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Optimization of best dose of chitosan to Mitigate stress-induced toxic effects of salinity and Nickel in rose

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Abstract

Rose is the most important ornamental flower and the main issue responsible for low rose yield is salinity and heavy metal stress. In this study, the best dose of chitosan was optimized to mitigate the stress-induced toxic effects of salinity and nickel in rose. Two resistant (Superstar, Blue moon) and two sensitive (King's ransom, Sterling silver) rose cultivars were sown in pots and after 60 days, salinity and nickel-stress were applied at intervals to avoid osmotic shock. Different doses of chitosan were sprayed on the plants 30 days after the induction of stress. A total of six treatments including control, salinity (6dSm-1), Nickle (100 M) and different concentrations of chitosan (50-200 mgL^{-1}) were used. Complete randomized design (CRD) with three replicates was followed and data was collected for root shoots of fresh and dry biomass and leaf area. All data were statistically analyzed by the Statistix 8.1 software. The results show that the tested rose cultivars responded positively to the exogenous application of chitosan under stress conditions. The best chitosan dose (150 mgL^{-1}) was found among all the treatments. Blue moon has proven to be a salt-tolerant cultivar that can be grown in salinity-prone soils without compromising aesthetic appearance or productivity. Superstar is Ni tolerant and ideal for growing in Nickel-rich soils. Chitosan can be used as a promising bio-stimulator to improve roses' ability to withstand salt and Ni stress.

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Introduction

Floriculture is an important industry with a large foreign market. Unfortunately, floriculture is a neglected sector in Pakistan, with only sporadic coverage in recent decades. Rose, carnation, tuberose, freesia, statice, gladiolus, lilies, gerbera, and iris, among others, are important horticultural crops in Pakistan as cut flowers (Riaz *et al.*, 2007; Younis *et al.*, 2007). In Pakistan, an estimated 10-12 thousand tonnes of horticultural commodities are generally produced from 6.9 thousand hectares (Khan and Ahmad, 2005). Rose is a famous cut flower comprised of around 18,000 cultivars with 200 species (Gudin, 2000).

It is grown as an ornamental plant in parks, homes, and gardens, as well as cultivated for the production of perfume, food, medicinal products, and volatile oil (Jabbarzadeh and Khosh-Khui, 2005). It has various pharmacological properties e.g., anti-HIV, anti-bacterial, hypnotic, antioxidant and anti-diabetic (Nayebi *et al.*, 2017). Roses are used on almost every occasion around the world, and the Netherlands, Italy, the United States of America, Israel, Kenya, Japan, and Colombia are among the top rose exporting nations (Evans, 2009).

Horticulture plays a significant role in Pakistan's economy, where a variety of agro-climatic conditions exist, allowing for the cultivation of cut roses. Cut roses are becoming a valuable consumer product in Pakistan. In Pakistan, roses are grown for cut flowers on 1,300 acres of land (Khan, 2005). Cut rose blooms can be seen in Punjab's Lahore, Pattoki, Chunian, Multan, and Rawalpindi, as well as Sindh's Hyderabad and Karachi, and Khyber Pakhtunkhwa's Mansehra, Peshawar, and Haripur, and Baluchistan's Quetta.

Nowadays, salinity is a major problem and limits the growth of plants. As salty soils are not considered suitable for plant growth, salinity reduces the cultivated area of agricultural land (Shrivastava *et al.,* 2015). Plants are subjected to a number of biotic and abiotic stresses during their lifetimes, such as salinity, erosion, heavy metal toxicity, and nutritional scarcity, as well as biotic stress caused by pathogenic microbes. Both forms of stress have a detrimental impact on plant development (Shaik and Ramakrishna, 2014). Abiotic stress has a variety of effects depending on the plant genotype, age, and severity of the stress. Salinity is a main abiotic environmental stress that has a significant impact on crop production and threatens agriculture's long-term viability (McKee *et al.*, 2004). Salinity is a big concern for economically important crops in arid and semi-arid regions around the world, affecting the plant by osmotic, oxidative, and basic ion toxicity (Pitman and Läuchli, 2002). Salt stress stunted plant development by disrupting a variety of biochemical and physiological pathways, including photosynthesis, mineral nutrition homeostasis, antioxidant metabolism, hormonal imbalance, and osmolytes accumulation (Masood *et al.,* 2012). Instead of NaCl, soil salinity induces salt stress, water stress, and ionic imbalance (Baghbani-Arani *et al.*, 2017). Sodium chloride is the most common source of salinity (Cui *et al.*, 2006). Plants exposed to such high sodium levels undergo major morphological, physiological, and developmental changes, and the inhibitory effects on plant establishment are lifted (Jampeetong and Brix, 2009).

Salinity greatly decreased plant growth and development of dry matter (Khalid and Cai, 2011; Shoresh *et al.*, 2011). Salt stress has adverse effects on plant health, distortion of flowers, marketability of flowers along with visual damage, and decreased stem length and growth (Sivanesan and Jeong, 2014). Salinity causes a decline in soil water uptake for plants, which leads to a decrease in growth, yield and quality attributes, and causes disruptions in soil structure (Yadav *et al.*, 2011).

Due to the extreme world's rising population, environmental pollution of toxic chemicals is becoming a more serious problem. Human activities have disrupted the environment and pollute it with organic or inorganic chemicals such as acids, heavy metals, petroleum products, pesticides and salts. Ni has gained attention among other heavy metals as a result of its high concentration in various environments around the world. Insoluble forms of Ni compounds enter plants via root cells and are transported to shoot via xylem and accumulated in the seeds and fruits.

The Ni transport and translocation processes are structured by metal legend centers such as histidine, nicotinamide, and organic acids or through some proteins for binding and transportation of Ni. It directly affects the activities of enzymes such as amylases, proteases and ribonucleases, directly affects mobilization and metabolism, in seed cotyledons, vegetative and reproductive stage, abnormal flower development, decrease dry weight production, leaf spot, chlorosis and necrosis at the excess level of Ni and ultimately, this leads to reduced crop yield.

Nickel toxicity is one of the most significant environmental strains of living organisms (Seregin and Kozhevnikova, 2006). After all, their products' chemical composition and concentration cause a lot of soil variation (Kramer *et al*., 2007). Nickel toxicity is phototoxic and ranges from 8 to 14 g g-1 on a dry mass basis (Nieminen *et al.,* 2007). Growth inhibition, mineral nutrition, photosynthesis, water regulation and water transport are all potential consequences of nickel toxicity (Seregin and Kozhevnikova, 2006). Reduced stomatal conductance and photosynthesis, as well as improvements in root morphology and growth (Seregin *et al*., 2003), are all typical symptoms of Ni toxicity (Piccini and Malavolta, 1992; Khalid and Cai, 2011).

Chitosan is a natural biopolymer consisting of polysaccharides and chitin segments, which is a biologically active and protected natural flora (Dias *et al*., 2013). Chitosan has been found to be one of the most effective treatments for abiotic stresses such as salinity, drought, and extreme heat or cold (Jabeen and Ahmad, 2013). Chitosan is produced on a large scale through chemical or enzymatic chitin deacetylation. Chitosan is an effective method for reducing the effects of abiotic stress on plants and microbial cell systems (Nguyen *et al.,* 2016). Chitosan and its oligosaccharides are natural polymers that are used to replace expensive and environmentally harmful chemicals in crops. Farmers can benefit from the use of chitosan in terms of lower application costs and increased yield. In view of the foregoing observations, it is thought that metal and salt stresses are the primary threats to the growth of plant growth and yield. All species of vegetables and flowers are the most vulnerable to abiotic stresses. Katiyar *et al.* (2014) investigated the effects of chitosan on tomato, rice, lettuce, and soybean growth.

The positive effects of 0.1 or 0.5 percent doses of chitosan were found beneficial for rice, lettuce, and soybean as it increased the leaf length index, fresh and dry biomass, while the 0.1 percent dose of chitosan was found positive only for tomato plants with the traits described above. Shouqiang and Langlai (2003) investigated the effects of 0.4-0.6 mg/g chitosan on Chinese cabbage seeds and found that spraying the leaves produced positive results in terms of plant height, fresh weight, root length, leaf area, protein, and soluble sugars, but crude fibre content was reduced.

This study was designed with the goal of determining the best chitosan concentration that can effectively alleviate stress-induced (effects of salinity and nickel) in roses.

Material and methods

Identification of the best level of Chitosan for combating the stress-induced toxic effects in roses

This experiment was performed on four rose genotypes such as Blue moon (T), sterling silver (S), king's ransom (S), superstar (T) collected from the Floriculture Section, Ayyub Agriculture Research Institute, Faisalabad, and some reliable private nurseries. Plants were planted in plastic pots placed in the nursery area of the Department of Horticulture, College of Agriculture, University of Sargodha. One plant per pot was sown and three replications were maintained that comprised of three pots. The cuttings were watered according to the need of plants by observing the moisture of growing media.

An optimization experiment was carried out to identify the best level of chitosan under both stressed (salinity and Nickel) conditions. Plants of one tolerant and one sensitive rose cultivar against each stress were sown in pots and after 60 days of planting, salinity (6 dSm^{-1}) and nickel-stress (100 μML^{-1}) were created. Thirty days after stress induction, different levels of chitosan were sprayed on the plants. Details for treatments are described in Table 1.

Fifteen days after the application of chitosan foliar spray, the following parameters were taken as stated below.

Salinity (NaCl) and Nickel (Ni) applications

In previous study Sodium chloride (NaCl) at various level of concentrations (0, 2, 4, 6, 8 and 10 dSm^{-1}) and nickel chloride ($NiCl_2$) (0, 50, 100, 150, 200 and 250 μML^{-1}) were applied by dissolving NaCl and $NiCl_2$ in distilled water 60 days after plantation of plants. The best concentrations of NaCl (6 dSm-1) and Nickel (100 µML-1) were selected for this experiment.

Data collection/sampling

Data for the growth parameters i.e. shoot and root length, plant fresh and dry weight, and leaf area per plant, were recorded and analyzed statistically.

Measurement of the shoot and root lengths

The plants were uprooted after two months and sprayed with purified water to remove the sand particles. The length of the shoot was determined using a meter rod (tip of the shoot to the base of hypocotyl) from three plants per replication. A similar approach was used to calculate root length.

Measurement of plant fresh and dry weight

Plant fresh and dry weights were taken by using a digital weighing balance. For the measurement of dry weights, shoots were separated from the root and wrapped with filter paper to remove excess moisture by drying in the oven for 1 day at 37°C. After drying the root/shoots were weighed separately and three replications for each were recorded for analysis.

Leaf area per plant (cm^2)

To calculate the area of the leaf per plant (LA) six leaves were randomly separated from each plant. (LA) was determined by putting different leaves on an automated leaf area meter. The average area of the leaf was measured using methods developed by Michael *et al.* (2002).

Experimental design and statistical analysis

The data was obtained two months after the rose plants were subjected to NaCl and $NiCl_2$ stress. The experimental units were arranged in a completely randomized design (CRD) of three replicates. The data obtained were statistically analyzed using Statistics 8.1.

Results

Effect of different concentration of chitosan on shoot length (cm) of rose cultivars under NaCl and Ni stress

According to results treatments of chitosan significantly affected the shoot length of rose cultivars. The maximum shoot length was recorded in the Blue moon (31.30 cm) and Sterling silver (30.25 cm) at control as shown in (Fig.1) which was reduced (19.25;14.37 cm) respectively at 6 dSm^{-1} salt stress. Treatment of chitosan @ 150 mgL^{-1} significantly changed the duration of the shoot among all other treatments. The plants are grown under chitosan level 150 mgL^{-1} presented the maximum increase in shoot length by the Blue moon (25.35 cm) and Sterling silver (19.95 cm) respectively.

However, in the case of nickel treatment maximum shoot length was observed in Superstar (34.28 cm) and King's Ransom (31.55 cm) in control (Healthy) plants. Whereas, it was reduced with the treatment of nickel (23.93; 18.73 cm) respectively. Chitosan treatment at 150 mg L^{-1} gave maximum shoot length in superstar (29.35 cm) and King's ransom (23.26 cm) as compared to other chitosan doses as described in (Fig.2).

Table 1. Details of all the treatments used to carry out this experiment.

Treatments	(Part-I) NaCl	(Part-2) Nickle
T1	Control (Non-saline + No foliar spray)	Control (No nickel + No foliar spray)
T2	Saline (6 dSm^{-1})	Nickel (100 µML^{-1})
T3	50 mg L^{-1} chitosan (Saline)	50 mg L^{-1} chitosan (Nickel)
T4	100 mg $^{L-1}$ chitosan (Saline)	100 mg L^{-1} chitosan (Nickel)
T5	150 mg L^{-1} chitosan (Saline)	150 mg L^{-1} chitosan (Nickel)
T6	200 mg L^{-1} chitosan (Saline)	200 mg L^{-1} chitosan (Nickel)

Effect of different concentration of chitosan on root length (cm) of rose cultivars under NaCl and Ni stress

Maximum root length was observed in a blue moon (17.26 cm) and minimum in sterling silver (16.25 cm) at control which was decreased (13.26; 12.85 cm) respectively with the salt treatment at 6 dSm^{-1}. The treatment of chitosan @ 150mg L^{-1} significantly affected on root length of plants while maximum root length was observed in Blue moon (17.26 cm) and Sterling silver (16.25 cm) respectively as shown in (Fig.3).

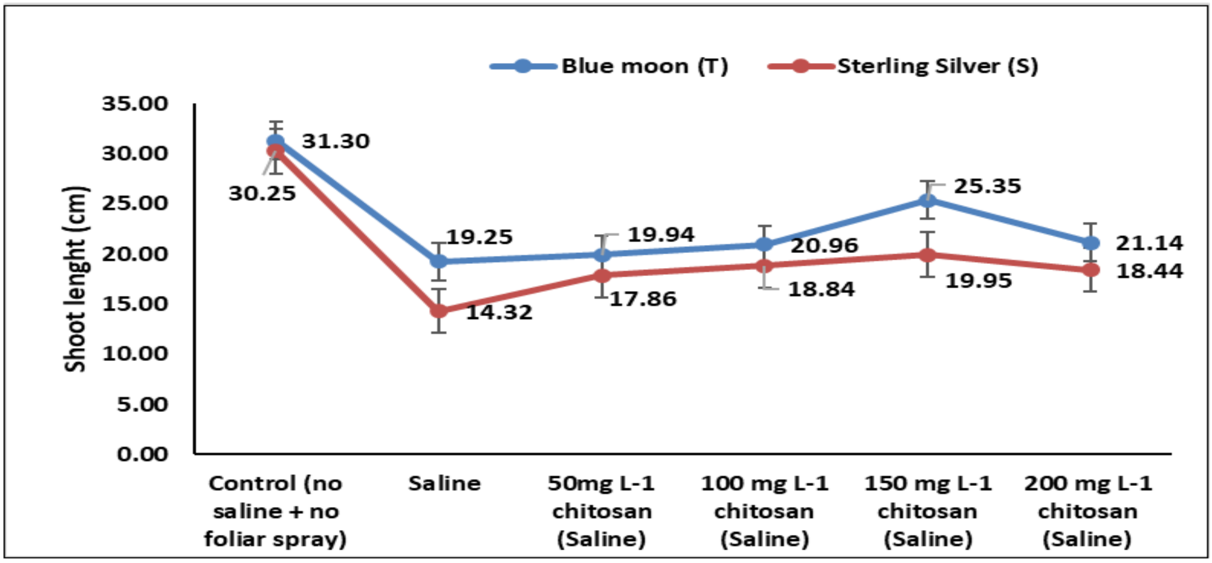

Fig. 1. Effect of salt stress on shoot length (cm) of tolerant (Blue moon) and sensitive (Sterling silver) cultivars of rose and its mitigation through chitosan.

Likewise, in the case of nickel treatment, maximum root length was observed in King's ransom (19.26 cm) and Superstar (17.42 cm) at control whereas it was reduced (14.25; 13.25 cm) with the application of nickel. However, 150 mg L^{-1} chitosan gave maximum root length in Superstar (16.84 cm) and King's ransom (15.44 cm) as compared to other treatments of chitosan as shown in (Fig. 4, Table 2).

Effect of different concentration of chitosan on fresh weight (g) of rose cultivars under NaCl and Ni stress

Data regarding fresh weight revealed that chitosan treatment (150 mg L^{-1}) effectively mitigated the adverse effects of salt stress by exhibiting the maximum fresh weight of Blue moon (58.24 g) and Sterling silver (56.51 g) at control which reduced (39.92;35.10 g) with the application of salt stress. Chitosan treatment at 150 mg L^{-1}, maximum fresh weight was maximum in the Blue moon (50.25 g) and minimum in sterling silver (43.4 g) respectively as shown in (Fig.5, Table 1).

On the contrary, in the case of nickel treatment, the maximum plant fresh weight was observed in Superstar (50.76 g) and King's ransom (50.23 g) at control than all other treatments and reduced (34.47;32.07 g) with nickel treatment. However, chitosan (150 mg L^{-1}) significantly affected on fresh weight of plants while maximum plant fresh weight was observed in Superstar (45.24 g) and King's ransom (38.42 g) as compared to all other treatments of chitosan as shown in (Fig.6, Table 2).

Effect of different concentration of chitosan on dry weight (g) of rose cultivars under NaCl and Ni stress

According to observations treatment of chitosan was most effective in the alleviation of salinity and Nickle stress in all the tested rose genotypes. However, the genotypes Blue moon (12.45 g) and Sterling silver (9.75 g) showed the highest dry weight when chitosan was applied @ (150 mg L^{-1}) as compared to all other treatments of chitosan than control (Fig. 7, Table 1).

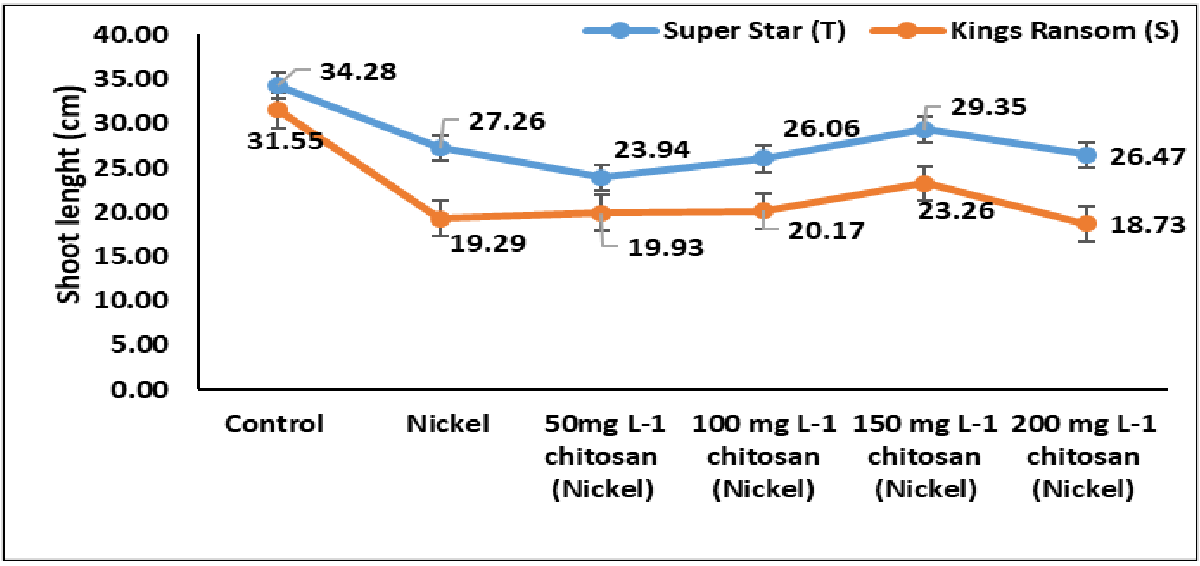

Fig. 2. Effect of Nickle stress on shoot length (cm) of tolerant (superstar) and sensitive (king's ransom) cultivars of rose and its mitigation through chitosan.

Also, in the case of nickel treatment maximum plant dry weight was observed in Superstar (11.72 g) and King's ransom (10.24 g) at control whereas it was reduced with the treatment of nickel.

However, 150 mg L^{-1} of chitosan treatment gave maximum plant dry weight in Superstar (9.65 g) and King's ransom (8.84 g) as compared to other chitosan levels as shown in (Fig.8, Table 2).

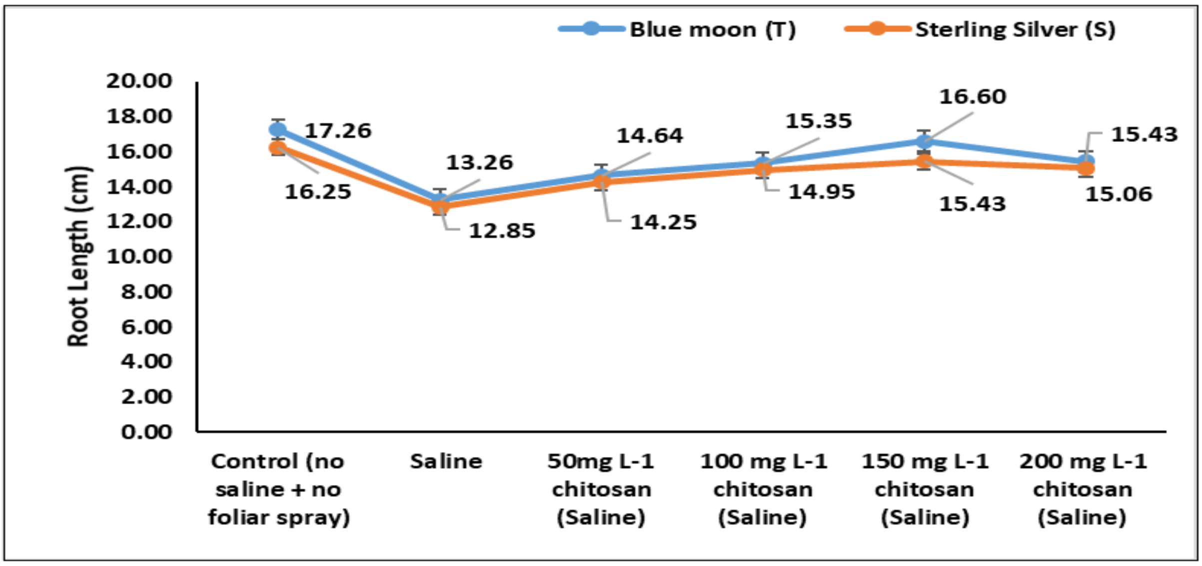

Fig. 3. Effect of salt stress on root length (cm) of tolerant (Blue moon) and sensitive (sterling silver) cultivars of rose and its mitigation through chitosan.

Effect of different concentration of chitosan on leaf area (cm^2) of rose cultivars under NaCl and Ni stress

The findings revealed that chitosan had a major impact on the leaf area of the plant as shown I n (Fig.9, Table 1). Leaf area is very important for the plants to absorbed light for photosynthesis. But in this study, Both Blue moon and Sterling silver after treatment with chitosan (150 mg L^{-1}) showed the highest plant leaf area (14.43 cm^2) and (11.64 cm^2) in contrast to other doses of chitosan and control.

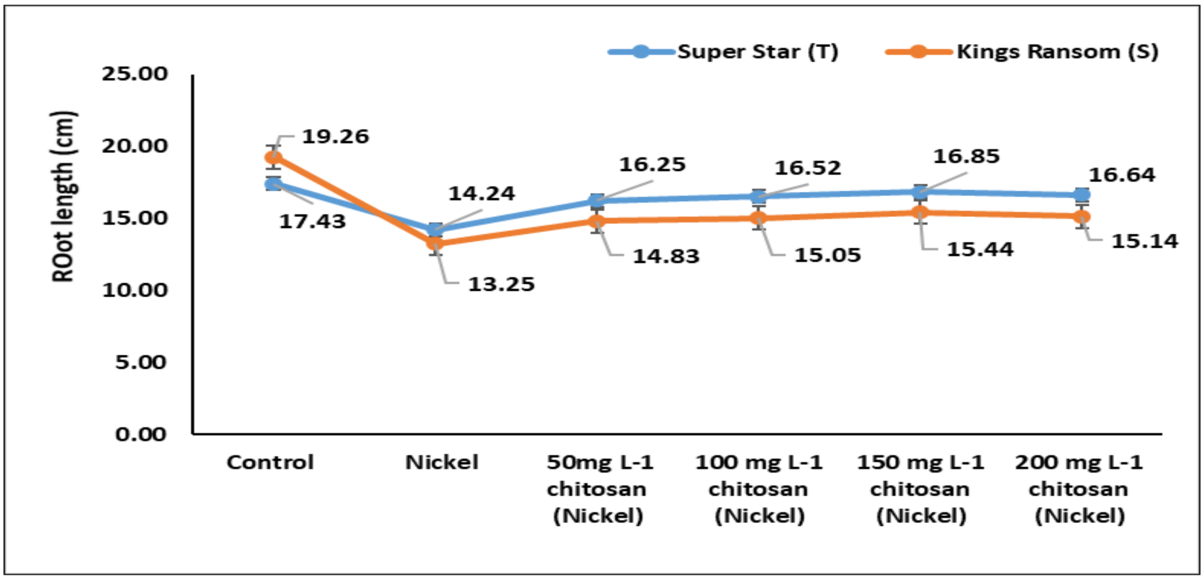

Fig. 4. Effect of salt stress on root length (cm) of tolerant (superstar) and sensitive (kings ransom) cultivars of rose and its mitigation through chitosan.

Similarly, in the case of nickel, the highest leaf area was observed in Superstar Star (13.94 cm^2) and King's ransom (12.35 cm^2) at control whereas it was reduced with the treatment of nickel. However, 150 mg L^{-1} chitosan more effective among all treatments of chitosan, and maximum leaf area was observed in Superstar (12.24 cm^2) and King's ransom (11.14 cm^2) as shown in (Table 2, Fig.10).

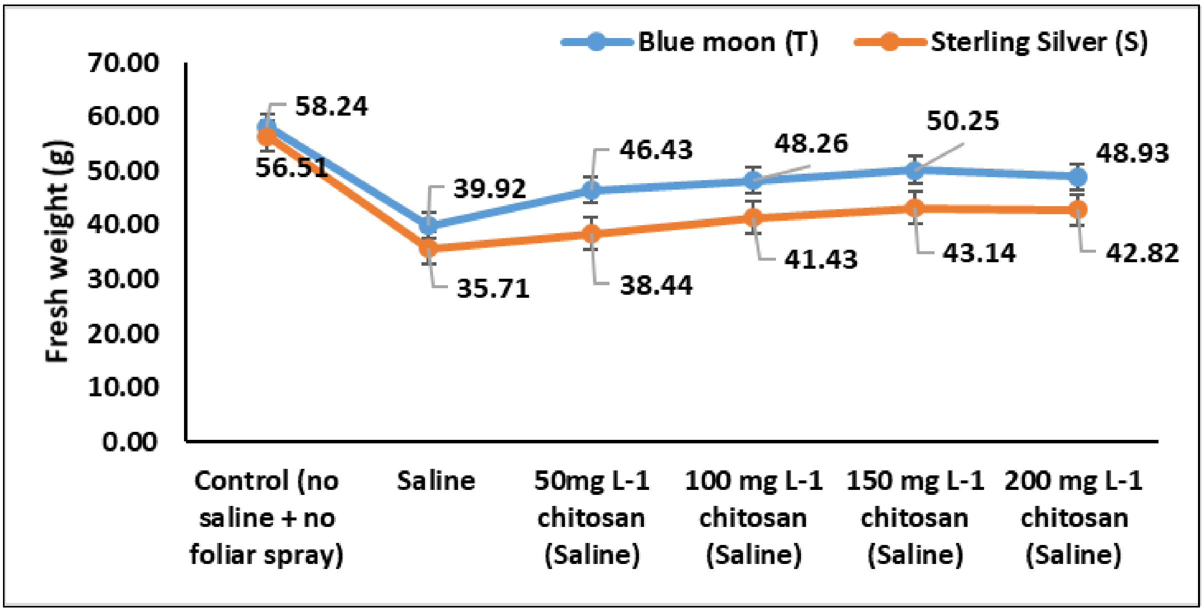

Fig. 5. Effect of salt stress on fresh weight (g) of tolerant (Blue moon) and sensitive (sterling silver) cultivars of rose and its mitigation through chitosan.

The high accumulation of Ni in tissues resulted in chlorosis in young leaves due to chloroplasts and mesophylls destruction, reduction in water potential due to reactive oxygen spp. (ROS).

It oxidizes macromolecules (lipids, protein, nucleic acids) and causes lipid peroxidation, protein denaturation, and pigment damage (Panday and Gautam, 2009; Habtamu, 2013). It is concluded from the current study that the rose cultivar Blue moon possessed a high genetic potential for salt tolerance to be exploited for cultivation under saline conditions. Similarly, the rose cultivar Superstar was found to be efficient as nickel tolerant.

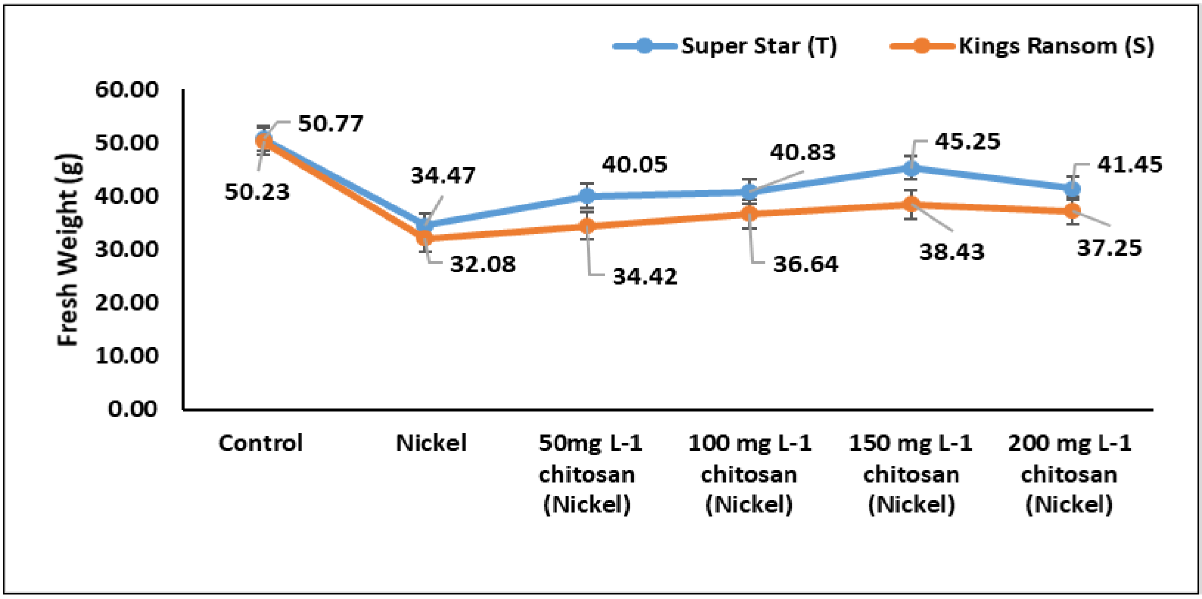

Fig. 6. Effect of salt stress on fresh weight (g) of tolerant (superstar) and sensitive (king's ransom) cultivars of rose and its mitigation through chitosan.

A significant difference was observed among the control and saline-treated cultivars and could be used as a successful tool for screening under stress conditions. Our results are confirmed by the findings of (Ashraf *et al.,* 2009) with respect to plant weight. The yield of plants is directly associated with the presence of fresh and dried biomass which becomes affected due to the accumulation of high salts in the plant body resultantly reduced productivity. A striking reduction in fresh and dry biomass was noted in both tolerant and sensitive rose cultivars.

This hindrance is may be due to impairments in plant metabolism, low transpiration rates, nutritional discrepancies, and ionic imbalances. And also may be due to NADPH, rubisco, and nicotinamide inactivity may directly responsible for low productivity. The findings of the present study are in line with previous reports of Benmahioul *et al.* (2009) and Hajlaoui *et al.* (2010). The salt-tolerant cultivars were efficacious in sustaining the high plant weight under saline regimes because of these accumulated low ratios of toxic ions in their tissues, while sensitive genotypes failed in this regard. Similarly, in the present investigation, the leaves of both tolerant and sensitive rose cultivars exhibited the highest necrosis under salt stress conditions while it was negligible in control plants.When a plant is exposed to salt stress, the first reaction is a reduction in leaf growth due to the osmotic impact of salinity on the root, which eventually contributes to a reduction in the water supply to the leaf cells (Chaves *et al*., 2009).

Root development is often inhibited by high salt concentrations in the root zones, which limits root mass, volume, and functions (Parida and Das, 2005). The plant's leaf area is also affected by salinity because of the decrease in cell division and elongation that happens as a result of this reduction in leaf size (Munns and Tester, 2008).

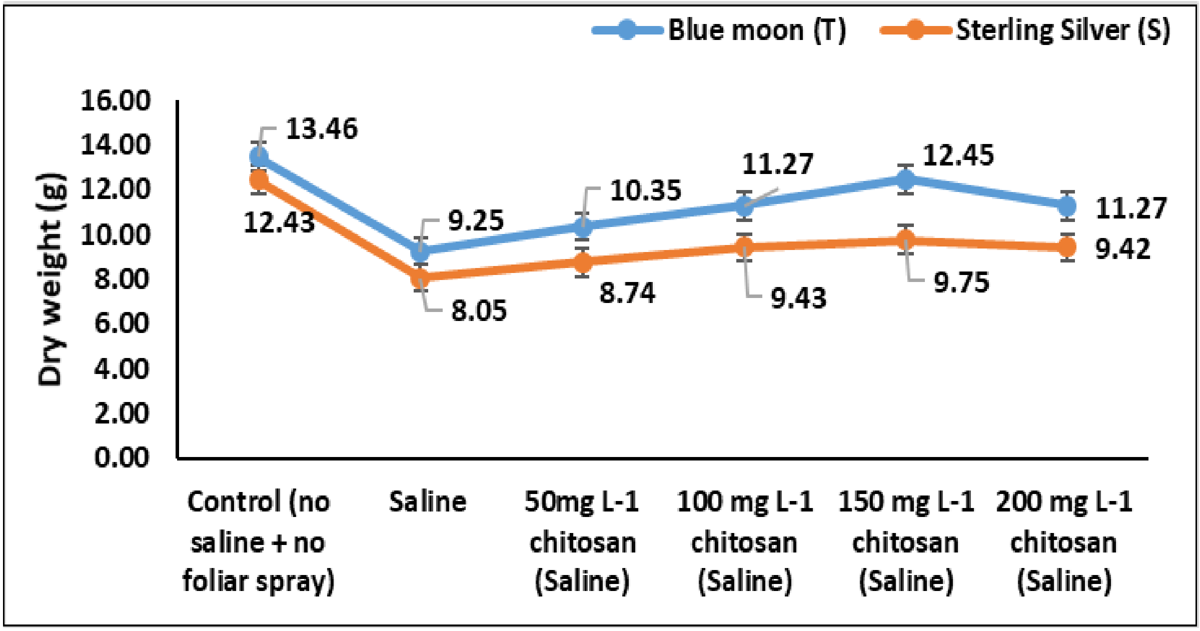

Fig. 7. Effect of salt stress on dry weight (g) of tolerant (Blue moon) and sensitive (sterling silver) cultivars of rose and its mitigation through chitosan.

The leaf area can be minimized due to a reduction in leaf turgor, as a result of altering the properties of the cell wall or reducing the photosynthetic rate (Cassaniti *et al*., 2009). This type of findings has been found in both sensitive and resistant ornamental plant species, such as *Eugenia myrtifolia* and *Cotoneaster lactus*, which have shown a decrease in leaf area and a dry shoot weight, respectively (Cassaniti *et al.,* 2012). Similarly, the influence of a high concentration of salt has been reported in ornamental plants such as *Salvia splendens* and *Coleus blumei* (Ljubojević *et al.,* 2017). It has been claimed earlier that roses can withstand saline levels up to 6 dS/m with the least toxicity on the productivity and flower quality (Chimonidou, 1997).

Ibrahim *et al.* (2007) reported about the lower shoot and root lengths of plants in presence of high salts.

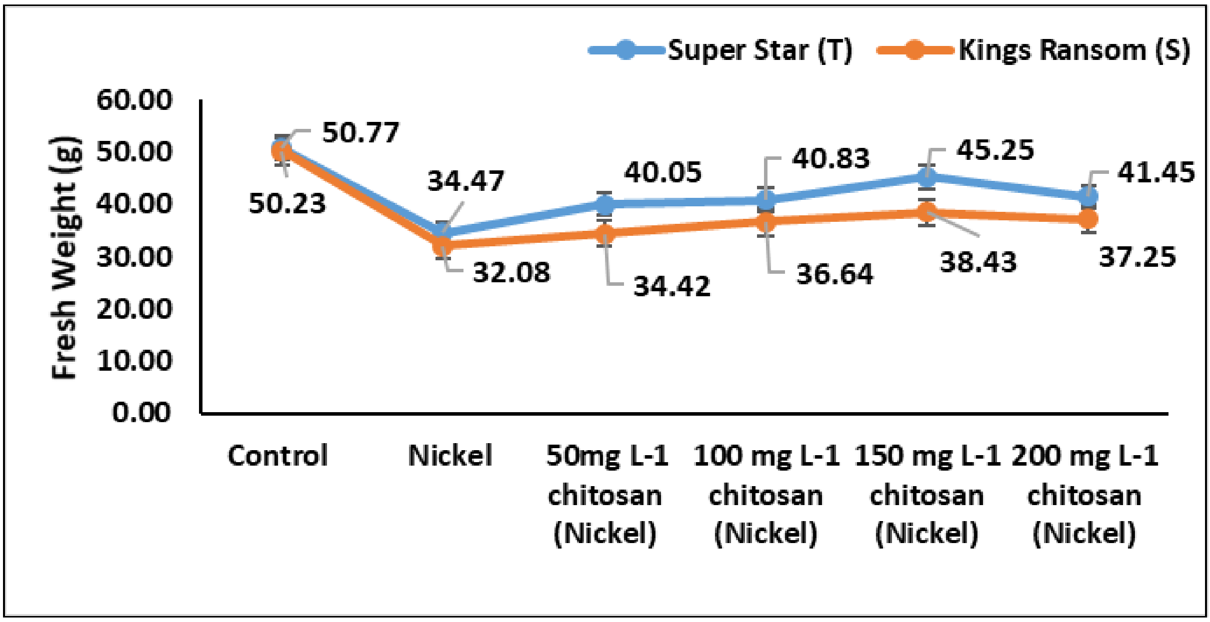

Fig. 8. Effect of salt stress on dry weight (g) of tolerant (superstar) and sensitive (king's ransom) cultivars of rose and its mitigation through chitosan.

The same findings were observed by Zeng *et al.* (2002) who claimed that shoot growth was reduced more than the root growth due to salt stress. Lower root and shoot growth are linked the reduced cell division and cell enlargement in the meristematic tissues (Mccue and Hanson, 1990). The results are in line with the observations of Ibrahim *et al.* (2007) with reduced root shoot ration in the saline medium.

Mohsen *et al.* (2014) reported that the plant biomass of Vicia faba has been affected profusely with salt levels Hussein and Abou-Baker (2014) noted a negative correlation between plant growth parameters and the amount of salts. The decreased dried weights may be attributed to increased Cl ions in plant tissues (Tavakkoli *et al.,* 2011).

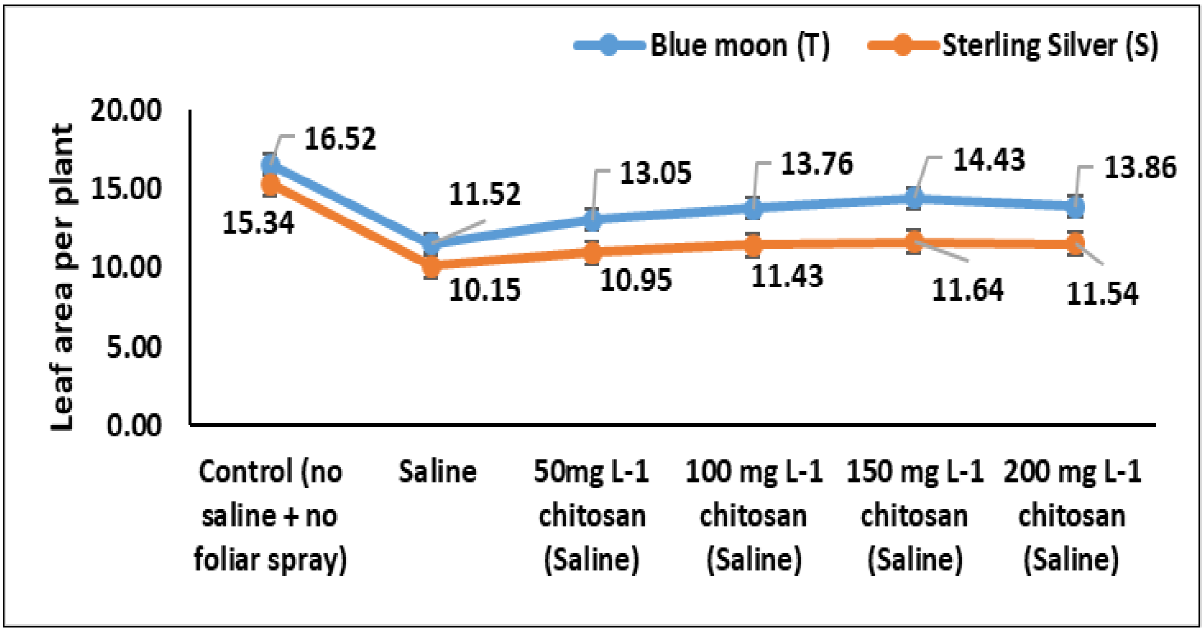

Fig. 9. Effect of salt stress on leaf area per plant (cm^2) of tolerant (Blue moon) and sensitive (sterling silver) cultivars of rose and its mitigation through chitosan.

In this study, salt stress significantly affected plant fresh and dry weight of both rose cultivars, and similar observations were noted on mulberry (Ahmad *et al.*, 2010), sunflower (Akram and Ashraf, 2011), mustard (Hayat *et al.,* 2011), and okra (Saleem *et al.*, 2011) salt stress reduced the net photosynthetic rate, stomatal conductance, and transpiration.

The limited water decreased the water status of the plant inside leaves that were found in this study due to the excess amount of salt present in the leaves cause closed stomata ultimately reduce the transpiration pull (Redondo-Gómez *et al.,* 2007) and lowers intercellular CO_2 levels (Wani *et al.*, 2013) and finally decreases plant growth-related parameters (Saleem *et al.,* 2011; Wani *et al.,* 2013). Ifediora *et al.* (2014) observed the effect of salinity on okra plants and found with the increase of salinity shoot height was reduced continuously with an increasing concentration of salinity as compared to control.

The plant under control may have adjusted osmotically to the growing conditions as a result of successfully required cell enlargement and maximum shoot height. Shahid *et al.* (2011) also recorded the visible symptoms of salinity on plants that were stunting the growth of roots and shoot with smaller leaf sizes. Due to the excess amount of salts in leaf and deficiency of water stomata close and which ultimately reduced the transpiration rate and decrease of CO_2 concentration inside leaf cells occurs and finally plant height, Root Length, Fresh and dry weight of plant reduced. Chitosan is widely used in agriculture to mitigate negative effects on plants during adverse conditions and to promote plant growth.

The salt stress was mitigated with an exogenous application of Chitosan. Different concentrations of chitosan were applied on stressed plants and the best concentration of chitosan was screened out. Among all the treatments of chitosan (150 µML-1.) was found to be the best one and it was applied on two salt-sensitive and tolerant cultivars. In this study, chitosan was sprayed on all four genotypes. According to the results, tested rose cultivars responded positively to the exogenous application of chitosan under stress conditions. Although the chitosan application successfully mitigates the salinity-induced drastic effects by improving the plant growth-related parameter under stress conditions.

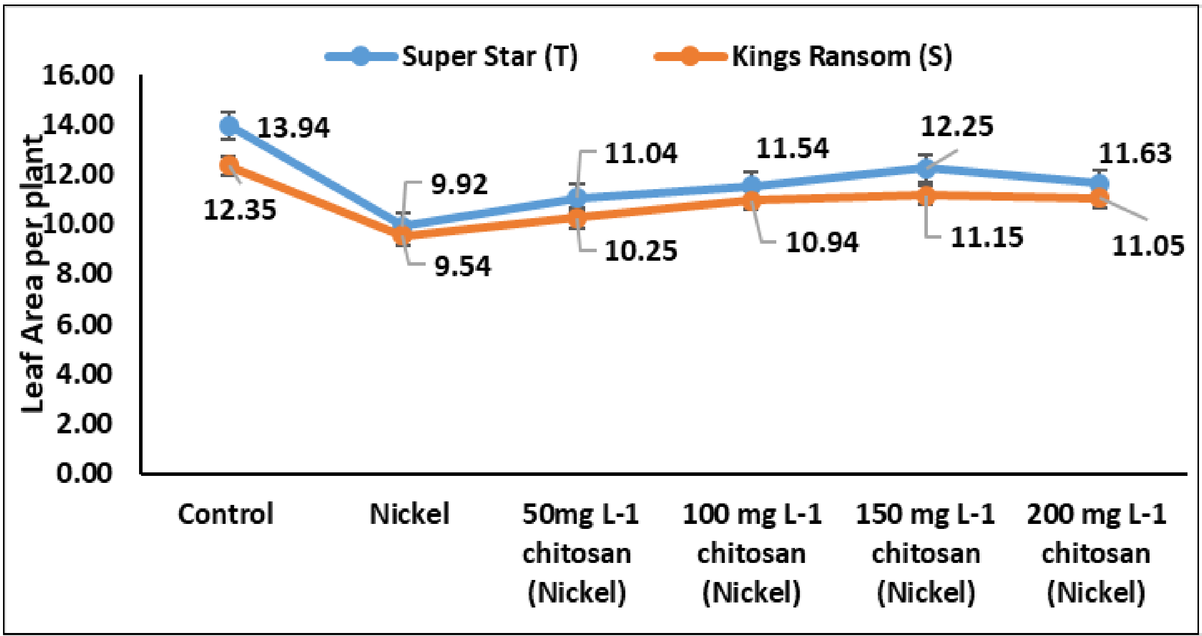

Fig. 10. Effect of salt stress on leaf area per plant (cm²) of tolerant (superstar) and sensitive (king's ransom) cultivars of rose and its mitigation through chitosan.

This observation is supported by the study results of El-Tantawy (2009) who reported that with chitosan application, the growth and development of tomato plants were enhanced.

Conclusion

The findings of this experiment depicted that chitosan significantly alleviates the drastic effects of salinity stress and nickel toxicity. Among all the treatments of chitosan (150 mgL-1.) was found to be the best one and it was applied on two salt-sensitive and tolerant cultivars. Overall, chitosan enhances the growth of rose plants under control conditions as well as under salinity stress. So, it is suggested that natural elicitor could be a promising element that can be used to mitigate the damaging effects of salinity stress on the growth and yield of rose plants. The current study is the first study to report this phenomenon in rose plants.

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Conflict of interest

The authors declare no conflict of interest

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