soluble sugars in the root cells of salt affected plants disturb osmotic potential of root cells but at the same time are very useful to protect root cells from plasmolysis because solubilization of sucrose into glucose and fructose or starch into glucose molecules enhance osmotic potential of a cell, keeping the cell turgid [60].

Presence of soluble sugars in the root cells enhances osmotic potential of cells while SA causes its rapid sinking. Hydrolysis of polysaccharides to soluble sugars by the application of foliar SA is mainly by activating polysaccharide degrading enzymes [18]. Cell immune responses are activated during salinity, as some type of sugars enhance membrane selectivity as well as tolerance to the entrance of Na^+ and Cl^- ions [61]. Foliar application of SA results in more proline and soluble sugars [62].

Total Proteins:
Hindering in the nitrate reductase activity, responsible for the availability of N_2 for the synthesis of amino acids, by high salt content is the actual cause of low proteins [63]. Drastic changes in the total protein content have been detected in the roots and shoots of *Oryza sativa* L. (rice) during salt stress, while leaf blades show negligible alteration in total proteins. Over all potential of protein synthesis also decreases at high salt content [64]. Low molecular weight molecules such as amino acids and polyamines, responsible for the reduction of water potential, are produced by some plants at high salt stress [3]. Salinity also accumulates certain proteins inside the cells which function as osmoregulators [65]. Enhanced amino acid contents were also reported by EL- Bassiouny and Bakheta in different wheat varieties during salt stress [66].Plants respond to biotic and abiotic stresses in the language of certain stress proteins while presence of SA causes to reduce their level [67]. SA also stimulates nitrate reductase activity but this stimulation depends on the presence or absence of some special inhibitors of nitrate [65]. In the present attempt, it is clear that foliar application of SA significantly enhances level of proteins during NaCl stress.

Ionic Composition of Different Plant Parts:
Sodium chloride stress cause accumulation of Na and Cl elements in the fennel plant while N, P, K, Ca and Mg elements are highly reduced [68]. Same type of results were also found for *Trachyspermum ammi* [69], *Achillea fragratissima* [70], peppermint and lemon verbena [71]. K^+ion concentration regulates osmotic potential of a cell while salinity alter K^+/Na^+ ratios, so some important physiological processes like photosynthesis, protein synthesis, transpiration and essential minerals uptake are highly affected [72].
Application of SA enhances the absorption of nitrogen, iron, manganese, magnesium and copper elements while inhibit the uptake of sodium and chlorine elements during high salinity in *zea mays* L. At high salinity, concentration of Fe element decreases while application of SA causes an increase in Fe content of leaves [44]. All these positive effects of exogenous application were also reported in maize [12, 44], cucumber [45], tomato [43] and barley plants [17]. Significant increase in the concentration of K and Ca elements by the application of SA could reduce deleterious effects of salt stress on plant growth and yield [73].

Conclusion

From the results, it is concluded that salinity significantly reduced the growth of *Cyamopsis tetragonoloba L. (guar)* and exogenous application of salicylic acid improves its growth under salinity stress. So it is recommended that salicylic acid should spray @0.5mM on leaves while growing this plant under saline irrigation water.

Acknowledgement

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