# USING AGRICULTURAL PLANT GROWTH REGULATORS AS A MITIGATING STRATEGY FOR HAIL-DAMAGED ROSE GERANIUM (*Pelargonium graveolens* L'Hér.)

**by**

**Zenzile Peter Khetsha**

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**Promoter: Prof MM Sedibe**

**Co-promoter: Dr RJ Pretorius**

**Co-promoter: Dr E Van Der Watt**

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# DECLARATION

I, **Zenzile Peter Khetsha** declare that this thesis: **Using agricultural plant growth regulators as a mitigating strategy for hail-damaged rose geranium (*Pelargonium graveolens* L'Hér.)** submitted to the Central University of Technology, Free State in fulfilment of the requirements for the degree **Doctor Technologiae: Agriculture** is my independent work, and I have not previously submitted the same work to obtain a qualification at another university. I further disclaim the copyright of this thesis in favour of the Central University of Technology, Free State.

**Signature:……………………** **Date:……………………..**

# DEDICATION

*"This work is dedicated to my late Grandma, Nuku Emily Khetsha, late Granduncle, Zenzile Petrus Khetsha and late Uncles Nkosana Benjamin, and Mahlomola John Khetsha."*

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## ABSTRACT

Hailstorms cause significant yield and financial losses to the agricultural sector. There are reports that hail can cause severe damage to rose geranium (*Pelargonium graveolens* spp.) plants. There are currently no mechanisms in place to alleviate hail-damaged essential oil plants, such as rose geranium. This study aims to develop a strategy for hail damage, using agricultural plant growth regulators (PGRs) as an alleviating remedies for hail-damaged rose geranium. The first experiment was conducted using combined agricultural PGRs (cytokinin [CK] + gibberellic acid [GA]) during the 2016/17 and 2017/18 growing seasons. Treatments comprised of three simulated hail damage levels: zero% defoliation (intact plants); 50%; and 100% defoliation, as well as two levels of PGRs: CK 0.32 mg/L + GA 150 mg/L (low); CK 0.64 mg/L + GA 300 mg/L (high); and distilled water as a control. The second experiment was run simultaneously, using four experimental treatments of abscisic acid (ABA) and methyl jasmonate (MeJA): 75 µM ABA; 150 µM ABA; 10 mM MeJA; 20 mM MeJA; and distilled water as a control. In this experiment, the treatment was applied daily for either seven or 14 days, and the hail damage simulation was 100% defoliation only. Both experiments had a factorial treatment design, laid out in a randomised complete block design with three replications. In all experiments, the herbage yield attributes, essential oil yield parameters, leaf trichomes, and essential oil quality were measured. As hypothesized, 100% simulated hail damage negatively affected the herbage yield in the first experiment, indicating that severely hail-damaged plants require attention to facilitate their recovery. The effect of simulated hail damage, and the subsequent application of CK + GA, did not provide conclusive results on the recovery of the herbage yield attributes (plant parameters), and essential oil yield parameters. However, citronellol content increased by 4.6% when a low concentration of combined agricultural PGRs was applied to plants subjected to 100% simulated hail damage. A principal component analysis (PCA) was then applied to provide an overview of the effects on the plant and essential oil yield and quality parameters. The PCA showed a strong association between plant height, number of branches, the branch to height ratio (B:H ratio) and foliar fresh mass (FFM). The biplot loading explained that plant height, number of branches, B:H ratio and FFM of the plants which sustained only 50% foliage loss could be characterised by all treatments, including distilled water in both seasons. Brevicollate trichome densities declined when the high concentration of combined agricultural PGRs was applied. In the second experiment, 75 µM ABA and 10 mM MeJA affected the biosynthesis of linalool, isomenthone, citronellol, geraniol, and the citronellol to geraniol ratio (C:G ratio). However, the variation in these

essential oil compounds were inconsistent between the treatments, and did not improve the overall oil quality. Leaf area decreased by 62% following the application of 20 mM MeJA for 14 days, during the 2016/17 growing season. This significant variation was not observed in the leaf area during the following season; however, variations between growing seasons showed a similar trend. Overall, the application of ABA and MeJA had an adverse effect on the herbage and essential oil yield. The results from the two repeated experiments did not solve the hail damage as espoused. Therefore, an additional experiment was conducted during the 2019 growing season to determine the effects of agricultural PGRs containing GA; brassinosteroids (BRs); and trace CKs on the recovery of three levels (low, medium and high), and a control of simulated hail-damaged rose geranium plants (as before). As expected, the mean number of branches per metre (B:H ratio) and mean FFM declined with increased simulated hail damage. Plants were less bushy (B:H ratio) with smaller leaves (leaf area) at 100% simulated hail damage compared to 50% simulated hail damage. It was also found that 100% defoliated rose geranium plants accumulate most of the phenolic compounds associated with defoliation and wounding, while those with only 50% simulated hail damage accumulate phenolic compounds to a level similar to intact plants. Overall, the highest mean FFM was recorded when the highest concentration of PGRs were applied. In addition, brevicollate trichome densities were higher when the PGR-mixture was applied to both leaf surfaces. Rose geranium plants that suffered 50% simulated hail damage had higher mean essential oil yield compared to plants that suffered 100% simulated hail damage and treated with medium PGR. High-quality essential oil, with a C:G ratio below three, was recorded on all defoliated plants compared to the intact plants. The PCA (71.34%) corroborated the use of low PGRs on rose geranium plants that lost 100% foliage, since the N, P, K, Mg, and Mn were higher at this level. It is therefore recommended to consider applying a low to medium concentration of GA, BRs and traces of CKs to improve, and recover the growth of the herbage and essential oil quality (C:G ratio <3) of rose geranium plants with 100% foliage loss from hail damage. Rose geranium plants are grown for essential oil production; therefore, the aerial herbage material is a crucial component of the crop. It has been shown from this study that hail can causes significant damage to these plants, reducing this valuable material, and directly affecting the essential oil yield and quality. This study contributed to the development of a mitigation strategy for hail-damaged rose geranium plants using agricultural PGRs. The study only focused on the use of existing agricultural PGRs, foliar-applied agricultural PGRs, the *in vivo* study approaches, and the application frequencies. It may be beneficial to focus on root-applied approach of the PGRs, *in vitro* studies, and test other sources of biostimulants (containing PGRs) for future studies.

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T2 with 95% confidence. The solid line describes zero% defoliation (0%), the dashed line describes 50% defoliation, and the dotted line describes 100% defoliation. The coding for zero% defoliation (undamaged plants) is indicated by the first 0; 50% defoliation is indicated by the first 50; 100% defoliation is indicated by the first 100. The PGRs-mixtures treatment levels are indicated within the second part of the coding, where 0 (distilled water) indicates level 1; 0.5 indicates level 2; 1.0 indicates level 2; 1.5 indicates level 4. Last part of the coding indicates the number of replications. (B) Loading plot of p[2] versus p[1] (Central University of Technology, Free State).

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## CHAPTER ONE

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## GENERAL INTRODUCTION

## 1.1 Background and motivation for the study

Globally, the annual cost of crop yield loss, due to natural hazards and disasters is estimated at ca. USD$ 13 billion (FAO 2015). Hailstorms are widely recognised as a natural hazard that result in significant crop yield losses (FAO 2017). In the USA alone, annual crop losses attributed to hail damage was USD$ 1.3 billion in 2009 (Jewell and Brimelow 2009). In Australia, ca. USD$ 1.8 billion is lost in agricultural production annually because of hail damage (Schuster *et al.* 2005).

Hail is pellets (in the form of small balls, or pieces of ice), which range in size from 0.5 mm to 100 mm in diameter (Conley *et al.* 2008). In southern Africa, hailstorms occur sporadically during the summer months, and rarely during winter (Piketh and Burger 2014). The severity of hail damage varies between plant species, and depends on several factors, such as the stage of development and plant height, as well as the leaf and stem morphology (Fernandes *et al.* 2012). Hail damage to plants is characterised by defoliation, tearing, bruising, breaking, and the loss and/or wounding of stems and flowers. The subsequent recovery of plants depends on the growth stage during which injury occurred, as well as the prevailing weather conditions following hail damage (Jamli 2014).

Severe hailstorm events have been reported in South Africa between 2016 and 2020 (Coleman 2016; Farmers weekly 2018; The Citizen 2020). A total of ca. 2.1% of the total annual agricultural production in South Africa is lost due to hail damage (Melville 2014; Piketh and Burger 2014). The areas most affected by hail damage are Mpumalanga Lowveld, KwaZulu-Natal, Western Cape, Limpopo, Gauteng, North West, and Eastern Cape Provinces (Melville 2014; Piketh and Burger 2014). Most of the essential oil plants, including rose geranium (*Pelargonium graveolens* spp.), are grown in these provinces. To date, there is no recorded data for the impact of hail damage on essential oil plants in South Africa; however, hail causes substantial damage to rose geranium plants (Swamy *et al.* 1960; Dermane and van der Walt 1989; Weiss 1997). The recovery response period for hail-damaged rose geranium plants has not been determined.

Changes in the secondary metabolites of essential oil plants vary depending on the type of damage incurred by the plant (Banchio *et al*. 2007). Hail wounding on these plants affect specific aromatic and therapeutic attributes that make these plants economically important (Banchio *et al*. 2007; Troncoso *et al.* 2011). It has also been shown that hail damage, mechanical damage, and insect herbivory cause essential oil compound changes (Troncoso *et al.* 2011). For example, hail damage simulation through leaf puncturing resulted in reduced menthone levels and increased pulegone concentrations in muña (*Minthostachys mollis* [Kunth] Griseb.). This subsequently altered the composition of the volatiles released from the damaged leaves (Banchio *et al*. 2007).

A common mitigation strategy for the loss in yield caused by hail damage is crop insurance (Potter *et al*. 2010). In South Africa, crop insurance against hail damage can be purchased for cotton (*Gossypium hirsutum* L.), maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), and soybean (*Glycine max* L. Merr.) (Marè 2014), but not for essential oil crops. Alternative strategies include the construction of hail nets as a preventative measure against hail damage. However, this strategy is often unfeasible as the high construction and maintenance costs require more herbage material to produce sufficient essential oil to recover these costs. This, in turn, requires more land for production. Some farmers increase the application of nitrogen after hail to facilitate the formation of new leaves and buds (Henson and Heichel 1986). It has been reported that increased nitrogen fertilisation increases rose geranium herbage material (Araya *et al.* 2006; Sedibe 2012); however, the essential oil quality is reduced (based on the ISO standard) (Sedibe 2012). In temperate regions where hail frequencies are high, agro-meteorologists implement strategies, such as hail forecasting and cloud seeding, to reduce the extent of hail damage (Potter *et al*. 2010).

Exogenous applications of natural and synthetic plant growth regulators (PGRs) have consistently demonstrated growth enhancement, yield and quality optimisation, as well as physiological efficiency in plants (Nemhauser *et al.* 2006; Giannakoula *et al.* 2012). PGRs are involved in diverse plant physiological processes, including the regulation of gene expression for adaptive responses to biotic and abiotic stresses (Anderson *et al*. 2004). Primary PGRs include auxins, abscisic acid (ABA), cytokinins (CKs), gibberellic acids (GAs) and ethylene (Drábková *et al.* 2015). Secondary PGRs include jasmonates and its analogues (methyl jasmonate, MeJA), brassinosteroids (BRs), salicylic acid, polyamines, sterols, and dehydrodiconiferyl alcohol glucosides (Drábková *et al.* 2015). The effect of foliar application of agricultural PGRs, as a mitigation strategy to improve hail damage recovery, has not been investigated in essential oil plants. There is no information on the response of hail-damaged rose geranium plants, ameliorated with PGRs.

Rose geranium is an indeterminate plant species, native to South Africa. It was developed from a cross between *P. radens* (H.E. Moore) and *P. capitatum* (L'Hér.) in 1690 (Araya 2012). Rose geranium oil is used globally in the aromatherapeutic, pharmaceutical, food, and perfumery industries (SEDA 2009; DAFF 2012). It ranks among the top 20 most valuable essential oil plant species in the world (Eiasu *et al.* 2009). In South Africa, the cultivation of rose geranium occurs in open field conditions, making it more susceptible to hail damage. Most South African rose geranium growers currently face production risks and uncertainties, which are further aggravated by climate change (FAO 2015).

### 1.2 Problem statement

Hail can cause significant damage to rose geranium plants (Swamy *et al.* 1960; Dermane and van der Walt 1989; Weiss 1997); however, this has never been investigated scientifically. Globally, essential oil crop producers primarily make use of agricultural crop insurance and costly mitigation strategies to recover lost production costs and alleviate hail-damaged plants. However, in South Africa, essential oil plants are not covered under agricultural crop insurance, and many commercial farmers are not able to regularly employ expensive alternative strategies. Therefore, hail damage may present a challenge to essential oil growers, including rose geranium growers. The use of natural and synthetic PGRs, as an alternative biological mitigation strategy against hail damage in essential oil crops, has not received much attention in South Africa.

### 1.3 Study objectives

The main objective of this study is to develop a mitigation strategy for hail-damaged rose geranium plants, using agricultural PGRs. To develop this strategy, the following specific objectives were undertaken:

- To evaluate the use of combined agricultural PGRs (CK + GA) as a potential recovery mechanism for the herbage yield, and the essential oil yield and quality of simulated hail-damaged rose geranium plants.
- To evaluate the use of ABA and MeJA as a potential mitigating mechanism on simulated hail-damaged rose geranium plants.
- To determine the recovery of simulated hail-damaged rose geranium plants, when treated with different levels of agricultural PGR-mixtures (GAs, BRs, and traces of

CKs: registered as *Lucky Plant*® [Agraforum, Germany]: EP1051075B1 & EP1933626).

- To evaluate the influence of agricultural PGR-mixtures on the leaf mineral status and phenolic composition of simulated hail-damaged rose geranium plants.

**1.4 Hypotheses**

It is hypothesised that simulated hail damage will negatively affect herbage and essential oil yields. The application of high concentrations of a mixture, or combination of agricultural PGRs with stimulatory effects, will lead to a rapid recovery of the vegetative mass and essential oil yield, as well as improving essential oil quality parameters.

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# CHAPTER TWO

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# LITERATURE REVIEW

## 2.1 Rose geranium

Rose geranium (*Pelargonium graveolens* L'Hér.) is one of 250 plant species in the family Geraniaceae. The name 'geranium' is derived from the Greek word meaning 'stork', because the seed case resembles the slender bill of these birds (ITC 2015). The most commonly cultivated species in the genus *Pelargonium* include *P. graveolens* (L'Hér.), *P. odoratissimum* (L'Hér.), *P. capitatum* (L'Hér.), and *P. radens* (Moore), all of which are grown for their essential oils (Lalli 2006). *Pelargonium* spp. differ slightly in terms of their morphology (Dermane 2002). The rose geranium cross, *P. radens* (Moore) x *P. capitatum* (L'Hér.), is polyploid ($2n = 77$) and octoploid ($2n = 88$), due to the high degree of male and female sterility.

### 2.1.1 Morphological description, scale of production, and uses

*South African rose geranium cultivars:* Globally, the cultivars with high-quality essential oil include 'Bourbon', 'Reunion', 'North Africa-type' (mostly called 'Egyptian' or 'Algerian'), 'Madagascar', and 'Chinese' (ISO 4731 2012). In South Africa, 'Bourbon', 'Reunion', and 'North Africa-type' are the most common cultivars (Motsa *et al.* 2006; Tembe and Deodhar 2010). The rose geranium (Figure 2.1) is described as large, bushy, upright, with branching shrubs, growing to a height of ca. 1.3 m (Dermane and Van Der Walt 1989; Motsa *et al.* 2006).

Generally, the stems of these cultivars are villous to densely villous, herbaceous with a soft green to grey colour, and covered with different types of bristles (long, short, and fine-textured) (Van Der Walt and Dermane 1988; Gupta *et al.* 2002). The external leaf morphology of rose geranium varies between cultivars; the main distinction being the alternate, opposite, or compound leaf types (Tembe and Deodhar 2010). Most of the cultivars have leaves with a deep-lobed fan shape, containing five to seven divided lobes, usually with palmate venation and stupules (Motsa *et al.* 2006).

'Reunion' has thick and less dentate teeth on the leaf margins, compared to 'Bourbon' and 'North Africa-type' cultivars (Figure 2.2) (Tembe and Deodhar 2010). In addition, it also has more pubescence on the leaf lamina, as a result of high glandular hair growth (Tembe and Deodhar 2010). The 'Bourbon' and 'North Africa-type' have similar leaf morphologies. 'North Africa-type' has 6 cm-12 cm petiolate leaves, with a triangular stipule 0.6 cm-0.10 cm long (El-Ghadban and Rizk 2006). In addition, the leaf is a simple blade, palmatisect with pinnatisect sections, and a distance of 0.3 cm-0.4 cm between the main vine and nearest lamina margin.

In its natural habitat, rose geranium flowers profusely during spring until the end of summer, particularly in the high-altitude regions of Africa (Rajeswara-Rao 2000). The flowers are light to dark pink in colour, with reddish-purple markings on the petals. The two posterior petals are larger than the three anterior petals, and contain seven stamens (Rajeswara-Rao 2000). Slight variations between the flowers of the different cultivars have been reported: the 'North African-type' is slender with dark pink flowers, while 'Bourbon' and 'Reunion' cultivars have light pink flowers and are more resistant to wet conditions (Rao *et al.* 1993).

Under the appropriate growing conditions, the oil yield for 'Reunion' is lower than that of the 'Bourbon' and 'North Africa-type' cultivars, as determined by chemical fingerprinting (Tembe and Deodhar 2010). Tembe and Deodhar (2010) reported an oil content of 0.2% for 'Reunion', 0.4% for 'Bourbon', and 0.6% for 'North Africa-type'. Rose geranium plants contain 80-120 known compounds, of which only a few contribute to the essential oil odour (Demarne 2002). From a total of 30 compounds that contribute to rose geranium oil quality, only seven (linalool, citronellol, geraniol, citronellyl formate, geranyl formate, guaia-6,9-diene, and isomenthone) have been identified as useful for commercial purposes (Rana *et al.* 2002; ISO 4731 2012). However, according to ISO 4731 (2012), *cis*-Rose oxide, *trans*-Rose oxide, menthone, $\alpha$-Terpineol, geranyl butyrate, geranyl tiglate, and $\beta$-phenylethyl tiglate are also important compounds, which contribute to the quality and characterisation of the oil. 'Bourbon' and 'Reunion' cultivars have a similar chemical fingerprint because Bourbon Island is the former name of Reunion Island (Rajeswara-Rao 2000; ISO 4731 2012). This followed after the change made by the ISO 4731 (2012) recently.

The most important compounds used in the perfume industry are geraniol, citronellol, and linalool, with a citronellol: geraniol ratio (C:G ratio) below 3:1 being preferred by the industry (Saxena *et al.* 2008). Essential oil composition differs between regions, for example, essential oil from Egypt ('North Africa-type') contains 10-*epi*-*y*-eudesmol, while this compound is not found in cultivars from Bourbon, China, and Madagascar (ISO 4731 2012). Tembe and Deodhar (2010) studied the chemical and molecular fingerprinting of 'Reunion', 'Bourbon', and 'North Africa-type'/'Egyptian' cultivars, and found the C:G ratios to be 1:1 ('Reunion'), 2:1 ('Bourbon'), and 4:1 ('North Africa-type'). According to the ISO 4731 [E] standard, the cultivars ‘Reunion’ and ‘Bourbon’ are recently merged, and classified based on a 3:1 C:G ratio standard (ISO 4731 2012).

*Scale of production:* Annual global production of rose geranium is between 350 and 400 tonnes, and is dominated by Egypt and China, which produce ca. 280-350 tonnes of essential oil per year (ITC 2015). Smaller quantities of essential oils are also produced by east and southern African countries (ITC 2015). India produces 25 to 35 tonnes, South Africa produces 5 to 10 tonnes, other central and southern African regions provide at least 10 tonnes, and Madagascar produces around 5 tonnes (ITC 2015). South African production takes place in the Mpumalanga Lowveld, KwaZulu-Natal, Western Cape, Limpopo, Gauteng, North West, Eastern Cape, and Free State Provinces (DAFF 2012; ITC 2015). South African production is mostly absorbed by the local market, although some of the production is intended for the international market (Araya 2012; Sedibe 2012).

*Uses:* The majority of rose geranium oil is used by the perfumery industry, which is dominated by France and Switzerland (DAFF 2012): rose geranium essential oil is used to produce expensive perfumes (Gomes *et al.* 2006). In food and flavouring industries, the USA and Japan dominate the soft drink industry, as it is also used as a flavouring agent in many soft drink and foods (Sedibe 2012). Rose geranium oil is also in demand from the aromatherapy, and the pharmaceutical industry as it helps blood circulation-related problems; it can be used to treat sore throats and tonsillitis; it stimulates the secretions of hormones in the adrenocortical glands and premenstrual syndrome; and the aroma of rose geranium has been shown to reduce nervous tension, stress, and neuralgia (Lawless 1995).

### 2.1.2 Rose geranium harvesting techniques

Under open field conditions, the first harvest of rose geranium can be obtained after three to six months, depending on the cutting size, location, and agronomic practices followed (Motsa *et al.* 2006). In protective structures with hydroponic systems, the first harvest can be obtained within three to four months, due to the controlled production system (Sedibe 2012). According to Haileslassie and Kebede (2015), the recommended harvesting period for improved herbage, as well as essential oil yield and quality, is between 12 and 20 weeks after planting. Rose geranium can be harvested three to five times per year, under both open field and greenhouse/protective structure conditions (ITC 2015). Rose geranium plants are kept for five years before replanting, but in areas experiencing high losses, replanting is usually done after three years.

## 2.2 Plant hail damage and hail mitigating strategies

Extreme climatic conditions can lead to significant losses in the agricultural sector (Bal *et al*. 2014; FAO 2017). These losses have drastically increased, by at least 400% from 1960 to 2005 (Mills 2005; Fernandes *et al*. 2012; Jamli 2014). Hailstorms are one of the most common global natural disasters (Jamli 2014), and temperate zones seem especially prone to hailstorm events (Bal *et al*. 2014). The following review focusses on hailstorm as one of the natural disasters responsible for crop loss and damage.

### 2.2.1 Extent of hail damage on plants

Hail is defined as precipitation in the form of small pellets, or pieces of ice, which vary in size from 0.5 mm to 100 mm (Steiner 1988). Hail can cause extensive damage to growing crops and other vegetation (Schubert 1991). Hail formation takes place in elongated convective cumulonimbus clouds (Figure 2.3), which are often associated with thunderstorms (Bal *et al*. 2014). Severe hail-related losses result from hailstones above H2 size (Powell 2013) (see Table 2.1 for details on hail sizes). Hailstones above H2 cause defoliation, tearing, bruising, breaking, and the loss of stems and flowers (Schubert 1991). Such wounding can also lead to plant stress, which affects growth and metabolic activities (Łukaszuk and Ciereszko 2012). This can further predispose plants to disease outbreaks since wounds provide an entry point for plant pathogens (Łukaszuk and Ciereszko 2012).

The extent of hail damage depends on several factors, such as the number of hailstones per unit area, wind velocity (Bal *et al.* 2014), and hailstone size (Table 2.1) (Changnon 1999). Changnon (1999) and Fernandes *et al.* (2012), reported that hail damage differs extensively amongst plant species, and is influenced by several factors, such as plant height, and leaf and stem morphology. Certain plant species, e.g. soybean (*Glycine max* [L.] Merr.), tea (*Camellia sinensis* [L.] Kuntze), and tobacco (*Nicotiana tabacum* L.), are susceptible to damage by hailstones of any size (>H0), due to their leaf and stem morphology (Changnon 1999; Klein and Shapiro 2011). In contrast, crops such as maize and wheat are mostly damaged by larger, windblown hailstones (>H3) because of their height, and differences in leaf area, the stem sizes and morphology (Changnon 1999; Klein and Shapiro 2011).

Plants also respond differently to wounding stress following hail damage. Physical wounding of plant tissue resulting from hail damage and defoliation initiates a cascade of biochemical or physiological processes, which results in the repair of damaged tissue and resistance to opportunistic pests and pathogens (Suttle *et al.* 2013). Such alterations occur both in the tissue immediately surrounding the wound, and in distal tissue not directly in contact with the damaged tissue (Suttle *et al.* 2013). At the wound site, cell division and the synthesis of cellular components that are required to isolate the damaged tissue, reduce water loss, and restore tissue integrity, is initiated (Suttle *et al.* 2013). In leaves, wounding also induces the synthesis and accumulation of anti-microbial compounds, and in the specific case of damage caused by herbivory, volatile metabolites are released to deter pests and attract their predators and parasitoids (Suttle *et al.* 2013).

Hail damage hinders plant growth, and affects the yield and quality by changing the cellular metabolic processes (Kundu and Gantait 2017). Defoliation and wounding stress in plants results in a knock-back effect that reduces the assimilation of carbon, ultimately affecting the rate of photosynthesis (Kundu and Gantait 2017). This stress stimulates the production of bio-inhibitors, reactive oxygen species levels, transient $Ca^{2+}$ influxes into the cytoplasm, and protein phosphorylation. It also causes irreversible injury to cells and tissue that eventually slows growth (Smékalová *et al.* 2014). This has been reported in potato plants (*Solanum tuberosum* L.), where ribonucleic acid (RNA) homology changed as a result of wounding (Sanchez-Serrano *et al.* 1986). Christopher *et al.* (2004) identified a suite of wound-regulated genes, indicating the diversity and multiplicity of the induced defence response in systemically wounded leaves of hybrid poplars (*Populus trichocarpa x P. deltoides*). In lettuce (*Lactuca sativa* L.), plant wounding induced the synthesis of phenylalanine ammonia-lyase compounds (Kang and Saltveit 2003).

The local and systemic plant responses activate and regulate defence mechanisms for localised tissue damage, such as those resulting from hail damage (Lèon *et al.* 2001). Plants can also positively adapt with altered growth habits to contradict the damaging effects of hail (Kundu and Gantait 2017). Thus, the responses are both reversible and irreversible modifications, such as cell division, alterations of membrane channels, and a change in the structure of the cell wall (Kundu and Gantait 2017). This has been demonstrated with muña (*Minthostachys mollis* [Kunth] Griseb.), where leaf puncturing resulted in reduced menthone levels, while the pulegone concentration increased during the first 48 hours of the experiment

(Banchio *et al.* 2007). Furthermore, an increased pulegone level and diminishing menthone emissions also altered the composition of volatiles released from damaged leaves. Depending on the phytohormonal crosstalk and proteins released, wound-inducible genes may either repair damaged plant tissue or produce inhibitory system (León *et al*. 2001).

### 2.2.2 Global hailstorm events and losses

The USA, Australia, South Africa, Canada, and India are most severely affected by hailstorms (Bal *et al.* 2014; FAO 2015), while China, France, Italy, Russia, Argentina, and Finland also experience moderate hail damage (Changnon 1999). According to Jewell and Brimelow (2009), the USA suffers the most severe hail-related crop losses annually, with ca. USD$ 1.3 billion in crop damage, and an average annual frequency of 158 days of hailstorms (Changnon 1999). In Alberta (Canada) annual crop losses, due to hail damage, amounts to 5% of the annual gross production value, with direct costs ranging from USD$ 393 million to US$ 800 million (Krauss and Santos 2004). In Argentina (Entre Rios), a loss of USD$ 270 million was reported on important commercial crops such as soybean, sunflower (*Helianthus annuus* L.), and maize, due to hail damage suffered in 2005 (Mezher *et al.* 2012).

Compared to the USA, hail occurrence in the EU is less frequent and less severe (Punge and Kunz 2016). However, in July 2013, an insurance amount of EUR€ 2.8 billion was claimed in Germany, representing the costliest global insurance event for that year (Punge and Kunz 2016). Hailstorms in June 2014 cost France, Belgium, and Germany a combined cost of EUR€ 2.3 billion in damages. Furthermore, in Germany, hailstorms, with hailstones measuring ca. 3 mm-6 mm in diameter, were recorded in July 2000 and June 2001 (Tartachnyk and Blanke 2002).

In the Southern Hemisphere, Australia experiences the most frequent hailstorm events (at an average cost of AUD$31 billion, annually), followed by South Africa, Kenya, Zimbabwe, Madagascar, South America, and Ecuador (Doswell 2001). Table 2.2 and Figure 2.4 reports on the notable hailstorm events recorded in South Africa. These hail events destroyed ca. 2.1% of the total annual agricultural production in South Africa during the 2014 production season, leading to damages worth millions of ZAR (Melville 2014; Piketh and Burger 2014). Severe hail damage incidents have primarily been recorded from the growing regions of Mpumalanga Lowveld, KwaZulu-Natal, Western Cape, Limpopo, Gauteng, North

West, and Eastern Cape Provinces, with between seven and 24 hailstorm events and an average of four hail days per annum (Melville 2014; Piketh and Burger 2014).

Schwarz (2014) reported an annual average of 26 hailstorms events in the Free State Province. Specific locations in the eastern Free State receive an average of three to five hail days per year (Visser and van Heerden 2000). These figures increase to five to seven days over the southern and eastern regions of the eastern Free State (Visser and van Heerden 2000). The North West Province experiences the lowest hailstorm frequencies in South Africa, with an average of only two events per annum (Schwarz 2014). Although globally, hail frequency and intensity changes over time due to climatic fluctuations, yearly losses are still within the 5% – 15% range (Powell 2013).

### 2.2.3 Hail damage simulations and assessment on plants

Agricultural crop insurance companies assess plant hail damage depending on the crop growth stage, which gives them an indication of recovery speed and plant health (Nielsen 2012). Klein and Shapiro (2011) state that the assessment of hail damage should be categorised based on the plant stand reduction, leaf defoliation, and stem damage. Agricultural crop insurance companies use various hail simulation techniques to mimic actual hail damage, and these are influenced by plant morphology, plant growth stage, and plant growth duration (Irigoyen *et al.* 2010). These simulations are implemented either through removing a certain amount, or a percentage of leaves and stems (either by hand or using commercial grass trimmers, hand-held flails, or staple removers), or the complete removal of whole plants. This involves the removal, defoliation, cutting-off, and bending of stems of aerial herbage parts (Irigoyen *et al.* 2010). These procedures are all based on the initial plant yield, prior to conducting the simulation, in order to assess hail-related losses quantitatively (Bueckert 2011). These simulations are also performed at different crop growth stages to determine the recovery rate of various crops.

Hail simulation techniques are more easily conducted on crops with determinant growth, compared to plants with indeterminate growth. This is because of the difficulty in defining the exact leaf area, as well as the number of leaves and stems, in indeterminate plants (Irigoyen *et al*. 2010; Bal *et al*. 2014). In agricultural production, various mitigation methods are used to alleviate and avoid hail-related risks (Klein and Shapiro 2011; Nielsen 2012).

### 2.2.4 Hail damage risk-mitigation strategies

Crop insurance is the most common risk-mitigation strategy used by commercial farmers. The following section discusses several forecasting, mitigation, and control strategies that are employed globally to alleviate the negative economic impact resulting from hail damage. These strategies require long-term planning, as well as an understanding of risk and uncertainty, and hail damage recovery strategies (Potter *et al.* 2010).

*Agricultural hail-risk insurance strategies:* It is difficult to compare the cost of agricultural losses from hail between countries due to variations in the value of different crops (Steiner 1988). However, globally, hail damage results in an annual estimated loss of ca. USD$ 1.3 billion in crop production (Potter *et al.* 2010). In 2018, a global record in crop loss cost of USD$11.23 billion was recorded in the USA alone (Prein and Holland 2018). Therefore, various methods are used to protect against hail damage, including agricultural crop insurance. Currently, there are two main hail insurance options; the Multi-Peril Crop insurances, and a range of cover provided by private companies (Potter *et al.* 2010). These insurance options are generally calculated by a loss ratio of the amount of money paid out in losses, divided by the amount of money collected in premiums (Potter *et al.* 2010). Growers with Multi-Peril Crop option covers enjoy lower premium costs for the insured because of the basis of an all-in-one type package.

*Hail control strategies:* Hail control strategies include cloud seeding, followed by a premature rainout strategy, the use of a cannon or techniques for creating loud noises or explosions to interfere with hail-embryo formations (Potter *et al.* 2010; Bal *et al.* 2014). Seeding is intended to melt hail into rain before it reaches the Earth's surface (Bal *et al.* 2014). Although in practise this approach is a viable option, detailed forecasting is needed to anticipate hailstorm events, and the costs associated with this technique are between USD$ 15 million and USD$ 90 million annually (Dordley 2019).

*Hail forecasting strategies:* Radio detection and ranging (radar) mechanisms are used to detect the formation of hailstones in potential storms (Visser and van Heerden 2000; Bal *et al.* 2014). Hail forecasting modelling in South Africa started in December 1971 in Nelspruit, Mpumalanga province (Visser and van Heerden 2000). This model is used as a guideline for severe climate forecasting and includes cloud top height and temperature as parameters for identifying severe hailstorms (Visser and van Heerden 2000; Bal *et al.* 2014). Following the hail-forecast modelling, radar makes use of algorithms to plot weather data and access detailed storm activities. Polarimetric radar is used to detect hail, using remote sensing and echo dimensions (Bal *et al.* 2014). Probability mapping is also an option for identifying hail-risk areas, but it requires long-term data of hailstorm events (Bal *et al.* 2014). Hail forecasting strategies are practical; however, they need exorbitant capital to develop, USD$300-400 (Bal *et al.* 2014). Therefore, it is not viable to establish these strategies for most essential oil production areas, as the industry is still developing.

*Hail preventative strategies:* Another method used for mitigating hail damage is the cultivation of crops under protective structures using hail nets. Hail nets provide protection against intense radiation, and as well reduces the intensity of the hailstones. Planting trees can also prevent hail damage, to some extent. Tree shelterbelts reduce wind speed, resulting in less kinetic energy and intensity from hailstones upon impact. However, they take a period of 10 to 30 years before they can provide any meaningful protection (Bal *et al.* 2014).

*Agronomic hail mitigating strategies:* Biological hail mitigation strategies include increased nitrogen (N) application following hailstorms, to facilitate the formation of new leaves and buds, as well as the use of pesticides for protection against pathogens (Henson and Heichel 1986). Araya *et al.* (2006) and Sedibe (2012) have reported that increased N fertilisation increases the herbage material of rose geranium under normal condition; however, it reduces the essential oil quality, based on the ISO standard (Sedibe 2012). Exogenous applications of natural and synthetic PGRs enhance growth, increase oil yield and quality, as well as improving physiological efficiency in plants (Torres *et al.* 2018). However, there is currently no information in the literature on hail-damaged rose geranium plants treated with PGRs.

## 2.3 PGRs as stimulatory substances

PGRs are molecules that influence the growth and development of plants, even at low concentrations. There are PGRs which are produced naturally by plants, as well as synthetic regulators, which are have been produced in biotechnology research as biostimulants (Banchio *et al.* 2007). Biostimulants are used in global crop production to improve field competitiveness, nutrient use efficiency, and stress resistance in plants (Torres *et al.* 2018). Most biostimulants and their products are extracted from algae, arbuscular mycorrhizal fungi, chitin and chitosan derivatives, fulvic and humic acids, synthetic phytohormones, and natural plant extracts

(Torres *et al.* 2018). This section focuses on the use of PGRs as biostimulants to recover defoliated, wounded, and hail-damaged plants.

### 2.3.1 History and classification of phytohormones

Plant metabolic activities are regulated by phytohormones (Mano and Nemoto 2012). Phytohormones are produced naturally by plants and are small organic molecules which, at low endogenous concentrations ($10^{-6}$ M to $10^{-9}$ M), induce metabolic activities within plant cells to modify growth and development (Drábková *et al.* 2015). However, synthetic chemicals, with the same properties and effects as natural phytohormones, can be produced (Banchio *et al.* 2007). A key research objective in plant biotechnology is to recognise the mechanism used by plants to respond to and overcome different environmental stressors (Kundu and Gantait 2017). Phytohormones are classified as either PGRs (CK, GA, BRs, and auxins) or as bio-inhibitors (ABA, jasmonates, and salicylic acid) (Giannakoula *et al.* 2012). PGRs are involved in a number of diverse plant physiological processes, including the regulation of gene expression for adaptive responses to biotic and abiotic stress (Anderson 2004) and in wound healing (Kundu and Gantait 2017; Han *et al*. 2018). Primary PGRs include auxins, ABA, CK, GA, and ethylene (Gasper *et al.* 1996). Other naturally occurring phytohormonal-like molecules include jasmonates, BRs, salicylic acid, polyamines, sterols, and dehydrodiconiferyl alcohol glucosides (Gasper *et al.* 1996). PGRs alter metabolic activities associated with cell division, cell enlargement, flowering, fruiting, and seed formation. Nemhauser *et al.* (2006) found that exogenous applications of PGRs regulate plant growth, and greatly influence plant stature and organ size. Bio-inhibitors are involved in the alleviation of biotic and abiotic stress that results from wounding, moisture stress, and temperature stress (Giannakoula *et al.* 2012).

### 2.3.2 Effects of PGRs on plant growth and development, and refoliation

PGRs promote growth and development, the production of secondary metabolites, as well as bio-inhibition, due to the extensive crosstalk and signal integration which affects the plants physio-morphological chemistry (Banchio *et al.* 2007). The recovery mechanism of defoliated, wounded, and hail-damaged plants has provided the rationale for investigating the potential of PGR application in this study. The following section examines the effects of natural and synthetic ABA, jasmonates, BRs, CK and GA on plant growth and development, and changes in primary and secondary metabolite biosynthesis.

*Abscisic acid:* ABA is a stress-signalling molecule, found to occur in all kingdoms, except Archea. The effect of foliar applications of ABA on plant growth is dependent on the plant species (Olds *et al.* 2018). It is a crucial regulator of important plant processes, including resilience to abiotic stressors, such as wounding, moisture, light, drought, and temperature (Saito *et al.* 2006). ABA is also involved in primary plant growth development. This includes buffering the day-night alterations of leaf growth rate, and regulating stomatal movement and transpiration rate (Tardieu *et al.* 2009). It also improves leaf growth by increasing tissue and whole-plant hydraulic conductivity (Punpee 2015). Dammann *et al*. (1997) found that hail damage, and defoliation causes increased levels of ABA in plants, which in turn activates the biosynthesis of jasmonates.

ABA is a $C_{15}$ sesquiterpenoid, formed by the joining of three isoprenoid units (Kundu and Gantait 2017; Olds *et al.* 2018). ABA occurs naturally as (S)-(+)-ABA, which is often called a *cis* isomer; a combination of 1:1 *cis* and *trans* abscisic acids, classed as isoprenoids (Punpee 2015). The ABA biosynthetic pathway starts from oxidative cleavage of epoxy-carotenoids and 9-*cis* violaxanthin, where xanthoxin is converted to abscisic acid (Kundu and Gantait 2017; Olds *et al.* 2018). The early $C_5$ precursor of ABA, isopentenyl pyrophosphate, is produced primarily in plastids, via 1-deoxy-D-xylulose-5-phosphate from pyruvate and glyceraldehydes-3-phosphate. This is then processed to farnesyl pyrophosphate, geranylgeranyl pyrophosphate, phytoene, carotene, and lycopene (Kundu and Gantait 2017; Olds *et al.* 2018). Subsequently, xanthoxin migrates from the plastid to the cytosol, where it is converted to ABA by abscisic aldehyde, xanthosis acid, or abscisic alcohol; clearly, abscisic aldehyde is an intermediary in the conversion of xanthoxin to ABA (Kundu and Gantait 2017; Olds *et al.* 2018).

Transportation of ABA primarily occurs through the vascular tissues of plants (Seo and Koshiba 2011). However, ABA responds to abiotic stress through the cells (Olds *et al.* 2018); this requires translocation from ABA-producing cells, via intercellular transport, to allow rapid distribution to other plant tissue (Olds *et al.* 2018). ABA is ubiquitous in plants; the endogenous levels in plant cells determine its homeostasis (Kundu and Gantait 2017). However, developmental and environmental factors such as light, wounding, salinity, and water stress affect ABA concentration levels (Kundu and Gantait 2017).

ABA is sensitive to direct sunlight and high temperatures, and rapidly deteriorates under these conditions (Tillberg 1992). Kong and Zhao (2014) found that foliar-applied ABA elevates the aroma content of aromatic rice (*Oryza sativa* L). Foliar application of ABA on yeba mate (*Ilex paraguariesnsis* A.St.-Hil.) increases plant growth, by reducing water stress through stomatal closure (Sansberro *et al.* 2004). It has also been reported that the exogenous application of ABA improves wounding tolerance in tomatoes and potatoes, and induces jasmonate gene expression in the leaves and roots of potatoes (Sansberro *et al.* 2004). The exogenous application of ABA should preferably be conducted during cool mornings, before sunrise and increasing temperatures.

As well as direct involvement in plant growth and development, ABA has a significant role in the regulation of environmental stress. Pena-Cortés *et al*. (1989) reported that there are two distinct pathways responsible for the developmental and environmental stress regulatory processes. Responses to plant wounding includes the local response at the site of the wound, and the systemic response, which occurs throughout the whole plant (León *et al.* 2001). The foliar concentrations of ABA applied to wounded plants varies from 0.001 mM to 1.5 mM solution per plant, with the optimal application rate and concentration differing between different crop species (Herde *et al.* 1999; Sansberro et al. 2004; Kong and Zhao 2014; Wang *et al.* 2018).

*Jasmonates:* Jasmonates and their methyl ester, MeJA, are natural-occurring growth regulators found in higher plants (Dar *et al.* 2015). Jasmonates were first discovered and isolated from a culture of the fungus *Lasiodiplodia theobromae* (Pat.), while MeJA is a component of the essential oils of *Jasminum grandiforum* (L.) and *Rosmanus officinalis* (L.) (Dar *et al.* 2015). Cyclopentanone-derived jasmonates are widespread in plants (Piotrowska and Bajguz 2011), while MeJA, and jasmonic acid and its hydroxylated derivatives are commonly used jasmonates for agricuclural purposes. In plants, *cis*-jasmone, jasmonyl-1-aminocyclopropane-1 carboxylic acid, and jasmonoyl isoleucine are also known to act as analogues of jasmonates (Piotrowska and Bajguz 2011).

Jasmonates are present throughout the plant body, with the highest concentration in growing tissues such as shoot tips, root tips, immature fruits, and young leaves (Piotrowska and Bajguz 2011). Jasmonate biosynthesis in plants ranges from 0.01 µg/g to 3 µg/g in fresh mass (Piotrowska and Bajguz 2011); however, it has been found to be as high as 95 µg/g in fresh mass of sagebrush (*Artemisia tridentate* [Nutt.]) (Dar *et al.* 2015). Jasmonates are derived from the fatty acid metabolism pathway and are harvested directly from jasmine (*J. grandiflorum* L.) (Dar *et al.* 2015). Huang *et al.* (2017) reported that biosynthesis of jasmonates takes place in three different cell membranes, and is activated by 13-lipoxygenase to form hydroperoxyoctadecatrienoic acid.

Jasmonates, along with their derivatives, control various aspects of plant growth and development, such as stamen development, root development, flowering, and leaf senescence (Huang *et al.* 2017). Jasmonates also induce a variety of physiological processes, such as seed germination, pollen development, ethylene synthesis, tuber formation, fruit ripening, and tendril coiling (Turner *et al.* 2002). However, when applied exogenously, jasmonates can modulate stress by either enhancing or suppressing plant development (Piotrowska *et al.* 2009).

Jasmonates also activate a signal transduction pathway in response to different kinds of stress (Piotrowska *et al.* 2009). Plant responses to abiotic stresses, such as wounding, are coordinated both locally and systemically by jasmonate signalling molecules (Turner *et al.* 2002). In addition, there is a causal link between wounding and jasmonates; wounding causes the release of linoleic acid, a jasmonate precursor, from the membrane lipids, in turn forming jasmonate (Turner *et al.* 2002). Thus, the jasmonate signalling pathway involves signal transduction events that are regulated by wounding shock, especially in relation to leaf defoliation (Turner *et al.* 2002).

The molecular mechanism of crosstalk between growth and immune-signalling networks are regulated and mediated through biosynthetic pathways of phenylpropanoids, polyketides, terpenoids, and N-containing compounds, which are directly associated with jasmonates (Guo *et al.* 2018). The effects of exogenously applied jasmonic acid on growth, changes in essential oil biosynthesis, and plants subjected to biotic and abiotic stresses has been tested (Koo and Howe 2009; Prins *et al.* 2010). In a study conducted by Xue and Zhang (2007), a high concentration of MeJA (1 mM) inhibited primary root growth of soybeans, while a low application concentration (0.01 µM) slightly stimulated root growth. Anderson (1988) also reported that low levels (1 µM-10 µM) of MeJA alters protein and mRNA populations, without inducing senescence in cell culture, while a high concentration of jasmonic acid or MeJA (50 µM) induces senescence in cell culture, and slows the primary root growth of soybeans (Xue and Zhang 2007).

MeJA applied at 0.5 mM increases the content of eugenol and linalool in basil plants compared to the control (Kim *et al.* 2006). In another study, MeJA applied to bigleaf marsh-elder (*Iva frutescens* L.) resulted in an increase in volatile compounds ($\alpha$-pinene, sabinene, and limonene emissions) (Degenhardt and Lincoln 2006). The increase in volatile emissions following MeJA treatment was ascribed to terpene synthase activation and *de novo* synthesis. In these studies, foliar application of MeJA varied between 0.01 mM and 0.5 mM solution, per plant, with the application rate and concentration differing between different crop species, under normal growing conditions (Thaler *et al.* 2002; Loivamäki *et al.* 2004; Konan *et al.* 2014; Jiang *et al.* 2017).

*Brassinosteroids:* BRs were named after the genus *Brassica* during the late 1970s (Hüster 2011), and they were initially extracted from maize pollen (Hüster 2011; Li *et al*. 2017): to date, there are more than 70 free BRs and conjugates, described from various plant species (Li *et al*. 2017). Trace amounts of BR's in a complex plant matrix are classed as polyhydroxylated steroid plant hormones and are widespread among plants (Li *et al*. 2017). In addition, BRs are structurally classed as $C_{27}$, $C_{28}$, or $C_{29}$ based on the different alkyl-substitution patterns of the side chains (Tang *et al.* 2016). All the BRs isolated from plants are produced through campesterol biosynthesis and belong to the $C_{28}$-BRs, with a 24$\alpha$-methyl group (Van Der Watt 2005).

The most effective BRs, which have been extracted from plants for agricultural use are brassinolide, castasterone, testosterone, and 6-deoxy castasterone (Van Der Watt 2005). Of all the BRs, brassinolide is biologically the most active (Tang *et al.* 2016); it is ubiquitous in plants and is produced in almost all plant parts, where it controls growth and developmental processes (Sirhindi *et al.* 2014). Plants synthesise excess brassinolide to meet the continuous need for growth and development, while inactive brassinolide is converted into active forms to maintain BRs homeostasis (Tang *et al.* 2016).

BRs are found in various plant species, including monoplast freshwater algae and brown algae, suggesting that they are ancient ubiquitous plant hormones (Tang *et al.* 2016). BRs are also found in pollen, immature seeds, roots, and flowers (Tang *et al.* 2016; Li *et al.* 2017). They range from 1 ng/g-100 ng/g fresh weight in flowers, while shoots and leaves have lower amounts of 0.01 ng/g-0.1 ng/g fresh mass (Tang *et al.* 2016; Li *et al.* 2017). BRs are not mobile within plants, they function by paracrine or autocrine signalling; however, long-distance transport of exogenously-applied BRs does occur in plants, particularly from the roots to shoots, but foliar-applied BRs (24-epibrassinolde) are fixed in the leaves (Tang *et al.* 2016). In addition, Rao *et al.* (2002) reported high mobility of BRs in a plant system.

BRs are involved in a wide variety of plant physiological activities. They regulate plant growth, at nanomolar to micromolar concentrations, for multiple developmental processes, including cell division, cell elongation, vascular differentiation, reproductive development, and modulation of gene expression (Piotrowska and Bajguz 2011). High metabolic activity, associated with growth, has been observed in rape (*Brassica napus* L.) treated with BRs (Hüster 2011). BRs are also involved in microbial infection recovery, hypocotyl growth, increased leaf lamina growth, increased shoot apex fresh weight, pollen tube growth, and stress tolerance (Van Der Watt 2005).

The application of BRs enhances plant biomass, secondary metabolites, antioxidant defence activities, and the accumulation of osmoprotectants under biotic and abiotic stress (Sirhindi *et al.* 2014). This has been demonstrated in soybean, where the application of 1 μmol/L BRs led to hypocotyl and epicotyl elongation. However, epicotyl elongation was affected by photoperiod, with no increase in length under dark conditions (Yin *et al*. 2019). Therefore, BRs applied at higher concentrations (≥1 μmol/L) in dark-grown plants suppress shoot and root development (Yin *et al*. 2019). Mung beans treated with the BR, 28-homobrasinolide, at $10^{-8}$ M, had increased leaf area, and plant height, as well as fresh and dry mass of shoots and roots over a 21-day growth period. This treatment also increased proline content (Alyemeni and Al-Quwaiz 2016). The foliar fresh matter of corn mint (*Mentha arvensis* L.f. Piperascens Malinv. Ex Holmes) and its menthol content increased when treated with lactonic spirostane-SABS (0.5 ng/L) and ketonic-SABS (0.5 ng/L) (Maia *et al.* 2007). BRs induce chlorophyll synthesis, through the activation of enzyme proteins (Kulaeva *et al*. 1991). Senthil *et al.* (2003) demonstrated this, with a foliar application of 0.5 ppm BRs increasing the chlorophyll content of soybean.

In light of these studies, it is evident that BRs can have a significant impact on plant growth and development, and therefore on recovering the yield and essential oil quality parameters of defoliated, wounded, or hail-damaged plants. The role of BRs in the alleviation of various abiotic and biotic stressors, such as temperature, salinity, moisture, and heavy metal exposure has been reported (Vardhini *et al.* 2010). However, there is limited information on the application of BRs on the recovery of defoliated, wounded, and grafted plants (Nanda and Melnyk 2018).

*Cytokinin:* CK was first discovered in the early 1940s when coconut milk was added to aid cell division in tobacco plants (Kieber 2002). Miller *et al.* (1955) formally described it as a hormone in 1955, when the role of kinetin was discovered in aspects of plant growth and development. Coconut milk, which is the liquid endosperm, contains substances that stimulate cell division, also contains an abundance of CK (Kieber 2002).

All CKs are adenine derivatives and mostly occur as either free compounds, glucosides, or ribosides in the plant root system, particularly the root apex (Punpee 2015; Koprna *et al.* 2016). According to Punpee (2015), CK biosynthesis occurs through biochemical modification of dimethylallyl diphosphate, which is initiated through the transference of the isopentenyl moiety from dimethylallyl diphosphate to the N6 position of adenosine triphosphate, catalysed by isopentenyl transferases. These form the isopentenyl transferases and the isoprene side chain, which is subsequently trans-hydroxylated by cytochrome P450 (CYP450) to yield zeatin ribosides (Punpee 2015). The metabolic storage and transport of CK is not yet fully understood, however, it is hypothesised that transport takes place via the vascular tissue (particularly the xylem), from the roots to the shoots of the plant (Kudoyarova *et al.* 2007).

Commonly-used CKs for agricultural purposes include zeatin, kinetin, 6-Benzylaminopurine (BA and BAP), 2-isopentenyl adenine, zeatin riboside, and dihydro-zeatin (Gasper *et al.* 1996; Santoro *et al.* 2013). The main functional properties of CKs for agricultural use are the stimulation of cell division, release of lateral bud dormancy, the induction of adventitious bud formation, retarded leaf senescence, and the promotion of chlorophyll synthesis (Egamberdieva *et al.* 2017). Exogenous application of CKs is currently used to optimise the internal concentrations of CKs for growth and development, organ regeneration after wounding damage, and changing the chemical compositions of essential oils (Egamberdieva *et al.* 2017).

The effects of exogenously-applied CKs to increase crop yield has been investigated in vegetables and medicinal plants. Zeatin and BA are the primary CKs that induce various plant responses (Koprna *et al*. 2016). When CK was applied to tobacco plants (1 mg/L), it altered metabolic activities and triggered biosynthesis of indole-3-acetic acid (IAA) (George *et al.* 2008). In peppermint (*Mentha piperita* L.), treatment with the CK, 6-Benzylaminopurine

(BAP), increased fresh shoot weight and total oil yield (Santoro *et al.* 2013). In another study, spraying kinetin (4 mM) at a rate of 860 mg/L improved shoot length, root length, leaf number, and leaf surface area of tomatoes (*Solanum lycopersicum* L.) (Niakan and Ahmadi 2014) and in kale (*Brassica oleraces* L.), maximum shoot numbers were observed in plants treated with CKs (1.5 BAP and 1 mg/L 1-Naphthaleneacetic acid [NAA]) (Ahmad and Spoor 1999).

In another study, the combined application of BAP (6 mg/L) and IAA (0.3 mg/L) increased the fresh weight of banana (*Musa acuminate* Colla.) callus (Ngomuo *et al.* 2013). Shoot regeneration in ciplukan (*Physalis angulata* L.) has been induced by supplementation with BAP (1.5 mg/ L) + indole-3-butyric acid (IBA) (0.5 mg/L) (Mastuti *et al.* 2017). Foliar application of CK (at an application rate of 0.001 mg/L-860 mg/L) has also been shown to improve the yield and quality 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).

Exogenous applications of CKs are via foliar sprays, soil drenching, or through irrigation systems in greenhouses (Punpee 2015). When plants have suffered defoliation and wounding, the physiological regeneration mechanism of the shoots is dependent on the interaction between exogenous CKs, GAs, BRs, auxin, and the availability of N (Nanda and Melnyk 2018). The response of plants to exogenous applications of CKs following simulated hail damage, with the co-occurrence of different abiotic stressors, under field and greenhouse conditions is unknown (Mittler 2006).

*Gibberellin*: GAs were first isolated from the fungus, *Fusarium moniliformae* Sheldon (*Gibberella fujikuroi* [Swada] Wollenweber), and named gibberellic acid ($GA_3$) (Hedden and Sponsel 2015). To date, ca. 126 naturally-occurring GAs have been discovered (Sharma *et al.* 2018), with each plant species containing at least six to ten GAs (Van Der Watt 2005). A wise decision was made early in GA research to number the various GAs, rather than naming them separately, as was done with chemical-related sterols. Therefore, GAs are known as $GA_1$, $GA_2$, and $GA_3$, etc. up to more than $GA_{126}$ (Hooykaas *et al*. 1999).

GAs are a large group of essential diterpenoid acids (Sharma *et al.* 2018). They are biosynthesised in shoot apices, young leaves, and flowers of plants, via the terpenoid pathway (Gupta and Chakrabarty 2013). Biosynthesis of GA requires three enzymes viz., terpene synthase, CYP450s, and 2-oxoglutarate (Gupta and Chakrabarty 2013). GAs are transported through plants by means of the vascular tissues, xylem, and phloem (Gupta and Chakrabarty 2013).

The most common molecular mechanisms of GA signalling in plants is through the GA receptor, *Gibberellin Insensitive Dwarf 1* (*GID1*) (Li *et al.* 2013; Punpee 2015). Upon binding, the receptors undergo conformation changes that favour the binding of DELLA proteins, a group of nuclear transcriptional regulators that repress GA signalling and plant growth (Li *et al.* 2013; Punpee 2015). Homeostasis and regulation of the GA biosynthetic pathway depends on the developmental and environmental signals, specifically the genes *GA 20-oxidases*, *GA 3-oxidase,* and *GA 2-oxidases* (Punpee 2015).

GAs are endogenous hormones functioning as PGRs that influence a wide range of developmental processes in higher plants (Weiss and Ori 2007). This includes plant growth and development through promoting leaf development, stem elongation, induction of seed germination, promotion of hypocotyls and stem elongation, regulation of pollen development, and flower initiation (Gupta and Chakrabarty 2013). Some GA-deficient mutants can cause dwarfism (Li *et al.* 2013). Different types of GAs are used to achieve specific agronomic objectives, for example, anti-flowering $GA_7$ and $GA_3$ are commonly used for promoting germination, seed development, leaf development, and stem elongation (Gupta and Chakrabarty 2013).

The mode of action of GAs depends on the endogenous and exogenous concentrations applied to plants (Aliyu *et al.* 2011; Hassanpouraghdam *et al.* 2011; Giannakoula *et al.* 2012). According to Ozhan and Hajibabaei (2013), GAs are activated in concentrations lower than 1 mM, and in some cases, at a concentration lower than 1 μM. However, an overdose of GAs can cause excessive plant growth and increased sterility (Ozhan and Hajibabaei 2013). Levels of exogenous $GA_3$ foliar applications vary between 10 mg/L-300 mg/L. Foliar application of $GA_3$ (200 mg/L) increases growth in lentils (*Lens culinaris* [Medik.]) (Giannakoula *et al.* 2012), and GA3 (50 mg/L to 100 mg/L) improves flowering, fruit retention, and nut size of cashews (*Anacardium occidentale* L.) (Aliyu *et al.* 2011). Hassanpouraghdam *et al.* (2011) also reported an increase in essential oil yield of French lavender (*Lavandula officinalis* [Chaix.]) following the exogenous application of $GA_3$ (at a rate of 300 mg/L).

*Combined CK and GA:* PGRs rarely function independently; they depend on a crosstalk network between their synergic and antagonistic metabolic processes (Gasper *et al.* 1996). GA primarily controls cell growth and division by stimulating the elongation of internodes (Taiz and Zeiger 2002). In a study by Cato *et al.* (2013), longer internodes and delayed flowering were observed in tomatoes treated with GA (5 mg/L), while plants treated with only CK (5 mg/L) formed no axillary buds. However, when GA and CK were combined, there was an increase in fresh matter (Cato *et al.* 2013). This demonstrates that there is a possible interaction between the major PGRs, with antagonistic relations, which improves plant growth and development. The crosstalk between GA and CK involves components from the GA biosynthesis pathways, which plays a central role in the regulation of plant growth and development (Weiss and Ori 2007). GAs and CKs are commonly used in agriculture, viticulture, gardens, and horticulture (Sharma *et al.* 2018).

Several studies have revealed a reciprocal developmental dependence between the two hormones, where the ratio between GA and CK affects the developmental processes of the plant (Weiss and Ori 2007). Cato *et al.* (2013) observed positive synergic crosstalk between GA and CK in tomatoes, and Koprna *et al*. (2016) reported that a combination of BA and GA induced longer tomato shoots, under different abiotic stress conditions. High CK and low GA signals are required for normal shoot apical meristem functioning (Sakamoto *et al.* 2001). In contrast to these findings, joint applications of GAs and CKs have been shown to exert antagonistic effects on numerous developmental processes, including shoot and root elongation, cell differentiation, shoot regeneration in culture, and meristem activity (Leite *et al.* 2003; Greenboim-Wainberg *et al.* 2005). Moreover, GA tends to inhibit CK-induced cell differentiation in plants (Weiss and Ori 2007). This inhibition is attributed to the loss of the SPINDLY protein function, which results from CK resistance.

CK activity is highest during early shoot initiation (controlling meristem activity) (Su *et al.* 2011); in contrast, GAs act at a later stage to regulate plant cell division and shoot elongation (Greenboim-Wainberg *et al.* 2005). The GA biosynthetic pathway from trans-geranylgeranyl diphosphate to $GA_{12}$-aldehyde, leads to the identification of positive and negative signalling components (Weiss and Ori 2007). In Arabidopsis (*Arabidopsis thaliana* L.), GA and CK signalling regulated the expression of ARR1 through repression of GA, via degradation of the DELLA protein RGA (Moubayidin *et al.* 2010). This indicates that reducing the GA concentration, five days after foliar application, releases ARR1 from repression, which in turn upregulates *SHY2*. This leads to an increase in cell differentiation, which balances with cell division to control plant growth. In addition, the regulation of *SHY2* by ARR1 also represents a point of crosstalk with auxin, thus connecting three hormones in a single network (Moubayidin *et al.* 2010). The homeostatic balance of GA and CK in plants may vary between species, and the response of plants to foliar treatments may differ as a result of external factors, such as change in environmental conditions, and stage of development (Verma and Sen 2008).

Changes in the ratios of GAs and CKs to both each other, and to other hormones, often results in distinct and divergent morphological features, such as dwarfism, contorted or twisted growth, weeping forms, or fastigiated and columnar forms (Barnes 2013). In addition, the ratios may cause extra-large leaves or elongated stems, and extensive shoot proliferation, especially under less favourable environmental conditions. Weeping forms have been observed in spruce (*Picea abies* Mill.), pine (*Pinus densiflora* L.), and sweet viburnum (*Viburnum odoratissimum* Ker Gawl.) treated with combined GA and CK (Schoene and Yeager 2005; Barnes 2013). Therefore, it is evident that under abiotic stress conditions, plants can be treated with combined GA and CK to induce foliage material development. However, it is crucial to plan the application scheduling and ratios for each of these hormones, as shown in studies by Leite *et al.* (2003) and Greenboim-Wainberg *et al*. (2005).

*Natural biostimulants-containing phytohormones:* Exogenous applications of synthetic PGRs have been shown to consistently enhance growth, yield optimisation and oil quality (Prins *et al.* 2010), as well as physiological efficiency in plants (Nemhauser *et al.* 2006). However, the production and availability of some synthetic phytohormones are expensive and not readily available. In addition, the practical use of these phytohormones is dependent on various environmental factors, such as temperature and light (Sezgin and Kahya 2018). The use of synthetic PGRs is a potential ecological hazard as they could pose a threat to the health of non-target organisms, especially when improperly used (Hüster 2011). As such, the use of less harmful and cheaper bioactive stimulants or PGRs are preferred over conventional synthetic phytohormones (Van Der Watt 2005).

There are numerous commercially available bio-fertilizers, plant conditioners, allelopathic preparations, biogenic stimulators, elicitors, plant strengtheners, and PGRs (Table 2.3). Most of these products are considered as biostimulants, containing PGRs. However, Bulgari *et al.* (2015) found that some biostimulants contain traces of natural phytohormones, but their biological action should not be attributed to them, unless registered as PGRs.

PGR products are developed based on the synergism between natural phytohormones (Verma and Sen 2008; Moubayidin *et al.* 2010). However, there are only a few published scientific reports on these products, since most industrial companies withhold information for market confidentiality purposes (examples in Table 2.3). Below are a few of the published reports to highlight the effects of registered PGRs and biostimulants (containing traces of phytohormones) on plant growth, recovery, and resistance against stressors.

Application of biostimulants (undisclosed brand-name) containing GA (50 mg/L) and CK (90 mg/L) increased the number of leaves, flower heads and the total flavonoid content of marigold plants inflorescences (Machado *et al.* 2014). Peppers (*Capsicum annuum* L.) treated with Megafol® (0.2% 40 mL; GA & CK content) had increased Ca uptake in the foliage material and fruits. Megafol® also increased the fruiting yield and the level of antioxidants in the same species (Parađiković *et al.* 2010). Hüster (2011) studied the stimulatory effects of ComCat® on wheat, maize, cabbage (*Brassica oleracea* L.), carrots (*Daucus carota* [Hoffm.] Schübl.), onions (*Allium cepa* L.), lettuce, beetroot (*Beta vulgaris* L.), and peas (*Pisum sativum* L.). They demonstrated that an optimum foliar application of 5 mg/L, significantly increased the foliar biomass of these crops. Marjoram (*Majorana hortensis* Moench.) treated with BRs (25 mg/L) was rich in *cis*-sabinene hydrate content (El-Khateeb *et al.* 2017). Foliar application of the tropical plant extract, Auxym® (2 ml/L), improved the yield of jute (*Corchorus olitorius* sp.) (Carillo *et al.* 2019). Auxym® also increased the chlorophyll content, enhanced the adaptation of jute plants to fluctuating light levels, and positively increased the starch, soluble proteins, and amino acids content when the plants received the full strength of nutrient solution (Carillo *et al.* 2019).

From these studies, it is evident that PGRs and biostimulants containing natural phytohormones can increase crop yield, enhance plant phytochemistry and secondary metabolites biosynthesis, and improve the recovery response mechanism of plants following biotic and abiotic stresses. However, no information on the response of hail-damaged plants, such as rose geranium, treated with natural PGRs could be found in the literature.

### 2.3.3 PGRs and production of secondary metabolites

Plant chemistry (e.g. the essential oil and phytohormone content) is altered following mechanical damage, as caused by hail and/or animal herbivory (Banchio *et al.* 2007). Endogenous phytohormones are the primary inducible defence response for this class of volatiles, signalling to the transduction pathway between wounding stress perception and induction (León *et al.* 2001; Brilli *et al.* 2011). These physiological response mechanisms occur within matter of minutes to several hours, resulting in the activation of wound-related defence genes (León *et al.* 2001).

The production and accumulation of essential oils is restricted to specialised structures (e.g. glandular trichomes, secretory cavities, and idioblasts) since they are toxic to healthy plant cells (Banchio *et al.* 2007; Prins *et al.* 2010). The production of these essential oils takes place in closely connected secretory structure formations. It has been shown that biotic and abiotic stress factors affect essential oil production (Banchio *et al.* 2007; Prins *et al.* 2010). Therefore, it has a direct effect on the stimulation of the essential oil biosynthesis, which directly benefits essential oil yield and quality.

According to Rajeswara-Rao (2000), increased biomass and essential oil yield was recorded in rose geranium plants when PGRs such as IAA, IBA, cycocel, cytozyne, biomyze, thephon, mepiquat chloride, triacontanol, and mixtalol were applied. However, the use of most of these natural or synthetic PGRs as a recovery method for hail-damaged plants has not been tested. Changes in the essential oil yield and quality have been found in most essential oil and medicinal plants, as a result of the exogenous application and endogenous triggering of PGRs (Prins *et al.* 2010). At least 60 essential oil constituents were identified in elderberry (*Sambucus ebulus* L.), with some of the components significantly increasing in content under different exogenous application of auxin (IAA and NAA) (Feibakhsh *et al.* 2014). Treatment with CK increased the total oil yield of peppermint plants by 40% (Santoro *et al.* 2013).

## 2.4 Conclusion

Hail damage causes wounding, tearing, defoliation, bruising, and breaking of plant morphological parts (Schubert 1991). This review has detailed the potential recovery response of plants to hail damage, the response of plants treated with synthetic PGRs, and the response of plants treated with natural PGRs extracted from plants. From this review, it is evident that the use of natural or synthetic phytohormones, as an alternative mitigation strategy against hail damage, might help in the recovery and improve essential oil plant yield following hail damage.

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**Table 2.1:** Hail intensity and magnitude chart (adapted from Changnon 1999; Bal *et al.* 2014).

| Size code | Intensity category | Size (mm) | Description | Typical damage |
|---|---|---|---|---|
| H0 | Hard hail | Up to 8.4 | Pea | No damage |
| H1 | Potentially damaging | 8.4 to 15.2 | Marble | Slight damage to plants |
| H2 | Potentially damaging | 15.2 to 20.3 | Dime | Slight damage to plants |
| H3 | Severe | 20.3 to 30.5 | Nickel | Severe damage to plants |
| H4 | Severe | 30.5 to 40.6 | Quarter | Widespread glass and auto damage |
| H5 | Destructive | 40.6 to 50.8 | Half dollar | Widespread destruction of glass, roofs, and risk of injuries |
| H6 | Destructive | 50.8 to 61 | Ping pong ball | Aircraft bodywork dented and brick walls pitted |
| H7 | Very destructive | 61 to 76.2 | Golf ball | Severe roof damage and risk of serious injuries |
| H8 | Very destructive | 76.2 to 88.9 | Hen egg | Severe damage to all structures |
| H9 | Super hailstorms | 88.9 to 101.6 | Tennis ball | Extensive structural damage could cause fatal injuries |
| H10 | Super hailstorms | >101.6 | Baseball | Extensive structural damage could cause fatal injuries |

**Table 2.2:** Noteworthy South African hailstorm events recorded during the past 10 years (2010-2020), along with agricultural losses suffered.

| Date | Location | Incident | Citation |
|---|---|---|---|
| 19-11-2013 | Witzenberg, Ceres and Koue Bokkeveld (Western Cape) | Hail caused extreme in fruit-growing areas and an estimated R 70 million was lost in production. | Barford (2006) |
| 16 -11-2014 | Wolseley and Worcester (Western Cape) | An average of 40-50% of the apple and pear crop was damaged by hail. | Tru-Cape (2014) |
| 17-03-2015 | Phokoane (Limpopo) | Crops were damaged. | SAWS (n.d) |
| 16-11-2015 | Hoedspruit (Limpopo) | Mango farmers estimated hail damage losses of 6-8 million Rands. | Du Preez (2015) |
| 5-12-2015 | Orange River and Northern Cape | Table grapes were blistered, forcing early harvest by hail damage. | Hendricks (2015) |
| 11-01-2016 | Modder river (Northern Cape);- Rouxville, and Smithfield (Free State) | Livestock killed. | SAWS (n.d) |
| 14-11-2016 | Wesselsbron and Bultfontein (Free State) | Maize and wheat plantings suffered hail damage. | Coleman (2016) |
| 27-09-2017 | Hoedspruit (Limpopo) | Strong winds and a hailstorm caused damage worth millions of ZAR to farms, in some cases, destroying 80-100% of crops. This included at least 80-90% of mango and citrus orchards. | Caxton News Service (2017) |
| 25-01-2018 | Mooi River (KwaZulu Natal) | Big hailstorm in Mooi River area, extensive damage to crops and buildings. | Storm Report Live |
| 28-02-2018 | Langkloof (Eastern Cape) | A 40-50% damage of the whole orchards plantation were recorded before harvest. | SABC News (2018) |
| 29-05-2018 | Onderberg region (Mpumalanga) | A hailstorm damaged large tracts of sugarcane, banana, citrus, and mango plantations. Assessment reports were not published, but Agri Mpumalanga CEO Robert Dave, estimated the damage to amount to millions of Rands. | Farmers weekly (2018) |
| 13-10-2018 | Johannesburg, Vereeniging, and Laudium (Gauteng) | Heavy hailstorms caused flooding, no agricultural losses estimates were published. | Eyewitness news (2018) |
| 04-12-2018 | Mbabane (Mpumalanga) | Crops were damaged. | Electroverse (2018) |
| 16-12-2018 | Rustenburg (North West) | Crops were damaged. | IOL (2018) |
| 12-03-2019 | Langkloof (Eastern Cape) | Some orchards suffered 100% loss. | Fresh Plaza (2019) |
| 20-03-2019 | Ladysmith (KwaZulu Natal) | Hail the size of golf balls and bigger through many areas of Ladysmith. | Ladysmith Gazette (2019) |
| 22-04-2019 | Johannesburg and Alberton (Gauteng) | Crops were damaged. | The Citizen (2019) |
| 23-07-2019 | Cape Town (Western Cape) | Houses and cars were damaged, no reports on agricultural losses. | SA People News (2019) |

**Table 2.3:** Examples of commercial PGRs and biostimulants containing phytohormones as declared on the labels.

| Product | Composition | Citation |
|---|---|---|
| *Lucky Plant®* | Gibberellic acid, BRs and traces of CKs | Agraforum, Germany |
| *ComCat®* | Brassinosteroids (2,4-Epibrassinolide) | Hüster (2011); Agraforum, Germany |
| *SilCat™* | Brassinosteroids (2,4-Epibrassinolide) | Agraforum, Germany |
| *AnnGro® EW* | Ethyl esters of fatty acids | Agraforum, Germany |
| *Stimplex®* | Cytokinin (kinetin 0.01%) | Bulgari *et al.* (2015) |
| *Fungicidal* | Salicylic acid | Pan African Farms, RSA |
| *PanAf®* | Salicylic acid | Pan African Farms, RSA |
| *Megafol®* | Amino acids, betaines, proteins, vitamins, auxin, GA, and cytokinins | Pađiković *et al.* (2019) |
| *Biozyme®* | Algae extract, PGRs (GAs, auxin and zeatin), and chelated micronutrients | Pađiković *et al.* (2019) |
| *Slavol* | N-fixing, and phosphate-mineralizing bacteria, and auxins | Pađiković *et al.* (2019) |
| *Algreen, Leili®* | Seaweed extract, PGRs, vitamins, free amino acids, and alginic acid | Pađiković *et al.* (2019) |
| *Agrispon®* | Natural plant extract with traces of phytohormones | Michalski (2008) |
| *Kelpak®* | Seaweed extract and traces of plant growth regulators | Michalski (2008) |
| *Stifun* | Complex biologically active substances of natural origin | Yakhin *et al.* (2011) |
| *Auxym®* | Plant growth regulators (auxins and CK), amino acids, peptides, vitamins, and essential micronutrients | Carillo *et al.* (2019) |

**Figure 2.1:** Rose geranium (*Pelargonium graveolens* spp.) shrub (from Narendra Geranium Farm, Kolhapur).

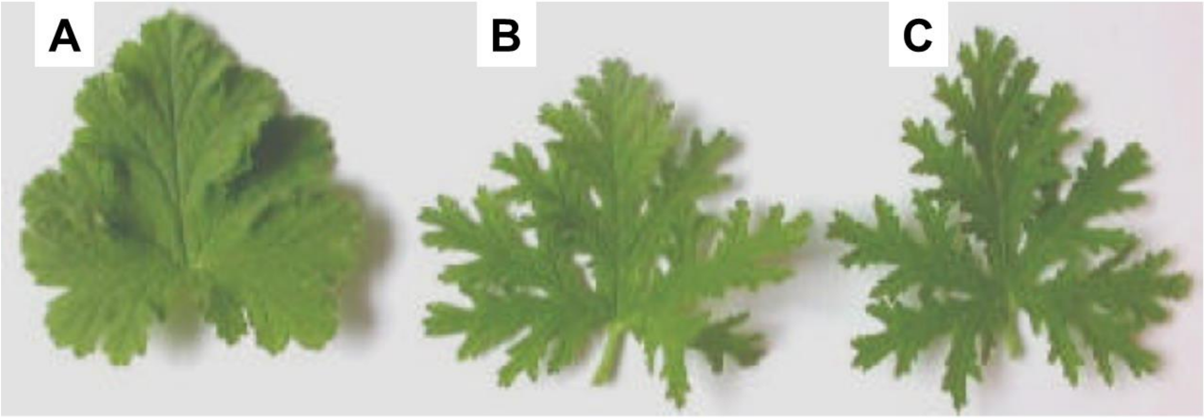


**Figure 2.2:** Different rose geranium cultivars (cv.) leaves (*Pelargonium graveolens* spp.). (A) cv.: ‘Reunion’, (B) cv.: ‘Bourbon’, and (C) cv.: ‘North Africa-type’ (from Tembe and Deodhar 2010).

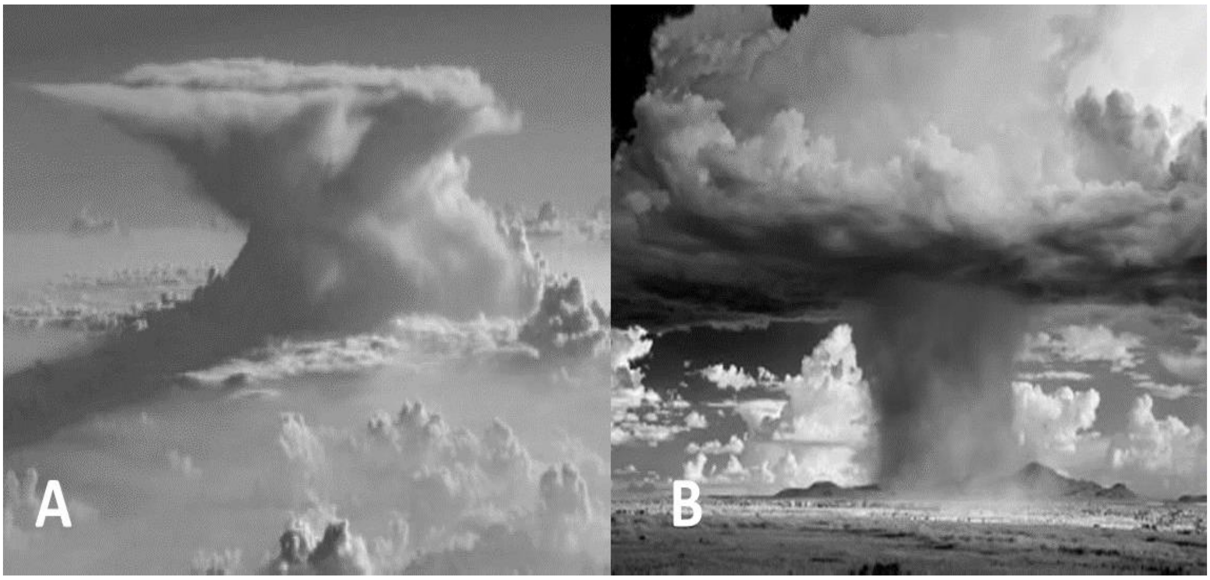


**Figure 2.3:** Anvil cumulonimbus clouds, illustrating the mechanism of (A) hail formation and (B) descending (from Bal *et al*. 2014).

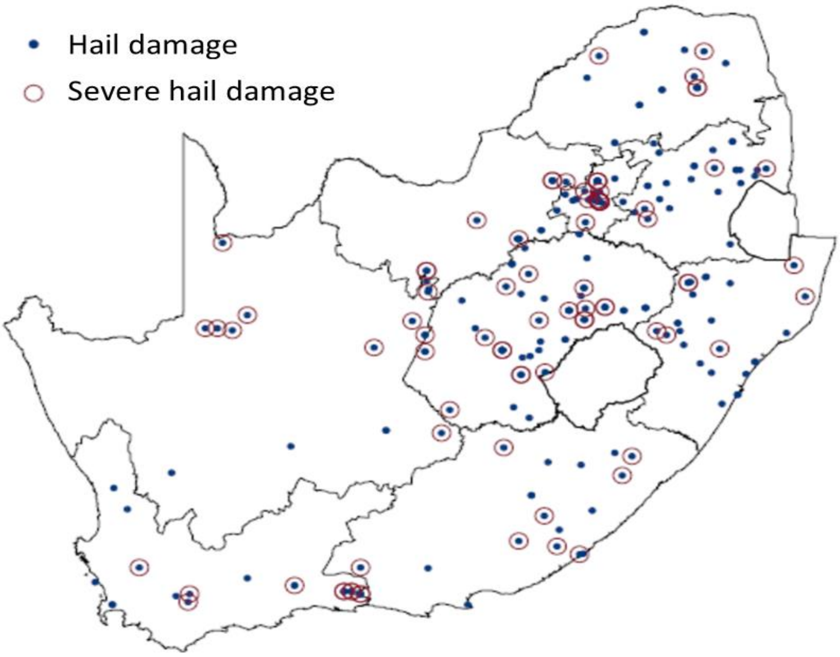


**Figure 2.4:** Hail damage and frequency reports from South Africa (from Piketh and Burger 2014).

# CHAPTER THREE

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# USING COMBINED AGRICULTURAL PLANT GROWTH REGULATORS AS A POTENTIAL MITIGATION STRATEGY FOR SIMULATED HAIL-DAMAGED ROSE GERANIUM (*Pelargonium graveolens* L'Her. cv. 'NORTH AFRICA-TYPE')

**Abstract**

This study evaluated the combined effects of cytokinin (CK), and gibberellic acid (GA) applications on rose geranium plants (*Pelargonium graveolens* L'Her.) following simulated hail damage. The experiment (arranged in a 3 x 3 factorial treatment design, with a randomised complete block design) was carried out in a tunnel structure covered with hail net, during the 2016/17 and 2017/18 growing seasons. Treatments comprised of three simulated hail damage levels (zero%, 50%, and 100% defoliation) and three levels of combined agricultural plant growth regulators (PGRs) (distilled water-only control, CK 0.32 mg/L + GA 150 mg/L; CK 0.64 mg/L + GA 300 mg/L) applied following the hail damage simulations. Simulated hail damage negatively affected the measured plant parameters, but, the application of combined agricultural PGRs did not improve any of the measured plant parameters. Brevicollate trichome densities declined on the adaxial (32.7%) and abaxial leaf surface (41.2%) of refoliated plants treated with the high concentration of combined agricultural PGRs. The essential oil yield was not affected by either PGR treatment. The low concentration PGR treatment increased the citronellol content by 47.4% on plants that sustained 100% defoliation. This study demonstrates that combined agricultural PGRs, in synthetic form, do not aid the recovery of the measured plant parameters, or the essential oil yield.

**Keywords:** Cytokinin; defoliation; gibberellic acid; hail damage, *Pelargonium graveolens*.

### 3.1 Introduction

Commercial production of rose geranium (*Pelargonium graveolens* L'Her.) occurs mostly under open field conditions in the Mpumalanga Lowveld, KwaZulu-Natal, Western Cape, Limpopo, Gauteng, North West, Eastern Cape, and Free State Provinces (Eiasu *et al*. 2009; Nilofer *et al.* 2018). Most of these provinces experience sporadic and severe hailstorm (Piketh and Burger 2014). Furthermore, rising temperatures in South Africa have significantly increased the frequency of hailstorms, and the subsequent impact on crops (Bal *et al.* 2014; Yu *et al*. 2016).

Most emerging growers, as well as some commercial growers who are unable to adapt appropriately to these adverse environmental conditions, are being displaced by the changing South African climate (Potter *et al*. 2010). There is currently no specific hail-mitigation strategy for most medicinal and aromatic crops. Therefore, the effects of potential hailstorm damage on essential oil and aromatic plants, as well as mitigation and control mechanisms, require further investigation by researchers.

Crop damage is caused by large hailstones (>H3) resulting in defoliation, tearing, bruising, breaking, and the loss of stems and flowers (Changnon 1999; Yu *et al.* 2016). Such wounding can also lead to stress that affects plant growth and metabolic activities (Troncoso *et al.* 2011). To produce a stress-response and recover metabolic functions, plants rely on the crosstalk between phytohormones (Banchio *et al.* 2007); the interplay between environmentally-activated cytokinin (CK) and gibberellin (GA) signals is crucial in the plant stress response (Drábková *et al.* 2015).

CK is a plant growth regulator (PGR), which primarily occurs as free compounds, glucosides, or as ribosides in the plant root system, particularly in the root apex (Punpee 2015). High CK concentrations occur during early shoot initiation, and controls meristem activity (Koprna *et al.* 2016; Egamberdieva *et al.* 2017). Following wounding stress, it is upregulated to control the physiological regeneration of the shoots through pectin secretion, forming a callus at the wounding site (Takei *et al*. 2002; Nanda and Melnyk 2018). It has been shown that foliar applications of CK improves yield and quality parameters of tobacco (*Nicotiana tabacum* L.), peppermint (*Mentha piperita* L.), and marigold (*Calendula officinalis* L.) when applied at a rate of 1mg/L-860 mg/L (Santoro *et al.* 2013; Machado *et al.* 2014; Niakan and Ahmadi 2014).

GA is also a PGR, and is biosynthesised in shoot apices and young leaves (Gupta and Chakrabarty 2013). GAs act at a later stage of plant growth and development to regulate plant cell division and shoot elongation (Greenboim-Wainberg *et al.* 2005). Exogenous applications of $GA_3$, between 10 mg/L and 300 mg/L, have been reported to improve yield and quality parameters of lentils (*Lens culinaris* [Medik.]), cashews (*Anacardium occidentale* L.), and French lavender (*Lavandula officinalis* [Chaix.]) (Aliyu *et al.* 2011; Giannakoula *et al.* 2012; Machado *et al.* 2014).

Treating plants with a combination of agricultural CK and GA following detrimental environmental conditions, such as hail damage, mechanical cutting back, or severe bruising, causes modifications to morphological features. These include the development of extra-large leaves or elongated stems, and extensive shoot proliferation (Barnes 2013). Application of CK and GA increases and alters the leaf morphology, biomass, photosynthesis in Japanese knotweed (*Polygonum cuspidatum* Houtt.) following environmental stress (Sugiura *et al.* 2016). However, the response of plants to this treatment may vary between species, varieties, ecological conditions, endogenous phytohormonal balance, and stage of development (Verma and Sen 2008).

The objective of this study was to determine the ability of combined (agricultural) synthetic CK and GA applications to recover simulated hail-damaged rose geranium plants. It is hypothesised that 100% simulated hail damage will reduce most of the selected essential oil yield parameters, and that a high concentration of combined agricultural PGRs (CK 0.64 mg/L + GA 300 mg/L) will recover and improve the essential oil yield and quality in rose geranium plants.

## 3.2 Materials and methods

### 3.2.1 Experimental site and crop management practices

*Experimental site:* The experimental site was located at Thitapoho farm (28°56'S; 26°19'E, 1307 amsl), situated ca. 90 km south of the city of Bloemfontein (Free State Province, RSA). Weather data was obtained from the Agricultural Research Council (ARC), Institute of Soil, Climate and Water (RSA), using an automated weather station (Netram Technologies, RSA) positioned at the experimental site. Temperature, relative humidity, and rainfall for both growing seasons are presented in Table 3.1. No hailstorm events were recorded during either of the cropping seasons.

The soil at this site has a clay loam texture, and a pH ranging from 4.6 to 4.8 (KCl). Morphologically, the soil is classified as a Klapmuts soil form, which is comprised of an orthic A-horizon, E-horizon, and a pedocutanic B-horizon (Soil Classification Working Group, 1991). Before applying fertilizers, top and subsoil samples were collected and analysed at the ARC, Small Grain Institute (Bethlehem, RSA) to determine the mineral content (Table 3.2). Beds were fertilized with N, P and K at a rate of 74, 111 and 74 kg/ha (2:3:2 [26]), respectively, before transplanting rose geranium rooted cuttings. Additionally, a once-off topdressing with 100 kg/ha of limestone ammonium nitrate (LAN) (28%) was done, 45 days after transplanting (Araya 2012; DAFF 2012).

*General crop management practices:* This experiment was conducted during the 2016/17 and 2017/18 growing seasons (September to March). Planting was done on September 5$^{th}$ for both seasons. The experiment was carried out in a 120 $m^2$ tunnel structure, covered with a hail net (Knittex® RSA). Rose geranium rooted cuttings ('North Africa-type') were obtained from the ARC, Tropical and Subtropical Crops (RSA). Only cuttings with two to four leaves (ca. 10 cm tall) were used for this study.

The soil was cultivated once with a disc plough (ca. 250 mm deep) to break the topsoil crust. This was done a week before planting (August 15th, both seasons). Disc ploughing was followed by irrigation for one hour to moisten the soil profile. The soil was then cultivated with a mouldboard plough to a depth of ca. 30 cm. Recycled tyres were used to construct elevated planting beds, each measuring ca. 20 cm x 1200 cm x 150 cm (h x l x w).

A green damp-proof course (DPC) plastic sheet (Archies Hardware, RSA) was used as a mulch to suppress weed germination and to preserve moisture. The irrigation system comprised of two dripper lines (Netafim, RSA) (300 mm spacing and 2 L/h delivery rate) per block, connected to a 20 mm mainline pipe (Figure 3.1). Plants were watered three times per week for two hours at a time (ca. 12 mm water per plant), as recommended by Eiasu *et al.* (2009) and DAFF (2012). Hunting for aphids (Hemiptera: Aphididae) was done during both growing seasons. Their presence necessitated the use of Malasol (Efekto, RSA), applied at 1.75 ml/L. These applications were repeated for three to six days, at four-week intervals, during both cropping seasons.

### 3.2.2 Experimental layout and treatment design

*Experimental layout and treatments:* The experiment had a 3 x 3 factorial design, arranged in a randomised complete block design with three replications. Treatments consisted of three hail damage simulation levels (zero% [intact plants], 50%, and 100% defoliation), and three combined agricultural PGR levels (distilled water-only control; low PGR, CK 0.32 mg/L + GA 150 mg/L; and high PGR, CK 0.64 mg/L + GA 300 mg/L), which were applied following the simulated hail damage treatments. Each experimental plot measured 1 $m^2$ in size and contained six plants. Row spacing and spacing between plants were ca. 50 cm and 30 cm, respectively (Kassahun *et al*. 2012).

*Hail damage simulations:* Hail damage was simulated using manually-operated garden secateurs, following the procedures described by Irigoyen *et al.* (2010) and Nielsen (2012). The 50% hail damage simulation level was achieved by defoliating the top half (average plant height per experimental unit) of each experimental plant (Obeso 2002). The 100% hail damage simulation treatment was achieved by the total removal of all leaves (Changnon 1999; Obeso 2002). This was followed by the removal of stem terminal buds and random bruising of the main stem and lateral branches (using a pair of hand secateurs) on the same day. The plants were subsequently sprayed with a fine water mist, using a nozzle calibrated precision sprayer (0.3 MPa pressure), to simulate humid summer afternoon conditions (Irigoyen *et al.* 2010; Bal *et al.* 2014).

*Agricultural PGR application:* Following the hail damage simulations, combined agricultural PGRs were applied between January 8th to 21st, during both the 2017 and 2018 growing seasons. Separate stock solutions for both CK and GA were prepared by adding 100 mg of each phytohormone into a 100 mL volumetric flask. Acetone (0.1% v/v) was subsequently added to dissolve the powder. Once dissolved, the solution was diluted with distilled water to a volume of 100 mL (with continuous stirring). Triton x100 (0.1% v/v) was then added to the stock solution as a wetting agent. CK (Kinetin) treatment solutions were prepared by adding 323 µL and 646 µL of the stock solution into 1 L distilled water to achieve a concentration of 0.32 mg/L and 0.64 mg/L, respectively (George *et al.* 2008; Santoro *et al.* 2013). The GA ($GA_3$) treatment solution was prepared by diluting 150 µL, and 300 µL of the stock solution into 1 L distilled water to obtain a concentration of 150 mg/L and 300 mg/L, respectively (Aliyu *et al.* 2011; Hassanpouraghdam *et al.* 2011). The same procedure was repeated for the control treatment, with the omission of the CK and GA solutions.

Synthetic CK was applied for seven days (January 8th to 14th, 2017 and 2018). This was followed by a seven-day application of synthetic GA (January 15th to 21st, 2017 and 2018), at ca. 6:00 am (Dokoozlian 2001; Leite *et al.* 2003; Greenboim-Wainberg *et al.* 2005; Kalawa *et al.* 2017). Distilled water and the PGR treatments were applied using a calibrated precision sprayer (0.3 MPa pressure) (Ferndale UK). Fifty mL of the solution was enough to cover all the leaves on a plant (Bano and Yasmeen 2010). To avoid the spray mixture drifting onto adjacent experimental plants, a mini plastic-covered tunnel, 650 mm x 480 mm x 1200 mm (h x l x w), was used as a cover during each application. A plastic floor cover was used to prevent possible soil contamination.

### 3.2.3 Data collection

The experimental plants were allowed to recover for a period of 75 days following the hail damage simulations. Data collection took place on the March 5th, both years. The following plant parameters were measured: leaf area, leaf chlorophyll content, foliar fresh mass (FFM), number of branches, plant height, branch to height ratio (B:H ratio), and the essential oil yield and content. The following essential oil quality parameters: *cis*-Rose oxide, *trans*-Rose oxide, epi-$\gamma$-eudesmol, isomenthone, citronellol, geraniol and the citronellol to geraniol (C:G) ratio were measured, as well as trichome parameters. Due to financial constraints, trichome parameters were only measured during the 2018 growing season.

Plant height was determined for six plants in each treatment unit, a day before harvesting. Rose geranium is a shrubby crop, therefore measurements were carried out on the middle main stem, using pieces of thread to keep the plants upright. Measurement started from the soil to the upright position of the plant, according to the procedures described by Wood and Roger (2000). The number of branches for each plant was determined during harvesting by counting the number of shoots developing from the main stem, continuing to the last top node (both old and new shoots), following the procedure of Pérez-Harguindeguy *et al.* (2013). The B:H ratio was used as an indication of bushiness, by dividing the number of branches by plant height (Sedibe 2012). The plant stand loss, which is the difference between the initial plant stand and the stand after recovery, was also determined (Sij *et al.* 2005).

Two plants were randomly selected from each treatment to determine the leaf area: all leaves were removed, and the leaf area for each plant was measured using a LI3000 leaf area meter (LI-COR Inc., USA). Before harvesting, the chlorophyll content was determined using a portable non-destructive chlorophyll meter (Opti-sciences CCM 200, USA), according to the procedure described by Chen and Black (1992). Readings were taken randomly from the upper-six fully-developed leaves of the crop.

Data for the external leaf morphology was collected from the new fourth leaf from the apical bud, seventy-five days following the recovery of simulated hail-damaged rose geranium plants. Data was collected on a cloudless day, between 10:00 am and 11:00 am. Pieces of leaf samples (1 $cm^2$) were collected from the leaf apex of young, fully-expanded rose geranium leaves. These were collected from each plant in the experimental units. Leaf samples were first fixed in 3% glutaraldehyde (0.1 M sodium phosphate buffer, pH 7) and then in osmium tetraoxide (1% for two hours), before rinsing with distilled water. Samples were dehydrated in a series of ethanol concentrations of 50%, 70%, 95%, and 100% (twice) for 15 mins each, followed by drying in a Tousimis critical point drier (Bio-Rad E300, Rockville).

Dried samples were then mounted on aluminium stubs using double-sided adhesive tape (Electron Microscopy Sciences, USA) and subsequently coated with gold using a vacuum unit (Bio-Rad microscience division coating system, UK). The critical point dryer was pressured with $CO_2$ liquid at 37°C to replace the ethanol. The samples were examined using a Shimadzu SSX-550 (Kyoto, Japan) scanning electron microscope (SEM) at x400 µm magnification. The morphology and density of trichome groups were determined from digital photos obtained from a computer connected to the SEM. Photos were analysed with Photoshop 7 Savvy (Sybex San Francisco, USA), then two-dimensional selections were made using the SEM scales printed on the photos.

Plant FFM was determined following the procedures of Wood and Roger (2000) by weighing foliar fresh plant material with a PGL 2002 Adam scale (USA). Rose geranium essential oil was extracted from three plants using a custom-built steam distillation unit (Sedibe 2012). Approximately 2 kg - 5 kg fresh plant material was distilled at ca. 98°C for one hour. Oil mass (yield) was determined by weighing the oil volume using a PGL 2002 Adam scale (USA) immediately following extraction, as described by 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. The compounds were identified using the NIST11 mass spectral library (https://www.nist.gov/system/files/documents/srd/Ver20Man.pdf). The ISO standard (ISO 4731 2012) was used to characterise rose geranium ('North Africa-type' cv.) essential oil quality parameters for the perfumery industry.

### 3.2.4 Statistical analysis

All parameters were statistically analysed and compared using PROC GLIMMIX, SAS version 9.4 (PROC GLIMMIX, SAS Institute 2013). 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 and homogeneity of variance were performed on standardised residuals to test for any deviations from normality and homoscedasticity, respectively (Shapiro and Wilk 1965; Lawal 2014). The plant parameters, essential oil yield, and the essential oil quality parameters, were subjected to multivariate data analysis, using principal component analysis (PCA-XLSTAT 2015) to identify and evaluate the groupings between the variables.

## 3.3 Results

### 3.3.1 Plant parameters, and essential oil yield

Plant height and the number of branches per plant were not affected by simulated hail damage and the subsequent application of PGRs during the 2016/17 season (Table 3.3). During the 2017/18 growing season, plant height and the number of branches per plant were both significantly reduced when the plants suffered 100% simulated hail damage (Table 3.4). FFM was reduced by the 100% simulated hail damage in both seasons (Tables 3.2 & 3.4). Leaf chlorophyll content was not affected by any of the treatments during either season (Tables 3.2 & 3.4). The B:H ratio; however, was affected by the interaction between simulated hail damage and PGRs application during 2017/18 (Table 3.4): in non-damaged plants, the B:H ratio was significantly higher when the high PGR treatment was applied, compared to the control ($F_{16.00} = 3.35$; $P<0.03$). No significant effects were observed between the plants which suffered 50% and 100% simulated hail damage and the control (Table 3.4). No other significant interactions were observed for the remaining yield parameters (Tables 3.2 & 3.4).

During the 2016/17 growing season, leaf area increased significantly (compared to the control) when the low concentration of PGRs was applied (Table 3.3): the same trend was observed during the 2017/18 season, however, it was not statistically significant (Table 3.4). Due to poor essential oil yield, the essential oil yield parameters were not recorded during the 2016/17 growing season. During the 2017/18 growing season, these parameters were not significantly affected by the treatments and interactions (Table 3.5).

A PCA was applied to reduce the dimensionality of the complex data from these variables, further increasing interpretability without the loss of any information (Mishra *et al.* 2018; Dube *et al*. 2018). The effects of simulated hail damage, and the subsequent application of PGRs, did not provide conclusive results on the recovery of the measured plant parameters, or on the improvement of the essential oil yield. Therefore, a PCA was applied to provide an overview of the associated plant parameters and the essential oil yield attributes (Table 3.6). The score plot and loading matrix accounted for 64.16% (PC1 = 41.12% and PC2 = 23.04%) of the total variance (Table 3.6). The PCA showed a strong correlation between plant height, number of branches, B:H ratio and FFM for both seasons. The biplot indicated that plant height and FFM were characterised by the application of the highest concentration of PGRs and the distilled water control (Figure 3.2), when plants endured 50% simulated hail damage. The B:H ratio for the intact plants was characterised by both low and high PGR treatments during the 2017/18 season, furthermore, the number of branches and the B:H ratio were characterised by the low PGR treatment and the control in all simulated hail-damaged rose geranium plants during the 2017/18 season.

### 3.3.2 Leaf trichomes

During this study, two groups of trichomes were observed on the rose geranium leaves. The first group consisted of a type of non-glandular trichome, regarded as an attenuated type of trichome: this type is characterised by a long, gradual taper, as described by Payne (1978) (Figure 3.3A). The second group consisted of three types of glandular trichomes, one of which is the peltate type: this type is characterised by a short neck with bigger, rounded tips, and is regarded as a brevicollate trichome (Figure 3.3B) (Payne 1978). Finally, the capitate type has two forms; an asciiform and elongated-capitate. The asciiform trichome is characterised by a short, segmented capitate, with a columnar hatchet-shaped tip that has a slightly bent apical cell pointing towards at the leaf apex (Figure 3.3 C) (Payne 1978). The elongated capitate consists of an elongated form, segmented flask-shaped bodies, and has a round head with similar characteristics to asciiform trichomes (Figure 3.3 D).

An interaction was observed between hail damage and PGRs for non-glandular trichomes on the adaxial leaf surface, but not on the abaxial leaf surface (Table 3.7). The highest densities of non-glandular trichomes were recorded on intact plants that received the distilled water treatment (control): the densities of the non-glandular trichomes significantly declined when PGRs were applied at lower level ($F_{18.00}$ = 3.74; P<0.01). However, increasing the level of

PGRs also increased the densities of the non-glandular trichomes, though not to the level of the control plants. On the 50% defoliated plants, the densities of the non-glandular trichomes were similar across all treatments. On plants that endured 100% simulated hail damage, the highest densities of the non-glandular trichomes was recorded when plants were treated with the highest level of PGRs; however, this was not significantly different to the control ($P>0.05$).

Asciiform trichome density, on both leaf surfaces, was affected by the interaction between simulated hail damage and the PGR application (Table 3.8). When the data was sliced in simulated hail damage, the densities of the asciiform trichomes were the same through the treatments of the combined agricultural plant growth regulators on intact plants. On the 50% defoliated plants, the highest densities of the asciiform trichomes were recorded when the high PGR treatment was applied ($F_{16.00} = 11.26$; $P<0.02$); however, this treatment did not differ to the control. On the 100% defoliated plants, the asciiform trichome densities were the same across all the treatments. Asciiform trichome densities differed between the different leaf surfaces; the abaxial leaf position had a significantly higher asciiform trichome density ($F_{16.00} = 6.14$; $P<0.02$).

Brevicollate trichome densities (both leaf surfaces) significantly declined with the application of the high concentration of PGRs (Table 3.8).

An interaction between the simulated hail damage and PGRs was observed for elongated-capitate trichome density, on both leaf surfaces (Table 3.8). The densities of elongated-capitate trichome were approximately the same for all PGR treatments on the intact plants and those that suffered 100% simulated hail damage. On the 50% defoliated plants, significantly higher densities of elongated-capitate trichome were recorded for both low and high PGR treatments ($F_{18.00} = 10.49$; $P<0.02$). Elongated-capitate trichome densities also differed between the leaf surfaces ($F_{16.00} = 5.81$; $P<0.02$), with the adaxial leaf surface having a higher density compared to the abaxial leaf surface, at both levels of simulated hail damage (50% and 100% defoliation).

### 3.3.3 Essential oil quality

An interaction was observed on the citronellol content between simulated hail damage levels and the different concentrations of combined agricultural PGRs (Table 3.9). The application of PGRs, at any level, did not affect the citronellol content of intact plants or those with 50% simulated hail damage. However, the highest citronellol content was recorded when a low concentration of PGRs was applied on plants that sustained 100% simulated hail damage

($F_{14.02}$ = 9.20; P<0.01); the citronellol content then declined by 5.4% when the level of combined agricultural PGRs was increased ($F_{14.02}$ = 9.20; P<0.01).

A multivariate data analysis was applied using a PCA to group the essential oil quality parameters. Similar results to the ANOVA were obtained from this method (Table 3.10 & Figure 3.4). The score plot and loading matrix, based on the first and second principal components (PC1 and PC2), accounted for 68.46% of the total variance. The biplot loading showed that citronellol content was characterised by the application of a low concentration of PGRs when plants sustained 100% simulated hail damage in PC1 (46.91%). On the other hand, *trans*-Rose oxide and isomenthone were tightly grouped by the application of the high concentration of PGRs and the distilled water control when plants suffered 100% simulated hail damage. In PC2 (21.55%), geraniol was the only essential oil compound characterised by 50% simulated hail damage and the application of a low concentration of PGRs, and distilled water.

## 3.4 Discussion

### 3.4.1 Plant parameters, and essential oil yield

Plant height, number of branches, leaf area and foliar biomass are essential parameters in determining the morphological characteristics, as well as the essential oil yield attributes of indeterminate crops, such as rose geranium (Pérez-Harguindeguy *et al.* 2013). There were no differences depending on the level of simulated hail damage or PGR treatment on number of branches per plant and plant height during the 2016/17 season: this could be explained by the high rainfall experienced close to harvest during January and February (Table 3.1). Eiasu *et al.* (2008) stated that high rainfall, or irrigation, tends to increase the herbage yield parameters of rose geranium, but reduces essential oil yield biosynthesis.

Hail damage is often associated with reduced yield (Bueckert 2011): according to Counce *et al.* (1994), the degree of yield loss depends on the extent of hail damage, the growth stage at the time of injury, and the physio-morphological response capacity of the crop to recover after hail damage. The significant reduction in plant parameters that occurs following hail damage is probably due to growth limitations, resulting from decreased metabolic activities after hail damage (Counce *et al.* 1994). In other studies, hail damage delayed the growth and maturity of canola (*Brassica napus* L.) and Alaska pea (*Pisum sativum* L.) (Miller and Muehlbauer 1984; McGregor 1987). The results from this study support the assumptions made by Swamy *et al.*

(1960), Dermane and Van Der Walt (1989) and Weiss (1997), that hail can cause significant damage to rose geranium plants, ultimately reducing the herbage yield.

The joint application of CK and GA exerts antagonistic effects on numerous developmental processes (Weiss and Ori 2007); however, this depends on the ratio between the two PGRs. In this study, synthetic CK was applied first, for seven days. This was followed by the application of synthetic GA, also for seven days. The high B:H ratio observed in intact plants treated with combined CK and GA could be associated with early shoot initiation, controlled by CK (Koprna *et al.* 2016), and the translocation of foliar-applied GA, which acts at a later stage to regulate shoot elongation (Egamberdieva *et al.* 2017). GA occurs mainly through the symplast, freely diffusing through the plant cell protoplast, increasing activities related to cell division and internodal elongation. This has a direct impact on plant height, and, therefore, the B:H ratios (Taiz and Zeiger 2002).

Plants require high GA and low CK concentrations to stimulate cell elongation, which can increase leaf area expansion (Weiss and Ori 2007). As indicated, GA acts at a later stage of plant growth and development to regulate plant cell division, inhibiting the action of CK during that time (Greenboim-Wainberg *et al.* 2005). Therefore, results for the leaf area obtained in this study could be attributed to GA effects, since it was exogenously applied a week later after CK. Leite *et al.* (2003) reported that the joint application of CK (30 mg/L) and GA (100 mg/L) tended to inhibit GA effects on soybean (*Glycine max* L.) during the vegetative stages, and this did not modify any of the yield parameters. Khan *et al.* (2006) reported that a relatively low level of GA (34.6 mg/L) increased the leaf area of two cultivars of tomato, Hyb-SC-3 and Hyb-Himalata.

Lower essential oil yield parameters during the 2016/17 growing season can be attributed to acidic soil conditions (pH 4.7 at 30 cm depth): Araya (2012) also found that a soil pH below 5.5 negatively affected the essential oil yield of rose geranium, and concluded that soil pH tends to respond slowly to applied lime. However, the poor essential oil yield during this season could also be attributed to the high rainfall recorded close to harvest during January and February (Table 3.1). According to Eiasu *et al.* (2008), high moisture or irrigation close to rose geranium harvest can reduce essential oil yield. The PCA from the two seasons demonstrates that the application of combined agricultural PGRs did not recover the plant parameters and the essential oil yield attributes**.**

### 3.4.2 Leaf trichomes

Trichomes play different roles in plant physiology and ecology, especially with regards to morphological, mechanical and phytochemical characteristics (Ma *et al.* 2016). In this study, all trichome types were densely grouped on the abaxial leaf surface, but scattered on the adaxial leaf surface (Figure 3.5: micrograph 1, 2 & 3). However, the attenuated trichome group was more dense near the midrib of the abaxial leaf surface (Figure 3.5: micrograph 1, 2 & 3 on B, D & F). The hemispherical peltate trichome group was evenly spaced on the abaxial leaf surface, but was mostly absent from the epidermis above the midrib vein.

Dense clusters of trichomes on the abaxial leaf surface are associated with the promotion of photosynthesis, and the establishment of phytochemical defence mechanisms in mature leaves (Ma *et al.* 2016). Interestingly, the developmental pattern of all identified and observed trichome types differed between the leaf surfaces. A similar heteroblastic series of trichomes has been found on intact rose geranium leaves, and this was claimed to be a genetic control (Bosabalidis and Exarchou 1995; Romitelli and Martins 2013).

Trichome density may vary with changes in environmental conditions (Ma *et al.* 2016). These variations may indicate trade-offs between the trichomes, subsequently increasing resistance against trichome production. In defoliated plants, the rate of leaf regeneration is slow, possibly due to the endogenous ratio between CK and GA, and regulated plant bio-inhibitors (Addicott and Lynch 1955; Dammann *et al*. 1997). Results from this study indicate that an increased level of combined agricultural PGRs decreases the density of the non-glandular trichomes, particularly on the adaxial leaf surface. Oosthuizen and Coetzee (1984) reported that non-glandular trichomes develop before glandular trichomes in the leaf primordial of *Pelargonium*, and cease development as the leaf expands. Therefore, the results obtained from this study could be ascribed to the combined CK and GA applications. CK stimulates cell division, and further regulates the leaf primordia by negatively affecting GA signalling through *IPT7* and *GA20* oxidases, at an early stage of leaf primordia (Kalve *et al.* 2014). Zhou *et al.* (2011) demonstrated this on Arabidopsis (*Arabidopsis thaliana* (L.) Heynh.), where the *C2H2* transcriptional factors regulated trichome cell differentiation through CK and GA pathways, suggesting that excess endogenous levels of these PGRs could be toxic for trichome development. In another study, Zhou *et al.* (2013) further reported that the *ZFP5* transcription factor of GA regulates trichome developmental actions, mainly through cell differentiation. In addition, GA is biosynthesised in young leaves and can translocate freely by diffusing through the plant cell protoplast when applied foliarly. Therefore, the densities of non-glandular trichomes in this study probably declined in response to excess GA applied onto undamaged lower leaves in the 50% simulated hail-damaged plants.

Reduced densities of asciiform trichomes on the adaxial leaf surface resulted from the transformation of asciiform trichomes into elongated-capitate trichomes (Table 3.8). A combination of endogenous development and external signals regulate the developmental distribution of trichomes on plant leaves (Xue *et al.* 2019). Therefore, under extreme external stimuli, such as complete defoliation, endogenous PGRs are only synchronised to regenerate lost material. This directly affects leaf expansion and trichome developmental rate. The development and densities of the asciiform trichome following simulated hail damage could also be attributed to the high concentration of GA (300 mg/L), applied at a later stage, in contrast to CK (0.64 mg/L) which was used earlier. According to Zhou *et al*. (2013), this could be due to the combined actions of *ZFP5* and *ZFP6* transcription factors from CK and GA, respectively. In combination, these transcription factors regulate trichome cell differentiation, an important metabolic mechanism associated with trichome development.

CK could have stimulated the auxin concentration, which acted to restore plant material, rather than the early stage development of the trichomes, while GA could have been involved in initiating trichome development (Ma *et al.* 2016). Jiao (2016) reported the accumulation of GA occuring through *TEMPRANILLO* (*TEM*) 1 and 2 genes in the mesophyll cells, but not in the epidermis, suggesting that the homeostasis and distribution of GA in mesophyll cells affects trichome formation in the epidermis. In addition, *TEMPRANILLO* 1 and 2 genes are involved in signalling CK at a later stage, a PGR (CK) that also promotes trichome formation.

No studies could be found in the literature on the effects of combined agricultural PGRs on brevicollate trichome density. However, Liu *et al*. (2017) reported that glandular trichome density on tobacco (*Nicotiana tabacum* L.) leaves, decreased with up to 50 trichomes per $cm^2$ when plants were treated with 17.3 mg/L GA. Thus, the decline in the brevicollate trichome density in this study could be due to high GA application, thereby directly affecting essential oil biosynthesis.

Although the developmental stages of capitate trichomes were not investigated in this study, the application of combined agricultural PGRs (both levels) could have stimulated the elongation of asciiform trichome stalks into elongated-capitate trichomes, mostly where plants sustained simulated hail damage. Barnes (2013) reported that changes in the endogenous ratios of CK and GA often cause alterations to plant morphological features, such as producing extra-large leaves or elongated stems, and extensive shoot proliferation, which is similar to the observations in this study.

Elongated-capitate trichomes are essentially stretched out asciiform trichomes with shrivelled heads, which could indicate a lack of stored essential oil in their sub-cuticular spaces. This was confirmed by Eiasu *et al.* (2009), who reported that capitate trichomes store less essential oil in their sub-cuticular areas compared to brevicollate trichomes. Therefore, the non-significant results for the essential oil yield attributes in this study are a result of the formation of paltate and capitate trichomes (Table 3.5).

### 3.4.3 Essential oil quality

Isomenthone, geraniol, *cis*-rose oxide, *trans*-rose oxide, epi-*γ*-eudesmol content, and the C:G ratio are associated with the physiological equilibrium of the plant (Ahmad *et al.* 2016). Essential oils are biosynthesised via the classical acetate-mevalonate pathway (MVA) and methyl-erythritol-4-phosphate pathways. Physiological processes associated with these pathways occur within immature leaves and inflorescences (Ganjewala and Luthra 2009). Therefore, it is possible that the recovery period (75 days) in this study was sufficient for the physiological processes associated with essential oil biosynthesis to reach equilibrium (Ganjewala and Luthra 2009).

Plants produce some essential oil compounds in response to physiological stress, pathogen attack, and other ecological factors (Prins *et al.* 2010). However, in this study, the overall citronellol levels were low in all treatments (including the control) and did not reach the minimum standard of 25% citronellol content for 'North Africa-type', as required by the ISO standard (ISO 4731 2012). According to Thakur *et al.* (2019), different types of mulching affects the production content and quality of essential oil compounds for different plant species. This was supported by de Carvalho Silva *et al.* (2014) with rose geranium, where citronellol content differed significantly between white, black, and silver coloured plastic mulches. Therefore, the low citronellol content in this study could be ascribed to the green plastic mulch used, but this hypothesis needs further testing.

### 3.5 Conclusion

PCA showed that the application of combined agricultural PGRs did not have any effects on the recovery of herbage and essential oil yield of rose geranium damaged by simulated hail. It was further demonstrated that refoliated rose geranium tends to develop a lower density of brevicollate trichomes (large trichomes) when PGRs are applied. It can be concluded from this study that hail damage reduces the yield and affects the essential oil quality of rose geranium. In addition, the application of combined agricultural PGRs may affect the transformation of trichome morphology, as well as change trichome densities. However, combined agricultural PGRs in synthetic form should not be applied on hail-damaged rose geranium plants to recover herbage material or improve the essential oil yield attributes.

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**Table 3.1:** Weather data obtained from the Thitapoho farm, Tweespruit weather station for the 2016/17 and 2017/18 growing seasons (Agricultural Research Council-Institute of Soil, Climate and Water, RSA).

| Month | Temperature (°C) | | Relative Humidity | Rainfall |
|---|---|---|---|---|
| | ***Aver. min** | ***Aver. max** | **(%)** | **(mm)** |
| **2016/17 season** | | | | |
| September 2016 | 2.56 ± 3.04 | 20.80 ± 4.19 | 52.75 ± 11.99 | 21.83 ± 2.85 |
| October 2016 | 7.57 ± 4.83 | 26.65 ± 4.17 | 40.77 ± 14.67 | 30.21 ± 2.73 |
| November 2016 | 12.56 ± 2.54 | 28.11 ± 3.63 | 57.50 ± 14.77 | 83.31 ± 8.25 |
| December 2016 | 14.54 ± 2.18 | 31.62 ± 2.62 | 47.47 ± 10.13 | 46.98 ± 5.96 |
| January 2017 | 14.35 ± 1.95 | 28.00 ± 4.17 | 69.13 ± 16.17 | 144.53 ± 13.46 |
| February 2017 | 15.04 ± 1.93 | 26.34 ± 4.05 | 78.21 ± 12.80 | 149.61 ± 10.94 |
| **2017/18 season** | | | | |
| September 2017 | 5.87 ± 3.99 | 26.95 ± 3.38 | 48.27 ± 11.20 | - |
| October 2017 | 6.89 ± 3.21 | 25.29 ± 3.78 | 49.90 ± 15.41 | 44.70 ± 4.14 |
| November 2017 | 9.31 ± 3.75 | 29.16 ± 4.38 | 40.97 ± 12.92 | 25.65 ± 3.08 |
| December 2017 | 12.85 ± 2.41 | 30.08 ± 2.70 | 51.57 ± 15.41 | 42.92 ± 3.02 |
| January 2018 | 13.52 ± 1.97 | 31.76 ± 3.01 | 48.27 ± 16.87 | 81.54 ± 5.56 |
| February 2018 | 13.68 ± 2.11 | 28.59 ± 2.53 | 70.01 ± 7.23 | 60.45 ± 4.06 |

*Aver. min = Average minimum temperature; Aver. max = Average maximum temperature

**Table 3.2:** Soil analysis for the experimental site located at Thitapoho farm, Tweespruit (conducted by the Agricultural Research Council-Small Grain Institute, RSA).

| **Minerals** | **Soil sample** | |
|---|---|---|
| | **Topsoil** | **Sub-soil** |
| Phosphate (mg/kg) | 16.30 | 10.30 |
| Potassium (mg/kg) | 162.00 | 90.60 |
| Calcium (mg/kg) | 620.00 | 431.00 |
| Magnesium (mg/kg) | 174.80 | 127.60 |
| Sulphate (mg/kg) | 6.66 | 9.69 |
| Sodium (mg/kg) | 9.90 | 13.10 |
| pH (KCl) | 4.80 | 4.60 |
| *Ca/Mg ratio | 2.16 | 2.06 |
| *CEC ($cmol_c$/kg) | 4.99 | 3.73 |
| Density ($g/cm^3$) | 1.02 | 0.95 |

*Ca/Mg ratio = Calcium to magnesium ratio; CEC ($cmol_c$/kg) = Cation exchangeable capacity

**Table 3.3:** The effects of simulated hail damage (zero%, 50%, and 100% defoliation) and the application of combined agricultural PGRs (a control, CK 0.32 mg/L + GA 150 mg/L, and CK 0.64 mg/L + GA 300 mg/L) on selected yield parameters of rose geranium during the 2016/17 growing season (Thitapoho farm, Tweespruit).

| **Treatments** | **Leaf area ($cm^2$/plant)** | **Chlorophyll (%)** | **FFM (kg/plant)** | **№ branches (per plant)** | **Plant height (cm/plant)** | **B:H ratio** |
|---|---|---|---|---|---|---|
| **Simulated hail damage (HD)** | | | | | | |
| 0% defoliation | 17.09 ± 2.63[a] | 18.58 ± 1.94[a] | 1.32 ± 0.17[a,b] | 24.03 ± 4.58[a] | 76.13 ± 8.60[a] | 31.56 ± 2.32[a] |
| 50% defoliation | 19.85 ± 3.43[a] | 19.12 ± 1.57[a] | 1.44 ± 0.37[a] | 25.18 ± 5.45[a] | 80.78 ± 11.53[a] | 31.17 ± 2.59[a] |
| 100% defoliation | 18.83 ± 3.44[a] | 19.58 ± 2.06[a] | 1.11 ± 0.43[b] | 24.83 ± 4.32[a] | 71.42 ± 8.46[a] | 34.76 ± 2.52[a] |
| *F-value* | 3.06 | 0.89 | 4.16 | 0.10 | 3.25 | 0.59 |
| *P-value* | 0.07[ns] | 0.43[ns] | ***0.03** | 0.90[ns] | 0.06[ns] | 0.56[ns] |
| **Combined PGRs levels** | | | | | | |
| Distilled water | 17.73 ± 2.62[b] | 18.67 ± 2.53[a] | 1.37 ± 0.31[a] | 25.76 ± 4.71[a] | 73.18 ± 10.10[a] | 35.34 ± 2.89[a] |
| CK0.32 + GA150 | 20.62 ± 2.62[a] | 19.04 ± 1.21[a] | 1.23 ± 0.23[a] | 24.25 ± 5.69[a] | 76.38 ± 10.74[a] | 32.55 ± 2.63[a] |
| CK0.64 + GA300 | 17.42 ± 3.76[b] | 19.57 ± 1.65[a] | 1.26 ± 0.25[a] | 24.03 ± 3.70[a] | 78.76 ± 9.64[a] | 30.75 ± 2.44[a] |
| *F-value* | 4.92 | 0.72 | 0.83 | 0.26 | 1.16 | 0.92 |
| *P-value* | ***0.02** | 0.49[ns] | 0.45[ns] | 0.77[ns] | 0.33[ns] | 0.41[ns] |
| **HD x Combined PGRs levels** | | | | | | |
| 0% x Distilled water | 18.47 ± 1.84[a] | 17.60 ± 2.93[a] | 1.39 ± 0.13[a] | 26.89 ± 5.75[a] | 70.75 ± 9.99[a] | 38.00 ± 2.42[a] |
| 0% x CK0.32 + GA150 | 18.30 ± 2.66[a] | 19.16 ± 1.30[a] | 1.34 ± 0.18[a] | 22.44 ± 3.65[a] | 77.27 ± 9.00[a] | 29.04 ± 2.41[a] |
| 0% x CK0.64 + GA300 | 14.51 ± 1.51[a] | 19.00 ± 1.63[a] | 1.23 ± 0.21[a] | 22.78 ± 4.39[a] | 80.37 ± 6.53[a] | 28.34 ± 2.41[a] |
| 50% x Distilled water | 18.16 ± 3.64[a] | 20.13 ± 1.22[a] | 1.61 ± 0.45[a] | 25.00 ± 6.36[a] | 81.66 ± 9.08[a] | 30.61 ± 2.48[a] |
| 50% x CK0.32 + GA150 | 21.34 ± 2.21[a] | 18.10 ± 1.13[a] | 1.25 ± 0.36[a] | 24.94 ± 7.02[a] | 75.31 ± 18.93[a] | 33.11 ± 3.67[a] |
| 50% x CK0.64 + GA300 | 16.99 ± 3.74[a] | 19.13 ± 2.01[a] | 1.45 ± 0.34[a] | 25.61 ± 5.37[a] | 85.38 ± 3.61[a] | 29.99 ± 2.57[a] |
| 100% x Distilled water | 16.56 ± 2.79[a] | 18.30 ± 3.23[a] | 1.12 ± 0.04[a] | 25.39 ± 3.50[a] | 67.15 ± 7.36[a] | 37.81 ± 2.19[a] |
| 100% x CK0.32 + GA150 | 22.24 ± 1.64[a] | 19.86 ± 0.75[a] | 1.09 ± 0.04[a] | 25.39 ± 7.70[a] | 76.56 ± 4.36[a] | 33.16 ± 3.09[a] |
| 100% x CK0.64 + GA300 | 20.76 ± 3.25[a] | 20.60 ± 1.34[a] | 1.11 ± 0.05[a] | 23.73 ± 0.30[a] | 70.55 ± 12.05[a] | 33.63 ± 2.47[a] |
| *F-value* | 2.46 | 1.50 | 0.54 | 0.22 | 1.22 | 0.83 |
| *P-value* | 0.08[ns] | 0.24[ns] | 0.71[ns] | 0.92[ns] | 0.34[ns] | 0.52[ns] |

Means followed by the same letter in the same column are statistically non-significant ($P$<0.05); ns = not significant; * = $F$-ratio probability of $P$<0.05; FFM = Foliar fresh mass; № branches = Number of branches; B:H ratio = Branch to height ratio

**Table 3.4:** The effects of simulated hail damage (zero%, 50%, and 100% defoliation) and the application of combined agricultural PGRs (a control, CK 0.32 mg/L + GA 150 mg/L, and CK 0.64 mg/L + GA 300 mg/L) on selected yield parameters of rose geranium during the 2017/18 growing season (Thitapoho farm, Tweespruit).

| Treatments | Leaf area ($cm^2$/plant) | Chlorophyll (%) | FFM (kg/plant) | № branches (per plant) | Plant height (cm/plant) | B:H ratio |
|---|---|---|---|---|---|---|
| **Simulated hail damage (HD)** | | | | | | |
| 0% defoliation | 9.74 ± 1.79[a] | 26.27 ± 11.29[a] | 1.34 ± 0.22[a] | 32.20 ± 6.67[a] | 75.38 ± 7.95[a] | 42.92 ± 2.70[a] |
| 50% defoliation | 10.01 ± 3.77[a] | 28.00 ± 5.86[a] | 1.12 ± 0.18[b] | 28.18 ± 3.60[a,b] | 72.35 ± 6.25[a] | 39.30 ± 2.67[a] |
| 100% defoliation | 7.86 ± 2.23[a] | 37.73 ± 11.46[a] | 0.75 ± 0.15[c] | 24.79 ± 3.69[b] | 63.27 ± 5.96[b] | 39.34 ± 2.93[a] |
| *F-value* | 3.57 | 1.98 | 22.98 | 7.32 | 9.22 | 1.25 |
| *P-value* | 0.06[ns] | 0.17[ns] | ***0.01** | ***0.01** | ***0.01** | 0.31[ns] |
| **Combined PGRs levels** | | | | | | |
| Distilled water | 8.67 ± 1.65[a] | 36.88 ± 11.64[a] | 1.11 ± 0.29[a] | 27.90 ± 4.50[a] | 68.74 ± 6.84[a] | 40.63 ± 2.88[a] |
| CK0.32 + GA150 | 9.97 ± 1.43[a] | 31.49 ± 10.13[a] | 1.12 ± 0.38[a] | 27.42 ± 5.41[a] | 73.38 ± 9.45[a] | 37.57 ± 2.74[a] |
| CK0.64 + GA300 | 8.96 ± 0.56[a] | 33.62 ± 10.03[a] | 0.99 ± 0.26[a] | 29.85 ± 6.98[a] | 68.88 ± 8.68[a] | 43.36 ± 2.35[a] |
| *F-value* | 1.21 | 0.53 | 1.32 | 0.87 | 1.62 | 2.43 |
| *P-value* | 0.32[ns] | 0.59[ns] | 0.29[ns] | 0.43[ns] | 0.22[ns] | 0.12[ns] |
| **HD x Combined PGRs levels** | | | | | | |
| 0% x Distilled water | 9.35 ± 0.84[a] | 37.65 ± 16.75[a] | 1.30 ± 0.14[a] | 26.66 ± 5.56[a] | 71.89 ± 8.44[a] | 36.93 ± 2.40[b] |
| 0% x CK0.32 + GA150 | 11.19 ± 4.21[a] | 37.85 ± 11.35[a] | 1.53 ± 0.28[a] | 32.16 ± 7.22[a] | 82.61 ± 5.19[a] | 38.75 ± 2.55[a,b] |
| 0% x CK0.64 + GA300 | 8.68 ± 1.32[a] | 33.30 ± 8.99[a] | 1.20 ± 0.11[a] | 37.78 ± 1.51[a] | 71.66 ± 6.12[a] | 53.08 ± 2.24[a] |
| 50% x Distilled water | 8.63 ± 1.25[a] | 30.06 ± 6.12[a] | 1.25 ± 0.16[a] | 30.77 ± 2.73[a] | 72.44 ± 3.09[a] | 42.46 ± 2.16[a,b] |
| 50% x CK0.32 + GA150 | 9.28 ± 0.67[a] | 24.33 ± 4.79[a] | 1.02 ± 0.25[a] | 25.05 ± 3.46[a] | 70.16 ± 10.57[a] | 36.64 ± 2.71[b] |
| 50% x CK0.64 + GA300 | 12.11 ± 0.61[a] | 29.60 ± 6.85[a] | 1.10 ± 0.97[a] | 28.72 ± 2.69[a] | 74.44 ± 4.62[a] | 38.82 ± 2.39[a,b] |
| 100% x Distilled water | 8.04 ± 1.52[a] | 42.93 ± 9.95[a] | 0.77 ± 0.18[a] | 26.28 ± 4.89[a] | 61.88 ± 0.76[a] | 42.51 ± 2.50[a,b] |
| 100% x CK0.32 + GA150 | 9.44 ± 3.44[a] | 32.29 ± 10.95[a] | 0.80 ± 0.11[a] | 25.05 ± 1.61[a] | 67.38 ± 4.68[a] | 37.32 ± 2.29[a,b] |
| 100% x CK0.64 + GA300 | 6.09 ± 0.54[a] | 37.97 ± 14.90[a] | 0.67 ± 0.16[a] | 23.05 ± 4.47[a] | 60.55 ± 8.96[a] | 38.19 ± 2.41[a,b] |
| *F-value* | 2.83 | 0.28 | 1.35 | 0.22 | 1.39 | 3.35 |
| *P-value* | 0.06[ns] | 0.88[ns] | 0.29[ns] | 0.89[ns] | 0.28[ns] | ***0.03** |

Means followed by the same letter in the same column are statistically non-significant ($P<0.05$); ns = not significant; * = $F$-ratio probability of $P<0.05$; FFM = Foliar fresh mass; № branches = Number of branches; B:H ratio = Branch to height ratio

**Table 3.5:** The effects of simulated hail damage (zero%, 50%, and 100% defoliation) and the application of combined agricultural PGRs (a control, CK 0.32 mg/L + GA 150 mg/L, and CK 0.64 mg/L + GA 300 mg/L) on the essential oil mass- and content of rose geranium during the 2017/18 growing season (Thitapoho farm, Tweespruit).

| Treatments | Oil mass (g/plant) | Oil content (%) |
|---|---|---|
| **Simulated hail damage (HD)** | | |
| 0% defoliation | 0.14 ± 0.04[a] | 0.10 ± 0.03[a] |
| 50% defoliation | 0.15 ± 0.07[a] | 0.13 ± 0.06[a] |
| 100% defoliation | 0.11 ± 0.05[a] | 0.14 ± 0.08[a] |
| *F-value* | 1.58 | 0.98 |
| *P-value* | 0.23[ns] | 0.39[ns] |
| **Combined PGRs levels** | | |
| Distilled water | 0.15 ± 0.07[a] | 0.14 ± 0.05[a] |
| CK0.32 + GA150 | 0.13 ± 0.04[a] | 0.12 ± 0.04[a] |
| CK0.64 + GA300 | 0.12 ± 0.06[a] | 0.12 ± 0.09[a] |
| *F-value* | 0.82 | 0.16 |
| *P-value* | 0.45[ns] | 0.85[ns] |
| **HD x Combined PGRs levels** | | |
| 0% x Distilled water | 0.18 ± 0.01[a] | 0.13 ± 0.04[a] |
| 0% x CK0.32 + GA150 | 0.14 ± 0.04[a] | 0.09 ± 0.01[a] |
| 0% x CK0.64 + GA300 | 0.11 ± 0.03[a] | 0.09 ± 0.01[a] |
| 50% x Distilled water | 0.19 ± 0.10[a] | 0.15 ± 0.06[a] |
| 50% x CK0.32 + GA150 | 0.13 ± 0.06[a] | 0.12 ± 0.05[a] |
| 50% x CK0.64 + GA300 | 0.14 ± 0.06[a] | 0.12 ± 0.04[a] |
| 100% x Distilled water | 0.10 ± 0.03[a] | 0.13 ± 0.06[a] |
| 100% x CK0.32 + GA150 | 0.12 ± 0.01[a] | 0.15 ± 0.08[a] |
| 100% x CK0.64 + GA300 | 0.11 ± 0.10[a] | 0.16 ± 0.07[a] |
| *F-value* | 0.57 | 0.26 |
| *P-value* | 0.69[ns] | 0.89[ns] |

Means followed by the same letter in the same column are statistically non-significant ($P<0.05$); ns = not significant; * = $F$-ratio probability of $P<0.05$

**Table 3.6:** Principal component analyses for the herbage biomass and related attributes of rose geranium (Thitapoho farm, Tweespruit).

| **Traits** | **Principal component 1** | **Principal component 2** |
|---|---|---|
| Eigenvalue | 5.34 | 2.99 |
| Variability % | 41.1 | 23.04 |
| Cumulative % | 41.1 | 64.16 |
| **Factor loading** | | |
| 2017Chloro | 0.00 | 0.11 |
| 2017LA | 0.26 | 0.22 |
| 2017PH | **0.67** | 0.00 |
| 2017No Brns | 0.11 | **0.53** |
| 2017B:H | **0.64** | 0.15 |
| 2017FFMg | **0.53** | 0.22 |
| 2018Chloro | 0.13 | 0.13 |
| 2018LA | 0.45 | 0.16 |
| 2018PH | **0.76** | 0.06 |
| 2018No Brns | **0.67** | 0.24 |
| 2018B:H | 0.14 | **0.61** |
| 2018FFMg | **0.71** | 0.05 |
| 2018Oilmass | 0.21 | 0.47 |

Values in bold correspond for each variable to the factor for which the squared cosine is the largest. **Abbreviations:** Chloro = Chlorophyll; LA = Leaf area; PH = Plant height; No Brns = Number of branches; B:H = Branch to height ratio; FFMg = Foliar fresh mass; Oilmass = Essential oil mass

**Table 3.7:** The effects of simulated hail damage (zero%, 50%, and 100% defoliation) and the application of combined agricultural PGRs (a control, CK 0.32 mg/L + GA 150 mg/L, and CK 0.64 mg/L + GA 300 mg/L) on the density of a non-glandular trichomes on the adaxial and abaxial leaf surfaces of rose geranium (Thitapoho farm, Tweespruit).

| **Treatments** | **Adaxial leaf surface** | **Abaxial leaf surface** |
|---|---|---|
| **Simulated hail damage (HD)** | | |
| 0% defoliation | 50.77 ± 3.31$^{a}$ | 16.22 ± 6.26$^{a}$ |
| 50% defoliation | 46.44 ± 7.28$^{a}$ | 12.44 ± 3.53$^{a}$ |
| 100% defoliation | 36.44 ± 2.95$^{b}$ | 18.77 ± 9.44$^{a}$ |
| *F-value* | 7.80 | 1.65 |
| *P-value* | ***0.01** | 0.22$^{ns}$ |
| **Combined PGRs levels** | | |
| Distilled water | 52.77 ± 3.94$^{a}$ | 16.00 ± 5.67$^{a}$ |
| CK0.32 + GA150 | 56.00 ± 7.26$^{a,b}$ | 15.33 ± 8.60$^{a}$ |
| CK0.64 + GA300 | 44.88 ± 0.70$^{b}$ | 16.11 ± 7.55$^{a}$ |
| *F-value* | 10.17 | 0.03 |
| *P-value* | ***0.01** | 0.97$^{ns}$ |
| **HD x Combined PGRs levels** | | |
| 0% x Distilled water | 65.33 ± 6.02$^{a}$ | 15.00 ± 6.92$^{a}$ |
| 0% x CK0.32 + GA150 | 38.00 ± 9.64$^{b,c}$ | 15.66 ± 10.01$^{a}$ |
| 0% x CK0.64 + GA300 | 49.00 ± 3.46$^{a,b}$ | 18.00 ± 1.00$^{a}$ |
| 50% x Distilled water | 50.33 ± 10.11$^{a,b}$ | 20.33 ± 5.03$^{a}$ |
| 50% x CK0.32 + GA150 | 54.00 ± 2.00$^{a,b}$ | 16.66 ± 13.42$^{a}$ |
| 50% x CK0.64 + GA300 | 35.00 ± 3.46$^{b,c}$ | 19.33 ± 11.84$^{a}$ |
| 100% x Distilled water | 42.66 ± 15.50$^{b}$ | 12.66 ± 3.05$^{a}$ |
| 100% x CK0.32 + GA150 | 16.00 ± 2.00$^{c}$ | 13.66 ± 2.88$^{a}$ |
| 100% x CK0.64 + GA300 | 50.33 ± 10.11$^{a,b}$ | 11.00 ± 5.19$^{a}$ |
| *F-value* | 9.83 | 0.20 |
| *P-value* | ***0.01** | 0.93$^{ns}$ |

Means followed by the same letter in the same column are statistically non-significant ($P$<0.05); ns = not significant; * = $F$-ratio probability of $P$<0.05

**Table 3.8:** The effects of simulated hail damage (zero%, 50%, and 100% defoliation) and the application of combined agricultural PGRs (a control, CK 0.32 mg/L + GA 150 mg/L, and CK 0.64 mg/L + GA 300 mg/L) on glandular trichome densities observed on rose geranium plant adaxial and abaxial leaf surfaces (Thitapoho farm, Tweespruit).

| | **Adaxial leaf surface** | | | **Abaxial leaf surface** | | |
|---|---|---|---|---|---|---|
| **Treatments** | **Asciiform** | **Brevicollate** | **Capitate** | **Asciiform** | **Brevicollate** | **Capitate** |
| **Simulated hail damage (HD)** | | | | | | |
| 0% defoliation | 5.55 ± 2.24$^{c}$ | 41.88 ± 15.16$^{a}$ | 17.77 ± 1.05$^{b}$ | 13.55 ± 5.63$^{b}$ | 17.88 ± 4.91$^{a}$ | 1.33 ± 3.22$^{a}$ |
| 50% defoliation | 9.88 ± 4.53$^{a}$ | 47.66 ± 12.45$^{a}$ | 24.00 ± 1.49$^{b}$ | 25.88 ± 6.64$^{a}$ | 18.55 ± 4.97$^{a}$ | 2.11 ± 2.08$^{a}$ |
| 100% defoliation | 7.66 ± 2.00$^{b}$ | 34.44 ± 11.59$^{a}$ | 38.66 ± 1.15$^{a}$ | 17.00 ± 7.33$^{b}$ | 16.77 ± 9.20$^{a}$ | 2.88 ± 1.40$^{a}$ |
| *F-value* | 10.50 | 2.72 | 12.97 | 12.40 | 0.35 | 3.44 |
| *P-value* | ***0.01** | 0.09$^{ns}$ | ***0.01** | ***0.01** | 0.71$^{ns}$ | 0.06$^{ns}$ |
| **Combined PGRs levels** | | | | | | |
| Distilled water | 10.22 ± 3.39$^{a}$ | 47.11 ± 16.35$^{a}$ | 18.88 ± 0.62$^{b}$ | 18.11 ± 5.15$^{a}$ | 21.00 ± 6.85$^{a}$ | 0.66 ± 0.51$^{b}$ |
| CK0.32 + GA150 | 5.77 ± 1.71$^{b}$ | 45.22 ± 13.18$^{a}$ | 24.77 ± 1.17$^{b}$ | 16.88 ± 9.70$^{a}$ | 19.88 ± 5.23$^{a}$ | 1.77 ± 0.76$^{b}$ |
| CK0.64 + GA300 | 7.11 ± 3.21$^{b}$ | 31.66 ± 11.15$^{b}$ | 36.77 ± 1.23$^{a}$ | 21.44 ± 9.35$^{a}$ | 12.33 ± 3.31$^{b}$ | 3.88 ± 1.11$^{a}$ |
| *F-value* | 11.64 | 4.39 | 9.37 | 1.70 | 9.53 | 15.23 |
| *P-value* | ***0.01** | ***0.02** | ***0.01** | 0.21$^{ns}$ | ***0.01** | ***0.01** |
| **HD x Combined PGRs levels** | | | | | | |
| 0% x Distilled water | 7.33 ± 2.08$^{b,c}$ | 49.00 ± 18.33$^{a}$ | 13.66 ± 0.47$^{b,c}$ | 17.66 ± 7.76$^{a,b}$ | 20.33 ± 4.50$^{a}$ | 1.33 ± 0.57$^{b,c}$ |
| 0% x CK0.32 + GA150 | 5.33 ± 2.08$^{b,c}$ | 31.00 ± 14.00$^{a}$ | 25.66 ± 1.69$^{a,b}$ | 11.00 ± 5.19$^{b}$ | 19.66 ± 5.03$^{a}$ | 2.33 ± 1.52$^{b,c}$ |
| 0% x CK0.64 + GA300 | 4.00 ± 1.73$^{c}$ | 45.66 ± 10.59$^{a}$ | 14.00 ± 1.23$^{b,c}$ | 12.00 ± 1.00$^{b}$ | 13.66 ± 3.21$^{a}$ | 0.33 ± 0.57$^{c}$ |
| 50% x Distilled water | 14.00 ± 4.35$^{a}$ | 34.33 ± 15.56$^{a}$ | 10.66 ± 0.98$^{c}$ | 18.66 ± 6.24$^{a,b}$ | 24.66 ± 11.50$^{a}$ | 0.33 ± 0.57$^{c}$ |
| 50% x CK0.32 + GA150 | 5.00 ± 1.00$^{b,c}$ | 28.33 ± 11.15$^{a}$ | 36.33 ± 1.63$^{a,b}$ | 28.66 ± 5.50$^{a}$ | 16.33 ± 4.16$^{a}$ | 1.66 ± 0.61$^{b,c}$ |
| 50% x CK0.64 + GA300 | 10.66 ± 0.57$^{a,b}$ | 40.66 ± 7.57$^{a}$ | 25.00 ± 1.38$^{a,b}$ | 30.33 ± 4.50$^{a}$ | 9.33 ± 3.51$^{a}$ | 4.33 ± 2.64$^{a,b}$ |
| 100% x Distilled water | 9.33 ± 1.52$^{a,b,c}$ | 58.00 ± 7.93$^{a}$ | 32.33 ± 1.52$^{a,b}$ | 18.00 ± 2.51$^{a,b}$ | 18.00 ± 1.00$^{a}$ | 0.33 ± 0.57$^{c}$ |
| 100% x CK0.32 + GA150 | 7.00 ± 1.73$^{b,c}$ | 35.66 ± 11.67$^{a}$ | 48.33 ± 1.54$^{a}$ | 11.00 ± 2.64$^{b}$ | 23.66 ± 5.13$^{a}$ | 1.33 ± 0.57$^{b,c}$ |
| 100% x CK0.64 + GA300 | 6.66 ± 2.08$^{b,c}$ | 49.33 ± 6.49$^{a}$ | 35.33 ± 0.88$^{a,b}$ | 22.00 ± 4.50$^{a,b}$ | 14.00 ± 1.00$^{a}$ | 7.00 ± 2.08$^{a}$ |
| *F-value* | 3.74 | 0.45 | 10.49 | 3.42 | 2.09 | 9.51 |
| *P-value* | ***0.02** | 0.77$^{ns}$ | ***0.02** | ***0.03** | 0.12$^{ns}$ | ***0.01** |

Means followed by the same letter in the same column are statistically non-significant ($P$<0.05); ns = not significant; * = $F$-ratio probability of $P$<0.05

**Table 3.9:** The effects of simulated hail damage (zero%, 50%, and 100% defoliation) and the application of combined agricultural PGRs (a control, CK 0.32 mg/L + GA 150 mg/L, and CK 0.64 mg/L + GA 300 mg/L) on selected essential oil quality parameters of rose geranium (Thitapoho farm, Tweespruit).

| Treatments | *cis*-Rose oxide | *trans*-Rose oxide | Epi-$\gamma$-eudesmol | Isomenthone | Citronellol | Geraniol | C:G ratio |
|---|---|---|---|---|---|---|---|
| **Simulated hail damage (HD)** | | | | | | | |
| 0% defoliation | 0.13 ± 0.05[a] | 0.04 ± 0.01[a] | 3.74 ± 0.83[a] | 5.25 ± 0.50[a] | 4.81 ± 1.52[b] | 0.90 ± 0.50[a] | 5.34 ± 1.51[a] |
| 50% defoliation | 0.19 ± 0.23[a] | 0.05 ± 0.01[a] | 3.60 ± 0.38[a] | 5.24 ± 0.44[a] | 2.90 ± 1.13[c] | 0.93 ± 0.01[a] | 3.11 ± 1.28[a] |
| 100% defoliation | 0.18 ± 0.12[a] | 0.17 ± 0.09[a] | 3.59 ± 0.30[a] | 6.39 ± 0.44[a] | 6.48 ± 2.99[a] | 1.21 ± 0.05[a] | 5.35 ± 1.26[a] |
| *F-value* | 0.13 | 3.34 | 0.20 | 2.25 | 7.06 | 0.55 | 2.60 |
| *P-value* | 0.87[ns] | 0.14[ns] | 0.81[ns] | 0.14[ns] | ***0.01** | 0.61[ns] | 0.16[ns] |
| **Combined PGRs levels** | | | | | | | |
| Distilled water | 0.11 ± 0.08[a] | 0.06 ± 0.03[a] | 3.46 ± 0.20[a] | 5.47 ± 0.44[a] | 4.37 ± 2.33[b] | 0.93 ± 0.57[a] | 4.69 ± 1.33[a] |
| CK0.32 + GA150 | 0.27 ± 0.23[a] | 0.09 ± 0.08[a] | 3.66 ± 0.85[a] | 5.85 ± 0.50[a] | 5.78 ± 3.45[a] | 0.74 ± 0.01[a] | 7.81 ± 1.55[a] |
| CK0.64 + GA300 | 0.13 ± 0.05[a] | 0.10 ± 0.11[a] | 3.83 ± 0.40[a] | 5.56 ± 0.44[a] | 4.04 ± 1.32[b] | 0.71 ± 0.02[a] | 5.64 ± 1.47[a] |
| *F-value* | 1.62 | 0.38 | 2.64 | 0.16 | 6.93 | 1.01 | 3.75 |
| *P-value* | 0.23[ns] | 0.70[ns] | 0.35[ns] | 0.85[ns] | ***0.01** | 0.42[ns] | 0.09[ns] |
| **HD x Combined PGRs levels** | | | | | | | |
| 0% x Distilled water | 0.17 ± 0.09[a] | 0.05 ± 0.01[a] | 3.33 ± 0.01[a] | 4.61 ± 0.80[a] | 5.86 ± 2.67[b] | 0.62 ± 0.81[a] | 9.44 ± 1.25[a] |
| 0% x CK0.32 + GA150 | 0.10 ± 0.01[a] | 0.06 ± 0.02[a] | 3.88 ± 0.01[a] | 5.32 ± 1.00[a] | 4.01 ± 0.02[b,c] | 0.30 ± 0.01[a] | 13.34 ± 1.96[a] |
| 0% x CK0.64 + GA300 | 0.12 ± 0.03[a] | 0.04 ± 0.01[a] | 4.03 ± 0.17[a] | 5.82 ± 0.36[a] | 4.56 ± 1.07[b,c] | 0.61 ± 0.04[a] | 7.46 ± 1.06[a] |
| 50% x Distilled water | 0.04 ± 0.01[a] | 0.06 ± 0.01[a] | 3.51 ± 0.27[a] | 4.94 ± 0.77[a] | 2.07 ± 0.14[c] | 0.62 ± 0.01[a] | 3.32 ± 1.21[a] |
| 50% x CK0.32 + GA150 | 0.31 ± 0.33[a] | 0.05 ± 0.01[a] | 3.41 ± 0.09[a] | 5.41 ± 0.08[a] | 3.48 ± 0.79[b,c] | 0.71 ± 0.01[a] | 4.92 ± 1.08[a] |
| 50% x CK0.64 + GA300 | 0.14 ± 0.09[a] | 0.05 ± 0.01[a] | 3.89 ± 0.56[a] | 5.38 ± 1.83[a] | 3.14 ± 1.68[b,c] | 0.69 ± 0.01[a] | 4.56± 1.58[a] |
| 100% x Distilled water | 0.14 ± 0.11[a] | 0.10 ± 0.01[a] | 3.50 ± 0.04[a] | 6.87 ± 0.87[a] | 5.18 ± 2.38[b] | 0.61 ± 0.03[a] | 8.48 ± 1.45[a] |
| 100% x CK0.32 + GA150 | 0.31 ± 0.15[a] | 0.19 ± 0.01[a] | 3.71 ± 0.58[a] | 6.82 ± 0.04[a] | 9.85 ± 0.70[a] | 0.62 ± 0.01[a] | 15.88 ± 1.60[a] |
| 100% x CK0.64 + GA300 | 0.13 ± 0.07[a] | 0.20 ± 0.14[a] | 3.57 ± 0.38[a] | 5.84 ± 0.41[a] | 4.41 ± 1.11[b,c] | 0.70 ± 0.01[a] | 6.29 ± 1.42[a] |
| *F-value* | 0.48 | 0.15 | 6.77 | 0.88 | 9.20 | 0.89 | 1.37 |
| *P-value* | 0.75[ns] | 0.81[ns] | 0.74[ns] | 0.50[ns] | ***0.01** | 0.46[ns] | 0.35[ns] |

Means followed by the same letter in the same column are statistically non-significant ($P<0.05$); ns = not significant; * = $F$-ratio probability of $P<0.05$. Abbreviation descriptions: C:G = C:G rate

**Table 3.10:** Principal component analyses for the essential oil quality attributes of rose geranium plants (Thitapoho farm, Tweespruit).

| **Traits** | **Principal component 1** | **Principal component 2** |
|---|---|---|
| Eigenvalue | 3.28 | 1.50 |
| Variability % | 46.9 | 21.55 |
| Cumulative % | 46.9 | 68.46 |
| **Factor loading** | | |
| *cis*-Rose oxide | 0.39 | 0.06 |
| *trans*-Rose oxide | **0.62** | 0.00 |
| Isomenthone | **0.70** | 0.18 |
| Citronellol | **0.81** | 0.02 |
| Geraniol | 0.07 | **0.71** |
| Epi-*γ*-eudesmol | 0.07 | 0.48 |

Values in bold correspond for each variable to the factor for which the squared cosine is the largest

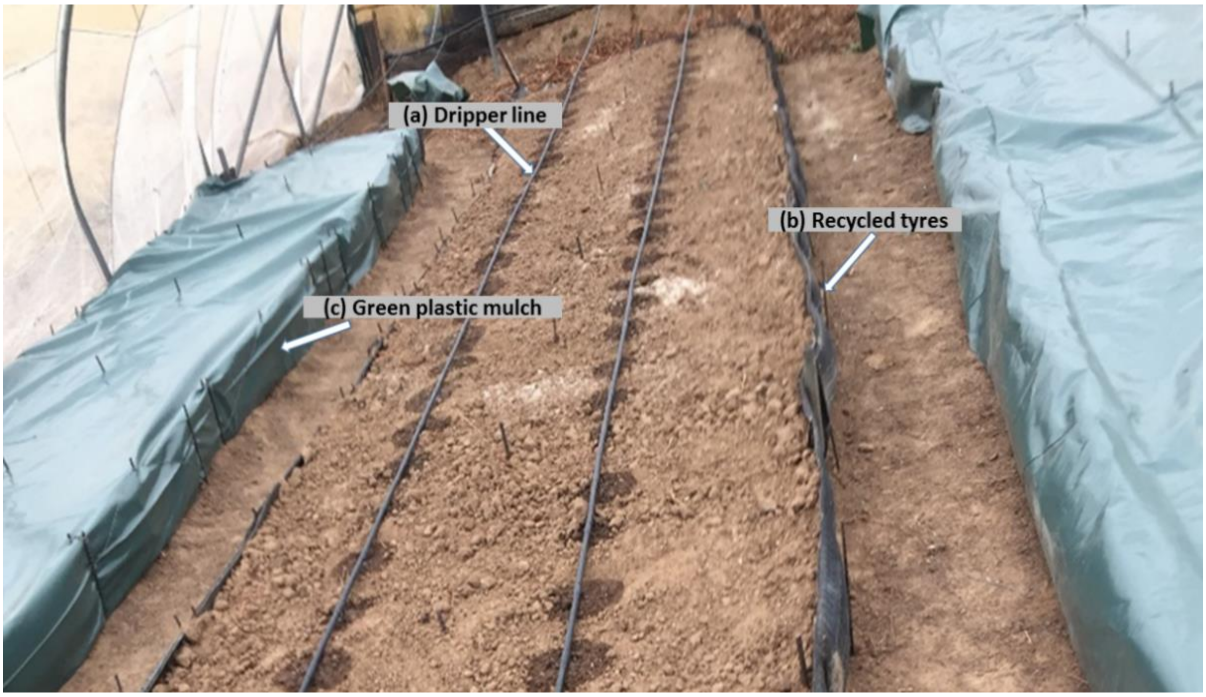


**Figure 3.1:** Recycled tyre beds: (a) dripper line; (b) recycled tyres; and (c) green plastic mulch (Thitapoho farm, Tweespruit).

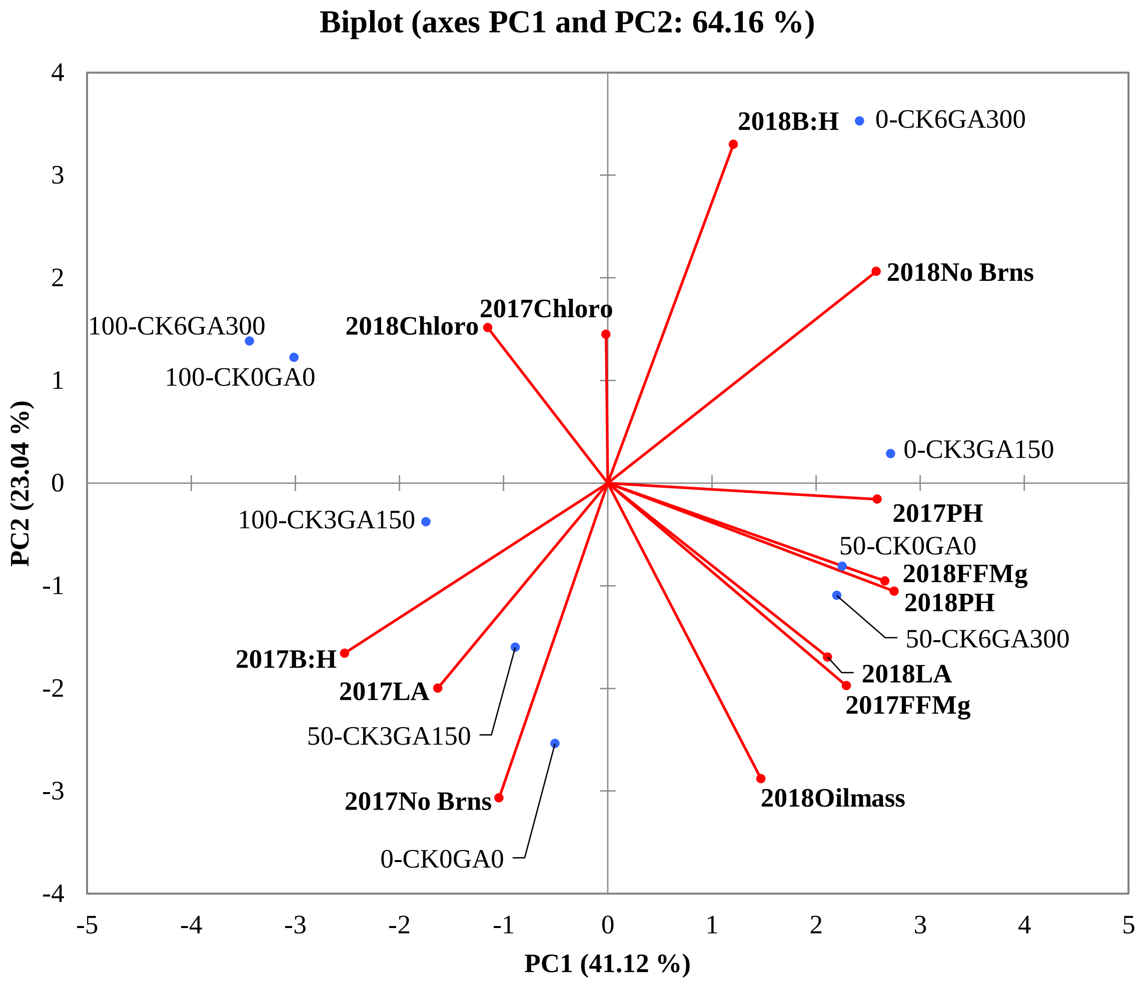


**Figure 3.2:** Principal component biplot illustrating the variations between rose geranium biomass and related attributes, as affected by simulated hail damage (zero%, 50%, and 100% defoliation) and the application of combined agricultural PGRs (a control, CK 0.32 mg/L + GA 150 mg/L, and CK 0.64 mg/L + GA 300 mg/L) (Thitapoho farm, Tweespruit). **Abbreviations:** Chloro = Chlorophyll; LA = Leaf area; PH = Plant height; No Brns = Number of branches; B:H = Branch to height ratio; FFMg = Foliar fresh mass; Oilmass = Essential oil mass; 0 = intact plants; 50 = 50% defoliation; 100 = 100% defoliation; CK0GA0 = Distilled water; CK3GA150 = CK0.32 + GA150; CK6GA300 = CK0.64 + GA300.

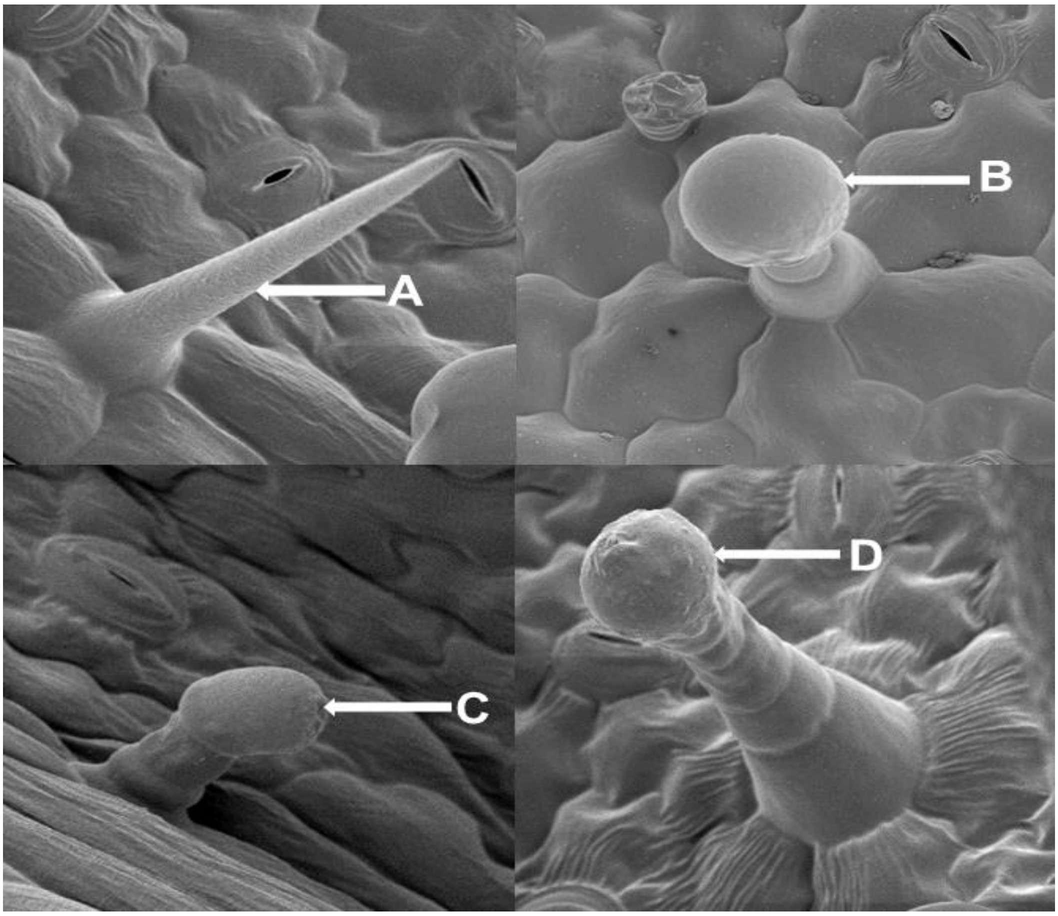


**Figure 3.3:** Four different types of trichomes observed on rose geranium leaves under a scanning electron microscope (x400 magnifications) during this study (Thitapoho farm, Tweespruit). (A) Non-glandular trichome, (B) Brevicollate trichome, (C) Asciiform trichome, (D) Capitate trichome.

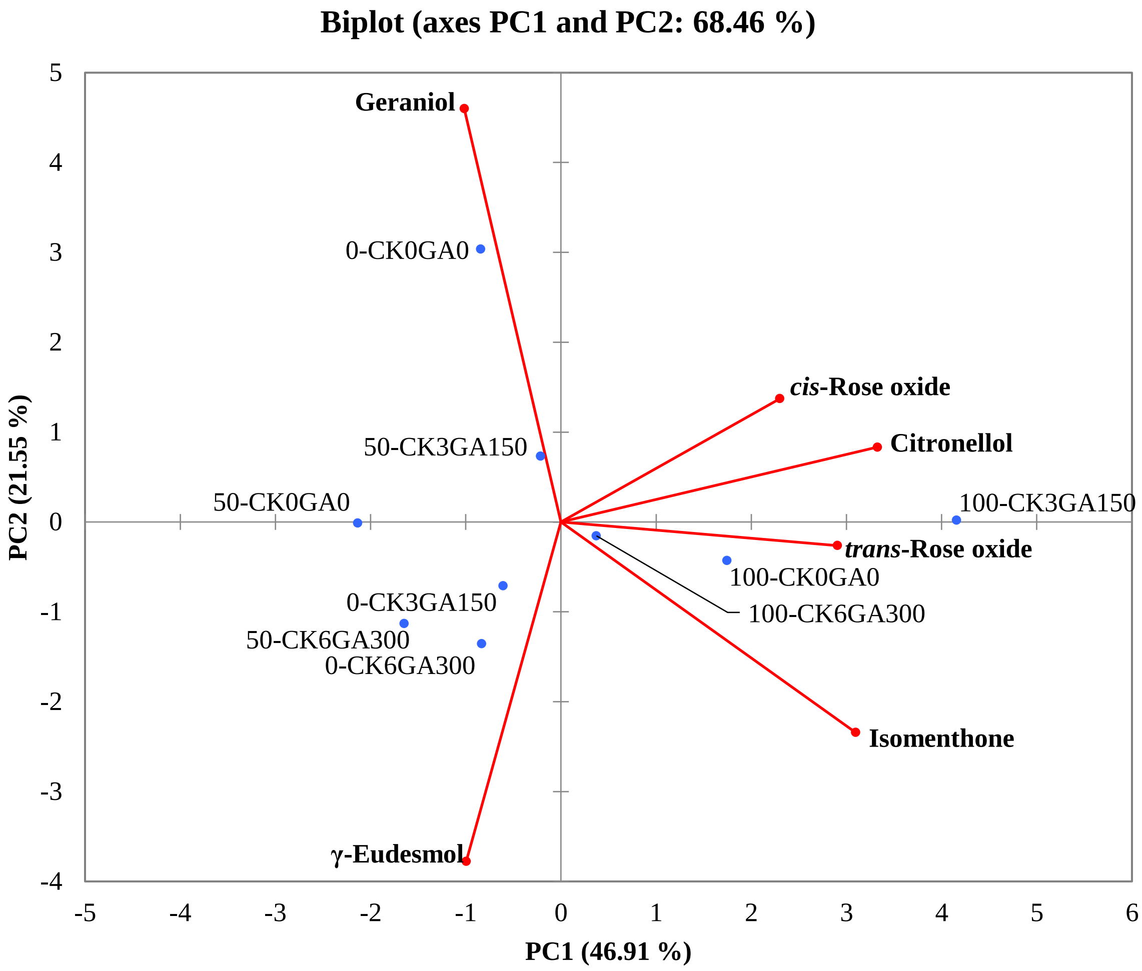


**Figure 3.4:** Principal component biplot illustrating the variations between rose geranium essential oil quality parameters as affected by simulated hail damage (zero%, 50%, and 100% defoliation) and the application of combined agricultural PGRs (a control, CK 0.32 mg/L + GA 150 mg/L, and CK 0.64 mg/L + GA 300 mg/L) (Thitapoho farm, Tweespruit). **Abbreviations:** 0 = intact plants; 50 = 50% defoliation; 100 = 100% defoliation; CK0GA0 = Distilled water; CK3GA150 = CK0.32 + GA150; CK6GA300 = CK0.64 + GA300.

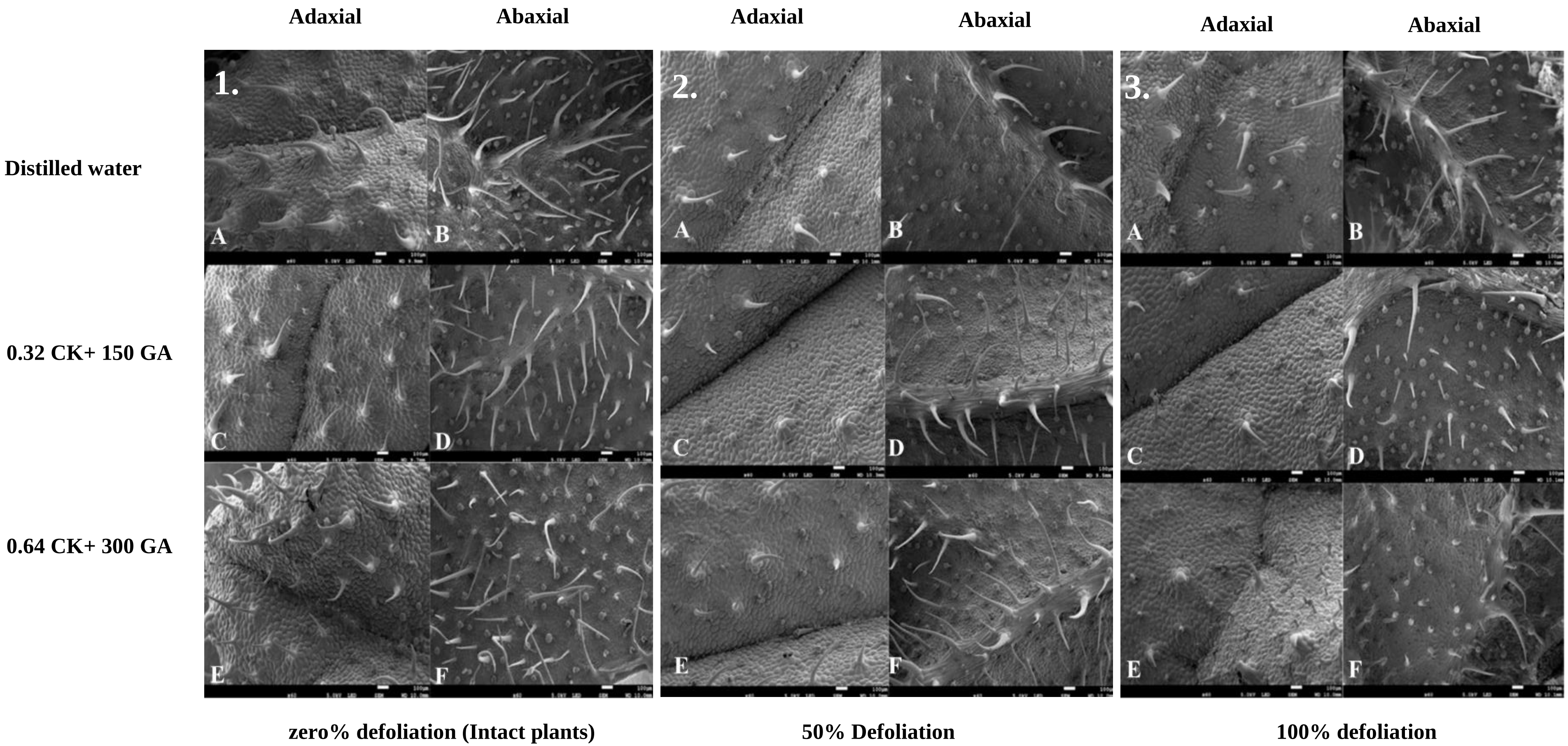


**Figure 3.5:** Newly developed leaf (adaxial and abaxial leaf surface) from the zero% (1.), 50% (2.) and 100% (3.) simulated hail-damaged rose geranium plants treated with distilled water (A and B), combined CK 0.32 mg/L + GA 150 mg/L (C and D) and combined CK 0.64 mg/L + GA 300 mg/L (E and F). Observed under a scanning electron microscope (x60 magnification; 100 $\mu$m).

# CHAPTER FOUR

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## POTENTIAL USE OF ABSCISIC ACID AND METHYL JASMONATE IN THE RECOVERY OF SIMULATED HAIL-DAMAGED ROSE GERANIUM (*Pelargonium graveolens* L'Hér. cv. 'BOURBON') PLANTS

## Abstract

Hail damage is an environmental stressor that can affect plant growth and essential oil biosynthesis in rose geranium (*Pelargonium graveolens* L'Hér.). This study evaluated the role of two agricultural plant growth regulators (PGRs), abscisic acid (ABA) and methyl jasmonate (MeJA), in the recovery of simulated hail-damaged rose geranium. The experiment was conducted in a temperature-controlled greenhouse, using a 5 x 2 factorial treatment design, arranged in a randomized complete block design with three replicates during the 2016/17 and 2017/18 growing seasons. Treatments comprised four levels of PGRs: 75 µM ABA, 150 µM ABA, 10 mM MeJA, 20 mM MeJA, and a control, and two application periods (daily application for either seven or 14 days). Hail damage was simulated through 100% defoliation and decapitation of the terminal buds. The results showed that using high concentrations of ABA and MeJA for a shorter period (seven days) increased recovery some of the essential oil yield. However, these results were inconclusive between the growing seasons. A multivariate analysis showed that neither ABA nor MeJA increased or improved plant parameters and essential oil biosynthesis of rose geranium. Geraniol content, along with some of its esters, was improved by the use of MeJA (10 mM), however, this treatment led to a low linalool content. ABA, in any concentration, negatively affected the citronellol to geraniol ratio, a determining factor in essential oil quality. This study shows that the use of ABA or MeJA inhibits the recovery of hail-damaged rose geranium herbage, and negatively affects the essential oil yield and quality.



**Keywords:** Abscisic acid, 'Bourbon-type', essential oil yield, hail damage simulation, methyl jasmonate


## 4.1 Introduction

Rose geranium (*Pelargonium graveolens* L'Hér.) is an indeterminate plant species, native to South Africa (Araya 2012). The essential oil derived from this plant is used globally in the aromatherapeutic, pharmaceutical, food, and perfume industries, and is considered one of the most valuable essential oil plant species (Nilofer *et al.* 2018). The chemical composition of rose geranium essential oil is complex, and is comprised of a large number of compounds contributing to the aroma, which is important to the lucrative perfumery industry (Bergman *et al.* 2019). The production and biosynthesis of rose geranium essential oil compounds can be affected by endogenous factors, as well as by environmental factors, such as hail damage (Prins *et al.* 2010).

Hail is a natural hazard that can cause significant loss to crop yields (Yu *et al.* 2016). Hailstones larger than 8.4 mm in diameter can result in defoliation, which, in turn, initiates cell division and the synthesis of cellular components (Changnon 1999; Bal *et al.* 2014). These metabolic activities are associated with the repair of damaged tissue and the slowing of plant growth activities around the wounding site (Nanda and Melnyk 2018). The process of plant wound healing is complex, and starts with an oxidative burst, followed by upregulation of plant growth regulators (PGRs) at the wound site (Ikeuchi *et al.* 2017).

PGRs are also used in the agricultural industry to mitigate against biotic and abiotic stresses (Han *et al.* 2018). Abscisic acid (ABA) and jasmonic acid are PGRs, classified as primary plant-stress relief hormones (Ju *et al.* 2016). They perform unique functions in plant development and stress repair, as well as improving the primary and secondary metabolite content of plants, which directly affects the essential oil biosynthesis and quality (Ju *et al.* 2016).

Foliar applications of ABA improve the medicinal and commercial value of plants by optimising the accumulation of essential oils (Ghassemia *et al.* 2017). For example, ABA (0.3 µM) increases the seed oil content in oilseed rape (*Brassica napus* L.) (Wilmer *et al.*, 1997), and Moustakime *et al.* (2017) reported that an exogenous application of ABA (1.1 mM) improves fat accumulation in olives (*Olea europaea* L.). In previous studies, foliar applications of ABA on wounded plants varies in concentration, from 0.001 mM to 1.5 mM per plant, with the application rate and level differing between different crop species (Wang *et al.* 2018).

Jasmonate and its methyl ester, methyl jasmonate (MeJA), are naturally present in plants (Drábková *et al.* 2015). Jasmonates primarily control various aspects of essential oil biosynthesis, including the formation of trichomes (Per *et al.* 2018): MeJA applied at 0.5 mM increases the eugenol and linalool content of basil (*Ocimum basilicum* L.) (Kim *et al.* 2006), while 50 µM applied to bigleaf marsh-elder (*Iva frutescens* L.), increases $\alpha$-pinene, sabinene, and limonene emissions. Foliar applications of MeJA vary in concentration from 0.01 mM to 20 mM solution per plant (Yu *et al.* 2016; Jiang *et al.* 2017; Liu *et al.* 2017).

The objective of this study was to evaluate the potential role of two agricultural PGRs (ABA and MeJA) on the recovery of simulated hail-damaged rose geranium plants. It is hypothesized that high concentrations of ABA and MeJA, applied daily for a period of seven days, will improve the essential oil yield and quality attributes of rose geranium.

## 4.2 Materials and methods

### 4.2.1 Study site and general crop management practices

*Study site:* This experiment was carried out in a 72 $m^2$ temperature-controlled greenhouse, located at the Central University of Technology, Free State campus (29°07'S, 26°12'E). The site is situated in a semi-arid region, at ca. 1390 amsl. The greenhouse temperature was maintained at a constant 26°C. This experiment was conducted during the 2016/17 and 2017/18 growing seasons.

*Plant material and agronomic practices:* In South Africa, 'Bourbon', 'Reunion', and 'North Africa-type' are the most common cultivars (Motsa *et al.* 2006; Tembe and Deodhar 2010). In the previous chapter, the 'North Africa-type' did not perform well with low essential oil yield and quality. Therefore, the rooted rose geranium cuttings 'Bourbon-type'(ca. 10 cm tall) were sourced and obtained from a reputable commercial grower (Siyakholwa Development Foundation, RSA). These rooted cuttings were transplanted into 5 L potting bags on September 2nd 2016 and September 1st 2017. The potting bags were filled with a sterile silica-sand root-medium, with a standard average grain size diameter of 2 mm. A single rooted cutting was transplanted into each potting container.

Water analysis was conducted during both growing seasons and taken into account during the formulation of the nutrient solution (Table 4.1). 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 mins, containing a balanced nutrient solution, as recommended for rose geranium (Table 4.2). 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, in order 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). No phytophagous pests were documented during the experiment, however, Malasol (an organophosphate) was preventatively sprayed at 1.75 ml/L throughout the cropping seasons. These applications were repeated for three to six days, at four-week intervals.

*Hail damage simulations:* Hail damage simulations were conducted on December 26th 2016, and December 25th 2017, which was 116 days after transplantation of the rooted cuttings. South African thunderstorms occur mostly during the afternoon (Powell 2013), therefore, hail damage simulation was performed at ca. 2:00 pm. Hail damage was simulated using a pair of garden secateurs to remove all the leaves (100% defoliation), however, all the sprouting primordial leaves were left intact. The terminal buds on the stems were then decapitated to create random stem bruises and wounds (Irigoyen *et al.* 2010). The simulated hail-damaged plants were then sprayed with a water mist (0.3 MPa pressure), using a nozzle-calibrated precision sprayer, to simulate humid summer afternoon conditions (Bal *et al.* 2014).

### 4.2.2 Experimental layout and treatment design

This study had a 5 x 2 factorial treatment design. Plants were treated with four different concentrations of PGRs: 75 µM ABA, 150 µM ABA, 10 mM MeJA, 20 mM MeJA, and distilled water as a control after the hail damage simulations. PGRs were applied for a duration of either seven or 14 days. The control plants were treated for 14 days only. Treatment combinations were arranged in a randomised complete block design with three replications. Each experimental unit measured 1 $m^2$ in size and contained six individual plants, spaced at ca. 50 cm x 30 cm.

The application of PGRs (Sigma-Aldrich, RSA) took place between December 26th 2016 and 8th January 2017, and December 25th 2017 and 7th January 2018. The ABA and MeJA treatments were applied daily at ca. 6:00 am, using a calibrated precision sprayer (0.3 MPa pressure) (Ferndale, UK). Treatment solution (50 mL) was applied to each plant in each unit plant. To avoid the spray mixture drifting onto adjacent experimental plants, a mini plastic-covered structure, measuring 650 mm x 480 mm x 1200 mm (h x w x l), was used to cover individual plants while they were sprayed.

Other studies have used widely different protocols for ABA and MeJA applications. For this study, we followed the following treatment regime: ABA treatment solutions were prepared by diluting 19.86 mL and 39.64 mL of stock solution in 1 L distilled water to obtain the final concentrations of 75 µM and 150 µM, respectively (e.g. Ruiz-Garciá *et al.* 2012; Ju *et al.* 2016). For the MeJA treatment solutions, 2.1 mL and 4.2 mL of the stock solution was diluted in 1 L distilled water to obtain final concentrations of 10 mM and 20 mM, respectively (e.g. Thaler *et al.* 2002; Loivamäki *et al.* 2004; Konan *et al.* 2014; Yu *et al.* 2016; Jiang *et al.* 2017; Liu *et al.* 2017). Triton x100 at 0.1% (v/v) was added to the stock solution as a wetting agent. The same procedure was repeated for the control plants, but ABA and MeJA were omitted. According to Kim *et al.* (2014), stress and defense-related phytorhomnes are significantly downregulated and also get irregular after a stress; thus, prolonged application (7 and 14 days) was followed in thus study to improve the plants recovery chances.

### 4.2.3 Data collection and statistical analysis

Data for all parameters were collected between the 6th and 11th of March 2017 and the 5th and 10th of March 2018, 75 days after the hail damage simulations (Kumar *et al.* 2001; Board 2003). All parameters and statistical analyses followed the same procedures as described in Chapter 3. Due to financial constraints, trichome parameters were only recorded during the 2017/18 season. The ISO standard (ISO 4731 [E] 2012) was used to characterise rose geranium ('Bourbon-type' cv.) essential oil quality parameters for the perfumery industry.

## 4.3 Results

### 4.3.1 Plant parameters, and essential oil yield attributes

During both growing seasons, the number of branches, plant height, plant stand loss, foliar fresh mass (FFM), and essential oil content were not improved by either PGR treatment, regardless of application frequency, and as well the interactions between PGR treatment and the application frequency (Tables 4.3 & 4.4). During the first growing season (2016/17), a significant interaction was observed between PGR treatment and application frequency for the leaf area ($F_{17.08}$ = 2.41; P<0.01) (Table 4.3). The leaf area tended to be smaller, when the application of 20 mM MeJA was increased from seven to 14 days compared to the control. This interaction was not observed in the leaf area during the second season (Table 4.4).

During the 2017/18 growing season, interactions were observed between the application period and PGR treatments on the essential oil yield (Table 4.4). The highest essential oil yield was recorded when 10 mM MeJA was applied for seven days ($F_{18.00}$ = 4.55; P<0.01); however, this was not significantly different to 20 mM MeJA, 75 µM ABA, or the control. When PGR applications were extended to 14 days, all treatments recorded higher essential oil mass, with the exception of 10 mM MeJA, which reduced the mean essential oil mass by 0.3 g. However, the mean essential oil mass was 0.2 g higher when the MeJA concentration was increased to 20 mM. The application of ABA at the lower concentration (75 µM) was better than the lower level of MeJA (10 mM) at 14 days of application period treatment (Table 4.4). The opposite effect was observed in the higher levels: 20 mM MeJA increased the mean essential oil yield by 50%, compared to 150 µM ABA. However, this was only recorded in the shorter application period of seven days. Although these differences were identified, none of the treatments were significantly different from the control (Table 4.3). A similar trend was shown in the first season, but this was also not significant.

Multivariate data analysis, using principal component analysis (PCA), is a suitable technique to increase the interpretability of complex and inconclusive data (Jolliffe and Cadima 2016; Mishra *et al.* 2018). For this reason, plant parameters and the essential oil yield variables were subjected to PCA to identify any possible associations between the variables and the PGR treatments applied for seven or 14 days. The score plot and loading matrix, based on the first and second principal components (PC1 and PC2), accounted for 57.7% of the total variance (Table 4.5 & Figure 4.1). In PC1 (37.8%), a biplot-loading indicated that the FFM, plant stand loss, the number of branches and the leaf area were characterised by the application of 150 µM ABA, as well as distilled water, during the 2016/17 season. During the second season, FFM declined as a result of the application of 150 µM ABA and 20 mM MeJA for seven and 14 days, respectively. Plant height was the only parameter positively affected by the application of 150 µM ABA and 20 mM MeJA during the 2017/18 season (PC2 = 19.9%). The number of branches per plant was lower for all MeJA treatments applied for 7 days. The PCA indicated that plants might grow taller following applications of 150 µM ABA and 20 mM MeJA when applied for 7 and 14 days, respectively.

### 4.3.2 Leaf trichomes

Two groups of trichomes were identified: glandular trichomes (peltate and capitate) and non-glandular trichomes (attenuate). The densities of trichomes were not affected by either PGR treatment, regardless of application frequency, or by interactions between simulated hail damage and the subsequent application of PGRs (Table 4.6).

### 4.3.3 Essential oil quality

Both MeJA treatment levels reduced the linalool content during both growing seasons; however, this was not always statistically significant (Tables 4.7 & 4.8). Both ABA treatments significantly increased the isomenthone content during the 2016/17 growing season, compared to the control and MeJA treatments (Table 4.7). The highest isomenthone content (3.1%) was measured when ABA was applied at a concentration of 75 µM. Even so, this isomenthone content was still lower than the required ISO 4731 2012 [E] standard. This effect was not observed during the 2017/18 growing season (Table 4.8).

During 2016/17 season, only the linalool and isomenthone were affected by PGR treatments (Table 4.7). This was not the case for the second season, all PGR treatments were similar compared with the control for citronellol, geraniol, citronellol to geraniol ratio (C:G ratio), linalool, and isomenthone (Table 4.8). Neither ABA nor MeJA improved geraniol content in both seasons (Table 4.7 & 4.8). The lowest geraniol content was recorded in plants treated with 150 µM ABA during the second season (Table 4.8). The C:G ratio was affected by biosynthesis of citronellol and geraniol. The C:G ratio was affected during the 2017/18 growing season, but not during 2016/17 (Table 4.7 & 4.8). The C:G ratio is used by the perfumery industry and ISO standard requirements to classify rose geranium essential oil quality. The application of ABA at any concentration level significantly increased the C:G ratio, however, the C:G ratio was >3, signifying essential oil with a low odour (Saxena *et al.* 2008).

In this study, geranyl tiglate was affected by the interaction between PGR treatment and application period during the 2016/17 growing season ($F_{17.68} = 4.14$; $P<0.03$), but not during the 2017/18 season (Table 4.9). The mean percentage of geranyl tiglate content increased by 49.7% when the 10 mM MeJA application was extended from seven to 14 days; however, this was not significantly different compared to the control. Both levels of ABA decreased the geranyl formate content during the 2017/18 growing season (Table 4.10). Moreover, geranyl formate content declined when the MeJA concentration was increased to 20 mM. The content of another geraniol ester, geranyl butyrate, increased when 10 mM MeJA was applied. However, this was not statistically different from the 150 µM ABA treatment (Table 4.10).

The essential oil parameters showed homogenous results over the two seasons, however, it was difficult to elucidate a conclusion. Therefore, the ANOVA results from the essential oil quality data were further subjected to a multivariate data analysis, using a PCA, to determine the association between essential oil quality variables and PGR treatment and application time. The score plot and loading matrix accounted for 54.5% of the total variance (Table 4.11 & Figure 4.2). In PC1 (35.9%), *trans*-Rose oxide and citronellol (2016/17 season), and linalool and guaia-6,9-diene (2017/18), were negatively correlated by the application of 150 µM ABA for 14 days. Geraniol esters, geranyl butyrate and geranyl tiglate, were positively correlated by the use of 150 µM ABA (seven days) and 10 mM MeJA (14 days) during the 2016/17 season. In PC2 (18.5%), geranyl formate was the only essential oil compound increased by the use of 150 µM ABA (seven days) and 10 mM MeJA (14 days) during the 2016/17 season.

## 4.4 Discussion

### 4.4.1 Plant parameters, and essential oil yield attributes

In this study, plants were completely defoliated (100%) to simulate severe hail damage. Therefore, all plant parameters which were not affected by defoliation could be attributed to a sufficient recovery period following the hail damage simulations. According to Haileslassie and Kebede (2015), the recommended harvesting period for improved herbage, as well as essential oil yield and quality, is between 12 and 20 weeks after planting. Therefore, the sufficient time allowed in this study led to a full recovery.

Endogenous accumulation of jasmonic acid in plant cells occurs within minutes of wounding, subsequently followed by the sequestering process (Han *et al.* 2018). Therefore, the findings of this study on the leaf area parameters could be ascribed to the prolonged exogenous application of MeJA (14 days), which could have accumulated to a toxic level, thereby inhibiting leaf growth. Glauser *et al.* (2009) reported a similar increase in jasmonic acid content in wounded leaves of Arabidopsis (*Arabidopsis thaliana* L.) within 30 seconds of application. In addition, repeated exogenous applications of jasmonates can cause transient changes in jasmonate concentration in most plant cells, before reaching internal equilibrium in tissues (Ahmad *et al.* 2016). Therefore, leaf growth inhibition in this study could also be a consequence of MeJA facilitating the arrest of cell metabolic activities, and repressing the expression of mitotic phase genes. A relatively low MeJA concentration (50 µM) reduces leaf area by ca. 80% in all developmental stages of Arabidopsis (Noir *et al.* 2013), and Donnelly *et al.* (1999) also reported that MeJA negatively affects the cell expansion of Arabidopsis by reducing cell proliferation during the transition phase (13 DAS).

In this study, neither ABA nor MeJA increased the essential oil yield, compared to the control (Tables 4.3 & 4.4). The reduced essential oil yield is attributed to a prolonged application (14 days) of MeJA at 10 mM, leading to toxic levels of MeJA in the plant cells. The toxicity of MeJA in the epidermal cells causes cell metabolism arrest, which represses metabolic activities associated with essential oil production (Misra *et al.* 2014). MeJA concentrations as low as 0.1 mM results in a lower essential oil yield when applied after a shorter period of 48 hours compared to the prolonged 72 hours on anise hyssop (*Agastache foeniculum* Nutt.) (Fard *et al.* 2012). It can therefore be concluded that neither ABA nor MeJA does not help in the recovery of essential oil yield variables following simulated hail damage.

**4.4.2 Leaf trichomes**

The recovery response mechanism of plants to hail damage, defoliation, wounding or grafting is complex, starting from upregulation of plant-stress hormones at the wound site, and later plant growth regulators to recover the lost organs (Ikeuchi *et al.*, 2017; Nanda and Melnyk, 2018). Therefore, the recovery of leaves following hail damage stress or related climate change affect the essential oil biosynthesis through the specialised structures called glandular trichomes, located on both surfaces of the leaf, and on tender stems and buds (Wang *et al.*, 2018). Moreover, Oosthuizen and Coetzee (1984) claimed that the densification of trichomes occurs as early as during leaf differentiation and continues throughout leaf development of *Pelargonium scabrum*. Since simulated hail damage was 100% in this study, results on the leaf trichome densities are ascribed to leaf ontogeny, because endogenous accumulation of the elicitors could have resulted in toxicity, thereby inhibiting leaf growth, and directly affecting plant leaf morphological growth.

**4.4.3 Essential oil quality**

Jasmonates are directly involved in the mevalonic acid pathway, through the enzyme mevalonate-5-diphosphate carboxylase, which directly affects the biosynthesis of linalool (Bruno *et al.* 2019). The decreased linalool levels measured in this study could be attributed to excess endogenous MeJA, which could have accumulated following simulated hail damage. In other studies, Rodriguez-Saona *et al.* (2002) and Van Schie *et al.* (2007) demonstrated that applications over 18 µM (MeJA) may significantly affect the accumulation of linalool content.

The high content of isomenthone during the 2016/17 season may be ascribed to leaf ontogeny. Biosynthesis of isomenthone occurs late in leaf development, when mature oil gland cells are in the post-secretory phase (Davis *et al.* 2005). At this late stage of leaf development, ABA is abundant in the epidermal cells, where it is involved in the abscission process (Wilmowicz *et al.* 2016). In this study, the daily application of ABA could have led to an excess of endogenous ABA content, causing toxicity affecting the biosynthesis of isomenthone. Prins *et al.* (2010) stated that the chemical composition of essential oil plants could be influenced by exogenous applications of ABA and MeJA.

All treatments, including the control, optimised the citronellol content to an acceptable range of between 18% and 26%, as required by the ISO standard (Table 4.8).

In plants, geraniol and ABA biosynthesis share a similar pathway (Olds *et al.* 2018): the ABA biosynthetic pathway starts from oxidative cleavage of the epoxy-carotenoids and 9-*cis* violaxanthin, where xanthoxin is converted to abscisic acid. The process is then followed by sequential production of farnesyl pyrophosphate, phytoene, carotene, lycopene, and geranyl pyrophosphate (Olds *et al.* 2018). ABA and geraniol biosynthesis occur at this stage through the isopentenyl diphosphate source (Burlat *et al.* 2004). Croteau and Purkett (1989) reported that geranyl pyrophosphate synthase activity is localised in the leaf epidermal glands of sage (*Salvia officinalis* L.), where monoterpenes are biosynthesised, suggesting that geranyl pyrophosphate synthase supplies the $C_{10}$ precursor for the production of monoterpenes. Although the second season was not significant, the repeated application of ABA could have reached a toxic level in the epidermal cells, causing disruptions of geraniol biosynthesis through the cytosolic mevalonate pathway (Burlat *et al.* 2004).

The physical and chemical similarities of citronellol and geraniol compounds make them inseparable (Weng *et al.* 2015). Moreover, biosynthesis pathways of these compounds are similar, which involves the conversion of mevalonic acid into isopentenyl pyrophosphate, for which the ABA is directly engaged through geranyl pyrophosphate (Burlat *et al.* 2004). Therefore, variations between citronellol and geraniol contents observed in this study could be related to exogenously applied ABA, increasing the C:G ratio, but reducing the essential oil quality standard.

Geraniol usually undergoes biotransformation into other terpenoids in aromatic plants, which influences the quality of the essential oil (Demyttenaere *et al.* 2000). Geranyl formate, geranyl butyrate, geranyl tiglate, and geranyl acetate are some of the acyclic monoterpenes derived from geraniol, which are regarded as geraniol esters (Liu *et al.* 2016). Results in this study may be ascribed to the prolonged application of a low concentration of MeJA, which could increase the content of geranyl tiglate. According to Zhang *et al.* (2019), jasmonates are upregulated by wounding stress and are directly involved in the biosynthesis of these terpenes. Therefore, the accumulation of geranyl tiglate in this study could be attributed to simulated hail damage, followed by the subsequent daily application of MeJA. To date, there are no reports on the effect of foliar applications of MeJA on the accumulation of geranyl tiglate.

Similar results were found for geraniol and its ester, geranyl formate, where both compounds decreased with exogenous applications of ABA. The accumulation of geranyl formate is attributed to the biosynthesis of geraniol, and the effects of subsequent daily use of MeJA (Liu *et al.* 2016). To date, there are no reports on the effect of foliar applications of MeJA on the accumulation of geraniol and its esters. Although the geraniol esters in this study were positively affected, the C:G ratio did not change.

As illustrated in Tables 4.7, 4.8, 4.9, and 4.10, the results for most of the essential oil compounds were inconsistent between the two seasons. A multivariate analysis was applied in conjunction with ANOVA to interpret the results between the seasons. The results from the PCA corroborated the observations from the ANOVA results, clearly indicating that the application of ABA and MeJA are not effective in improving the quality of essential oil in rose geranium plants, following severe hail damage.

## 4.5 Conclusion

The multivariate analysis provided conclusive results between the seasons. The use of PGRs: ABA and MeJA, at lower concentrations (75 µM & 10 mM) affected the biosynthesis of rose geranium essential oil; however, it did not improve the essential oil quality. In addition, the application of these PGRs at any level has an adverse effect on the recovery of the essential oil yield and related attributes. Therefore, from this study it can be concluded that any exogenous application of ABA and MeJA will inhibit the recovery of hail-damaged rose geranium, and negatively affect the essential oil yield and quality.

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**Table 4.1**: Mineral water analysis for the Central University of Technology, Free State experimental site during the 2016/17 and 2017/18 growing seasons (Testit-labs, RSA).

| **Minerals, pH, conductivity and alkalinity** | **Quantity** | |
|---|---|---|
| | **2016/17** | **2017/18** |
| Ammonium (mg/L) | 1.6 | 1.6 |
| Calcium (mg/L) | 10 | 16.8 |
| Nitrate (mg/L) | <1 | <1 |
| Phosphate (mg/L) | 0.05 | 0.1 |
| Potassium (mg/L) | 1.8 | 2.3 |
| Sulphate (mg/L) | 5.7 | 8.6 |
| Magnesium (mg/L) | 0.5 | 3.2 |
| Copper (µg/L) | <500 | <500 |
| Manganese (µg/L) | <200 | <200 |
| Zinc (µg/L) | 0.04 | 0.07 |
| Molybdenum (µg/L) | <20 | <20 |
| Boron (µg/L) | <40 | <40 |
| Chloride (mg/L) | 11.5 | 9.4 |
| Sodium (mg/L) | 2.2 | 6 |
| pH (25°C) | 7.37 | 7.9 |
| Conductivity (25°C) (mS/m) | 14.7 | 15.7 |
| Alkalinity ($CaCO_3$) (mg/L) | 15 | 19.2 |

**Table 4.2**: Macronutrient composition used to fertigate hydroponically-grown rose geranium at the Central University of Technology, Free State experimental site (Combrink 2005; Sedibe and Allemann 2012).

| Ions | | Concentrations |
|---|---|---|
| **Macronutrients (meq/L)** | | |
| | $NH_4$ | 0.91 |
| | K | 5.35 |
| | Ca | 5.85 |
| | Mg | 2.36 |
| | $NO_3$ | 9.18 |
| | $H_2PO$ | 2.09 |
| | $SO_4$ | 3.20 |
| **Micronutrients (mg/L)** | | |
| | Fe (EDTA) | 1.12 |
| | Mn | 0.54 |
| | Zn | 0.18 |
| | B | 0.03 |
| | Cu | 0.02 |
| | Mo | 0.05 |

**Table 4.3:** The effects of abscisic acid (ABA) and methyl jasmonate (MeJA) concentrations (75 µM ABA, 150 µM ABA, 10 mM MeJA, 20 mM MeJA, and a control) and application period (daily application for a duration of 7 and 14 days, respectively) on selected plant parameters of simulated hail-damaged rose geranium during the 2016/17 growing season (Central University of Technology, Free State).

| Treatment | Leaf area ($cm^2$/plant) | № of branches (per plant) | Plant height (cm/plant) | Plant stand loss | FFM (g/plant) | Oil mass (g/plant) | Oil content (%) |
|---|---|---|---|---|---|---|---|
| **PGRs** | | | | | | | |
| Control (Distilled water) | 53.77 ± 8.50[a] | 40.37 ± 6.44[a] | 66.65 ± 6.59[a] | 4.83 ± 0.71[a] | 252.22 ± 113.50[a] | 0.29 ± 0.22[a] | 0.12 ± 0.11[a] |
| ABA (75 µM) | 49.68 ± 22.90[a] | 37.24 ± 10.33[a] | 65.88 ± 9.90[a] | 4.16 ± 1.65[a] | 220.83 ± 147.26[a] | 0.16 ± 0.08[a] | 0.09 ± 0.05[a] |
| ABA (150 µM) | 54.20 ± 16.48[a] | 40.59 ± 5.01[a] | 68.03 ± 2.64[a] | 4.66 ± 1.33[a] | 280.55 ± 89.67[a] | 0.24 ± 0.13[a] | 0.09 ± 0.04[a] |
| MeJA (10 mM) | 41.75 ± 14.65[a] | 32.18 ± 12.84[a] | 60.21 ± 7.71[a] | 4.16 ± 1.40[a] | 162.77 ± 71.31[a] | 0.17 ± 0.22[a] | 0.11 ± 0.15[a] |
| MeJA (20 mM) | 36.29 ± 18.64[a] | 29.95 ± 11.65[a] | 63.20 ± 4.70[a] | 3.00 ± 1.52[a] | 168.16 ± 88.11[a] | 0.13 ± 0.09[a] | 0.07 ± 0.03[a] |
| *F-value* | 1.86 | 1.73 | 0.57 | 1.32 | 1.98 | 1.08 | 0.21 |
| *P-value* | 0.16[ns] | 0.18[ns] | 0.69[ns] | 0.30[ns] | 0.14[ns] | 0.40[ns] | 0.92[ns] |
| **Appl. period** | | | | | | | |
| Seven days | 45.18 ± 17.12[a] | 34.89 ± 11.05[a] | 63.80 ± 12.51[a] | 4.06 ± 1.30[a] | 209.00 ± 98.62[a] | 0.20 ± 0.16[a] | 0.10 ± 0.08[a] |
| Fourteen days | 50.01 ± 17.31[a] | 37.77 ± 8.70[a] | 65.97 ± 5.54[a] | 4.35 ± 1.43[a] | 228.86 ± 121.45[a] | 0.21 ± 0.16[a] | 0.09 ± 0.09[a] |
| *F-value* | 0.68 | 0.58 | 0.42 | 0.20 | 0.21 | 0.01 | 0.06 |
| *P-value* | 0.42[ns] | 0.45[ns] | 0.52[ns] | 0.66[ns] | 0.64[ns] | 0.99[ns] | 0.80[ns] |
| **PGRs x App** | | | | | | | |
| Distilled water x 14 days | 56.87 ± 9.85[a] | 39.70 ± 5.03[a] | 63.73 ± 7.56[a] | 5.00 ± 1.00[a] | 218.61 ± 139.78[a] | 0.12 ± 0.06[a] | 0.05 ± 0.01[a] |
| ABA (75 µM) x 7 days | 41.73 ± 32.52[a,b,c] | 35.05 ± 10.49[a] | 59.94 ± 10.58[a] | 3.66 ± 2.08[a] | 202.78 ± 163.79[a] | 0.17 ± 0.03[a] | 0.12 ± 0.07[a] |
| ABA (75 µM) x 14 days | 57.63 ± 8.03[a] | 39.43 ± 11.92[a] | 71.71 ± 5.26[a] | 4.66 ± 1.52[a] | 238.89 ± 162.51[a] | 0.16 ± 0.12[a] | 0.07 ± 0.03[a] |
| ABA (150 µM) x 7 days | 54.14 ± 17.12[a,b] | 38.71 ± 5.93 [a] | 68.63 ± 2.21[a] | 4.66 ± 1.52[a] | 225.55 ± 62.03[a] | 0.17 ± 0.03[a] | 0.08 ± 0.01[a] |
| ABA (150 µM) x 14 days | 54.25 ± 19.65[a,b] | 42.47 ± 4.13[a] | 67.43 ± 3.38[a] | 4.66 ± 1.54[a] | 335.55 ± 84.74[a] | 0.32 ± 0.14[a] | 0.10 ± 0.06[a] |
| MeJA (10 mM) x 7 days | 31.14 ± 12.05[b,c] | 32.15 ± 18.63[a] | 59.62 ± 27.94[a] | 3.66 ± 1.52[a] | 161.67 ± 105.63[a] | 0.07 ± 0.07[a] | 0.04 ± 0.07[a] |
| MeJA (10 mM) x 14 days | 52.37 ± 7.33[a,b] | 32.21 ± 8.08[a] | 60.81 ± 1.55[a] | 4.66 ± 1.52[a] | 163.89 ± 39.38[a] | 0.24 ± 0.29[a] | 0.15 ± 0.19[a] |
| MeJA (20 mM) x 7 days | 48.21 ± 2.54[a,b] | 27.48 ± 10.62[a] | 61.23 ± 5.32[a] | 3.66 ± 1.52[a] | 169.17 ± 43.75[a] | 0.09 ± 0.03[a] | 0.05 ± 0.03[a] |
| MeJA (20 mM) x 14 days | 18.41 ± 17.63[c] | 33.66 ± 16.49[a] | 66.16 ± 1.64[a] | 2.00 ± 1.41[a] | 166.67 ± 164.98[a] | 0.27 ± 0.24[a] | 0.10 ± 0.01[a] |
| *F-value* | 2.41 | 0.19 | 0.73 | 0.74 | 0.80 | 3.12 | 1.41 |
| *P-value* | ***0.01** | 0.94[ns] | 0.58[ns] | 0.57[ns] | 0.54[ns] | 0.06[ns] | 0.27[ns] |

Means followed by the same letter in the same column are statistically non-significant (Tukey; $P$<0.05); № of branches = Number of branches; FFM = Foliar fresh mass; Plant-stress hor. = Plant growth regulators; Appl. period = Application period

**Table 4.4:** The effects of abscisic acid (ABA) and methyl jasmonate (MeJA) concentrations (75 µM ABA, 150 µM ABA, 10 mM MeJA, 20 mM MeJA, and a control) and application period (daily application for a duration of 7 and 14 days, respectively) on selected plant parameters of simulated hail-damaged rose geranium during the 2017/18 growing season (Central University of Technology, Free State).

| Treatment | Leaf area ($cm^2$/plant) | № of branches (per plant) | Plant height (cm/plant) | Plant stand loss | FFM (g/plant) | Oil mass (g/plant) | Oil content (%) |
|---|---|---|---|---|---|---|---|
| **PGRs** | | | | | | | |
| Control (Distilled water) | 33.62 ± 13.25[a] | 20.36 ± 5.85[a] | 49.60 ± 11.88[a] | 4.45 ± 1.04[a] | 363.19 ± 54.43[a] | 0.22 ± 0.13[a] | 0.06 ± 0.03[a] |
| ABA (75 µM) | 43.34 ± 8.22[a] | 18.68 ± 6.05[a] | 58.08 ± 9.52[a] | 5.00 ± 0.89[a] | 373.47 ± 37.05[a] | 0.25 ± 0.07[a] | 0.07 ± 0.02[a] |
| ABA (150 µM) | 37.61 ± 6.73[a] | 23.57 ± 6.14[a] | 46.20 ± 11.95[a] | 4.50 ± 1.04[a] | 309.72 ± 147.00[a] | 0.19 ± 0.10[a] | 0.09 ± 0.09[a] |
| MeJA (10 mM) | 39.36 ± 10.78[a] | 20.03 ± 8.85[a] | 53.88 ± 12.94[a] | 4.16 ± 1.83[a] | 378.05 ± 104.38[a] | 0.22 ± 0.16[a] | 0.05 ± 0.03[a] |
| MeJA (20 mM) | 37.01 ± 8.02[a] | 23.00 ± 2.64[a] | 52.62 ± 13.31[a] | 4.00 ± 1.67[a] | 438.88 ± 70.25[a] | 0.27 ± 0.10[a] | 0.06 ± 0.02[a] |
| *F-value* | 1.82 | 1.20 | 1.97 | 0.81 | 1.85 | 1.02 | 0.87 |
| *P-value* | 0.16[ns] | 0.34[ns] | 0.14[ns] | 0.53[ns] | 0.16[ns] | 0.42[ns] | 0.90[ns] |
| **Appl. period** | | | | | | | |
| Seven days | 36.73 ± 9.81[a] | 21.53 ± 6.26[a] | 50.40 ± 8.25[a] | 4.40 ± 1.24[a] | 397.77 ± 62.61[a] | 0.24 ± 0.12[a] | 0.06 ± 0.03[a] |
| Fourteen days | 39.65 ± 9.43[a] | 20.72 ± 6.09[a] | 53.75 ± 11.95[a] | 4.46 ± 1.40[a] | 347.55 ± 115.22[a] | 0.22 ± 0.11[a] | 0.07 ± 0.06[a] |
| *F-value* | 1.55 | 0.23 | 1.38 | 0.03 | 2.76 | 0.22 | 1.36 |
| *P-value* | 0.22[ns] | 0.63[ns] | 0.25[ns] | 0.86[ns] | 0.15[ns] | 0.64[ns] | 0.40[ns] |
| **PGRs x App** | | | | | | | |
| Distilled water x 14 days | 37.35 ± 12.94[a] | 24.27 ± 3.71[a] | 47.61 ± 12.69[a] | 4.66 ± 1.15[a] | 410.00 ± 79.37[a] | 0.33 ± 0.04[a,b] | 0.09 ± 0.02[a] |
| ABA (75 µM) x 7 days | 38.85 ± 8.91[a] | 19.66 ± 8.69[a] | 55.45 ± 13.52[a] | 4.66 ± 1.15[a] | 346.11 ± 133.71[a] | 0.26 ± 0.08[a,b] | 0.07 ± 0.02[a] |
| ABA (75 µM) x 14 days | 47.83 ± 5.81[a] | 17.71 ± 3.63[a] | 60.71 ± 4.89[a] | 5.33 ± 0.57[a] | 458.33 ± 84.57[a] | 0.25 ± 0.08[a,b] | 0.07 ± 0.03[a] |
| ABA (150 µM) x 7 days | 40.07 ± 4.07[a] | 26.81 ± 6.46[a] | 46.33 ± 11.93[a] | 4.00 ± 1.00[a] | 419.44 ± 63.64[a] | 0.15 ± 0.07[c,d] | 0.04 ± 0.02[a] |
| ABA (150 µM) x 14 days | 35.15 ± 8.47[a] | 20.33 ± 4.60[a] | 46.07 ± 14.66[a] | 5.00 ± 1.00[a] | 383.05 ± 67.75[a] | 0.22 ± 0.13[b,c] | 0.15 ± 0.10[a] |
| MeJA (10 mM) x 7 days | 36.59 ± 14.20[a] | 20.90 ± 5.13[a] | 50.01 ± 8.87[a] | 4.33 ± 1.15[a] | 368.05 ± 66.36[a] | 0.37 ± 0.04[a] | 0.09 ± 0.01[a] |
| MeJA (10 mM) x 14 days | 42.14 ± 8.11[a] | 19.17 ± 12.93[a] | 57.69 ± 17.21[a] | 4.00 ± 2.64[a] | 378.89 ± 57.89[a] | 0.07 ± 0.02[d] | 0.02 ± 0.01[a] |
| MeJA (20 mM) x 7 days | 38.23 ± 6.53[a] | 23.87 ± 3.14[a] | 48.55 ± 3.45[a] | 4.66 ± 2.30[a] | 409.16 ± 25.04[a] | 0.30 ± 0.15[a,b] | 0.07 ± 0.03[a] |
| MeJA (20 mM) x 14 days | 35.79 ± 10.63[a] | 22.13 ± 2.29[a] | 56.69 ± 7.01[a] | 3.33 ± 0.57[a] | 210.27 ± 154.05[a] | 0.24 ± 0.06[a,b] | 0.06 ± 0.01[a] |
| *F-value* | 1.39 | 1.91 | 0.68 | 1.18 | 1.87 | 4.55 | 3.53 |
| *P-value* | 0.27[ns] | 0.15[ns] | 0.61[ns] | 0.35[ns] | 0.15[ns] | ***0.01** | 0.45[ns] |

Means followed by the same letter in the same column are statistically non-significant (Tukey; $P<0.05$); № of branches = Number of branches; FFM = Foliar fresh mass; PGRs = Plant growth regulators; Appl. period = Application period

**Table 4.5:** Principal component analyses for the selected plant parameters of simulated hail-damaged rose geranium (Central University of Technology, Free State).

| Traits | Principal component 1 | Principal component 2 |
|---|---|---|
| Eigenvalue | 4.53 | 2.38 |
| Variability % | 37.79 | 19.89 |
| Cumulative % | 37.79 | 57.68 |
| **Factor loading** | | |
| LA2017 | **0.59** | 0.01 |
| NoBrc2017 | **0.71** | 0.02 |
| PH2017 | 0.38 | 0.02 |
| Plt St2017 | **0.56** | 0.03 |
| FFMg2017 | **0.80** | 0.06 |
| Oil mass2017 | 0.19 | 0.07 |
| LA2018 | 0.10 | 0.45 |
| NoBrc2018 | 0.05 | **0.55** |
| PH2018 | 0.01 | **0.93** |
| Plt St2018 | 0.41 | 0.00 |
| FFMg2018 | **0.59** | 0.00 |
| Oil mass2018 | 0.09 | 0.20 |

Values in bold correspond for each variable to the factor for which the squared cosine is the largest. **Abbreviations:** LA = Leaf area; No Brns = Number of branches; PH = Plant height; Plt = Plant stand loss; FFMg = Foliar fresh mass; Oilmass = Essential oil mass

**Table 4.6:** The effects of abscisic acid (ABA) and methyl jasmonate (MeJA) concentrations (75 µM ABA, 150 µM ABA, 10 mM MeJA, 20 mM MeJA, and a control) and application period (daily application for a duration of 7 and 14 days, respectively) on trichome density of simulated hail-damaged rose geranium during the 2017/18 growing season (Central University of Technology, Free State).

| | **Abaxial leaf surface** | | | | **Adaxial leaf surface** | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Non-Glandular** | **Elongated Capitate** | **Asciiform** | **Brevicollate** | **Non-Glandular** | **Elongated Capitate** | **Asciiform** | **Brevicollate** |
| **PGRs** | | | | | | | | |
| Control (Distilled water) | 40.00 ± 5.00$^{a}$ | 0.20 ± 0.01$^{a}$ | 70.66 ± 5.50$^{a}$ | 42.33 ± 13.05$^{a}$ | 66.33 ± 22.81$^{a}$ | 1.00 ± 1.00$^{a}$ | 110.00 ± 46.03$^{a}$ | 75.33 ± 16.65$^{a}$ |
| ABA (75 µM) | 66.00 ± 62.41$^{a}$ | 0.33 ± 0.81$^{a}$ | 92.83 ± 19.91$^{a}$ | 41.33 ± 6.62$^{a}$ | 86.33 ± 39.33$^{a}$ | 1.66 ± 2.25$^{a}$ | 83.66 ± 46.40$^{a}$ | 62.83 ± 26.36$^{a}$ |
| ABA (150 µM) | 44.66 ± 19.28$^{a}$ | 0.16 ± 0.40$^{a}$ | 86.33 ± 13.95$^{a}$ | 50.33 ± 11.09$^{a}$ | 77.83 ± 19.00$^{a}$ | 1.50 ± 1.22$^{a}$ | 97.33 ± 24.93$^{a}$ | 64.50 ± 9.83$^{a}$ |
| MeJA (10 mM) | 39.33 ± 15.05$^{a}$ | 0.50 ± 0.54$^{a}$ | 67.66 ± 21.48$^{a}$ | 34.16 ± 10.55$^{a}$ | 66.16 ± 15.76$^{a}$ | 1.33 ± 1.96$^{a}$ | 82.00 ± 34.62$^{a}$ | 95.33 ± 36.65$^{a}$ |
| MeJA (20 mM) | 50.33 ± 18.93$^{a}$ | 0.93 ± 0.50$^{a}$ | 93.83 ± 18.49$^{a}$ | 44.33 ± 17.87$^{a}$ | 86.83 ± 25.23$^{a}$ | 0.83 ± 1.16$^{a}$ | 97.00 ± 33.89$^{a}$ | 67.83 ± 25.42$^{a}$ |
| *F-value* | 0.58 | 0.42 | 2.00 | 1.17 | 0.71 | 0.24 | 0.37 | 1.32 |
| *P-value* | 0.68$^{ns}$ | 0.79$^{ns}$ | 0.14$^{ns}$ | 0.35$^{ns}$ | 0.59$^{ns}$ | 0.91$^{ns}$ | 0.82$^{ns}$ | 0.30$^{ns}$ |
| **Appl. period** | | | | | | | | |
| Seven days | 57.00 ± 44.84$^{a}$ | 0.25 ± 0.62$^{a}$ | 85.33 ± 21.81$^{a}$ | 41.33 ± 12.58$^{a}$ | 72.66 ± 19.80$^{a}$ | 1.66 ± 1.87$^{a}$ | 93.16 ± 38.03$^{a}$ | 74.83 ± 30.78$^{a}$ |
| Fourteen days | 42.53 ± 15.72$^{a}$ | 0.40 ± 0.63$^{a}$ | 82.13 ± 18.78$^{a}$ | 43.46 ± 13.01$^{a}$ | 82.00 ± 29.58$^{a}$ | 1.00 ± 1.25$^{a}$ | 91.46 ± 34.05$^{a}$ | 71.40 ± 24.18$^{a}$ |
| *F-value* | 1.00 | 0.75 | 0.01 | 0.20 | 1.41 | 0.97 | 0.15 | 0.15 |
| *P-value* | 0.63$^{ns}$ | 0.39$^{ns}$ | 0.96$^{ns}$ | 0.65$^{ns}$ | 0.25$^{ns}$ | 0.33$^{ns}$ | 0.69$^{ns}$ | 0.70$^{ns}$ |
| **PGRs x App** | | | | | | | | |
| Distilled water x 14 days | 40.00 ± 5.00$^{a}$ | 0.33 ± 0.81$^{a}$ | 70.66 ± 5.50$^{a}$ | 42.33 ± 13.05$^{a}$ | 66.33 ± 22.81$^{a}$ | 1.00 ± 1.00$^{a}$ | 110.00 ± 46.03$^{a}$ | 75.33 ± 16.65$^{a}$ |
| ABA (75 µM) x 7 days | 85.66 ± 19.22$^{a}$ | 0.50 ± 0.54$^{a}$ | 92.00 ± 29.46 | 43.33 ± 7.50$^{a}$ | 65.66 ± 16.80$^{a}$ | 2.66 ± 3.05$^{a}$ | 97.33 ± 69.13$^{a}$ | 67.66 ± 39.80$^{a}$ |
| ABA (75 µM) x 14 days | 46.33 ± 16.04$^{a}$ | 0.16 ± 0.40$^{a}$ | 93.66 ± 11.01$^{a}$ | 39.33 ± 6.42$^{a}$ | 70.00 ± 15.40$^{a}$ | 0.66 ± 0.57$^{a}$ | 70.00 ± 6.55$^{a}$ | 58.00 ± 9.16$^{a}$ |
| ABA (150 µM) x 7 days | 56.00 ± 19.69$^{a}$ | 0.66 ± 1.15$^{a}$ | 91.33 ± 18.14$^{a}$ | 52.66 ± 12.01$^{a}$ | 78.66 ± 23.96$^{a}$ | 2.00 ± 1.73$^{a}$ | 97.66 ± 23.79$^{a}$ | 63.00 ± 12.28$^{a}$ |
| ABA (150 µM) x 14 days | 33.33 ± 12.50$^{a}$ | 0.33 ± 0.57$^{a}$ | 81.33 ± 9.07$^{a}$ | 48.00 ± 12.12$^{a}$ | 77.00 ± 18.08$^{a}$ | 1.00 ± 0.00$^{a}$ | 97.00 ± 31.43$^{a}$ | 66.00 ± 9.16$^{a}$ |
| MeJA (10 mM) x 7 days | 41.66 ± 17.95$^{a}$ | 0.33 ± 0.57$^{a}$ | 70.66 ± 22.50$^{a}$ | 30.66 ± 0.57$^{a}$ | 57.66 ± 11.50$^{a}$ | 0.66 ± 1.15$^{a}$ | 88.66 ± 18.00$^{a}$ | 92.66 ± 43.14$^{a}$ |
| MeJA (10 mM) x 14 days | 37.00 ± 15.09$^{a}$ | 0.66 ± 0.57$^{a}$ | 64.66 ± 24.90$^{a}$ | 37.66 ± 15.53$^{a}$ | 74.66 ± 16.50$^{a}$ | 2.00 ± 2.64$^{a}$ | 75.33 ± 50.40$^{a}$ | 98.00 ± 38.43$^{a}$ |
| MeJA (20 mM) x 7 days | 44.66 ± 14.57$^{a}$ | 0.66 ± 1.15$^{a}$ | 87.33 ± 21.54$^{a}$ | 38.66 ± 17.03$^{a}$ | 88.66 ± 23.54$^{a}$ | 1.33 ± 1.52$^{a}$ | 89.00 ± 46.60$^{a}$ | 76.00 ± 29.13$^{a}$ |
| MeJA (20 mM) x 14 days | 56.00 ± 24.24$^{a}$ | 0.33 ± 0.57$^{a}$ | 100.33 ± 16.25$^{a}$ | 50.00 ± 20.29$^{a}$ | 85.00 ± 32.04$^{a}$ | 0.33 ± 0.57$^{a}$ | 105.00 ± 22.53$^{a}$ | 59.66 ± 23.86$^{a}$ |
| *F-value* | 0.63 | 0.53 | 0.38 | 0.55 | 0.88 | 1.09 | 0.33 | 0.20 |
| *P-value* | 0.60$^{ns}$ | 0.66$^{ns}$ | 0.76$^{ns}$ | 0.65$^{ns}$ | 0.47$^{ns}$ | 0.38$^{ns}$ | 0.80$^{ns}$ | 0.89$^{ns}$ |

Means followed by the same letter in the same column are statistically non-significant (Tukey; $P<0.05$)

**Table 4.7:** The effects of abscisic acid (ABA) and methyl jasmonate (MeJA) concentrations (75 µM ABA, 150 µM ABA, 10 mM MeJA, 20 mM MeJA, and a control) and application period (daily application for a duration of 7 and 14 days, respectively) on essential oil compounds of simulated hail-damaged rose geranium during the 2016/17 growing season (Central University of Technology, Free State).

| Treatment | Citronellol | Geraniol | C:G ratio | Linalool | Isomenthone | *cis*-Rose oxide | *trans*-Rose oxide |
|---|---|---|---|---|---|---|---|
| **PGRs** | | | | | | | |
| Control (Distilled water) | 20.15 ± 1.61[a] | 10.88 ± 0.65[a] | 1.85 ± 0.04[a] | 2.00 ± 0.53[a] | 1.07 ± 0.33[b] | 0.57 ± 0.12[a] | 0.25 ± 0.06[a] |
| ABA (75 µM) | 21.19 ± 5.87[a] | 10.95 ± 1.35[a] | 1.91 ± 0.28[a] | 2.15 ± 0.38[a] | 3.10 ± 0.91[a] | 0.58 ± 0.18[a] | 0.25 ± 0.07[a] |
| ABA (150 µM) | 18.87 ± 1.73[a] | 9.75 ± 0.74[a] | 1.92 ± 0.10[a] | 2.16 ± 0.44[a] | 2.93 ± 0.45[a] | 0.57 ± 0.12[a] | 0.25 ± 0.04[a] |
| MeJA (10 mM) | 17.64 ± 2.46[a] | 9.91 ± 1.21[a] | 1.78 ± 0.17[a] | 1.58 ± 0.88[a,b] | 1.26 ± 1.61[b] | 0.40 ± 0.18[a] | 0.17 ± 0.06[a] |
| MeJA (20 mM) | 18.47 ± 1.76[a] | 10.39 ± 1.40[a] | 1.78 ± 0.15[a] | 0.97 ± 0.88[b] | 1.59 ± 0.42[b] | 0.34 ± 0.18[a] | 0.16 ± 0.07[a] |
| *F-value* | 1.02 | 1.62 | 0.94 | 4.43 | 9.42 | 2.41 | 2.47 |
| *P-value* | 0.43[ns] | 0.22[ns] | 0.47[ns] | ***0.01** | ***0.01** | 0.11[ns] | 0.10[ns] |
| **Appl. period** | | | | | | | |
| Seven days | 18.48 ± 1.44[a] | 10.06 ± 0.86[a] | 1.83 ± 0.08[a] | 1.97 ± 0.70[a] | 2.08 ± 0.83[a] | 0.49 ± 0.17[a] | 0.21 ± 0.07[a] |
| Fourteen days | 19.95 ± 4.58[a] | 10.56 ± 1.37[a] | 1.88 ± 0.24[a] | 1.67 ± 0.77[a] | 2.34 ± 1.36[a] | 0.50 ± 0.18[a] | 0.22 ± 0.06[a] |
| *F-value* | 2.54 | 1.76 | 1.50 | 2.54 | 0.37 | 0.34 | 0.40 |
| *P-value* | 0.13[ns] | 0.20[ns] | 0.24[ns] | 0.13[ns] | 0.55[ns] | 0.56[ns] | 0.53[ns] |
| **PGRs x App** | | | | | | | |
| Distilled water x 14 days | 20.15 ± 1.61[a] | 10.88 ± 0.65[a] | 1.85 ± 0.04[a] | 2.00 ± 0.53[a] | 1.07 ± 0.38[a] | 0.57 ± 0.12[a] | 0.25 ± 0.06[a] |
| ABA (75 µM) x 7 days | 18.28± 0.29[a] | 10.18 ± 0.68[a] | 1.80 ± 0.13[a] | 2.30 ± 0.53[a] | 2.35 ± 0.17[a] | 0.61 ± 0.21[a] | 0.26 ± 0.10[a] |
| ABA (75 µM) x 14 days | 24.11 ± 7.79[a] | 11.72 ± 1.53[a] | 2.02 ± 0.38[a] | 2.00 ± 0.07[a] | 3.85 ± 0.59[a] | 0.49 ± 0.10[a] | 0.24 ± 0.02[a] |
| ABA (150 µM) x 7 days | 17.63 ± 0.57[a] | 9.44 ± 0.15[a] | 1.86 ± 0.05[a] | 2.01 ± 0.52[a] | 2.94 ± 0.65[a] | 0.49 ± 0.09[a] | 0.23 ± 0.04[a] |
| ABA (150 µM) x 14 days | 20.10 ± 1.60[a] | 10.15 ± 0.99[a] | 1.98 ± 0.12[a] | 2.32 ± 0.38[a] | 2.92 ± 0.28[a] | 0.63 ± 0.10[a] | 0.28 ± 0.04[a] |
| MeJA (10 mM) x 7 days | 16.00 ± 1.76[a] | 11.03 ± 0.54[a] | 1.82 ± 0.07[a] | 2.48 ± 0.47[a] | 0.79 ± 0.06[a] | 0.56 ± 0.10[a] | 0.22 ± 0.02[a] |
| MeJA (10 mM) x 14 days | 20.11 ± 0.67[a] | 9.17 ± 0.84[a] | 1.75 ± 0.23[a] | 0.98 ± 0.33[a] | 1.57 ± 1.53[a] | 0.30 ± 0.13[a] | 0.14 ± 0.06[a] |
| MeJA (20 mM) x 7 days | 18.54 ± 2.16[a] | 9.91 ± 1.24[a] | 1.86 ± 0.06[a] | 1.26 ± 0.79[a] | 1.80 ± 0.17[a] | 0.34 ± 0.18[a] | 0.15 ± 0.06[a] |
| MeJA (20 mM) x 14 days | 18.47 ± 0.12[a] | 11.85 ± 0.13[a] | 1.56 ± 0.14[a] | 0.80 ± 0.10[a] | 0.99 ± 0.11[a] | 0.42 ± 0.10[a] | 0.21 ± 0.01[a] |
| *F-value* | 1.57 | 2.31 | 0.94 | 0.56 | 1.41 | 1.98 | 1.06 |
| *P-value* | 0.24[ns] | 0.11[ns] | 0.46[ns] | 0.58[ns] | 0.27[ns] | 0.17[ns] | 0.41[ns] |

Means followed by the same letter in the same column are statistically non-significant (Tukey; $P<0.05$); PGRs = Plant growth regulators; Appl. period = Application period

**Table 4.8:** The effects of abscisic acid (ABA) and methyl jasmonate (MeJA) concentrations (75 µM ABA, 150 µM ABA, 10 mM MeJA, 20 mM MeJA, and a control) and application period (daily application for a duration of 7 and 14 days, respectively) on essential oil compounds of simulated hail-damaged rose geranium during the 2017/18 growing season (Central University of Technology, Free State).

| Treatment | Citronellol | Geraniol | C:G ratio | Linalool | Isomenthone | *cis*-Rose oxide | *trans*-Rose oxide |
|---|---|---|---|---|---|---|---|
| **PGRs** | | | | | | | |
| Control (Distilled water) | 23.16 ± 3.35$^{a,b}$ | 8.60 ± 0.42$^{a,b}$ | 2.68 ± 0.28$^{c}$ | 1.49 ± 0.31$^{a,b}$ | 2.90 ± 1.67$^{a}$ | 0.74 ± 0.16$^{a}$ | 0.35 ± 0.09$^{a}$ |
| ABA (75 µM) | 25.66 ± 2.52$^{a}$ | 7.42 ± 0.79$^{b,c}$ | 3.48 ± 0.46$^{a}$ | 1.90 ± 0.43$^{a}$ | 3.28 ± 1.75$^{a}$ | 0.29 ± 0.38$^{a}$ | 0.38 ± 0.13$^{a}$ |
| ABA (150 µM) | 23.15 ± 1.40$^{a,b}$ | 7.33 ± 0.72$^{c}$ | 3.16 ± 0.15$^{a,b}$ | 1.69 ± 0.52$^{a}$ | 3.27 ± 1.02$^{a}$ | 0.48 ± 0.46$^{a}$ | 0.46 ± 0.22$^{a}$ |
| MeJA (10 mM) | 21.32 ± 2.22$^{b}$ | 7.99 ± 1.57$^{a,b,c}$ | 2.72 ± 0.34$^{c}$ | 1.11 ± 0.30$^{b}$ | 3.48 ± 0.83$^{a}$ | 0.27 ± 0.28$^{a}$ | 0.28 ± 0.12$^{a}$ |
| MeJA (20 mM) | 26.09 ± 2.79$^{a}$ | 8.96 ± 1.26$^{a}$ | 2.96 ± 0.55$^{b,c}$ | 1.43 ± 0.26$^{a,b}$ | 2.73 ± 0.93$^{a}$ | 0.96 ± 0.22$^{a}$ | 0.44 ± 0.09$^{a}$ |
| *F-value* | 3.43 | 3.43 | 4.01 | 3.38 | 0.42 | 1.44 | 1.22 |
| *P-value* | ***0.03** | ***0.05** | ***0.03** | ***0.03** | 0.79$^{ns}$ | 0.26$^{ns}$ | 0.33$^{ns}$ |
| **Appl. period** | | | | | | | |
| Seven days | 23.10 ± 3.08$^{a}$ | 7.88 ± 1.78$^{a}$ | 3.06 ± 0.69$^{a}$ | 1.49 ± 0.61$^{a}$ | 2.88 ± 1.05$^{a}$ | 0.42 ± 0.14$^{a}$ | 0.93 ± 0.32$^{a}$ |
| Fourteen days | 24.32 ± 3.04$^{a}$ | 7.83 ± 1.09$^{a}$ | 3.13 ± 0.44$^{a}$ | 1.49 ± 0.34$^{a}$ | 3.34 ± 1.14$^{a}$ | 0.35 ± 0.15$^{a}$ | 0.77 ± 0.31$^{a}$ |
| *F-value* | 1.90 | 0.01 | 0.38 | 0.05 | 0.85 | 1.33 | 1.01 |
| *P-value* | 0.18$^{ns}$ | 0.92$^{ns}$ | 0.54$^{ns}$ | 0.82$^{ns}$ | 0.36$^{ns}$ | 0.26$^{ns}$ | 0.32$^{ns}$ |
| **PGRs x App** | | | | | | | |
| Distilled water x 14 days | 23.16 ± 3.35$^{a}$ | 8.60 ± 0.42$^{a}$ | 2.68 ± 0.28$^{a}$ | 1.49 ± 0.31$^{a}$ | 3.66 ± 2.26$^{a}$ | 0.80 ± 0.07$^{a}$ | 0.38 ± 0.07$^{a}$ |
| ABA (75 µM) x 7 days | 24.60 ± 3.00$^{a}$ | 7.42 ± 0.29$^{a}$ | 3.33 ± 0.52$^{a}$ | 2.10 ± 0.47$^{a}$ | 3.66 ± 1.27$^{a}$ | 0.95 ± 0.41$^{a}$ | 0.41 ± 0.19$^{a}$ |
| ABA (75 µM) x 14 days | 26.73 ± 1.85$^{a}$ | 7.43 ± 1.22$^{a}$ | 3.64 ± 0.44$^{a}$ | 1.69 ± 0.33$^{a}$ | 2.91 ± 0.92$^{a}$ | 0.82 ± 0.17$^{a}$ | 0.35 ± 0.08$^{a}$ |
| ABA (150 µM) x 7 days | 22.18 ± 1.16$^{a}$ | 6.86 ± 0.52$^{a}$ | 3.23 ± 0.07$^{a}$ | 1.76 ± 0.79$^{a}$ | 3.34 ± 1.33$^{a}$ | 1.09 ± 0.44$^{a}$ | 0.49 ± 0.19$^{a}$ |
| ABA (150 µM) x 14 days | 24.13 ± 0.87$^{a}$ | 7.80 ± 0.60$^{a}$ | 3.10 ± 0.19$^{a}$ | 1.62 ± 0.21$^{a}$ | 3.19 ± 0.91$^{a}$ | 0.96 ± 0.61$^{a}$ | 0.44 ± 0.29$^{a}$ |
| MeJA (10 mM) x 7 days | 22.18 ± 1.49$^{a}$ | 9.33 ± 0.62$^{a}$ | 2.38 ± 0.01$^{a}$ | 1.25 ± 0.31$^{a}$ | 2.61 ± 0.18$^{a}$ | 0.88 ± 0.14$^{a}$ | 0.41 ± 0.07$^{a}$ |
| MeJA (10 mM) x 14 days | 20.75 ± 2.74$^{a}$ | 7.10 ± 1.33$^{a}$ | 2.94 ± 0.39$^{a}$ | 1.02 ± 0.32$^{a}$ | 4.06 ± 0.31$^{a}$ | 0.40 ± 0.41$^{a}$ | 0.19 ± 0.03$^{a}$ |
| MeJA (20 mM) x 7 days | 26.82 ± 1.62$^{a}$ | 9.69 ± 0.12$^{a}$ | 2.31 ± 0.50$^{a}$ | 1.21 ± 0.18$^{a}$ | 2.57 ± 1.17$^{a}$ | 1.02 ± 0.33$^{a}$ | 0.47 ± 0.14$^{a}$ |
| MeJA (20 mM) x 14 days | 25.36 ± 3.91$^{a}$ | 8.23 ± 1.55$^{a}$ | 3.31 ± 0.50$^{a}$ | 1.64 ± 0.04$^{a}$ | 2.90 ± 0.85$^{a}$ | 0.90 ± 0.04$^{a}$ | 0.41 ± 0.03$^{a}$ |
| *F-value* | 0.35 | 2.64 | 2.86 | 1.27 | 0.93 | 0.57 | 0.63 |
| *P-value* | 0.83$^{ns}$ | 0.07$^{ns}$ | 0.06$^{ns}$ | 0.32$^{ns}$ | 0.46$^{ns}$ | 0.68$^{ns}$ | 0.64$^{ns}$ |

Means followed by the same letter in the same column are statistically non-significant (Tukey; $P<0.05$); PGRs = Plant growth regulators; Appl. period = Application period

**Table 4.9:** The effects of abscisic acid (ABA) and methyl jasmonate (MeJA) concentrations (75 µM ABA, 150 µM ABA, 10 mM MeJA, 20 mM MeJA, and a control) and application period (daily application for a duration of 7 and 14 days, respectively) on essential oil compounds of simulated hail-damaged rose geranium during the 2016/17 growing season (Central University of Technology, Free State).

| Treatment | Citronellyl formate | Geranyl formate | Geranyl butyrate | Geranyl tiglate | Guaia-6,9-diene |
|---|---|---|---|---|---|
| **PGRs** | | | | | |
| Control (Distilled water) | 17.11 ± 1.40$^{a}$ | 11.25 ± 0.67$^{a}$ | 1.85 ± 0.04$^{a}$ | 2.03 ± 0.45$^{a}$ | 10.17 ± 0.17$^{a}$ |
| ABA (75 µM) | 19.75 ± 7.68$^{a}$ | 13.20 ± 3.93$^{a}$ | 1.91 ± 0.28$^{a}$ | 2.18 ± 0.33$^{a}$ | 9.75 ± 1.73$^{a}$ |
| ABA (150 µM) | 17.75 ± 7.68$^{a}$ | 10.93 ± 0.37$^{a}$ | 1.92 ± 0.10$^{a}$ | 2.16 ± 0.33$^{a}$ | 9.60 ± 0.52$^{a}$ |
| MeJA (10 mM) | 17.91 ± 2.67$^{a}$ | 13.00 ± 0.89$^{a}$ | 1.52 ± 0.51$^{a}$ | 2.77 ± 1.27$^{a}$ | 9.73 ± 0.82$^{a}$ |
| MeJA (20 mM) | 16.59 ± 1.10$^{a}$ | 11.86 ± 1.04$^{a}$ | 1.53 ± 0.26$^{a}$ | 2.97 ± 0.85$^{a}$ | 8.95 ± 0.52$^{a}$ |
| *F-value* | 0.54 | 1.20 | 0.33 | 1.72 | 0.31 |
| *P-value* | 0.71$^{ns}$ | 0.35$^{ns}$ | 0.85$^{ns}$ | 0.21$^{ns}$ | 0.86$^{ns}$ |
| **Appl. period** | | | | | |
| Seven days | 17.12 ± 1.24$^{a}$ | 11.54 ± 0.84$^{a}$ | 1.89 ± 0.43$^{a}$ | 2.51 ± 0.67$^{a}$ | 9.53 ± 0.41$^{a}$ |
| Fourteen days | 18.60 ± 5.54$^{a}$ | 12.73 ± 2.87$^{a}$ | 1.74 ± 0.37$^{a}$ | 2.37 ± 0.89$^{a}$ | 9.68 ± 1.37$^{a}$ |
| *F-value* | 0.94 | 1.91 | 0.48 | 0.89 | 0.16 |
| *P-value* | 0.35$^{ns}$ | 0.19$^{ns}$ | 0.50$^{ns}$ | 0.36$^{ns}$ | 0.69$^{ns}$ |
| **PGRs x App** | | | | | |
| Distilled water x 14 days | 17.11 ± 1.40$^{a}$ | 11.25 ± 0.67$^{a}$ | 1.25 ± 0.15$^{a}$ | 2.03 ± 0.45$^{b,c}$ | 10.17 ± 0.17$^{a}$ |
| ABA (75 µM) x 7 days | 16.94 ± 1.12$^{a}$ | 11.58 ± 0.64$^{a}$ | 1.49 ± 0.14$^{a}$ | 2.35 ± 0.34$^{a,b,c}$ | 9.47 ± 0.03$^{a}$ |
| ABA (75 µM) x 14 days | 22.56 ± 11.08$^{a}$ | 14.81 ± 5.52$^{a}$ | 1.26 ± 0.12$^{a}$ | 1.94 ± 0.12$^{c}$ | 10.04 ± 2.69$^{a}$ |
| ABA (150 µM) x 7 days | 16.41 ± 0.38$^{a}$ | 10.81 ± 0.38$^{a}$ | 1.51 ± 0.14$^{a}$ | 2.42 ± 0.27$^{a,b,c}$ | 9.88 ± 0.17$^{a}$ |
| ABA (150 µM) x 14 days | 17.90 ± 0.76$^{a}$ | 11.04 ± 0.39$^{a}$ | 1.29 ± 0.08$^{a}$ | 1.91 ± 0.14$^{c}$ | 9.33 ± 0.65$^{a}$ |
| MeJA (10 mM) x 7 days | 19.96 ± 0.86$^{a}$ | 12.21 ± 0.44$^{a}$ | 1.16 ± 0.12$^{a}$ | 1.74 ± 0.17$^{c}$ | 9.74 ± 0.01$^{a}$ |
| MeJA (10 mM) x 14 days | 17.22 ± 3.48$^{a}$ | 13.53 ± 0.68$^{a}$ | 1.77 ± 0.54$^{a}$ | 3.46 ± 1.20$^{a}$ | 9.72 ± 1.16$^{a}$ |
| MeJA (20 mM) x 7 days | 16.78 ± 1.27$^{a}$ | 11.79 ± 1.26$^{a}$ | 1.56 ± 0.31$^{a}$ | 3.27 ± 0.75$^{a,b}$ | 9.09 ± 0.55$^{a}$ |
| MeJA (20 mM) x 14 days | 16.02 ± 0.12$^{a}$ | 12.08 ± 0.14$^{a}$ | 1.43 ± 0.10$^{a}$ | 2.08 ± 0.11$^{b,c}$ | 8.54 ± 0.10$^{a}$ |
| *F-value* | 0.47 | 0.53 | 1.96 | 4.14 | 0.46 |
| *P-value* | 0.75$^{ns}$ | 0.71$^{ns}$ | 0.15$^{ns}$ | ***0.03** | 0.76$^{ns}$ |

Means followed by the same letter in the same column are statistically non-significant (Tukey; *P*<0.05)

**Table 4.10:** The effects of abscisic acid (ABA) and methyl jasmonate (MeJA) concentrations (75 µM ABA, 150 µM ABA, 10 mM MeJA, 20 mM MeJA, and a control) and application period (daily application for a duration of 7 and 14 days, respectively) on essential oil compounds of simulated hail-damaged rose geranium during the 2017/18 growing season (Central University of Technology, Free State).

| Treatment | Citronellyl formate | Geranyl formate | Geranyl butyrate | Geranyl tiglate | Guaia-6,9-diene |
|---|---|---|---|---|---|
| **PGRs** | | | | | |
| Control (Distilled water) | 16.27 ± 1.29[a] | 7.32 ± 1.23[a] | 1.15 ± 0.05[b] | 1.83 ± 0.07[a,b] | 8.28 ± 1.14[a] |
| ABA (75 µM) | 17.37 ± 1.62[a] | 6.06 ± 0.28[b] | 1.09 ± 0.15[b] | 1.61 ± 0.32[b] | 9.05 ± 1.14[a] |
| ABA (150 µM) | 16.03 ± 0.95[a] | 6.21 ± 0.29[b] | 1.28 ± 0.12[a,b] | 1.98 ± 0.35[a,b] | 8.57 ± 0.97[a] |
| MeJA (10 mM) | 16.21 ± 1.31[a] | 7.27 ± 0.37[a] | 1.42 ± 0.25[a] | 2.27 ± 0.42[a] | 8.05 ± 1.66[a] |
| MeJA (20 mM) | 16.90 ± 1.06[a] | 6.52 ± 0.92[a,b] | 1.16 ± 0.07[b] | 1.69 ± 0.11[b] | 7.95 ± 0.75[a] |
| *F-value* | 1.00 | 3.96 | 4.24 | 3.72 | 0.78 |
| *P-value* | 0.43[ns] | ***0.01** | ***0.01** | ***0.02** | 0.55[ns] |
| **Appl. period** | | | | | |
| Seven days | 16.36 ± 1.30[a] | 6.41 ± 0.69[a] | 1.23 ± 0.15[a] | 1.87 ± 0.35[a] | 8.18 ± 0.99[a] |
| Fourteen days | 16.76 ± 1.27[a] | 6.72 ± 0.80[a] | 1.23 ± 0.20[a] | 1.90 ± 0.37[a] | 8.58 ± 1.26[a] |
| *F-value* | 0.70 | 1.09 | 0.03 | 0.01 | 0.90 |
| *P-value* | 0.41[ns] | 0.31[ns] | 0.85[ns] | 0.97[ns] | 0.35[ns] |
| **PGRs x App** | | | | | |
| Distilled water x 14 days | 15.95 ± 1.48[a] | 7.32 ± 1.29[a] | 1.15 ± 0.05[a] | 1.83 ± 0.07[a] | 8.63 ± 0.46[a] |
| ABA (75 µM) x 7 days | 17.28 ± 1.97[a] | 6.11 ± 0.38[a] | 1.07 ± 0.20[a] | 1.49 ± 0.34[a] | 8.26 ± 1.13[a] |
| ABA (75 µM) x 14 days | 17.46 ± 1.62[a] | 6.00 ± 0.21[a] | 1.11 ± 0.12[a] | 1.73 ± 0.32[a] | 9.84 ± 0.30[a] |
| ABA (150 µM) x 7 days | 15.84 ± 1.31[a] | 6.09 ± 0.34[a] | 1.31 ± 0.14[a] | 2.07 ± 0.47[a] | 8.57 ± 1.03[a] |
| ABA (150 µM) x 14 days | 16.23 ± 0.64[a] | 6.33 ± 0.23[a] | 1.24 ± 0.10[a] | 1.90 ± 0.24[a] | 8.56 ± 1.14[a] |
| MeJA (10 mM) x 7 days | 15.02 ± 0.33[a] | 7.17 ± 0.23[a] | 1.24 ± 0.15[a] | 2.04 ± 0.39[a] | 8.64 ± 0.31[a] |
| MeJA (10 mM) x 14 days | 17.01 ± 0.99[a] | 7.33 ± 0.49[a] | 1.54 ± 0.24[a] | 2.43 ± 0.44[a] | 7.65 ± 2.21[a] |
| MeJA (20 mM) x 7 days | 16.63 ± 0.10[a] | 6.41 ± 1.32[a] | 1.21 ± 0.07[a] | 1.77 ± 0.08[a] | 7.67 ± 0.67[a] |
| MeJA (20 mM) x 14 days | 17.17 ± 1.61[a] | 6.62 ± 0.60[a] | 1.12 ± 0.04[a] | 1.60 ± 0.05[a] | 8.32 ± 0.86[a] |
| *F-value* | 0.64 | 0.48 | 2.43 | 1.01 | 0.77 |
| *P-value* | 0.64[ns] | 0.75[ns] | 0.08[ns] | 0.43[ns] | 0.55[ns] |

Means followed by the same letter in the same column are statistically non-significant (Tukey; $P<0.05$)

**Table 4.11:** Principal component analyses for the essential oil quality attributes of rose geranium (Central University of Technology, Free State).

| Traits | Principal component 1 | Principal component 2 |
|---|---|---|
| Eigenvalue | 8.63 | 4.45 |
| Variability % | 35.99 | 18.54 |
| Cumulative % | 35.99 | 54.53 |
| **Factor loading** | | |
| 2017Linalool | 0.22 | 0.04 |
| 2017*cis*-Rose oxide | **0.54** | 0.04 |
| 2017*trans*-Rose oxide | **0.66** | 0.00 |
| 2017Isomenthone | 0.23 | 0.40 |
| 2017Citronellol | **0.65** | 0.08 |
| 2017Geraniol | 0.40 | 0.01 |
| 2017Citronellyl formate | 0.25 | 0.42 |
| 2017Geranyl formate | 0.00 | **0.50** |
| 2017Guaia_6,9_diene | 0.00 | 0.36 |
| 2017Geranyl butyrate | **0.58** | 0.02 |
| 2017Geranyl tiglate | **0.66** | 0.01 |
| 2017C:G ratio | 0.20 | 0.25 |
| 2018Linalool | **0.51** | 0.00 |
| 2018*cis*-Rose oxide | 0.38 | 0.29 |
| 2018*trans*-Rose oxide | 0.32 | 0.38 |
| 2018Isomenthone | 0.18 | 0.20 |
| 2018Citronellol | 0.39 | 0.02 |
| 2018Geraniol | 0.00 | 0.46 |
| 2018Citronellyl formate | 0.00 | 0.16 |
| 2018Geranyl formate | 0.37 | 0.01 |
| 2018Guaia_6,9_diene | **0.63** | 0.21 |
| 2018Geranyl butyrate | **0.69** | 0.10 |
| 2018Geranyl tiglate | **0.50** | 0.13 |
| 2018C:G ratio | 0.18 | 0.26 |

Values in bold correspond for each variable to the factor for which the squared cosine is the largest

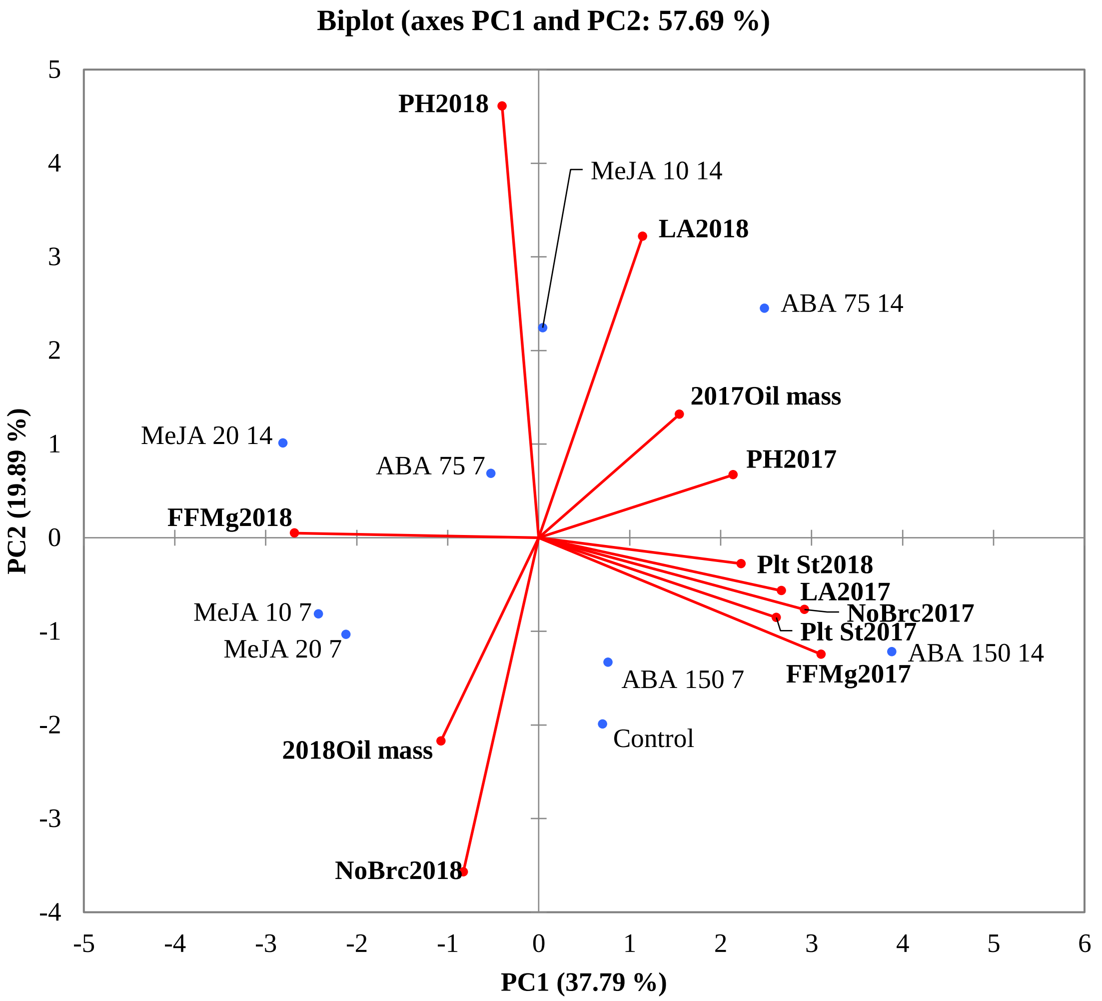


**Figure 4.1** Principal component biplot illustrating the variations between simulated hail-damaged rose geranium plant parameters, as affected by application of abscisic acid (ABA) and methyl jasmonate (MeJA) concentrations (75 µM ABA, 150 µM ABA, 10 mM MeJA, 20 mM MeJA, and a control) and application period (daily application for a duration of 7 and 14 days, respectively) (Central University of Technology, Free State). **Abbreviations:** LA = Leaf area; NoBrnc = Number of branches; PH = Plant height; Plt St = Plant stand loss; FFMg = Foliar fresh mass; Oilmass = Essential oil mass; 7 = 7 days; 14 = 14 days; ABA = Abscisic acid (75 and 150 $\mu$M); MeJA = Methyl jasmonate (10 and 20mM).

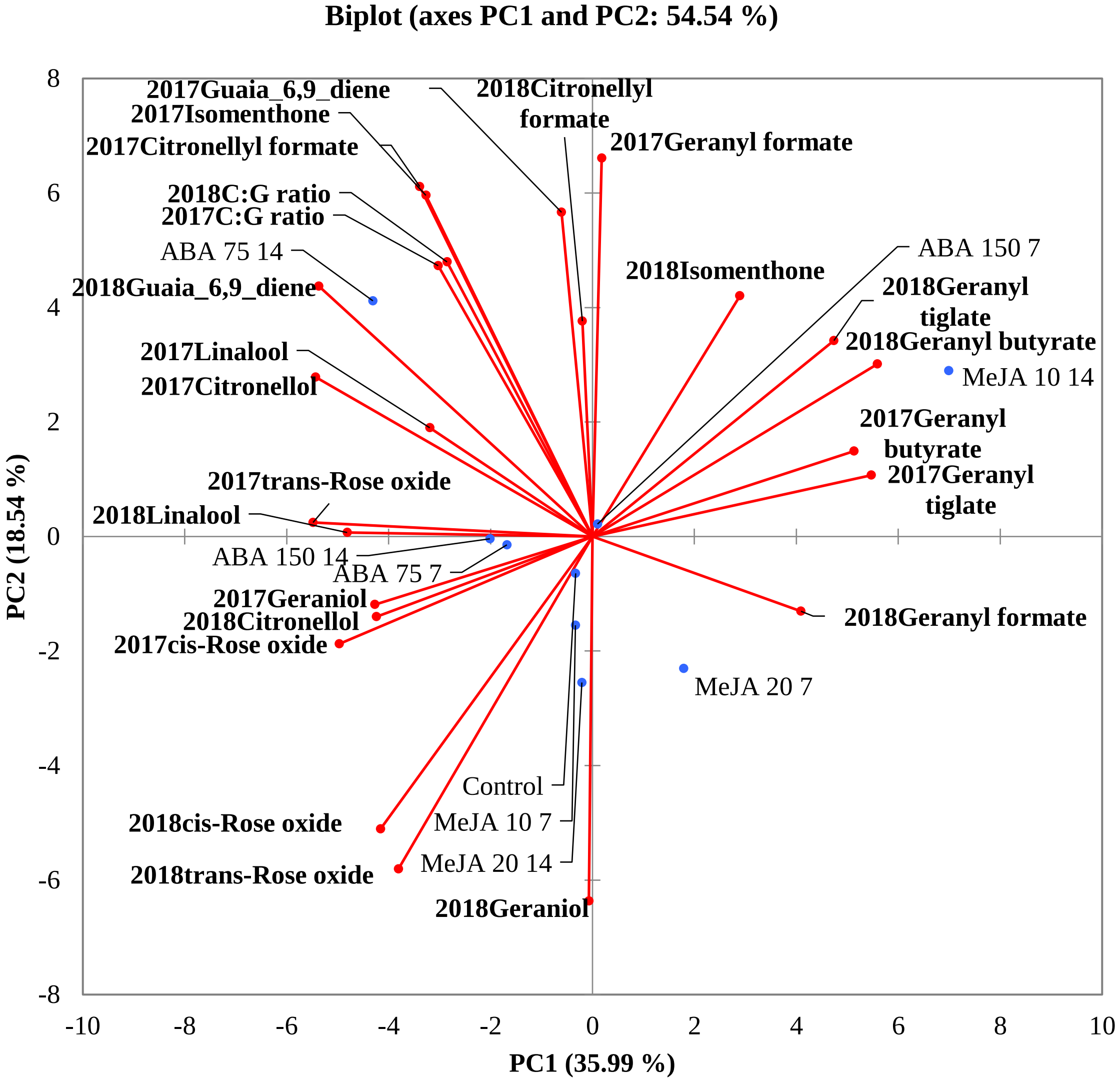


**Figure 4.2** Principal component biplot illustrating the variations between simulated hail-damaged rose geranium essential oil quality parameters, as affected by application of abscisic acid (ABA) and methyl jasmonate (MeJA) concentrations (75 µM ABA, 150 µM ABA, 10 mM MeJA, 20 mM MeJA, and a control) and application period (daily application for a duration of 7 and 14 days, respectively) (Central University of Technology, Free State).

**Abbreviations:** 7 = 7 days; 14 = 14 days; ABA = Abscisic acid (75 and 150 µM); MeJA = Methyl jasmonate (10 and 20mM).

# CHAPTER FIVE

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## RECOVERY RESPONSE OF SIMULATED HAIL-DAMAGED ROSE GERANIUM (*Pelargonium graveolens* L'Hér. cv. 'BOURBON') TO DIFFERENT MIXTURES OF AGRICULTURAL PLANT GROWTH REGULATORS

## Abstract

The aim of this study was to determine the potential ability of plant growth regulators-mixtures (PGRs) to recover the herbage and essential oil yield, as well improve the essential oil quality, of simulated hail-damaged rose geranium (*Pelargonium graveolens* L'Her.) plants. The experiment was carried in a 3 x 4 factorial treatment design, laid out in a randomised complete block design in a temperature-controlled greenhouse during the 2019 growing season. The treatments consisted of three hail damage simulation levels: 0% defoliation (intact plants), and 50% or 100% defoliation, and three PGR-mixtures and a control: level 1, distilled water control; level 2 gibberellic acid (GA) 1.26 mg/kg, brassinosteroids (BRs) 0.51 mg/kg, and traces of cytokinins (CKs) 0.025 mg/kg; level 3, GA 2.55 mg/kg, BRs 1.02 mg/kg, and traces of CKs 0.05 mg/kg; level 4 GA 3.83 mg/kg, BRs 1.53 mg/kg, and traces of CKs 0.075 mg/kg. The number of branches, the branches to height ratio, leaf area, foliar fresh mass (FFM), and the relative growth rate of the stem were all negatively affected by the 100% simulated hail damage treatment. However, these plants grew taller when PGRs-mixtures were applied from levels 2 to 3. The FFM started to increase when PGRs-mixtures were applied from level 3. The change in herbage yield parameters did not affect the essential oil yield for plants that sustained more than 50% foliage loss. The level 3 treatment increased the essential oil yield of the intact plants, as well as plants which sustained up to 50% foliage loss. The essential oil yield results could be associated with the changes in brevicollate trichome; however, this started to occur when PGRs were applied from level 2. Citronellol and geraniol content, and as well the citronellol to geraniol ratio (C:G ratio), improved with the level 2 treatment, in plants that sustained 100% foliage loss. It can be concluded that the application of PGR-mixture should be applied at the concentrations of the level 3 treatment, in order to improve the herbage yield and the essential oil quality of rose geranium plants with more than 50% foliage loss. Plants with less than 50% foliage loss can be treated with a PGR-mixture from level 2 to improve the essential oil yield and the densities of the brevicollate trichome.



**Keywords:** 'Bourbon-type', brassinosteroids, cytokinin, essential oil, gibberellic acid, hail damage simulation, trichome density


## 5.1 Introduction

Prolonged droughts, increased floods and frequent extreme weather events are evidence of climate change, as a consequence of global warming (Tack *et al.* 2015). Agriculture is adapting to the variability in global climatic conditions, with farmers continually developing strategies to respond to changing weather patterns (Burke *et al.* 2015). Aromatic and medicinal plants are among those plants which are negatively affected by climate change (Cavaliere 2009). In addition, there is a concern over climate change affecting the secondary metabolites of many medicinal and aromatic plants (Das *et al.* 2016; Kundu and Gantait 2017). This has been demonstrated with muña (*Minthostachys mollis* [Kunth] Griseb.), where leaf puncturing resulted in reduced menthone levels, while the pulegone concentration increased during the first 48 hours of the experiment (Banchio *et al.* 2007).

Loss of plant yield, associated with hail damage, is often variable and dependent on the plant species, the timing of injury, relative growth rate, and the prevailing conditions following the damage (Atkinson *et al.* 2014; Conley *et al.* 2008). Hailstones >H2 (15.2 to 20.3 mm) can cause extensive damage to growing crops through defoliation, leaf shredding, and stand losses (Changnon 1999; Conley *et al.* 2008). Hailstones may cause significant damage to aromatic and medicinal plants, such as rose geranium (*Pelargonium graveolens* L'Hér.): post-hail damage strategies are therefore required to recover the lost herbage material. Studies have shown that some species recover slowly from hail damage due to delayed growth patterns, i.e. canola (*Brassica napus* L.), Alaska pea (*Pisum sativum* L.), guar (*Cyamopsis tetragonoloba* [L.] Taub.), and lentil (*Lens culinaris* L.) (Miller and Muehlbauer 1984; McGregor 1987; Sij *et al.* 2005; Bueckert 2011).

Following hail damage, the tissue around the damaged site initiates a cascade of biochemical or physiological processes to repair the damaged tissue: this further depletes energy stores to rebuild photosynthetic material and regenerate lost plant tissue (Suttle *et al.* 2013; Atkinson *et al.* 2014). These physiological activities are primarily driven by the upregulation of endogenous plant growth regulators (PGRs) (Ikeuchi *et al.* 2017; Nanda and Melnyk 2018). Extensive studies have demonstrated that exogenous applications of agricultural PGRs on plants may effectively stimulate vegetative growth, improve nutrient acquisition, and increase the antioxidant capacity of the plant tissue (Torres *et al.* 2018; Parađiković *et al.* 2019).

The effects of PGRs are based on the synergism to improve the growth and development, as well as the recovery, resistance and survival of stressed plants (Prins *et al.* 2010; Parađiković *et al.* 2019). Therefore, the objective of this study was to determine the ability of PGR-mixtures to recover the herbage and essential oil yield attributes, and as well improve the essential oil quality of simulated hail-damaged rose geranium plants. It is hypothesized that high concentrations of PGR-mixtures will improve the essential oil yield and quality of simulated hail-damaged rose geranium plants.

## 5.2 Materials and methods

### 5.2.1 Experimental sites

This experiment was conducted in a 72 $m^2$ temperature-controlled, plastic-covered greenhouse, on the campus of the Central University of Technology, Free State (29°07'S 26°12'E), Republic of South Africa (RSA). The temperature in the greenhouse was maintained by a fan, which was triggered at ca. 26° C. Rose geranium cuttings (cv. 'Bourbon': ca. 10 cm tall), obtained from the South African Essential Oil Business Incubator (RSA) were transplanted on September 1st 2019. Plants were grown for a single cycle (122 days) using a hydroponic drain to waste system, as described in Chapter 4.

### 5.2.2 Experimental design and procedures

*Experimental layout and treatment design:* The experiment had a 3 x 4 factorial design, arranged in a randomized complete block design with three replications. Treatments consisted of three hail damage simulation levels: 0% defoliation (intact plants), 50%, and 100% defoliation, and three PGR-mixtures (registered as *Lucky Plant*® [Agraforum, Germany]: patent number – EP1051075B1 & EP1933626) and a control: level 1, distilled water control; level 2, GA 1.26 mg/kg, BRs 0.51 mg/kg, and (trace) CKs 0.025 mg/kg; level 3, GA 2.55 mg/kg, BRs 1.02 mg/kg, (trace) CKs 0.05 mg/kg; level 4, GA 3.83 mg/kg, BRs 1.53 mg/kg, (trace) CKs 0.075 mg/kg (Table 5.1). Row spacing and spacing between plants were ca. 50 cm and 30 cm, respectively (Kassahun *et al*. 2012).

Hail damage simulation treatments were performed at ca. 14:00 on October 31st 2019, 61 days after transplantation of the rose geranium cuttings. Hail was mechanically simulated using a pair of manually-operated garden secateurs, as described in Chapters 3 & 4 (Nielsen 2012). The application treatments commenced one hour after hail damage simulation (ca. 15:00).

As described by Franks and Faquhar (2001), approximately 50 mL of the PGR treatment solution was applied directly onto each plant, until the solution dripped from the plants. Treatments were repeated every 14 days, on sunny days between 13:00 and 14:00 (lasting for ca. 1 hour), until harvest. To avoid the spray mixture drifting onto adjacent experimental plants a mini plastic-covered tunnel, measuring 650 mm x 480 mm x 1200 mm (h x l x w), was used as a cover during each application. Moreover, each plant was placed on a 30 cm terracotta plastic saucer to avoid cross-contamination from the drainage.

### 5.2.3 Parameters

Foliar fresh mass (FFM) and related attributes, including trichome density parameters and essential oil attributes (yield and quality), were determined following the procedures described in Chapter 3. In this chapter, only the relative growth rate (RGR) of the main stem and the free proline parameters are described in detail. The RGR was measured every week, measurement only commenced after the first application of PGRs (Pérez-Harguindeguy *et al.* 2013). Weekly plant height data were used to determine the RGR (cm $cm^{-1}$/day) by means the following formula:

$$RGR = In\,(height2) - In\,(height1)_{/}\, time2 - time1$$

Where: *in (height1)* = height at the start of the interval; *in (height2)* = height at the end of the interval; *time1* = time at the start of the interval (in days); *time2* = time end of the interval (Meyer 1998).

Free proline, and leaf micro-morphology studies were also determined. Free proline was extracted and analysed using a ninhydrin-based method (Gibon *et al.* 2000; Carillo and Gibon 2011). The material was mixed with an ethanol:water (70:30 v/v) buffer, equivalent to 20 times the fresh weight (FW) in volume. The mixture was placed in a 50 mL falcon tube and centrifuged at 12000 g for 5 mins. After centrifugation, 0.5 mL of the supernatant was added to a reaction mixture of 0.5 mL ninhydrin solution (1%), prepared in glacial acetic acid:water (60:40 v/v) and 0.5 mL ethanol (20% v/v). After homogenisation of the mixture by vortexing, it was heated in a water bath at 95°C for 20 mins, then centrifuged for 2 mins at 12000 g. The supernatant was poured into a disposable cuvette, and the absorbance was read at 520 nm. The standard curve was prepared using standard proline solution (0.04 mM-0.1 mM). The free proline concentration in the extract was derived using the following equation (Carillo and Gibon 2011):

$$Proline = (Abs\,extract - blank)/slope * Vol\,extract/Vol\,aliquot * 1/FW$$

Where: *Abs extract* is the absorbance of the extract, the absorbance of the *blank*, and the *slope* (expressed as absorbance/nmol) is determined by linear regression, *Vol extract* is the total volume of the extract, *Vol aliquot* is the volume used in the assay and FW (expressed in mg) is the amount of plant material extracted.

For the leaf micro-morphology analysis, leaves were sampled 28 days after simulated hail damage. Because the younger leaves have a denser indumentum than the older ones, only the uppermost, young, fully-expanded leaves were selected (Oosthuizen and Coetzee 1984). A sample (1 $cm^2$) was cut from the middle part of the leaf blade apex with a surgical blade and prepared for scanning electron microscopy, following the procedures described in Chapters 3.

### 5.2.4 Statistical analysis

All parameters were statistically analysed following the methods described in Chapters 3 & 4. Analysis of variance was applied using combined data on the weekly interval for the RGR. The Shapiro-Wilk test was also applied to test for any deviations from normality (Shapiro and Wilk 1965).

## 5.3 Results

### 5.3.1 Plant parameters, leaf trichomes and essential oil yield attributes

The number of branches, branches to height ratio (B:H ratio), leaf area, and chlorophyll content were not affected by interactions between simulated hail damage and the subsequent application of PGRs-mixture (Table 5.2). Only the plant height was affected by interactions between simulated hail damage and the subsequent application of PGRs ($F_{19.00}$ = 6.19; $P<0.001$) (Table 5.2). Intact plants tended to grow taller when PGR-mixtures (all levels) were applied, compared to the control: only level 2 was not statistically different from the control. In the 50% defoliated plants, plant height was similar with all PGR treatments, including the control. In 100% defoliated plants, the results were inconsistent between the levels: a marginal increase in growth was recorded when the PGR-mixture was applied at level 3 compared to the control; however, this application was not statistically different to levels 2 and 4.

The number of branches per plant, and the FFM declined by 37.9% and 56.1%, respectively when the simulated hail damage was intensified up to 100% defoliation compared to intact plants (control) (Table 5.2). Intact plants continued with normal straight elongation of the terminal shoots (Figure 5.1); however, this was not the case in 50% and 100% simulated hail-damaged plants, in which the number of branches were affected. Branches of the 50% and 100% defoliated plants tended to be plagiotropic, with the terminal bud tips drying and not showing any signs of recovery (Figure 5.1). Plants that suffered 100% simulated hail damage were less bushy, with significantly smaller leaves compared to the intact plants and those that suffered 50% simulated hail damage. This is shown by the lower B:H ratio and smaller leaf area in Table 5.2.

The application of PGR-mixtures at levels 3 and 4 increased the FFM for all hail damage levels (Table 5.2): this could also be ascribed to the proline content (Figure 5.2). A significantly lower proline content was recorded when levels 3 and 4 were applied, compared to the control and the level 2 treatment ($F_{19.00}$ = 6.34; P<0.02).

The stem RGR was affected by the interaction between hail damage simulation and time (week factor) (Table 5.3 & Figure 5.3). During the first week, the rate of stem development did not differ between the simulated hail damage intensities. The RGR tended to vary significantly from the second week until the sixth week for all hail damage levels; however, this trend remained the same throughout the recovery period. A consistent RGR was recorded on intact plants; however, a relatively constant slow RGR was recorded on plants that endured simulated hail damage.

Two groups of trichomes were identified: glandular (peltate and capitate) and non-glandular trichomes (attenuate) (Table 5.4). These trichomes were similar to those described in Chapters 3 & 4. However, in this study, the elongated-capitate trichomes were rarely observed on rose geranium leaves and therefore are not discussed any further. The attenuate trichome type is insignificant to essential oil biosynthesis; therefore, they are also not discussed any further.

An interaction was observed between simulated hail damage and the subsequent application of PGR-mixtures for the asciiform trichome densities on both leaf surfaces of the rose geranium plant (Table 5.4). On intact plants, the densities of the asciiform trichome were significantly ($F_{24.00}$ = 10.10; P<0.01) high when PGR-mixtures were applied at level 2 on the adaxial leaf surface compared with the control; however, this treatment was not significantly different to level 3. The density of the asciiform trichome started to decline when the PGR-mixture was applied from level 3 to level 4. When plants sustained 50% simulated hail damage, PGR-mixture application produced inconsistent results: a marginally higher density of the asciiform trichomes were recorded when PGRs-mixture were applied at levels 3 and 4, however, these applications were not significantly different to the control. On the 100% defoliated plants, the asciiform trichome densities declined by 60.6% when PGRs were applied at level 2 compared with the control.

On the abaxial leaf surface, the opposite effect was recorded on refoliating plants that had lost up to 50% defoliation (Table 5.4), with the asciiform trichome densities increasing by 39% when the PGR-mixture was applied at level 2. Asciiform trichome densities gradually declined when the PGR-mixture was increased from level 2 up to level 4 ($F_{24.00}$ = 3.18; $P<0.001$). The intact plants, and those that sustained 100% simulated hail damage, had similar asciiform trichome densities following all PGR treatments, including the control. The asciiform trichome densities were increased by 32.1% on the 50% simulated hail-damaged plants compared to 100% defoliated plants when PGR-mixture was applied at level 2.

Brevicollate trichome densities were significantly ($F_{24.00}$ = 17.56; $P<0.001$) higher when PGRs were applied at level 3 on the adaxial leaf surface (Table 5.4). However, this was not significantly different from level 4. On the abaxial leaf surface, the densities of the brevicollate trichome were affected by the interation between simulated hail damage and the subsequent application of PGR-mixtures (Table 5.4). On the intact plants, the densities of brevicollate trichome were higher when the PGR-mixtures were applied compared with the control, from level 3 to level 4 ($F_{24.00}$ = 6.23; $P<0.005$). On the 50% defoliated plants, the PGR-mixture applied at level 4 resulted in marginally higher densities of brevicollate trichomes, compared to the control ($F_{24.00}$ = 6.23; $P<0.005$). With the 100% defoliated plants, all PGR treatments were similar to the control.

Essential oil mass and essential oil content were significantly affected by the interaction between reduced simulated hail damage levels and the subsequent application of PGRs (Table 5.5). In the intact plants, the highest essential oil mass was recorded when PGR-mixtures were applied at level 3 ($F_{24.00}$ = 3.28; $P<0.006$); however, the essential oil content was similar in all PGR-mixtures levels. In 50% defoliated plants, the essential oil mass was marginally increased ($F_{24.00}$ = 3.76; $P<0.01$) when the PGRs-mixtures were applied at level 3 compared with the control; however, this was not statistically different from levels 2 and 4. A similar trend was recorded for the essential oil content on the 50% defoliated plants: there were no statistical differences between all PGR treatments for both essential oil yield attributes when plants sustained 100% simulated hail damage.

### 5.3.2 Essential oil quality

The levels of *cis*-Rose oxide and *trans*-Rose oxide declined by 75% and 80%, respectively, with increasing defoliation intensity, while the linalool content increased in plants that had lost 100% foliage. None of the PGR treatments had an effect on the concentrations of *cis*-Rose oxide, *trans*-Rose oxide, isomenthone and linalool compounds following hail damage (Table 5.6).

Interactions were observed between simulated hail damage and the subsequent application of different levels of PGRs for citronellol, geraniol, and the citronellol to geraniol (C:G) ratio (Table 5.6). The highest ($F_{20.23}$ = 4.22; P<0.006) citronellol content was recorded when PGRs were applied at level 4 on the intact plants compared with the control; however, this PGR level was not significantly different from level 3. On 50% and 100% defoliated plants, the citronellol content was similar in all PGR treatments. Although citronellol and geraniol share a similar biosynthetic pathway, the geraniol results were not the same: the geraniol content of the intact plants, and those that suffered 50% simulated hail damage were not significantly affected by the subsequent application PGRs, at any treatment level (Table 5.6). In the 100% defoliated plants, a higher ($F_{22.00}$ = 5.63; P<0.02) geraniol content was recorded when the level 2 treatment was applied compared with the control: however, this treatment level was not significantly different to level 4.

As expected, changes in citronellol and geraniol content affected the C:G ratio (Table 5.6). The perfumery industry prefers a C:G ratio of <3:1 (Saxena *et al.* 2008). In the intact plants, the C:G ratio was higher than 3:1 when PGRs-mixtures were applied at levels 3 and 4 ($F_{22.00}$ = 4.44; P<0.01). In the 50% defoliated plants, the lowest C:G ratio was recorded when PGRs were applied at level 4, compared to the control. On plants that suffered 100% simulated hail damage, the C:G ratio was lower when the level 2 treatment was applied, compared to the control ($F_{22.00}$ = 4.44; P<0.01). A significantly lower C:G ratio (<3:1) was recorded on all defoliated plants (50 & 100%) compared to the intact plants ($F_{22.00}$ = 4.44; P<0.01).

In this study, the geraniol esters and the citronellyl formate content were significantly higher in plants that sustained 100% simulated hail damage (Table 5.7). The citronellyl formate content accumulated ($F_{22.00}$ = 9.77; P<0.007) up to 20.2% when PGRs were applied at level 4; however, this was not significantly different compared to the control.

## 5.4 Discussion

### 5.4.1 Plant parameters, leaf trichomes and essential oil yield attributes

Taller plants in this study after addition of PGRs could be ascribed to increased plasticity of the cell wall, regulated by the action of GA and BRs (Yamaguchi *et al.* 2010): Matusmoto *et al.* (2016) indicated that GA and BRs also regulate stem elongation. Therefore, the accumulation of BRs following the PGR application could have facilitated GA to promote stem elongation. According to Sun (2010), an increase in cell elongation and cell division occurs during stem growth because GA induces the transcription of genes involved in growth processes. Dayan *et al*. (2012) found that endogenous concentrations of bioactive GA in young internodes of defoliated tobacco plants (*Nicotiana tabacum* L.) were reduced by 60% compared to those of intact plants, however, 0.8 mg/L GA restored the cambium and xylem fibre differentiation. In the 100% defoliated plants, taller plants could be attributed to the action of BRs, since this also regulates abiotic stress (Yamaguchi *et al.* 2010). The stem terminal buds were removed following defoliation to simulate hail bruising in this study, therefore, the accumulation of BRs could have been slower in the stem as the plant recovered from defoliation.

Defoliation and the removal of the shoot apex through hail damage simulations may affect the apical dominance, and stimulate the growth of branches from auxiliary buds (Prins and Verkaar 1992). Therefore, the alteration of branching patterns following apex removal is not uncommon, as was the case with the simulated hail-damaged plants (50% and 100%) in this study. This could be due to delayed plant growth, as auxiliary branches must first form new leaves before the photosynthetic rate can reach the same levels as pre-defoliation (Prins and Verkaar 1992).

As expected, the B:H ratio decreased in plants that sustained 100% defoliation. The removed stem terminal buds showed no signs of regrowth (Figure 5.2). This could be attributed to the nutrients and carbohydrates requirements between the auxiliary buds and the shoot apex, since plants need to recuperate following defoliation (Prins and Verkaar 1992). In this study, the bushiness of rose geranium plants did not recover following the application of PGRs.

Plant leaf development follows a standard basic sequence that is flexible and adjusted according to species, developmental stage, and environmental conditions (Bar and Ori 2014). Meyer (1998) also reported that the plant response to extreme defoliation is affected by its capacity to draw upon sufficient resources to maintain growth recovery, which directly affects the leaf maturation process. In a study with perennial ryegrass (*Lolium perenne* L.), decreased leaf area could be ascribed to decreased cell production and expansion. In this study, the intact and 50% simulated hail-damaged plants had a significantly higher number of mature leaves at harvest, compared to plants that endured 100% simulated hail damage (Figure 5.2). Therefore, the reduction in the leaf area in the 100% simulated hail-damaged plants could be because of reduced photosynthetic capacity following complete defoliation (Bar and Ori 2014). Contrary results were reported for goldenrod (*Solidago altissima* L.), where refoliating plants had bigger leaves compared to the control plants (Meyer 1998).

According to Pérez-Harguindeguy *et al.* (2013), agronomic parameters such as plant height, the number of branches, leaf area, and FFM are essential parameters in determining the morphological characteristics and yield components of indeterminate crops, such as rose geranium. Reduced FFM following simulated hail is due to fewer branches per plant, a reduced number of branches per meter, and a smaller leaf area. This confirms the hypothesis of resultant decrease in biomass and related attributes where rose geranium plants endure extreme simulated hail damage intensity (Swamy *et al.* 1960; Dermane and van der Walt 1989; Weiss 1997). Similar findings were recorded in a study by Bueckert (2011) on lentils (*Lens culinaris* L.), which is also an indeterminate crop species.

Plants accumulate proline as an adaptive mechanism for stress, but it is also beneficial under normal plant growth conditions (Zhang and Becker 2015). Besides its proteogenic function, proline also plays a role in energy utilization (Hare and Cress 1997), protein unfolding (Liang *et al.* 2013), as well as cell reprogramming and development (D'Aniello *et al.* 2015). Moreover, recent studies have also shown that proline plays a vital role in plant growth and differentiation at different growth stages (Kishor *et al.* 2015). In this study, the application of PGRs could have increased energy utilization, and improved cell development, subsequently increasing the FFM (Hare and Cress 1997). Therefore, results on the increased FFM and declined proline content could be ascribed to the PGRs and proline in the chloroplast and cytoplasm. In addition, Li *et al.* (2012) reported that a BRs and GA interact at the signalling level to promote plant growth and development. This occurs through the regulation of BRs, which directly affects plant growth by modulating GA levels. Amiri *et al.* (2014) reported that FFM was significantly increased by the application of GA (100 ppm) on German chamomile (*Matricaria recutita* L.).

Growth rate, as an indicator of life history and ecological strategy, may be crucial to post-defoliation recovery strategies. According to Atkinson *et al.* (2014), plants deploy stored resources to rebuild photosynthetic material following defoliation. Meyer (1998) reported similar results on goldenrod, where less damaged plants recovered more rapidly than completely defoliated plants. In this study, fortnightly applications of PGRs did not affect the rate of relative stem growth (Table 5.3).

In this study, the densities of asciiform trichomes on both leaf surfaces differed between the simulated hail damage levels. Generally, the intact plants, and those that suffered 50% foliage loss on both leaf surfaces recorded higher asciiform trichome densities than those that suffered 100% simulated hail damage. Prins *et al.* (2010) also demonstrated that the development of trichomes, and the biosynthesis of essential oil could be influenced by exogenous applications of PGRs. GA positively contributes to the regulation of trichome development (Zhou *et al.* 2013). According to Traw and Bergelson (2003), GA modifies the epidermal cell surface area, and also the cell number per unit area. However, in this study, the activities associated with asciiform trichome development on refoliated plants could be ascribed to *TEM*s (Jiao 2016). *TEM*s regulate the signalling of CK at the later developmental stage compared to GA, signalling in earler developmental stage. CK is a PGR that also promotes trichome formation under normal growth conditions. Moreover, since BRs and jasmonic acid are directly involved in the formation and variation of trichome density (Campos *et al.* 2009): the *dpy* mutant (BR-deficient) is the one which enhances pubescence, while the jasmonic acid *jai1-1* mutant produces the opposite phenotypic effect (Campos *et al.* 2009; Fambrini and Pugliesi 2019). The Arabidopsis *bls1* mutant, which is impaired in the BR response, developed fewer trichomes on both the abaxial and adaxial leaf surfaces, indicating the possible involvement of BR in trichome development (Pattanaik *et al.* 2014). There are no studies on refoliating plants treated with PGR. Therefore, the changes in asciiform trichome densities is attributed to the traces of CKs.

The increased density of brevicollate trichomes on the intact plants and those suffering 50% simulated hail damage could also be ascribed to the GA and CK in the PGR-mixture. Zhou *et al.* (2011, 2013) reported similar findings in Arabidopsis (*Arabidopsis thaliana* [L.])

Heynh.), where GA and CK, at concentrations as low as 100 µM, increased the density of glandular trichomes. According to the author, this was ascribed to GA and CK molecules, which regulated the development of glandular trichome through the combined action of *ZFP5* and *ZFP6* transcription factors (Zhou *et al*. 2013). As for the 50% defoliated rose geranium, plants deploy stored resources to rebuild photosynthetic material and regenerate new organs or tissues following defoliation and wounding (Stobbe *et al.* 2002; Nanda and Melnyk 2018). During the refoliation, it is possible that the endogenous CK content in 50% defoliated plants was already too high; then GA accumulation occurred at the later stage to regulate growth. GA and CK could have combined during these periods via antagonistic crosstalk effects following the fortnightly applications of PGRs. According to Barnes (2013), the amalgamation of CK and GA following hail damage may cause alterations in morphological features, such as increased trichome density. Other than GA and CK, BRs and jasmonic acid directly affect trichome formation through the accumulation of *zgb* and *PI-I* transcripts, indicating the importance of BRs in leaf recovery following defoliation or wounding (Campos *et al.* 2009). This was not the case for the 100% defoliated plants, as most energy was focused on recovering plant leaf material.

Asciiform trichome type is characterised by a short segmented capitate with a columnar hatchet-shaped tip, that has a slightly bent apical cell pointing at the leaf apex (Payne 1978). On the other hand, breviocollate trichomes are characterised by a short neck with bigger round tips (Payne 1978). Eiasu *et al.* (2009) reported that the capitate group (asciiform trichome) lack stored essential oil in their sub-cuticular areas compared to the brevicollate trichome. Thus, in this study, PGRs-mixture should be applied at a rate of level 3 on rose geranium plants that have lost up to 50% foliage to increase the densities of the brevicollate trichomes, with the aim of improving the essential oil yield.

PGRs stimulate plant growth and terpene biosynthesis in a large number of aromatic plant species, which result in beneficial changes in terpene accumulation (Farooqi and Shukla 1990). There are no reports in the literature where a combination of GA, BRs, and CK affected essential oil yield attributes of refoliating plants, however, Poyh and Ono (2006) recorded higher essential oil content for sage (*Salvia officinalis* L.) treated with 100 mg/L GA. Fraternale *et al.* (2003) reported a higher essential oil yield of Spanish marjoram (*Thimus mastichina* L.) using a medium culture with CK as low as 0.1 mg/L. In the leaves of Spanish marjoram treated with CK, there were a greater number of glandular trichomes recorded at the later leaf developmental stage, which could be ascribed to increased essential oil yield. Foliar application of a PGR-28-homobrassinolide ($10^{-6}$ M) also enhanced the essential oil yield of mint (*Mentha arvensis* L.) (Naeem *et al.* 2012). In this study, it is evident that only the 50% hail-damaged plants yield higher essential oil, following treatment with PGRs at level 3.

### 5.4.2 Essential oil quality

Croteau *et al.* (2000) reported that molecules could change before undergoing enzymatic oxidation, with a vast array of compounds, of remarkably different structures formed under various environmental conditions. *cis*-Rose oxide and *trans*-Rose oxide can be synthesised from the conversion of citronellol, through citronellyl diphosphate (phosphorylated citronellol) in rose geranium (Wüst *et al.* 1999). This may also occur because of a photo-oxygenation mechanism, including singlet oxygen as the oxidising agent (Yamaguchi 1981). In this study, the enzymatic oxidation of citronellol could have gradually declined as a result of defoliation, directly affecting the cyclization of the diol to *cis*-Rose oxide and *trans*-Rose oxide (Wüst *et al.* 1999). Takana *et al.* (2010) reported that when plant tissues undergo physio-morphological changes, such as wounding or defoliation, some chemical compounds are converted to secondary polyphenols by enzymatic and non-enzymatic reactions. This could have been the case in this study; however, there are no similar cases in the literature to support this hypothesis.

Linalool content tends to increase with an increase in the intensity levels of defoliation and wounding, and this could be due to the ontogeny of the leaf factor (Maia *et al.* 2007). Severely simulated hail-damaged plants could contain younger leaves at the time of recovery, compared to intact and those plants with at least 50% leaf loss. With the ontogeny of the leaf factor, this metabolic process affect the primary and secondary metabolic processes during refoliation. This was demonstrated on balsam fir (*Abies balsamea* L.), where linalool content was higher in defoliated plants compared to intact plants (Deslauriers *et al.* 2015). In sweet basil (*Ocimum basilicum* L.), younger leaves had higher linalool content compared to mature leaves (Fischer and Hammerschmidt 2011).

Citronellol shares a similar biosynthesis pathway with geraniol and its esters (Bergman *et al.* 2019). Chacón *et al.* (2019) reported that the biotransformation of geraniol occurs through endogenous enzymatic changes. On the intact plants in this study, increased citronellol content could be ascribed to the exogenous application of agricultural PGRs (Suga and Shishibori 1973). The results on increased geraniol in this study could be ascribed to leaf ontogeny as well as the effects of GA in the PGR-mixture. According to Prins *et al.* (2010), plants produce essential oils in response to physiological stress, pathogen attack, and other ecological factors. Therefore, simulated hail damage in this study could be responsible for increased geraniol content. Geraniol content fluctuates significantly during leaf development, which subsequently affects its esters and citronellol biosynthesis (Chacón *et al.* 2019). In this study, 100% simulated hail-damaged plants had younger leaves compared to intact plants at the time of harvest. It is also possible that as plants refoliated, the fortnightly-applied PGRs could have also affected the biosynthesis of the geraniol. According to Burlat *et al.* (2004), geraniol and GA share a similar biosynthetic route: the mevalonic acid pathway. This pathway could have been affected at a lower concentration of PGR treatment (level 2), and improved the accumulation of geraniol and citronellol as plants refoliated. However, an increase in the PGR-mixture up to level 3 & 4 could have led to the toxicity of the GA, causing the decline of the geraniol content.

The C:G ratio is used to classify rose geranium essential oil quality, and is a requirement of the perfumery industry and ISO standards (Saxena *et al.* 2008). Biosynthesis of citronellol and geraniol affected the C:G ratio in this study. The higher citronellol and low geraniol contents observed in this study after the application of PGRs could be related to the metabolic pathway of geraniol biosynthesis, GA concentration, and the leaf ontogeny (Suga and Shishibori 1973; Burlat *et al.* 2004; Motsa *et al.* 2006). In this study, it may be beneficial to apply mixtures of PGRs at level 2 only when plants have suffered complete defoliation, resulting in a lower and favourable C:G ratio <3. This may improve the essential oil quality.

Geranyl formate, geranyl butyrate, and geranyl tiglate are geraniol esters (Liu *et al.* 2016). The equal accumulation of these esters could be attributed to the biosynthesis of geraniol, and leaf development (Motsa *et al.* 2006). Plant defoliation affects leaf ontogeny and biosynthesis of secondary metabolites (Burlat *et al.* 2004; Motsa *et al.* 2006). Therefore, the conversion rate of geraniol into geraniol esters could have differed between leaf developmental stages in this study. Ganjewala and Luthra (2009) reported similar findings, with geraniol esters declining from 59% to 3% during the leaf growth period from day 10 to day 50 after transplanting. In addition, Motsa *et al.* (2006) also demonstrated that geraniol content declines as rose geranium plants age. Therefore, it may be concluded that the variations between the geraniol esters changed with leaf age factor, especially following the defoliation treatment.

Citronellyl formate is an ester to citronellol. Both constituents, along with geraniol and its esters, share the same chemical structure (citronellyl moiety) and biosynthetic pathway (Lis-Balchin 2002; Sedibe 2012; Bergman *et al.* 2019). In this study, citronellol, as well as geraniol and its esters, were improved by leaf ontogeny factor. Interestingly, this was also the case with citronellyl formate: Malatova *et al.* (2011) reported a similar situation for rose geranium, where a change in citronellol content affected the citronellyl formate content. Therefore, refoliating plants could have accumulated high content of these compounds (citronellol and geraniol esters), including citronellyl formate.

### 5.5 Conclusion

In this study, simulated hail damage affected the recovery and growth of the measured rose geranium foliage parameters. However, it can be concluded that the application of a mixture of PGRs should be applied at 2.55 mg/kg (GA), 1.02 mg/kg (BRs), and 0.05 mg/kg (CKs) (level 3 in this study) in order to improve the foliage yield and essential oil quality (to <3, as per industry standards) for plants suffering more than 50% foliage loss. Plants with less than 50% foliage loss can be treated with a lower level of PGRs: level 2 to level 3 in this study, to improve the essential oil yield and brevicollate trichome density.

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**Table 5.1:** Treatment levels used as PGRs-mixtures in this study.

| Component | PGRs treatments (mg/kg) | | | |
|---|---|---|---|---|
| | **Level 1** | **Level 2** | **Level 3** | **Level 4** |
| Gibberellic acid | Zero | 1.26 | 2.55 | 3.83 |
| Brassinosteroids | Zero | 0.51 | 1.02 | 1.53 |
| Cytokinins: | | | | |
| 6-Benzylaminopurine | Zero | 0.025 | 0.05 | 0.075 |
| Kinetin | Zero | 0.025 | 0.05 | 0.075 |
| *trans*-Zeatin | Zero | 0.025 | 0.05 | 0.075 |

**Table 5.2:** The effects of hail-simulated damage (0%, 50%, and 100% defoliation) and the application of PGRs-mixtures (level 1 consisted of distilled water; level 2 had gibberellic acid (GA)-1.26 mg/kg, brassinosteroids (BRs)-0.51 mg/kg, and traces of cytokinins (CKs)-0.025 mg/kg; level 3 had GA-2.55 mg/kg, BRs-1.02 mg/kg, and traces of CKs-0.05 mg/kg; level 4 had GA-3.83 mg/kg, BRs-1.53 mg/kg, and traces of CKs-0.075 mg/kg) on selected plant growth parameters of rose geranium (Central University of Technology, Free State).

| Treatments | Plant height | № of branches | B:H ratio | Leaf area | Chlorophyll (%) | FFM |
|---|---|---|---|---|---|---|
| **Simulated hail damage** | | | | | | |
| 0% defoliation | 59.10 ± 5.53$^{a}$ | 32.51 ± 6.62$^{a}$ | 55.00 ± 10.93$^{a}$ | 44.61 ± 7.21$^{a}$ | 14.65 ± 2.87$^{a}$ | 692.60 ± 116.23$^{a}$ |
| 50% defoliation | 47.37 ± 4.62$^{b}$ | 27.53 ± 7.27$^{b}$ | 58.11 ± 15.85$^{a}$ | 42.33 ± 3.81$^{a}$ | 14.66 ± 2.17$^{a}$ | 556.04 ± 97.37$^{b}$ |
| 100% defoliation | 46.45 ± 6.80$^{b}$ | 20.18 ± 6.97$^{c}$ | 44.44 ± 17.38$^{b}$ | 27.82 ± 4.69$^{b}$ | 13.33 ± 2.30$^{a}$ | 304.08 ± 108.23$^{c}$ |
| *F-value* | 10.78 | 6.83 | 3.98 | 8.52 | 1.83 | 9.89 |
| *P-value* | ***0.001** | ***0.002** | ***0.03** | ***0.001** | 0.18$^{ns}$ | ***0.01** |
| **Plant growth regulators (PGRs)** | | | | | | |
| Level 1 | 44.93 ± 6.41$^{b}$ | 23.37 ± 7.64$^{a}$ | 52.00 ± 13.74$^{a}$ | 35.51 ± 9.30$^{a}$ | 13.63 ± 1.97$^{a}$ | 401.80 ± 175.86$^{b}$ |
| Level 2 | 51.83 ± 6.15$^{a}$ | 25.21 ± 6.98$^{a}$ | 48.64 ± 10.29$^{a}$ | 38.15 ± 10.28$^{a}$ | 14.63 ± 3.04$^{a}$ | 505.55 ± 209.23$^{a,b}$ |
| Level 3 | 55.16 ± 7.06$^{a}$ | 27.88 ± 9.42$^{a}$ | 50.54 ± 16.29$^{a}$ | 39.92 ± 9.13$^{a}$ | 14.57 ± 3.01$^{a}$ | 554.58 ± 213.95$^{a}$ |
| Level 4 | 51.80 ± 9.72$^{a}$ | 30.50 ± 9.72$^{a}$ | 60.88 ± 20.72$^{a}$ | 39.44 ± 9.05$^{a}$ | 14.03 ± 1.97$^{a}$ | 608.35 ± 133.14$^{a}$ |
| *F-value* | 9.51 | 2.63 | 1.60 | 1.32 | 0.53 | 14.89 |
| *P-value* | ***0.001** | 0.08$^{ns}$ | 0.22$^{ns}$ | 0.29$^{ns}$ | 0.66$^{ns}$ | ***0.001** |
| **HD x PGRs levels** | | | | | | |
| 0% x Level 1 | 51.75 ± 1.08$^{b,c}$ | 29.36 ± 7.68$^{a}$ | 56.72 ± 15.69$^{a}$ | 42.74 ± 8.08$^{a}$ | 11.86 ± 2.26$^{a}$ | 565.41 ± 53.79$^{a}$ |
| 0% x Level 2 | 57.41 ± 1.52$^{a,b}$ | 32.25 ± 2.51$^{a}$ | 56.17 ± 5.66$^{a}$ | 43.75 ± 13.79$^{a}$ | 17.15 ± 3.17$^{a}$ | 717.50 ± 47.18$^{a}$ |
| 0% x Level 3 | 64.08 ± 1.46$^{a}$ | 35.75 ± 8.56$^{a}$ | 55.78 ± 14.32$^{a}$ | 46.61 ± 4.11$^{a}$ | 14.58 ± 2.27$^{a}$ | 759.16 ± 187.37$^{a}$ |
| 0% x Level 4 | 63.16 ± 3.95$^{a}$ | 32.69 ± 8.48$^{a}$ | 51.73 ± 12.15$^{a}$ | 45.36 ± 0.83$^{a}$ | 15.21 ± 1.59$^{a}$ | 728.33 ± 17.95$^{a}$ |
| 50% x Level 1 | 43.58 ± 1.44$^{c,d}$ | 24.50 ± 6.16$^{a}$ | 56.21 ± 14.18$^{a}$ | 38.63 ± 1.61$^{a}$ | 14.16 ± 1.27$^{a}$ | 446.25 ± 110.00$^{a}$ |
| 50% x Level 2 | 51.50 ± 2.83$^{b,c}$ | 25.64 ± 4.25$^{a}$ | 49.78 ± 8.66$^{a}$ | 42.13 ± 2.47$^{a}$ | 14.88 ± 0.94$^{a}$ | 546.66 ± 52.44$^{a}$ |
| 50% x Level 3 | 50.08 ± 3.53$^{b,c,d}$ | 26.77 ± 7.88$^{a}$ | 53.45 ± 19.83$^{a}$ | 43.51 ± 4.53$^{a}$ | 17.06 ± 2.76$^{a}$ | 582.91 ± 38.75$^{a}$ |
| 50% x Level 4 | 44.33 ± 4.80$^{c,d}$ | 33.22 ± 10.20$^{a}$ | 74.93 ± 14.48$^{a}$ | 45.04 ± 4.12$^{a}$ | 12.54 ± 0.32$^{a}$ | 648.33 ± 61.04$^{a}$ |
| 100% x Level 1 | 39.83 ± 1.76$^{d}$ | 16.25 ± 2.16$^{a}$ | 40.79 ± 9.03$^{a}$ | 25.17 ± 4.97$^{a}$ | 12.08 ± 2.23$^{a}$ | 193.75 ± 25.82$^{a}$ |
| 100% x Level 2 | 46.58 ± 7.25$^{c,d}$ | 17.75 ± 3.55$^{a}$ | 38.10 ± 8.90$^{a}$ | 28.56 ± 4.19$^{a}$ | 11.86 ± 2.26$^{a}$ | 252.50 ± 64.55$^{a}$ |
| 100% x Level 3 | 51.33 ± 2.15$^{b,c}$ | 21.14 ± 7.54$^{a}$ | 41.18 ± 16.64$^{a}$ | 29.63 ± 7.12$^{a}$ | 14.35 ± 2.71$^{a}$ | 321.66 ± 33.78$^{a}$ |
| 100% x Level 4 | 47.91 ± 6.27$^{b,c,d}$ | 25.61 ± 10.75$^{a}$ | 53.45 ± 30.72$^{a}$ | 27.94 ± 3.45$^{a}$ | 15.05 ± 0.40$^{a}$ | 448.41 ± 67.06$^{a}$ |
| *F-value* | 6.19 | 0.52 | 0.85 | 0.13 | 1.57 | 1.11 |
| *P-value* | ***0.001** | 0.46$^{ns}$ | 0.54$^{ns}$ | 0.99$^{ns}$ | 0.22$^{ns}$ | 0.39$^{ns}$ |

Means followed by the same letter in the same column are statistically non-significant ($P<0.05$); ns = not significant; * = $F$-ratio probability of $P<0.05$. Plant height (cm/plant); Leaf area ($cm^2$/plant); FFM (g/plant)

**Table 5.3** Summary of ANOVA for the relative growth of the stem (RGR) as affected by hail-simulated damage (0%, 50%, and 100% defoliation) and the application of PGRs-mixtures (level 1 consisted of distilled water; level 2 had gibberellic acid (GA)-1.26 mg/kg, brassinosteroids (BRs)-0.51 mg/kg, and traces of cytokinins (CKs)-0.025 mg/kg; level 3 had GA-2.55 mg/kg, BRs-1.02 mg/kg, and traces of CKs-0.05 mg/kg; level 4 had GA-3.83 mg/kg, BRs-1.53 mg/kg, and traces of CKs-0.075 mg/kg) in different weeks (week 1, week 2, week 3, week 4, week 5, and week 6) on rose geranium plants (Central University of Technology, Free State).

| **Treatments** | **Df** | ***F*-value** | ***P*-value** |
|---|---|---|---|
| **Week** | 5 | 0.48 | 0.78 |
| **Simulated hail damage** | 2 | 5.83 | ***0.001** |
| **PGRs-mixtures** | 3 | 5.01 | ***0.002** |
| **Week x Simulated hail damage** | 10 | 1.96 | ***0.04** |
| **Week x PGRs-mixtures** | 15 | 0.59 | 0.88 |
| **Simulated hail damage x PGRs-mixtures** | 6 | 2.06 | 0.06 |
| **Week x Simulated hail damage x PGRs-mixtures** | 30 | 0.64 | 0.92 |

**Table 5.4:** The effects of hail-simulated damage (0%, 50%, and 100% defoliation) and the application of PGRs-mixtures (level 1 consisted of distilled water; level 2 had gibberellic acid (GA)-1.26 mg/kg, brassinosteroids (BRs)-0.51 mg/kg, and traces of cytokinins (CKs)-0.025 mg/kg; level 3 had GA-2.55 mg/kg, BRs-1.02 mg/kg, and traces of CKs-0.05 mg/kg; level 4 had GA-3.83 mg/kg, BRs-1.53 mg/kg, and traces of CKs-0.075 mg/kg) on the leaf surfaces of rose geranium leaves (Central University of Technology, Free State).

| **Treatments** | **Adaxial leaf surface** | | **Abaxial leaf surface** | |
|---|---|---|---|---|
| | **Asciiform** | **Brevicollate** | **Asciiform** | **Brevicollate** |
| **Simulated hail damage** | | | | |
| 0% defoliation | 27.55 ± 7.39[a] | 21.11 ± 4.15[a] | 41.33 ± 6.03[a] | 47.33 ± 15.28[a] |
| 50% defoliation | 24.66 ± 7.77[a] | 29.66 ± 5.81[a] | 33.77 ± 10.64[b] | 44.99 ± 8.67[a] |
| 100% defoliation | 15.99 ± 5.40[b] | 27.77 ± 6.83[a] | 37.55 ± 5.68[a,b] | 47.22 ± 17.66[a] |
| *F-value* | 25.92 | 14.12 | 7.25 | 0.38 |
| *P-value* | ***0.001** | 0.69[ns] | ***0.001** | 0.69[ns] |
| **Plant growth regulators (PGRs)** | | | | |
| Level 1 | 22.44 ± 4.09[a] | 21.55 ± 4.33[c] | 39.55 ± 9.27[a] | 30.88 ± 10.19[b] |
| Level 2 | 20.33 ± 13.58[a] | 25.33 ± 4.87[b,c] | 39.00 ± 7.93[a] | 47.88 ± 5.15[a] |
| Level 3 | 25.11 ± 7.89[a] | 30.11 ± 6.07[a] | 37.66 ± 4.84[a] | 55.00 ± 11.09[a] |
| Level 4 | 23.00 ± 5.67[a] | 27.55 ± 8.60[a,b] | 33.00 ± 9.98[a] | 52.22 ± 14.54[a] |
| *F-value* | 1.98 | 17.56 | 0.39 | 7.56 |
| *P-value* | 0.14[ns] | ***0.001** | 0.80[ns] | ***0.001** |
| **HD x PGRs levels** | | | | |
| 0% x Level 1 | 22.00 ± 7.00[b,c] | 18.33 ± 2.51[a] | 48.66 ± 2.08[a] | 27.33 ± 10.96[c,d] |
| 0% x Level 2 | 37.66 ± 1.52[a] | 21.33 ± 3.78[a] | 35.66 ± 2.88[a,b,c] | 46.66 ± 8.96[a,b,c,d] |
| 0% x Level 3 | 27.33 ± 0.57[a,b,c] | 24.66 ± 4.16[a] | 41.66 ± 5.50[a,b] | 63.66 ± 5.50[a] |
| 0% x Level 4 | 23.33 ± 4.72[b,c] | 20.33 ± 5.03[a] | 38.50 ± 2.12[a,b,c] | 51.66 ± 5.03[a,b] |
| 50% x Level 1 | 25.00 ± 1.00[b,c] | 24.33 ± 3.21[a] | 29.33 ± 1.52[b,c] | 25.66 ± 9.71[d] |
| 50% x Level 2 | 15.33 ± 4.04[c,d] | 30.66 ± 1.52[a] | 48.33 ± 3.78[a] | 48.00 ± 1.00[a,b,c,d] |
| 50% x Level 3 | 32.00 ± 6.24[a,b] | 37.00 ± 3.00[a] | 35.00 ± 4.00[a,b,c] | 47.00 ± 14.79[a,b,c,d] |
| 50% x Level 4 | 26.33 ± 7.76[a,b,c] | 25.00 ± 1.41[a] | 22.33 ± 6.65[c] | 68.33 ± 6.02[a] |
| 100% x Level 1 | 20.33 ± 0.57[c,d,e] | 22.00 ± 5.56[a] | 40.66 ± 7.37[a,b] | 39.66 ± 5.03[b,c,d] |
| 100% x Level 2 | 8.00 ± 1.73[f] | 24.00 ± 3.00[a] | 33.00 ± 5.29[b,c] | 49.00 ± 4.58[a,b,c] |
| 100% x Level 3 | 16.00 ± 2.64[c,d] | 28.66 ± 1.52[a] | 36.33 ± 3.21[a,b,c] | 54.33 ± 5.77[a,b] |
| 100% x Level 4 | 19.33 ± 3.05[c,d] | 36.33 ± 5.68[a] | 40.00 ± 5.29[a,b] | 36.66 ± 5.68[b,c,d] |
| *F-value* | 10.10 | 0.62 | 3.18 | 6.23 |
| *P-value* | ***0.001** | 0.08[ns] | ***0.001** | ***0.005** |

Means followed by the same letter in the same column are statistically non-significant ($P<0.05$); ns = not significant; * = $F$-ratio probability of $P<0.05$.

**Table 5.5:** The effects of hail-simulated damage (0%, 50%, and 100% defoliation) and the application of PGRs-mixtures (level 1 consisted of distilled water; level 2 had gibberellic acid (GA)-1.26 mg/kg, brassinosteroids (BRs)-0.51 mg/kg, and traces of cytokinins (CKs)-0.025 mg/kg; level 3 had GA-2.55 mg/kg, BRs-1.02 mg/kg, and traces of CKs-0.05 mg/kg; level 4 had GA-3.83 mg/kg, BRs-1.53 mg/kg, and traces of CKs-0.075 mg/kg) on essential oil mass yield of rose geranium (Central University of Technology, Free State).

| Treatments | Oil mass (g/plant) | Oil content (%) |
|---|---|---|
| **Simulated hail damage** | | |
| 0% defoliation | $3.06 \pm 0.77^{a}$ | $0.44 \pm 0.09^{a}$ |
| 50% defoliation | $2.24 \pm 0.81^{b}$ | $0.39 \pm 0.12^{a}$ |
| 100% defoliation | $0.55 \pm 0.42^{c}$ | $0.17 \pm 0.12^{b}$ |
| *F-value* | 3.58 | 9.81 |
| *P-value* | ***0.001** | ***0.001** |
| **Plant growth regulators (PGRs)** | | |
| Level 1 | $1.29 \pm 1.02^{c}$ | $0.27 \pm 0.14^{b}$ |
| Level 2 | $1.78 \pm 1.07^{b,c}$ | $0.32 \pm 0.13^{a,b}$ |
| Level 3 | $2.45 \pm 1.66^{a}$ | $0.38 \pm 0.10^{a}$ |
| Level 4 | $2.29 \pm 0.99^{a,b}$ | $0.36 \pm 0.14^{a,b}$ |
| *F-value* | 13.07 | 3.96 |
| *P-value* | ***0.01** | ***0.02** |
| **HD x PGRs levels** | | |
| 0% x Level 1 | $2.44 \pm 0.46^{b}$ | $0.43 \pm 0.06^{a,b,c}$ |
| 0% x Level 2 | $2.51 \pm 0.49^{b}$ | $0.35 \pm 0.09^{a,b,c,d}$ |
| 0% x Level 3 | $3.98 \pm 0.47^{a}$ | $0.53 \pm 0.06^{a}$ |
| 0% x Level 4 | $3.31 \pm 0.44^{a,b}$ | $0.45 \pm 0.06^{a,b}$ |
| 50% x Level 1 | $1.18 \pm 0.53^{c,d}$ | $0.25 \pm 0.05^{b,c,d,e}$ |
| 50% x Level 2 | $2.39 \pm 0.50^{b,c}$ | $0.43 \pm 0.04^{a,b}$ |
| 50% x Level 3 | $3.02 \pm 0.49^{a,b}$ | $0.52 \pm 0.10^{a}$ |
| 50% x Level 4 | $2.38 \pm 0.47^{b,c}$ | $0.37 \pm 0.11^{a,b,c}$ |
| 100% x Level 1 | $0.25 \pm 0.23^{d}$ | $0.12 \pm 0.09^{d,e}$ |
| 100% x Level 2 | $0.44 \pm 0.24^{d}$ | $0.19 \pm 0.13^{c,d,e}$ |
| 100% x Level 3 | $0.35 \pm 0.10^{d}$ | $0.10 \pm 0.02^{e}$ |
| 100% x Level 4 | $1.17 \pm 0.26^{c,d}$ | $0.26 \pm 0.04^{b,c,d,e}$ |
| *F-value* | 3.76 | 3.82 |
| *P-value* | ***0.01** | ***0.01** |

Means followed by the same letter in the same column are statistically non-significant ($P<0.05$); ns = not significant; * = *F*-ratio probability of $P<0.05$.

**Table 5.6:** The effects of hail-simulated damage (0%, 50%, and 100% defoliation) and the application of PGRs-mixtures (level 1 consisted of distilled water; level 2 had gibberellic acid (GA)-1.26 mg/kg, brassinosteroids (BRs)-0.51 mg/kg, and traces of cytokinins (CKs)-0.025 mg/kg; level 3 had GA-2.55 mg/kg, BRs-1.02 mg/kg, and traces of CKs-0.05 mg/kg; level 4 had GA-3.83 mg/kg, BRs-1.53 mg/kg, and traces of CKs-0.075 mg/kg) on *cis*-Rose oxide, *trans*-Rose oxide, isomenthone, linalool, citronellol, geraniol, and the C:G ratio of rose geranium (Central University of Technology, Free State).

| Treatments | *cis*-RO | *trans*-RO | Isomenthone | Linalool | Citronellol | Geraniol | C:G ratio |
|---|---|---|---|---|---|---|---|
| **Simulated hail damage** | | | | | | | |
| 0% defoliation | 0.20 ± 0.05[a] | 0.10 ± 0.02[a] | 1.75 ± 0.81[a] | 0.41 ± 0.19[b] | 23.23 ± 4.14[a] | 8.37 ± 0.80[a] | 2.77 ±0.72[a] |
| 50% defoliation | 0.13 ± 0.03[b] | 0.06 ± 0.02[b] | 1.83 ± 1.13[a] | 0.63 ± 0.15[a] | 24.26 ± 2.08[a] | 8.98 ± 1.04[a] | 2.70 ± 0.35[a] |
| 100% defoliation | 0.05 ± 0.02[c] | 0.02 ± 0.01[c] | 1.07 ± 0.68[a] | 0.76 ± 0.11[a] | 19.97 ± 2.27[b] | 9.11 ± 1.89[a] | 2.19 ± 0.36[b] |
| *F-value* | 8.83 | 3.12 | 2.63 | 11.11 | 11.26 | 2.23 | 3.86 |
| *P-value* | ***0.001** | ***0.01** | 0.10[ns] | ***0.009** | ***0.003** | 0.13[ns] | ***0.001** |
| **Plant growth regulators (PGRs)** | | | | | | | |
| Level 1 | 0.11 ± 0.07[a] | 0.05 ± 0.04[a] | 1.36 ± 0.89[a] | 0.61 ± 0.25[a] | 20.26 ± 3.84[b] | 8.14 ± 1.47[c] | 2.48 ± 0.60[a] |
| Level 2 | 0.15 ± 0.06[a] | 0.08 ± 0.02[a] | 1.91 ± 0.84[a] | 0.67 ± 0.19[a] | 23.50 ± 1.94[a] | 9.76 ± 1.30[a] | 2.40 ± 0.42[a] |
| Level 3 | 0.13 ± 0.07[a] | 0.06 ± 0.03[a] | 1.32 ± 1.20[a] | 0.54 ± 0.17[a] | 23.31 ± 2.94[a] | 8.30 ± 0.53[a,c] | 2.81 ± 0.35[a] |
| Level 4 | 0.12 ± 0.07[a] | 0.05 ± 0.03[a] | 1.71 ± 0.77[a] | 0.61 ± 0.22[a] | 23.28 ± 3.91[a] | 9.24 ± 1.26[a,b] | 2.51 ± 0.78[a] |
| *F-value* | 0.63 | 1.19 | 0.66 | 0.71 | 5.29 | 7.02 | 5.35 |
| *P-value* | 0.60[ns] | 0.34[ns] | 0.58[ns] | 0.56[ns] | ***0.02** | ***0.03** | 0.09[ns] |
| **HD x PGRs levels** | | | | | | | |
| 0% x Level 1 | 0.17 ± 0.09[a] | 0.08 ± 0.04[a] | 2.00 ± 0.98[a] | 0.80 ± 0.09[a] | 17.36 ± 1.01[d] | 9.15 ± 0.33[a,b] | 1.89 ± 0.18[f] |
| 0% x Level 2 | 0.22 ± 0.02[a] | 0.11 ± 0.01[a] | 2.23 ± 0.74[a] | 0.73 ± 0.01[a] | 24.24 ± 1.73[b] | 8.83 ± 0.47[b] | 2.75 ± 0.32[b,c,d] |
| 0% x Level 3 | 0.20 ± 0.04[a] | 0.10 ± 0.03[a] | 0.99 ± 0.66[a] | 0.69 ± 0.08[a] | 24.99 ± 1.61[a,b] | 7.84 ± 0.21[b] | 3.18 ± 0.16[a,b] |
| 0% x Level 4 | 0.22 ± 0.04[a] | 0.10 ± 0.01[a] | 1.78 ± 0.36[a] | 0.88 ± 0.22[a] | 27.87 ± 0.09[a] | 7.30 ± 0.30[b] | 3.81 ± 0.16[a] |
| 50% x Level 1 | 0.13 ± 0.02[a] | 0.06 ± 0.01[a] | 1.34 ± 0.97[a] | 0.68 ± 0.19[a] | 24.26 ± 2.09[b] | 7.65 ± 0.75[b] | 3.17 ± 0.18[b,c] |
| 50% x Level 2 | 0.13 ± 0.01[a] | 0.06 ± 0.01[a] | 1.65 ± 1.10[a] | 0.71 ± 0.16[a] | 23.85 ± 1.79[b] | 9.37 ± 0.50[a,b] | 2.54 ± 0.09[c,d,e] |
| 50% x Level 3 | 0.15 ± 0.01[a] | 0.07 ± 0.01[a] | 2.11 ± 1.89[a] | 0.56 ± 0.10[a] | 25.09 ± 1.66[a,b] | 8.78 ± 0.29[b] | 2.85 ± 0.09[b,c,d] |
| 50% x Level 4 | 0.11 ± 0.07[a] | 0.05 ± 0.04[a] | 2.24 ± 0.72[a] | 0.60 ± 0.16[a] | 23.86 ± 3.45[b] | 10.11 ± 0.53[a,b] | 2.36 ± 0.26[d,e,f] |
| 100% x Level 1 | 0.04 ± 0.01[a] | 0.01 ± 0.01[a] | 0.73 ± 0.25[a] | 0.35 ± 0.21[a] | 19.17 ± 3.86[d,c] | 7.61 ± 2.39[b] | 2.51 ± 0.43[b,c,d,e] |
| 100% x Level 2 | 0.08 ± 0.04[a] | 0.05 ± 0.01[a] | 1.83 ± 0.96[a] | 0.53 ± 0.39[a] | 21.86 ± 2.54[b,c] | 11.74 ± 0.25[a] | 1.86 ± 0.16[f] |
| 100% x Level 3 | 0.03 ± 0.01[a] | 0.01 ± 0.01[a] | 0.85 ± 0.60[a] | 0.36 ± 0.15[a] | 19.85 ± 1.54[d,c] | 8.29 ± 0.57[b] | 2.39 ± 0.06[d,e,f] |
| 100% x Level 4 | 0.06 ± 0.01[a] | 0.03 ± 0.01[a] | 1.14 ± 0.78[a] | 0.46 ± 0.14[a] | 19.65 ± 0.69[d,c] | 9.68 ± 0.32[a,b] | 2.02 ± 0.01[e,f] |
| *F-value* | 0.61 | 0.87 | 0.84 | 0.54 | 4.22 | 5.63 | 4.44 |
| *P-value* | 0.71[ns] | 0.53[ns] | 0.55[ns] | 0.76[ns] | ***0.006** | ***0.02** | ***0.01** |

Means followed by the same letter in the same column are statistically non-significant ($P$<0.05); ns = not significant; RO = Rose oxide; * = $F$-ratio probability of $P$<0.05.

**Table 5.7:** The effects of hail-simulated damage (0%, 50%, and 100% defoliation) and the application of PGRs-mixtures (level 1 consisted of distilled water; level 2 had gibberellic acid (GA)-1.26 mg/kg, brassinosteroids (BRs)-0.51 mg/kg, and traces of cytokinins (CKs)-0.025 mg/kg; level 3 had GA-2.55 mg/kg, BRs-1.02 mg/kg, and traces of CKs-0.05 mg/kg; level 4 had GA-3.83 mg/kg, BRs-1.53 mg/kg, and traces of CKs-0.075 mg/kg) on citronellyl formate, geranyl formate, geranyl butyrate, geranyl tiglate and guaia-6,9-diene of rose geranium (Central University of Technology, Free State).

| Treatments | Geranyl formate | Geranyl butyrate | Geranyl tiglate | Citronellyl formate | Guaia-6,9-diene |
|---|---|---|---|---|---|
| **Simulated hail damage** | | | | | |
| 0% defoliation | $5.40 \pm 0.69^{b}$ | $1.01 \pm 0.23^{b}$ | $1.93 \pm 0.45^{b}$ | $18.71 \pm 0.77^{b}$ | $11.35 \pm 0.49^{a}$ |
| 50% defoliation | $5.85 \pm 0.55^{b}$ | $0.93 \pm 0.15^{b}$ | $1.98 \pm 0.37^{b}$ | $18.65 \pm 0.84^{b}$ | $11.92 \pm 0.43^{a}$ |
| 100% defoliation | $8.37 \pm 0.98^{a}$ | $1.30 \pm 0.28^{a}$ | $2.74 \pm 0.61^{a}$ | $19.87 \pm 1.37^{a}$ | $12.23 \pm 0.88^{a}$ |
| *F-value* | 5.10 | 9.51 | 11.16 | 10.12 | 0.84 |
| *P-value* | ***0.001** | ***0.002** | ***0.001** | ***0.001** | $0.20^{ns}$ |
| **Plant growth regulators (PGRs)** | | | | | |
| Level 1 | $6.46 \pm 1.33^{a}$ | $1.16 \pm 0.28^{a}$ | $2.32 \pm 0.53^{a}$ | $19.39 \pm 1.04^{a}$ | $11.86 \pm 0.82^{a}$ |
| Level 2 | $6.40 \pm 0.96^{a}$ | $0.99 \pm 0.13^{a}$ | $1.97 \pm 0.27^{a}$ | $18.23 \pm 0.51^{b}$ | $11.59 \pm 0.43^{a}$ |
| Level 3 | $6.61 \pm 1.84^{a}$ | $1.15 \pm 0.34^{a}$ | $2.42 \pm 0.80^{a}$ | $18.52 \pm 0.62^{b}$ | $12.16 \pm 0.82^{a}$ |
| Level 4 | $6.72 \pm 2.05^{a}$ | $0.99 \pm 0.26^{a}$ | $2.04 \pm 0.59^{a}$ | $20.15 \pm 1.26^{a}$ | $11.67 \pm 0.63^{a}$ |
| *F-value* | 0.10 | 1.82 | 2.08 | 9.77 | 2.23 |
| *P-value* | $0.95^{ns}$ | $0.18^{ns}$ | $0.14^{ns}$ | ***0.007** | $0.12^{ns}$ |
| **HD x PGRs levels** | | | | | |
| 0% x Level 1 | $5.84 \pm 0.94^{a}$ | $1.23 \pm 0.39^{a}$ | $2.33 \pm 0.75^{a}$ | $18.32 \pm 0.50^{a}$ | $11.29 \pm 0.87^{a}$ |
| 0% x Level 2 | $5.62 \pm 0.58^{a}$ | $0.97 \pm 0.07^{a}$ | $1.79 \pm 0.16^{a}$ | $18.33 \pm 0.78^{a}$ | $11.26 \pm 0.47^{a}$ |
| 0% x Level 3 | $5.31 \pm 0.28^{a}$ | $1.01 \pm 0.05^{a}$ | $1.91 \pm 0.13^{a}$ | $18.72 \pm 0.13^{a}$ | $11.47 \pm 0.33^{a}$ |
| 0% x Level 4 | $4.56 \pm 0.13^{a}$ | $0.78 \pm 0.03^{a}$ | $1.57 \pm 0.05^{a}$ | $19.87 \pm 0.79^{a}$ | $11.38 \pm 0.43^{a}$ |
| 50% x Level 1 | $5.60 \pm 0.68^{a}$ | $1.02 \pm 0.24^{a}$ | $2.03 \pm 0.28^{a}$ | $19.44 \pm 0.19^{a}$ | $11.89 \pm 0.58^{a}$ |
| 50% x Level 2 | $6.48 \pm 0.39^{a}$ | $0.94 \pm 0.02^{a}$ | $1.96 \pm 0.22^{a}$ | $17.93 \pm 0.26^{a}$ | $11.90 \pm 0.32^{a}$ |
| 50% x Level 3 | $5.53 \pm 0.20^{a}$ | $0.89 \pm 0.08^{a}$ | $1.92 \pm 0.11^{a}$ | $17.81 \pm 0.24^{a}$ | $12.09 \pm 0.70^{a}$ |
| 50% x Level 4 | $5.77 \pm 0.29^{a}$ | $0.89 \pm 0.20^{a}$ | $2.03 \pm 0.79^{a}$ | $19.41 \pm 0.41^{a}$ | $11.88 \pm 0.20^{a}$ |
| 100% x Level 1 | $7.93 \pm 0.91^{a}$ | $1.27 \pm 0.22^{a}$ | $2.74 \pm 0.34^{a}$ | $20.43 \pm 0.83^{a}$ | $12.65 \pm 0.53^{a}$ |
| 100% x Level 2 | $7.46 \pm 1.20^{a}$ | $1.10 \pm 0.29^{a}$ | $2.27 \pm 0.33^{a}$ | $18.53 \pm 0.05^{a}$ | $11.61 \pm 0.23^{a}$ |
| 100% x Level 3 | $8.99 \pm 0.85^{a}$ | $1.55 \pm 0.27^{a}$ | $3.42 \pm 0.50^{a}$ | $19.02 \pm 0.53^{a}$ | $12.92 \pm 0.70^{a}$ |
| 100% x Level 4 | $8.79 \pm 0.77^{a}$ | $1.27 \pm 0.22^{a}$ | $2.37 \pm 0.48^{a}$ | $21.07 \pm 1.71^{a}$ | $11.66 \pm 1.05^{a}$ |
| *F-value* | 2.46 | 1.73 | 1.91 | 1.44 | 0.49 |
| *P-value* | $0.07^{ns}$ | $0.18^{ns}$ | $0.14^{ns}$ | $0.25^{ns}$ | $0.30^{ns}$ |

Means followed by the same letter in the same column are statistically non-significant ($P<0.05$); ns = not significant; * = $F$-ratio probability of $P<0.05$.

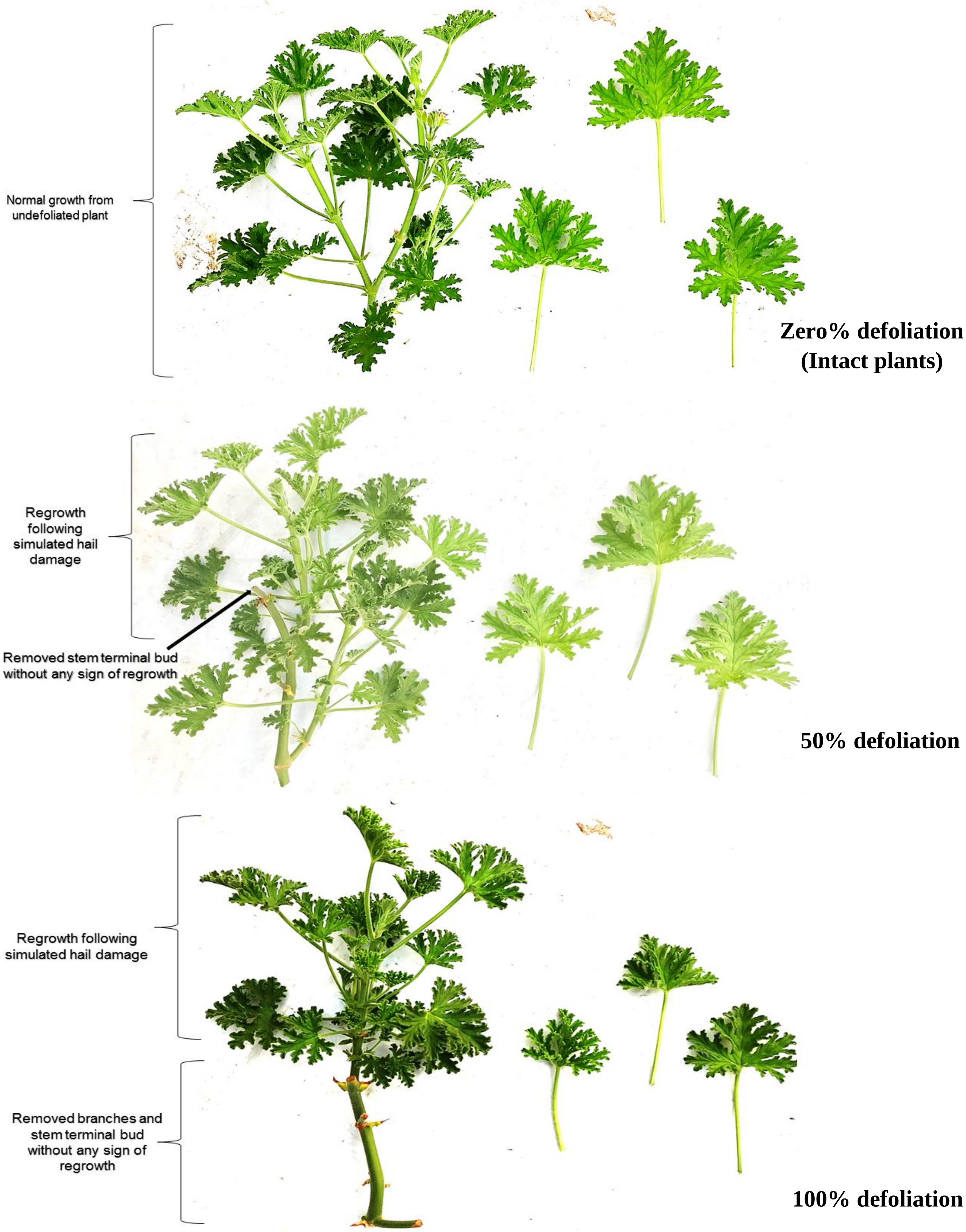


**Figure 5.1:** Terminal buds from simulated hail-damaged rose geranium plant (Central University of Technology, Free State).

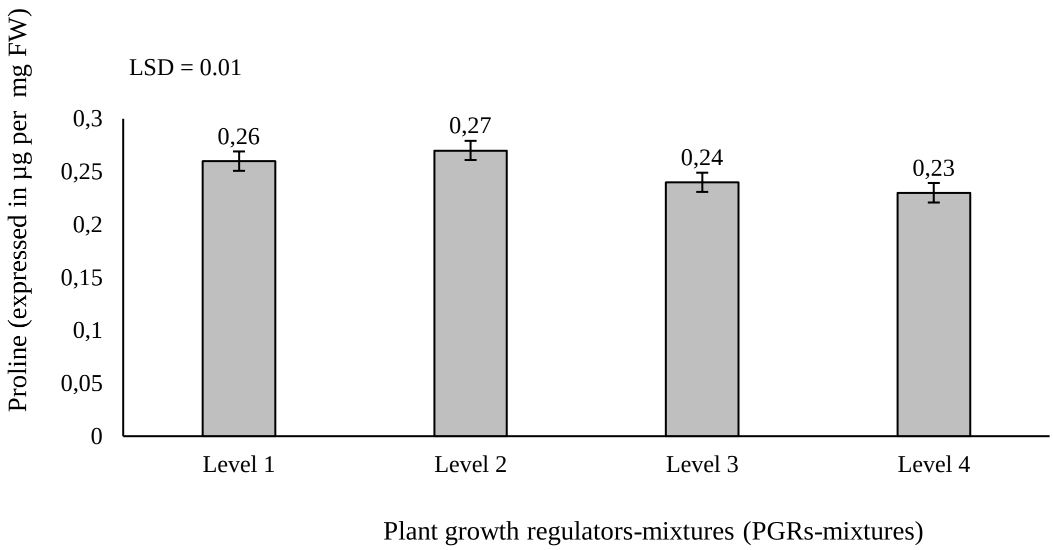


**Figure 5.2:** The effects of PGRs-mixtures (level 1 consisted of distilled water; level 2 had gibberellic acid (GA)-1.26 mg/kg, brassinosteroids (BRs)-0.51 mg/kg, and traces of cytokinins (CKs)-0.025 mg/kg; level 3 had GA-2.55 mg/kg, BRs-1.02 mg/kg, and traces of CKs-0.05 mg/kg; level 4 had GA-3.83 mg/kg, BRs-1.53 mg/kg, and traces of CKs-0.075 mg/kg) on free proline of rose geranium (Central University of Technology, Free State).

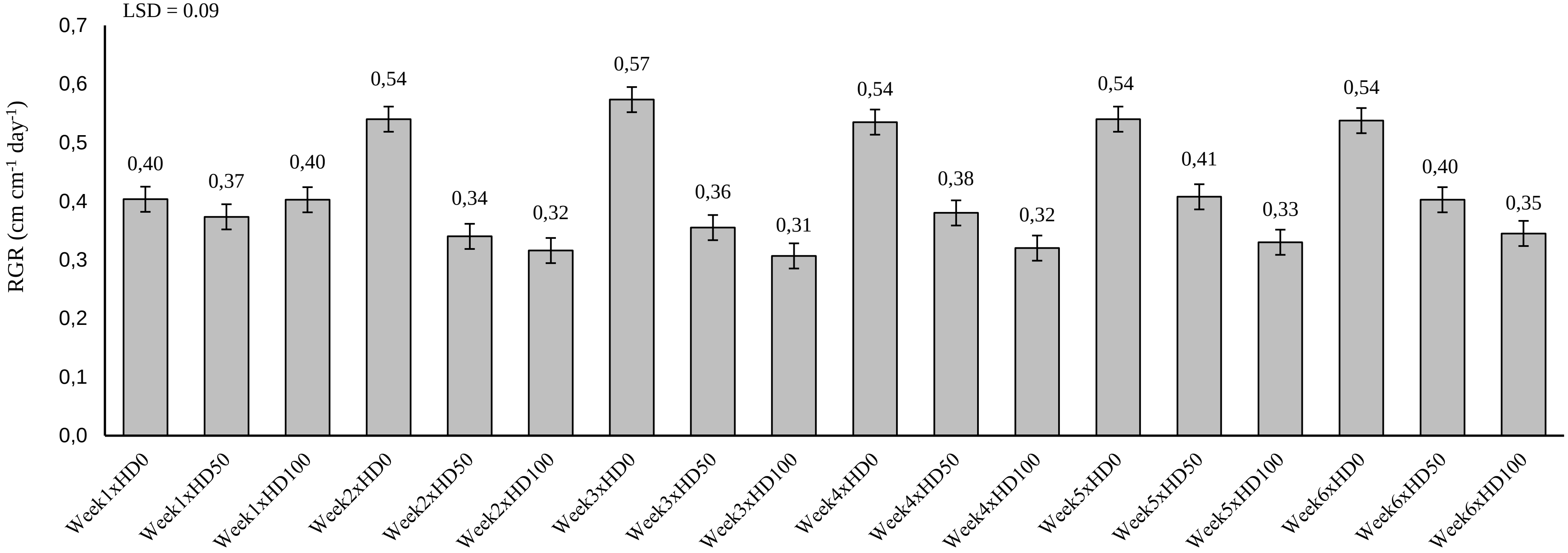


**Figure 5.3:** The effects of simulated hail damage (zero%, 50%, and 100% defoliation) between the weeks (week 1, week 2, week 3, week 4, week 5, week 6) on the relative growth rate of rose geranium stems (Central University of Technology, Free State). **Abbreviations:** HD0 = zero% defoliation; HD50 = 50% defoliation; HD100 = 100% defoliation.

# CHAPTER SIX

## THE EFFECTS OF AGRICULTURAL PLANT GROWTH REGULATORS-MIXTURES ON LEAF MINERAL STATUS AND PHENOLIC COMPOSITION OF SIMULATED HAIL-DAMAGED ROSE GERANIUM (*Pelargonium graveolens* L'Hér. cv. 'BOURBON')

## Abstract

Phytohormones regulate growth, improve nutrient acquisition, and increase the antioxidant capacity of plant tissue following external stress, therefore the ability to stimulate them is crucial to their cultivation. The aim of this study was to determine the ability of exogenous applications of plant growth regulators (PGRs) to improve the leaf mineral status, the plant carbon to nitrogen ratio, and the concentrations of phenolic compounds in rose geranium (*Pelargonium graveolens* L'Hér.) exposed to simulated hail damage. This experiment was conducted under a temperature-controlled greenhouse in a 3 x 4 factorial treatment design with three replications. The treatments consisted of three hail damage simulation levels (zero%, 50%, and 100% defoliation), and four PGRs-mixtures. The principal component analysis plot showed that simulated hail damage caused an accumulation of caffeic acid, caffeoyl tartaric acid, p-Coumaroyl tyrosine, and protocatechuic acid O-hexoside. The application of the PGRs-mixtures on plants which sustained 100% foliage loss strongly correlated with the levels of nitrogen, phosphate, potassium, magnesium and manganese. However, this treatment caused a decline in the carbon to nitrogen ratio by 16.7%. It can be concluded that a mixture of PGRs may be applied to enhance the accumulation of minerals associated with the recovery of the foliar material in hail-damaged rose geranium. This study provide useful information to growers that hail-damaged rose geranium plants can be recovered using PGRs.



**Keywords:** Brassinosteroids; cytokinin; defoliation; gibberellic acid, plant growth regulators.


## 6.1 Introduction

Abiotic stresses affect plant growth and development at the physiological, biochemical, and molecular levels, which can lead to a reduction in crop productivity (Wani *et al.* 2016). Amongst the abiotic stress conditions, hail damage is one of the most severe environmental stress factors, causing defoliation and wounding of stems and flowers (Wani *et al.* 2016). Defoliation and wounding stress results in mineral shock, subsequently reducing the assimilation of carbon (Smékalová *et al.* 2014). In addition, this may affect the physiological processes such as the photosynthesis rate, the nutrient balance, also the N acquisition.

Plants respond to defoliation and wounding through the induction of phenylpropanoids metabolism to accumulate phenolic compounds (Bernards and Båstrup-Spohr 2008). Phenolics provide cytotoxic effects, as well as the building blocks for polyphenolic-based cell wall modifications (Kim *et al.* 2019). Polyphenolic-based cell wall modifications assist with organ regeneration in defoliated or wounded plants (Bernards and Båstrup-Spohr 2008; Nanda and Melnyk 2018). The level of endogenous phenolics in refoliated plants influences plant growth and development, clearly indicating the relation of the leaf refoliation with the leaf ontogeny (Maia *et al.* 2007).

The age of the plant is associated with the level of the total phenolics. On wampee (*Clausena lansium* Lour.), Chang *et al.* (2018) reported high total leaf phenolics in early growth stages compared to the later growth stages. A high phenolic content was also observed in the early stages of leaf development of yerba-mate (*Ilex paraguariesnsis* A.St Hil.), which affected the development and quality of these plants (Blum-Silva *et al.* 2015). High phenolics level in young leaves may be associated with defence and refoliation following defoliation (Yoshioka *et al.* 2004).

The complexity of the total minerals and phenolic pathways data from the treated plants may result in substantial and complex data (Mishra *et al.* 2018). Multivariate data analysis, using the principal component analysis (PCA), is a suitable technique to reduce the dimensionality of such datasets, increasing interpretability but at the same time minimizing information loss. The PCA transforms the data into a new set of variables, the principal components, which are uncorrelated, and which are ordered so that the first few retain most of the variation present in all of the original variables (Mishra *et al.* 2018).

Previous studies have demonstrated that exogenous application of agricultural plant growth regulators (PGRs) on plants may effectively stimulate vegetative growth, improve nutrient acquisition, and increases the antioxidant capacity of plant tissue (Parađiković *et al.* 2019). Aspects of phenolics accumulation are driven by upregulation of endogenous phytohormones. However, this occurs through crosstalk networks between these phytohormones, for which some commercially available (Wani *et al.* 2016). A combination of spermine, methyl jasmonate, and epibrassinolide was found to induce secondary metabolites, including phenolics, in sweet basil (*Ocimum basilicum* L.) (Koca and Karaman 2014). From these studies, it is evident that the accumulation of the plant phenolics may be associated with the mineral balance in the plant. According to Treutter (2010), maintenance of minerals is a prerequisite for providing co-factors for many enzymes of the phenolic pathway.

After defoliation, and during the process of leaf refoliation, there are trade-offs between plant growth, mineral acquisition, and secondary metabolism, which may counteract with the management of phenolic compounds for agricultural purposes. The response of plants to PGRs have been investigated; however, the regulatory mechanism of induced phenolics compounds by the same growth regulators on refoliating plants are not well understood. Therefore, this study aimed to determine the ability of exogenous application of PGRs to improve the leaf mineral status, plant carbon to nitrogen ratio, and the phenolic compounds of a hail-damaged rose geranium.

## 6.2 Materials and methods

This study was conducted concurrently with the experiment in Chapter 5. For a detailed description of the field trial and agronomic practices, please refer to Chapter 5.

### 6.2.1 Parameters

For the leaf mineral analysis, top, middle and below leaves were oven-dried (ECOtherm labotec oven, RSA) at a temperature of 60°C for 48 hours. After the drying process, ca. 2 g of the leaf material was mechanically milled to ca. 0.25 mm diameter, using a micro hammer mill (Culatti, Zurich) to produce powdery content for major and minor mineral analysis (B, Ca, Cu, Fe, K, Mg, Na, P, Mn, and Zn). An inductively coupled plasma Optical Emission Spectroscopy (ICP-OES) instrument (Thermo iCAP 6000 series, Thermo Scientific, USA) was used for the determination of major and minor mineral analysis in the rose geranium leaves. The operating condition for the ICP-OES instrument was established after optimization of the instrumental parameters (Table 6.1).

Nitric acid (6 mL) and hydrogen peroxide (1 mL) were used for sample digestion. These chemicals were added to ca. 0.5 g of dried homogenous sample, and digested using a CEM MARS microwave digester (CEM Corporation, MARS 6 classic, USA) with operating condition shown in (Table 6.1). The vessels were cooled, and samples were made up to a final volume of 50 mL containing 1% of hydrochloric acid. Chromatograms for 11 elements were obtained simultaneously following specific wavelengths (Table 6.2) (Masson *et al.* 2010).

The analysis of N and S were carried out using Vario EL Cube Elemental analyser (Elementar Vario PYRO cube, Elementary, Hanau, Germany). Samples were oxidized in the combustion tube (in the presence of $O_2$) at a high temperature (1050°C) using tungsten oxide

($WO_3$) as a catalyst. The combustion gas ($O_2$) was free from foreign gases (volatile halogens). Nitrogen ($N_2$), $NO_x$, $SO_2$ and $SO_3$ were separated from each other with the help of specific adsorption columns and determined in succession with a thermal conductivity detector. The Argon carrier gas carried the $N_2$, $NO_x$, $SO_2$ and $SO_3$ through the reduction tube filled with Cu wire.

The reduction column reduced the $NO_x$ to $N_2$ and $SO_3$ to $SO_2$. All the volatile halogen-bound compounds produced during combustion were attached to the silver wool in the reduction column. The operating conditions for the Vario EL Cube Elemental analyser instrument were established after optimization of the instrumental parameters (Table 6.3). Quality control was carried out by analysing certified reference material (CRM) for every sample batch after every 20 samples, and as well at the end of each batch (Dhaliwal *et al.* 2015).

For the phenolic profile assays, the plant leaf material was oven-dried (ECOt herm labotec oven, RSA) at a temperature of 60°C, for 48 hours. Approximately 2 g of dry leaves were ground in a micro hammer mill (Culatti, Zurich). The finely milled leaf material was extracted with 50% methanol in water containing 1% formic acid (15 mL). The extraction was performed in a 50 mL polypropylene centrifuge tube. This step was followed by a 60 min extraction in an ultrasonic bath (0.5 Hz, Integral Systems, RSA) at room temperature. The extracts were centrifuged (Hermle Z160m, 3000g for 5 min) and transferred to vials. Stock solutions were prepared quantitatively in the dilution series of the stock ranging from 1 to 100 $\mu$g/ml in concentration. The solvent used for the preparation of the cocktails was 50% methanol in water, containing 1% formic acid, which was the same as that used for extraction of the leaf samples.

A Waters Synapt G2 Quadrupole time-of-flight (QTOF) mass spectrometer (MS) connected to a Waters Acquity ultra-performance liquid chromatography (UPLC) (Waters, Milford, MA, USA) was used for analysis. Electrospray ionization was used in negative mode with a cone voltage of 15 V, and a desolvation temperature of 275°C, desolvation gas at 650 L/h. The rest of the MS settings optimized for best resolution and sensitivity. Data were acquired by scanning from 150 to 1500 m/z in resolution mode, as well as in MSE mode. In MSE mode, two channels of MS data were acquired, one at low collision energy (4 V) and the second using a collision energy ramp (40-100 V) also to obtain fragmentation data. Leucine enkephalin was used as lock mass (reference mass) for accurate mass determination, and the instrument was calibrated with sodium formate. The separation was achieved on a Waters HSS T3, 2.1 × 100 mm, 1.7 μm column.

An injection volume of 2 μL was used, and the mobile phase consisted of 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid as solvent B. The gradient started at 100% with solvent A for 1 min. It was linearly followed by 28% solvent B for 22 min . The gradient further went to 40% B over 50 s and a wash step of 1.5 min at 100% B, followed by re-equilibration to initial conditions for 4 min. The flow rate was 0.3 ml/min, and the column temperature was maintained at 55°C. Ion mobility data were obtained using the same UPLC gradient and column as above, and IMS Wave velocity was set at 332 m/s, and wave height at 20.2 V. Polyalanine was used for the calibration and calculations (Stander *et al.* 2017).

### 6.2.2 Statistical analysis

N, C and C:N ratio parameters were statistically analysed following the procedures described in Chapters 3 & 4. The Pearson's correlation coefficient was used to determine the relationships between the leaf minerals. A PCA was used to identify and evaluate the groupings between the variables following the description in Chapters 3 & 4 for the leaf minerals and the phenolic profiling. Waters Acquity ultra-performance liquid chromatography combined with a PCA was used to profile the phenolics. Scatter score plots and loading plots from the PCA were constructed to identify and evaluate groupings, trends, and influential outliers.

## 6.3 Results

*Leaf minerals:* A significant interaction was observed between the simulated hail damage and the subsequent application of PGRs-mixtures on the C content, the N content and C:N ratio (Table 6.4). For the intact plants and 50% defoliated plants, the C content was the same in all PGR treatments. However, in 100% defoliated plants, the mean value of the C content decreased by 11.8% when the level 2 PGR-mixture was applied, compared with level 1 (control). In addition to this, when the PGR-mixture was applied at levels 3 & 4, the C content was higher than level 2; however, this increase was not significantly different compared with the control. Nitrogen content from the 50% simulated hail-damaged plants was increased by the application of PGR-mixture from as low as level 2; the 0 and 100% were not necessarily affected by any application of the PGRs-mixtures. The lowest C:N ratio was observed when level 2 PGR-mixture was applied to plants that suffered 100% simulated hail damage. However, higher levels of the PGR-mixture did not necessarily improve the C:N ratio.

A Pearson correlation was applied to correlate the leaf mineral composition (Table 6.5). Overall, a strong positive correlation was observed between N, Ca, S and Fe. Ca was positively correlated to B, while P was correlated to K, Mg and Na. Mg was positively correlated to S, Cu and Mn, with B correlated to S and Fe. Cu was positively correlated to Zn, while Mn was positively correlated to Ca and Zn. On the other hand, a strong negative correlation was observed between Ca and Mn; Mg and Fe; S and Mn; B and Mn. Another negative correlation was observed between Fe, Mg and Mn. Mn was also negatively correlated to B. The PCA method was applied to further characterise the concentrations of leaf minerals, following hail damage simulations, and the application of PGR-mixtures (Table 6.6 & Figure 6.1). The biplot loading in PC 1 explained 42.31% of the correlation between Ca, S, B, and Fe (Figure 6.1). These minerals were found in lowest concentrations in plants applied with level 2 PGR-mixture at both intact plants and 50% defoliated plants. There was a strong positive correlation between Mg and Mn, following application with all PGR treatments on plants with 100% foliage loss (PC 1). PC 2 (29.03%) showed that N, P, and K content were also positively correlated to the application of PGR-mixture, from as low as level 2 when plants lost 100% foliage (Figure 6.1).

*Leaf phenolic profile:* The phenolic compounds on the rose geranium samples were identified using retention indices and the detected masses (Table 6.7 & Figure 6.2). Plants which were re-foliating following 100% simulated hail damage and treatment with the PGR-mixtures were shown with dotted lines are located on the right-hand region of the y axis in PCA (t[1] and t[2]) score plot (Figure 6.3 A). Overall, the PCA score and loading plots shows that all PGR-mixtures and 100% hail damage simulation were characterised by high levels of caffeic acid (9.17_179), caffeoyl tartaric acid (9.18_311), p-Coumaroyl tyrosine (12.86_327), protocatechuic acid O-hexoside (8.35_315), and other unidentified compounds (Figure 6.3 A&B).

The phenolic compounds detected on undamaged and plants with 50% foliage loss and treated with the PGR-mixtures were shown with solid, and dashed line, respectively, are located on the left-hand region of the y axis in the PCA score plot in Figure 6.3A. All the PGR levels, including the control (distilled water), were characterised by high levels of 5-Heptadecylresorcinol (12.45_349), 5-Pentacosylresorcinol (14.59_461), chicoric acid (16.10_475), citric acid (2.11_191), kaempferol-O-hexoside-O-deoxy-hexose (18.53_593), p-Coumaroyl tyrosine (12.86_327), and other unidentified compounds (Figure 6.3B).

## 6.4 Discussion

*Leaf mineral status:* Bryant *et al.* (1993) reported that C:N ratio declines following severe defoliation, and this is caused by nutritional stress resulting from severe defoliation. In addition, the levels of phytohormones with inhibitory effects rises following severe defoliation and wounding. The rise in these phytohormones suppress the levels of stimulatory phytohormones associated with the mineral acquisition, and photosynthetic activities (Ikeuchi *et al.* 2017; Nanda and Melnyk 2018; Pommerrenig *et al.* 2019). Therefore, a low C:N ratio in plants treated with level 2 PGR-mixture could be due to the antagonistic crosstalk effects between the phytohormones with inhibitory effects such as abscisic acid, and growth stimulatory phytohormones such as GA, BR and CK (Pommerrenig *et al.* 2019).

Bryant *et al.* (1993) and Dada and Oworu (2010) demonstrated that a common reaction by plants to defoliation is decline of leaf minerals, which is similar to the decline of Ca, S, B, and Fe. Nitrogen, P, K, Mg and Mn were increased with applications of PGRs where plants endured complete defoliation. According to Kudoyarova *et al.* (2015), the changes in availability of plant minerals influence the expression of genes controlling hormones metabolism, and signalling. This mechanism is depend primarily on the shoot-to-root or root-to-shoot signalling. Therefore, the application of PGRs may directly improve the acquisition of minerals associated with growth and development on refoliating plants (Pankoke and Müller 2013). This correlates with the findings of a number of previous studies, for example, Oliveira *et al.* (2010) reported that CK stimulated the accumulation of Mg in pond apple (*Annona glabra* L.). Janowska *et al.* (2018) found that GA applied to black velvet (*Gladiolus communis* L.) improved Mn acquisition. Vinković *et al.* (2012) also reported that tomatoes (*Lycopersicon esculentum* Mill.) treated with biostimulants containing PGRs increased the content of N, P, and K, compared to control plants.

*Leaf phenolic profile:* The accumulation of caffeic acid, caffeoyl tartaric acid, p-Coumaroyl tyrosine and protocatechuic acid O-hexoside are a typical plant response to defoliation, including wounding stress (Bryant *et al.* 1993; Nanda and Melnyk 2018). As hypothesised, the PGRs did not necessarily affect these phenolics. The findings on 5-Heptadecylresorcinol, 5-Pentacosylresorcinol, chicoric acid, citric acid, kaempferol-O-hexoside-O-deoxy-hexose and p-Coumaroyl tyrosine, as shown in Figure 6.3B, clearly illustrates the similarities between the non-defoliated plants and those that lost less than 50% foliage material. The hail damage simulation level of 50% was achieved by defoliating the top half (average plant height per experimental unit) of each experimental plant. Therefore, the remaining bottom leaves could have sustained the plants to recover similarly to the non-defoliated plants. As hypothesised, the PGRs did not necessarily affect these phenolics.

## 6.5 Conclusion

This study shows that 100% hail-damaged rose geranium plants accumulate most of the phenolic compounds associated with defoliation and wounding, while those with only 50% defoliation amass similar phenolic compounds as non-defoliated plants. Moreover, the C:N ratio declines with increasing intensity of the defoliation. A level 2 PGR-mixture (1.26 mg/kg GA, 0.51 mg/kg BR, and traces of CKs) improves the acquisition of N, P, K, Mg and Mn content on plants that lost 100% of their foliage. Since these minerals are directly associated with plant growth and development, this study provide useful information to growers that hail-damaged rose geranium plants can be treated with this PGR-mixture to improve recovery. This study provide useful information to growers that hail-damaged rose geranium plants can be recovered using PGRs.

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**Table 6.1:** The operating conditions for the Thermo iCAP 6000 series instrument.

| Operation | Condition |
|---|---|
| ***Operating conditions for instrument:*** | |
| RF power (W) | 1350 |
| Carrier gas (L/min) (Argon) | 0.65 |
| Aux gas (L/min) (Argon) | 1 |
| Nebuliser | 2 ml/min Micro mist |
| Internal standard used | 1 ppm Yttrium |
| ***Microwave method used for digestion:*** | |
| Power level | 1600 W, 100% |
| Ramp time | 25 min |
| Pressure | 800 psi |
| Hold time | 10 min |

**Table 6.2:** Analytical wavelengths for the mineral elements (B, Ca, Cu, Fe, K, Mg, Na, P, Mn, and Zn), ICP-OES.

| Element | Wavelength nm |
|---|---|
| B | 249.60 |
| Ca | 315.80 |
| Cu | 224.70 |
| Fe | 239.50 |
| K | 769.80 |
| Mg | 285.20 |
| Na | 330.20 |
| P | 177.40 |
| Mn | 257.60 |
| Zn | 206.20 |

**Table 6.3:** The operating conditions for the Vario EL Cube Elemental analyser (Elementar Vario PYRO cube).

| **Operating conditions for instrument** | **Condition** |
|---|---|
| Carrier gas used | Ar 5 at ca.1 bar |
| Dosing gas for combustion | $O_2$ at 2 bar |
| Combustion temperature | 1150-1050ºC (Sleep is at 800ºC) |
| Reduction column temperature | 850ºC (Sleep is at 600ºC) |
| Thermal conductivity detector (TCD) temperature | 60ºC |
| TCD airflow | 220 ml/min |

**Table 6.4:** The effects of hail-simulated damage (zero%, 50%, and 100% defoliation) and the application of PGRs-mixtures (level 1 consisted of distilled water; level 2 had gibberellic acid (GA)-1.26 mg/kg, brassinosteroids (BRs)-0.51 mg/kg, and traces of cytokinins (CKs)-0.025 mg/kg; level 3 had GA-2.55 mg/kg, BRs-1.02 mg/kg, and traces of CKs-0.05 mg/kg; level 4 had GA-3.83 mg/kg, BRs-1.53 mg/kg, and traces of CKs-0.075 mg/kg) on carbon, nitrogen, and the C:N ratio of rose geranium plant leaves (Central University of Technology, Free State).

| Treatments | Carbon | Nitrogen | C:N ratio |
|---|---|---|---|
| **Simulated hail damage** | | | |
| 0% defoliation | $44.41 \pm 0.91^{a}$ | $3.57 \pm 0.17^{c}$ | $12.43 \pm 0.60^{a}$ |
| 50% defoliation | $43.56 \pm 2.60^{a}$ | $3.73 \pm 0.27^{b}$ | $11.67 \pm 0.87^{b}$ |
| 100% defoliation | $41.95 \pm 1.12^{b}$ | $3.95 \pm 0.18^{a}$ | $10.62 \pm 0.86^{c}$ |
| *F-value* | 11.93 | 20.27 | 33.16 |
| *P-value* | ***0.003** | ***0.001** | ***0.001** |
| **Plant growth regulators (PGRs)** | | | |
| Level 1 | $43.73 \pm 1.04^{a}$ | $3.74 \pm 0.29^{a}$ | $11.69 \pm 0.98^{a}$ |
| Level 2 | $41.79 \pm 2.68^{b}$ | $3.70 \pm 0.27^{a}$ | $11.29 \pm 1.36^{a}$ |
| Level 3 | $43.36 \pm 1.23^{a,b}$ | $3.79 \pm 0.17^{a}$ | $11.44 \pm 0.72^{a}$ |
| Level 4 | $44.35 \pm 1.73^{a}$ | $3.76 \pm 0.32^{a}$ | $11.79 \pm 1.23^{a}$ |
| *F-value* | 6.80 | 0.67 | 1.72 |
| *P-value* | ***0.002** | $0.57^{ns}$ | $0.19^{ns}$ |
| **HD x PGRs levels** | | | |
| 0% x Level 1 | $44.29 \pm 0.28^{a}$ | $3.46 \pm 0.13^{e}$ | $12.80 \pm 0.46^{a}$ |
| 0% x Level 2 | $43.67 \pm 0.51^{a}$ | $3.62 \pm 0.28^{b,c,d,e}$ | $12.06 \pm 0.82^{a,b}$ |
| 0% x Level 3 | $44.24 \pm 0.21^{a}$ | $3.67 \pm 0.13^{b,c,d,e}$ | $12.05 \pm 0.38^{a,b}$ |
| 0% x Level 4 | $45.45 \pm 1.30^{a}$ | $3.53 \pm 0.15^{c,d,e}$ | $12.87 \pm 0.40^{a}$ |
| 50% x Level 1 | $43.23 \pm 1.83^{a}$ | $4.02 \pm 0.25^{a,b}$ | $10.75 \pm 0.33^{b,c,d}$ |
| 50% x Level 2 | $43.19 \pm 0.21^{a}$ | $3.52 \pm 0.19^{d,e}$ | $12.26 \pm 0.67^{a,b}$ |
| 50% x Level 3 | $43.52 \pm 0.21^{a}$ | $3.80 \pm 0.23^{a,b,c,d,e}$ | $11.45 \pm 0.69^{a,b,c}$ |
| 50% x Level 4 | $44.28 \pm 1.51^{a}$ | $3.59 \pm 0.14^{b,c,d,e}$ | $12.33 \pm 0.78^{a,b}$ |
| 100% x Level 1 | $43.65 \pm 0.24^{a}$ | $3.74 \pm 0.21^{a,b,c,d,e}$ | $11.67 \pm 0.67^{a,b,c}$ |
| 100% x Level 2 | $38.51 \pm 2.03^{b}$ | $3.96 \pm 0.11^{a,b,c}$ | $9.72 \pm 0.27^{d}$ |
| 100% x Level 3 | $42.32 \pm 1.79^{a}$ | $3.92 \pm 0.05^{a,b,c,d}$ | $10.79 \pm 0.48^{b,c,d}$ |
| 100% x Level 4 | $43.32 \pm 2.16^{a}$ | $4.16 \pm 0.01^{a}$ | $10.41 \pm 0.53^{c,d}$ |
| *F-value* | 2.69 | 5.92 | 6.28 |
| *P-value* | ***0.04** | ***0.008** | ***0.006** |

Means followed by the same letter in the same column are statistically non-significant ($P<0.05$); ns = not significant; * = *F*-ratio probability of $P<0.05$.

**Table 6.5:** Pearson's correlation coefficient between leaf minerals (B, Ca, Cu, Fe, K, Mg, Mn, N, Na, P, and Zn) of rose geranium (Central University of Technology, Free State).

| Variables | N | P | K | Ca | Mg | S | B | Cu | Fe | Mn | Zn | Na |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **N** | **1** | | | | | | | | | | | |
| **P** | -0,18 | **1** | | | | | | | | | | |
| **K** | 0,01 | **0,78** | **1** | | | | | | | | | |
| **Ca** | **0,77** | 0,13 | 0,40 | **1** | | | | | | | | |
| **Mg** | -0,39 | **0,77** | **0,69** | -0,20 | **1** | | | | | | | |
| **S** | **0,82** | -0,24 | -0,05 | **0,80** | **-0,52** | **1** | | | | | | |
| **B** | 0,44 | 0,13 | 0,20 | **0,65** | -0,32 | **0,71** | **1** | | | | | |
| **Cu** | -0,09 | 0,35 | 0,14 | -0,19 | **0,58** | -0,01 | -0,08 | **1** | | | | |
| **Fe** | **0,54** | -0,20 | 0,01 | **0,74** | **-0,52** | **0,90** | **0,75** | -0,11 | **1** | | | |
| **Mn** | -0,36 | 0,36 | 0,19 | **-0,57** | **0,77** | **-0,67** | **-0,62** | **0,60** | **-0,83** | **1** | | |
| **Zn** | 0,36 | 0,23 | 0,20 | 0,00 | 0,48 | -0,04 | -0,17 | **0,51** | -0,40 | **0,70** | **1** | |
| **Na** | 0,03 | **0,78** | **0,56** | 0,39 | 0,40 | 0,24 | 0,48 | 0,44 | 0,36 | -0,07 | -0,03 | **1** |

Bolded = Variable factor for which the Pearson's correlation coefficient is the largest

**Table 6.6:** Principal component analyses for the leaf mineral composition of rose geranium plant leaves (Central University of Technology, Free State).

| Traits | Principal component 1 | Principal component 2 |
|---|---|---|
| Eigenvalue | 5.07 | 3.48 |
| Variability % | 42.31 | 29.03 |
| Cumulative % | 42.31 | 71.34 |
| **Factor loading** | | |
| N | 0.01 | **0.72** |
| P | 0.13 | **0.69** |
| K | 0.02 | **0.65** |
| Ca | **0.57** | 0.27 |
| Mg | **0.56** | 0.38 |
| S | **0.80** | 0.07 |
| B | **0.54** | 0.19 |
| Cu | 0.17 | 0.25 |
| Fe | **0.84** | 0.05 |
| Mn | **0.84** | 0.03 |
| Zn | 0.16 | 0.14 |
| Na | 0.44 | 0.06 |

Values in bold correspond for each variable to the factor for which the squared cosine is the largest

**Table 6.7:** Phenolic compounds seperated from the rose geranium leaves (Central University of Technology, Free State).

| Comp. ID | Compounds name | Quant. Mass (*m/z*) | RT (Min) |
|---|---|---|---|
| 2.11_191 | Citric acid | 191 | 2.11 |
| 8.35_315 | Protocatechuic acid O-hexoside | 315 | 8.35 |
| 9.16_623 | Isorhamnetin-O-hexoside-O-deoxy-hexose | 623 | 9.16 |
| 9.17_179 | Caffeic acid | 179 | 9.17 |
| 9.18_311 | Caffeoyl tartaric acid | 311 | 9.18 |
| 9.60_165 | Coumaroyl hexose | 165 | 9.60 |
| 10.33_299 | Avenanthramide 2p | 299 | 10.33 |
| 12.45_349 | 5-Heptadecylresorcinol | 349 | 12.45 |
| 12.86_327 | p-Coumaroyl tyrosine | 327 | 12.86 |
| 13.50_329 | Avenanthramide 2f | 329 | 13.53 |
| 13.53_431 | 5-Tricosenylresorcinol | 431 | 13.84 |
| 14.59_461 | 5-Pentacosylresorcinol | 461 | 14.59 |
| 15.70_539 | Oleuropein | 539 | 15.70 |
| 16.10_475 | Chicoric acid | 475 | 16.10 |
| 17.12_377 | 5-Nonadecylresorcinol | 377 | 17.12 |
| 18.33_433 | Ellagic acid arabinoside | 433 | 18.33 |
| 18.53_593 | Kaempferol-O-hexoside-O-deoxy-hexose | 593 | 18.53 |
| 18.65_373 | 5-Nonadecylresorcinol | 373 | 18.65 |
| 18.77_525 | Ligstroside | 525 | 18.77 |
| 19.32_527 | Demethyloleuropein | 527 | 19.32 |
| 20.87_419 | Oleoside dimethylester | 419 | 20.87 |

Comp. ID = Compound identity; Quant. Mass = quantitative mass

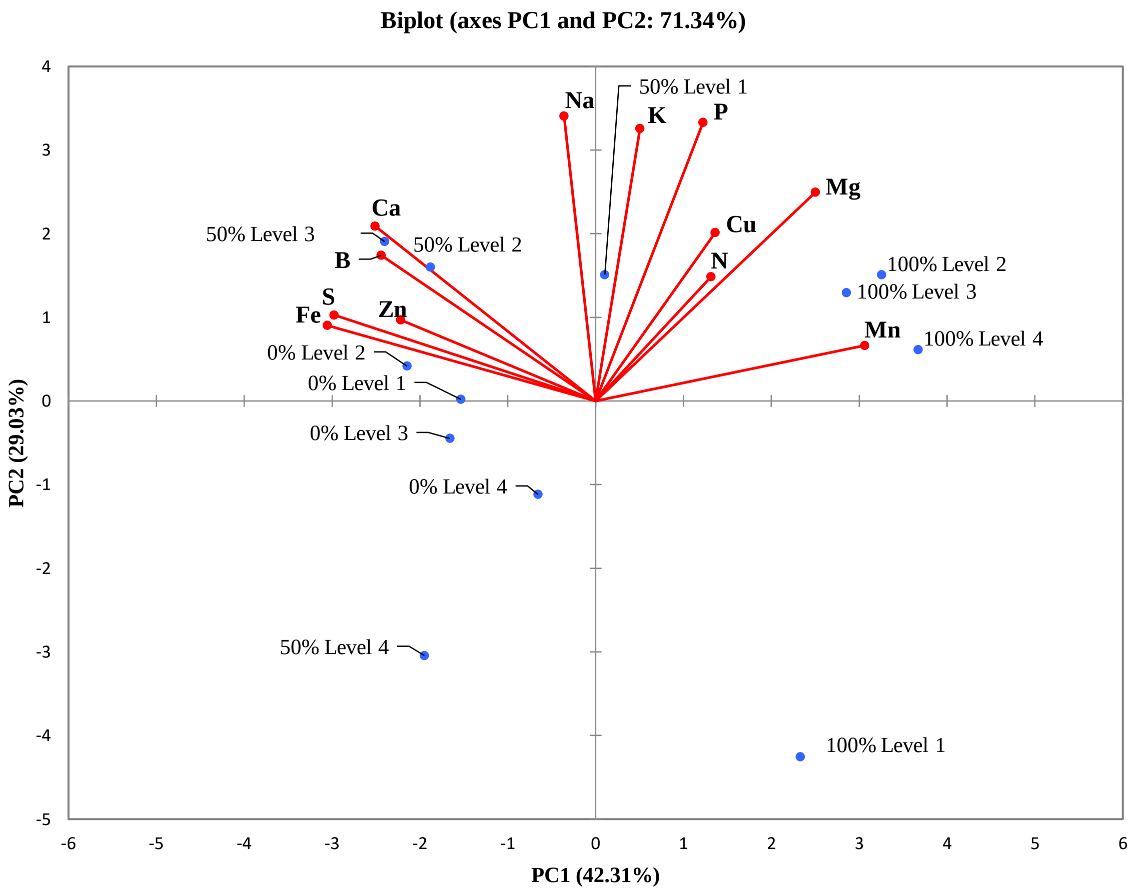


**Figure 6.1:** Principal component biplot showing the variations between leaf mineral content (B, Ca, Cu, Fe, K, Mg, Mn, N, Na, P, and Zn) in response to hail-simulated damage (zero%, 50%, and 100% defoliation) and the application of PGRs-mixtures (level 1 consisted of distilled water; level 2 had gibberellic acid (GA)-1.26 mg/kg, brassinosteroids (BRs)-0.51 mg/kg, and traces of cytokinins (CKs)-0.025 mg/kg; level 3 had GA-2.55 mg/kg, BRs-1.02 mg/kg, and traces of CKs-0.05 mg/kg; level 4 had GA-3.83 mg/kg, BRs-1.53 mg/kg, and traces of CKs-0.075 mg/kg) (Central University of Technology, Free State).

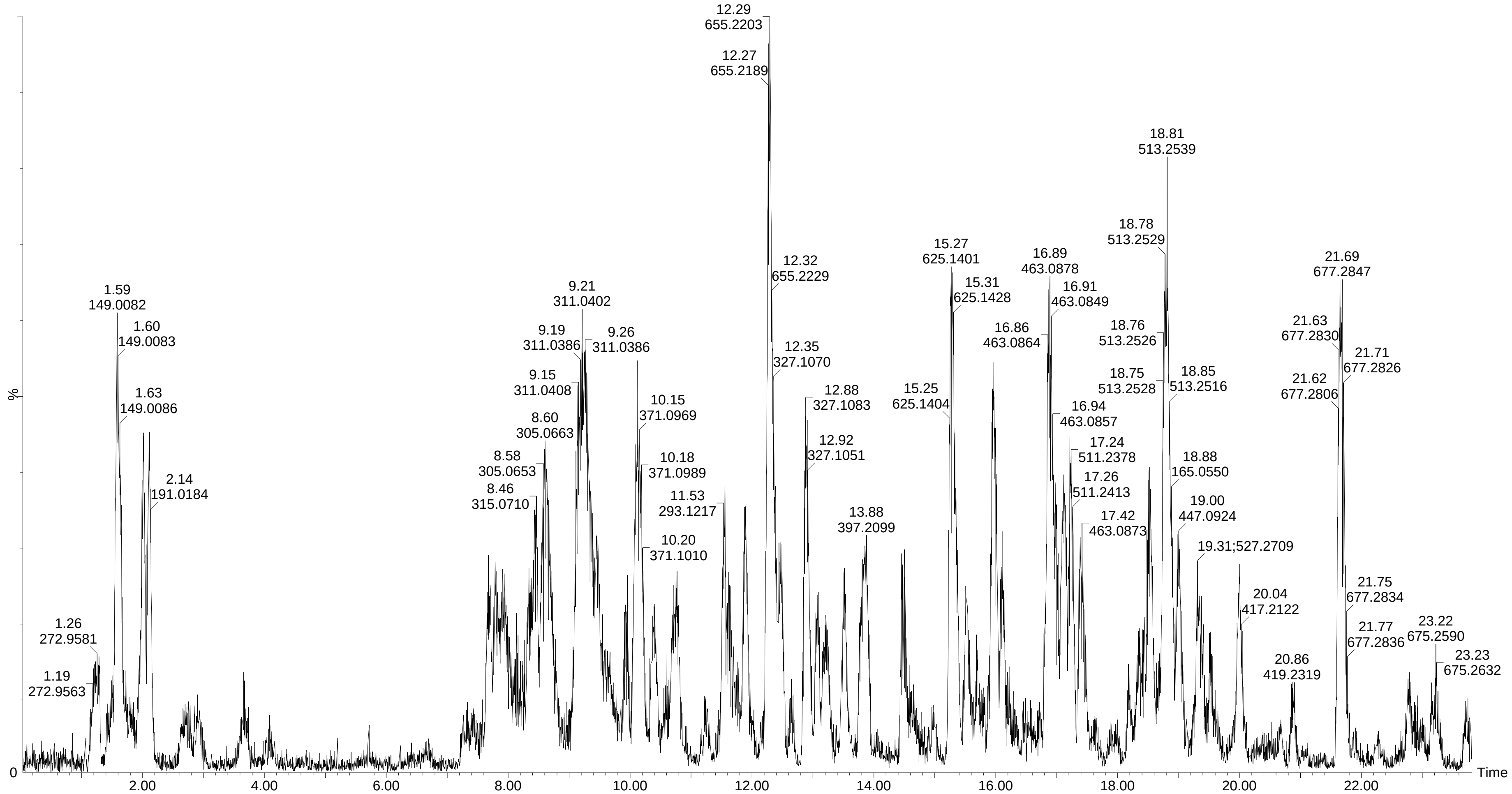

**Figure 6.2:** Total chromatograms of rose geranium leaf phenolic profile.

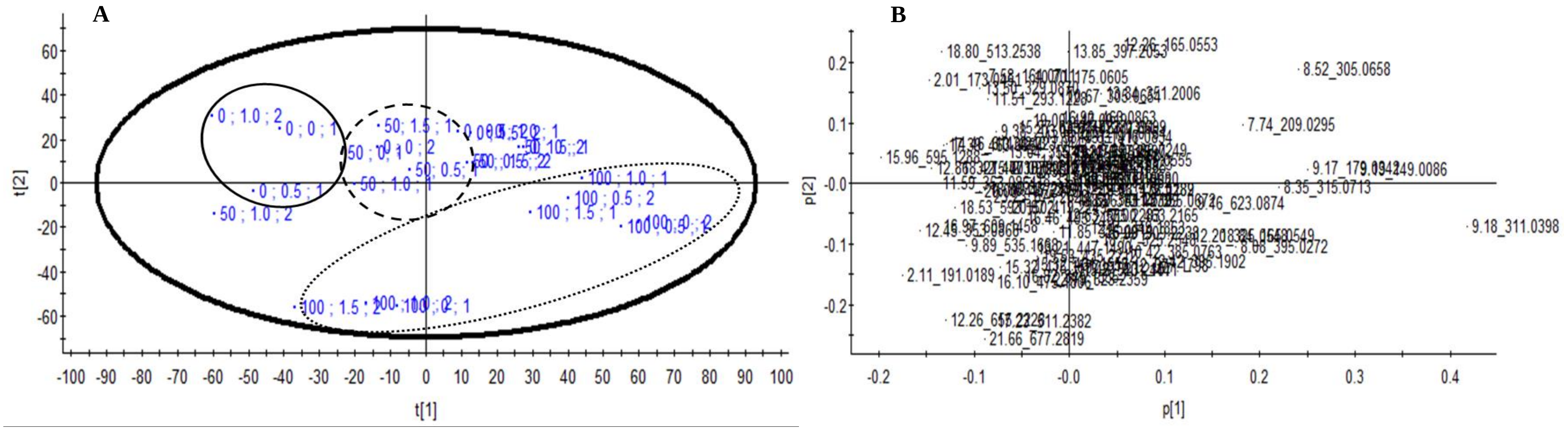


**Figure 6.3:** Principal component analysis (PCA) model of simulated hail-damaged rose geranium (zero%, 50%, and 100% defoliation) treated with PGRs-mixtures (level 1 [0.] consisted of distilled water; level 2 [0.5.] had gibberellic acid (GA)-1.26 mg/kg, brassinosteroids (BRs)-0.51 mg/kg, and traces of cytokinins (CKs)-0.025 mg/kg; level 3 [1.0.] had GA-2.55 mg/kg, BRs-1.02 mg/kg, and traces of CKs-0.05 mg/kg; level 4 [1.5.] had GA-3.83 mg/kg, BRs-1.53 mg/kg, and traces of CKs-0.075 mg/kg). (A) Score plot t[2] versus t[1] (R2X[1] = 0.22, R2X[2] = 0.15). The bold ellipse represents the Hostelling T2 with 95% confidence. The solid line describes 0% defoliation, the dashed line describes 50% defoliation, and the dotted line describes 100% defoliation. The coding for zero% defoliation (intact plants) is indicated by the first 0; 50% defoliation is indicated by the first 50; 100% defoliation is indicated by the first 100. The PGRs-mixtures treatment levels are indicated within the second part of the coding, where 0 (distilled water) indicates level 1; 0.5 indicates level 2; 1.0 indicates level 3; 1.5 indicates level 4. Last part of the coding indicates the number of replications. (B) Loading plot of p[2] versus p[1] (Central University of Technology, Free State).

# CHAPTER SEVEN

# CONCLUSIONS AND RECOMMENDATIONS

## 7.1 Study summary

Rose geranium (*Pelargonium graveolens* L'Her.) plants are grown for essential oil production; therefore, the aerial herbage material is a crucial component of the crop. Environmental stress factors, such as hail damage, can cause significant damage to these plants, reducing this valuable material, and directly affecting the essential oil yield and quality (Swamy *et al.* 1960; Dermane and van der Walt 1989; Weiss 1997). Natural and synthetic plant growth regulators (PGRs) have been extensively investigated on plant growth and development, and also on the recovery of plants following stress (Gasper *et al.* 1996; Prins *et al.* 2010). Based on Gasper *et al.* (1996) and Prins *et al.* (2010), it was hypothesised that the application of agricultural PGRs will recover the herbage yield and improve the biosynthesis of essential oil of rose geranium plants subjected to simulated hail damage. Therefore, this study was conducted to develop a mitigation strategy using agricultural PGRs on hail-damaged rose geranium plants.

To achieve this goal, this study was sectioned into four specific objectives:

**Objective 1:** To evaluate the use of combined PGRs (cytokinin [CK] + gibberellic acid [GA]) as a potential recovery mechanism of the herbage yield, and the essential oil yield and quality of simulated hail-damaged rose geranium plants

**Objective 2:** To evaluate the use of abscisic acid (ABA) and methyl jasmonate (MeJA) as a potential mitigating mechanism on simulated hail-damaged rose geranium plants.

**Objective 3:** To determine the recovery of simulated hail-damaged rose geranium when treated with different levels of agricultural PGRs-mixtures (GA, brassinosteroids [BRs], and traces of CKs: registered as *Lucky Plant*® [Agraforum, Germany]: EP1051075B1 & EP1933626).

**Objective 4:** To evaluate the influence of agricultural PGRs-mixtures on the leaf mineral status, and phenolic composition of simulated hail-damaged rose geranium.

## 7.2 Conclusions

This study verified the assertions made by Swamy *et al.* (1960), Dermane and van der Walt (1989) and Weiss (1997) that hail can cause significant damage to rose geranium plants. It can be concluded from this study that hail intensity >H3 (20.3 mm-30.5 mm) will cause damage to rose geranium leaves. Interestingly, it was also found that 100% defoliated rose geranium plants accumulate most of the phenolic compounds associated with defoliation and wounding, while those with only 50% simulated hail damage accumulate phenolic compounds to a similar level of intact plants. This study has also found that the use of combined agricultural PGRs (CK + GA) applied in different weeks does not recover the herbage yield and essential oil yield, regardless of the rate of application: this could be attributed to the decline in densities of the brevicollate trichomes. It was further discovered that refoliated rose geranium develop higher densities of elongated-capitate trichomes following the application of a high concentration of combined PGRs (CK 0.64 mg/L + GA 300 mg/L). Multivariate analysis, through principal component analysis (PCA), also demonstrated that treatment with 75 µM ABA and 10 mM MeJA affected the biosynthesis of linalool, isomenthone, citronellol, geraniol, and the citronellol to geraniol ratio (C:G ratio). However, alterations to these essential oil compounds did not improve the essential oil quality. Furthermore, the application of ABA and MeJA has an adverse effect on the recovery of the herbage yield and essential oil yield. This study further revealed that using a mixture of agricultural PGRs (containing GA, BRs, and traces of CKs) from level 3 (medium level) will improve the recovery and growth of the herbage in rose geranium plants with more than 50% foliage loss. Interestingly, this finding was also corroborated by the accumulation of primary minerals (N, P, K, Mg and Mn) associated with plant growth and development when PGRs-mixture was applied as low as level 2 on rose geranium plants with more than 50% foliage loss. This study also found that the essential oil quality (C:G ratio <3) is high when the PGR-mixture is applied at level 3 on rose geranium plants with more than 50% foliage loss. In addition, it was also demonstrated that the change in essential oil yield is ascribed to changes in brevicollate trichome densities, induced from the lowest PGR concentration (level 2) in rose geranium plants with less than 50% foliage loss. Lastly, it was also found that the application of low levels of PGRs will negatively affect the essential oil yield of intact plants, as well as plants that have sustained up to 50% foliage loss.

### 7.3 Recommendations and future studies

Based on these findings and conclusions, the following recommendations can be made to rose geranium growers:

- Estimate the intensity of the hail damage using an average plant height by randomly selecting damaged and undamaged plants from each row to measure. For any defoliation less than the top half of damaged plants, growers can approximate that the hail damage intensity is less than 50% (<H2). However, any defoliation beyond the top half of the plants, can be classified as severely hail-damaged plants (>50%), or >H3. The treatment of these plants should be similar to that of plants which have suffered complete defoliation (100% or >H3).

- Consider using *Lucky Plant*® (mixture of GA, BRs, and traces of CKs (PGRs-mixture) as a source of agricultural PGRs, rather than combined CK + GA (split applied in different weeks), ABA, or MeJA (applied separately).
- Treat intact plants, and those that sustained less than 50% foliage loss (<H2) with a mixture of GA (1.26 mg/kg), BRs (0.51 mg/kg), and CKs (0.0025 mg/kg) per plant, or *Lucky Plant*® at 50 g/ha to improve the essential oil yield.
- To improve and recover the mineral acquisition, the growth of the herbage material and the essential oil quality (C:G ratio <3) of rose geranium plants with more than 50% foliage loss (H3>), consider applying PGR-mixtures between GA (1.26 mg/kg), BRs (0.51 mg/kg), and CKs (0.025 mg/kg) per plant (or *Lucky Plant*® at 50 g/ha); and GA (2.55 mg/kg), BRs (1.02 mg/kg), and CKs (0.05 mg/kg) per plant (or *Lucky Plant*® at 100 g/ha).
- Rose geranium re-growth may be slow for the first 4-8 weeks following hail damage; therefore, it is also recommended to consider applying a PGR-mixture (GA, BRs, and traces of CKs) immediately after a hail damage event. The application should be done fortnightly following hail damage to facilitate the rapid recovery of damaged plants.
- Expect a regrowth period of 4-6 weeks before considering harvest.

Future studies should focus on the following:

- Determining the effects of root-applied synthetic CK on the recovery of essential oil yield attributes, and the essential oil yield and quality of hail-damaged rose geranium.
- Determining the effects of CK and the auxin ratio on hail-damaged rose geranium plants, *in vivo* study.
- Identifying the effects of combined PGRs with stimulatory and inhibitory properties, applied at different growth stages of hail-damaged rose geranium plants.
- Identifying the effects of synthetic PGRs as a contaminant of essential oil quality: A perfumery industry study.

## References


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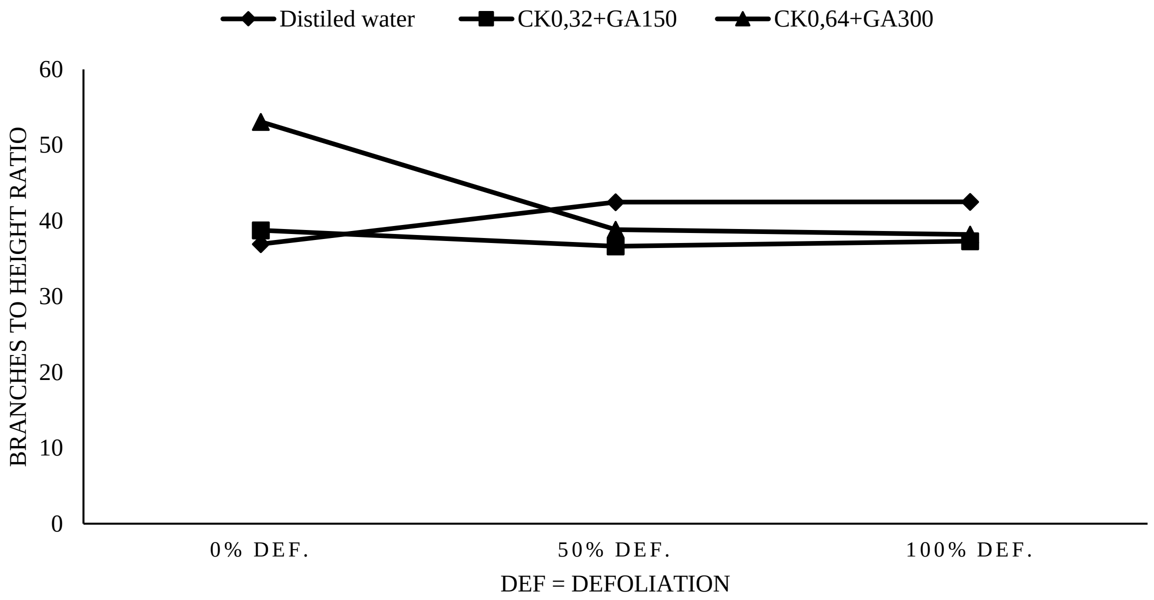
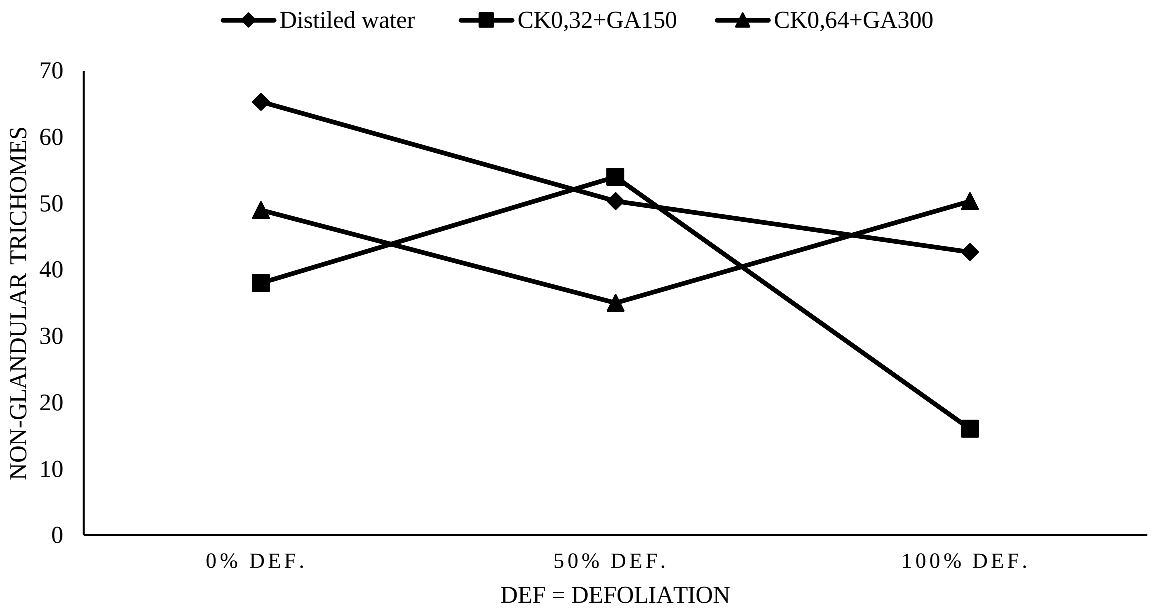
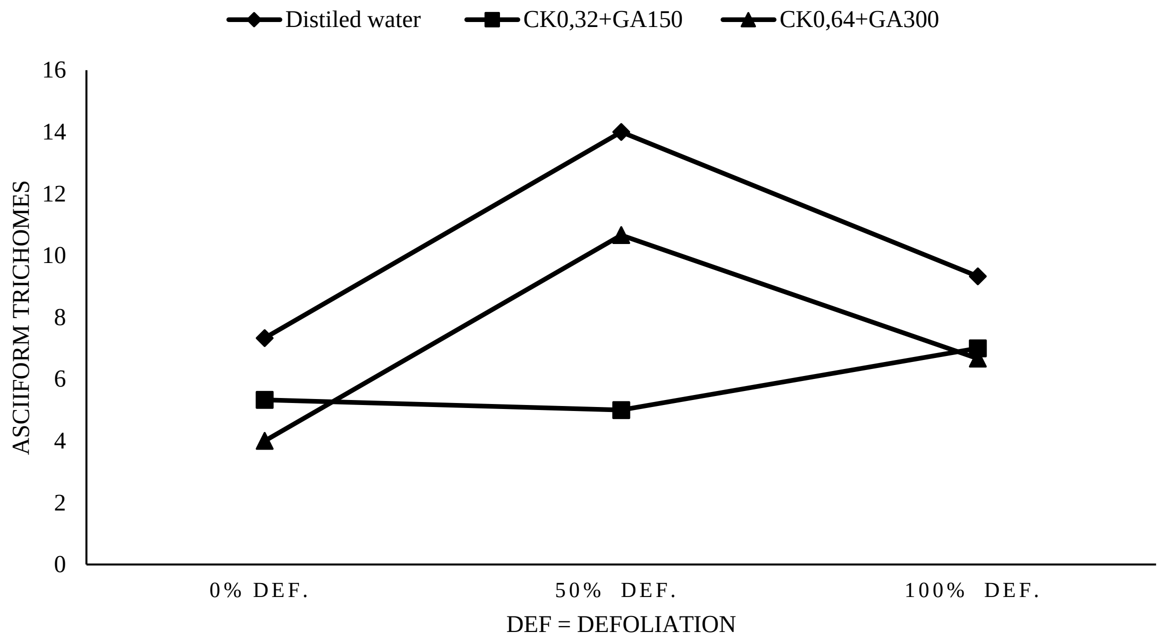
## APPENDIX



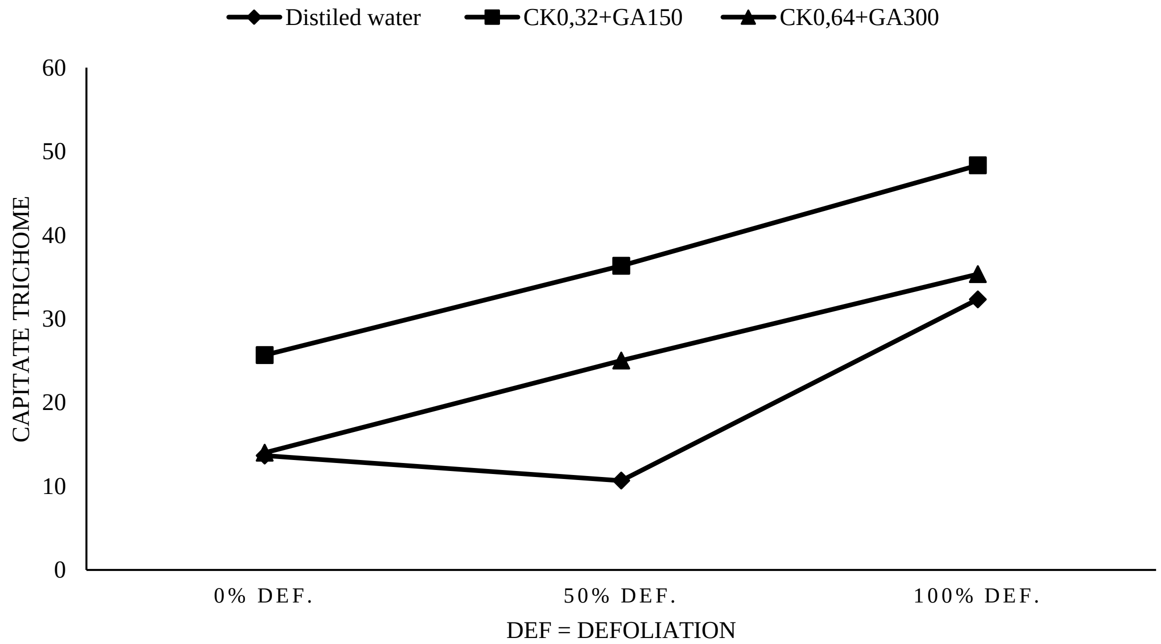

**Appendix 3.1:** Interaction plots for number of branch to height ratio as affected by simulated hail damage and combined agricultural plant growth regulators (2017/18 production season) – Chapter 3.



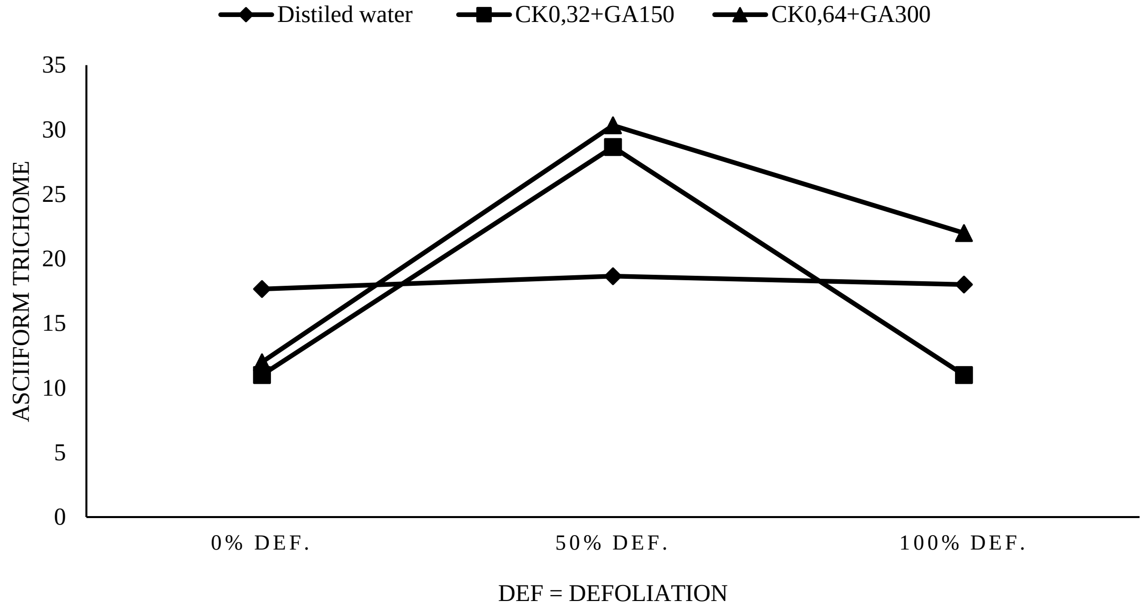

**Appendix 3.2:** Interaction plots for the non-glandular trichomes (adaxial leaf surface) as affected by simulated hail damage and combined agricultural plant growth regulators – Chapter 3.



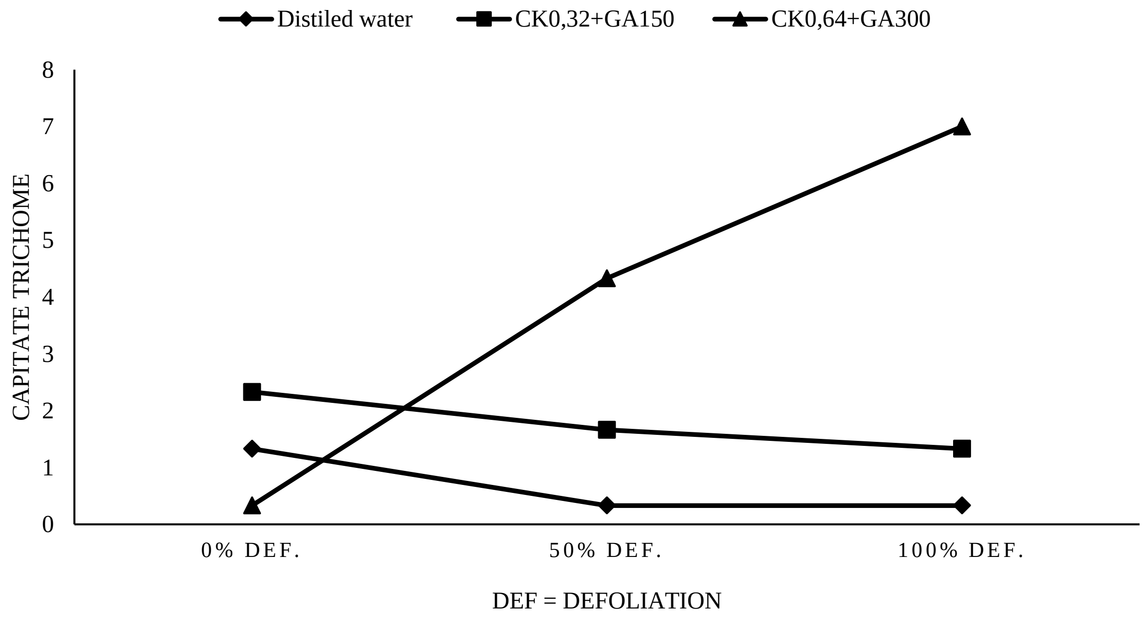

**Appendix 3.3:** Interaction plots for the asciiform trichomes (adaxial leaf surface) as affected by simulated hail damage and combined agricultural plant growth regulators – Chapter 3.



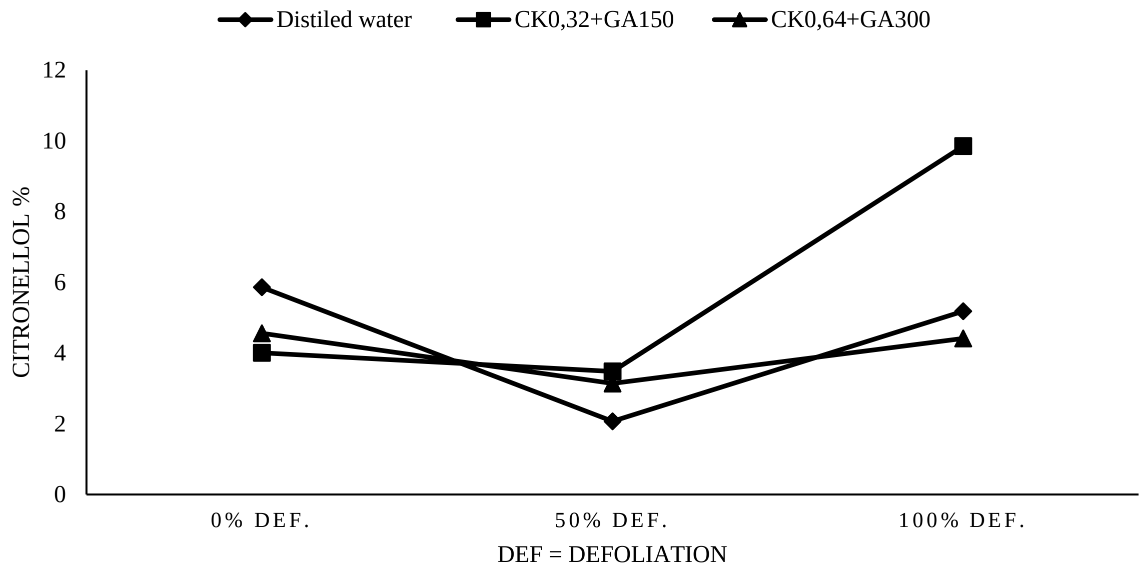

**Appendix 3.4:** Interaction plots for the capitate trichomes (adaxial leaf surface) as affected by simulated hail damage and combined agricultural plant growth regulators – Chapter 3.



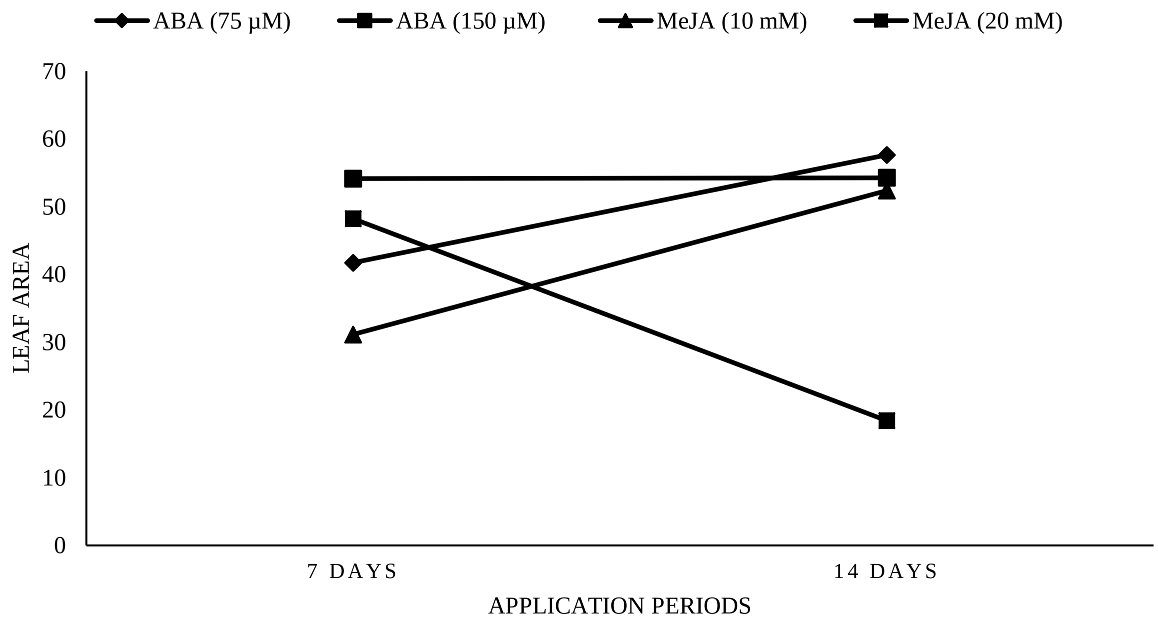

**Appendix 3.5:** Interaction plots for the asciiform trichomes (abaxial leaf surface) as affected by simulated hail damage and combined agricultural plant growth regulators – Chapter 3.



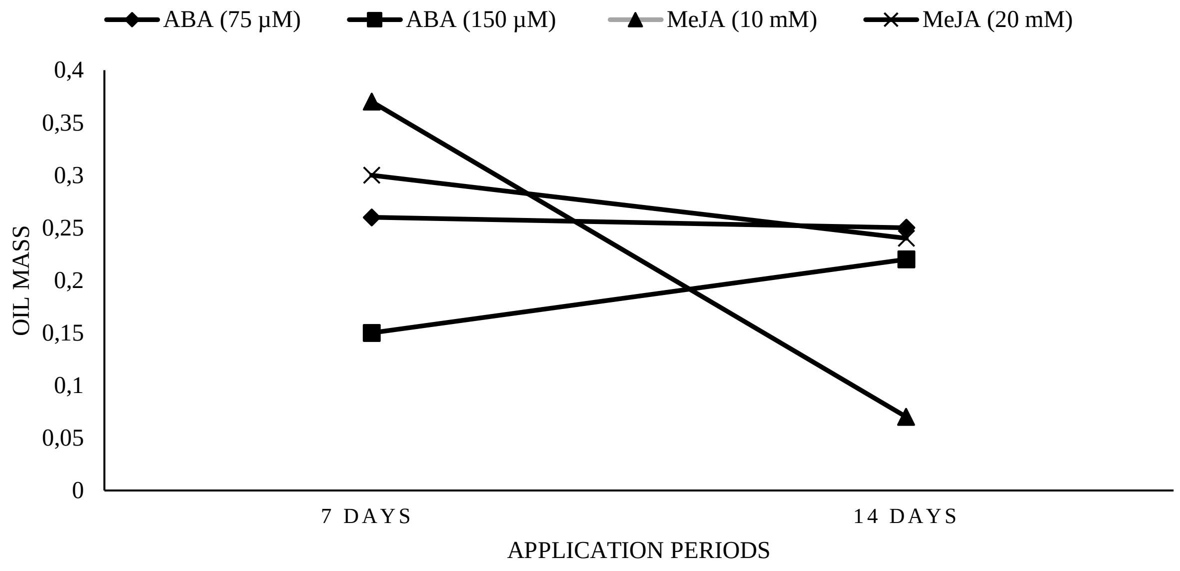

**Appendix 3.6:** Interaction plots for the capitate trichomes (abaxial leaf surface) as affected by simulated hail damage and combined agricultural plant growth regulators – Chapter 3.



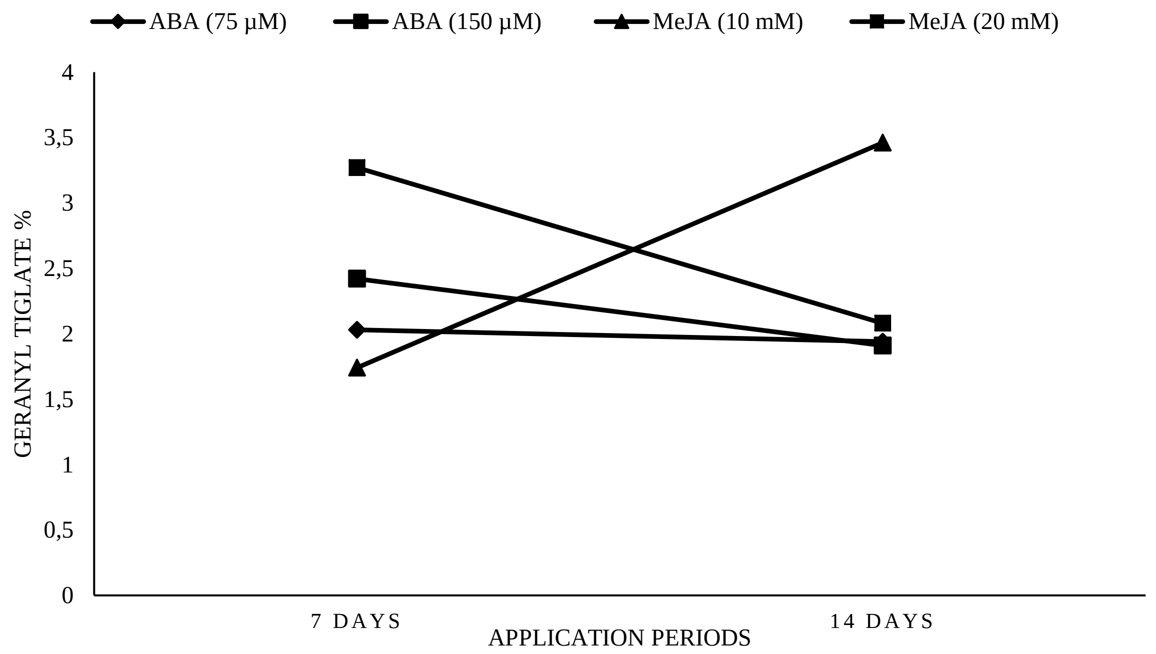

**Appendix 3.7:** Interaction plots for the citronellol content as affected by simulated hail damage and combined plant agricultural growth regulators (2017/18 production season) – Chapter 3.



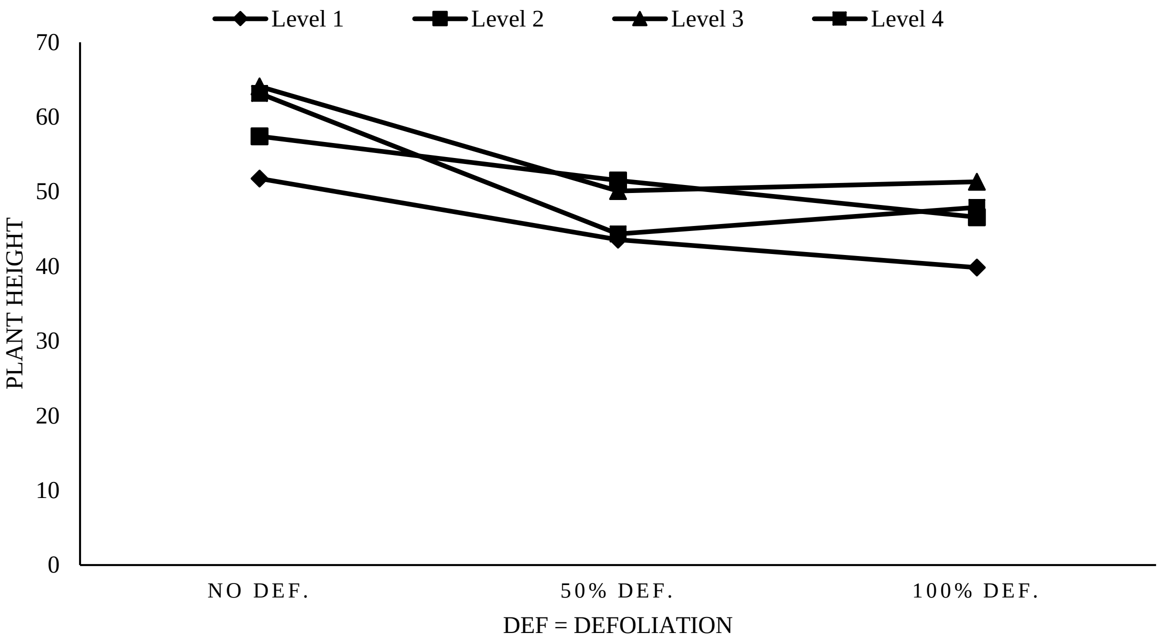

**Appendix 4.1:** Interaction plots for the leaf area as affected by plant growth regulators applied in different periods (2016/17 production season) – Chapter 4.



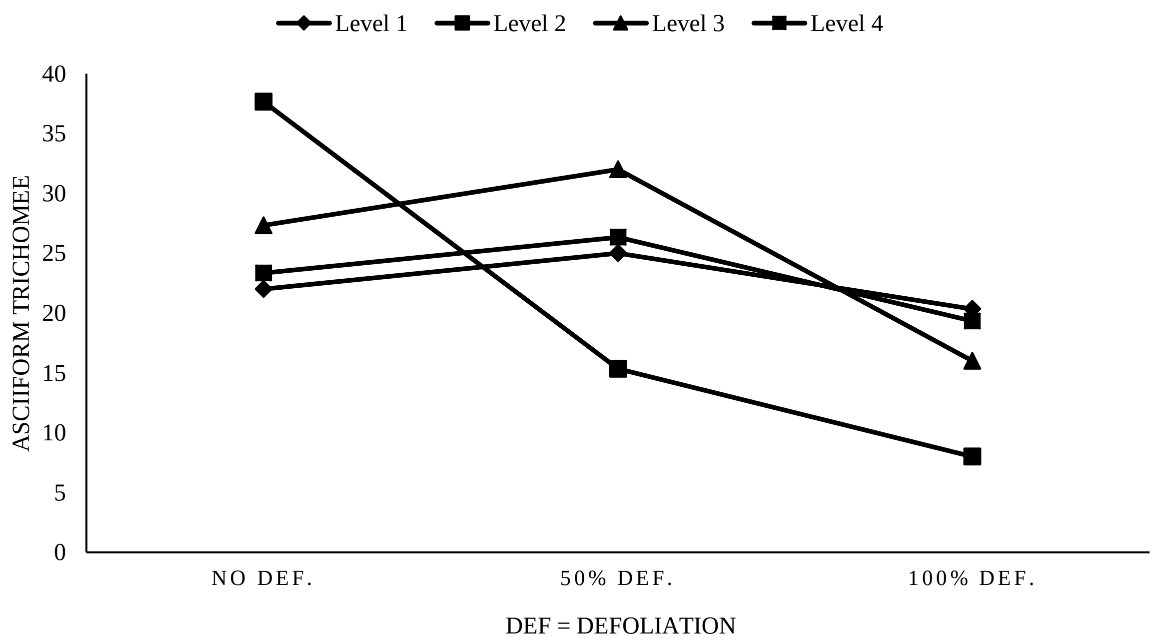

**Appendix 4.2:** Interaction plots for the oil mass as affected by plant growth regulators applied in different periods (2017/18 production season) – Chapter 4.



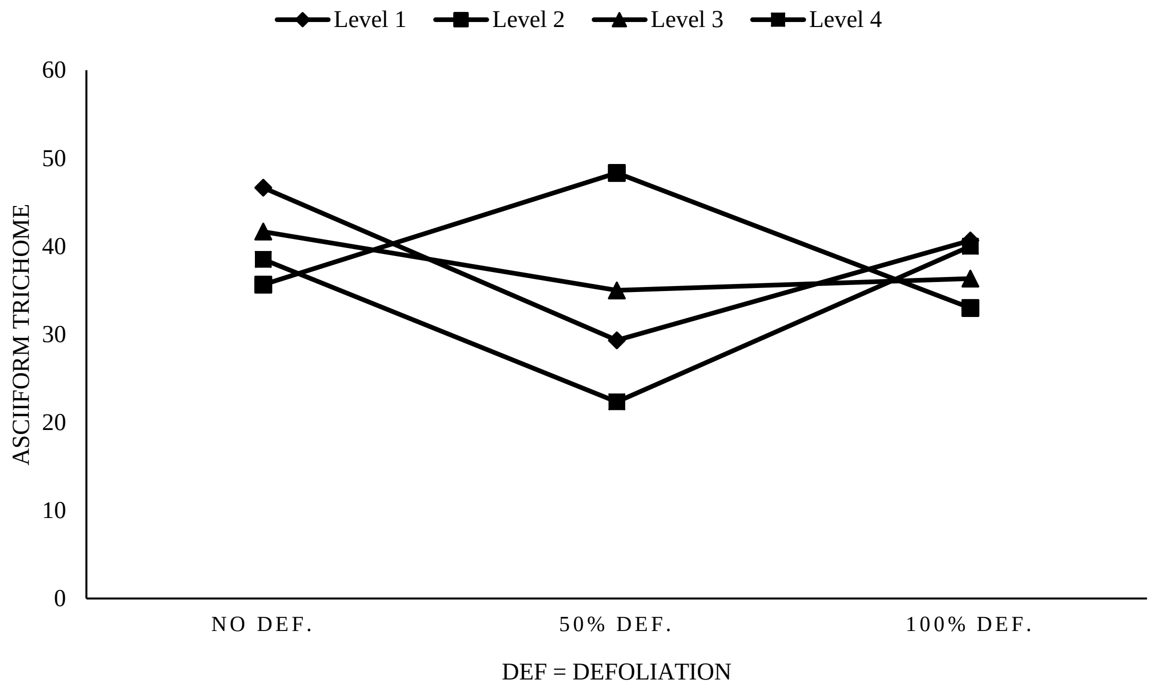

**Appendix 4.3:** Interaction plots for geranyl tiglate as affected by plant growth regulators applied in different periods (2016/17 production season) – Chapter 4.



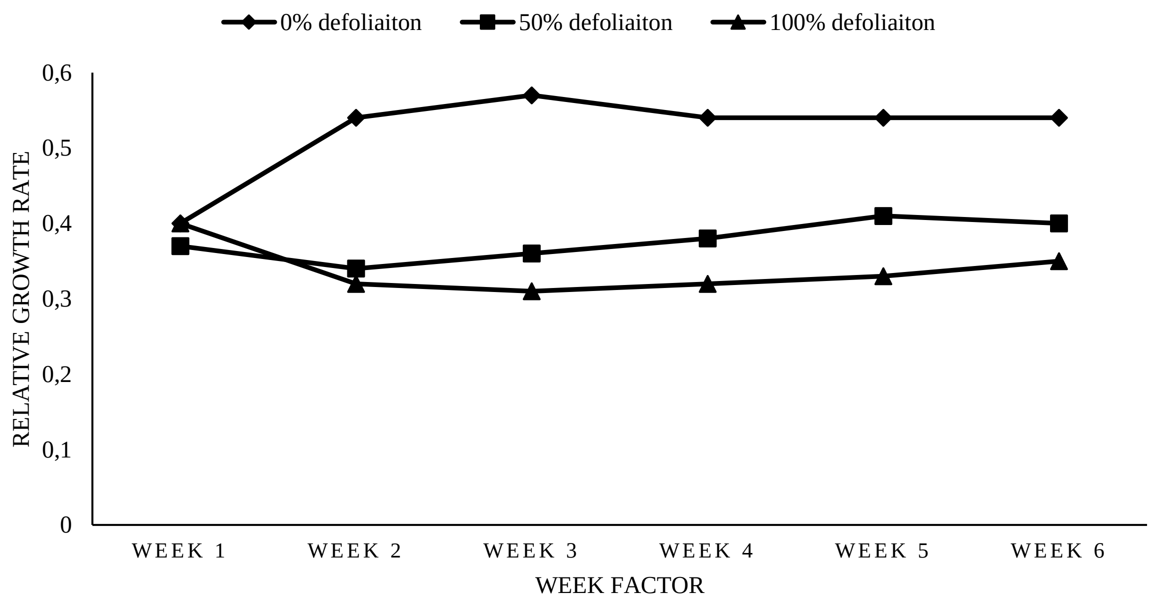

**Appendix 5.1:** Interaction plots for the plant height as affected by simulated hail damage and plant growth regulators – Chapter 5.



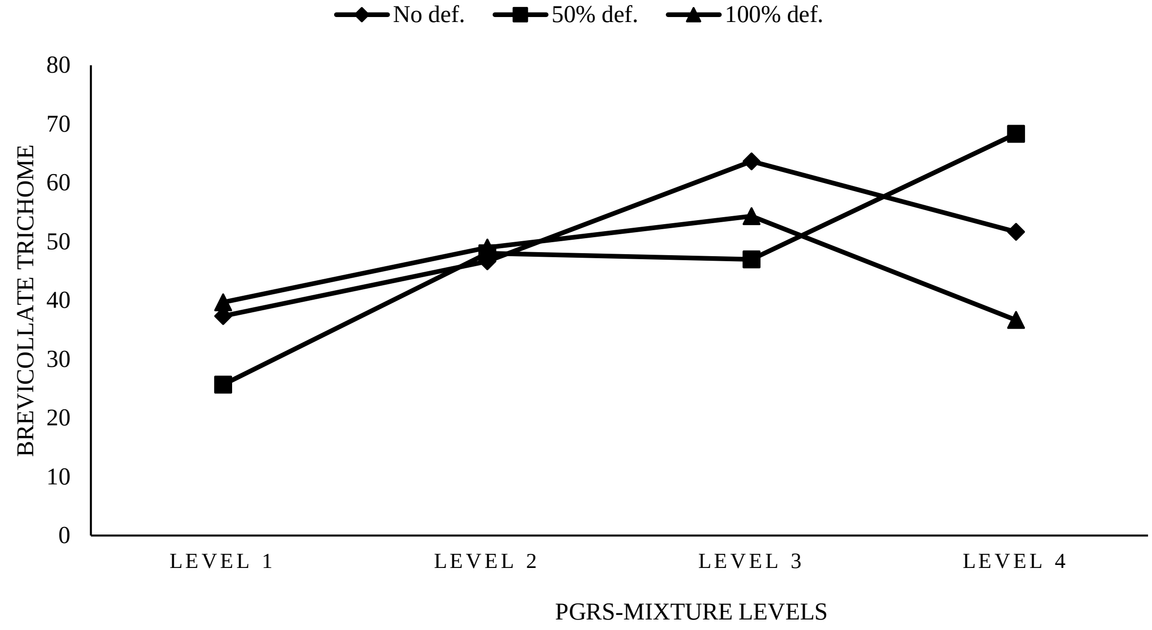

**Appendix 5.2:** Interaction plots for the asciiform trichome (adaxial) as affected by simulated hail damage and plant growth regulators – Chapter 5.



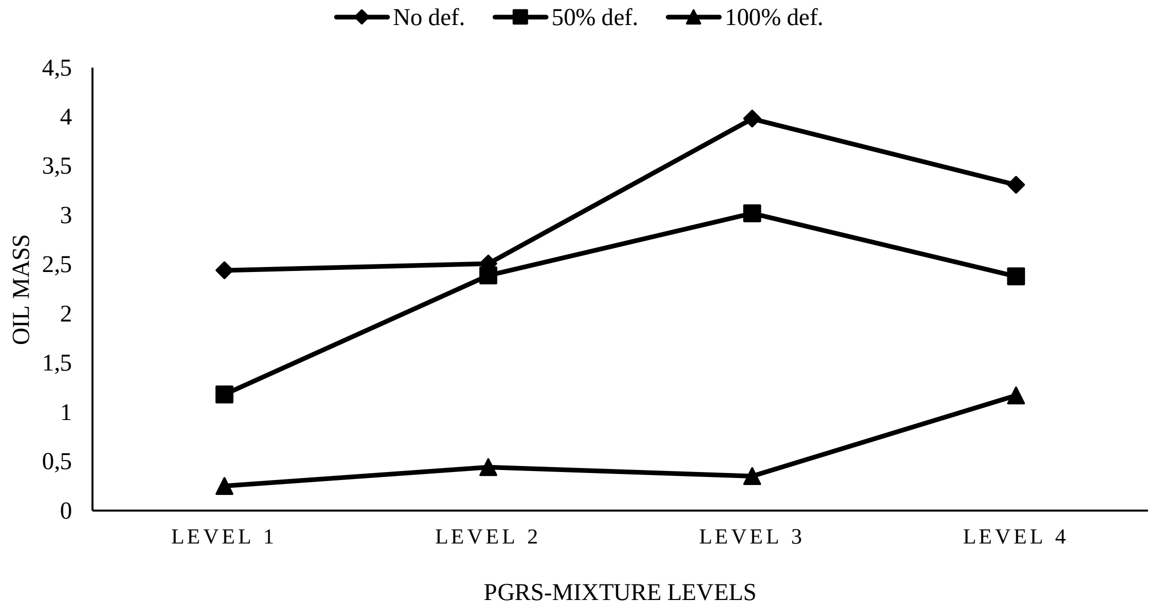

**Appendix 5.3:** Interaction plots for the asciiform trichome (abaxial) as affected by simulated hail damage and plant growth regulators – Chapter 5.



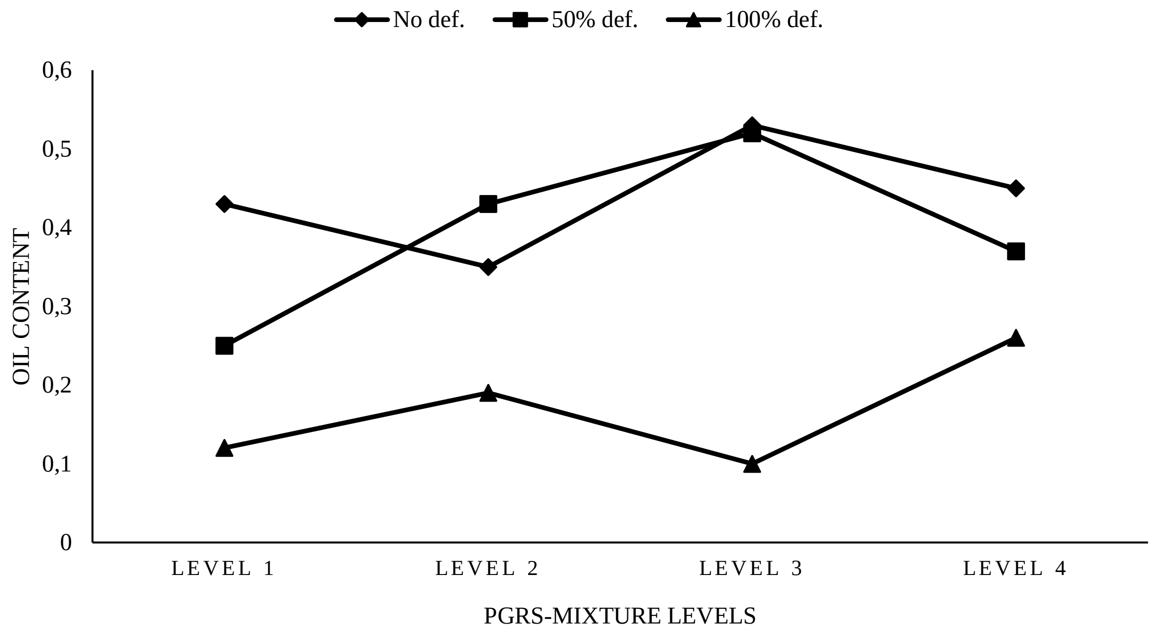

**Appendix 5.4:** Interaction plots for the relative growth rate of rose geranium stems as affected by simulated hail damage and week factor – Chapter 5.



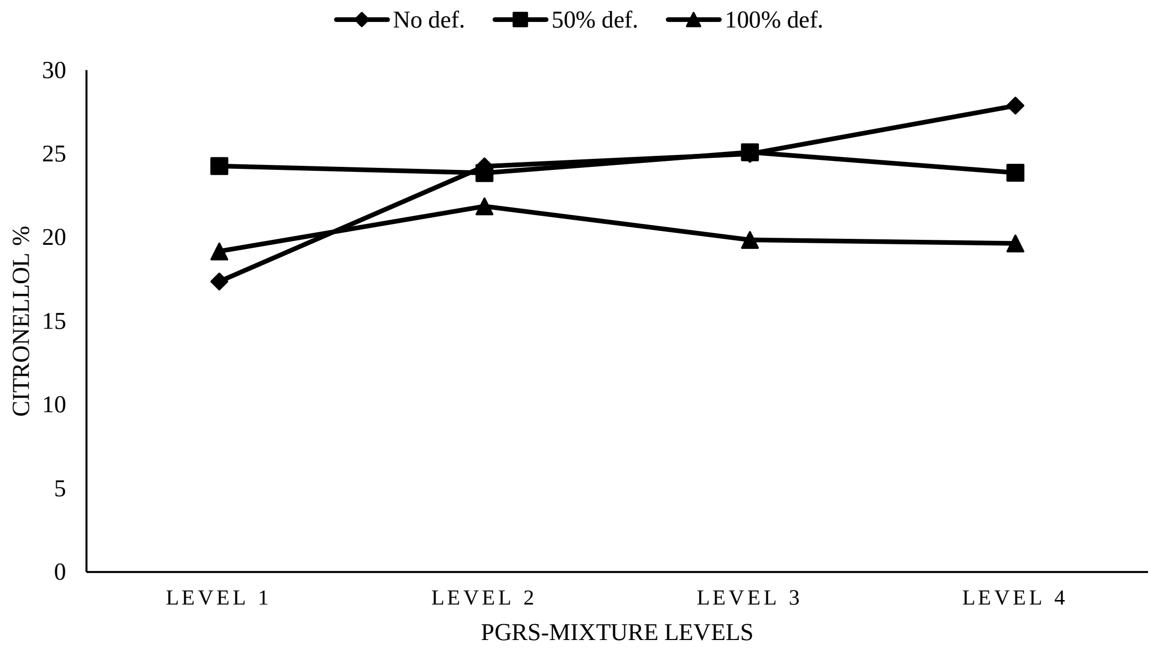

**Appendix 5.5:** Interaction plots for the brevicollate trichome (abaxial) as affected by simulated hail damage (def) and plant growth regulators – Chapter 5.



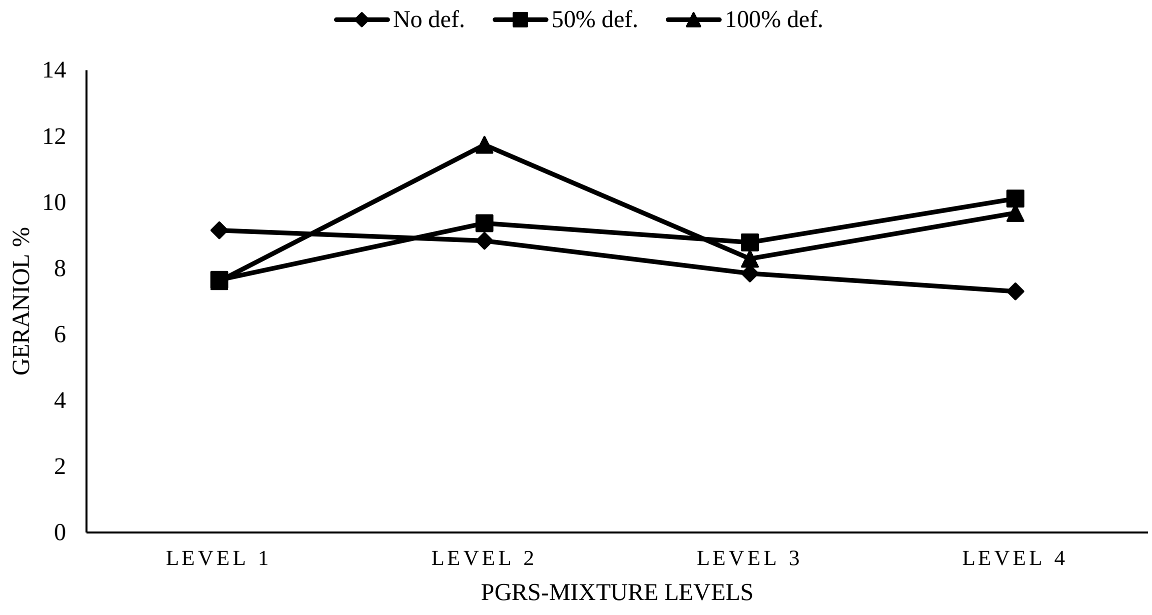

**Appendix 5.6:** Interaction plots for the oil mass as affected by simulated hail damage (def) and plant growth regulators – Chapter 5.



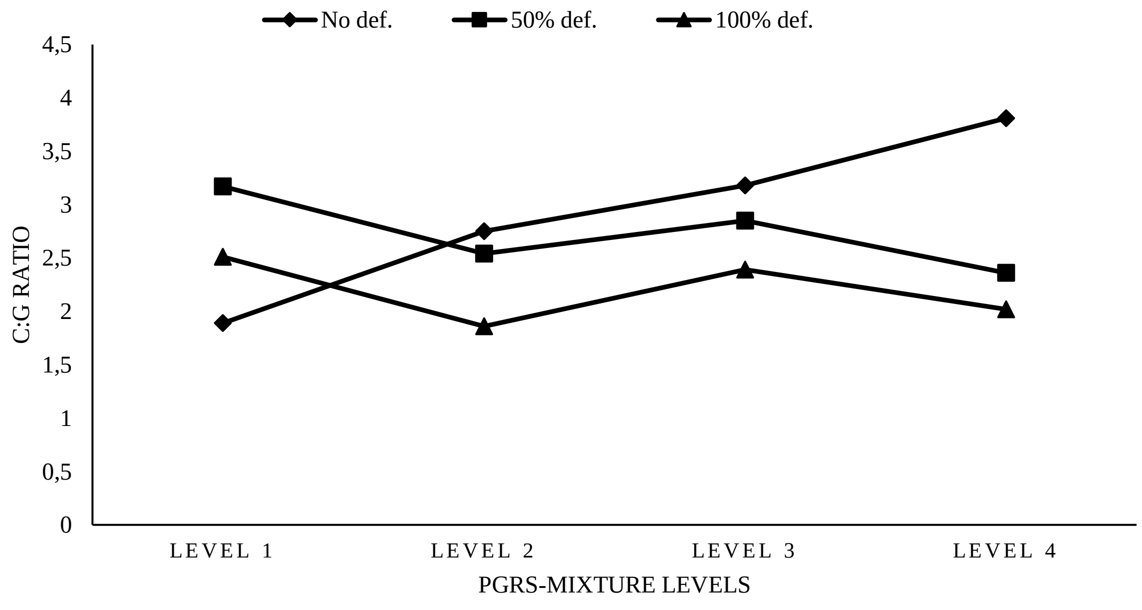

**Appendix 5.7:** Interaction plots for the oil content as affected by simulated hail damage (def) and plant growth regulators – Chapter 5.



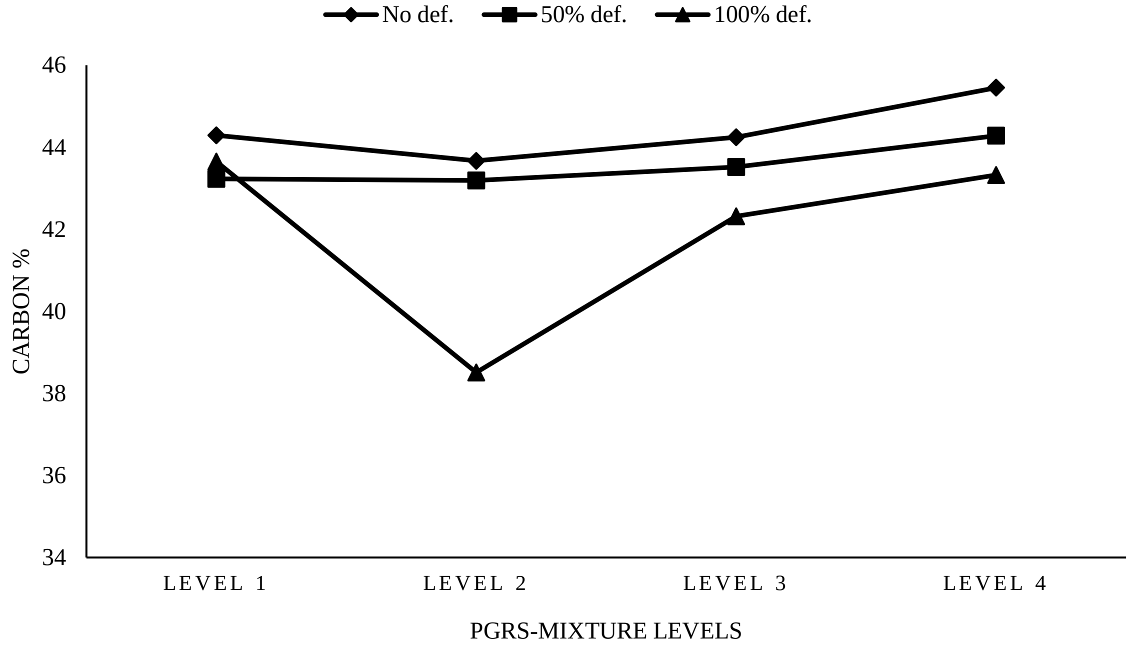

**Appendix 5.8:** Interaction plots for the citronellol content as affected by simulated hail damage (def) and plant growth regulators – Chapter 5.



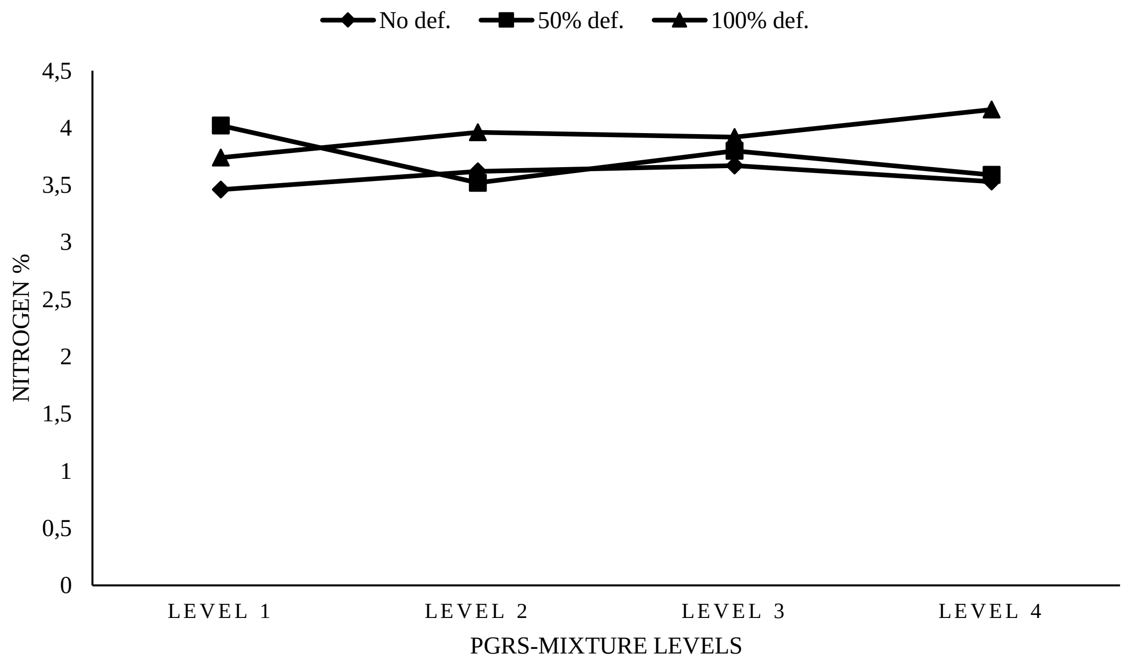

**Appendix 5.9:** Interaction plots for the geraniol content as affected by simulated hail damage (def) and plant growth regulators – Chapter 5.



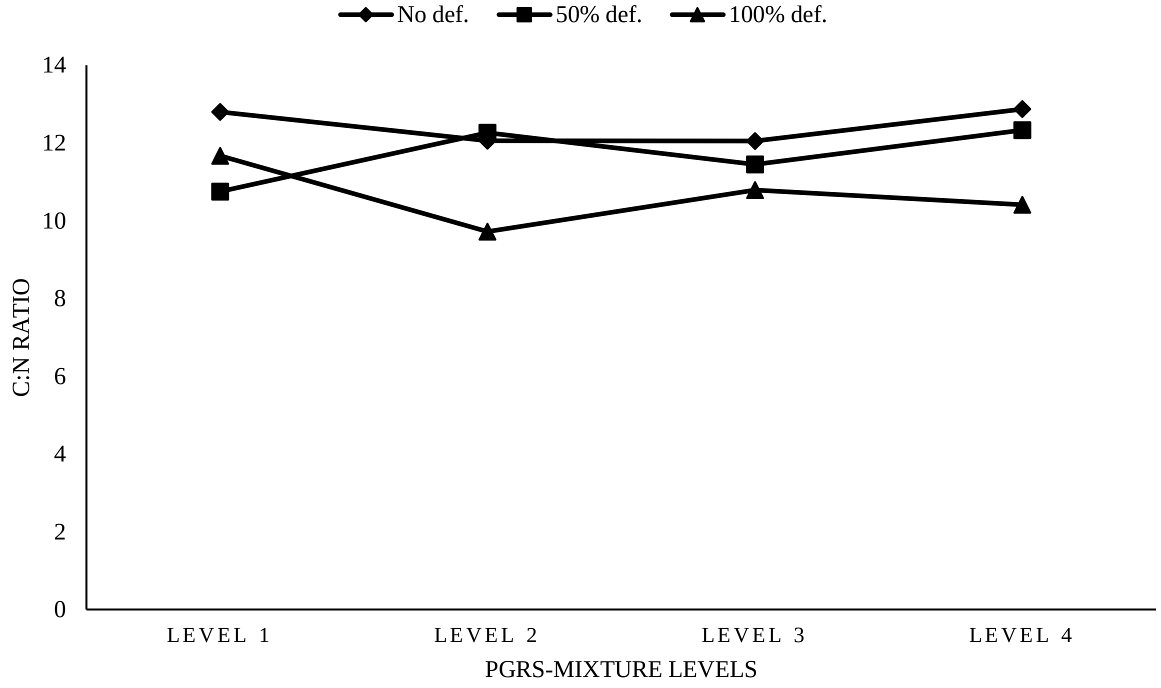

**Appendix 5.10:** Interaction plots for the citronellol to geraniol ratio as affected by simulated hail damage (def) and plant growth regulators – Chapter 5.




**Appendix 6.1:** Interaction plots for the carbon content as affected by simulated hail damage (def) and plant growth regulators – Chapter 6.




**Appendix 6.2:** Interaction plots for the nitrogen content as affected by simulated hail damage (def) and plant growth regulators – Chapter 6.




**Appendix 6.3:** Interaction plots for the carbon to nitrogen ratio as affected by simulated hail damage (def) and plant growth regulators – Chapter 6.

# Effects of abscisic acid and methyl jasmonate on the recovery of hail damaged rose geranium (*Pelargonium graveolens*) plants

Z.P. Khetsha, M.M. Sedibe and R.J. Pretorius

Department of Agriculture, Faculty of Health and Environmental Sciences, Central University of Technology, Free State, Bloemfontein, South Africa.

*Abstract*

An annual global crop yield loss due to natural hazards and disaster is currently significantly affecting emerging farmers. Hail produced by hailstorm has been identified as one of the natural hazards that causes yield loss in crops. The level of hail damage varies across plant species depending on stages of development, plant height, the leaf and stem. Hail injure plants by defoliation, tearing, bruising, breaking, loss and wounding of stems and flowers. Farmers use various, strategies to mitigate against hail and to prevent a possible loss of income. In South Africa, agricultural crop insurance companies do not have a product that cover essential oil plants against hail damage. The use of phytohormones as alternative biological mitigating strategy against hail damaged essential oil crops has not received much attention in South Africa. The aim of this study was to determine the effect of foliar application and frequencies (7 and 14 days) of bio-inhibitors (abscisic acid [ABA] and methyl jasmonate [MeJA]) on yield recovery and wounding healing response time of hail damaged rose geranium. The study was set up in a randomized block design with three replications. Treatments were laid out in a 3×3×2 factorial design totalling 18 treatment combinations. Plants were grown in a temperature controlled greenhouse. Treatments were assigned to different levels of ABA (0, 75 and 150 µM $L^{-1}$), MeJA (0, 10 and 20 mM $L^{-1}$) and the subsequent hail damage application frequency (7 and 14 days). Hail was simulation using 100% defoliation. Herbage and oil yield parameters were measured in 2016 to determine the recovery response mechanisms after hail damage. Tukey's Student Range test was used to determine significant differences between experimental means ($\alpha$=0.05). Results for 2016 season showed that an interaction between ABA (150 µM $L^{-1}$), MeJA (0 mM $L^{-1}$) and the application frequency (14 days) increased the leaf area ($P$=0.05) significantly. Furthermore, where MeJA was applied alone significantly reduced the leaf area ($P$=0.05), plant recovery rate ($P$=0.01), foliar fresh mass ($P$=0.01) and oil yield ($P$=0.05) of hail damaged rose geranium plants. Based on the preliminary results obtained in the first trial; hail damaged rose geranium plants have to be applied with an ABA at 150 µM $L^{-1}$ for 14 days without any MeJA administration (0 mM $L^{-1}$) to improve herbage mass.

**Keywords:** abscisic acid, application frequency, hail damage, methyl jasmonate, rose geranium

## INTRODUCTION

Hail produced by hailstorms has been identified as one of the natural hazards that causes yield loss in crops (Changnon, 1999; FAO, 2015). It is described as pellets in the form of small balls, or pieces of ice, which varies in size ranging from 0.5 to 100 mm (Bueckert, 2011). Hail stones larger than an American nickel (size code: H3) damage plants through physical wounding and defoliation, tearing and bruising of plant tissue, and loss of stems and flowers (Changnon, 1999; Bueckert, 2011). Physical wounding by hail damage on plants, result in a cascading of biochemical and physiological processes that result in repair of a damaged tissue and inducing resistance of plants to respond against opportunistic pathogens (Suttle et al., 2013). Plant physical wounding slower plant growth and development rate resulting from the site of trauma that initiate cell division and synthesis of cellular components to repair the damaged tissue through water loss reduction (Suttle et al., 2013).

Plant wound healing is a complex process that begins with an oxidative burst, followed by secondary metabolism at the wounded site (Han et al., 2018). Phytohormones are part of the secondary metabolites, which are associated with regulation response of plants against wounding (León et al., 2001). Suttle et al. (2013) reported that the response of wounded leaves is mediated by increasing the synthesis of phytohormones and activation of their cognate signal transduction pathways. The response is often within minutes resulting in an increased content of bio-inhibitors such as jasmonates, abscisic acid (ABA) and ethylene (Han et al., 2018; Hildmann et al., 1992). This response has been confirmed in the leaf tissue of *Arabidopsis thaliana* ([L.] Heynh.), where jasmonic acid (JA) accumulated within two minutes of wounding. Although, the response of phytohormones synthesis occur rapidly, the recovery mechanism of many plants based on their compositional nature, time course of recovery and the regulation of wound-healing are still unknown for both endogenous and exogenous applications of phytohormones (Floková et al., 2014).

Jasmonic acid is a plant stress hormone known for the activation of the signal transduction pathway in response to biotic and abiotic stresses (Piotrowska and Bajguz, 2011). It has been reported to exert both stimulatory and inhibitory effects in different plants. Furthermore, when applied exogenously, it can modulate stress by either enhancing or suppressing plant development (Guo et al., 2018; Small and Degenhardt, 2018). It is known to promote senescence and stomatal closure of many plants (Guo et al., 2018). In addition, Guo et al. (2018) reported that JA signaling network plays a major role in the defense reflex response subsequent to plant wounding. An increased endogenous application of methyl jasmonate (MeJA) resulted in the slower development of soybean (*Glycine max* L.) primary root growth. This was reported by Xue and Zhang (2007), where MeJA at the highest concentration of $10^{-3}$ mol $L^{-1}$ caused an 83% inhibition of the primary root growth, while low concentration of $10^{-7}$ mol $L^{-1}$ resulted in slightly stimulated root growth.

Abscisic acid is a bio-inhibitor performing unique functions of stress alleviation in plant development and the adaptation to biotic and abiotic stress (Small and Degenhardt, 2018). It belongs to the isoprenoid group and it is also a by-product which is formed from the plastidal 2-C methyl-D-erythritol-4-phosphate pathway (Kundu and Gantait, 2017). It is known to be directly involved in certain physiological processes such as stomatal closure, embryo morphogenesis, seed development, germination and leaf senescence (Gaspar et al., 1996). Developmental functions were reported by Sansberro et al. (2004), where foliar application of ABA on yeba mate (*Ilex paraguariesnsis* A.St.-Hil.) plants increased growth by reducing water stress through stomatal closure. In addition, León et al. (2001) and Dammann et al. (1997) further reported that the exogenous application of ABA improved wounding tolerance of tomatoes (*Solanum lycopersicum* L.) and potatoes (*Solanum tuberosum* L.), and, subsequently, induced different sets of JA gene expressions in the leaves and roots of potatoes. Contrary to these findings, some studies showed greater involvement of ABA as an environmental stress regulator, rather than being directly involved in plant growth and development. Pēna-Cortés et al. (1989) reported that separate pathways for the developmental and environmental regulation of *pin2* gene expression is responsible for little influence in the tissue-specific expression in plant development. Therefore, ABA and JA crosstalk are identified as signaling molecules in local and systemic wounding (Pena-Cortes et al., 1989).

Rose geranium (*Pelargonium graveolens* spp.) is a native indeterminate plant species to South Africa (Araya et al., 2011). Little is known about the response of hail damaged rose geranium plants following exogenous application of ABA and MeJA, especially since Guo et al. (2018), Small and Degenhardt (2018) and Sansberro et al. (2004), reported that modulation of plant stress could either be enhancing or suppressing plant development. Therefore, the aim of this study is to determine the effect of foliar application and frequencies (7 and 14 days) of bio-inhibitors (ABA and MeJA) on yield recovery and wounding healing response time of hail damaged rose geranium.

## MATERIALS AND METHODS

### Study site

This study was conducted hydroponically during 2016 cropping season under natural light in a temperature controlled greenhouse at Central University of Technology, Free State campus situated in a semi-arid area at a latitude and longitude of 29°07'16.78"S and 26°12'45.95"E, respectively. The temperature of the tunnel was maintained at a maximum of 28°C.

### Experimental design and methods used

The study was set up in a randomized block design with three replications. The treatment layout was a 3×3×2 factorial design totalling 18 treatment combinations. Treatment combinations comprised of ABA at 0, 75 and 150 µM $L^{-1}$, MeJA at 0, 10 and 20 mM $L^{-1}$, and phytohormonal application frequencies for 7 and 14 days, respectively. Each experimental unit was 1 $m^2$ in size, containing six plants per unit. The row spacing and spacing between plants was 50 and 30 cm, respectively. Within the tunnel structure, a boarder row of rose geranium plants was cultivated around the treated plants in drainage troughs to eliminate any possible edge effects.

Rooted cuttings (±10 cm) of rose geranium (Bourbon type) were obtained from a commercial grower (Siyakholwa, RSA). Potting bags (5 L) sourced from Kloppers and Sons (RSA) were filled with a sterile silica sand root medium with a standard average texture of 2 mm in diameter (Sedibe and Allemann, 2012). One rooted cutting was transplanted into each pot and allocated a dripper tubing with a flow rate of 2 L $h^{-1}$. Plants were fertigated with a "drain to waste" drip system thrice a day (8:00, 12:00 and 16:00) for five minutes with a balanced nutrient solution prepared according to the levels described by Combrink (2005) and Sedibe and Allemann (2012, 2013) for rose geranium. As plants developed and the demand for more water and nutrients increased, the applied volumes were increased to ensure that 10 to 25% solution drained to waste to prevent salt accumulation in the potting bags as recommended by Combrink (2005) and Sedibe and Allemann (2012, 2013). The electric conductivity and pH of the nutrient solutions were maintained at 1.63 mS $cm^{-1}$ and 5.5, respectively (Combrink, 2005).

The main aim of rose geranium production is to increase the herbage mass. Therefore, hail-induced damage was simulated through 100% defoliation of the potted rose geranium plants on the third month of growth to assess yield recovery period. Since South African thunderstorms occur mostly in the afternoon, hail damage was simulated at approximately 14:00 pm.

A water suspension of ABA and MeJA (Sigma-Aldrich, RSA) elicitor was prepared following Pena-Cortés et al. (1989) and Ruiz-García et al. (2012) where Triton x100 was used as a wetting agent (0.1% v/v). Control plants were sprayed with an aqueous suspension of Triton x100 (Sigma-Aldrich, RSA) wetting agent and acetone (0.1% v/v). Following 10 days after the hail simulations (Van Bockhaven et al., 2013), each ABA and MeJA treatment level were applied once a day and repeated daily following the foliar application frequency treatment of 7 and 14 days. Foliar application of ABA and MeJA treatments were applied in the morning at 6:00 am to avoid degradation of the ABA and MeJA molecules (Melgoza et al., 2014). Abscisic acid and MeJA solutions were sprayed on each plant at 50 mL per pot, using a nozzle calibrated precision sprayer (0.3 MPa pressure) (Franks and Farquhar, 2001). To avoid spray mixture drifting onto the adjacent plants, a mini plastic covered tunnel (650×480×1200 mm) was used as a cover during each application.

### Parameters

Data was collected on the third month following hail simulation. Plant height, number of branches, branch and height ratio (B:H), foliar fresh mass, leaf area, chlorophyll content, oil mass and oil content were measured according to the procedures of Pérez-Harguindeguy et al. (2013) and Sedibe and Allemann (2012, 2013) to determine yield recovery following hail damage and foliar application of ABA and MeJA. Plant recovery rate was determined by the difference in the initial plant population and post exogenous application of ABA and MeJA recovery plant population as described by Sij et al. (2005).

### Statistical methods

Experimental data was analyzed using SAS GLM, version 9.2, SAS Institute Inc. (2008). The Shapiro-Wilks test was performed on the standardized residuals to test for deviations from normality (Shapiro and Wilk, 1965). The data was then subjected to an appropriate analysis of variance (ANOVA). Tukey's Studentized Range test was used to determine significant difference between the means ($\alpha$=0.05). Correlation was compared using Microsoft Excel software 2016.

## RESULTS AND DISCUSSION

Results presented in this study form part of the ongoing repeated study. Chlorophyll, leaf area, number of branches, plant height, B:H ratio, recovery rate and foliar fresh mass parameters were measured and attributed to the herbage yield, while oil mass and oil content are ascribed to oil yield.

During 2016 cropping season, there were no significant effects where ABA was exclusively applied in all herbage and oil yield parameters evaluated. Results from this study suggest that ABA could have been more involved in wounding stress regulation, rather than being directly involved in plant growth and development. This was reported by Pēna-Cortés et al. (1989), where the author suggests that separate pathways for the developmental and environmental regulation of *pin2* gene expression have little influence in the tissue-specific expression in plant development. The subsequent hail damage application frequency of 7 and 14 days had no significant effects in all yield parameters.

No significant effects were recorded where MeJA was applied on the chlorophyll content, number of branches, plant height, B:H ratio and the oil content parameters. However, a gradual increase in foliar application of MeJA showed a significant decline in the leaf area ($P$=0.01), recovery rate of plants ($P$=0.05), foliar fresh mass ($P$=0.05) and oil yield ($P$=0.01) of rose geranium plants as shown in Figures 1-4, respectively. This results highly confirm the key function of the jasmonates hormones on abiotic stress regulation. In line with the study, it is shown that the response of plants to wounding after simulated hail damage differ from one plant to another. Sanchez-Serrano et al. (1986) reported that there is an RNA homologous change as a result of wounding when compared with non-wounded leaf, whereas normally the expression of this gene is under strict developmental control. Key part of the developmental control include regulation of bio-inhibitors to regulate wounding. Methyl jasmonate has been reported to exert both stimulatory and inhibitory effects in different plants endogenously and through exogenous application (Guo et al., 2018; Small and Degenhardt, 2018). In this study, MeJA gradually reduced key yield parameters proportionally to the increase in application up to 20 mM $L^{-1}$. This response was also reported by Xue and Zhang (2007) on soy bean, where MeJA at the highest concentration of $10^{-3}$ mol $L^{-1}$ caused an 83% inhibition of the primary root growth, while low concentration of $10^{-7}$ mol $L^{-1}$ resulted in slightly stimulated root growth.

There was a significant interaction between ABA, MeJA and application frequency on the leaf area ($P$=0.05) and oil mass ($P$=0.05). Leaf area was increased by an interaction between ABA (150 µM $L^{-1}$), MeJA (0 mM $L^{-1}$) and the subsequent application frequency (14 days) ($P$=0.05) as shown in Figure 5. The results recorded in this study for leaf area showed an antagonistic relation between bio-inhibitors. This antagonistic relationship is ascribed to the ABA and MeJA signaling molecules in local and systemic wounding. Wounding activates systemin and propagates an electric signal which serves as a systemic and local signal that is involved in the accumulation of ABA which in turn stimulates the biosynthesis of JA (Pēna-Cortés et al., 1989; Hildmann et al., 1992). In contrast, Anderson et al. (2004) reported that an exogenous application of ABA suppressed both basal and JA-ethylene activated transcription from defense genes. Anderson et al. (2004) and Suttle et al. (2013) reports suggest that reduced plant growth and development rate are associated with antagonistic interactions between multiple components of ABA and the JA-ethylene signaling pathways which modulate defense and stress responsive gene expression in response to biotic and abiotic stresses.

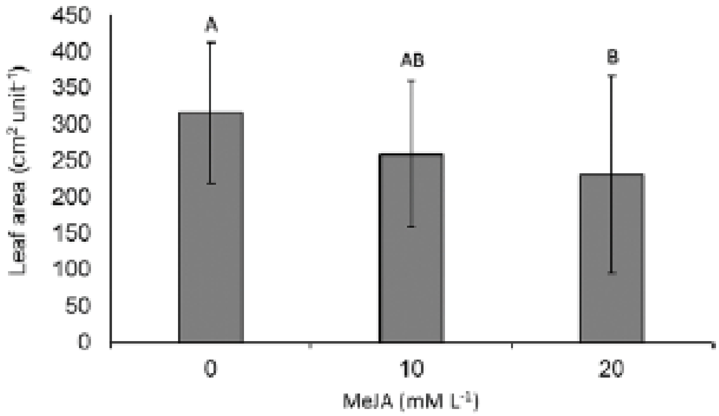



Figure 1. The effects of MeJA (0, 10 and 20 mM $L^{-1}$) on leaf area of hail damaged rose geranium.

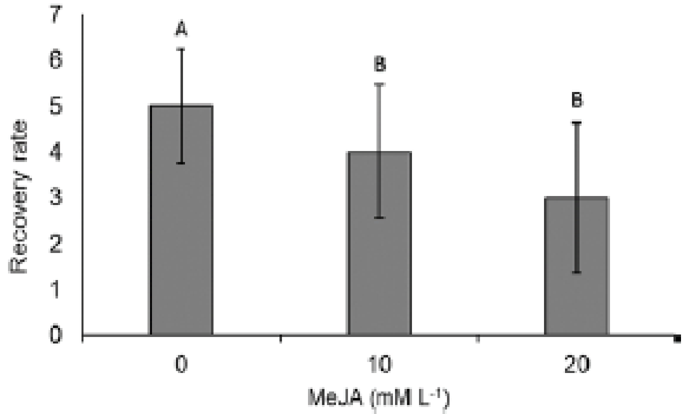



Figure 2. The effects of MeJA (0, 10 and 20 mM $L^{-1}$) on recovery rate of hail damaged rose geranium plants.

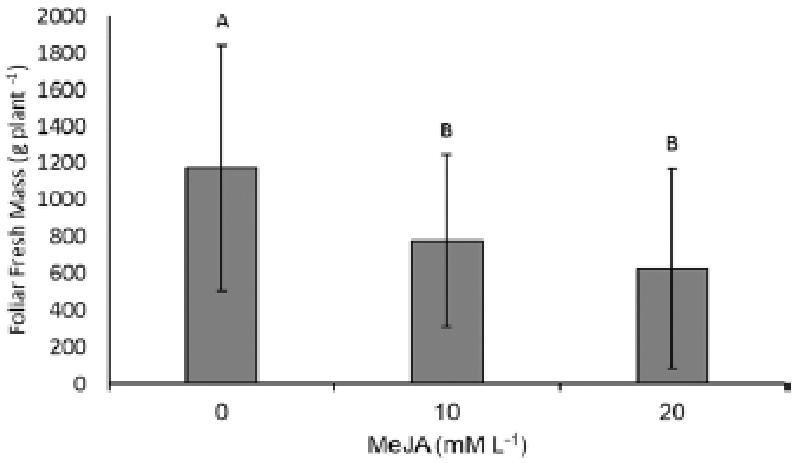



Figure 3. The effects of MeJA (0, 10 and 20 mM $L^{-1}$) on foliar fresh mass hail damaged rose geranium.

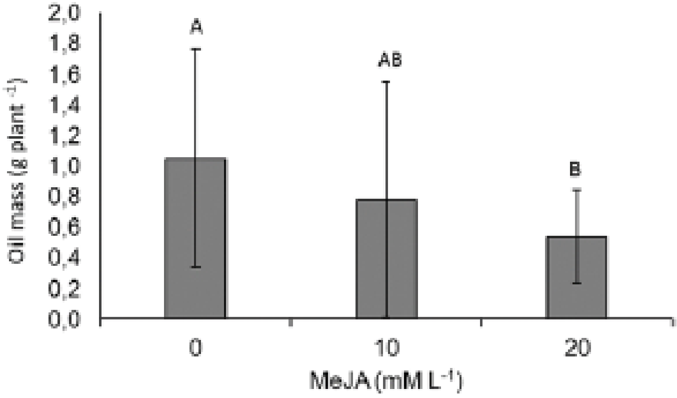



Figure 4. The effects of MeJA (0, 10 and 20 mM $L^{-1}$) on oil mass of hail damaged rose geranium.

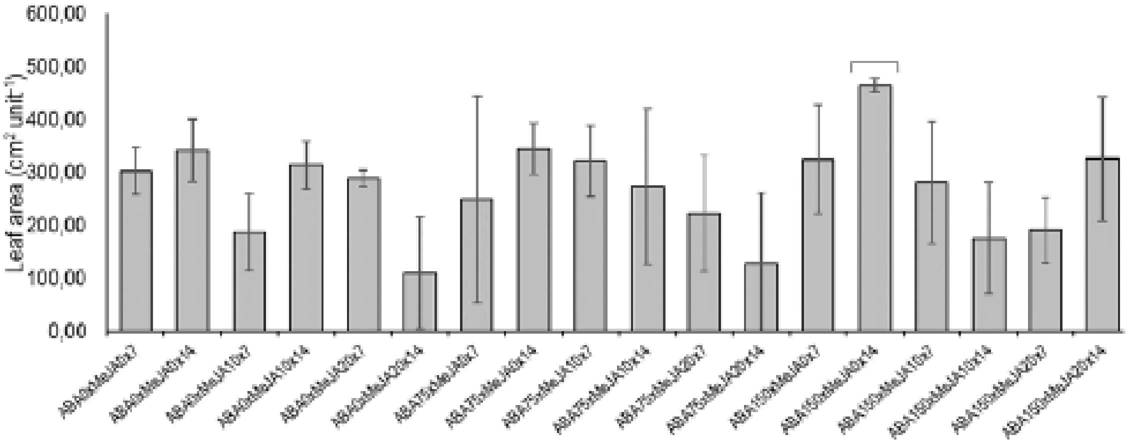



Figure 5. The interaction between ABA (0, 75 and 150 µM $L^{-1}$), MeJA (0, 10 and 20 mM $L^{-1}$) and application frequency (7 and 14 days) on leaf area of hail damaged rose geranium. A cap indicates a significant difference ($P$=0.05) between the ABA, MeJA and application frequencies.

Oil yield was rather increased where there was no administration of any of the bio-inhibitors (ABA [0 µM $L^{-1}$] and MeJA [0 mM $L^{-1}$]) for 7 days (Figure 6). It is a known phenomenon that yield and biosynthesis of oil are directly influenced by the plant foliage mass. Contrary, results in this study are attributed to the wounding repair rate and key functions of the bio-inhibitors. Bio-inhibitors are part of the secondary metabolites which are associated with regulation response of plants against wounding (León et al., 2001). Plant physiological chemistry such as the essential oil and phytohormonal content are changed under various mechanical damage (Banchio et al., 2007). The composition of volatiles released from damaged leaves were altered by an increased pulegone and diminishing menthone emissions as reported by Banchio et al. (2007). Moreover, depending on the phytohormonal crosstalk and proteins released, wound-inducible genes may either repair damaged plant tissue or produce inhibitor growth defense system (Sanchez-Serrano et al., 1986; León et al., 2001). According to Prins et al. (2010), the production and accumulation of essential oils as elaborated by Banchio et al. (2007), are related to specialized structures (glandular trichomes, secretory cavities and idioblasts) since they are very toxic to normal plant cells. The production and accumulation occurs through closely connected secretory structure formations. Banchio et al. (2007) and Prins et al. (2010) further suggested that under various mechanical damage, essential oils production is altered as a result of the direct effect on the stimulation of the essential oil biosynthesis, which directly benefits the essential oil yield and quality of the plant.

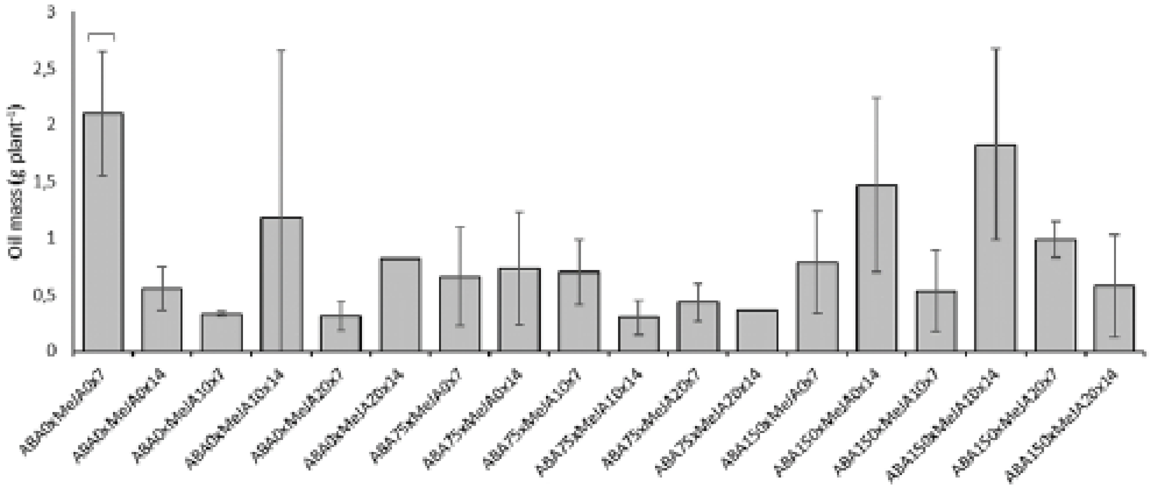



Figure 6. The interaction between ABA (0, 75 and 150 µM $L^{-1}$), MeJA (0, 10 and 20 mM $L^{-1}$) and application frequency (7 and 14 days) on oil mass of hail damaged rose geranium. A cap indicates a significant difference (*P*=0.05) between the ABA, MeJA and application frequencies.

## CONCLUSIONS

Rose geranium is a herbaceous crop and yield and biosynthesis of oil is affected by the mass of plant foliage, therefore the main focus in its production should be to increase the number of leaves, branches and the amount of herbage mass per unit area, as this is where most of the trichomes are located. Based on the results obtained from 2016 trial; ABA at 150 µM $L^{-1}$ for 14 days without any MeJA application (0 mM $L^{-1}$) improved herbage yield of hail damaged rose geranium plants. However, foliar application of bio-inhibitors is not recommended since biomass was not impacted by any application. Further study on essential oil quality analysis is recommended to conclude on the effects of foliar application of bio-inhibitors under hail wounding stress before rejecting the application of bio-inhibitors.

## ACKNOWLEDGEMENTS

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