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Interactive Role of Potassium Nitrate and Sodium Chloride on Growth and Biochemical Constituents of *Brassica Junce*a**l.**

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ABSTRACT

In semi-arid and arid zones of the world, salinity act as important adverse factor that can lower leaf water potential, reduced turgor and other responses and finally results in lower crop productivity. Many workers indicate that nutrient's foliar application increases tolerance to salinity. In present paper experiment was carried out in pots to study the response of foliarly applied potassium on *Brassica juncea* grown under sodium chloride stress. *Brassica juncea* plants were grown under four salinity levels (0, 50, 100 and 150ppm NaCl) and three concentrations of potassium nitrate (0, 2% and 4%).Salinity treatment decreased plant height, root length, number of leaves, fresh and dry biomass, number of pods/plant, chlorophyll contents, carotenoids and total proteins while showed increase in reducing, non-reducing sugars and total carbohydrates of plant. Increase in potassium concentration from 2% to 4% counteracted the deleterious effect of salinity on above mentioned parameters.
KEYWORDS: Salinity, chlorophyll, proteins, Reducing sugars, Carbohydrates, Biomass

INTRODUCTION

Negative effect of any non-living and living factor on the living organisms in a definite environment is known as abiotic stress[1]. Abiotic factors are uneven and control the environment away from its normal deviation range to the extent where it severely and badly affect the individual physiology of any organism and the population act in a significant way [2]. Salinity is the saltiness or melted salt content of water. Presence of salt in lakes, ponds, rivers, sea and ocean is very simple phenomenon but its definition and precise calculation is technically challenging. Some definition presented salinity as presence of melted salt content in water. In most agricultural land of the world presence of salt in water and soil adversely affect the plant growth and decrease crop production. More than 76 M ha of the world's land has been salt affected as a result of human causes and this figure confirmed by [3] and [4]. Dubey [5] and Yeo [6] explained that salt in soil and water causes both osmotic and ionic and effects on plants. Ghoulam et al., [7] (2002) confirmed reduction in plant growth as general response of plant to salinity in their studies. Photosynthesis is a major process responsible for growth and improvement of plants and most of the stresses affect this process and ultimately reduce growth of plant [5, 8].

Potassium supply to plant in the form of foliar application is an efficient method to avoid interaction with essential macro and micro elements both antagonistically and synergistically. This method also acts as supplemental nutrient management practice under any stress condition where potassium uptake from soil becomes reduce. So this method may act as possible management tool to alleviate reduce yield under potassium deficiency under saline soil and saline irrigation. This nutrient play major role in biochemistry and other processes of plants such as leaf movements, opening and closing of stomates and other plant tropisms that are driven by potassium generated potentials [9, 10].

Brassica juncea (L.) Czern. belongs to the Cruciferae (old name Brassicaceae) family which is also known as the mustard family and with its unclear origin. Spect and Diederichsen [11] support the view that *B. juncea* probably evolved somewhere between Eastern Europe and China, where the progenitor species are sympatric. Shape of the flowers that have four obliquely opposed petals forming a cross is the main sign to give this family the name Crucifer. Fertilization of ovules normally done by self-pollination, although inter-plant out crossing rates of 20-30% have been reported [12]. This family is used for the treatment of muscular, skeletal and bronchitis pains. Members of this family stimulate circulation in pain area and help to relieve pain. The seeds of most of the members are used for tumor treatment in China. The main purpose of such researches is to use such salts to improve growth and productivity of crops under saline soil and saline irrigation water. So, this research was undertaken with the aim to observe the effects of foliar application and different concentration of nutrients (KNO_3) on growth and biochemical aspects of *Brassica juncea* plant grown under different sodium chloride concentrations.

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MATERIAL AND METHODS

Seeds of *Brassica juncea* were obtained from Agriculture Research Institute Tarnab, Peshawar, Khyber Pukhtunkhwa. This experiment was comprised of 36 pots which were divided into three sets.

1st Set: Without potassium, comprising of non-saline control and three salinity treatments of (50, 100 and 150 ppm NaCl irrigation).
2thSet: Potassium provided as potassium nitrate @ 2%, the set comprises of non-saline control and three salinity treatments of (50, 100 and 150 ppm NaCl irrigation.
3th Set: Potassium provided as potassium nitrate @ 4%, the set comprises of non-saline control and three salinity treatments of (50, 100 and 150 ppm NaCl irrigation).

Plastic pots used in this experiment were of 17.5 cm in diameter and 6.5 cm deep having basal outlet for drainage. Out of 36 pots 12 pots present in each set and 3 replicates were maintained for each treatment i) control (non-saline), ii) 50 mM, iii) 100 mM and iv) 150 mMNaCl concentrations. Every pot was filled with 1 Kg of thoroughly washed sandy loam soil. Soil in each pot was saturated with full strength Hoagland's solution. Approximately uniform size and equal number of seeds were surface sterilized with 0.1% $HgCl_2$ for 1 minute and then washed with distilled H_2O. 5 seeds were sown in each pot. Pots were then daily irrigated with an equal amount i.e., 50 ml of tap H_2O. When seedlings were reached at 3 leaves stage they were thinned out as one seedling per pot. 36 pots were then arranged in a completely randomized design (CRD) in the Botanical Garden of Department of Botany, Abdul Wali Khan University, Mardan. NaCl treatment was started at this stage and concentrations of NaCl were gradually increased in irrigation water till it reached to the desired salinity of each treatment. Each pot was irrigated with 1.5L of tap water / NaCl solution twice a week. When sodium chloride salinity was maintained in pots different concentrations of potassium nitrate and were applied foliarly in different sets.

GROWTH ANALYSIS

At termination of experiment plant harvested and height of plant, root length, number of leaves, fresh and dry biomass, number of pods per plant was recorded.

BIOCHEMICAL ANALYSIS

Chlorophyll content:

Chlorophyll concentration (Chl) was determined in fresh leaves following the protocol of Maclachlam, and Zalik, [13].

Reducing Sugars Estimation:

The reducing sugars were estimated by employing arsenomolyb date reagent[14] for cuprous oxide determination formed in oxidation process of the reducing-sugars by alkaline cooper tartarate reagent.

Total Carbohydrate Content:

Determination of total carbohydrate was carried out according to modified anthrone reagent [15].

Analysis of Total Protein:

Total protein was extracted and analyzed by Bradford method [16].

Total Phenol Estimation:

The total phenols present in leaves were determined according to the method described by Malik and Singh [17].

Statistical analysis

In present project experiment was maintained with three 3 salt levels and 3 replicates. Experimental design was completely randomized Design (CRD). Data collected and analyzed statistically by using SPSS version 21 for analysis of variance (ANOVA) and the means compared by Duncan's multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

Plant height

Brassica juncea plant height markedly decrease ($P<0.0001$) under different salinity regimes which correspond with the findings of [18] (Figure 1). Munns [19] explained that increase in salinity cause decrease in plant height and common cause of this reduction is the accumulation of toxic ions in cells which adversely affect cell division and expansion. Foliar application of KNO_3proved to be very effective and showed significant ($P<0.05$) increase in plant height in this study and showed resemblance with the result of [20], who observed the same phenomenon in pepper plants.

Root length

Root length in present investigation showed marked and significant (P<0.0001) decrease in salt stressed *Brassica juncea* plants (Figure 2). Asif *et al.,*[21] observed significant reduction in root length as a result of salt concentrations increase as well as significant difference in absolute and relative root lengths of maize accession. Exogenous application of KNO_3had synergetic effect in terms of significant(P<0.0001) increase in root length. Our observations showed resemblance with the result of Jason and Menzies[22] who observed and stated that when potassium was applied, there was no significant difference in the plant uptake. They also explained that applied treatment had a smaller root length and a lower root length density in the concentrated soil section than the other treatments.

Number of leaves

Number of leaves under salinity was significantly (P<0.0001) reduced when compared with control plants (Figure 3). Cuartero *et al*., [23] observed measures of ion contents and water relations in their study and stated that highest heritability exhibited in leaf area showed as compared to shoot dry weight. Yasar *et al.,*[24] also observed that application of elevated salt treatment showed significant decrease in leaf area of tomato leaves. Foliar application of different levels of KNO_3exhibited marked improvement and showed significant (P<0.0001) increase in number of leaves. This phenomenon was previously studied by Hegazi *et al.,*[25] and stated that potassium content in leaves increased markedly when potassium nitrate applied foliarly.

Number of fruits

Salt presence in saline irrigation water caused a significant (P<0.0001) decrease in number of fruits (Figure 4). Navarro [26] observed the same phenomenon and stated that increased salinity caused significant decrease in fruit size of plants. Salt stress cause reduction in fruit size and ultimately reduces total yield of a plant[27]. Values of above mentioned parameter were enhanced due to foliar application of KNO_3. Our observation showed resemblance with the result of Bhuvaneswari *et al*., [20] who observed non-significant enhancement in fruit number/plant with the increase of potassium application. Potassium fertilizer application is responsible for highest fruit number/plant [28].

Biomass

Salinity exhibited significant (P<0.0001) and considerable reduction in both fresh and dry weights of plants (Figure 5-6). Misra and Dwivedi[29]observed the same finding as did in our experiment length, fresh and dry biomass showed decrease as salt concentrations increased in the experiment. Misra and Dwivedi [29] also observed remarkable decrease in germination of seed and ultimately pronounced reduction in seed vigor and growth of mung bean Foliar application of KNO_3partially mitigate salt effect and showed significant (P<0.0001) increase in biomass in our observations. Salt stress exhibited non-significant reduction in leaf area, fresh and dry weight of leaves of plants. Fresh and dry biomass of leaves and leaf area of both the plants was markedly promoted by foliarly appliedKNO_3 irrespective to the plant growth under non saline or saline conditions [30].

Total chlorophyll

Plant pigments (chl a, chl b and total chlorophyll) were significantly (P<0.0001) decreased in *Brassica juncea* plants due to salinity (Figure 7-10). Our observations having similarity with the findings of Mitra and Banerjee [31] they observed chlorophyll content decrease under high salinity. According to Iyenger and Reddy [32] the reason for decreased chlorophyll content under high salinity might be changes in protein lipid ratio of pigment-protein complexes. Plant pigments were generally increased after exogenous application of KNO_3 in our observation. Mohsen Kazemi[33] observed potassium application caused significant (P<0.001) increase in chlorophyll of tomato leaves as compared to control. These findings have similarity with the previous investigations by different researchers[34, 35]. Potassium affects chlorophyll development was studied [36]. Our observations are in correspondence with the findings of Dhanapackiam and Ilyas[37]. Chlorophyll a content is predominated over chlorophyll b content which is clear from the results. Another phenomenon confirmed that chlorophyll a/b ratio reduced under increasing salt concentrations. It is reported that causes of chlorophyll content decrease under salt stress are different and it may be due to deterioration of membrane contents[38].KNO_3 showed non-significant increase in chlorophyll in our observation that show resemblance with the findings of Mitra and Banerjee[31], who observed that potassium application caused a non-significant increase in chlorophyll a:braio.

Carotenoids

Salt stress cause marked and significant (P<0.0001) reduction in carotenoids in *Brassica juncea* plants (Figure 11). Our observations are in correspondence with the findings of Mitra and Banerjee, [31] who observed significant decrease in carotenoids under increasing salinity. The reduction of the different pigments with aquatic salinity is significant when analyzed statistically. Exogenously applied varying levels of KNO_3slightly

enhance the carotenoids in plants. Aslam et al., [39] stated that carotenoids of different lines of wheat were also promoted after foliar application of potassium with different concentrations.

Carbohydrates

Salinity regimes in the root zone caused significant (P<0.0001) decrease in carbohydrates in *Brassica juncea* leaves (Figure 12). Our observations are in correspondence with the findings Wttana and Maysaya [40] who observed that carbohydrates composition were differently altered by salinity stress. Significant (P<0.05) improvement in total carbohydrates with enhanced levels of exogenously applied $KNO_3$was observed in present investigation. Effects of different nutrients (e.g. Mg, K and Ca) deficiencies on carbohydrate metabolism has been investigated in Citrus leaves by Ruth *et al*., [41] and under their observations K nutrient deficiency strong effects as compare to other nutrients. Its deficiency results less starch and more soluble sugars.

Reducing sugar and non reducing sugar

Values of reducing and non-reducing sugars were significantly (P<0.0001) increased in leaves of *Brassica juncea* under salt stress (Figure 13-14). Beltrano[42]studied rice leaves and observed significant influence on the reducing and non-reducing sugars under different salinity levels. Polynomial response was observed in reducing sugars with increasing salinity. Exogenous application of KNO_3partially alleviate deleterious effect of salt and showed non-significant promotion in reducing sugar and non-reducing sugar indicated by improvements under both non-saline and saline conditions. Application of nitrogen through soil showed better results because application of high amount of nitrogen fertilizers reduced the reducing sugar and non-reducing sugars or sometimes it exhibited nearly similar values as compared to those applied with low concentrations of nitrogen fertilizer. Another study on spinach plants [43] reported that application of low concentration of nitrogen showed increased concentrations of total sugars and reducing sugars.

Total protein

The values of total proteins in the leaves of *Brassica juncea* showed significant (P<0.0001) decrease under different salt regimes (Figure 15). Our observations are in correspondence with the findings of Rajakumar [44] who observed that after increasing salinity plant showed reduced protein content. It is also observed that at low concentrations of sodium chloride plants exhibited higher protein content. Protein content significantly decreased in plant treated in different concentration of sodium [45]. Foliar treatment of KNO_3had a small positive effect and showed non- significant increase in protein in present investigation. Barneix and Causin, [46] stated that generally NO_3^- assimilation in plants formed plant proteins. In both sunflower and safflower leaves soluble protein concentrations decreased under high salinity. Reasons for this phenomenon may be i- change in the balance between proteins and soluble aminoacids, ii- Increased breakdown of proteins through proteolytic process. Different researchers [47, 48, 49, 50] also reported this phenomenon in different plant leaves that high salinity impose reduction in protein contents. Foliar application of potassium nitrate showed increase in soluble protein concentrations in plants grown under normal and saline conditions.

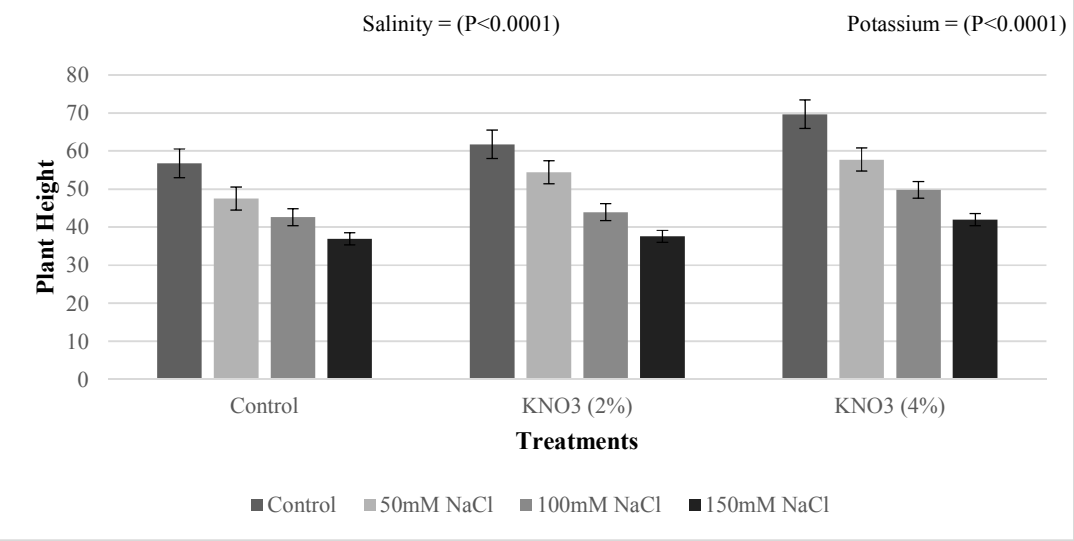

Figure 1. Effect of potassium on plant height (cm) of *Brassica junceae* grown under different concentrations of sodium chloride.

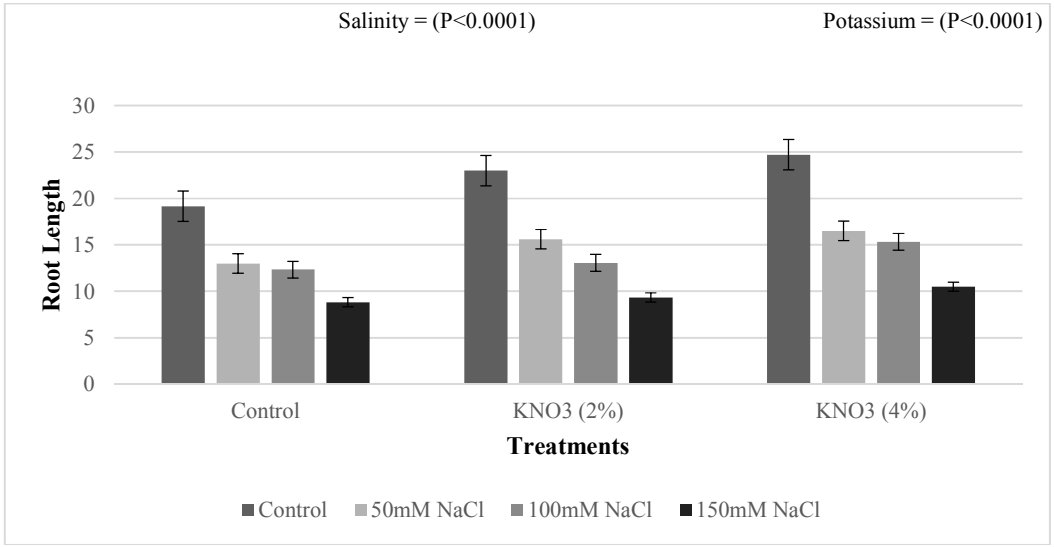

Figure 2. Effect of potassium on root length (cm) of *Brassicajunceae* grown under different concentrations of sodium chloride.

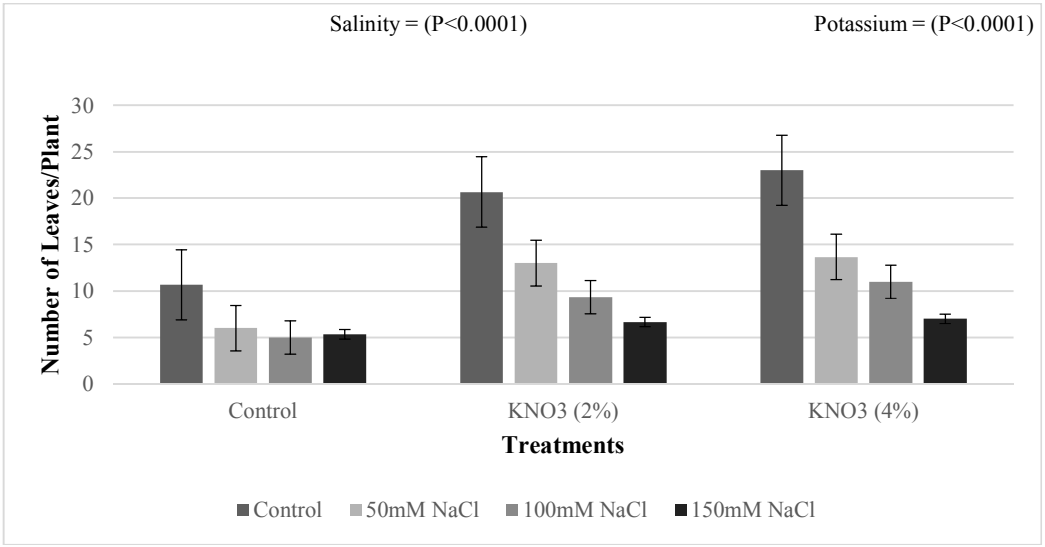

Figure 3. Effect of potassium on number of leaves/plant of *Brassica junceae* grown under different concentrations of sodium chloride.

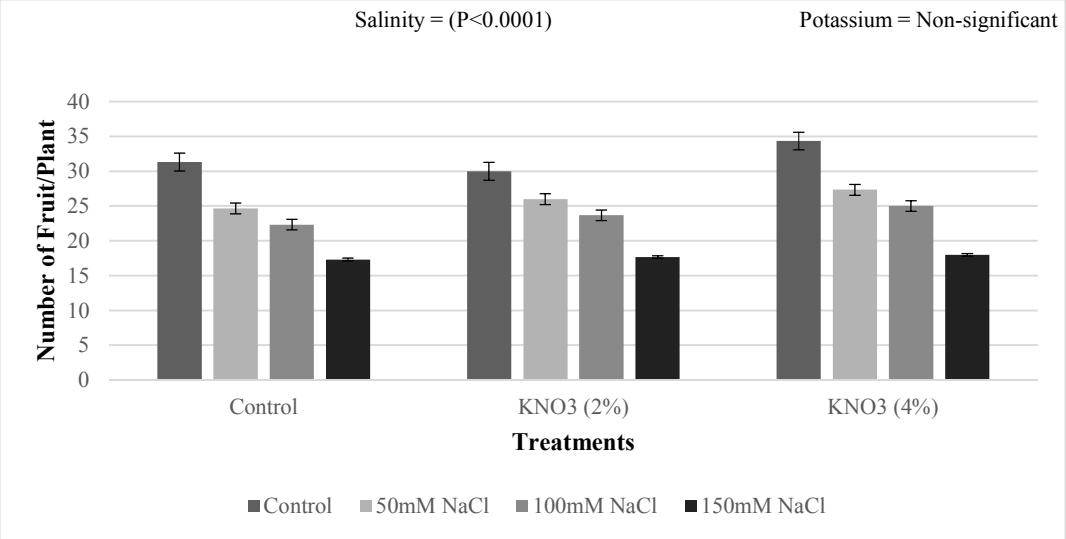

Figure 4. Effect of potassium on number of fruits/plant of *Brassica junceae* grown under different concentrations of sodium chloride

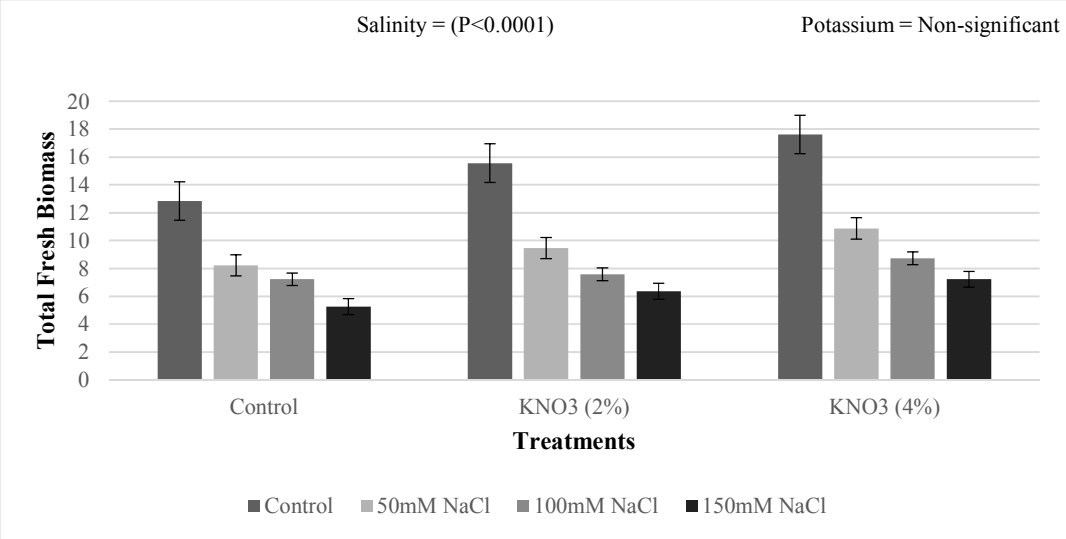

Figure 5. Effect of potassium on fresh biomass (gms) of *Brassica junceae* grown under different concentrations of sodium chloride.

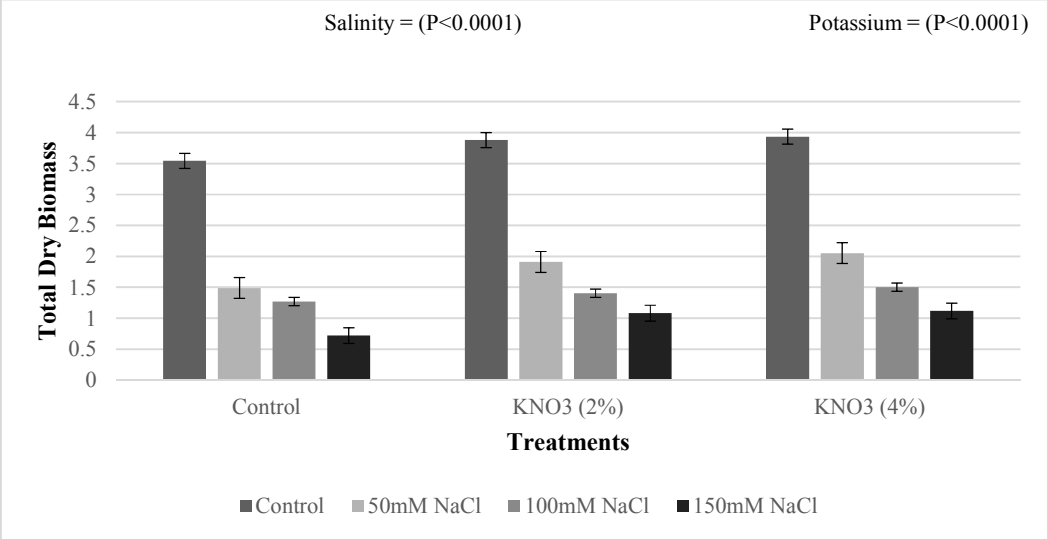

Figure 6. Effect of potassium on dry biomass (gms) of *Brassicajunceae* grown under different concentrations of sodium chloride

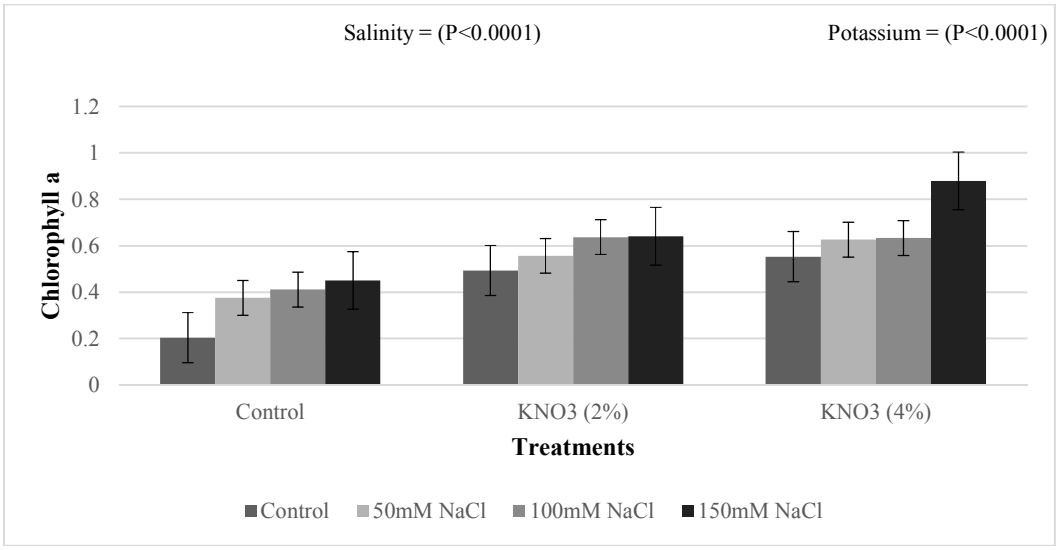

Figure 7. Effect of potassium on chlorophyll a (mg/gmfr.Wt) of *Brassicajunceae* grown under different concentrations of sodium chloride.

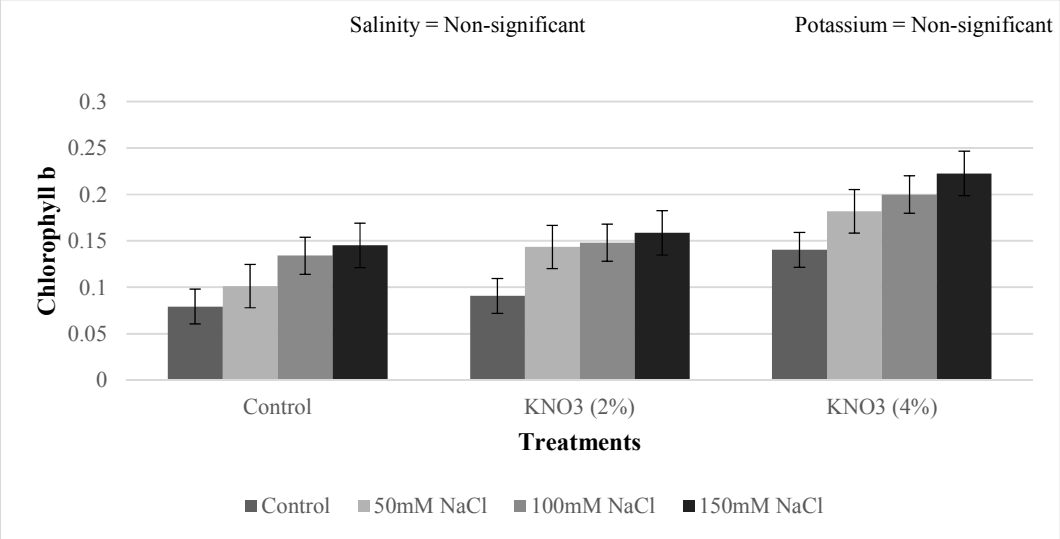

Figure 8. Effect of potassium on chlorophyll b (mg/gmfr.wt) of *Brassicajunceae* grown under different concentrations of sodium chloride.

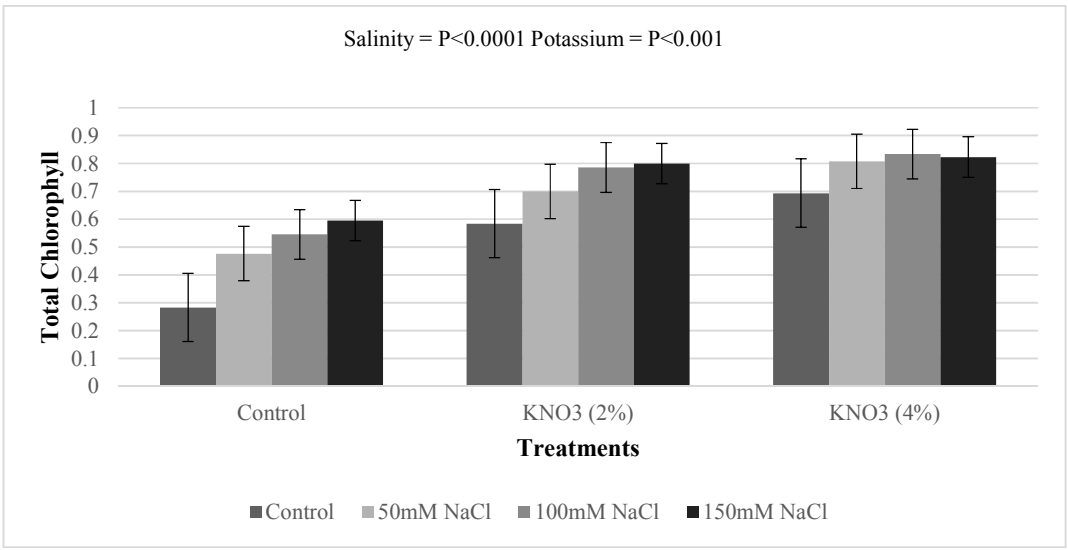

Figure 9. Effect of potassium on total chlorophyll (mg/gmfr.wt) of *Brassicajunceae* grown under different concentrations of sodium chloride.

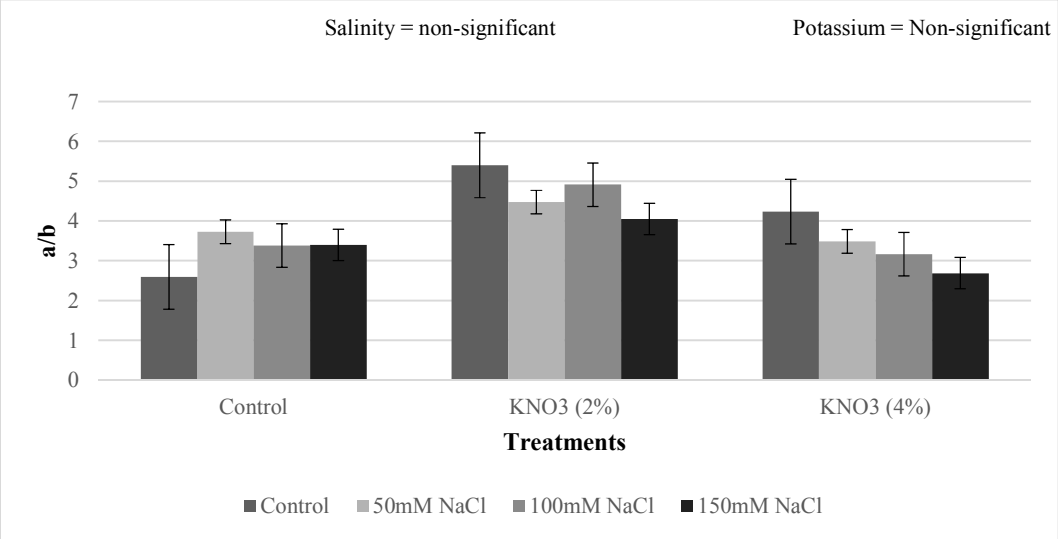

Figure 10. Effect of potassium on chlorophyll ab ratio of *Brassicajunceae* grown under different concentrations of sodium chloride.

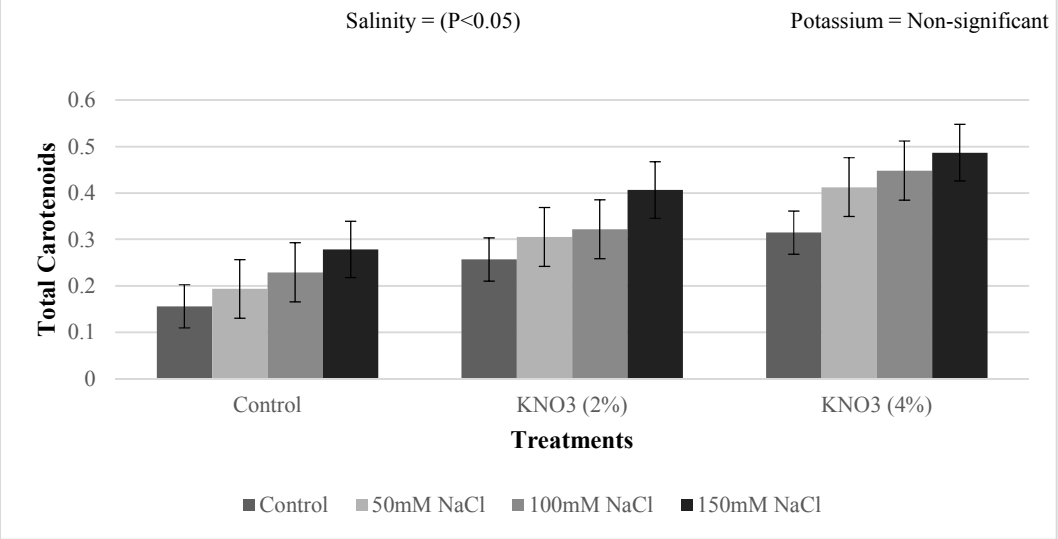

Figure 11. Effect of potassium on total carotenoids (mg/gmfr.wt) of *Brassicajunceae* grown under different concentrations of sodium chloride.

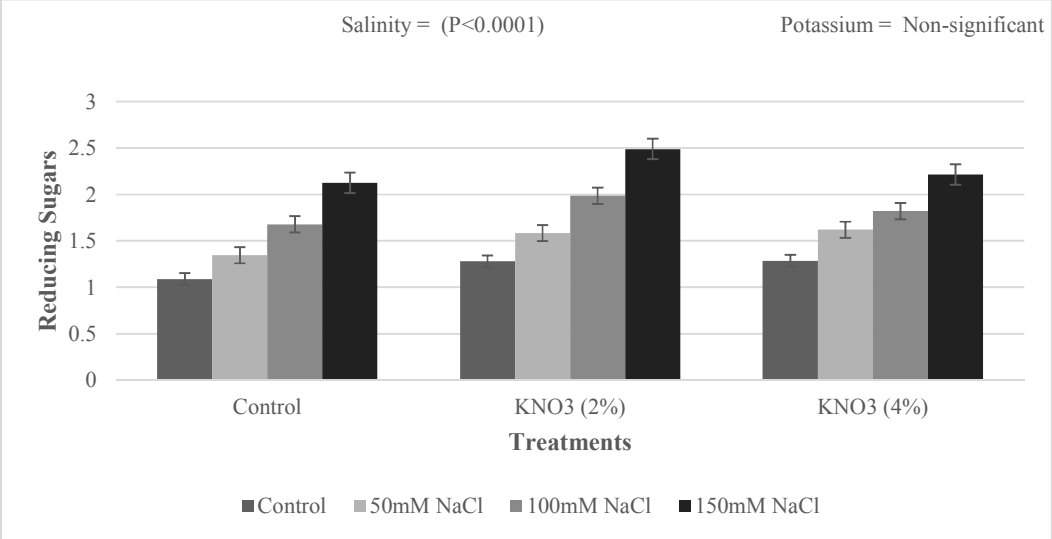

Figure 12. Effect of potassium on reducing sugars (mg/gmfr.wt) of *Brassicajunceae* grown under different concentrations of sodium chloride.

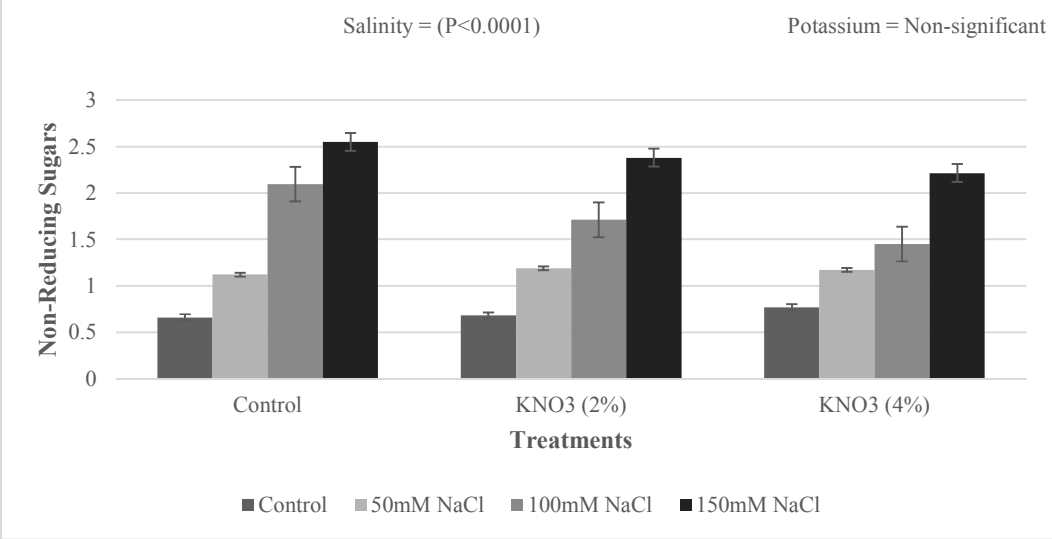

Figure 13. Effect of potassium on non-reducing sugars (mg/gmfr.wt) of *Brassicajunceae* grown under different concentrations of sodium chloride.

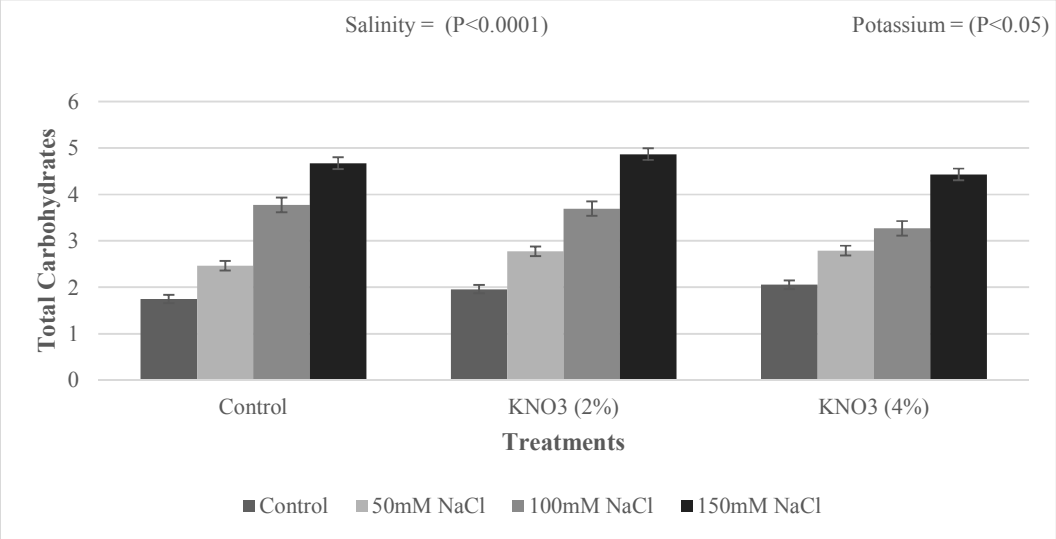

Figure 14. Effect of potassium on total carbohydrates (mg/gmfr.wt) Of *Brassicajunceae* grown under different concentrations of sodium chloride.

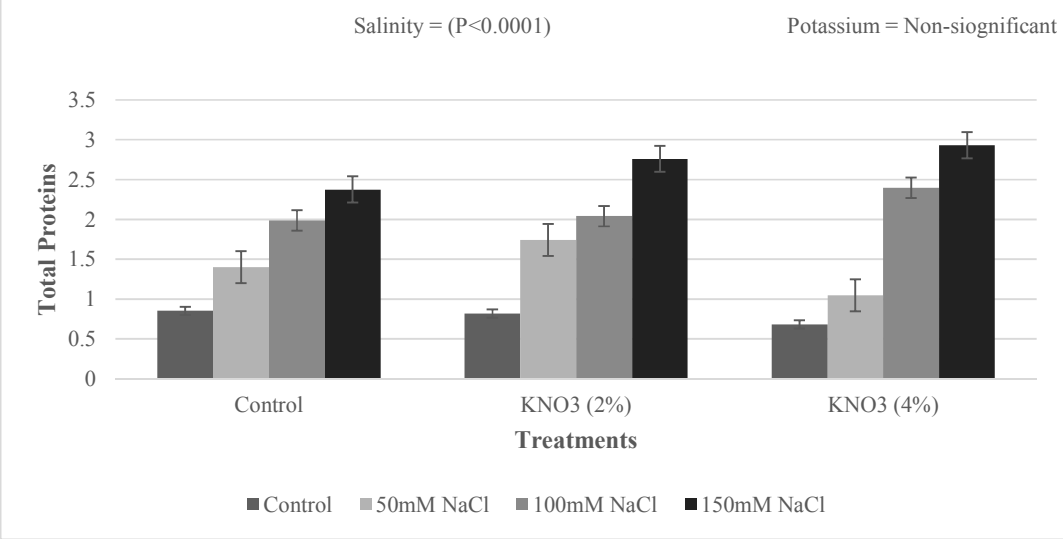

Figure 15. Effect of potassium on total proteins (mg/gmfr.wt) Of *Brassicajunceae* grown under different concentrations of sodium chloride.

CONCLUSION

Brassica junceae plant showed reduced growth under salt stress condition and increase in potassium concentration from 2% to 4% counteracted the deleterious effect of salinity when applied foliarly.

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