

Salt-Induced Damage Alleviation in *Tanacetum Balsamita* L. by Foliar Application of Dobogen Biofertilizer, Glucose and KNO_3

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Abstract

The effects of NaCl salinity stress and foliar application of KNO₃, glucose and Dobogen were tested on *Tanacetum balsamita*. The results showed the significant interaction effects of salinity and foliar sprays on chlorophyll a, K⁺, Na⁺, Mg²⁺, Fe²⁺, Zn²⁺, Mn²⁺ and Si content, K/Na ratio and total phenolic and flavonoid contents. The highest phenolic content was acquired with 100 mM salinity and foliar spray of Dobogen and glucose, 50 mM NaCl × KNO₃ application and 50 mM salinity × nonfoliar application. The highest K/Na ratio was observed in control plants and controls × KNO₃ and/or Dobogen application. The greatest Si content was recorded with controls × Dobogen and KNO₃ applications and no saline × no foliar control plants. Malondialdehyde, flavonoid and proline contents as well as catalase activity were influenced by the independent effects of treatments. Chlorophyll b and superoxide dismutase were affected by salinity. Total soluble solids and Ca²⁺ were influenced by foliar applications. Malondialdehyde and proline were the highest at 150 mM salinity. Salinity adversely affected the physiological responses of costmary. However, foliar treatments partially ameliorated the salinity effect, and the results would be advisable to the extension section and pioneer farmers.

Introduction

Costmary (*Tanacetum balsamita* L. from Asteraceae), a traditional medicinal plant of Iranian origin, has been in common endemic use as flavoring, cardiogenic and flatulence¹. Costmary is a volatile oil-bearing plant, and essential oil is a major source of flavoring in the food industry. The crop is under production in many parts of Iran and some European countries^{1,2}.

Salinity stress has been defined as the predominant abiotic stressor that limits the growth, development and productivity of plants by reducing the rhizosphere osmotic potential, ionic imbalances, oxidative stress, damage to the cellular membranes, photosynthesis impedance and/or a great increase in light-dependent respiration^{3,4,5}. Salinity tolerance is a complicated phenomenon controlled by a cluster of genes mediating several physiological and biochemical processes^{6,7}.

In general, the common way to compensate for nutrient shortages in soils is to use chemical fertilizers. However, several surveys demonstrate the deteriorative effects of continuous soil-based chemical fertilizers on ecosystems, with often negative impacts such as nitrate and heavy metal accumulation and eutrophication. Foliar application is beneficial in reducing chemical fertilizer inputs and more influential in meeting emergent plants and sometimes meeting long-term needs and hence promoting plant growth and development^{5,8,9}. Potassium is a major nutrient element with unsubstitutable roles in several vital physiological processes, such as stomatal movement, osmotic regulation, enzymatic activity, cell growth, water balance maintenance, carbohydrate translocation, membrane polarity & stability, and pH equilibrium, and plays chief roles in assimilate translocation in phloem^{10,11,12,13} and secondary metabolisms¹⁴. Currently, sugars are commonly used as growth regulators that mediate plant development and gene expression under stressful environments^{15,16}. Luo et al.¹⁵ noted that trehalose, a nonreduced disaccharide, had an antioxidant role, protected proteins and elicited genes involved in the detoxification process in response to environmental stress factors. Plants treated with salicylic acid (SA) were more tolerable to reactive oxygen species (ROS) deterioration under stressful conditions^{17,18}. In costmary, SA application greatly reduced the salinity effects from NaCl and CaCl₂ and improved the growth and productivity of plants². Therefore, resorting to protocols reducing the dangerous effects of salinity is of special interest. It seems that with the progressed precipitation declines and the coincident salinity incidence in most localities of Iran, the study of foliar applications with promising reagents under saline conditions to overcome salinity depressions is crucially important. Therefore, the aim of the present study was to evaluate i) salinity effects on costmary and ii) the foliar implementation of Dobogen biostimulant, KNO₃ and glucose on the growth and some physiological traits of costmary under salinity stressful conditions.

Material And Methods

This experiment was conducted during the spring and summer of 2019 at the Research Greenhouse of Azarbaijan Shahid Madani University, Tabriz, Iran. The greenhouse growing conditions were as follows: lightening period: 16:8 day and night, temperature regime, 30°C and 25°C day and night, and relative humidity of approximately 65±5% in the greenhouse.

Plant material and experimental setup. The homogeneous costmary (*Tanacetum balsamita* L.) rhizomes were provided by a commercial cultivation site in Azar-shahr County, Northwest Iran, in accordance with the relevant institutional and national guidelines and legislations. The rhizomes were divided into uniform sections (approximately 10 cm in size) and surface sterilized with sodium hypochloride (NaCl; 5% v/v) for 20 min followed by washing with distilled water. Rhizomes were planted in 5-liter pots containing medium-sized perlite. During the early establishing growth stage, the plants were nourished with half-strength Hoagland's nutrient solution. Afterwards, following a period of 3 weeks, salinity treatments were imposed. The salinity levels were 0, 50, 100 and 150 mM NaCl. Salinity began at 25 mM and then gradually increased to reach the final level within 10 days. Salinity levels were based on the high salinity damage record in Northwest Iran and previous studies⁵. To avoid salinity standup on the pot surfaces, the pots were regularly washed with tap water once every week. Fertigation was applied during the daytime adapted according to the plant needs and growth stage. The optimal pH of the nutrient solution (NS) was 5.8 and was recorded every day and adjusted accordingly by using H₂SO₄ (5% v/v). Following salinity application, the electrical conductivity (EC) of NS was 2.1 mS cm⁻¹ (0 mM NaCl), 6.0 mS cm⁻¹ (50 mM NaCl), 12.0 mS cm⁻¹ (100 mM NaCl), and 19.0 mS cm⁻¹ (150 mM NaCl). Foliar treatments were applied as sprays, and the solutions used included dH₂O, 2 g L⁻¹ KNO₃, 2 g L⁻¹ glucose and 2% Dobogen biofertilizer (Arman Sabz Adineh, Iran). Concentration levels were chosen based on preliminary experimentation and previous records (unpublished data from our lab). Dobogen contains 10% SA, 0.05% soluble boron and 0.005% soluble molybdenum. Foliar applications were applied twice during the plant growing period. The first application was just after salinity exposure, and the second foliar spray was 3 weeks later. One month after the second foliar treatments (at seven weeks of salt stress), the plant samples were taken for

further analysis. Every pot contained a single rhizome of 10 cm in length × approximately 1 cm in diameter. Each experimental unit consisted of two pots, and the treatments had three replications.

Fresh and dry weight of plants (biomass). The plants were harvested at the early flowering stage. The aboveground and belowground parts of the plants were separated, weighed, and air-dried until reaching a constant weight. The fresh and dry biomass of plant organs was recorded by a digital scale (BB141, Boeco, Germany). Furthermore, plant height, root dry weight (oven-dried until constant weight), petiole length and the length and width of leaves were recorded at the harvest time.

Chlorophyll content. Chlorophylls a and b were quantified by the method of Prochazkova et al.¹⁹ by a spectrophotometer (T80⁺, Beijing, China) at 645 and 665 nm. Leaf samples (0.5 g) were extracted by dimethyl sulfoxide (DMSO, Sigma Aldrich, Germany) in the dark for 4 h at 65°C, and the results were expressed in mg per g of fresh weight (mg/g Fwt).

Soluble solids content of the leaves. Soluble solid content (TSS) was quantified by a hand refractometer (Erma, Tokyo, Japan) from the extract obtained by squeezing the leaves, and the data are presented as °Brix.

Elemental composition. Leaves were dried at 75°C for 4 d, weighed, and ground in a Wiley mill to particles less than 0.42 mm. Subsamples (0.2-0.3 g) were acid digested (2 N HCl) and analyzed for nutrient content as described in Chrysargyris et al. (2016). The contents of sodium (Na) and potassium (K) were quantified by the flame photometric method (Corning, 410, England). The contents of magnesium (Mg), calcium (Ca) and iron (Fe) were measured by atomic absorption spectroscopy (Shimadzu, AA6300, Tokyo, Japan) as previously described by Honarjoo et al.²¹, phosphorus (P) by vanadate molybdate (Honarjoo et al., 2016) and nitrogen (N) content by the Kjeldahl method²¹.

Hydrogen peroxide and lipid peroxidation. The content of hydrogen peroxide (H₂O₂) was assessed according to Arjunan et al.²¹. Leaf tissue (0.2 g) was powdered in liquid N₂ and then ground in ice-cold 0.1% trichloroacetic acid (TCA) and centrifuged at 12000 *g* for 15 min. Aliquots (0.5 mL) of the supernatant were mixed with 0.5 mL of 10 mM potassium phosphate buffer (pH=7.5) and 1 mL of 1 M potassium iodide. The H₂O₂ concentration was evaluated using standards of 5 to 1000 µM H₂O₂, and a calibration curve was plotted accordingly. The absorbance of the samples and standards was measured at 390 nm, and the results were expressed as µmol H₂O₂/g fresh weight.

Lipid peroxidation was determined as described by Azevedo-Neto et al.²² in terms of malondialdehyde content (MDA). Leaf tissue (0.2 g) was homogenized in 0.1% TCA, and the extract was centrifuged at 12000 *g* for 15 min. The reaction mixture of 0.5 mL extract and 1.5 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA was incubated at 95°C for 30 min and then cooled in an ice bath. The absorbance was determined at 520 nm and corrected for nonspecific absorbance at 600 nm. The MDA amount was determined using an extinction coefficient of 155 mM/cm. The results were expressed as nmol of MDA/g fresh weight.

Superoxide dismutase (SOD) and catalase (CAT) activity. SOD activity was traced by recording the inhibition of nitroblue tetrazolium (NBT) photoreduction by the enzyme. The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 0.1 mM EDTA, 50 mM sodium carbonate, 12 mM L-methionine, 50 µM NBT, 10 µM riboflavin and 100 µL of plant sample extract in a final volume of 3.0 mL. SOD activity was recorded at 560 nm by a spectrophotometer. One unit (U) of SOD activity was defined as the amount of enzyme causing 50% inhibition of photochemical reduction of NBT (Alici & Arabaci, 2016). CAT activity was recorded by monitoring the absorbance decline at 240 nm as a consequence of H₂O₂ scavenging. The activity was expressed as units (U) of catalase, which caused an absorbance change of 0.001 per min. The reaction mixture contained 100 mM sodium phosphate buffer (pH 7.0), 30 mM H₂O₂ and 100 µL of the plant extract with a final volume of 3.0 mL²³.

Total phenolics and flavonoids content. A methanolic extract of plant tissue (0.5 g) was used to quantify the phenolic content by Folin-Ciocalteu reagent at 755 nm according to Kim et al.²⁴. The results were expressed as equivalents of gallic acid (Scharlau, Barcelona, Spain) per g of plant dry weight (mg of GAE/g dry weight).

Total flavonoids were assayed according to the aluminum chloride colorimetric method²⁴, and the absorbance was recorded at 510 nm. The content of total flavonoids is expressed as rutin equivalents (mg rutin/g dry tissue).

Proline content. The proline content was assayed according to the method of acid-ninhydrin and toluene at 520 nm, as described by Fedina et al.²⁵. The proline content was computed using a standard curve of proline, and the results were expressed as micrograms of proline per gram of plant fresh weight.

Essential oil extraction and analysis. Air-dried plant samples (50 g) were hydrodistilled by a Clevenger-type apparatus from the European pharmacopoeia for 3 hrs. The oils were dried over anhydrous sodium sulfate and kept in sealed airtight amber vials until analysis. The EO yield was measured (mL m⁻²), oils were analyzed by gas chromatography-mass spectrometry (GC/MS- Shimadzu GC2010 gas chromatograph interfaced with Shimadzu GC/MS QP2010 plus mass spectrometer), and the constituents were determined as described previously²⁶.

Experimental design and data analysis. The experiment was as factorial based on completely randomized design with three replications and each replication was a pool of two plant tissue samples as biological replications. Data were analyzed by MSTATC and SPSS (ver.15) and means were compared by Duncan's multiple range test at p≤0.05 and p≤0.01%. The graphs were drawn by Microsoft Excel, 2013.

Results And Discussions

Growth related traits. Root dry weight, petiole length and leaf length and width were affected by salinity (P≤0.01) (Table 1). Leaf length was increased in the control and 50 mM NaCl treatments (Table 2). Meanwhile, the top plant height, petiole length and leaf width were acquired by control plants, while salinity of

≥ 50 mM decreased those parameters (Table 2). Similar results were reported by Valizadeh-Kamran et al.²⁷ on *Lavandula stoechas* and by Chrysargyris et al.²⁸ in *Mentha spicata* grown under saline conditions. Moreover, a reduction in plant height due to salinity has been reported in *Solanum nigrum*²⁹ and *Tanacetum parthenium*². Other studies have shown negative salinity effects on yield and morphological traits as well as on plant height. The reasons ascribed to this are reduced photosynthesis, chlorophyll structural breakdown, diminished vital metabolite biosynthesis and the toxic effects of Na^+ and Cl^- , which all meaningfully reduce cell turgor and plant productivity^{3,4,30}.

Table 1

ANOVA for the effect of salinity (0, 50, 100 and 150 mM NaCl) and foliar applications (no foliar, KNO_3 , glucose and Dobogen) on the root and aerial parts dry weight, plant height, leaf length and width, petiole length, total soluble solid content and essential oil yield of *Tanacetum balsamita* plants grown hydroponically in perlite. ns, nonsignificant; *significant difference at $P \leq 5\%$, following two-way ANOVA.

Significance	Biomass DM	Root DM	Plant height	Leaf length	Leaf width	Petiole length	Total soluble solid content
Salinity (S)	ns	*	*	*	*	*	ns
Foliar (F)	ns	ns	ns	ns	ns	ns	*
S×F	ns	ns	ns	ns	ns	ns	ns

Total soluble solids content. Salinity effects were not significant on TSS levels (Table 1). In contrast, foliar treatments had significantly different impacts, and the lowest TSS content belonged to the no foliar treatment ($P \leq 0.05$) (Table 4). Soluble solids act as osmolytes and as cell structure protectors against oxidative stresses. In costmary, with increasing salinity, the carbohydrate content was greatly increased²⁷. Chang et al.³¹ demonstrated that trehalose application improved photosynthesis capability, transpiration and stomatal conductance in *Catharanthus roseus*. Carbohydrates play a parental role in metabolic processes and in gene expression and hence improve plant tolerance versus stressors³². Soluble solids are able to nourish metabolic pathways by producing NADPH and motivate the pentose-phosphate oxidative pathway, which scavenges and controls ROS radical levels³³.

Chlorophyll's content. Figure 1 shows that the highest chl a content was recorded with 50 mM NaCl × foliar application of glucose and KNO_3 and with 100 mM NaCl × KNO_3 treatment ($P \leq 0.01$). Chl b was also affected by salinity ($P \leq 0.05$) (Table 3). Up to 100 mM NaCl, there was no difference in chl b content (Table 2). With increasing photosynthetic pigment content and accumulation, photochemical energy and metabolic activities increase, and hence, growth and productivity improve³⁴. In research on coriander (*Coriandrum sativum*), foliar treatment with KNO_3 under salinity stress improved photosynthesis potential, ionic equilibrium, relative water content, and protein biosynthesis; hence, potassium treatment regulates cell turgor and polarity, xylem translocation, and nitrogen metabolism and ameliorates salinity side effects³⁵. Chlorophyll content in plants is an indicator of abiotic stressor tolerance³⁴. Abdallah et al.¹⁶ noted that rice seed pretreatment with trehalose increased the chlorophyll content under salinity conditions. It seems that the increase in Rubisco enzyme activity and the consequent enhanced chlorophyll biosynthesis are the major reasons for the improved photosynthesis potential in the plants treated with trehalose, which ultimately led to the enhanced yield and productivity³⁶.

Table 2

Mean comparisons for the effects of salinity (0, 50, 100 and 150 mM NaCl) on root DM, plant height, leaf length, leaf width, petiole length, chlorophyll b content, proline and MDA content as well as on SOD and CAT activity of *Tanacetum balsamita* plants grown hydroponically in perlite. Significant differences among salinity treatments are indicated by the different Latin letters.

Salinity levels (mM)	Root DM (g)	Plant height (cm)	Chlorophyll b ($\text{mg}^{-1}\text{g Fwt}$)	SOD activity (Units $\text{mg}^{-1}\text{protein}$)	Proline content ($\mu\text{g}^{-1}\text{g Fwt}$)	Catalase activity ($\mu\text{mol H}_2\text{O}_2^{-1}\text{mg/min}$)	MDA content ($\text{nmol}^{-1}\text{g Fwt}$)	Petiole length (cm)	Leaf width (cm)	Leaf length (cm)
0	21.8 ^a	42 ^a	0.78 ^a	3.9 ^a	40.7 ^d	2.13 ^c	58.8 ^b	7.9 ^a	3.9 ^a	9.1 ^a
50	23 ^b	37 ^b	0.72 ^a	3.47 ^b	105.4 ^c	2.74 ^b	56.3 ^c	3.9 ^b	3.3 ^b	8.7 ^a
100	26.4 ^a	36 ^b	0.69 ^a	3.53 ^b	129.2 ^b	3.1 ^{ab}	58.9 ^b	2.8 ^b	3.2 ^b	6.8 ^b
150	26.2 ^a	36.2 ^b	0.49 ^b	3.43 ^b	151.4 ^a	3.24 ^a	82.1 ^a	2.7 ^b	3.0 ^b	6.5 ^b

Table 3

ANOVA for the effects of salinity (0, 50, 100 and 150 mM NaCl) and foliar applications (no foliar, KNO₃, glucose and Dobogen) on the chlorophyll a and b, total phenolics, flavonoids, proline, H₂O₂ and MDA content as well as on SOD and CAT activity of *Tanacetum balsamita* plants grown hydroponically in perlite. ns, nonsignificant; *significant difference at $P \leq 5\%$, **significant difference at $P \leq 1\%$, following two-way ANOVA

Significance	Chlorophyll a content	Chlorophyll b content	H ₂ O ₂ content	MDA content	Flavonoids content	Total phenolics content	SOD activity	CAT activity	Proline content
Salinity (S)	**	*	**	**	*	**	*	**	**
Foliar (F)	**	ns	**	**	ns	**	ns	**	**
S×F	**	ns	ns	ns	**	**	ns	ns	ns

Table 4

Mean comparisons for the effects of 2 g L⁻¹ glucose, 2 g L⁻¹ KNO₃ and 2% Dobogen foliar application on TSS, proline and MDA content as well as catalase activity of *Tanacetum balsamita* plants grown hydroponically in perlite. Significant differences among treatments are indicated by different Latin letters.

Foliar application	TSS (°Brix)	Proline content (µg ⁻¹ g FWt)	Catalase activity (µmol H ₂ O ₂ ⁻¹ mg ⁻¹ min)	MDA content (nmol ⁻¹ g FWt)
No foliar spray	2.4 ^b	100 ^b	3.13 ^a	69.1 ^a
Glucose	3.2 ^a	104 ^b	2.9 ^{ab}	65.2 ^b
KNO ₃	2.8 ^a	115 ^a	2.7 ^{bc}	59.7 ^d
Dobogen	2.7 ^a	106 ^b	2.41 ^c	62.2 ^c

Total phenolics and flavonoids content. Both total phenolic and flavonoid contents were impacted by salinity × foliar application ($P \leq 0.01$) (Table 3). The highest phenolic content was traced by 50 mM NaCl without foliar spray and KNO₃ foliar application as well as with 100 mM NaCl × foliar application of Dobogen and glucose (Fig. 2). The highest data for flavonoids were devoted to control plants foliar sprayed with Dobogen, 50 mM NaCl × KNO₃ and glucose foliar treatment and 100 mM salinity + no foliar application (Fig. 3). In rosemary, with salinities of up to 50 mM, the total flavonoid content was increased⁵. Phenolics and flavonoids are the major secondary metabolites that nullify oxidants, especially hydroxyl, peroxy and peroxynitrite radicals^{29,37}. In *Catharanthus roseus*, the application of SA improved dry weight, water content, photosynthetic pigments and proline content as well as increased phenylalanine ammonia lyase (PAL) activity, which was coincident with phenolic biosynthesis stimulation in plant². Furthermore, in *Solanum nigrum*, the expression of genes related to carotenoid and flavonoid biosynthesis (PAL, chalcone synthase and flavonol synthase) was affected by salinity and further enhanced the accumulation of lutein and quercetin-3-β-D-glucoside. With 150 mM salinity, the amounts of quercetin-3-β-D-glucoside were elevated. Meanwhile, lutein and β-carotene were negatively influenced by the mentioned salinity levels²⁹, depicting the side effects of salinity on antioxidant compound biosynthesis²⁹. Overall, salinity stress, by imposing osmotic and ionic stresses and by ionic toxicity, influences the physiological, biochemical and cellular dynamics of plants. Therefore, plants need to combat salinity deteriorative effects by modifying the genetic pathway and ion selection, distribution and substitution as well as by activating antioxidant systems^{29,38}.

Proline. The proline content was affected by the independent effects of salinity and foliar application (Table 3). The highest proline content was recorded at 150 mM NaCl and was approximately 26% higher than that of the control (Table 2). For the foliar application treatments, KNO₃ foliar spray was the most responsive (Table 4). In coriander, with salinity, proline content was increased, and KNO₃ foliar application reduced proline content³⁵. Under salinity, the Na⁺ load considerably increases in the vacuoles. Therefore, the cells need parallel compounds with the same charge to control the osmotic potential of the cells. K⁺ availability within plant cells induces proline biosynthesis by protein hydrolysis³⁹. Proline is able to scavenge free hydroxyl radicals and hence protects and stabilizes macromolecules such as DNA and proteins and furthermore secures cell membranes⁴⁰.

MDA content. MDA content was independently affected by salinity and foliar application (Table 3). The salinity of 150 mM NaCl contained the highest MDA content (4% more than the control) (Table 2). Table 4 shows that again, the control plants with no foliar spray treatments had the highest MDA content. Foliar treatments efficiently reduced MDA accumulation, and the lowest MDA amount belonged to the Dobogen foliar application, which was nearly 10% lower than the control (Table 4). In a study conducted on rosemary⁵ with increasing salinity, MDA accumulated more in the plant tissue. Under salinity conditions, ROS generation propagates via the enhanced enzymatic activity of membrane-anchored NADPH oxidases and peroxidases (Durner and Klessig, 1996). In costmary, salinity added up MDA production²⁷. The simultaneous application of salinity and SA led to reduced amounts of MDA, showing the ameliorative effects of SA on membrane integrity by the reduced genesis of ROS molecules⁴¹.

SOD activity. SOD activity was influenced by the salinity treatments (Table 3). The highest data was recorded for controls. There was no difference between salinity treatments considering SOD activity (Table 2). Plant survival under saline conditions is dependent upon antioxidant enzyme activity, which scavenges ROS molecules. Salinity negatively influenced SOD activity in *Gossypium hirsutum*⁴². Foliar application of SA improved SOD activity in saline environments⁴².

SOD is the frontal barrier struggling the damage caused by ROS radicals and acts by converting O_2^- into H_2O_2 ⁴³. The produced H_2O_2 is then disassociated to H_2O and O_2 by the action of catalase. Otherwise, peroxidases neutralize H_2O_2 by the help and mediation of phenolics or other antioxidants⁴³.

CAT activity. The highest CAT activity was recorded with the control plants + glucose (Table 4). Furthermore, CAT activity was responsive to the independent effects of salinity, and with salinities from 100-150 mM, the activity was increased (Table 2). The lowest data for CAT were devoted to control plants (Table 2). Valizadeh Kamran et al.²⁷ reported that salinity enhanced CAT activity in costmary. CAT is responsible for the catalysis of H_2O_2 with the help of ascorbate, guaiacol and phenolics⁴⁴. The CAT, peroxidase and ascorbate activities in response to SA application meaningfully reduced membrane deterioration and amended plant tolerance and productivity under stressful environments⁴⁵.

Na⁺, K⁺ amounts and K⁺/Na⁺ ratio. Na⁺ and K⁺ amounts and the K⁺/Na⁺ ratio were influenced by the interaction effects of salinity and foliar application (Table 5). The highest K⁺ content was traced with 150 mM NaCl × KNO₃ foliar application (77% more than control) (Fig. 4b). Na⁺, 100 and 150 mM salinity × no foliar spray and 150 mM NaCl treatment with KNO₃ foliar application were the statistically significant treatments (Fig. 4a). The highest K⁺/Na⁺ ratio belonged to the control treatments (without salinity × without foliar spray) or without salinity × KNO₃ and Dobogen foliar spray (Fig. 4c). Under saline-sodic conditions, Na⁺ enters the apoplastic lumens and with substitution of Ca²⁺ ions in the cell membranes, depolarizes membranes and interferes with the selective absorption of essential minerals^{46,47}. Salinity stress denatures membranes, breakdowns membrane integrity and, by K⁺ ions, out-leakage stimulates polarization/activation of outward rectifying (KOR) K⁺ channels⁴⁶. Keeping low Na⁺ and high K⁺ levels as well as the increased K⁺/Na⁺ ratios are goal-oriented criteria that mediate tolerance to salinity stress³⁸. It seems that foliar treatment of plants with KNO₃ is a feasible and reliable way to reduce the adverse effects of salinity via the lessened competition between Na⁺ and K⁺.

Mg²⁺ content. Mg²⁺ content was influential by the interaction of 50 mM salinity × glucose foliar application (38% more than control), 50 and 100 mM NaCl salinity × KNO₃ and 50 mM NaCl salinity × Dobogen (Fig. 4d). Elhindi et al. (2016) noted that foliar application of KNO₃ improved Mg²⁺ content under salinity but reduced iron uptake and hence stimulated the dissociation of chlorophyll via photooxidation, blockage of chlorophyll biosynthesis, and overactivation of chlorophyll-catalyzing enzymes¹⁸.

Table 5
Effect of salinity (0, 50, 100 and 150 mM NaCl) and foliar applications (no foliar, KNO₃, glucose and Dobogen) on the mineral content of *Tanacetum balsamita* plants grown hydroponically in perlite. ns, nonsignificant; *significant difference at P ≤ 5%, **significant difference at P ≤ 1%, following two-way ANOVA.

Significance	Na ⁺	K ⁺	K ⁺ /Na ⁺ ratio	Si	Mn ²⁺	Zn ²⁺	Fe ²⁺	Mg ²⁺	Ca ²⁺
Salinity (S)	**	*	**	**	**	**	**	**	ns
Foliar (F)	**	**	**	*	**	**	**	**	**
S×F	**	**	**	**	**	**	**	**	ns

Mn²⁺ content. For Mn²⁺ content, the uppermost data belonged to foliar application with glucose × without salinity stress (Fig. 4f). Salinity interferes with the intake of nutrients from the soil. Specifically, Na⁺ triggers strong osmotic effects and, by its impact on soil structure, markedly decreases water and nutrient acquisition. Furthermore, Na⁺ replaces Ca²⁺ seating places in the cell structure and hence impedes the regular functions^{48,49}.

Iron and silicon content. The iron amount was increased by the interactions of no salinity × glucose and 150 mM NaCl × glucose (Fig. 4g). The top amount for Si was recorded with no-salinity × no-foliar spray and with foliar spray of KNO₃ and Dobogen (Fig. 4h). With salinity, the Fe content of soybean plants drastically declines (Weisany et al., 2014). Iron plays crucial roles in plant growth, development, chlorophyll biosynthesis, thylakoid formation and chloroplast development³⁵. Overall, abiotic stressors impact plants by their effects on enzymatic, physiological and biochemical activities along with their influences on the antioxidant pool, photosynthesis and ion homeostasis. Moreover, ionic imbalances caused by the overaccumulation of Cl⁻ and Na⁺ hamper the absorption of other essential nutrient elements^{5,8,48}.

Zn²⁺ content. Zn²⁺ content was impacted by the interaction effects of no saline + no foliar spray treatments, no saline × glucose and Dobogen, 150 mM salinity with no foliar spray, and with glucose and KNO₃ foliar spray × 100 mM salinity with glucose foliar application (Fig. 4e). Zn²⁺ plays a pivotal role in membrane integrity and maintains a dominant leadership role in the regulated entrance of Na⁺ and other toxic ions inside the cells. Appropriate Zn²⁺ availability is crucial for the survival of plants under saline-stressful environments since, with optimized Zn²⁺ availability, the activity of NADPH, an enzyme responsible for the generation of some ROS types, greatly declines⁵⁰. Salinity greatly lessens Zn²⁺ absorption by plants and concurrently diminishes the photosynthetic potential, stomatal conductance, respiration rate, chlorophyll content and hormonal balance in plants^{5,35}.

Ca²⁺ content. Salinity do not influence Ca²⁺ content. The highest Ca²⁺ content was recorded in no-foliar and foliar sprays with glucose and KNO₃ (Fig. 5). Weisany et al.⁴⁸ noted that with increasing salinity exposure, Ca²⁺ absorption and accumulation declined in soybean roots. Aerial parts of plants are more sensitive to the unbalanced distribution of nutrients than the root system. The discrepancy in salt sensitivity and/or tolerance is species-dependent, and even in a defined plant taxon, it is quite linked with genetic make-up and specific gene expression under stressful environments. The availability of appropriate amounts of Ca²⁺ is vital for cell membrane integrity and osmotic potential adjustment under saline environments. Excessive Na⁺ in the rhizosphere medium and the subsequent intake of Na⁺ ions substitutes cell wall bonded Ca²⁺ with fake Na⁺ ions, persuades magnificent devastation on cells, tissue and plant

organs and, subsequently, on the growth potential and productivity⁴⁸. Elevated Ca²⁺ content as a secondary cellular messenger regulates the expression of specific salinity-dependent genes in favor of disciplined osmotic regulation, water absorption, ionic balances and hence more acclimation to harsh saline environments in main part by the appropriate responses of antioxidants, which improve Ca²⁺ intake for the maintenance of cell intactness and viability against oxidative damages^{42,51}.

Essential oil yield and constituents. Essential oil yield was not affected by salinity, foliar application or the interaction of salinity × foliar application (Table 6). Salinity at 0 to 150 mM NaCl did not affect EO yield (averaged at 1.40 mL m⁻²) for plants grown under saline conditions (Table 6). Similarly, foliar application of glucose (averaged at 1.43 mL m⁻²) or Dobogen (averaged at 1.36 mL m⁻²) did not change the EO yield, while KNO₃ application decreased the EO yield at a salinity of 50 mM NaCl compared with the relevant control (0 mM NaCl+ KNO₃).

The effects of different salinity levels and foliar application on the chemical composition of the EO of costmary are given in Table 7. Considering the EO analysis, 39 components for salinity, 40 components for glucose and KNO₃ application and 41 components for Dobogen application were identified, showing that treatments represented 97.64-99.36% of the oils (Table 7). Oxygenated (monoterpenes and sesquiterpenes) compounds ranged from 89.38–94.50% and 0.82–3.11%, respectively, while hydrocarbon (monoterpenes and sesquiterpenes) compounds ranged from 0.53–1.71% and 1.13–3.44%, respectively (Table 7). The major constituents of the examined costmary EOs in decreasing order were carvone, cis-thujone, eucalyptol, trans-thujone, n-dodecane, tetradecane, trans-carvone oxide, and β-Bisabolene, while the rest of the compounds were identified in amounts lower than 1% of the total volatile component content (Table 7). Following statistical analysis, salinity affected the content of eucalyptol, trans-carvone oxide, and β-Bisabolene, while neither foliar application nor the interaction of salinity × foliar treatment affected the EO composition (Table 6).

Foliar Dobogen application in nonsaline-grown plants increased the eucalyptol content compared with that in the nonsprayed plants. Similarly, KNO₃ foliar application in nonsaline-grown plants increased the trans-carvone oxide content compared to the control treatment (no foliar) (Table 7). Terpenoids, as the major secondary metabolites of essential oil-bearing plants, respond to agricultural practices and environmental stimuli. Plants react to divergent environmental conditions by variations in the quantity and proportional ratio of chemical constituents. Under varying agricultural practices, the fluctuations in the phytochemical profile of plants are quite logical as well. The genetic make-up and phytochemical potential of plants inevitably mediate the chemical profile, albeit in coordination with environmental cues. Therefore, all these sensing and signaling events lead to different physiological, biochemical and yield-related responses. These fluctuations in the chemical profiles verify the enhanced adaptation process under stressful conditions. Therefore, the proportional variations in the oil constituents in response to the studied treatments are fully acceptable.

Table 6
Effect of salinity levels (0, 50, 100 and 150 mM NaCl) and foliar applications (no foliar, 2 g L⁻¹ glucose, 2 g L⁻¹ KNO₃, and 2% Dobogen) on the essential oil yield (mL/m²) and components (with > 1% content) in costmary grown in hydroponics. ns, *, **, and *** indicate nonsignificant or significant differences at P< 5%, 1% and 0.1%, respectively, following two-way ANOVA.

Factors	Salinity (S)	Foliar (F)	Interaction S × F
EO yield	ns	ns	ns
carvone	ns	ns	ns
cis-Thujone	ns	ns	ns
Eucalyptol	*	ns	ns
trans-Thujone	ns	ns	ns
n-Dodecane	ns	ns	ns
Tetradecane	ns	ns	ns
trans-Carvone oxide	**	ns	ns
β-Bisabolene	**	ns	ns

Table 7

Chemical composition (%) of essential oils of costmary plants exposed to salinity (0, 50, 100 and 150 mM NaCl) and foliar applications (no foliar, 2 g L⁻¹ glu followed by the same letter are not significantly different, $P \leq 0.05$.

	Foliar	No-foliar	D-Glucose						KNO ₃			
	NaCl	Control	50 mM	100 mM	150 mM	Control	50 mM	100 mM	150 mM	Control	50 mM	1
Compound	RI	1	2	3	4	5	6	7	8	9	10	1
Camphene	948	-	-	-	-	-	-	-	-	-	-	-
Sabinene	973	-	-	-	-	-	-	-	-	-	-	-
n-Decane	997	0.96ab	0.79ab	0.93ab	0.78ab	0.67ab	0.47ab	1.13ab	0.90ab	0.53ab	0.40b	0
p-Cymene	1006	0.21ab	0.23ab	0.25ab	0.29ab	0.26ab	0.29ab	0.38a	0.30ab	0.33ab	0.13b	0
Limonene	1028	0.05b	0.05b	0.06ab	0.10ab	0.08ab	0.09ab	0.19a	0.09ab	0.09ab	0.00b	0
Eucalyptol	1031	2.32bc	2.58bc	2.51bc	2.95ab	2.61bc	3.18ab	2.89ab	2.68abc	3.28ab	1.72c	2
Butanoic acid, 2-methyl-, 3-methylbutyl ester	1098	0.09abc	0.10abc	0.08abc	0.13a	0.04c	0.11abc	0.14a	0.13a	0.11abc	0.06bc	0
Butanoic acid, 2-methyl-, 2-methylbutyl ester	1102	0.17de	0.18de	0.17de	0.26ab	0.17e	0.22abcde	0.26ab	0.21abcde	0.22abcde	0.25abc	0
cis-Thujone	1106	20.89a	20.09a	18.66a	20.69a	20.71a	18.50a	21.15a	20.74a	18.68a	18.60a	1
trans-Thujone	1116	2.20a	2.13a	2.07a	2.39a	2.25a	2.08a	2.45a	2.35a	2.15a	2.15a	2
trans-p-Mentha 2,8-dien-1-ol	1119	0.31b	0.34ab	0.48ab	0.41ab	0.40ab	0.61a	0.53ab	0.53ab	0.57ab	0.45ab	0
cis-p-Mentha -2,8-dien-1-ol	1133	0.11b	0.14ab	0.20ab	0.17ab	0.16ab	0.27a	0.23ab	0.23ab	0.25ab	0.19ab	0
trans-Pinocarveol	1139	0.08c	0.18abc	0.22abc	0.26ab	0.22abc	0.29a	0.25ab	0.25ab	0.29a	0.11bc	0
Sabina ketone	1159	0.02bc	0.06abc	0.07abc	0.08abc	0.09abc	0.15a	0.12abc	0.14a	0.14a	0.00c	0
Pinocarvone	1163	0.37c	0.42bc	0.50abc	0.58ab	0.51abc	0.53abc	0.57ab	0.50abc	0.55ab	0.49abc	0
Thujol (3-thujanol)	1165	0.00b	0.06ab	0.05ab	0.15a	0.06ab	0.06ab	0.12ab	0.06ab	0.16a	0.00b	0
cis-Pinocarveol	1186	0.00c	0.00c	0.17bc	0.68ab	0.45abc	0.52abc	0.51abc	0.21bc	0.78a	0.35abc	0
a-Terpineol	1191	0.05ab	0.11ab	0.18a	0.12ab	0.18a	0.00b	0.12ab	0.00b	0.10ab	0.00b	0
cis-Dihydro carvone	1198	0.57d	0.59cd	0.62bcd	0.67abcd	0.71ab	0.66abcd	0.66abcd	0.66abcd	0.73a	0.62bcd	0
n-Dodecane	1200	1.57a	0.83a	1.38a	1.15a	0.72a	0.42a	1.06a	0.91a	0.50a	0.50a	1
Verbenone	1211	0.00a	0.00a	0.00a	0.04a	0.04a	0.12a	0.07a	0.09a	0.05a	0.00a	0
trans-Carveol	1219	0.19c	0.19c	0.34abc	0.41abc	0.27bc	0.60ab	0.41abc	0.46abc	0.53abc	0.57abc	0
cis-Carveol	1231	0.06c	0.09bc	0.23abc	0.23abc	0.17abc	0.30a	0.29a	0.25abc	0.29a	0.33a	0
cis-Ocimenone	1232	0.09ef	0.09f	0.12cdef	0.16bcdef	0.11def	0.22ab	0.19abcde	0.20abcd	0.19abcd	0.23ab	0
Cumin aldehyde	1241	0.09ab	0.05bc	0.14a	0.13ab	0.12ab	0.00c	0.00c	0.00c	0.07abc	0.00c	0
Carvone	1244	63.33a	65.52a	64.47a	61.56a	63.32a	64.32a	60.15a	60.80a	63.90a	61.68a	6
cis-Chrysanthenyl acetate	1259	0.07bc	0.06c	0.10abc	0.13abc	0.13abc	0.13abc	0.18a	0.15a	0.12abc	0.16a	0
cis-Carvone oxide	1262	0.00d	0.03cd	0.00d	0.04bcd	0.09abc	0.10abc	0.06abcd	0.11abc	0.11abc	0.00d	0
Perilla aldehyde	1275	-	-	-	-	0.03ab	0.00b	0.00b	0.00b	0.03ab	0.00b	0

	Foliar	No-foliar	D-Glucose						KNO ₃			
trans-Carvone oxide	1276	0.93b	1.10ab	1.08ab	1.07ab	1.25ab	1.43a	1.23ab	1.31a	1.31a	1.36a	1
trans-Carvyl acetate	1335	0.00a	0.00a	0.04a	0.04a	0.06a	0.05a	0.05a	0.00a	0.05a	0.00a	0
cis-Carvyl acetate	1360	0.02d	0.05cd	0.14abcd	0.13abcd	0.16abc	0.14abcd	0.18ab	0.08bcd	0.17abc	0.24a	0
Tetradecane	1397	1.14a	0.47a	1.01a	0.70a	0.38a	0.19a	0.55a	0.52a	0.20a	0.37a	0
β-Bisabolene	1520	1.38bc	1.38bc	1.27bc	1.19bc	1.06bc	0.95c	1.08bc	1.48bc	1.56bc	2.98a	2
trans-Calamenene	1531	0.22bc	0.21bc	0.18bc	0.21bc	0.18c	0.18c	0.24abc	0.26abc	0.25abc	0.46a	0
Spathulenol	1581	0.11bcd	0.08cd	0.12bcd	0.16bcd	0.18bcd	0.19bcd	0.22bcd	0.31bcd	0.16bcd	0.62a	0
Caryphyllene oxide	1587	0.00b	0.01b	0.03b	0.00b	0.02b	0.11ab	0.00b	0.13ab	0.05ab	0.17a	0
Hexadecane	1597	0.37a	0.16a	0.32a	0.18a	0.12a	0.00a	0.17a	0.17a	0.00a	0.15a	0
1 epi-Cubenol	1628	0.08cd	0.06cd	0.04cd	0.09cd	0.08cd	0.06cd	0.07cd	0.17abcd	0.00d	0.30a	0
β-Cedren-9-one	1634	0.00b	0.00b	0.00b	0.05b	0.00b	0.00b	0.00b	0.12ab	0.00b	0.22a	0
a epi-cadinol	1638	0.42abc	0.39abc	0.35abc	0.35abc	0.22abc	0.15bc	0.36abc	0.43abc	0.15bc	0.65a	0
a Cadinol	1657	0.65a	0.54a	0.57a	0.64a	0.66a	0.63a	0.71a	0.90a	0.57a	1.15a	1
Total Identified		99.13	99.38	99.17	99.36	98.96	98.31	98.97	98.80	99.20	97.64	9
Monoterpene hydrocarbons		1.22abc	1.06abc	1.26abc	1.17abc	1.01abc	0.84bc	1.70a	1.29abc	0.95abc	0.53c	1
Oxygenated Monoterpenes		91.87ab	94.05a	92.42ab	93.22ab	94.03a	94.25a	92.48ab	91.82ab	94.50a	89.38b	8
Sesquiterpenes hydrocarbons		1.59bc	1.59bc	1.46bc	1.40bc	1.24bc	1.13c	1.32bc	1.75bc	1.81bc	3.44a	2
Oxygenated Sesquiterpenes		1.27bc	1.09bc	1.12bc	1.29bc	1.17bc	1.14bc	1.36bc	2.05abc	0.93c	3.11a	2
Others		3.27a	1.64a	3.06a	2.41a	1.63a	0.94a	2.12a	1.90a	1.07a	1.18a	2

Conclusions

The overall results show the ameliorative impacts of foliar treatments on the salinity depression of costmary. Salinity profoundly influenced plant height, leaf length and width, proline content, CAT and SOD activity and MDA and chlorophyll b amounts. Moreover, total phenolics and flavonoids, chlorophyll a, Si, Fe, Mn, and Mg content as well as the K⁺/Na⁺ ratio were impacted by the treatments. Na⁺ content was responsive to the interaction of salinity × foliar applications. Altogether, costmary was relatively tolerant to salinity depression, and foliar application of KNO₃ and glucose would be cost-effective feasible alternatives to enhance salt tolerance and to improve the growth responses and productivity of costmary.

Declarations

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Author Contributions

All listed authors contributed equally to this work,and all authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare that they no competing interests.

Data Availability

Allnew research data are presented in this contribution.

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Figures

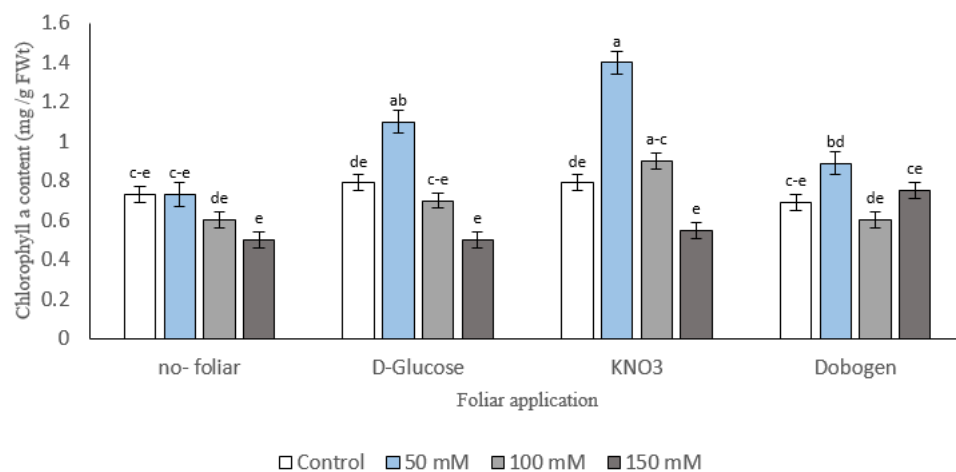


Figure 1

Interaction effect of salinity (0, 50, 100 and 150 mM NaCl) and foliar applications (no foliar, 2 g L⁻¹ glucose, 2 g L⁻¹ KNO₃ and 2% Dobogen biofertilizer) on the chlorophyll a content of *Tanacetum balsamita* plants grown in perlite. Significant differences among treatments are indicated by the different Latin letters.

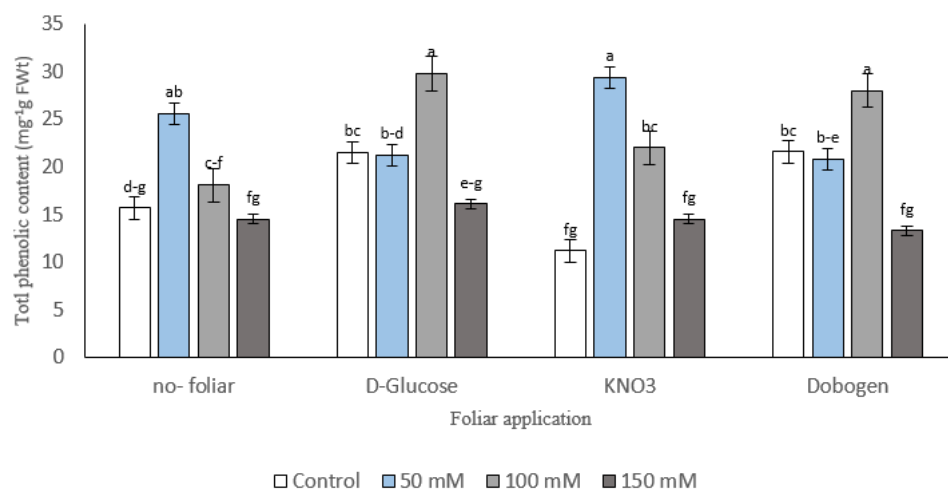


Figure 2

Interaction effects of salinity (0, 50, 100 and 150 mM NaCl) and foliar applications (no foliar, 2 g L⁻¹ glucose, 2 g L⁻¹ KNO₃ and 2% Dobogen biofertilizer) on the total phenolic content of *Tanacetum balsamita* plants grown in perlite. Significant differences among treatments are indicated by different Latin letters

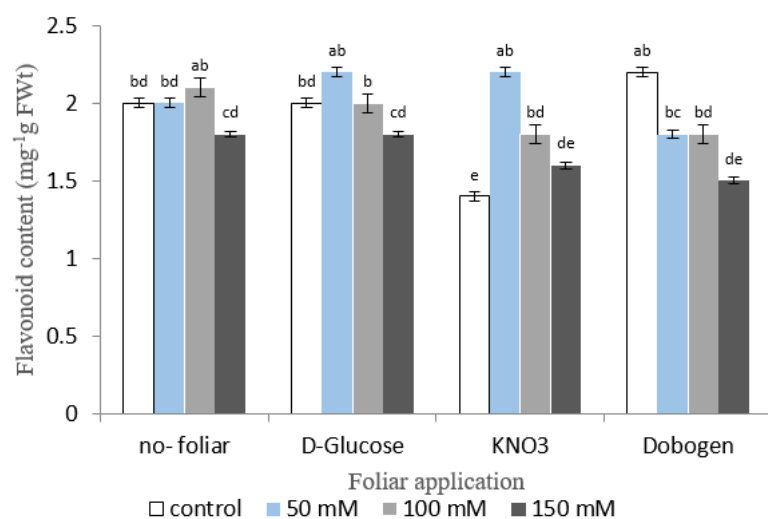


Figure 3

Interaction effects of salinity (0, 50, 100 and 150 mM NaCl) and foliar applications (no foliar, 2 g L-1 glucose, 2 g L-1 KNO3 and 2% Dobogen biofertilizer) on flavonoid content of *Tanacetum balsamita* plants grown in perlite. Significant differences among treatments are indicated by different Latin letters.

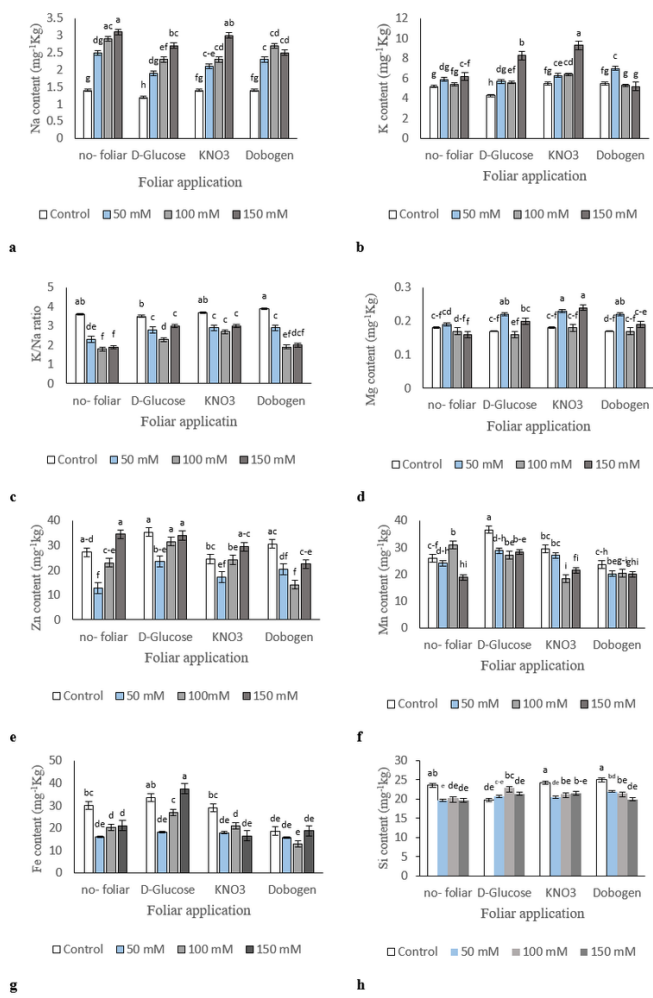


Figure 4

Interaction effect of salinity (0, 50, 100 and 150 mM NaCl) and foliar applications (no foliar, 2 g L-1 glucose, 2 g L-1 KNO3 and 2% Dobogen biofertilizer) on the elemental content of *Tanacetum balsamita* plants grown in perlite. Significant differences among treatments are indicated by different Latin letters (P ≤ 0.01).

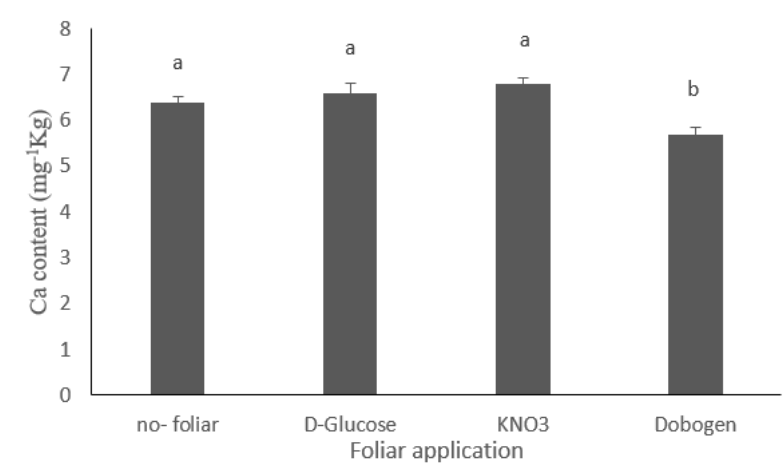


Figure 5

Mean comparisons for the effects of 2 g L⁻¹ glucose, 2 g L⁻¹ KNO₃ and 2% Dobogen foliar application on Ca content of *Tanacetum balsamita* plants grown hydroponically in perlite. Significant differences among treatments are indicated by different Latin letters ($P \leq 0.01$).