# THE PHYSIOLOGICAL AND QUALITY RESPONSE OF PELARGONIUM GRAVEOLENS (L.) GROWN ON NITRATE AND AMMONIUM NUTRIENT SOLUTIONS

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(Received 29th Jun 2020; accepted 17th Sep 2020)

**Abstract.** The objective of this study was to evaluate the effects of nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>) on the yield, mineral content and oil quality of rose geranium. Nitrate, at concentrations between eight and 14 meq L<sup>-1</sup>, and, ammonium at concentrations between 0.0 and 1.5 meq L<sup>-1</sup>, was applied to rose geranium. Nitrate (10 meq L<sup>-1</sup>) produced the most significant increase in plant height, foliar fresh mass, foliar dry mass, oil yield and oil content. Ammonium applied at 1.0 meq L<sup>-1</sup> had no significant effects on agronomic-related attributes (e.g. plant height, foliar fresh mass, and foliar dry mass, etc.), only folia N (5%), P (10%) and S (22%) levels were increased. Principal component analysis was used to reduce the redundancy of the NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> data, and the eigenvalue of the correlation matrix. For the NO<sub>3</sub><sup>-</sup> study, PC1 and PC2 accounted for most of the variability, showing a cumulative variability> 55.5%: PC1 accounted for 42.20%, while PC2 accounted for 13.31% of the total variance. For NH<sub>4</sub><sup>+</sup>, PC1 and PC2 accounted for most of the variability> 47.1%: PC1 accounted for 26.54%, while PC2 accounted for 20.56% of the total variance. For successful cultivation of rose geraniums, nutrient solutions should contain optimal concentrations of NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>.

Keywords: chlorophyll, geranium oil, hydroponically, ion, mineral

### Introduction

Production of rose geranium (*Pelargonium graveolens* L.) oil occurs in South Africa (Sedibe and Allemann, 2012); local producers export geranium oil to the USA, Japan, and Europe, with an annual income estimated between 34 and 57 million USD (DAFF, 2017). In South Africa, geranium oil is a key component in the production of many food and beverage products (Rao et al., 1996; Sedibe, 2012), and is an active ingredient in skin care products (Sedibe, 2012).

In order to obtain a profitable income, high quality standards of rose geranium oil have to be met. This is an on-going challenge for South African producers, since weather parameters such as temperature and rainfall affect oil quality and these effects are profound on geraniol, citronellol and citronellylformate compounds of rose geranium oil (Prakasa Rao et al., 1995). Growers mitigate the effects of weather by management of the plants' nutrient regime (Sedibe and Allemann, 2012; Nyakane et al., 2019).

Rose geraniums need between 100 kg to 200 kg N ha<sup>-1</sup>, depending on the soil nutrient status (Singh et al., 1996, 1999; Ram et al., 2003; Araya et al., 2006). Nitrogen is the only nutrient element that can be formulated in ionic solution either as the cation ( $NH_4^+$ ) or the anion ( $NO_3^-$ ). For greenhouse crops, most of the N is supplied in the form of  $NO_3^-$ . However, small quantities of  $NH_4^+$ , are used by soil-less growers to acidify their nutrient solution, consequently preventing precipitating Fe and Zn (Combrink, 2019). The objective of this study was to evaluate the effects of  $NO_3^-$  and  $NH_4^+$  on the yield, the mineral content and the oil quality of rose geranium oil, using a multivariate principle component analysis (PCA).

### Material and methods

### **Research** site

This trial was conducted in a greenhouse at the west campus facility at the University of the Free State in Bloemfontein, situated in the Free State province of South Africa. The site is at an altitude of 1351 m above sea level ( $29^{\circ}07'16.78''$  S,  $26^{\circ}12'45.95''$  E). A wet wall pad and two fan systems were used to control temperature to  $\pm 26^{\circ}$  in the greenhouse.

### Experimental design

Rose geranium cuttings were rooted for eight weeks by a commercial grower (Pico-gro, RSA) prior to planting. Plants were then planted in 5 L pots filled with 2 mm sterile silica sand. Plants were grown for five months during 2016 growing season. Each treatment was replicated five times in a randomised complete block design. Each experimental unit had eight pots containing one plant (Sedibe, 2012). *Table 1* shows concentrations of each ion in the nutrient solutions, in order to fulfil the experimental concentrations, NO<sub>3</sub><sup>-</sup> levels were applied at 8, 10, 12 and 14 meq L<sup>-1</sup> concentrations and NH<sub>4</sub><sup>+</sup> were applied using 0.0, 0.5, 1.0 and 1.5 meq L<sup>-1</sup> concentrations.

**Table 1.** Ion ratio used in the nutrient solutions to test the effect of  $NO_3^-$  and  $NH_4^+$  concentrations on rose geranium growth and oil quality parameters

Nitrate ion (meq L <sup>-1</sup> )									
$\mathrm{NH_{4}^{+}}$	$\mathbf{K}^+$	Ca <sup>2+</sup>	$Mg^{2+}$	NO <sub>3</sub> -	$H_2PO_4^-$	$SO_4^=$			
1.00	5.50	6.50	2.50	8.00	2.11	4.83			
1.00	5.50	6.50	2.50	10.00	1.50	3.44			
1.00	5.50	6.50	2.50	12.00	0.89	2.05			
1.00	5.50	6.50	2.50	14.00	0.28	0.66			
Ammonium ion (meq L <sup>-1</sup> )									
$\mathrm{NH_{4}^{+}}$	$\mathbf{K}^+$	Ca <sup>2+</sup>	$Mg^{2+}$	NO <sub>3</sub> -	$H_2PO_4^-$	$SO_4^=$			
0.00	5.88	6.95	2.67	10.00	1.50	3.44			
0.50	5.69	6.72	2.59	10.00	1.50	3.44			
1.00	5.50	6.50	2.50	10.00	1.50	3.44			
1.50	5.31	6.28	2.41	10.00	1.50	3.44			

A "drain-to-waste" drip system was used to fertigate all the pots, using four irrigation cycles per day, scheduled at 08:00, 11:30, 14:00 and 18:00 for 30 min (Sedibe et al., 2005). The emitter flow rate was set to 4 L hour<sup>-1</sup>. As plants developed and the demand for water and nutrients increased, volumes were increased to ensure that 10% to 25% solution drained-to-waste, to prevent salt accumulation in the potting bags (Combrink, 2019). A fresh solution was mixed every two weeks.

During the experimentation period no diseases occurred. Aphids were controlled with organophosphate, using levels prescribed for ornamentals in South Africa (0.5 ml L<sup>-1</sup>). A full cover spray was applied and then this application was repeated after three and six days, following the method of Nyakane et al. (2019) and Sedibe and Allemann (2012).

The nutrient solutions were maintained at pH 6.98 (±0.02), with electrical conductivity (EC) of 1.60 (± 0.1) mS cm<sup>-1</sup>. The EC level of the solution was maintained by proportionally lowering the concentrations of phosphate and sulphate with increased

nitrate concentrations (Table 1). Micronutrients (mg L<sup>-1</sup>) used in all the experiments were 6.0 Fe; 0.21 B; 0.03 Cu; 0.2 Zn; 0.55 Mn; and 0.05 Mo (Sedibe and Allemann. 2012: Combrink, 2019). The nutrient solutions were made up in 1000 L tanks, which served as reservoirs for the treatments. The feeding water had an EC of 0.2 mS cm<sup>-1</sup> and a pH of 6.92. Expressed as meq L<sup>-1</sup>, it contained 0.48 Na; 0.05 K; 1.0 Ca; 0.45 Mg; 0.01 N; 0.15 SO<sub>4</sub>; 0.21 Cl; and a total alkalinity of 1.18 (HCO<sub>3</sub>). The alkalinity was lowered to 0.04 meq  $L^{-1}$  by applying 60% nitric acid at 0.78 meq  $L^{-1}$ .

Plant height, foliar fresh mass (FM), and foliar dry mass (DM) were measured at harvesting. DM was determined by drying the harvested material at 68°C for 72 hours. The dried leaves were milled to 0.25 mm diameter using a micro hammer mill (Culatti, Zurich) (Jones et al., 1991). These leaves were subsequently used for the analyses of Ca, Mg, P, K, S, and N. Content of Ca, Mg, P, and K in the plant tissue was determined using inductively coupled plasma optical emission spectrometry (ICP-OES) (Optima 4300 DV, PerkinElmer Inc. USA) (Van Maarschalkerweerde and Husted, 2015). Sulphur content was also determined by ICP-OES using an extract solution (ICP-OES; JY Horiba Ultima, USA) and set at a wavelength of 181.978 nm (Van Maarschalkerweerde and Husted, 2015). The chlorophyll content was determined from the upper six mature leaves on the crop, using a chlorophyll meter (Optisciences CCM 200, USA), following the procedure described by Chen and Black (1992).

Nitrogen content was determined following the Dumas combustion method in a Leco FP-528 combustion N analyser (LecoCorp. St. Joseph, MI, USA) (Matejovic, 1995; Etheridge et al., 1998). Fresh biomass (2 kg - 5 kg) was collected for the extraction of essential oil. The oil was extracted from the stem and leaves using water and steam distillation for one hour (Motsa et al., 2006) in a customised 5 kg test distiller (PA Pretorius). Oil quality was determined by gas chromatography (GC); an Aligent GC (FID; model 6890N) fitted with a 30 mm x 0.25 mm DB-5 fused silica capillary column, and film thickness of 0.25 µm (Novák et al., 2001; Adams, 2004).

### Statistical analysis

Analyses of variance were conducted using the general linear model (GLM) procedure of statistical analysis system (SAS) version 9.3 (SAS, 2017). A regression analysis was also run using the SAS program. Significant results were analysed using Tukey's while Fischer's test was used to calculate the least significant difference test (LSD), described by Steel and Torrie (1980). Statistically significant differences between treatment means was determined at the 5% level of significance.

Basic formulae were used in performing PCA, by adjusting the data matrix, X, which consists of *n* observations (rows) on *p* variables (columns) as described by NCSS (2019).

The basic equation of PCA is, in matrix notation, given by:

$$Y=WX'$$
(Eq.1)

where W is a matrix of coefficients that is determined by PCA.

These equations are also written as:

$$Y_{ij} = {}_{W1i} x_{1j} + {}_{W2i} x_{2j} + \dots + {}_{Wpi} x_{pj}$$
 (Eq.2)

As seen, the components are a weighted average of the original variables. The weights, W, are constructed so that the variance of  $y_1$ ,  $Var(y_1)$ , is maximized. Also, so that  $Var(y_2)$  is maximized and that the correlation between  $y_1$  and  $y_2$  is zero. The remaining yi's are calculated so that their variances are maximized, subject to the constraint that the covariance between  $y_i$  and  $y_j$ , for all i and j (i not equal to j), is zero.

## Results

### Yield, mineral and oil quality attributes

The yield attributes, mineral content and oil quality parameters of rose geranium are shown in *Table 2*. Plant height, FM, DM, oil yield and oil content were all significantly affected by nitrate content (P<0.05). The optimal NO<sub>3</sub><sup>-</sup> level for all of these parameters was 10.0–12.0 meq L<sup>-1</sup>. Increases of 24% (plant height,) 40% (FM), 50% (DM), 60% (oil yield) and 17% (oil content) were observed at 10.0 NO<sub>3</sub><sup>-</sup> meq L<sup>-1</sup>. Only chlorophyll content had an optimal NO<sub>3</sub><sup>-</sup> concentration that was significantly different (12.0 meq L<sup>-1</sup>; P<0.001). Geraniol and geranyl formate were significantly affected by NO<sub>3</sub><sup>-</sup>, though the optimum concentration could not be established as concentrations varied in an inconsistent manner. The optimal NO<sub>3</sub><sup>-</sup> concentration for citronellyl formate was also 10.0–12.0 meq L<sup>-1</sup>, an increase of 8% compared to other treatments (P<0.05).

Nitrate (meq L <sup>-1</sup> )	Plant height (cm)	Foliar fresh mass (g plant <sup>-1</sup> )	Oil yield (g plant <sup>-1</sup> )	Oil content (%)	Foliar dry mass (g plant <sup>-1</sup> )	Chlorophyll
8.00	46.60±2.88°	585.00±112.64 <sup>b</sup>	$0.68 \pm 0.68^{b}$	$0.12 \pm 0.04^{b}$	$124.93{\pm}15.82^{b}$	$18.33 \pm 0.74^{b}$
10.00	57.00±6.16 <sup>b</sup>	820.20±159.63ª	1.09±1.09 <sup>a</sup>	$0.14{\pm}0.02^{ab}$	187.12±29.79 <sup>a</sup>	20.14±1.13 <sup>b</sup>
12.00	$55.80{\pm}7.40^{ab}$	822.80±59.44 <sup>a</sup>	1.16±1.16 <sup>a</sup>	$0.14{\pm}0.04^{ab}$	192.04±22.38 <sup>a</sup>	24.24±3.33ª
14.00	$49.40 \pm 1.67^{bc}$	455.20±101.12 <sup>b</sup>	$0.75 \pm 0.75^{b}$	$0.17 \pm 0.02^{a}$	$120.79 \pm 13.07^{b}$	$23.13 \pm 1.49^{a}$
LSD <sub>T0.05</sub>	7.10*	147.18***	$0.26^{*}$	0.03*	29.80***	2.87***
Nitrate	Ν	Р	K	Ca	Mg	S
8.00	3.36±0.22ª	0.51±0.06 <sup>a</sup>	2.91±0.31 <sup>ab</sup>	2.02±0.15 <sup>a</sup>	0.35±0.03ª	0.49±0.05ª
10.00	3.39±012 <sup>a</sup>	$0.43 \pm 0.02^{b}$	2.61±0.27 <sup>b</sup>	1.77±0.89 <sup>b</sup>	$0.35{\pm}0.02^{a}$	0.43±0.01ª
12.00	$3.35{\pm}0.08^{a}$	$0.38 \pm 0.03^{b}$	2.97±0.28 <sup>a</sup>	2.00±0.18 <sup>a</sup>	$0.36{\pm}0.04^{a}$	$0.34{\pm}0.07^{b}$
14.00	$3.39{\pm}0.37^{a}$	0.27±0.03°	$2.78{\pm}0.26^{ab}$	1.73±0.08 <sup>b</sup>	$0.21{\pm}0.01^{a}$	0.26±0.03°
LSD <sub>T0.05</sub>	0.35 <sup>NS</sup>	0.06***	0.32**	0.19*	0.34 <sup>NS</sup>	$0.08^{***}$
Nitrate	Linalool	Citronellol	Geraniol	Citronellyl- formate	Geranyl- formate	Guaia6,9diene
8.00	1.96±1.09 <sup>a</sup>	31.91±1.22 <sup>a</sup>	13.94±0.88 <sup>a</sup>	19.66±2.07 <sup>b</sup>	8.61±1.03 <sup>a</sup>	9.69±0.71ª
10.00	1.52±0.41ª	33.46±1.39 <sup>a</sup>	10.39±1.05 <sup>b</sup>	21.39±0.96 <sup>ab</sup>	$7.98{\pm}0.45^{ab}$	$9.49{\pm}0.24^{a}$
12.00	1.89±0.61ª	$32.31{\pm}1.87^{a}$	10.25±0.63 <sup>b</sup>	$22.31 \pm 0.48^{a}$	$7.47 \pm 0.44^{b}$	$10.39{\pm}0.54^{a}$
14.00	1.25±1.18 <sup>a</sup>	31.50±1.50 <sup>a</sup>	12.87±1.32 <sup>a</sup>	20.20±1.36 <sup>b</sup>	$8.44{\pm}0.28^{a}$	$10.17 \pm 0.59^{a}$
LSD <sub>T0.05</sub>	0.72 <sup>NS</sup>	2.17 <sup>NS</sup>	$1.40^{***}$	$1.87^{*}$	0.85*	0.74 <sup>NS</sup>

*Table 2.* Yield, mineral content and oil quality of rose geraniums cultivated using a nutrient solution with variable nitrate content

NS not significant at P<0.05; \*significant at P<0.05; \*\* significant at P<0.01; \*\*\* significant at P<0.001. Means ( $\pm$ standard deviation). The same superscript letter within a column denotes non significance (P>0.05) As shown in *Table 3*, ammonium had no significant effect on agronomic-related attributes (e.g. plant height, FM, DM, oil yield, oil and chlorophyll content). Significant changes were mostly observed on the foliar mineral contents.  $NH_4^+$  at 1.0 meq L<sup>-1</sup> increased folia levels of N (5%), P (10%) and S (22%). Foliar Mg content and linalool were also significantly affected by ammonium concentrations (P<0.05), but the optimal nutrient level was unclear. Citronellol, geraniol and citronellyl formate were not significantly affected by ammonium content (P>0.05). The optimal NH<sub>4</sub><sup>+</sup> concentration for geranyl formate and guai6,9diene was 1.00 meq L<sup>-1</sup> (P<0.01 and P<0.05, respectively).

Ammonium (meq L <sup>-1</sup> )	Plant height (cm)	Foliar fresh mass (g plant <sup>-1</sup> )	Oil yield (g plant <sup>-1</sup> )	Oil content (%)	Foliar dry mass (g plant <sup>-1</sup> )	Chlorophyll
0.00	67.00±6.51ª	760.20±119.88ª	$0.85{\pm}0.18^{a}$	$0.112{\pm}0.02^{a}$	170.34±8.92 <sup>a</sup>	$21.90{\pm}1.51_{a}$
0.50	$63.20{\pm}5.35^{a}$	803.20±118.20 <sup>a</sup>	$0.99{\pm}0.20^{a}$	$0.124{\pm}0.02^{a}$	$170.00 \pm 14.42^{a}$	$24.266 \pm 3.20^{a}$
1.00	$60.20{\pm}5.80^{\mathrm{a}}$	790.40±90.50ª	1.00±0.11ª	$0.127{\pm}0.12^{a}$	$158.28{\pm}14.02^{a}$	26.17±4.155ª
1.50	$60.60{\pm}10.18^{a}$	777.80±123.33ª	$1.00{\pm}0.09^{a}$	$0.130{\pm}0.01^{a}$	161.05±42.57ª	23.70±2.41ª
LSD <sub>T0.05</sub>	9.39 <sup>NS</sup>	168.53 <sup>NS</sup>	0.20 <sup>NS</sup>	0.01 <sup>NS</sup>	11.12 <sup>NS</sup>	4.41 <sup>NS</sup>
Ammonium	Ν	Р	K	Ca	Mg	S
0.00	3.12±0.14 <sup>b</sup>	0.40±0.01 <sup>b</sup>	2.29±0.15 <sup>a</sup>	1.77±0.10 <sup>a</sup>	$0.37 \pm 0.01^{b}$	0.32±0.01°
0.50	$3.11 \pm 0.16^{b}$	0.41±0.03ª	2.53±0.24 <sup>a</sup>	1.74±0.11ª	$0.40{\pm}0.02^{a}$	0.33±0.05°
1.00	3.29±0.12 <sup>a</sup>	0.44±0.01ª	$2.38{\pm}0.10^{a}$	$1.85 \pm 0.07^{a}$	$0.37{\pm}0.01^{b}$	$0.39{\pm}0.01^{b}$
1.50	3.31±0.17 <sup>a</sup>	$0.40{\pm}0.03^{b}$	2.40±0.14 <sup>a</sup>	1.76±0.12 <sup>a</sup>	0.30±0.01°	$0.43{\pm}0.02^{a}$
LSD <sub>T0.05</sub>	0.12***	0.03***	0.23 <sup>NS</sup>	0.13 <sup>NS</sup>	$0.02^{***}$	$0.04^{***}$
Ammonium	Linalool	Citronellol	Geraniol	Citronellyl- formate	Geranyl- formate	Guaia6,9diene
0.00	1.77±0.28 <sup>a</sup>	33.46±2.32ª	12.68±1.22 <sup>a</sup>	20.48±0.34a	9.18±0.58 <sup>ab</sup>	9.17±0.61 <sup>b</sup>
0.50	$1.80{\pm}0.36^{a}$	33.38±1.09ª	11.78±1.03 <sup>a</sup>	20.70±0.58a	$9.14{\pm}0.34^{ab}$	9.16±0.42 <sup>b</sup>
1.00	1.06±0.32 <sup>b</sup>	31.86±1.14 <sup>a</sup>	10.82±2.31ª	20.65±2.39a	$9.97{\pm}0.68^{a}$	$10.27 \pm 0.69^{a}$
1.50	1.71±0.26 <sup>a</sup>	33.79±2.47ª	11.99±1.10 <sup>a</sup>	20.36±1.90a	$8.58 {\pm} 0.75^{b}$	9.43±0.27 <sup>b</sup>
LSD <sub>T0.05</sub>	$4.28^{*}$	2.75 <sup>NS</sup>	2.16 <sup>NS</sup>	2.41 <sup>NS</sup>	$0.88^{**}$	0.69**

*Table 3.* Yield, mineral content and oil quality of rose geraniums cultivated using a nutrient solution with variable ammonium content

NS not significant at P<0.05; \*significant at P<0.05; \*\* significant at P<0.01; \*\*\* significant at P<0.001. Means ( $\pm$ standard deviation). The same superscript letter within a column denotes non significance (P>0.05)

### Nitrate and ammonium principal component analysis

*Table 4* shows the PCA used to reduce the redundancy of the NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> data, and the eigenvalue of correlation matrix. *Table 4* and *Fig. 1B* show that for the nitrate treatment, out of nineteen principal components (PC) used, the first two (PC1 and PC2) accounted for most of the variability, with a cumulative variability >55.5%. PC1 accounted for 42.2%, while PC2 accounted for 13.31% of the total variance. *Table 4* and *Fig. 1B* show that plant height, FM, oil yield, DM, total biomass, citronellol, geraniol and Mg content were loaded positively on PC1, however linalool, Ca, P and S were loaded on PC2. This confirms that the majority of the tested variables were significantly affected by NO<sub>3</sub><sup>-</sup> levels between 10 and 12 meq L<sup>-1</sup>. Geraniol was the only compound was positively affected by the application of NO<sub>3</sub><sup>-</sup> at 8 meq L<sup>-1</sup>.

Nitrate				Ammonium				
Principal component	PC1	PC2	PC3	Principal component	PC1	PC2	PC3	
Eigenvalue	13.083	4.125	2.652	Eigenvalue	7.433	5.757	3.475	
% of variance	42.204	13.307	8.555	% of variance	26.545	20.561	12.409	
Cumulative % of total variance	42.204	55.511	64.066	Cumulative % of total variance	26.545	47.106	59.515	
Factor loadings				Factor loadings				
Height	0.398	0.099	0.130	Height	0.289	0.214	0.018	
Foliar fresh mass	0.704	0.164	0.000	Foliar fresh mass	0.583	0.028	0.092	
Oil yield	0.665	0.000	0.257	Oil yield	0.187	0.694	0.026	
Oil content	0.002	0.338	0.434	Oil content	0.050	0.652	0.009	
Foliar dry mass	0.862	0.000	0.013	Foliar dry mass	0.726	0.081	0.043	
Total biomass	0.903	0.005	0.007	Total biomass	0.736	0.144	0.089	
Chlorophyll	0.102	0.297	0.069	Chlorophyll	0.008	0.329	0.065	
Linalool	0.000	0.293	0.219	Linalool	0.070	0.408	0.226	
isoMenthone	0.046	0.181	0.011	isoMenthone	0.001	0.146	0.002	
Citronellol (C)	0.272	0.143	0.015	Citronellol (C)	0.316	0.018	0.175	
Geraniol (G)	0.572	0.089	0.114	Geraniol (G)	0.080	0.236	0.107	
Citronellyl- formate	0.269	0.188	0.025	Citronellyl- formate	0.001	0.053	0.012	
Geranyl-formate	0.347	0.038	0.127	Geranyl-formate	0.204	0.066	0.356	
Guaia6,9diene	0.000	0.024	0.220	Guaia6,9diene	0.011	0.178	0.210	
C:G ratio	0.668	0,029	0.071	C:G ratio	0.200	0.203	0.033	
Ν	0,038	0,018	0.000	Ν	0.006	0.011	0.304	
Mg	0.314	0.198	0.015	Mg	0.009	0.013	0.012	
Ca	0.054	0.236	0.002	Ca	0.097	0.012	0.613	
Р	0.001	0.731	0.017	Р	0.015	0.017	0.566	
К	0.004	0.084	0.013	K	0.086	0.044	0.003	
S	0.005	0.587	0.018	S	0.083	0.220	0.006	

**Table 4.** Eigenvalues and principal component factor loading of parameters affected by nitrateand ammonium

Values in bold correspond to the factor with the greatest squared cosine

In the ammonium treatment, the first two principal components (PC1 and PC2) accounted for most of the variability, with a cumulative variability > 47.1%. PC1 accounted for 26.54%, while PC2 accounted for 20.56% of the total variance. *Table 4* and *Fig. 1B* show that plant height, FM, DM, total biomass, and citronellol were loaded at PC1, while, in contrast to the NO<sub>3</sub><sup>-</sup> results, oil yield, oil content, chlorophyll and linalool were loaded on PC2.



*Figure 1.* Rotated principal component analysis of measured yield, mineral and oil quality parameters of rose geraniums grown under A) variable ammonium  $(NH_4^+)$  concentrations; and B) variable nitrate  $(NO_3^-)$  concentrations

#### Discussion

In South Africa, growers use both nitrate and ammonium in nutrient solutions as a source of N, however, most of N comes from NO<sub>3</sub><sup>-</sup>. Owing to its small size, and single charge ion, NH4<sup>+</sup> is easily absorbed by roots, resulting in the secretion of H<sup>+</sup>. However, it is often applied in smaller quantities, used mainly to reduce pH at the roots. In other countries, greenhouse crops are grown using nitrate as the sole N source, and the pH of these nutrient solutions tend to increase towards the root zone. Where organic substrates are used, bicarbonate is released during decomposition, thus increasing the alkalinity and raising the substrate pH (Benoit, 2003). Increasing the NH<sub>4</sub><sup>+</sup> level can counteract this, especially on crops that are sensitive to Ca-deficiency (Combrink, 2019), however, Kafkaffi (2003) reported that damage by  $NH_4^+$  increases at high root zone temperatures. Ammonium is taken up by plant cells by means of  $NH_4^+$  transporters in the plasma membrane and distributed to intracellular compartments, such as chloroplasts, mitochondria and vacuoles (Howitt and Udvardi, 2000). The recommended optimum ratio of  $NH_4^+$  to  $NO_3^-$  for most summer crops is 1:10. For acid-loving crops, the recommendation is 1:1 (Pienaar, 2005; Combrink, 2019), and for blueberries it is 3:1 (Sonneveld, 2002; Combrink, 2019). Plant growth is impaired by using  $NH_4^+$  as the sole source of N (Claussen and Lenz, 1999).

The optimum NO<sub>3</sub><sup>-</sup> concentration for most parameters measured in this study was between 10 and 12 meq L<sup>-1</sup>. Steiner's universal nutrient solution contains 12, 1 and 7 meq L<sup>-1</sup> of NO<sub>3</sub><sup>-</sup>, H<sub>2</sub>PO<sub>4</sub> and SO<sub>4</sub>, respectively (Steiner, 1984). Thus, nitrate represents 60% of Steiner' total anion application. In this trial the optimum anion ratio can be estimated at about 11 meq L<sup>-1</sup> (74%), with 1.2 meq H<sub>2</sub>PO<sub>4</sub> L<sup>-1</sup> and 2.75 meq SO<sub>4</sub> L<sup>-1</sup>. The level of NO<sub>3</sub><sup>-</sup> in rose geranium cultivation is crucial for optimising yield, in terms of height, FM, DM, oil and oil content, and for foliar content of P, K, S, geraniol, citronellyl-formate and geranyl-formate. Nitrogen promotes optimum plant growth by increasing cytokinin production. The synthesized cytokinin encourages cell wall elasticity, by increasing the number of meristematic cells, and cell growth (Razaq et al., 2017). There is a positive relationship between chlorophyll and N-content of the leaves (Abasi et al., 2016; Gholizadeha et al., 2017) and chlorophyll content can be used as an alternative measure for N status of most plant species (Fontes and de Araujo, 2006). This relationship between chlorophyll and foliar N content was not found in this study, possibly due to the increase in biomass production that occurred at increased nitrate levels. It is interesting to note that higher ammonium increased foliar N content and leaf chlorophyll was not affected.

It is known that high concentrations of  $NH_4^+$  in nutrient solutions may suppress the uptake of calcium (Abasi et al., 2016). In this study foliage Ca content was not affected by  $NH_4^+$  at levels of up to 1.5 meq L<sup>-1</sup>. Environmental conditions varied in the greenhouse that could have stimulated or suppressed transpiration, so causing differences in foliar Ca concentrations. As a result, as well as the fact that foliage Ca concentrations were well within limits for healthy plant growth (1.72%-2.06%), inconsistent effects of  $NO_3^-$  concentrations on foliage Ca occurred. Calcium accumulation in plant tissues is affected by Ca uptake by the roots as well as the transpiration rate of the involved plant species. (Adams and Holder, 1992; Combrink, 2019). High humidity, high temperature, low temperature and low transpiration rates may induce Ca deficiency (Olle and Bender, 2009).

To ensure high herbage and oil yields, rose geraniums need to be grown at a relatively high nitrate concentration, of between 10 to 12 meq L<sup>-1</sup>. At these nitrate concentrations, plant height, FM, oil yield, DM, total biomass, citronellol, geraniol, C:G ratio and Mg content were loaded positively on PC1, however linalool, Ca, P and S were loaded on PC2. The ammonium concentrations evaluated in this study had no effect on herbage and oil yield, but affected foliar N and S content. Given that some oil quality parameters were also affected by ammonium; this provides useful information to growers that 1.00 meq L<sup>-1</sup> ammonium might be sufficient for hydroponically grown rose geraniums. The principal component analysis also revealed that plant height, FM, DM, total biomass, and citronellol were loaded at PC1, while oil yield, oil content, chlorophyll and linalool were loaded on PC2.

### Conclusion

Unlike  $NH_4^+$ , a relatively high concentration of  $NO_3^-$  of 10 to 12 meq L<sup>-1</sup> ensures high herbage and oil yields. Further investigation of the ratio of  $NH_4^+$  to  $NO_3^-$  is required for rose geranium grown using nutrient solution.

Acknowledgements. This work was supported by the Central University of Technology, Free State and the National Research Foundation- Thuthuka funding.

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http://www.aloki.hu • ISSN 1589 1623 (Print) • ISSN 1785 0037 (Online)

DOI: http://dx.doi.org/10.15666/aeer/1806\_80818090

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