Scientific Papers. Series B, Horticulture. Vol. LXVIII, No. 1, 2024
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

USING CYTOKININ TO ENHANCE ESSENTIAL OIL BIOSYNTHESIS OF TWO ROSE GERANIUM (*PELARGONIUM GRAVEOLENS* L.) VARIETIES: REUNION AND MADAGASCAR-TYPE

Zenzile KHETSHA

Central University of Technology, Free State, 20 President Brand Street; +27515073130, Bloemfontein, 9300, South Africa

Corresponding author email: zkhetsha@cut.ac.za

Abstract

Previous studies have demonstrated that exogenous application of phytohormone-based biostimulants and subjecting aromatic plants to environmental stresses improves the biosynthesis of secondary metabolites. This experiment was laid out in a 5×2 factorial design, arranged in a randomised complete block design, and replicated three times to determine the effects of cytokinin (CK) on essential oil biosynthesis. Treatments consisted of four CK (221.6, 443.2, 664.8, 886.4 µM and control); and two varieties of rose geranium (Pelargonium graveolens L.): Reunion-type, and Madagascar-type. Analysis of variance and multivariate analysis showed that CK between 221.6 and 664.8 µM may improve the essential oil biosynthesis for the two varieties. Linalool and geranyl tiglate were increased by CK-886.4 µM following simulated wounding on the Bourbon-type, and Madagascar-type, respectively. This study demonstrated that the Bourbon type remains better than the Madagascar type. The author concludes that the application of CK-664.8 µM closer to harvest may improve the Rhodinol and essential oil biosynthesis of both varieties.

***Key words**: Biostimulant, citronellol to geraniol ratio, essential oil quality, phytohormone, Rhodinol.*

INTRODUCTION

The global essential oil market is projected to expand between 8.4% and 11.3% on an annual basis until 2025, accumulating an amount of \$14.0 and \$15.8 billion, ranging from 500 – 750 tons annually (Kumar et al., 2022; CBI, 2018). In recent years, production of high-quality rose geranium oil in Reunion Island has been immensely declining, thereby affording opportunities to emerging producers globally. South Africa produces rose geranium oil like that of Reunion Island, and the country has increased its rose geranium oil production by 25 tons in the past five years, making it 5 tons annually (CBI, 2018). Despite the increase, South Africa, as the origin country of the crop, could potentially contribute 50 tons of geranium oil annually to the global essential oil market (Araya, 2012). However, to optimise this espoused production yield and essential oil quality, the research to enhance and optimise the essential oil yield and quality of rose geranium is crucial.

Rose geranium is a native aromatic crop to South Africa and is globally cultivated for its leaf-extractable high-value essential oil, which is utilized in the aroma and fragrance industry, a billion-dollar market (CBI, 2018). The rose geranium essential oil comprises a complex mixture of more than 120 volatile organic compounds (VOC's) (Demarne and Van der Walt, 1989; Araya, 2012), of which few are key ingredients in cosmetics, perfumery, agrochemicals, pharmaceuticals, and aromatherapy; namely citronellol, geraniol, linalool, citronellyl formate, geranyl formate, guaia-6, 9-diene and rose-oxide (Sedibe and Allemann, 2012). These compounds occur in various proportions depending on the country of origin. The Reunion-type oil from the Island of Reunion (Reunion-type includes Reunion Island origin), for instance, is the only geranium oil with the citronellol to geraniol (C:G) ratio close to 1:1 and is seen as endowed with the highest quality (Khetsha and Sedibe, 2015). On the other hand, the C:G ratio for Madagascar type varies from 1.3 to 1.8 (ISO 4731E, 2012).

Cultivation in South Africa is dominated by populated rural areas, which are poverty-stricken (DAFF, 2012). Most of these communities depend on farming for survival, mainly cultivating rose geranium due to its demand, drought tolerance and low risk (Eiasu et al., 2022). However, a usual setback in the cultivation of rose geranium is the quantitative and qualitative difference at harvest. This results in decreased oil market value and uncertainty in the agri-businesses and a loss of opportunity afforded to emerging farmers by the market suppliers' inability to meet the continuous global demand growth for rose geranium oil.

According to Eiasu et al. (2022), Zigene and Kassahun (2023), and Khetsha et al. (2022), the low oil quality of rose geranium at harvest owes to the reduced content of essential oil compounds, at most leading to high C:G ratio, which is greatly affected by the age of plant shoots, the different environmental factors. Geraniol and linalool levels have been found to decline by 40-70% in older leaves, while citronellol and its esters remain at constant levels (Gomes et al., 2005). In addition, Eiasu et al., (2022) and Motsa et al. (2006) further corroborated that shorter growth cycles may increase essential oil biosynthesis, depending on the harvesting stage and techniques as reported by Gebremeskel (2014) and Malatova et al. (2011). Typically, hot sunny days, with average temperatures between 10 and 22ºC are suitable for high yield and essential oil biosynthesis (Zigene and Kassahun, 2023; Rao et al., 2001). In addition, since it was reported that, the essential oils are involved in plants' response to abiotic stresses (Aqeel et al., 2023); therefore, exposing aromatic plants to water stress and wounding has also been key in improving secondary metabolites accumulation (Khetsha et al., 2022; Thakur et al., 2019; Eiasu et al., 2012). Therefore, to improve the essential oil yield and quality of rose geranium at harvest, researching innovative production techniques is important.

Phytohormone-based biostimulants, such as cytokinins (CK), have been proven to control entire plant life processes, even under unfavourable growing conditions (Asghar et al., 2023; Svolacchia and Sabatini, 2023; Abualia et al., 2023). For this reason, they have been used to improve the essential oils' accumulation (Prins et al. 2010).

Norouzi et al. (2021), Khetsha et al. (2021), Thakur and Kumar (2020) and Pal et al. (2016) reported that foliar application phytohormones could influence the essential oil biosynthesis of aromatic plants, such as thyme (*Thymus vulgaris* L.), rose geranium, and damask rose (*Rosa damascena* Mill.). In addition, foliar application of CK (up to 1.18 mM) has also been shown to improve secondary metabolites and yield parameters of tobacco (*Nicotiana tabacum* L.), peppermint (*Mentha piperita* L.), and marigold (*Calendula officinalis* L.) (Santoro et al., 2013; Machado et al., 2014; Niakan and Ahmadi, 2014). Fraternale et al. (2003) in the review of Prins et al. (2010) reported an increase in the essential oil yield of *Thimus mastichina* (L.) after spraying with benziladenine (BA).

Phytohormone-based biostimulants are derived from phytohormones, and produce signalling molecules that, either alone or combined, play a key role in regulating all aspects of plant growth and development, as well as in mediating plants' responses to biotic and abiotic stresses (Singh et al., 2022; Xu et al., 2018). These compounds act in small concentrations at or near the site of synthesis and distant tissues. Their actions affect processes of signal transduction, a relay through which intracellular or extracellular signals convert into plant responses, such as essential oil biosynthesis (Khetsha et al., 2022; Wang and Irving, 2011; Prins et al., 2010). However, the responses of plants to the foliar application of these compounds depend on the species, variety, environmental conditions, developmental stage, physiological and nutritional status, hormone concentration as well as type of phytohormone and time of application (Sharafzadeh and Zare, 2011). According to Prins et al. (2010), plants produce essential oils in response to physiological stress, pathogen attack, and other ecological factors, and CK is involved in the biosynthesis of essential oil compounds. Therefore, by simulating wounding, limiting moisture, and administering CK towards harvest in this study, it is hypothesised that CK at high concentration in simulated wounding and limited moisture towards harvest may enhance the essential oil biosynthesis of the two rose geranium varieties, Reunion-type, and Madagascar-type cv.

MATERIALS AND METHODS

Experimental site and description. This experiment was carried out under a 72 m^2 plastic-covered greenhouse tunnel located at Central University of Technology, Free State, Bloemfontein. Bloemfontein (29°07’16.78’’S 26°12’45.95’’E) is a semi-arid area situated in the Free State province at an altitude of 1380 m above sea level. An air extraction fan system linked to a thermometer adapter was set at 26°C to regulate the day temperature in the tunnel; however, at night the temperature was not regulated.

Growing system and planting culture. Stem-propagated cuttings of rose geranium varieties, Reunion-type, and Madagascar-type with a height of ±15 cm and a diameter of 0.75-1.0 cm, were sourced from Agricultural Research Council (Tropical and Subtropical Crops, RSA), and Siyakholwa Developmental Foundation Nursery (Eastern Cape, RSA), respectively. The two varieties of stem-propagated cuttings were transplanted on the 1st of November 2018.

Experimental design. The experiment was laid out in a 5×2 factorial design, arranged in a randomised complete block design, and replicated three times. Treatments consisted of four CK (221.6, 443.2, 664.8 and 886.4 μM) and the control (distilled water); and two varieties: Reunion-type, and Madagascar-type. Each treatment unit consists of 10 plants, and each type of treatment consists of five plants.

Treatments. A nine-month experiment was conducted from November 2018 to August 2019 to determine the effects of CK (Kinetin, Sigma-Aldrich, RSA) on two rose geranium varieties. The same transplanted plant material was harvested for three growing cycles, on the 31st of January 2019, the 30th of April 2019, and the 1st of August 2019. For each growing cycle, CK treatment was administered on the same plants throughout the three harvesting cycles. In all harvesting cycles, plants were treated with CK daily, for seven days before the beginning of each harvest.

A protective dividing plastic cover was used to divide the experimental units (Figure 1), as well as a mini-plastic tunnel was utilized as a protective cover during the application in all treatments to avoid spillage and drifting over other treatments. All treatments were exogenous in the morning between 6:00 and 7:00 am to avoid the deterioration of the phytohormonal molecules (Melgoza et al., 2014). A nozzle-calibrated precision sprayer (0.3 MPa pressure) will be utilized to spray the combination treatment solutions at 20 mL/plant (Franks and Faquhar, 2001; Bano and Yasmeen, 2010).

Foliar application of CK was achieved by preparing a 1 mg/mL stock solution CK, and then adding 100 mg of the CK in a 100 mL volumetric flask. Between 3-5 mL of solvent was prepared and added to the volumetric flask to dissolve the CK powder. Once completely dissolved, it was added to a volume with distilled/deionized water. The solution was stirred while adding water to keep the material in solution. The stock solution was stored in a cool environment to avoid deterioration.

Figure 1. The demonstration of the rose geranium treatment combination divisions between treatments

During the first harvest, intact rose geranium plants were harvested by mechanically cutting off the stem using a pair of handheld secateurs at about ±15 cm from the ground, specifically on the last seventh day after CK treatment. Plants were allowed to regrow for three more months.

For the second growing cycle, a day before harvest, rose geranium plants were mechanically wounded by cutting off all the apical dominance, and roughly shattering the foliage material using a hand-held designed flail (Fugate et al., 2016). The mechanical wounding was applied uniformly across all plants. Plants were then harvested on the last seventh day after CK treatment, following the same procedure as in the first growing cycle.

During the third last growing cycle, plants were subjected to moisture stress, seven days before harvest. All plants were uniformly subjected to moisture stress using polyethylene glycol (PEG 6000) at -0.15 MPa of osmotic stress. To reach the -0.15 MPa of osmotic pressure, PEG 6000 was diluted in the nutrient solution by adding 100g/L (Sedibe and Allemann, 2011). Plants were then harvested on the last seventh day after CK treatment, following the same procedure as in the first and second growing cycles.

Growing conditions. Plants were fertigated three times per day (8:00 am, 12:00 pm, and 4:00 pm) using a 'drain to waste' drip irrigation system, where each dripper supplied 2 L/h of water and nutrient solution (Sedibe and Allemann, 2012). Initially, fertigation intervals for the young plants lasted for five minutes, containing a balanced nutrient solution, as recommended for rose geranium (Table 1a and 1b).

Table 1a. Recommended macronutrient (ions) composition used to fertigate rose geranium plants in this experiment

Ions (meq/L)	Concentrations
NH_4^+	0.91
K^+	5.35
Ca^{2+}	5.85
Mg^{2+}	2.36
NO_3^-	9.18
$H_2PO_4^-$	2.09
SO_4^{2-}	3.20

Table 1b. Recommended micronutrient composition used to fertigate rose geranium plants in this experiment

Micronutrient (mg/L)	Chemical form	Concentrations
Fe	Libfer; 13% Fe-EDTA	1.12
Mn	Manganese sulphate	0.54
Zn	Zinc sulphate	0.18
B	Boric acid	0.03
Cu	Copper sulphate	0.02
Mo	Ammonium molybdate	0.05

As the plants matured and the demand for water and nutrients increased, the applied volumes were increased to ensure that ca. 10% to 25% of the solution drained to waste, to prevent salt accumulation in the growing media (Combrink, 2005; Sedibe and Allemann, 2012). The electric conductivity (EC) and pH of the nutrient solutions were maintained at 1.6 mS/cm and 5.5, respectively. Using a pH and EC meter (Hanna HI 98129 Digital meter), the desired pH and EC levels were achieved by using nitric acid and adjusting the nutrient solution concentration to reach the desired EC (Combrink, 2005). Malasol was preventatively sprayed for three to six days, at four-week intervals, at 1.75 ml/L throughout the cropping seasons.

Parameters. After three full months, the harvesting of the crops took place and five fully grown plants per experimental unit were cut at a height of ±15 cm above ground level (Araya, 2012) and placed tenderly on the paper towel to remove any remains of free surface water. The weighing of the herbage mass was conducted rapidly before wilting could occur. By weighing the fresh plant materials making use of a PGL 2002 Adam Scale as described in the procedures of Wood and Roger (2000), the fresh herbage mass (FHM) was determined.

A custom-made distiller with a capacity of 5kg was utilized to extract the essential oil from the herbage material of rose geranium (Khetsha et al., 2021). The plant material lifted per treatment unit for oil extraction was then distilled for one hour at a temperature of ±98°C. The total oil yield per treatment was computed after weighing the oil quantity with a PGL 2002 Adam scale (USA) as soon as possible after the distillation and separation processes (Swamy and Rao, 2009).

The extracted essential oil was analysed using gas chromatography (GC) (Agilent 7890B), equipped with a 30 mm x 0.25 mm x 0.25 µm column (Agilent 19091S 433 UI, HP5-MS UI) and a mass selective detector (Agilent 5977A). The oven temperature program was maintained at 60°C for 10 mins. The temperature was increased to 100°C at a rate of 2°C/min, and then to 145°C at a rate of 1°C/min. Finally, the temperature was increased to 300°C at a rate of 20°C/min and then run for two mins. Helium was used as carrier gas at a constant flow of 0.67 ml/min. Spectra were obtained by electron impact at 70 eV, scanning from 35/mz to 550/mz. The peak areas of the selected GC constituents were individually expressed as percentages of the total of all the Total Ion Chromatogram (TIC) peak areas, as determined by mass spectrometry detection (MSD at 250°C) without using correction factors, illustrated in Figure 2 (a and b) for the two varieties. The compounds were identified using the NIST11 mass spectral library. The ISO standard (ISO 4731E, 2012) was used to characterise rose geranium (Reunion-type and Madagascar-type cv.) essential oil quality parameters for the perfumery industry (Table 2).

Table 2. Chromatographic profiles of Reunion-type and Madagascar-type commercially recognised essential oil compounds according to ISO 4731[E] (2012) with retention time

Component	Retention time (min)	[1]Min. %	[2]Max. %	Min. %	Max. %
		Reunion-type		Madagascar-type	
cis-Rose oxide	14.85	0.3	1.1	0.4	1.4
trans-Rose oxid	15.85	0.1	0.5	0.1	0.6
Isomenthone	17.73	5.0	10.0	5.0	10.0
Geranyl formate	25.37	4.0	8.0	3.7	7.0
[3]Citro. formate	25.46	6.5	11.0	6.5	11.0
Guaia-6,9-diene	38.90	5.0	8.5	5.0	9.0
Geranyl butyrate	50.78	0.7	2.0	0.7	1.7
Geranyl tiglate	64.84	0.7	2.0	0.7	2.0
Citronellol	22.37	18.0	26.0	18.0	26.0
Geraniol	24.00	12.0	20.0	10.0	20.0
Linalool	14.30	8.0	11.0	4.0	10.0
[4]β-Phen. tiglate	52.77	0.4	1.0	0.4	1.0

[1]Min. = Minimum; Max. = Maximum; [3]Citro. formate = Citronellyl formate; [4]β-Phen. tiglate = β-phenylethyl tiglate.

Data analysis. The essential oil yield parameters, major essential oil compounds and the essential oil quality-determining compounds were statistically analysed and compared using PROC GLIMMIX, SAS version 9.4 (PROC GLIMMIX, SAS Institute). Significantly different means among the treatments were separated using Tukey's least significant difference *ad hoc* mean comparison tests, at the 0.05 level of significance (Steel and Tourie, 1980). The Shapiro-Wilks test was performed on standardised residuals to test for any deviations from normality (Shapiro and Wilk, 1965). Only the total essential oil composition was subjected to multivariate data analysis, using principal component analysis (PCA) (PCA-XLSTAT 2015) to identify and evaluate the groupings between the variables.

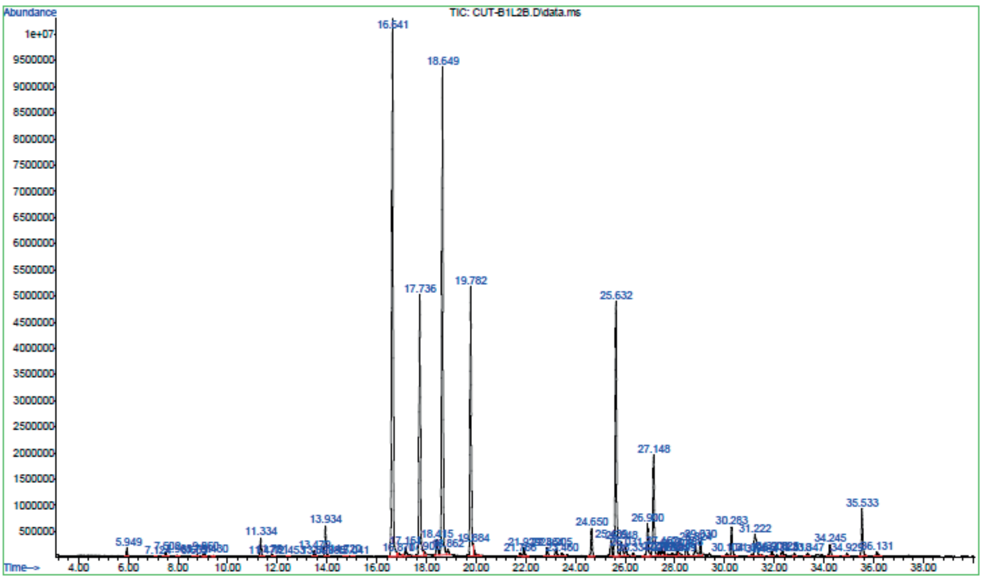

Figure 2a. Total chromatograms of rose geranium essential oil profile for the Reunion-type cv

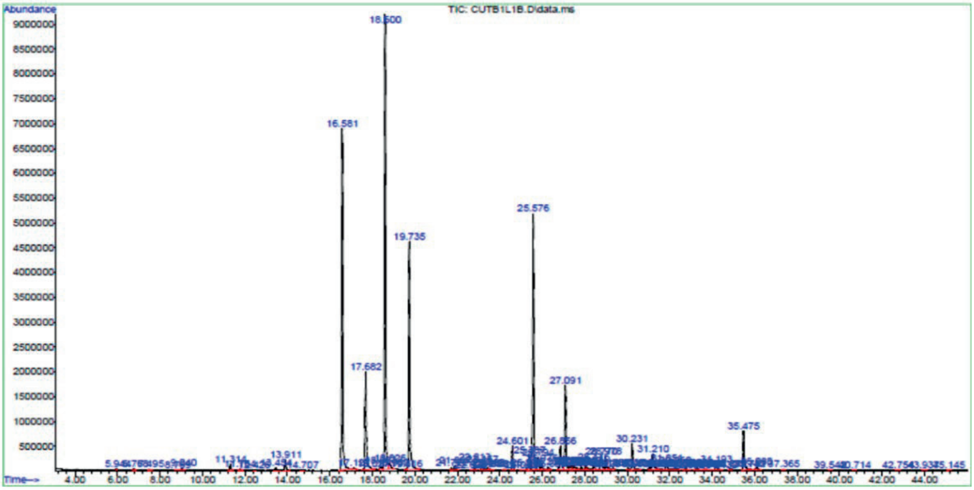

Figure 2b. Total chromatograms of rose geranium essential oil profile for the Madagascar-type cv

RESULTS AND DISCUSSIONS

Essential oil yield parameters. Foliar herbage mass, essential oil mass, and content were all affected by the interactions between rose geranium varieties and the CK application in all production cycles (Table 3).

Table 3. The effects of CK application on rose geranium varieties (Reunion and Madagascar-type) on the essential oil yield parameters for three production cycles, November 2018 - January 2019; February 2019 - April 2019; May 2019 - July 2019

Treatments	Essential oil yield parameters								
	November 2018 - January 2019			February 2019 - April 2019			May 2019 - July 2019		
	[1]FHM	Oil mass	Oil %	FHM	Oil mass	Oil %	FHM	Oil mass	Oil %
Cytokinin (μM)									
Control	350.91^{b}	0.10^{d}	0.04^{d}	261.44^{d}	0.23^{a}	0.10^{c}	210.31^{d}	0.12^{c}	0.06^{c}
221.6	506.55^{a}	0.37^{c}	0.09^{c}	337.40^{c}	0.68^{d}	0.20^{b}	291.24^{c}	0.56^{c}	0.22^{b}
443.2	503.66^{a}	0.94^{b}	0.20^{b}	497.55^{b}	0.95^{c}	0.24^{b}	328.81^{b}	0.93^{b}	0.30^{a}
664.8	516.03^{a}	1.25^{a}	0.26^{a}	402.76^{a}	1.50^{b}	0.32^{a}	448.45^{a}	1.22^{a}	0.30^{a}
886.4	520.64^{a}	1.25^{a}	0.24^{a}	504.70^{a}	1.31^{a}	0.25^{b}	458.36^{a}	1.28^{a}	0.32^{a}
LSD	37.54	0.15	0.04	34.45	0.10	0.04	35.99	0.20	0.07
P-value	0.005	0.001	0.001	0.003	0.001	0.001	0.002	0.001	0.005
Variety									
Reunion	404.75^{b}	1.02^{a}	0.23^{a}	360.32^{b}	1.10^{a}	0.29^{a}	287.92^{b}	1.13^{a}	0.36^{a}
Madagascar	568.90^{a}	0.58^{b}	0.10^{b}	454.08^{a}	0.73^{b}	0.15^{b}	420.98^{a}	0.55^{b}	0.12^{b}
LSD	23.69	0.09	0.02	21.74	0.06	0.03	22.71	0.13	0.04
P-value	0.001	0.004	0.001	0.001	0.003	0.001	0.001	0.003	0.001
Cytokinin x Variety									
ControlxReunion	272.68^{g}	0.16^{f}	0.06^{e}	224.81^{f}	0.31^{f}	0.14^{f}	186.50^{e}	0.16^{e,f}	0.08^{d}
ControlxMadagascar	468.25^{d,e}	0.02^{f}	0.00^{f}	316.38^{e}	0.10^{g}	0.04^{g}	246.02^{c,d}	0.07^{f}	0.03^{c,d}
221.6xReunion	379.51^{f}	0.54^{e}	0.14^{c,d}	331.70^{e}	0.81^{d}	0.24^{c,d}	222.85^{d,e}	0.72^{c}	0.33^{b}
221.6xMadagascar	633.59^{a}	0.19^{f}	0.03^{e,f}	343.10^{d,e}	0.54^{e}	0.16^{e,f}	359.62^{c}	0.40^{d,e}	0.11^{c,d}
443.2xReunion	423.97^{e,f}	1.15^{b}	0.27^{b}	386.82^{c,d}	1.03^{c}	0.27^{c}	296.78^{b}	1.25^{b}	0.42^{a,b}
443.2xMadagascar	583.36^{a,b}	0.74^{d,e}	0.13^{d}	418.69^{b,c}	0.82^{d}	0.21^{c,d,e}	360.83^{b}	0.61^{c,d}	0.17^{c}
664.8xReunion	446.09^{e}	1.71^{a}	0.38^{a}	441.21^{b}	1.97^{a}	0.45^{a}	369.38^{b}	1.68^{a}	0.46^{a}
664.8xMadagascar	585.96^{a,b}	0.78^{c,d}	0.13^{c,d}	553.89^{a}	1.02^{c}	0.18^{d,e,f}	527.51^{a}	0.75^{c}	0.14^{c}
886.4xReunion	501.48^{c,d}	1.51^{a}	0.30^{b}	417.09^{b,c}	1.51^{b}	0.34^{b}	364.10^{b}	1.82^{a}	0.50^{a}
886.4xMadagascar	539.79^{b,c}	0.98^{b,c}	0.18^{c}	592.41^{a}	1.12^{c}	0.15^{e,f}	552.61^{a}	0.73^{c}	0.13^{c}
LSD	53.35	0.21	0.05	48.96	0.14	0.06	51.14	0.28	0.10
P-value	0.002	0.005	0.002	0.006	<.0001	0.008	0.005	0.003	0.002

[1]FHM = Foliar Herbage Mass

The foliar application of CK at any level between the two varieties, Reunion-type and Madagascar-type was found to differ significantly in FHM, essential oil mass and content (±P<0.01) in all three growing cycles. For example, during the first production cycle (November 2018 - January 2019), the FHM was recorded significantly higher when CK-221.6 µM was administered on the Madagascar type compared to the control and the Reunion type. However, the significantly higher FHM where CK-221.6 µM was administered did not differ significantly from CK-443.2 and -664.8 µM. The lowest FHM was recorded where CK-221.6 µM was administered on the Reunion-type. In the second growth cycle (February 2019 - April 2019), the Madagascar type was significantly higher when CK was administered from CK-664.8 to -886.4 µM compared to the control.

A similar trend was recorded in the second growing cycle and during the third growing cycle (May 2019 - July 2019). In this study, it could be observed that administering CK significantly increases the FHM; however, the effects of simulated wounding and stress required high concentrations of CK to record higher herbage mass.

According to Verma et al. (2016), based on the two distinct geranium oil types, the African type, and the Reunion type, the two varieties in this study, did not possess 10-epi-γ-eudesmol; thus, they possess the same traits; Reunion type. Tembe and Deodhar (2010a), Motsa et al. (2006), Gupta et al. (2002) and Van Der Walt and Dermane (1988) reported the distinct morphological differences between the Reunion type and the Bourbon type, where the Reunion type possessed long and fine-textured bristles with distinct deep-lobed fan shape containing at least five to seven divided lobes. Therefore, high FHM in all production cycles in this study could first be attributed to the leaf morphology since the Madagascar type is highly associated with the Bourbon type morphologically (Juliani et al., 2006). It has been reported widely that CK increases the herbage material of aromatic plants such as rose geranium. For example, El-Sayed et al. (2023) reported enhanced vegetative growth when CK was applied in the form of benzyladenine on *P. zonale* (L.) L'Hér. ex Aiton. With amino acids, CK increased the development of the uniform growth of shoots in *P. hederaefolium* (spp.) and *P. hotorum* (spp.); subsequently increasing the herbage material (Wojtania and Gabryszewska, 2001). On lemongrass (*Cymbopogon citratus* (DC.) Stapf.), when CK was applied, the plants exhibited highly branching morphology (Camas-Reyes et al., 2022). Shoot regeneration in ciplukan (*Physalis angulata* L.) has been induced by supplementation with different types of CK (Mastuti et al., 2017).

As illustrated in Table 3, the essential oil mass was recorded significantly (±P<0.01) higher when CK was administered on the Reunion type compared to the control and the Madagascar type in all production cycles. Interestingly, the highest recorded was when the CK was applied at a concentration of CK-664.8 µM in all production cycles; however, when the CK applications were increased to CK-886.4 µM in the first and third growing cycles, the essential oil did not change significantly. It could be deduced that an application of CK-664.8 µM improves the essential oil yield, irrespective of any stress-induced closer to harvest. Farooqi and Sharma (1988) and Khetsha et al. (2021) reported that the administration of CK (kinetin) increased the essential oil yield of Japanese mint (*Mentha arvensis* (L.) var. piperascens Mal.), and rose geranium, respectively; however, the applications were much lesser compared to the administered CK in this study. On the other hand, during the second production cycle, a day before harvest, all rose geranium plants were uniformly mechanically wounded by cutting off all the apical dominance, and roughly shattering the foliage material using a hand-held designed flail (Fugate et al., 2016). The increased essential oil yield during the second growing cycle when CK was applied at CK-886.4 µM could be attributed to the interaction between CK and simulated wounding. When plants have endured wounding, the physiological regeneration mechanism of the shoots is dependent on the interaction between exogenous CKs, brassinosteroids, auxin and gibberellic acid, (Nanda and Melnyk, 2018); therefore, in this study, upregulation of CK (886.4 µM) in synergistic relation with other plant growth regulators may have improved the essential oil yield at this concentration.

Essential oil content is another important parameter used to determine the essential oil yield based on the FHM and the essential oil mass (Table 3). In this study, the essential oil content was similar to the findings recorded in the essential oil in the first two production cycles. As shown in Table 3, 0.38 and 0.45% essential oil contents were recorded when CK-664.8 μM was administered. During the last production cycle, the highest essential oil content (0.5%) was recorded when CK-886.4 μM was administered; however, this was not significantly different compared to CK-664.8 μM therefore, it could be deduced that an administration of CK-664.8 μM could be recommended for the essential oil yield (essential oil mass and content) on Reunion-type. As widely reported, the Reunion-type bears smaller shrub compared to a majority of rose geranium varieties and yet produces significantly higher essential oil yield (Tembe and Deodhar, 2010a), which were similar findings in this study. El-Keltawi and Croteau (1987) reported partially similar results, where different types of CK were administered on *M. piperita* (L.), *M. spicata* (L), *M. suaveolens* (Ehrh.) and *Salvia officinalis* (L.), improving biosynthesis of essential oil; in this case, increasing the yield and essential oil content.

Major essential oil compounds. Table 2 and Figure 2 depict the chromatographic profiles of Reunion-type and Madagascar-type commercially recognised essential oil compounds and the C:G ratio (essential oil quality determining parameter for aromatic and perfumery industry) according to ISO 4731[E] (2012) with retention time. Therefore, all essential oil compounds discussed in this study were based on the accepted indexes as described in Table 2, with accepted concentrations ranging from minimum to maximum indexes. Tables 4, 5 and 6 illustrate the major essential oil compounds for production cycles from November 2018 to January 2019; February 2019 to April 2019; and May 2019 to July 2019, respectively.

Table 4. The effects of CK application on rose geranium varieties (Reunion and Madagascar-type) on major essential oil compounds for the November 2018 to January 2019 production cycle

Treatments	Major essential oil compounds November 2018 – January 2019								
	cis-Rose oxide	*trans*-Rose oxide	[1]Isom.	Guaia-6,9-diene	[2]*β*-Phen. tiglate	Citronellyl formate	Geranyl formate	Geranyl butyrate	Geranyl tiglate
Cytokinin (μM)									
Control	0.12^{a,b}	0.05^{a}	6.26^{b}	11.78^{a}	1.13^{a}	18.89^{a}	7.37^{a}	0.63^{b}	1.19^{a}
221.6	0.13^{a}	0.06^{a}	7.38^{a}	12.22^{a}	1.29^{a}	19.33^{a}	7.00^{a}	0.68^{a,b}	1.44^{a}
443.2	0.10^{a,b}	0.04^{a}	7.20^{a}	11.96^{a}	1.32^{a}	19.31^{a}	7.01^{a}	0.82^{a,b}	1.24^{a}
664.8	0.13^{a}	0.06^{a}	8.03^{a}	12.28^{a}	1.69^{a}	20.47^{a}	6.81^{a}	0.96^{a}	1.41^{a}
886.4	0.09^{b}	0.04^{a}	7.73^{a}	12.76^{a}	1.23^{a}	20.31^{a}	7.56^{a}	0.83^{a,b}	1.31^{a}
LSD	0.04	0.02^{a}	0.92	1.44	0.43	1.47	0.83	0.22	0.28
P-value	0.07	0.33^{a}	0.01	0.65	0.12	0.16	0.30	0.04	0.34
Variety									
Reunion	0.13^{a}	0.06^{a}	6.66^{b}	12.41^{a}	1.00^{b}	19.22^{b}	6.62^{b}	0.83^{a}	1.19^{b}
Madagascar	0.09^{b}	0.04^{b}	8.11^{a}	12.01^{a}	1.71^{a}	20.19^{a}	7.70^{a}	0.75^{a}	1.45^{a}
LSD	0.02	0.01	0.58	0.91	0.27	0.93	0.53	0.14	0.18
P-value	0.01	0.02	0.01	0.31	<.0001	0.05	0.01	0.22	0.01
Cytokinin x Variety									
ControlxReunion	0.15^{a}	0.06^{a}	5.27^{a}	12.07^{a}	0.96^{a}	18.72^{a}	7.06^{a}	0.57^{a}	1.09^{a}
ControlxMadagascar	0.08^{a}	0.04^{a}	7.75^{a}	11.35^{a}	1.39^{a}	19.15^{a}	7.83^{a}	0.72^{a,b}	1.33^{a}
221.6xReunion	0.16^{a}	0.07^{a}	6.47^{a}	11.99^{a}	0.63^{a}	19.63^{a}	6.38^{a}	0.59^{b}	1.28^{a}
221.6xMadagascar	0.10^{a}	0.04^{a}	8.30^{a}	12.45^{a}	1.95^{a}	19.04^{a}	7.63^{a}	0.77^{a,b}	1.59^{a}
443.2xReunion	0.11^{a}	0.05^{a}	6.50^{a}	12.21^{a}	1.00^{a}	19.46^{a}	6.40^{a}	0.87^{a,b}	1.11^{a}
443.2xMadagascar	0.08^{a}	0.03^{a}	7.90^{a}	11.70^{a}	1.64^{a}	19.17^{a}	7.62^{a}	0.78^{a,b}	1.36^{a}
664.8xReunion	0.13^{a}	0.06^{a}	8.10^{a}	13.01^{a}	1.49^{a}	18.91^{a}	6.41^{a}	1.18^{a}	1.23^{a}
664.8xMadagascar	0.13^{a}	0.06^{a}	7.97^{a}	11.54^{a}	1.90^{a}	22.02^{a}	7.21^{a}	0.74^{a,b}	1.58^{a}
886.4xReunion	0.10^{a}	0.05^{a}	6.97^{a}	12.75^{a}	0.91^{a}	22.02^{a}	6.87^{a}	0.93^{a,b}	1.26^{a}
886.4xMadagascar	0.07^{a}	0.04^{a}	8.50^{a}	12.77^{a}	1.55^{a}	21.23^{a}	8.25^{a}	0.73^{a,b}	1.36^{a}
LSD	0.06	0.03	1.31	2.05	0.61	2.08	1.19	0.31	0.40
P-value	0.34	0.60	0.11	0.66	0.22	0.08	0.95	0.04	0.89

[1]Isom. = Isomenthone.
[2]*β*-Phen. tiglate = *β*-phenylethyl tiglate

Table 5. The effects of CK application on rose geranium varieties (Reunion and Madagascar-type) on major essential oil compounds for the February 2019 - April 2019 production cycle

Treatments	Major essential oil compounds February 2019 – April 2019							
	cis-Rose oxide	*trans*-Rose oxide	Isomenthone	Guaia-6,9-diene	[1]β-Phen. tiglate	Citronellyl formate	Geranyl formate	Geranyl tiglate
Cytokinin (µM)								
Control	0.09^{a}	0.08^{a}	11.84^{a}	11.87^{a}	0.78^{a}	21.17^{a}	6.40^{b}	1.31^{c}
221.6	0.05^{a}	0.02^{a}	10.95^{a}	13.01^{a}	1.16^{a}	18.57^{a,b}	10.78^{a}	2.85^{a}
443.2	0.09^{a}	0.04^{a}	8.03^{a}	13.52^{a}	1.08^{a}	18.87^{a,b}	10.07^{a}	2.86^{a}
664.8	0.05^{a}	0.02^{a}	9.21^{a}	13.53^{a}	1.11^{a}	18.20^{b}	10.82^{a}	2.15^{b}
886.4	0.05^{a}	0.02^{a}	5.76^{a}	14.05^{a}	1.26^{a}	16.40^{b}	11.07^{a}	3.18^{a}
LSD	0.05	0.06	6.48	1.47	0.44	2.76	1.25	0.55
P-value	0.28	0.22	0.35	0.08	0.50	0.04	<.0001	0.001
Variety								
Reunion	0.08^{a}	0.04^{a}	9.14^{a}	12.58^{b}	0.92^{b}	19.81^{a}	9.52^{a}	2.03^{b}
Madagascar	0.05^{a}	0.03^{a}	8.99^{a}	13.95^{a}	1.26^{a}	17.21^{b}	10.37^{a}	3.02^{a}
LSD	0.03	0.04	4.09	0.92	0.28	1.74	0.79	0.35
P-value	0.06	0.44	1.00	0.01	0.01	0.009	0.09	<.0001
Cytokinin x Variety								
ControlxReunion	0.07^{a}	0.09^{a}	7.85^{b,c,d}	12.03^{a}	0.80^{a}	21.10^{a}	6.37^{a}	0.73^{e}
ControlxMadagas car	0.11^{a}	0.06^{a}	17.83^{a}	11.62^{a}	0.75^{a}	21.27^{a}	6.44^{a}	2.17^{e}
221.6xReunion	0.06^{a}	0.02^{a}	6.23^{c,d}	12.62^{a}	0.87^{a}	19.68^{a}	10.59^{a}	2.91^{b,c}
221.6xMadagascar	0.04^{a}	0.03^{a}	15.67^{a,b}	13.40^{a}	1.44^{a}	17.45^{a}	11.07^{a}	2.79^{c,d}
443.2xReunion	0.14^{a}	0.04^{a}	9.87^{a,b,c,d}	12.17^{a}	0.76^{a}	20.88^{a}	9.67^{a}	2.27^{b,c}
443.2xMadagascar	0.03^{a}	0.03^{a}	6.20^{c,d}	14.86^{a}	1.39^{a}	16.86^{a}	10.48^{a}	3.44^{c,d}
664.8xReunion	0.05^{a}	0.02^{a}	12.53^{a,b,c}	12.80^{a}	1.02^{a}	19.43^{a}	10.39^{a}	1.44^{a,b}
664.8xMadagascar	0.04^{a}	0.03^{a}	5.88^{c,d}	14.25^{a}	1.19^{a}	16.98^{a}	11.25^{a}	2.86^{c}
886.4xReunion	0.04^{a}	0.02^{a}	9.22^{a,b,c,d}	13.26^{a}	1.14^{a}	17.95^{a}	10.60^{a}	2.82^{a}
886.4xMadagascar	0.06^{a}	0.03^{a}	2.30^{d}	14.84^{a}	1.37^{a}	14.84^{a}	11.53^{a}	3.54^{a}
LSD	0.08	0.08	9.21	2.08	0.63	3.92	1.79	0.78
P-value	0.11	0.91	0.02	0.35	0.67	0.64	0.93	0.03

[1]β-Phen. tiglate = β-phenylethyl tiglate

Table 6. The effects of CK application on rose geranium varieties (Reunion and Madagascar-type) on major essential oil compounds for the May 2019 - July 2019 production cycle

Treatments	Essential oil compounds May 2019 – July 2019						
	cis-Rose oxide	*trans*-Rose oxide	Isomenthone	Guaia-6,9-diene	Citronellyl formate	Geranyl formate	Geranyl tiglate
Cytokinin (µM)							
Control	0.08^{a}	0.05^{a}	14.20^{a}	13.72^{a}	17.41^{a}	5.92^{a}	2.78^{a}
221.6	0.10^{a}	0.05^{a}	12.47^{a}	13.55^{a}	17.34^{a}	5.86^{a}	2.69^{a}
443.2	0.11^{a}	0.06^{a}	10.90^{a}	14.04^{a}	18.04^{a}	5.77^{a}	2.61^{a}
664.8	0.10^{a}	0.06^{a}	13.77^{a}	14.31^{a}	16.84^{a}	5.99^{a}	2.79^{a}
886.4	0.09^{a}	0.06^{a}	9.08^{a}	14.52^{a}	16.56^{a}	5.67^{a}	2.72^{a}
LSD	0.05	0.03	9.43	1.38	2.55	1.49	0.58
P-value	0.83	0.94	0.76	0.49	0.73	0.98	0.97
Variety							
Reunion	0.11^{a}	0.06^{a}	12.96^{a}	13.47^{b}	18.05^{a}	5.53^{a}	2.44^{b}
Madagascar	0.08^{a}	0.05^{a}	10.99^{a}	14.65^{a}	16.36^{a}	6.17^{a}	3.01^{a}
LSD	0.03	0.02	5.95	0.87	1.61	0.94	0.36
P-value	0.13	0.24	0.54	0.02	0.06	0.13	0.005
Cytokinin x Variety							
ControlxReunion	0.09^{a}	0.05^{a}	12.17^{a}	13.06^{a}	18.47^{a}	5.91^{a}	2.42^{a}
ControlxMadagas car	0.08^{a}	0.05^{a}	17.25^{a}	14.71^{a}	15.84^{a}	5.59^{a}	3.32^{a}
221.6xReunion	0.11^{a}	0.05^{a}	9.23^{a}	12.89^{a}	18.63^{a}	5.47^{a}	2.49^{a}
221.6xMadagascar	0.08^{a}	0.04^{a}	15.70^{a}	14.20^{a}	16.05^{a}	6.25^{a}	2.88^{a}
443.2xReunion	0.15^{a}	0.07^{a}	12.70^{a}	12.83^{a}	20.29^{a}	5.95^{a}	2.11^{a}
443.2xMadagascar	0.07^{a}	0.04^{a}	9.10^{a}	15.26^{a}	15.79^{a}	5.59^{a}	3.11^{a}
664.8xReunion	0.10^{a}	0.05^{a}	18.43^{a}	14.14^{a}	17.15^{a}	6.48^{a}	2.50^{a}
664.8xMadagascar	0.10^{a}	0.06^{a}	9.10^{a}	14.48^{a}	16.53^{a}	6.48^{a}	3.08^{a}
886.4xReunion	0.09^{a}	0.06^{a}	12.27^{a}	14.43^{a}	15.72^{a}	4.84^{a}	2.67^{a}
886.4xMadagascar	0.09^{a}	0.05^{a}	5.90^{a}	14.61^{a}	17.40^{a}	6.49^{a}	2.77^{a}
LSD	0.07	0.04	13.40	1.97	3.63	2.11	0.82
P-value	0.36	0.24	0.33	0.44	0.16	0.68	0.52

Rose oxide (*cis*- and *trans*-), guaia-6,9-diene, *β*-phenylethyl tiglate, citronellyl formate, and geranyl formate were not affected by interactions between rose geranium varieties and the CK in all production cycles (Tables 4, 5 and 6).

During the first growing cycle, both Rose oxide compounds, *cis*- and *trans*- were significantly affected by the variety. In both compounds, *cis*-Rose oxide, and *trans*-Rose oxide, Reunion-type recorded 0.04% (P<0.01) and 0.02% (P<0.02) higher compared to the Madagascar-type.

According to ISO 4731[E] (2012) (Table 2), both compounds were above the index range, recording 0.13% and 0.06; thus, the compounds may not be considered for use in the high-quality perfumery industry. As illustrated in Tables 5 and 6, the two compounds were not significantly different for the two growing cycles; however, a similar trend was recorded, and at most, these compounds were within the range required for the industry during these periods.

Isomenthone compound was recorded significantly (P<0.01) lower where plants were treated with distilled water compared to all CK treatments; therefore, confirming that any application of CK improves the isomenthone content in both varieties (Table 4). In a study conducted by Zielińska et al. (2011) on *Agastache rugosa* (Fischer & C.A.Meyer) O. Kuntze, administering CK in the form of kinetin showed less differences between the treatments on isomenthone content; however, these treatments were better than the control. On the other hand, the Madagascar type recorded a significantly higher content of isomenthone compared to the Reunion type, and both varieties were within the range as described in ISO 4731[E] (2012) (Table 4). Significant interactions between rose geranium varieties and the CK application were recorded during the second growing cycle; however, data between the interactions were inconsistent to conclude (Table 5). No significant effects, including the interactions were recorded for the third growing cycle for isomenthone (Table 6).

As illustrated in Tables 5 and 6, only the varieties were recorded as significant for the guaia-6,9-diene compound; however, this was not the case during the first growing cycle, Table 4. Madagascar-type had the highest (P<0.01) guaia-6,9-diene content compared to Reunion-type during the second growing cycle, with the same recording (P<0.02) during the third growing cycle, respectively. During these production cycles, the content of guaia-6,9-diene recorded was higher than the recommended ISO 4731[E] (2012); therefore, this compound could mostly be considered for other aromatic and cleaning industries, not the perfumery industry.

During the two cycles where *β*-phenylethyl tiglate was recorded, the variety was the only factor significantly affected. In both growing cycles one (P<.0001) and two (P<0.01), Madagascar-type recorded higher *β*-phenylethyl tiglate content; however, the content was higher to be recommended for the perfumery industry (ISO 4731[E], 2012). Amongst the treatments applied, *β*-phenylethyl tiglate was recorded in traces during the last treatments, and therefore this compound was excluded in Table 6.

In the second and third production cycles, simulated wounding and moisture stress were introduced closer to harvest. Da Silva et al. (2023) and Khetsha and Sedibe (2015) reported that various abiotic and biotic factors like moisture stress, salinity, temperature, heavy minerals, wounding, defoliation, light, and living organisms significantly affect the biosynthesis of essential oil compounds. Therefore, the increased content of guaia-6,9-diene and *β*-phenylethyl tiglate in Madagascar-type could be attributed to the two stress factors induced closer to harvest, the simulated wounding, and moisture stress during the second and third growing cycles, respectively. There are no published articles to corroborate the differences in guaia-6,9-diene and *β*-phenylethyl tiglate in varieties of rose geranium.

The administration of CK at any concentration significantly reduced the citronellyl formate content (Table 5). Citronellyl formate content was recorded as significantly (P<0.01) higher in the Madagascar type compared to the Reunion type during the first growing cycle. However, this was not the case during the second growing cycle: citronellyl formate content tended to be 0.97% significantly (P<0.009) higher in the Reunion type compared to the Madagascar type (Table 5). Nonetheless, the citronellyl formate content was higher than the recommended content, ISO 4731[E] (2012). There were no significant differences recorded in the citronellyl formate content during the third growing cycle (Table 6). The Madagascar type and the Reunion type resemble the same requirements of between 6.5 and 11% of citronellyl formate content (ISO 4731[E], 2012); thus, changes between the two varieties could be attributed to the uniform simulated wounding close to the harvest. The results of this study demonstrated that the Madagascar type may be sensitive to any type of wounding closer to harvest, especially to produce citronellyl formate. On the other hand, the Reunion type produced a higher content of citronellyl formate when subjected to simulated wounding closer to harvest, indicating a favourable strategy to produce citronellyl formate; nonetheless, growers need to be cautious as the strategy tends to increase the content of citronellyl formate beyond the acceptable index for perfumery industry. Steele et al. (1998) reported that monoterpene synthases arise earlier when grand fir (*Abies grandis* (Douglas ex D. Don) Lindley) endured simulated wounding. Therefore, since citronellyl formate is the monoterpene type present mostly in *P. graveolens* (Džamić et al., 2014), the attributes of the changes in the content of this monoterpene during the second production cycle are attributed to simulated wounding.

According to Khetsha et al. (2022), Liu et al. (2016), and Demyttenaere et al. (2000), geranyl acetate, geranyl butyrate, geranyl formate and geranyl tiglate formed from biotransformation of the geraniol as esters, subsequently changing the essential oil composition and quality. In this study, the geraniol esters were mostly recorded; however, at most, they appeared in traces. For example, during the second and third growing cycles (Tables 5 and 6), geranyl butyrate was recorded in traces amongst the treatments, while geranyl acetate was in traces across all growing cycles; thus, these compounds were excluded.

As illustrated in Table 4, geranyl formate and geranyl tiglate were marginally ($\pm P<0.01$) within the acceptable index as recommended by ISO 4731[E] (2012) in Madagascar-type compared to Reunion-type. Interestingly, as illustrated in Tables 5 and 6, a similar trend was recorded, where the Madagascar-type accumulated significantly ($P<.0001$) higher geranyl tiglate content compared to the Reunion-type. The results of this study showed that irrespective of treatments, Madagascar-type accumulated more geranyl tiglate; however, it could not be associated with any published literature.

Interestingly, when CK was applied to the two varieties, the geranyl tiglate content tended to be significantly ($\pm P<0.03$) higher, because of the interactions recorded (Table 5). The geranyl tiglate content increase only occurred when CK concentration was applied at 886.4 μM compared to the controls. Punpee (2012) reported biosynthesis of CK, where the author described the process occurring through biochemical modification of dimethylallyl diphosphate, which eventually through other metabolic processes yields zeatin ribosides, an important type of CK. In addition, Nogués et al. (2006) reported that geranyl esters and dimethylallyl diphosphate share the same novel chloroplastic pathway, the intermediate methylerythritol phosphate. On the other hand, Lulai et al. (2016) reported a quantifiable level of CK when potatoes (*Solanum tuberosum* L.) were subjected to simulated wounding; justifying the possible effects of the wounding on the metabolomes. Egamberdieva et al. (2017) also reported that the exogenous application of CKs can optimize the endogenous levels of CKs for growth and development and have significant effects on the biosynthesis of essential oils after wounding damage. Therefore, in this study, the significant changes in the geranyl tiglate content are associated with high CK concentration application and the uniform simulated wounding closer to the harvest for the two varieties.

The recorded significant ($\pm P<0.01$) interactions between rose geranium varieties and the CK application of geranyl butyrate were not consistent for a meaningful conclusion (Table 4). During the second cycle, irrespective of the CK application concentration, the geranyl formate tended to be significantly better compared to the control (Table 5); this was not the case for the third cycle, no significant differences were recorded. Although a significant change was recorded during the second growing cycle, it was unclear how the CK affected the geranyl butyrate; nonetheless, attributes could be associated with the simulated wounding, the biosynthesis pathway between the CK and the geranyl esters.

Essential oil quality-determining compounds. The most important compounds commonly used in the perfume industry are linalool, geraniol, and citronellol, with a C:G ratio below 3:1 being preferred by the industry for many of the varieties like Reunion-type and Madagascar-type (ISO 4731[E], 2012; Saxena et al., 2008). As illustrated in Table 7 in this study, significant interactions were recorded between rose geranium varieties and the CK application for linalool, citronellol, geraniol and the C:G ratio; however, this differed between the growing seasons.

In all growing cycles, linalool was recorded as significantly high where CK was administered at 886.4 µM on the Reunion-type compared to the control. However, during the second and third growing, there were no significant differences where CK was administered at 886.4 µM on the Madagascar-type; yet the content of linalool was still better than the control. Interestingly, throughout the three cycles, only during the second production cycle linalool was within the acceptable range for the perfumery industry (ISO 4731[E], 2012). There is an association between wounding and the biosynthesis of linalool (Zhang et al., 2022), as well as enhancement of linalool when CK is administered (Tavares et al., 2004). On lemon (*Citrus jambhiri* Lush.), Yamasaki et al. (2007) reported that wounded rough lemons recorded a linalool content that was 14.5 times higher than that of unwounded rough lemon leaves. Ono et al. (2011) and Tavares et al. (2004) reported that linalool was increased by the administration of CK on basil (*Ocimum basilicum* L.) and lemon balm (*Lippia alba* Mill. N. E. Br.) compared to the control, respectively.

According to Weng et al. (2015), the physical and chemical similarities of citronellol and geraniol compounds make them inseparable. In this study, citronellol content was much higher ($P<.0001$) in the Reunion type compared to the Madagascar type; however, I noticed that only in the second season citronellol content was within the acceptable range for the perfumery industry (Table 7).

According to Tembe and Deodhar (2010b), the chemical composition of the Reunion type remains the best, with a desirable rosy aroma and considerably better citronellol, geraniol and C:G ratio compared to other varieties, including the Madagascar type. On the other hand, the citronellol within the range could be attributed to the simulated wounding; this was also reported by Yamasaki et al. (2007) on wounded rough lemons. During the last season, the application of CK reduced the content of citronellol significantly ($P<.0001$) in all treatments for the Reunion-type compared to the control. Findings in this study were similar to results reported by Farooqi et al. (1993), wherein damask rose (*Rosa damascene* Mill.) CK application resulted in an increased essential oil content and citronellol content in the essential oil. This was not the case for the Madagascar type, results were inconsistent across all treatments.

Table 7. The effects of CK application on rose geranium varieties (Reunion and Madagascar-type) on the essential oil quality-determining compounds parameters for three production cycles, November 2018 - January 2019; February 2019 - April 2019; May 2019 - July 2019

Treatments	Rhodinol and [1]C:G ratio											
	November 2018 - January 2019				February 2019 - April 2019				May 2019 - July 2019			
	[2]Lin.	[3]Citro.	[4]Ger.	C:G	Lin.	Citro.	Ger.	C:G	Lin.	Citro.	Ger.	C:G
Cytokinin (µM)												
Control	5.70^{d}	26.74^{a}	6.93^{c}	3.90^{a}	2.78^{d}	23.54^{a}	8.06^{c}	2.92^{a}	3.14^{d}	23.62^{a}	7.95^{c}	2.98^{a}
221.6	7.39^{c}	27.24^{a}	8.47^{b}	3.25^{b}	6.06^{c}	24.08^{a}	9.16^{b}	2.65^{a,b}	4.65^{c}	21.50^{b}	12.09^{a}	1.78^{b}
443.2	8.57^{b}	26.86^{a}	11.45^{a}	2.37^{c}	6.73^{c}	22.02^{a}	8.94^{b}	2.32^{b,c}	5.22^{c}	21.43^{b}	10.20^{b}	2.12^{b}
664.8	8.48^{b}	27.16^{a}	10.93^{a}	3.02^{b}	8.45^{b}	22.74^{a}	10.68^{a}	2.21^{c}	7.82^{b}	19.89^{c}	10.73^{b}	1.91^{b}
886.4	13.47^{a}	27.03^{a}	8.95^{b}	3.17^{b}	10.83^{a}	22.77^{a}	10.16^{a}	2.28^{c}	12.90^{a}	22.75^{a}	11.24^{a,b}	2.08^{b}
LSD	0.84	2.66	1.54	0.63	0.93	2.81	0.77	0.33	1.26	0.91	1.31	0.34
P-value	0.001	0.99	0.001	0.01	<.0001	0.25	0.001	0.05	<.0001	0.001	0.003	0.05
Variety												
Reunion	9.67^{a}	29.69^{a}	11.22^{a}	2.85^{b}	8.01^{a}	24.60^{a}	10.54^{a}	2.37^{a}	7.28^{a}	22.87^{a}	11.31^{a}	2.11^{a}
Madagascar	7.91^{b}	24.15^{b}	7.52^{b}	3.40^{a}	6.25^{b}	21.32^{b}	8.38^{b}	2.55^{a}	6.44^{b}	20.60^{b}	9.69^{b}	2.19^{a}
LSD	0.53	1.68	0.97	0.40	0.59	1.23	0.49	0·21	0.79	0.58	0.83	0.22
P-value	<.0001	<.0001	0.002	0.01	0.004	<.0001	<.0001	0.06	0.01	<.0001	<.0001	0.22
Cytokinin x Variety												

ControlxReunion	4.75^{e}	29.70^{a}	$7.65^{d,e}$	$3.94^{a,b}$	2.83^{e}	24.71^{a}	$8.30^{d,e}$	2.98^{a}	$3.61^{d,e}$	26.83^{a}	$8.49^{e,f}$	3.21^{a}
ControlxMadagascar	7.14^{d}	22.31^{a}	5.84^{e}	$3.85^{a,b}$	2.70^{e}	21.78^{a}	7.69^{e}	2.83^{a}	2.45^{e}	18.86^{d}	7.14^{f}	2.64^{b}
221.6xReunion	7.59^{d}	31.08^{a}	$9.19^{c,d}$	$3.39^{a,b,c}$	8.15^{b}	25.59^{a}	9.77^{c}	2.65^{a}	5.66^{c}	23.62^{b}	12.77^{a}	$1.85^{c,d,e}$
221.6xMadagascar	7.19^{d}	23.40^{a}	$7.76^{d,e}$	$3.10^{b,c,d}$	3.97^{e}	22.58^{a}	$8.55^{d,e}$	2.64^{a}	$3.65^{d,e}$	19.38^{d}	$11.40^{a,b,c}$	$1.72^{d,e}$
443.2xReunion	11.76^{b}	30.76^{a}	12.95^{b}	$2.41^{d,e}$	$7.98^{b,c}$	24.82^{a}	9.96^{c}	2.30^{a}	$4.83^{c,d}$	21.36^{c}	$10.55^{b,c,d}$	$2.04^{c,d}$
443.2xMadagascar	5.37^{e}	22.95^{a}	9.95^{c}	$2.32^{d,e}$	5.91^{d}	19.22^{a}	$8.26^{d,e}$	2.35^{a}	5.60^{c}	21.49^{c}	$9.86^{c,d,e}$	$2.19^{b,c,d}$
664.8xReunion	10.04^{c}	28.75^{a}	15.73^{a}	1.83^{e}	10.12^{a}	24.08^{a}	13.07^{a}	1.84^{a}	9.28^{b}	18.74^{d}	$12.11^{a,b}$	1.55^{e}
664.8xMadagascar	6.91^{d}	25.58^{a}	6.13^{e}	4.22^{a}	$6.79^{c,d}$	21.41^{a}	$8.28^{d,e}$	2.58^{a}	6.37^{b}	21.04^{c}	$9.35^{d,e}$	$2.27^{c,d,e}$
886.4xReunion	14.23^{a}	28.18^{a}	10.55^{c}	$2.68^{c,d,e}$	10.98^{a}	23.79^{a}	11.42^{b}	2.10^{a}	13.00^{a}	23.80^{b}	12.63^{a}	$1.89^{b,c}$
886.4xMadagascar	12.70^{b}	25.89^{a}	$7.34^{d,e}$	$3.66^{a,b}$	10.68^{a}	21.76^{a}	$8.90^{c,d}$	2.45^{a}	12.79^{a}	21.69^{c}	$9.85^{c,d,e}$	2.27
LSD	1.20	3.78	2.18	0.90	1.33	4.75	1.10	0.47	1.79	1.30	1.87	0.49
P-value	<.0001	0.09	0.02	0.01	0.001	0.38	0.01	0.07	0.04	<.0001	0.34	0.01

[1]C:G = Citronelol to Geraniol ratio.
[2]Lin. = Linalool.
[3]Citro. = Cintronellol.
[4]Ger. = Geraniol.

The recorded significant (±P<0.01) interactions between rose geranium varieties and the CK application on geraniol were not consistent for a meaningful conclusion during the last growing cycles as illustrated in (Table 7). Therefore, it could be deduced that by applying CK at 664.8 µM following normal production systems and where simulated wounding is administered, the geraniol content may increase. However, when moisture stress is introduced closer to harvest, this is not the case. However, Eiasu et al. (2009) reported that the decline in geraniol is due to the conversion of geraniol into citronellol, and this was also described by Weng et al. (2015).
High-quality essential oil is based on the C:G ratio (ISO 4731[E], 2012; Saxena et al., 2008). In all production cycles, the C:G ratio was best when CK was administered at 664.8 µM on the Bourbon type; however, this interaction was not significantly different during the second growing cycle. The essential oil quality based on the C:G ratio could be associated with the citronellol, geraniol, and CK biosynthesis pathway and the conversions with time as described by Farooqi et al. (1993), Eiasu et al. (2009) and Weng et al. (2015), respectively.
Therefore, in this study, it could be concluded that administration of synthetic CK-664.8 µM closer to harvest following moisture stress and under normal conditions may improve the Rhodinol and essential oil quality. However, it may be beneficial to increase the application level of CK to 886.4 µM following simulated wounding where linalool is targeted on the Bourbon type.

Total essential oil composition. From over 50 organic compounds listed in rose geranium essential oil (Shellie and Marriott, 2003), chromatographic analysis made it possible to identify 39 (74.47% total oil), 44 (91.18% total oil) and 46 (90.94% total oil) compounds for growing cycle one, two, and three, respectively.
The pattern of diversity amongst all essential oil compounds as shown in Figures 3, 4 and 5 was determined using the PCA for each respective production cycle. The grouping of essential oil compounds was affected by the CK and the variety of rose geranium varieties in all growing cycles. Out of nine principal components used in each PCA, the first two principal components (PC1 and PC2) accounted for most of the variability.
The score plot and loading matrix accounted for 61.34%, 63.28%, and 54.93% of the total variances as illustrated in Figures 3, 4 and 5, respectively. In all growing cycles, the two rose geranium varieties were mostly grouped separately (categorization illustrated in black circles in all figures: Figures 3, 4 and 5) irrespective of the CK treatment.
When the CK treatment was considered as a factor, increasing the CK concentration from 221.6 up to 443.2 µM, grouped the Reunion type in the same quadrant; however, this gradual increase in CK application was not different compared to the control (Figure 3). On the other hand, the two higher CK concentrations, 664.8 and 886.4 µM in the Reunion type were grouped separately, indicating significant differences compared to the control and where lower CK was administered. Therefore, increasing CK from 664.8 to 886.4 µM in the Reunion type may improve these essential oil compounds.
In this study, only essential oil compounds contributing most of the variability amongst (squared cosine [r^2]) the essential oils loadings were discussed. Therefore, CK applied at 664.8 and 886.4 µM tended to adversely affect the content of citronellol, *α*-Copaene, (*E*)-*β*-

Caryophyllene, germacrene D, and (*Z*)-Geranyl acetate as illustrated in PC 1 accounting for 43.21% (Figure 3). Findings on the citronellol when PCA was applied assisted with the conclusion on possible adverse effects of CK on rose geranium (Bourbon type) grown under normal conditions.

As illustrated in PC 1 (Figure 3), the Madagascar type was grouped in the same quadrant irrespective of the CK applications (excluding the control). This grouping was shown to increase the content of isomenthone, *α*-Cubebene, *β*-cubenene, *cis*-Muurola-3,5-diene, caryophyllene oxide, and *trans*-Calamenene geranyl tiglate (PC1).

Principal component 2 accounted for 18.12% and grouped *δ*-Cadinene and citronellyl butyrate, which were shown to be increased by any CK treatment except the control for Madagascar type only; thus, administering CK closer to harvest may improve these essential oil compounds of the Madagascar type under normal production system. On the other hand, it was interesting to note that the two varieties bear significantly different essential oil compositions as described by Tembe and Deodhar (2010a) and Tembe and Deodhar (2010b).

During the second cycle, as illustrated in Figure 4, *β*-Myrcene, 3,7-Dimethyl-1,3,6-octatriene, neral, geranial and citronellyl formate content in the Reunion type treated with all CK treatments including the control were all adversely affected as shown in PC 1 (48.36%). Humelene, *α*-Copaene, *β*-Caryophyllene, *trans*-muurola-4(14),5-diene, *α*-muurolene, citronellyl butyrate, 1-epi-cubenol, geranyl tiglate, geranyl butanoate, and citronellyl tiglate content were increased by the increasing the concentration of CK to 886.4 μM on both varieties, Reunion, and Madagascar type. Results on the geranyl tiglate corroborate findings in the analysis of variance, Table 5. On the other hand, increasing CK concentration from 221.6 up to 664.8 μM tended to increase the content of geranyl formate, *α*-Guaiene, guaia-6,9-diene, geranyl propionate, cubenol, germacrene D, 2-Phenylethyl tiglate, geranyl 2-methyl butanoate, and 1,10-di-epi-cubenol on Madagascar type (PC 1). Although the two key compounds were not significantly different when the analysis of variance was applied, the PCA on the other hand showed that geranyl formate and guaia-6,9-diene can be increased by CK concentration from 221.6 up to 664.8 μM.

In PC 2, accounting for a 14.92% level of variation, CK at 886.4 μM increased the content of citronellyl acetate and *β*-Cadinene on both varieties: Reunion and Madagascar type; thus, increasing CK to 886.4 μM improves citronellyl acetate and *β*-Cadinene for the two varieties.

In the third production cycle, PC 1 and PC 2 accounted for 35.61% and 19.32%, respectively (Figure 5). Citronellyl formate and *cis*-Rose oxide were associated with the application of CK at 443.2 μM on the Reunion type; however, this was not significantly different from the control. On the other hand, applying CK at 221.6 and 664.8 μM on the Reunion type adversely affected citronellyl acetate, *β*-Guaiene and *δ*-Cadinene content. There were no significant differences between the CK applied at 221.6, 664.8, and 886.4 μM and the control on Madagascar type.

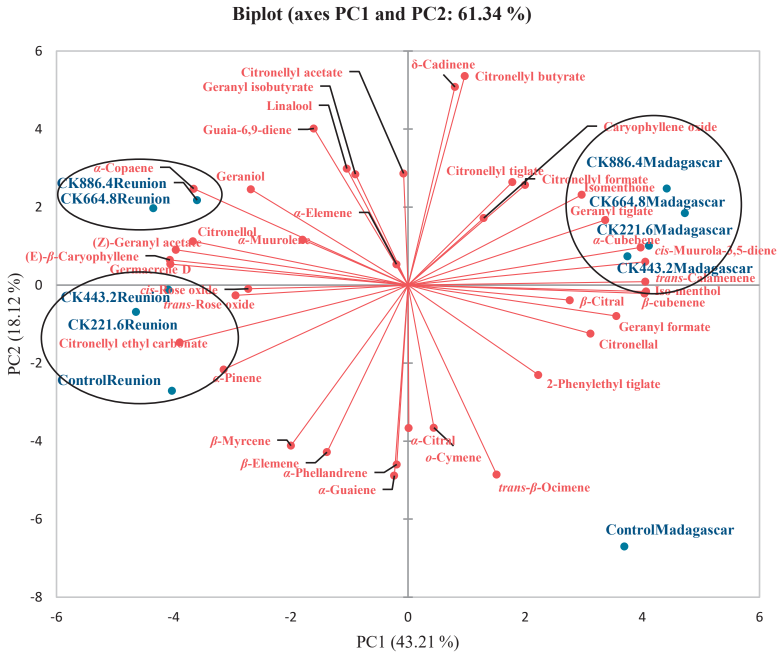

Figure 3. Principal component biplot showing the variations between total essential oil composition content in response to CK application (221.6, 443.2, 664.8, 886.4 µM and the control) and rose geranium varieties (Reunion and Madagascar-type) during the November 2018 to January 2019 production cycle

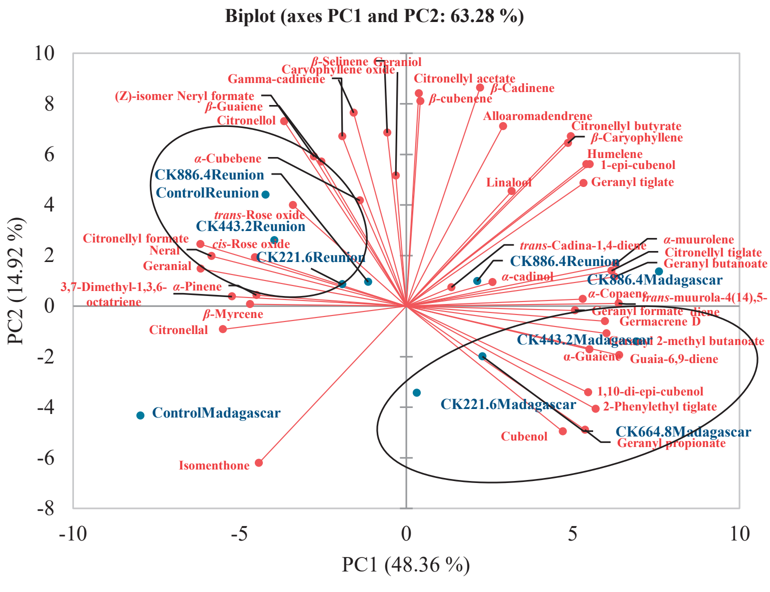

Figure 4. Principal component biplot showing the variations between total essential oil composition content in response to CK application (221.6, 443.2, 664.8, 886.4 µM and the control) and rose geranium varieties (Reunion and Madagascar-type) during February 2019 to April 2019 production cycle

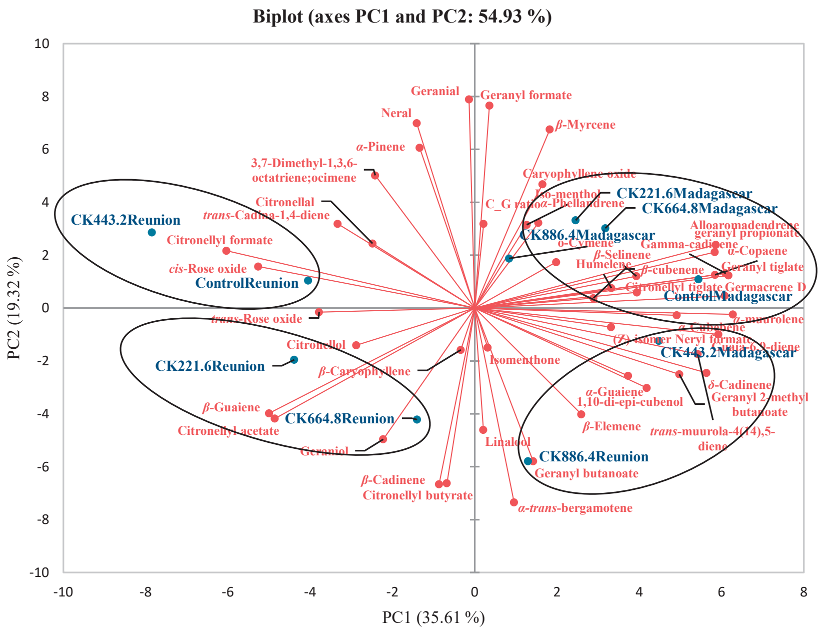

Figure 5. Principal component biplot showing the variations between total essential oil composition content in response to CK application (221.6, 443.2, 664.8, 886.4 µM and the control) and rose geranium varieties (Reunion and Madagascar-type) during the May 2019 to July 2019 production cycle

Interestingly, the highest application of CK (886.4 µM) on Reunion type showed to increase α-Cubebene, guaia-6,9-diene, *trans*-muurola-4(14),5-diene, α-muurolene and geranyl 2-methyl butanoate content. On the other hand, these compounds were also increased by administering CK at 443.2 µM on the Madagascar type.

In PC 2, by applying CK at 221.6, 664.8, 886.4 µM on Madagascar type increased β-Myrcene, geranyl formate, alloaromadendrene, germacrene D, gamma-cadinene and geranyl tiglate; however, this was not different from the control. Administering CK at 443.2 and 886.4 µM on Madagascar and Reunion type, respectively, caused a decline of α-trans-bergamotene, guaia-6,9-diene, *trans*-muurola-4(14),5-diene, α-muurolene, δ-Cadinene and geranyl 2-methyl butanoate content. Based on the analysis of variance, the guaia-6,9-diene was not affected by the interaction between the rose geranium varieties and CK application during the third production cycle; however, when PCA was applied, the two levels were shown to be adverse in the production of guaia-6,9-diene. For this reason, CK may be applied at 221.6 µM for a possible increased content of guaia-6,9-diene in both varieties.

In all production cycles, a trend was observed where uniform simulated wounding with CK administered between 221.6 to 664.8 µM came out to improve most of the essential oil compounds. Although this study focused on the major essential oil compounds and quality, it was interesting to note significant differences between the two varieties and as well how CK improved some of the key compounds, mostly corroborating the analysis of variance.

CONCLUSIONS

Environmental stress factors, such as wounding, temperature, water deficit, salinity and moisture stress have been reported widely to improve the secondary metabolites across wide species. However, this at most caused a significant reduction of the herbage material for aromatic plants such as rose geranium, directly affecting the essential oil yield and quality (Rhodinol).

In this study, the author observed that a uniform simulation of wounding and moisture stress closer to the harvest with CK applied between 221.6 to 664.8 µM concentrations may improve the essential oil yield, quality, and most of the major essential oil compounds for the two varieties: Reunion and Madagascar type cv. In some cases, it may be beneficial to increase the application of CK to 886.4 µM following simulated wounding where linalool and geranyl tiglate content are targeted on the Bourbon type, and Madagascar type, respectively.

Bourbon type remains better than the Madagascar type with the essential oil yield and maintains higher essential oil quality (Tembe and Deodhar, 2010a; Tembe and Deodhar, 2010b); however, both varieties were at most within the high-quality range as prescribed by ISO 4731[E] (2012). Therefore, based on these findings, the author concludes that the administration of any biostimulant containing CK at a concentration of 664.8 µM can be considered by growers, especially closer to harvest to improve the essential oil biosynthesis and quality of both varieties.

Future studies should focus on other cheaper biostimulants containing CK as well as determining the distinctions between normal production systems (as control) and introducing simulated wounding and moisture stress closer to harvest across the three production cycles as a repeated study, as this was the limitation of this study.

ACKNOWLEDGEMENTS

The author would like to thank Mr Mabuso for the cultivation, nurturing and for assisting with parts of the data collection; Dr Olivier, Dr Nieuwenhuis, and Mr Malatji (SEOBI) for providing the cuttings and facilities for the essential oil analysis; the Department of Agriculture, at the Central University of Technology, Free State for providing all the resources, and the National Research Foundation (Thuthuka Funding Instrument: PhD Track) for financial assistance.

REFERENCES

Abad Farooqi, A. H., & Sharma, S. (1988). Effect of growth retardants on growth and essential oil content in Japanese mint. *Plant Growth Regulation*, 7, 39-45.

Abad Farooqi, A. H., Sharma, S., Naqvi, A. A., & Khan, A. (1993). The effect of kinetin on flower and oil production in *Rosa damascena*. *Journal of Essential Oil Research*, 5(3), 305-309.

Abualia, R., Riegler, S., & Benkova, E. (2023). Nitrate, auxin and cytokinin—A trio to tango. *Cells*, 12(12), 1613.

Aqeel, U., Aftab, T., Khan, M. M. A., & Naeem, M. (2023). Regulation of essential oil in aromatic plants under changing environment. *Journal of Applied Research on Medicinal and Aromatic Plants*, 32, 100441.

Araya, H. T. (2012). Rose-scented geranium (*Pelargonium* spp.) herbage yield, essential oil yield and composition as influenced by nitrogen nutrition and liming (Doctoral dissertation, University of Pretoria).

Asghar, S., Ghori, N., Hyat, F., Li, Y., & Chen, C. (2023). Use of auxin and cytokinin for somatic embryogenesis in plant: a story from competence towards completion. *Plant Growth Regulation,* 99(3), 413-428.

Camas-Reyes, A., Vuelvas-Nolasco, R., Cabrera-Ponce, J. L., Pereyra-Alférez, B., Molina-Torres, J., & Martínez-Antonio, A. (2022). Effect of different cytokinins on shoot outgrowth and bioactive compounds profile of lemograss essential oil. *International Journal of Plant Biology,* 13(3), 298-314.

CBI (Centre for the promotion of imports) Ministry of Foreign Affairs. (2018). Exporting rose geranium oil to Europe. Web: https://www.cbi.eu/market-information/natural-ingredients-cosmetics/rose-geranium-oil/ [Accessed 04 February 2018].

Combrink, N. J. J. (2005). *Nutrient solutions and greenhouse management*. Combrink Familietrust: Stellenbosch.

Da da Silva, T. I., Lopes, A. S., da Silva Ribeiro, J. E., Nóbrega, J. S., de Melo Gonçalves, A. C., de Melo Filho, J. S., Sales, G. N. B., Blank, A. F., da Costa, F. B. & Dias, T. J. (2023). Salicylic acid decreases salt stress damage on photosynthetic processes and increases essential oil content in basil 'cinnamon'. *Journal of Soil Science and Plant Nutrition*, 23(3), 4318-4327.

DAFF (Department of Agriculture, Forestry and Fishery). (2012). Rose-geranium production. Web: www.nda.agric.za/docs/Bronchures/ProGuRosegeranium [Accessed 28 January 2018].

Demarne, F., & Van der Walt, J. J. A. (1989). Origin of the rose-scented *Pelargonium* cultivar grown on Réunion Island. *South African Journal of Botany*, 55(2), 184-191.

Demyttenaere, J. C., del Carmen Herrera, M., & De Kimpe, N. (2000). Biotransformation of geraniol, nerol and citral by sporulated surface cultures of *Aspergillus niger* and *Penicillium* sp. *Phytochemistry,* 55(4), 363-373.

Džamić, A. M., Soković, M. D., Ristić, M. S., Grujić, S. M., Mileski, K. S., & Marin, P. D. (2014). Chemical composition, antifungal and antioxidant activity of *Pelargonium graveolens* essential oil. *Journal of Applied Pharmaceutical Science*, 4(3), 001-005.

Egamberdieva, D., Wirth, S. J., Alqarawi, A. A., Abd_Allah, E. F., & Hashem, A. (2017). Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. *Frontiers in Microbiology*, 8, 2104.

Eiasu, B. K., Dyafta, V., & Araya, H. T. (2022). Effect of leaf age on essential oil yield and composition in rose-scented geranium. *HortScience*, 57(12), 1524-1528.

Eiasu, B. K., Steyn, J. M., & Soundy, P. (2009). Rose-scented geranium (*Pelargonium capitatum* × *P. radens*) growth and essential oil yield response to different soil water depletion regimes. *Agricultural Water Management*, 96(6), 991-1000.

Eiasu, B. K., Steyn, J. M., & Soundy, P. (2012). Physiomorphological response of rose-scented geranium (*Pelargonium* spp.) to irrigation frequency. *South African Journal of Botany,* 78, 96-103.

El-keltawi, N. E., & Croteau, R. (1987). Influence of foliar applied cytokinins on growth and essential oil content of several members of the Lamiaceae. *Phytochemistry*, 26(4), 891-895.

El-Sayed, B. A., Noor El-Deen, T. M., Diab, R. I., & Abdelsadek, O. A. (2023). Impact of vermicompost and benzyladenine on geranium plants quality. *Scientific Journal of Flowers and Ornamental Plants,* 10(2), 151-161.

Fraternale, D., Giamperi, L., Ricci, D., Rocchi, M. B. L., Guidi, L., Epifano, F., & Marcotullio, M. C. (2003). The effect of triacontanol on micropropagation and on secretory system of *Thymus mastichina*. *Plant Cell, Tissue and Organ Culture*, 74, 87-97.

Fugate, K. K., Ribeiro, W. S., Lulai, E. C., Deckard, E. L., & Finger, F. L. (2016). Cold temperature delays wound healing in postharvest sugarbeet roots. *Frontiers in Plant Science,* 7, 499.

Gebremeskel, H. (2014). Growth, biomass and oil yield of rose-scented geranium (*Pelargonium graveolens* L. Herit) as influenced by different harvesting stages. *International Journal of Novel Research in Life Sciences*, 1(1), 48-55.

Gomes, P. C. B. M. S. (2005). *Engineering perfumes*. Universidade do Porto (Portugal).

Gupta, R., Banerjee, S., Mallavarapu, G. R., Sharma, S., Khanuja, S. P. S., Shasany, A. K., & Kumar, S. (2002). Development of a superior somaclone of rose-scented geranium and a protocol for inducing variants. *HortScience*, 37(4), 632-636.

International Organization for Standardization (ISO) 4731. (2012). *Essential oil of geranium (Pelargonium* x ssp*.)*. International Standard, Geneva: Switzerland.

Juliani, H. R., Koroch, A., Simon, J. E., Hitimana, N., Daka, A., Ranarivelo, L., & Langenhoven, P. (2006). Quality of geranium oils (*Pelargonium* species): case studies in Southern and Eastern Africa. *Journal of Essential Oil Research*, 18, 116-121.

Khetsha, Z. P., & Sedibe, M. M. (2015). Effect of NaCl and root-media selection on yield attributes, oil composition and mineral composition of rose geranium (*Pelargonium graveolens* L.). *In Proceeding of the 2nd International Conference on Agriculture and Forestry*, l, 152-166.

Khetsha, Z. P., Sedibe, M. M., Pretorius, R. J. & van der Watt, E. (2021). Cytokinin, gibberellic acid and defoliation on density and morphology of trichome of *Pelargonium graveolens* L'Hér for essential oil biosynthesis. *Agrociencia*, 55(4), pp.331-346.

Khetsha, Z. P., Sedibe, M. M., Pretorius, R. J., Rathebe, P. C., & Moloantoa, K. (2022). *Using biostimulants containing phytohormones to recover hail-damaged essential oil plants*. In Revisiting Plant Biostimulants: IntechOpen.

Kumar, A., Verma, N., Nilofer, A., Kaur, P., Kumar, D., Ghosh, D., Singh, A., Siddiqui, A., Kumar, N., Singh, A. K., Khare, P., & Singh, S. (2022). Physiological and chemical changes induced by transparent polythene+ green net shed on *Pelargonium graveolens* L. mother plants during monsoon season. *Industrial Crops and Products,* 188, 115686.

Liu, L. W., Xu Xin, X. X., Zhang RuBing, Z. R., Cheng Tao, C. T., Cao YuJin, C. Y., Li XiaoXiao, L. X., Guo JianTao, G. J., Liu HuiZhou, L. H., & Xian Mo, X. M. (2016). Engineering Escherichia coli for high-yield geraniol production with biotransformation of geranyl acetate to geraniol under fed-batch culture. *Biotechnology for Biofuels*, 9, 1-8.

Lulai, E. C., Suttle, J. C., Olson, L. L., Neubauer, J. D., Campbell, L. G., & Campbell, M. A. (2016). Wounding induces changes in cytokinin and auxin content in potato tuber, but does not induce formation of gibberellins. *Journal of Plant Physiology*, 191, 22-28.

Machado, V. P. D. O., Pacheco, A. C., & Carvalho, M. E. A. (2014). Effect of biostimulant application on production and flavonoid content of marigold (*Calendula officinalis* L.). *Revista Ceres*, 61, 983-988.

Malatova, K., Hitimana, N., Niyibizi, T., Simon, J. E. & Juliani, H. R., (2011). Optimization of harvest regime and post-harvest handling in geranium production to maximize essential oil yield in Rwanda. *Industrial Crops and Products*, 34(2), pp.1348-1352.

Mastuti, R., Munawarti, A., & Firdiana, E. R. (2017). *The combination effect of auxin and cytokinin on in vitro callus formation of Physalis angulata L.–A medicinal plant*. In AIP Conference Proceedings, l. 1908, 1: AIP Publishing.

Melgoza, F. J., Kusakabe, A., Nelson, S. D., & Melgar, J. C. (2014). Exogenous applications of abscisic acid increase freeze tolerance in citrus trees. *International Journal of Fruit Science*, 14(4), 376-387.

Motsa, N.M., Soundy, P., Steyn, J.M., Learmonth, R.A., Mojela, N., & Teubes, C. (2006). Plant shoot age and temperature effects on essential oil yield and oil composition of rose-scented geranium (*Pelargonium* sp.) grown in South Africa. *Journal of Essential Oil Research*, 18, 106-110.

Nanda, A. K., & Melnyk, C. W. (2018). The role of plant hormones during grafting. *Journal of Plant Research*, 131(1), 49-58.

Niakan, M., & Ahmadi, A. (2014). Effects of foliar spraying kinetin on growth parameters and photosynthesis of tomato under different levels of drought stress. *Iranian Journal of Plant Physiology*, 4(2) 939-947.

Nogués, I., Brilli, F., & Loreto, F. (2006). Dimethylallyl diphosphate and geranyl diphosphate pools of plant species characterized by different isoprenoid emissions. *Plant Physiology*, 141(2), 721-730.

Norouzi, Y., Ghobadi, M., Saeidi, M. & Dogan, H. (2021). Effect of nitrogen and cytokinin on quantitative and qualitative yield of thyme (*Thymus vulgaris* L.). *Agrotechniques in Industrial Crops*, 1(1), pp.52-60.

Ono, E. O., Rodrigues, J. D., Barreiro, A. P., & Marques, M. O. M. (2011). Yield and composition of the essential oil of basil on plant growth regulators application. *Journal of Essential Oil Research*, 23(1), 17-22.

Pal, P.K., Mahajan, M. & Agnihotri, V.K. (2016). Foliar application of plant nutrients and kinetin modifies growth and essential oil profile in *Rosa damascena* under acidic conditions. *Acta Physiologiae Plantarum*, 38, pp.1-14.

Prins, C. L., Vieira, I. J., & Freitas, S. P. (2010). Growth regulators and essential oil production. *Brazilian Journal of Plant Physiology*, 22, 91-102.

Punpee, P. (2015). Conditional up-regulation of cytokinin status increases growth and survival of sugarcane in water-limited conditions. [PhD thesis]. Australia: University of Queensland.

Rao, B.R., Bhattacharya, A., Kaul, P. & Ramesh, S., (2001). Yield and chemical composition of rose-scented geranium (*Pelargonium* species) oil at different times of harvesting. *Journal of Essential Oil Research*, 13(6), pp.456-459.

Santoro, V. M., Nievas, F. L., Zygadlo, J. A., Giordano, W. F., & Banchio, E. (2013). Effects of growth regulators on biomass and the production of secondary metabolites in peppermint (*Mentha piperita*) micropropagated *in vitro*. *American Journal of Plant Sciences,* 4 (05), 49.

Saxena, G., Verma, P. C., Banerjee, S., & Kumar, S. (2008). Field performance of somaclones of rose scented geranium (*Pelargonium graveolens* L'Her Ex Ait.) for evaluation of their essential oil yield and composition. *Industrial Crops and Products*, 27(1), 86-90.

Sedibe, M. M. (2012). Yield and quality response of hydroponically grown rose geranium (*Pelargonium* sp.) to changes in the nutrient solution and shading (Doctoral dissertation, University of the Free State).

Sedibe, M. M., & Allemann, J. (2011). Effects of shading and moisture stress on yield and oil quality of hydroponically grown rose geranium. *In International Symposium on Medicinal and Aromatic Plants*, 1023, 249-254.

Sedibe, M. M., & Allemann, J. (2012). Yield and quality response of rose geranium (*Pelargonium graveolens* L.) to sulphur and phosphorus application. *South African Journal of Plant and Soil*, 29(3-4), 151-156.

Sharafzadeh, S., & Zare, M. (2011). Influence of growth regulators on growth and secondary metabolites of some medicinal plants from Lamiaceae family. *Advances in Environmental Biology*, 5(8), 2296-2302.

Shellie, R. A., & Marriott, P. J. (2003). Comprehensive two-dimensional gas chromatography-mass spectrometry analysis of *Pelargonium graveolens* essential oil using rapid scanning quadrupole mass spectrometry. *Analyst*, 128(7), 879-883.

Singh, A. L., Singh, S., Kurella, A., Verma, A., Mahatama, M. K., & Venkatesh, I. (2022). Plant bio-stimulants, their functions and use in enhancing stress tolerance in oilseeds. New and Future Developments in Microbial Biotechnology and Bioengineering. *Sustainable Agriculture Revitalization through Organic Products*, 239.

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, 52, 591-611.

Steel, R. G., & Torrie, J. H. (1980). *Principles and procedures of statistics*. Mcgraw-hill book: New York.

Steele, C. L., Katoh, S., Bohlmann, J., & Croteau, R. (1998). Regulation of oleoresinosis in grand fir (*Abies grandis*) differential transcriptional control of monoterpene, sesquiterpene, and diterpene synthase genes in response to wounding. *Plant Physiology*, 116(4), 1497-1504.

Svolacchia, N. and Sabatini, S. (2023). Cytokinins. *Current Biology*, 33(1), 10-13.

Swamy, K. N., & Rao, S. S. R. (2009). Effect of 24-epibrassinolide on growth, photosynthesis, and essential oil content of *Pelargonium graveolens* (L.) Herit. *Russian Journal of Plant Physiology*, 56, 616-620.

Tavares, E. S., Lopes D., Bizzo, H. R., Celso, L. S. & Leitão, S. G. (2004). Kinetin enhanced linalool production by *in vitro* plantlets of *Lippia alba*. *Journal of Essential Oil Research*, 16, 405-408.

Tembe, R. P., & Deodhar, M. A. (2010a). Chemical and molecular fingerprinting of different cultivars of *Pelargonium graveolens* (L'Herit.) viz., Reunion, Bourbon and Egyptian. *Biotechnology*, 9(4), 485-491.

Tembe, R. P., & Deodhar, M. A. (2010b). Clonal propagation of different cultivars of *Pelargonium graveolens* (L'Herit.) viz., Reunion, Bourbon and Egyptian. *Biotechnology*, 9(4), 492-498.

Thakur, M., & Kumar, R. (2020). Foliar application of plant growth regulators modulates the productivity and chemical profile of damask rose (*Rosa damascena* Mill.) under mid hill conditions of the western Himalaya. *Industrial Crops and Products*, 158, 113024.

Thakur, M., Bhatt, V., & Kumar, R. (2019). Effect of shade level and mulch type on growth, yield and essential oil composition of damask rose (*Rosa damascena* Mill.) under mid hill conditions of Western Himalayas. *PloS One*, 14(4), 0214672.

Van der Walt, J. J. A., & Demarne, F. (1988). *Pelargonium graveolens* and *P. radens*: A comparison of their morphology and essential oils. *South African Journal of Botany*, 54(6), 617-622.

Verma, R. S., Padalia, R. C., & Chauhan, A. (2016). *Rose-scented geranium* (*Pelargonium* sp.) *oils*. In Essential oils in food preservation, flavor and safety: Academic Press.

Wang, Y. H., & Irving, H. R. (2011). Developing a model of plant hormone interactions. *Plant Signaling & Behavior*, 6(4), 494-500.

Weng, D. C. J., Latip, J., Hasbullah, S. A., & Sastrohamidjojo, H. (2015). Optimal extraction and evaluation on the oil content of citronella oil extracted from *Cymbopogon nardus*. *Malaysian Journal of Analytical Sciences*, 19(1), 71-76.

Wojtania, A., & Gabryszewska, E. (2001). Effect of cytokinins and amino acids on multiplication of *Pelargonium* cultivars. *Acta-Societatis Botanicorum Poloniae*, 70(3), 203-208.

Wood, A. J., & Roger J. (2000). A simple and non-destructive technique for measuring plant growth and development. *American Biology Teacher* 62: 215-217.

Xu, L., Wu, C., Oelmüller, R., & Zhang, W. (2018). Role of phytohormones in *Piriformospora indica*-induced growth promotion and stress tolerance in plants: more questions than answers. *Frontiers in Microbiology*, 9, 1646.

Yamasaki, Y., Kunoh, H., Yamamoto, H., & Akimitsu, K. (2007). Biological roles of monoterpene volatiles derived from rough lemon (*Citrus jambhiri* Lush) in citrus defense. *Journal of General Plant Pathology*, 73, 168-179.

Zhang, P., Li, X., Cui, Z., & Xu, D. (2022). Morphological, physiological, biochemical and molecular analyses reveal wounding-induced agarwood formation mechanism in two types of *Aquilaria sinensis* (Lour.) Spreng. *Industrial Crops and Products*, 178, 114603.

Zielińska, S., Piątczak, E., Kalemba, D., & Matkowski, A. (2011). Influence of plant growth regulators on volatiles produced by in vitro grown shoots of *Agastache rugosa* (Fischer & CA Meyer) O. Kuntze. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 107, 161-167.

Zigene, Z. D., & Kassahun, B. M. (2023). Agro-morphological characters, essential oil content and essential oil yield of rose-scented geranium (*Pelargonium graveolens* L.) under different environmental conditions of Ethiopia. *Journal of Plant Biology and Crop Research*, 6, 1080.