

EFFECT OF NaCl AND ROOT-MEDIA SELECTION ON YIELD ATTRIBUTES, OIL COMPOSITION AND MINERAL COMPOSITION OF ROSE GERANIUM

(*Pelargonium graveolens* L.)

Zenzile P. Khetsha¹ and Moosa M. Sedibe²

^{1,2} Department of Agriculture, Unit for Drug Discovery Research (UDDR), Faculty of Health and Environmental Sciences, Central University of Technology, Free State

Abstract

Rose geranium is an essential oil crop that is commercially produced worldwide for use in perfumery, medicinal and aromatherapy industries. A study was carried out in a greenhouse tunnel at the University of the Free State to evaluate the effect of salinity levels (1.6, 2.4, 3.2 and 4.0 mS cm⁻¹) and root-media (sand and sawdust) on the yield, mineral and oil composition of rose geranium. Treatments were laid out in a split plot design which was arranged in a randomised complete block. Salinity levels were allocated to the main plots and root-media to the subplots replicated three times. Salinity at 4.0 mS cm⁻¹ level significantly reduced the yield attributes and also oil quality which is ascribed to C:G ratio. Sawdust root-media had better yield attributes compared to sand root-media. Significant interaction was found between salinity at 4.0 mS cm⁻¹ and sand root-media for the Na content. Mineral contents of P, K, Mg and Cl were significantly affected by salinity levels. It is evident from the study that rose geranium should be grown using nutrient solution with salinity below 4.0 mS cm⁻¹ for better yield and oil quality using sawdust as a root-media.

Keywords: Mineral composition, root-media, rose geranium, salinity levels

Introduction

Recent reports of most studies indicate that medicinal and aromatic plants have been receiving much attention in perfumery, pharmaceutical, cosmetic, food and agroalimentary industries [15; 20; 31]. Medicinal and aromatic plants contain essential oil compounds which are a complex mixture of volatile compounds. Biosynthesis of essential oils is highly dependent on the biotic and abiotic environmental stress factors [20]. Beside climate and nutrients abiotic stress factors effects, salinity has posed as one of the most common essential oil yield and quality reducer. This has been shown on peppermint (*Mentha piperita* L.), where high salinity stress reduced the stem length, root length, shoot fresh mass, root fresh mass and dry mass and also the oil yield [20]. Ashraf et al. [3] also reported the

reduction of yield and yield attributes when salinity concentration was high. In the same study, fresh mass and dry mass of both shoots and roots of snapdragon (*Ammolei majus* L.) was reduced significantly. Moreover, on the study conducted by [15], high salinity reduced the methyl chavicol biosynthesis and accumulation of basil (*Ocimum basilicum* L.). It was also found that high salinity stress significantly reduced the growth parameters of chamomile (*Matricaria chamomile* L.) [15].

Contrary to the reduction of yield and yield attributes of essential oil plants, high salinity concentrations have been found to improve quality of most essential oil plants instead. This has been shown on basil and coriander (*Coriandrum sativum* L.) where linalool content was increased by 57% and 45%, respectively [4; 25]. Moreover, improved oil quality was reported on

Corresponding Author. Email: zkhetsa@cut.ac.za

sage (*Salvia officinalis* L.), where salinity increased oxygenated monoterpene by 48% [43]. Hendawy and Khalid [16] also stated the effects of salinity conditions on the plants physiological and biochemical potentials, which in turn affect the plants primary and secondary metabolism.

According to the report of [45], around 20-30% of the cultivated land is affected by the salinity globally and this inhibits plant production. Razmjoo et al. [29] indicated that the correction of these affected areas is a main issue in agriculture. In South Africa, the demand for good quality water is escalating due to the presence of specific concentrations of ions such as Na and Cl and this made the water quality to become a key factor [36; 45]. Hassanpouraghdam et al. [15] also reported that saline sodic being a primary restraining problem for plants physiological process limiting field and horticultural crops particularly in arid and semi-arid region with NaCl being the principal salts sources. Moreover, these excess soluble salts contribute to the sodicity of the soil and increased salinity of the feeding water leading to osmotic stress, specific ion toxicity and ionic imbalances [23]. It has been noted that high salinity in the feeding water induces problems relating to metabolic activities in plants from many studies focusing on salinity stress. The effect of salinity varies between plants species and also according to the type of production system used [18; 40]. Said-Al Ahl and Omer [33] reported that accumulation of salts in the root media was caused by rates of evapo-transpiration and poor leaching of water.

Rose geranium oil is produced commercially worldwide for use in perfumery, medicinal and aromatherapy industries [11; 47]. Rose geranium is characterised by lobed leaves, small pink flowers and a rosy aroma [21]. Leaves, flowers and branches are important parts of the plant because this is where most of the oil bearing trichomes are found [1]. Sedibe and Allemann [35] and [12] reported that the yield of rose geranium is affected by nutritional requirements and other environmental factors. Therefore, an integrated nutrient solution management regime with efficient root-media is necessary to ensure proper growth and normal development of rose geranium to improve the yield and essential oil quality. The aim of the experiment was to

evaluate the effect of NaCl (salinity) and root-media selection on yield attributes, oil composition and mineral composition of rose geranium.

Material and Methods

Site and climate descriptions

This experiment was carried out in a greenhouse at Bloemfontein, campus of the University of the Free State located in the semi-arid area with coordinates of 29°10'S and 26°17'E at an altitude of 1395 m above sea-level. The experiment was conducted during summer season and the temperature was kept at maximum 26°C using two axial fans and a wet-wall of the greenhouse which were triggered by a climate adapter (Climate adapter Johnson A419 series USA).

Experimental field plan and crop management

The experiment was laid out in a split plot design, where the main plots consisted of four different salt levels (1.68, 2.40, 3.20 and 4.0 mS cm⁻¹) and the sub-plots consisted of two root media types (silica sand [with a diameter of 2 mm] and sawdust) and the units were replicated three times, arranged in a randomised complete block design. Each salinity concentration level contained 12 potted plants that were split-plotted into two cultivation units (Figure 1). In each unit, six potted plants were grown in different root media, sand and sawdust.

Nutrient composition was prepared according to the levels described by [9] with varying concentrations of Na and Cl (Table 1). The pH of the nutrient solution was maintained at 5.5 in all the experimental units. Salinity shock was reduced by initiating NaCl application gradually in a weekly sequence in the third month after transplanting at 25, 50, 75 and 100% until constant levels were met in all treatment levels.

Rooted cuttings (± 10 cm) Bourbon cultivar of rose geranium was obtained from a commercial grower (Pico-gro RSA). One rooted cutting was planted per each pot of 5 L used [19]. Aphids and red spider mites were problematic during the experimental period. With no registered insecticide for rose geranium, aphids and

red spider mites were controlled by a full cover spray of malasol at 1.75 mL L⁻¹ and abamectin at 1.20 mL L⁻¹. These applications were repeated for three to six days at four-week intervals.

Description of irrigation systems used

A customized small-scale growing units (450 x 800 x 215 cm) adapted from the unit used by [37] was used to grow rose geranium (Figure 1). The irrigation systems had six-dripper tubing with a flow rate of 4 L hour⁻¹; these drippers were allocated to six potted plants. An irrigation pump with a flow rate capacity of 700 L hour⁻¹ was mounted to a 20 mm tubing pipe distributed to the pot-holding tank of the growing unit. All nutrient solutions used were recirculated and replaced with fresh solution every 14 days. Three irrigation system cycles were scheduled for one hour per cycle at 7:00, 11:00 and 15:00.

Parameters measured

Number of leaves, plant height, number of branches and foliar fresh mass (FFM) were determined at harvest following techniques of [27].

Stomatal conductance

Stomatal conductance was determined twice at 10:00 and 14:00 for four days before harvesting. Data were collected on clear days, using a leaf porometer (Decagon SC-1 USA). Measurements were taken on six selected mature leaves [7].

Relative water content

Three leaves on each plant were collected to determine relative water content (RWC). Leaf fresh mass (LFM) was determined immediately at harvesting and subsequently the leaves were immersed in distilled water for 12 hours to determine leaf turgid mass (LTM). Afterwards these samples were dried in an oven set at 60°C for 24 hours to determine the leaf dry mass (LDM). The relative water content was determined by procedure described by [38];

$$\text{RWC}\% = (\text{LFM} - \text{LDM}) \div (\text{LTM} - \text{LDM}) \times 100$$

Chlorophyll content and leaf area

Chlorophyll content was determined according to the procedure described by [8] before harvest using portable non-destructive chlorophyll meter (Optisciences CCM 200 USA). Readings were taken randomly from the upper six mature leaves on the crop. Leaf area was measured using a portable leaf area meter (CI-202 USA) used by [14]. Six mature leaves were harvested from the crop in the following sequence; two from the top, two from the middle and two at the bottom of the plant. Measurements were taken immediately after harvest.

Essential oil extraction and oil composition analysis

Rose geranium oil was extracted from the leaves and stems using a custom-built steam distillation unit [38]. About ±5 kg of fresh plant material was distilled for oil at a temperature of ±98°C for one hour. The mass of the oil volume (yield) was determined by weighing the oil volume using PGL 2002 Adam scale (USA) immediately after extraction as described by [42].

Essential oil compounds were primed by comparing the retention times of the chromatogram peaks. Key oil components determined for oil composition were citronellol, geraniol, linalool, iso-menthone, citronellyl formate, geranyl formate and guaia-6,9-diene. The retention indices were computed from a gas chromatogram that was logarithmically interpolated between the n-alkanes. A homologous series of n-alkanes (C8-C22 Polyscience USA) was used as a standard. The oil concentration data were obtained by electronic integration of peak areas as described by [17].

Mineral analysis

Leaf nitrogen content was determined using the Dumas combustion nitrogen analyzer [22]. Phosphorus, Zn, Fe, Cu and B were measured using a high resolution atomic absorption spectrometer as described by [38], [24] and [32], respectively. Sulphur content was measured following the procedure of [38]. Potassium and Na concentrations were measured by flame photometry [2], while Ca and Mg were determined using a Laser

induced breakdown spectroscopy [24]. Chlorine was estimated by silver ion-titration with a Corning 926 chloridometer [30]. Molybdenum was determined using ultraviolet-visible spectrophotometer [5].

Data analysis

Data was analyzed with a SAS statistical software version 9.2 [34]. Significant differences were compared using Tukey's least significant difference (LSDT) at 5% level of significance [41].

Results and Discussion

Yield and yield attributes

The results on the number of leaves, plant height, number of branches, leaf area, RWC, chlorophyll content, FFM, oil yield and oil content are shown in Table 2. No significant effect of salinity was found on RWC, FFM (herbage yield), oil yield and oil content as tabulated in Table 2. However, the number of leaves ($p < .01$), plant height ($p < .05$), and number of branches ($p < .05$), leaf area ($p < .01$) chlorophyll content ($p < .05$) and FDM ($p < .01$) were significantly affected by salinity. This significant parameters are attributed to yield attributes. Moreover, there was a strong polynomial relationship between number of leaves ($r^2=0.99$), plant height ($r^2=0.88$), number of branches ($r^2=0.97$), leaf area ($r^2=0.99$), chlorophyll content ($r^2=0.92$) and the salinity levels shown in Figure 2, 3, 4, 5 and 6, respectively. Yield attributes of rose geranium were significantly reduced at 4.0 mS cm⁻¹ salinity level and this is associated with the toxicity effects of NaCl within the plant cell. High salinity levels in feeding water induces severe ion toxicity by depositing high levels of Na in plant cells, causing the plant membrane to disorganise and thereafter inhibit cell division and cell expansion [48]. Vacuole is an antiporter and it regulates Na uptake; excess Na ions will be transported and stored in the vacuole [6; 40]. In high levels of Na ions also disrupt the activities of enzymes in the plant cell. According to [26], plants can tolerate salinity up to a certain threshold without any reduction in yield and in this case rose geranium could not withstand high salinity level at 4.0 mS cm⁻¹. High

salinity conditions has been shown to reduce the number of branches, plant height and number of flowers of chamomile [10].

Plant height, number of branches, RWC, chlorophyll content, oil yield and oil content were also not significantly affected by the root-media (Table 2). However, root-media affected the number of leaves ($p < .01$), leaf area ($p < .01$) and FFM ($p < .01$) of rose geranium. The number of leaves, leaf area and FFM were better when using sawdust root-media with an increase of 451.83 plant⁻¹, 799.08 cm² and 511.24 g plant⁻¹, respectively, compared to the sand as shown in Table 2. No significant interactions were found in all yields and yield attributes parameters. The effect of salinity varies between plants species and also according to the type of production system used [18; 40]. Said-Al Ahl and Omer [33] reported that accumulation of salts in the root-media was caused by rates of evapo-transpiration and poor leaching of water. In this case, attributes of sawdust results compared to sand are due to better leaching of water although the parameter was not measured. Sawdust has better and sufficient root-zone moisture, temperature and lower bulk density compared to sand as root-media. According to [38] and [28], sawdust offers better drainage and good aeration and it also provides plants with sufficient moisture, aeration and an optimum ratio between elements in the root-zone. Softwood cuttings (*Ficus binnendijkii* L.) and gypsophila (*Gypsophila paniculata* L.) developed better sprouts when sawdust was used as a substrate [39; 46].

Mineral composition

Figure 7 illustrate the interactions between the salinity levels and root-media. The results found on the data not presented showed that salinity levels, root-media and the interactions were all found significant at $p < .01$. Significant interaction was found between salinity at 4.0 mS cm⁻¹ and using sand as a root-media. Furthermore, leaf mineral content of P ($p < .05$), K ($p < .01$) and Mg ($p < .01$) were significantly high where salinity levels were low at 1.6 mS cm⁻¹ and consequently gradually reduced when salinity levels increased up to 4.0 mS cm⁻¹ (Figure 8). Dissimilar to P,

K and Mg; the leaf mineral content of Na ($p < .01$) and Cl ($p < .01$) were significantly increased at high salinity level (Figure 8). The interaction between salinity at 4.0 mS cm⁻¹ and sand root-media is ascribed to the high application of salinity at 4.0 mS cm⁻¹ and also the accumulation of Na in sand with poor water leaching compared to sawdust [33]. The current study results are in agreement with [13] study on eucalyptus species, whereby high salinity concentration increased the Na and Cl leaf-tissue content. The decrease in K and Mg content is associated to the cation uptake competition between Na, K and Mg which will consequently be followed by the mineral deficiency [9].

Oil composition

As shown in Table 3, salinity had no significant effects on the linalool, iso-menthone, citronellol, geraniol, citronellyl formate and guaia-6,9-diene contents of rose geranium. However, significant results were found on geranyl formate ($p < .05$) and C:G ratio ($p < .01$) where salinity level were high, 4.0 mS cm⁻¹. Furthermore, root-media had no significant on most oil composition content except iso-menthone ($p < .05$). Geranyl formate content was reduced at a high salinity level of 4.0 mS cm⁻¹; consequently the C:G ratio was increased, thus lowering the oil quality based on literature. No significant interaction between salinity and root media was recorded either.

In most salinity studies, it has been shown that salinity affects the biosynthesis of essential oils [37]. Furthermore, it has also been shown that these biosynthesis activities occur inside the palisade cells and are stored in the vacuole. Sodium at high levels tends to accumulate in the plant cell and directly interferes with the biosynthesis of essential oils in the vacuole [6; 37]. Literature was not found that explains in details how salinity affects the synthesis of geranyl formate. However, since salinity is involved in the biosynthetic activities associated with those of essential oil biosynthesis, the significant effects of salinity on geranyl formate and C:G ratio is ascribed to directly interferes with the biosynthesis of essential oils in the vacuole [6; 37]. Beside geranyl formate; other essential oil compounds has been shown to be affected by

salinity. Linalool content of basil and coriander was increased to respectively 57% and 45% at high salinity levels [4]. Good oil quality was reported on sage, where high salinity increased oxygenated monotepernes [43].

Conclusion

Rose geranium yield attributes were found to be significantly reduced by salinity at 4.0 mS cm⁻¹ and also using sand as a root-media although no interactions were found. Oil yield was found statistically non-significant but the trend showed that at salinity at 4.0 mS cm⁻¹ reduced oil yield. Furthermore, essential oil quality was reduced by the increase in salinity levels compared to industrial requirements of 1:1 and 2:1 C:G ratio for Bourbon cultivar. It is evident from the study that rose geranium should be grown using nutrient solution with salinity below 4.0 mS cm⁻¹ for better yield and oil quality using sawdust as a root-media

Table 1 Nutrient solution concentrations used to study the response of rose geranium to salinity and root-media (1.6, 2.4, 3.2 and 4.0 mS cm⁻¹)

Salinity (mS cm ⁻¹)	Ions															
	Micro-nutrients (mmol L ⁻¹)					Micro-nutrients (mg L ⁻¹)										
	Na ⁺	NH ₄ ⁺	K ⁺	Ca ⁺	Mg ²⁺	NO ₃ ⁻	H ₂ PO ₄ ⁻	SO ₄ ²⁻	Cl ⁻	HCO ₃ ⁻	Fe ²⁺	Mn ³⁺	B	Cu ²⁺	Mo ²⁺	Zn ²⁺
1.6	1.31	1.00	5.5	6.50	2.5	11.04	0.10	3.80	1.31	0.40	1.12	0.54	0.03	0.02	0.05	0.18
2.4	8.49	1.00	5.5	6.50	2.5	11.04	0.10	3.80	8.49	0.40	1.12	0.54	0.03	0.02	0.05	0.18
3.2	16.5	1.00	5.5	6.50	2.5	11.04	0.10	3.80	16.5	0.40	1.12	0.54	0.03	0.02	0.05	0.18
4.0	24.5	1.00	5.5	6.50	2.5	11.04	0.10	3.80	24.5	0.40	1.12	0.54	0.03	0.02	0.05	0.18

Table 2 The effect of salinity and root-media on the number of leaves, plant height, number of branches, leaf area, relative water content, chlorophyll, foliar fresh mass, oil yield and oil content of rose geranium

Notes. Means followed by the same letter in the same column are statistically non-significant with ns = not significant at $p < .05$.

* F-ratio probability of $p < .01$.

Treatments	Parameters								
	Number of leaves (plant ⁻¹)	Plant height (cm)	Number of branches (plant ⁻¹)	Leaf area (cm ²)	Relative water content	Chlorophyll (%)	Foliar fresh mass (g plant ⁻¹)	Oil yield (g plant ⁻¹)	Oil content (%)
NaCl level (mS cm ⁻¹)									
1.6	448.44 ^a	43.44 ^a	38.88 ^a	931.16 ^a	84.56 ^a	28.11 ^a	490.83 ^a	1.49 ^a	.31 ^a
2.4	482.11 ^a	42.83 ^a	33.33 ^{ab}	811.05 ^{ab}	81.27 ^a	26.05 ^{ab}	430.27 ^a	1.44 ^a	.35 ^a
3.2	425.44 ^a	43.49 ^a	32.11 ^b	686.11 ^b	86.57 ^a	25.87 ^{ab}	502.50 ^a	1.87 ^a	.37 ^a
4.0	249.94 ^b	36.27 ^b	30.50 ^b	459.44 ^c	83.13 ^a	22.92 ^b	373.33 ^a	1.28 ^a	.35 ^a
LSD _(0.05)	88.64 [*]	4.75 ^{**}	5.91 ^{**}	155.09 [*]	ns	3.22 ^{**}	ns	ns	ns
Root-media									
Sand	351.13 ^b	40.10 ^a	32.66 ^a	644.80 ^b	83.62 ^a	24.92 ^a	387.22 ^b	1.34 ^a	.35 ^a
Sawdust	451.83 ^a	42.91 ^a	34.75 ^a	799.08 ^a	84.15 ^a	26.55 ^a	511.24 ^a	1.70 ^a	.34 ^a
LSD _(0.05)	62.68 [*]	ns	ns	109.66 [*]	ns	ns	85.62 [*]	ns	ns

** F-ratio probability of $p < .05$.

Table 3 Effect of salinity-induced nutrient solution and root-media on oil composition of rose geranium

Notes. Means followed by the same letter in the same column are statistically non-significant with ns = not significant at $p < .05$.

Treatments	Oil composition (%)							
	Linalool	Iso-menthone	Citronellol	Geraniol	Citronellyl formate	Geranyl formate	Guaia-6, 9-diene	C:G ratio
NaCl level (mS cm ⁻¹)								
1.6	1.23 ^a	1.78 ^a	31.51 ^a	13.71 ^a	21.21 ^a	7.98 ^a	9.37 ^a	2.35 ^a
2.4	1.46 ^a	1.95 ^a	31.84 ^a	12.70 ^a	21.55 ^a	7.61 ^a	9.37 ^a	2.54 ^a
3.2	1.48 ^a	2.18 ^a	32.48 ^a	12.62 ^a	21.66 ^a	7.63 ^a	9.51 ^a	2.60 ^a
4.0	1.10 ^a	1.48 ^a	35.19 ^a	11.10 ^a	22.73 ^a	6.53 ^b	9.49 ^a	3.18 ^b
LSD (0.05)	ns	ns	ns	ns	ns	.94 ^{**}	ns	.40 [*]
Root-media								
Sand	1.04 ^a	2.54 ^a	32.58 ^a	12.57 ^a	21.97 ^a	7.53 ^a	9.71 ^a	2.66 ^a
Sawdust	1.39 ^a	1.66 ^b	32.98 ^a	12.50 ^a	21.60 ^a	7.32 ^a	9.16 ^a	2.68 ^a
LSD (0.05)	ns	.79 ^{**}	ns	ns	ns	ns	ns	ns

* F-ratio probability of $p < .01$.

** F-ratio probability of $p < .05$.

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Appendix

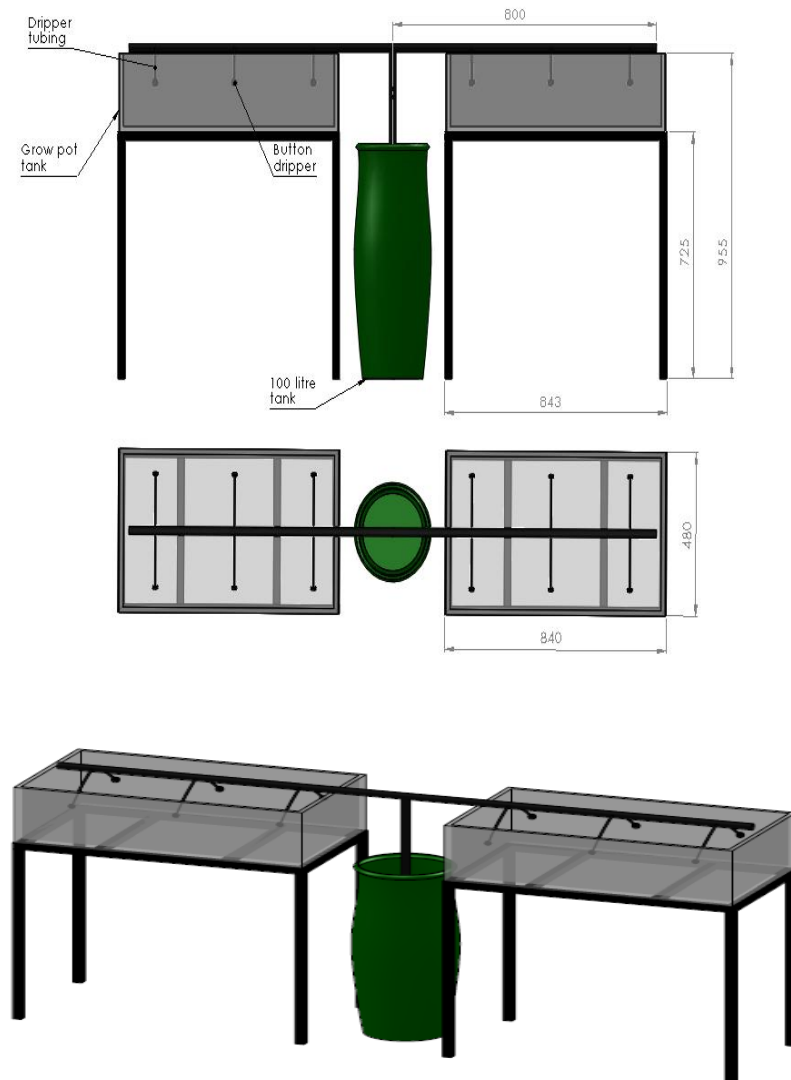


Figure 1. Schematic representation of small-scale growing unit and irrigation system used to grow rose geranium plants

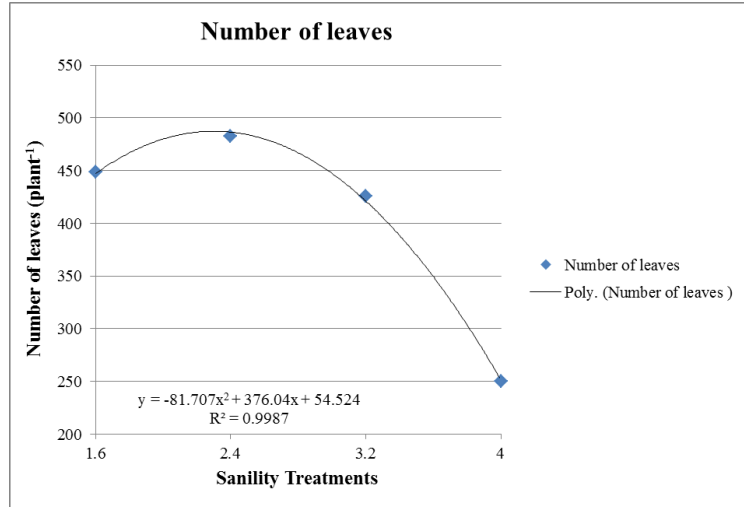


Figure 2. Polynomial relationship between number of leaves and salinity levels (1.6, 2.4, 3.2 and 4.0 mS cm⁻¹)

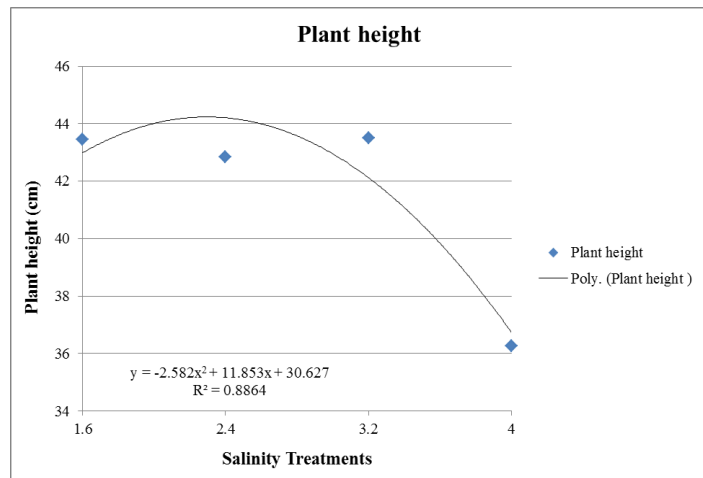


Figure 3. Polynomial relationship between plant height and salinity levels (1.6, 2.4, 3.2 and 4.0 mS cm⁻¹)

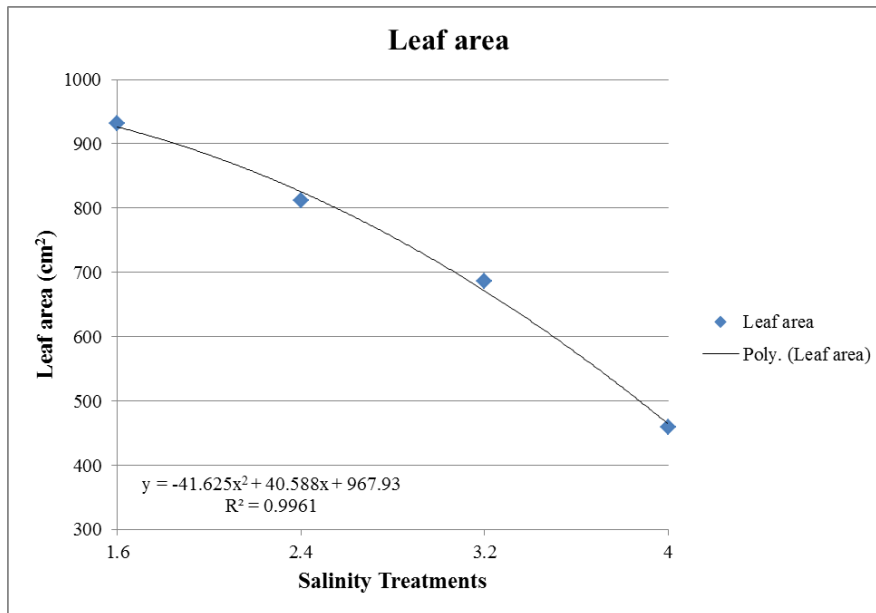


Figure 4. Polynomial relationship between leaf area and salinity levels (1.6, 2.4, 3.2 and 4.0 mS cm⁻¹).

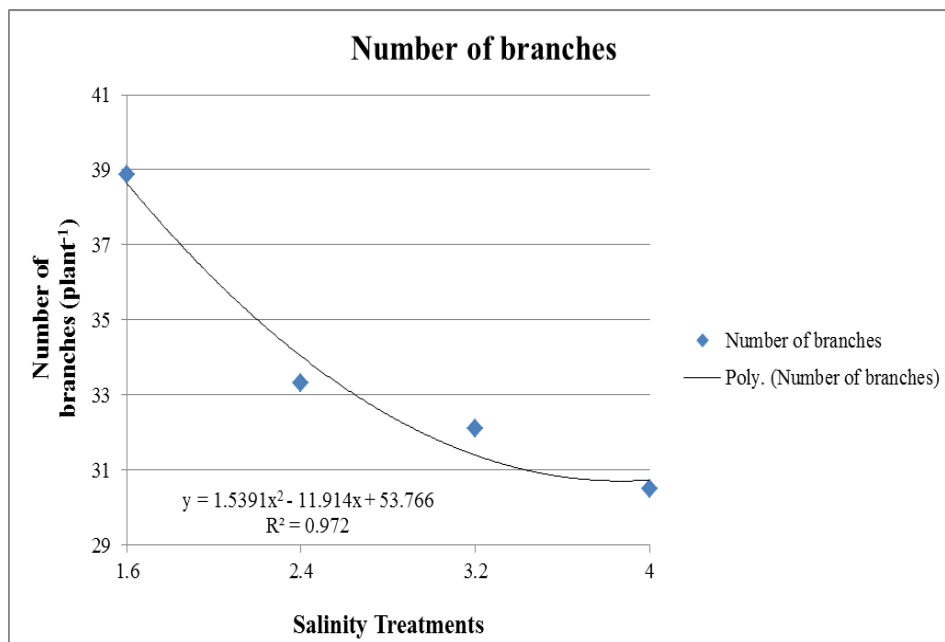


Figure 5. Polynomial relationship between number of branches and salinity levels (1.6, 2.4, 3.2 and 4.0 mS cm⁻¹).

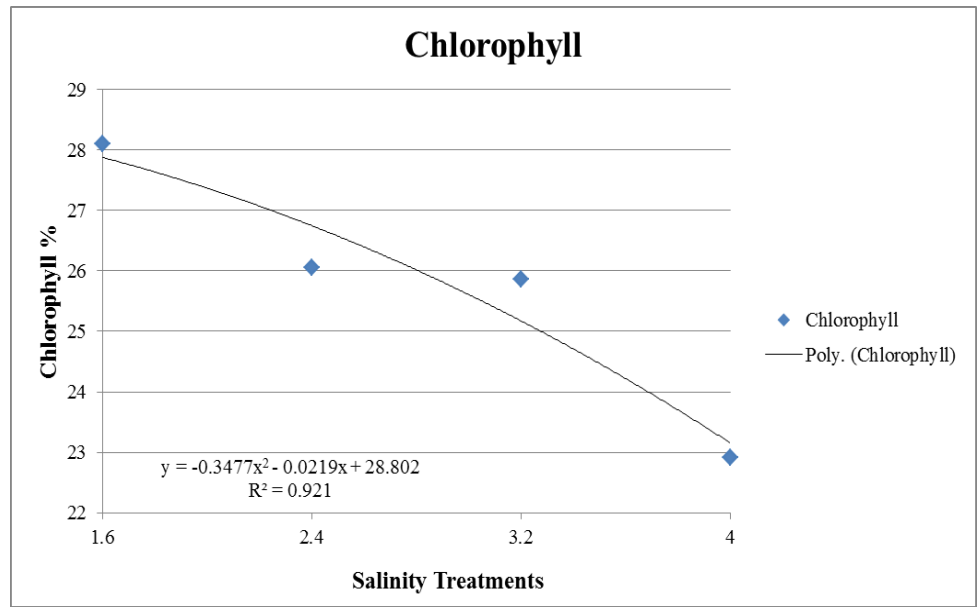


Figure 6. Polynomial relationship between chlorophyll content and salinity levels (1.6, 2.4, 3.2 and 4.0 mS cm⁻¹).

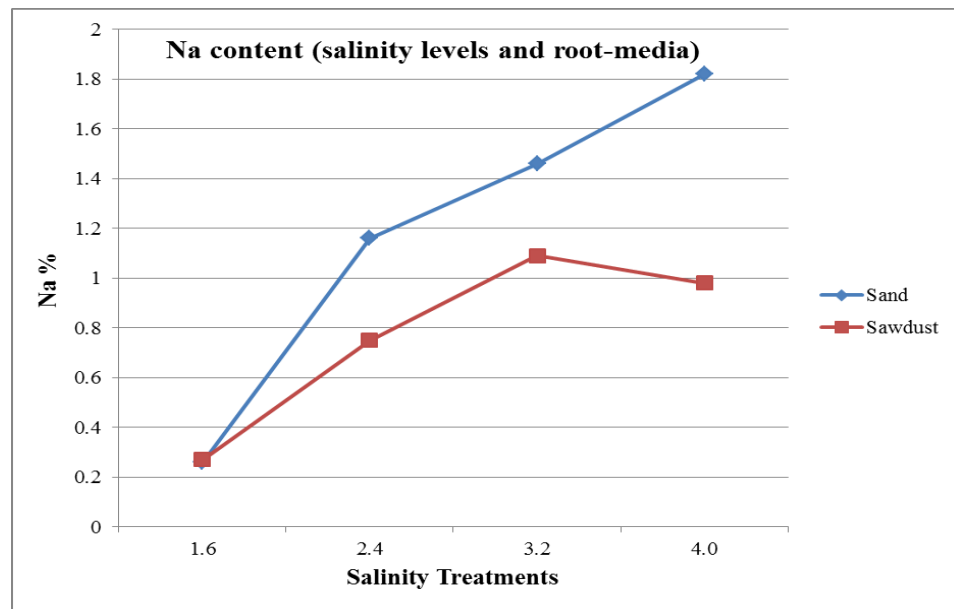


Figure 7. Interaction effects of Na content between salinity concentrations and root-media of rose geranium.

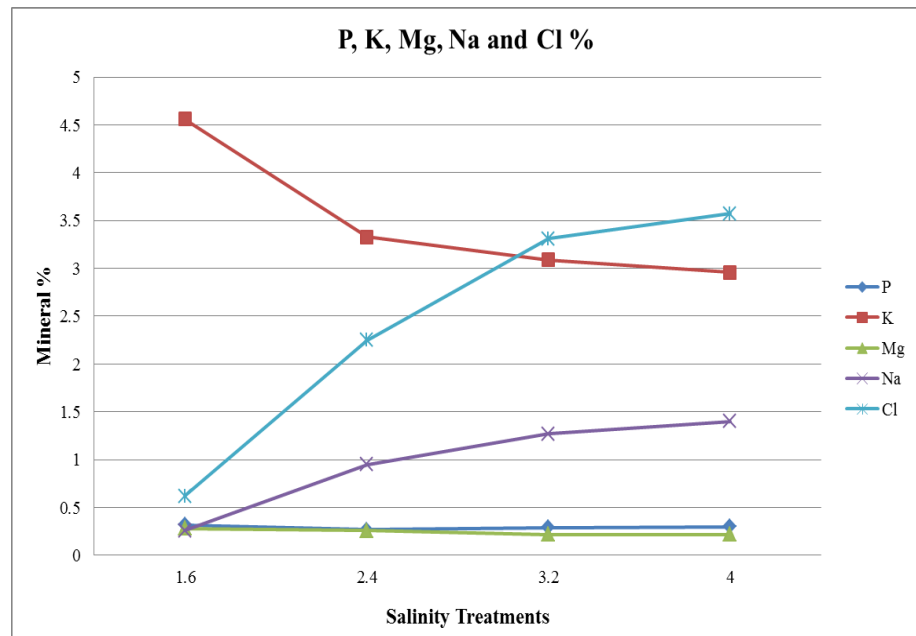


Figure 8. Effects of salinity-induced nutrient solution and root-media on P, K, Mg, Na and Cl content of rose geranium