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Foliar Application of Potassium Nitrate Affects the Growth and Nitrate Reductase Activity in Sunflower and Safflower Leaves under Salinity

Nusrat JABEEN*, Rafiq AHMAD

*University of Karachi, Department of Botany, Biosaline Research Laboratory, Karachi, Pakistan; jabeennusrat@yahoo.com (*corresponding author)*

Abstract

Effect of foliar application of KNO_3 on growth and the activity of nitrate reductase were studied in the leaves of sunflower (*Helianthus annuus* L.*)* and safflower (*Carthamus tinctorius* L.) plants growing under different levels of salinity. The seeds were sown in pots under non saline condition and saline water irrigation was started at three leaf stage after germination. Different concentration of saline water (i.e. 0.3% and 0.6%, equivalent to an EC of 4.8 and 8.6 dS/m respectively) were made by dissolving sea salt per litre of tap water. Nutrient solution of KNO_3 was sprayed at the rate of 250 ppm. The concentration of Na^+ and Cl^- rapidly increased in the leaves of both the plants under salinity stress. In contrast the nitrate (NO_3^-) and soluble protein concentration were decreased with the increasing salinity. Salinity reduced leaf area, its fresh and dry weight per plant and also inhibited the activity of Nitrate reductase (NRA) enzyme. The application of KNO_3 significantly reduced the increasing tendency of Na^+ and Cl^- and increased leaf area, its fresh and dry weight per plant, NO_3^- and soluble protein concentration and NR activity in leaves irrespective to the growth of plant under non saline or saline conditions.

Keywords: foliar spray, growth, nutrient solution, nitrate reduction, salinity stress

Introduction

The availability of good quality water is one of the major limiting factors for plant growth as irrigation water may often contain salts and ions that can have negative impacts on the plant growth and development. Salt water in the root zone induces osmotic changes and directly affect nutrient uptake as Na^+ reducing K^+ uptake or by Cl^- reducing NO_3^- uptake (Cornillon and Palloix, 1997; Halperin *et al.,* 2003). Besides limiting the acquisition of nitrate by roots salinity may also restrict the ability of plants to reduce and assimilate nitrogen (N), as a result of the inhibition of the activity of enzymes involved in N metabolism. The cytosolic NADH nitrate reductase (NR; EC 1.6.6.6), the first enzyme in the pathway of nitrate assimilation (Flores *et al.,* 2002), is one of the enzymes whose activity has been shown to decline in salt stressed leaves of various plant species, including *Sorghum vulgare* (Rao and Ganaham, 1990), bean and cotton (Gouia *et al.,* 1994), maize (Abd-El Baki *et al.,* 2000), *Prosopis alba* (Meloni *et al.,* 2004), sunflower (Azedo-Silva *et al.,* 2004) and tomato (Debouba *et al.,* 2007). The information regarding the effect of salinity on N uptake and assimilation are limited and controversial (Viegas *et al.,* 1999). Stimulation in nitrate reductase activity (NRA) has been reported in *Phaseolus aureus* (Misra and Dwiverdi, 1990) and in annual ryegrass (Sagi *et al.,* 1997) under salt stress condition. These contradictory results highlight the need to pursue the investigation on the effects of salt stress on leaves NR activity, extending these studies to a wider number of species.

Nitrates is one of the major sources of N, taken up by roots of higher plant, translocate to the shoot, store in vacuole and assimilate into reduced N products. The processes of nitrate uptake, translocation, and assimilation are interdependent and closely regulated in higher plants (Huber *et al.,* 1996; Sivasankar and Oaks, 1996). An increase in the amount and activity of nitrate reductase leads to a corresponding increase in the potential for nitrate reduction and confers a greater capacity for amino acid synthesis, protein synthesis, or total N assimilation (Barneix and Causin, 1996; Lopez-Cantarero *et al.,* 1997). Nitrate availability, growth regulators, light, and other physiological and environmental parameter are the factors which effect the regulation of nitrate assimilation (Crawford 1995; Lillo, 1994; Ruiz *et al.,* 1998). Nitrate uptake and transport appear sensitive to salinity and modification in nutrient supply can alleviate the negative effect of salinity. It is well known that exogenous application of NO_3^- may increase the nitrate content of salt stressed plant and improves the tolerance of plant to salinity to various extents (Ebert *et al.,* 2002; Kaya and Higgs, 2003). Foliar application of KNO_3 was found to increase nitrate content of ryegrass leaves under non-saline or saline condition (Tabatabaei and Fakhrzad, 2008) and NR activity and protein content in tomato and maize (Maritinez and Cerda ,1989; Panday, 2000).

In the present study, the effect of foliar application of KNO_3 was assessed on growth, amount of nitrate, its reduction and assimilation in the leaves of sunflower and safflower subjected to different levels of salinity.

Materials and methods

Plant material and culture conditions

A moderately salt tolerant variety of sunflower (*Helianthus annuus* L. cv 'NuSun 636') and safflower (*Carthamus tinctorius* L. cv 'Spiny 321') were sown in plastic pot. These pots were 0.28 m in diameter, and 0.30 m deep, having basal holes for leaching irrigation water, filled with 20 kg of sandy loam and cow dung manure (9:1) having pH 7.4 dS/m. The air temperature and relative humidity throughout the growing period was 25-32°C and 60-80% respectively. Nitrogen, Phosphorous, Potassium (NPK) ratio in fertilizer was given 4:3:2 through urea, diammonium phosphate (DAP) and sulphates of potash (SOP) for sunflower as recommended by Nawaz *et al.* (2003), which amounts to 0.744 g Nitrogen (N), 0.558 g Phosphorus (P) and 0.372 g Potassium (K) per pot, and 7:15:7 for safflower as recommended by Naik *et al.* (2007), which amounts to 0.217 g (N), 0.465 g Phosphorus (P) and 0.217 g Potassium (K) per pot, given at the time of sowing and at the time of flowering. A certain amount of micronutrients were given in soil vide Hoagland solution (Hoagland and Arnon, 1938) twice along with irrigation water.

It has been conducted two separate experiments for sunflower and safflower in November 2008 in a randomized complete block design with five replications. 30 pots, of each experiment, were divided in 02 sets comprising of 15 pots each. One set was of control (non spray) and other set was treated with KNO_3. Out of 15 pots of each set, 5 pots of each were subjected to following different levels of saline water irrigation.

a) Non saline water (control) (EC 0.5 dS/m)
b) 0.3% sea salt solution* (EC 4.8 dS/m)
c) 0.6% sea salt solution (EC 8.6 dS/m)

The seeds were sown in pots under non saline condition and saline water irrigation was started at three leaf stage after germination to get seedlings of equal size. After thinning only one seedling was kept in each pot for further work. They were irrigated with gradual increasing sea salt concentration weekly up to reaching the desired salinity levels of the experiment mentioned above. To maintain the required soil medium salt levels the EC of the soil medium was measured periodically by portable EC meter. Plants were sprayed with nutrient solution of KNO_3 at the time of seedlings establishment, grand period of growth and incipient of floral heads at the rate of 250 ppm. The calculated amount of K^+ and NO_3^- in their respective solution, applied to the plants through foliar application was 96.5 ppm, and 153.5 ppm respectively. Tween-20 (0.1%) was used as a wetting agent for each treatment. A volume 300 ml/plant, of the solution was sprayed on all pots with a manual sprayer. Spray was carried out between 09:00 and 11:00 AM. The plants were sprayed with solutions with uniform coverage until the leaves were completely wet and the solution ran off the leaves. At the time of spray other plants were covered with plastic sheet to prevent the contamination of sprayed nutrients. Control (non saline) plants were irrigated with 3.5 l of tap water and plants under saline treatments were irrigated with 3.5 l of their respective sea salt solution ensuring about 40% leaching.

Plant sampling and analysis

Leaves were sampled at flowering stage. Leaf samples were standardized by using only plants with the same size of fully expanded leaves almost from the middle part of each replicate plant. The material was rinsed with distilled water. Fresh leaf matter was used for NRA assay, proteins and nitrate analysis. A subsample was prepared for Na and K analysis according to Chapman and Pratt (1982), where the weighed plant sample were dry ashed in Muffle furnace at 550°C, then extracted by 2N HCl. Total Na and K content were determined through flame photometer (JENWAY PFP7). Chloride in samples was extracted in deionized distilled water and determined with a chloride analyzer (Corning, 925).

Measurement of vegetative characteristics

Leaf area, fresh and dry weight of leaves per plant was measured at the grand period of growth. Leaf area was measured with the apparatus AM-Licor 1300 (Lincoln, Nebraska, USA), after taking the fresh weight the leaves samples were oven-dried at 60°C for 72 hours and dry weight was recorded.

Nitrate (NO_3^-) determination

Nitrate in leaf tissue was determined by the method of Cataldo *et al.* (1975). 0.1 g of leaf disc was boiled for 10 minutes in 5 ml of distilled water. 0.2 ml of salicylic acid was added in 0.05 ml of extract. After incubation at room temperature for 20 minutes, 4.75 ml of 2N NaOH was added to solution. Final volume was made up to 5 ml. Absorbance was taken at 410nm.

Detection of in vivo Nitrate Reductase Activity (NRA)

NR (EC 1.6.6.6) activity was determined by the method of Silveira *et al.* (1998). Leaf disc from the second youngest fully expanded leaves (200 mg fresh mass) were infiltrated twice for two minutes with 5 ml of reaction mixture containing 100 mmol/l Potassium Phosphate buffer (pH 7.5) ; 25 mmol/l KNO_3 ; and 1% isopropanol. The reaction mixture was incubated at 35°C for 30 minutes in the dark. NR activity was estimated from the amount of NO_2^- formed during the incubation period and released from the leaf discs to the medium after boiling for 5 minutes. Aliquots were mixed with 2 ml of (1:1) 1% sulfanilamide in 2.4 mol /l HCl ; 0.02% N-1-naphtyl-ethylenediamine and the absorbance was taken at 540 nm.

Soluble proteins determination

Soluble protein concentrations were determined using Coomassie brilliant blue (Bradford, 1976) with bovine serum albumin (BSA) as a protein standard.

* Sea salt solutions for irrigation were prepared by adding required amount of sea salt in tap water per liter. Sea salt is available in crude form in market. About 4% of its concentration was found equivalent to concentration of salts in the water of Indian Ocean (Castro and Huber, 2005).

Statistical analyses

SPSS version 13 was used for data analysis. Data sets were subjected to two-way analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was used to measure least significant differences (LSD) between treatment methods and controls (Duncan, 1955).

Results and discussion

Effect of foliar application of KNO_3 on vegetative characteristics (leaf area, fresh and dry weight of leaves) of sunflower and safflower plant under different salinity levels is presented in Tab. 1. Leaf area, fresh and dry weight of leaves of both the plants was significantly reduced by the increasing of salinity. In sunflower, leaf area, fresh and dry weight of leaves were reduced by 37.6%, 48% and 51.5% and in safflower 66.9%, 47.3% and 51.8% respectively, at the highest salinity level (ECe:9.9dS/m) in comparison with non saline control. Leaf area, fresh and dry weight of leaves of both the plants was substantially increased by foliar spray of potassium nitrate irrespective to the plant growth under non saline or saline conditions. In sunflower, leaf area, fresh and dry weight of leaves were increased by 32%, 36.4%, and 43.4% and in safflower 31.3%, 41%, 43.1% respectively at the highest salinity level (ECe:9.9dS/m) in comparison with their respective non sprayed control.

The opposing effects of salinity on the plants growth are a serious problem reported by many workers (Greenway and Munns, 1980; Tester and Davenport, 2003). Ion toxicity and imbalanced nutrition in saline conditions are the main constraints for plant growth. The decline in leaf growth is an earliest response of the plants to salinity (Cramer, 2002; Munns and Termaat, 1986). Both the reduction in leaf growth and increasing of dead leaves at highest salinity level i.e. 0.6% sea salt solution, led to further reduction in leaf area. This may be caused by ions accumulation in the leaves, particularly old leaves (Greenway and Munns, 1980). Foliar supply of KNO_3 to the salt treated plants may reduce toxic ions uptake as well improve K and N status of salt treated plants. The role of potassium in ionic balance is reflected in nitrate metabolism (Jeschke and Wolf, 1985). Nitrogen being an active participant of chlorophyll and protein is an essential element for plant growth. Spray with potassium result an increase in leaf potassium content which was accompanied by increased rates of photosynthesis, photorespiration and RuBP carboxylase activity. Hence there was considerable improvement in growth even under saline strata in present investigation. Ebert *et al.* (2002) found that supplying of $Ca(NO_3)_2$ at 10 mM had a beneficial effect on growth and metabolism of NaCl treated guava seedlings. Akram *et al.* (2009) observed an improvement in growth of sunflower due to the foliar spray of K_2SO_4 and KNO_3 at 1.25% under saline concentration of 150 mM NaCl.

The data of leaf ion concentration of both the plants in relation to salinity and KNO_3 levels are presented in Fig. 1. Salinity in the root zone led to a significant decrease in K concentration and an increase in Na^+ and Cl^- concentration. Lacerda *et al.* (2003) reported high levels of Na^+ inhibited the K^+ concentration in sorghum plants which result an increased Na^+/K^+ ratio under salt stress. Mohamedin *et al.* (2006) reported in sunflower that high levels of external Na^+ interfere with K^+ acquisition by the roots and disturb the root membranes and alter selectivity. In present investigation foliar application of KNO_3 alleviate the toxicity of Na^+ by decreasing the chances of its accumulation in plant parts. The results of present investigation are in agreement with the findings of many workers in different plant species (Abdel-Rehman, 1999; Cha-um *et al.*, 2010; Sultana *et al.,* 2001; Tabatabaei and Fakhrzad, 2008) who found that nutrients were absorbed by the leaves when applied onto the shoot. Treated leaves contained higher element concentration compared to non sprayed plants even under saline condition.

In present investigation salt stressed resulted an increase in Cl^- concentration, about 72% increase was recorded in leaves of both the plants at the highest salinity

Tab. 1. Effect of foliar application of KNO_3 on vegetative characteristics (leaf area, fresh and dry weight of leaves) of sunflower and safflower plant under irrigation of different salinity levels

		Sunflower			Safflower		
Sea salt concentration (%)	Foliar spray treatment	Leaf area index (cm^2)	Leaves fresh weight (g)	Leaves dry weight (g)	Leaf area index (cm^2)	Leaves fresh weight (g)	Leaves dry weight (g)
0 (ECiw:0.5dS/m, ECe:1.8dS/m)	Control-1(non spray)	2894.4^{ab}	65.8^{ab}	13.9^{ab}	5065.2^{ab}	63.6^{ab}	13.7^{ab}
	KNO_3	3857.0^{a}	97.5^{a}	22.6^{a}	6968.0^{a}	102.8^{a}	22.2^{a}
0.3 (ECiw:4.8dS/m, ECe:6.1dS/m)	Control-2 (non spray)	2424.4^{ab}	52.5^{bc}	10.5^{b}	3569.8^{b}	50.5^{ab}	10.2^{b}
	KNO_3	3465.0^{b}	80.4^{b}	17.8^{ab}	5041.8^{ab}	82.8^{b}	17.2^{ab}
0.6 (ECiw:8.6dS/m, ECe:9.9dS/m)	Control-3 (non spray)	1806.0^{c}	34.2^{c}	6.74^{c}	1675.0^{c}	33.5^{c}	6.6^{bc}
	KNO_3	2652.0^{abc}	53.8^{bc}	11.9^{bc}	2438.8^{c}	56.7^{bc}	11.6^{ac}
LSD at level 0.05	Salt	16.082	1.339	0.561	15.503	1.495	0.815
	Spray	13.131	1.094	0.458	12.658	1.221	0.666
	Interaction (spray x salinity)	***	***	***	***	***	**

ECiw, electrical conductivity of irrigation water, ECe, electrical conductivity of soil extract, LSD: Least Significant Difference, ns: not significant, different letters indicate significant differences among treatments at 5% level of significance in Duncan's Multiple Range Test

level (i.e. ECe: 9.9dS/m) in comparison with their non saline control (Fig. 1) and a decrease of NO_3^- concentration in leaves of both sunflower and safflower plants (Tab. 2). This repression of NO_3^- was directly proportional to the increasing concentrations of salt. The decrease in NO_3^- concentration by salt treatment could be attributed to the disruption of root membrane integrity (Carvajal *et al.,* 1999), an inhibition of nitrate uptake (Parida and Das, 2004) and low NO_3^- loading into root xylem (Abd-El Baki *et al.,* 2000). The direct competition of chloride with nitrate may also inhibited the uptake of nitrate by nitrate transporters (Deane-Drummond, 1986) or nitrate transporters may be inactivated by the toxic effects of salt ions (Lin *et al.,* 1997) which results higher accumulation of chloride accumulation in leaves. The observed changes in foliar nitrate were negatively correlated with chloride concentration in both sunflower (r^2=0.852, NO_3= - 0.219 × [chlorine] + 31.00) and safflower (r^2 + 0.864, NO_3= -0.352 × [chlorine] + 28.82). The concentration of Cl^- was significantly reduced when KNO_3 nutrient solution was supplied through foliar spray. This negative relationship between NO_3^- and Cl^- has been previously reported by Kafkafi *et al.* (1992) and Pearez-Alfocea *et al.* (1993). Foliarly supplied NO_3^- through KNO_3 decreased Cl^- concentration and offset its toxic effects, thereby lessening growth inhibition (Bar *et al.,* 1997). Tabatabaei *et al.* (2004) also reported that the decreased of nitrate is accompanied by a high chloride uptake and low rate of xylem exudation in high osmotic condition either by salts or other nutrients. It leads to reduced concentration of nitrate in leaves, consequently reducing NR activity of leaves under salinity conditions.

It appeared from the present results of both sunflower and safflower plant that increasing levels of salinity induced a substantial decline in NRA (Tab. 2). The reduction of NRA in leaves, under conditions of restricted nitrate flux induced by salt stress, could be due to the enzyme degradation/inactivation and the reduction in gene expression and NR protein synthesis (Ferrario *et al.,* 1998). Ferraio *et al.* (1998) suggested that the reduction in NR mRNA levels is related to lower levels of NO_3^- and glutamine in leaves. Salt reduced NO_3^- fluxes from roots to leaves and impaired the NRA in leaves (Debouba *et al.,* 2007; Foyer *et al.,* 1998). Cramer and Lips (1995) indicated that salinity may control NRA through nitrate uptake since NRA is largely determined by nitrate flux into the metabolic pool. As NR is highly regulated enzyme, its activity being dependent on several internal signals and nitrate is the first signal that induces that transcription of NR genes (Crawford, 1995; Kaiser *et al.,* 2002). Similar reduction under salinity in NO_3^- and NRA was also reported in leaves of olive trees by Tabatabaei (2006), in tomato by Debouba *et al.* (2007), in algarrobo by Meloni *et al.* (2004) and in soybean by Moussa (2004).

Application of foliar mineral, KNO_3 significantly increased nitrate content and NR activity in sunflower and safflower plants irrespective to their growth under non saline or saline conditions. The minimizing effects of the activity of nitrate reductase under salinity were offset by foliar application of mineral under various extant in present investigation. The observed changes in foliar NRA induced by both salinity and foliar spray were linearly correlated with the concomitant variation in foliar nitrate content in both sunflower (r^2 = 0.952, NRA= 0.238 + 0.364 × [nitrate]) and safflower (r^2 = 0.916, NRA= 0.738 + 0.204 × [nitrate]). Tabatabaei and Fakhrzad (2008) found in perennial ryegrass that 0 to 10 mM KNO_3 in the solution applied through soil or foliar increased NO_3^- concentration in leaves irrespective to plants growth under non saline or saline conditions. Maritinez and Cerda (1989) observed

Tab. 2. Effect of foliar application of KNO3 on amount of nitrate, nitrate reductase activity and soluble proteins of sunflower and safflower plant under irrigation of different salinity levels

		Sunflower			Safflower		
Sea salt concentration (%)	Foliar spray treatment	Nitrate µmole (g. f.w)$^{-1}$	Nitrate reductase activity µmole NO_2^- (g. f.w.hr)$^{-1}$	Soluble proteins mg/g f.wt	Nitrate µmole (g. f.w)$^{-1}$	Nitrate reductase activity µmole NO_2^- (g.f.w.hr)$^{-1}$	Soluble proteins mg/g f.wt
0 (ECiw:0.5dS/m, ECe:1.8dS/m)	Control-1 (non spray)	25.6^{ab}	9.42^{a}	25.8^{ab}	24.0^{ab}	5.45^{ab}	11.70^{ab}
	KNO_3	29.4^{a}	10.95^{a}	29.2^{a}	27.1^{a}	6.30^{a}	13.26^{a}
0.3 (ECiw:4.8dS/m, ECe:6.1dS/m)	Control-2 (non spray)	23.5^{b}	8.71^{b}	24.0^{ab}	19.8^{b}	4.65^{b}	10.00^{b}
	KNO_3	27.5^{ab}	10.29^{ab}	27.9^{b}	23.0^{ab}	5.49^{ab}	11.90^{ab}
0.6 (ECiw:8.6dS/m, ECe:9.9dS/m)	Control-3 (non spray)	18.7^{c}	7.10^{c}	19.0^{c}	13.5^{c}	3.48^{c}	7.33^{c}
	KNO_3	22.5^{bc}	8.50^{bc}	23.3^{abc}	15.8^{c}	4.10^{bc}	9.10^{bc}
LSD at level 0.05	Salt	0.544	0.328	0.179	0.436	0.297	0.174
	Spray	0.769	0.464	0.253	0.617	0.420	0.247
	Interaction (spray x salinity)	ns	ns	*	ns	ns	ns

ECiw, electrical conductivity of irrigation water, ECe, electrical conductivity of soil extract, LSD: Least Significant Difference, ns: not significant, different letters indicate significant differences among treatments at 5% level of significance in Duncan's Multiple Range Test

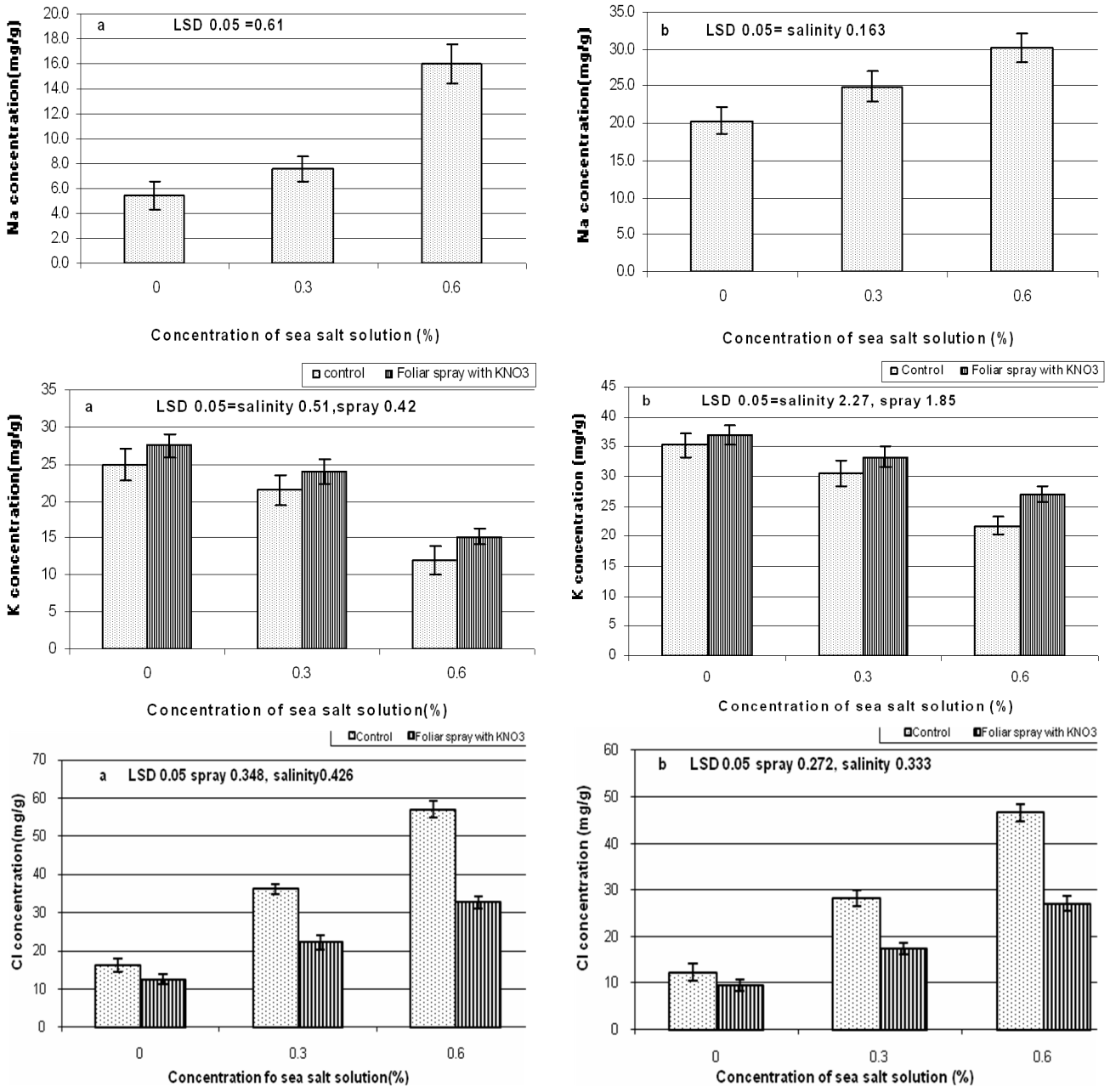

Fig. 1. Effect of salinity and foliar spray of nutrient solution of potassium on K and Cl concentration in sunflower (a) and safflower (b) leaves. ECiw: 0.5 dS/m, ECe: 1.8d S/m (non saline); ECiw: 4.8 dS/m, ECe: 6.1 dS/m (0.3% sea salt solution); ECiw: 8.6 dS/m, ECe: 9.9 dS/m (0.6% sea salt solution). Vertical bars mean ± S.E. (n=3), LSD: Least Significant Difference; ECiw: electrical conductivity of irrigation water; ECe: electrical conductivity of soil extract

increased NR activity with the exogenous supply of NO_3^- in tomato and cucumber.

Proteins are generally the products of NO_3^- assimilation (Barneix and Causin, 1996). The soluble protein concentrations of leaves of sunflower and safflower decreased in salinity as compare to their respective control (Tab. 2) This decrease may be due to the change in the balance between soluble amino acids and proteins by salinity or high salinity may increase break down of protein by proteolytic process. Decrease in protein contents of leaves has been reported in many plants under salt stress irrespective of their salt tolerance (Ashraf and Fatima, 1995; Ahmad and Jabeen, 2009; Moussa, 2004; Parida and Das, 2005). The soluble protein concentrations increased with the foliar application of KNO_3 irrespective to the plant growth under non saline or saline conditions. It may be due to the direct involvement of K in several steps of translation process, including the binding of tRNA to ribosomes (Evans and Wildes, 1971). The exogenous application of KNO_3 is related to increased NO_3^- absorption, its reduction and assimilation (Ruiz and Romero, 1999).

Conclusions

The experiments clearly demonstrated the beneficial effects of KNO_3 application on growth, nutrients concentration, NRA and soluble proteins of sunflower and safflower plants, irrespective to their growth under non saline or saline conditions.

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