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COMPARATIVE EFFECTS OF TWO ELICITORS ON THE ESSENTIAL OIL BIOSYNTHESIS OF SIMULATED HAIL-DAMAGED ROSE GERANIUM (*Pelargonium graveolens* L'Hér. cv. 'BOURBON')

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ABSTRACT

Extreme abiotic stress factors such as hail, defoliation, salinity, and moisture stress can affect the biosynthesis of essential oils. To mitigate this, most commercial farmers globally use agricultural crop insurance; however, this is not the case for most smallholder and emerging farmers in southern Africa, as agricultural insurance tends to be expensive when included in the production system. Therefore, this study aimed to investigate the comparative effects between two elicitors: abscisic acid (ABA) and methyl jasmonate (MeJA) on essential oil biosynthesis of simulated hail-damaged rose geranium (*Pelargonium graveolens* L'Hér.) as an alternative strategy to improve the essential oil biosynthesis following severe hail damage of rose geranium. The study was conducted in a 72 m^2 temperature-controlled greenhouse, using a 4 x 2 factorial treatment design arranged in a randomized complete block design. Treatments comprised four levels of two elicitors at 75 µM (ABA), 150 µM (ABA), 10 mM (MeJA), and 20 mM (MeJA), applied in two different application periods (daily application for either 7 or 14 days). The simulation of hail damage was through 100% defoliation, with decapitation of the terminal buds. A significant interaction was observed between the elicitor treatment, and length of the period of application on essential oil yield (mass), where the highest yield was observed when MeJA (10 mM) was applied for seven consecutive days compared to the 14-day treatment. Geraniol esters were partially improved by the application of MeJA at 10 mM. Application of the two elicitors improved citronellol, geraniol, and linalool, regardless of the length of the period of application. The citronellol to geraniol ratio, a determining factor of essential oil quality, was 2.7:1 when MeJA was applied at 10 mM, significantly better than any application level of ABA. Therefore, it could be concluded that the application of MeJA at 10 mM may improve the essential oil biosynthesis of hail-damaged rose geranium plants compared to ABA at any level, and this may be adopted as a possible mitigating strategy by the emerging farmers and growers in southern Africa for improving the biosynthesis of rose geranium post hail damage. On the other hand, where growers are interested in higher contents of linalool and citronellol, ABA may be used at any level.

Key words: Defoliation, essential oil, yield, phytohormone, citronellol, geraniol, linalool

INTRODUCTION

The production of rose geranium (*Pelargonium graveolens* L'Hér.) globally remains under threat as the industry faces challenges relating to the unevenness of the essential oil quality due to abiotic factors such as hail damage [1]. The use of agricultural crop insurance is a common practice to mitigate the loss in yield caused by 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. Some emerging and advanced commercial farmers tend to increase nitrogen fertilizer to facilitate the formation of new leaves and buds after hail; however, this has been shown to significantly reduce the essential oil quality based on International Standard ISO 4731(E) [2]. Essential oil quality is mostly important in the food industry, medicinal and perfumery, and aromatherapy industry, primarily produced by smallholder, and emerging farmers widely in the Mpumalanga, Limpopo, Eastern Cape, and KwaZulu Natal provinces of South Africa [3]. However, the most prominent use of essential oil is in the perfumery industry, and strictly requires a high standard that depends on the citronellol and geraniol ratio (C:G ratio), which should be <3:1 [4].

The chemical composition of rose geranium essential oil is complex and comprises many compounds contributing to the aroma, which is important to the lucrative perfumery industry [5]. Thus, the production and biosynthesis of rose geranium essential oil compounds can physiologically be affected by environmental factors, as well as by endogenous factors, which significantly affect the variation of the essential oil quality and yield [6]. Amongst the environmental factors, plant wounding and defoliation in the form of hail damage have been extensively reported to improve the yield and essential oil quality of rose geranium [7]. Upon enduring the wounding and defoliation, the first metabolic response of plants is the secretion of plant bio-inhibitors, which initiate the repair of damaged tissue, subsequently slowing the plant growth activities around the wounding site, and is later followed by upregulation of plant growth regulators at the wound site [8].

In a pilot study in 2016 and 2017, abscisic acid (ABA) and methyl jasmonate (MeJA) were reported to significantly improve the yield and essential oil biosynthesis of rose geranium when compared to the control [9]. However, it remained unclear as to which of the two elicitors may improve the essential oil biosynthesis to meet the International Standard ISO 4731(E) [2]. Therefore, in this study, ABA and MeJA were explored and compared for their ability to improve the essential oil yield and quality of simulated hail-damaged rose geranium. To date, biostimulants in the form of phytohormones have been extensively researched, and various patented products are sold commercially. However, at most, a majority has not been tested by essential oil growers under various adverse growing conditions and plant stress [10].

There is a high correlation between biomass and oil yield [7]. Changes in the secondary metabolites of essential oil plants vary depending on the type of damage incurred by the plant. Exogenous applications of natural and synthetic phytohormones have consistently demonstrated growth enhancement, yield, and quality optimisation, as well as physiological efficiency in plants. For example, the essential oil and phytohormone content (plant chemistry) is altered following mechanical damage that is caused by hail. Therefore, endogenous phytohormones are the primary inducible defence response for this class of volatiles, signalling the transduction pathway between wounding stress perception and induction [8]. These physiological response mechanisms occur within a matter of minutes to several hours, resulting in the activation of wound-related defence genes [10].

The production and accumulation of essential oils are restricted to specialised structures (for example glandular trichomes, secretory cavities, and idioblasts) since they are toxic to healthy plant cells [6]. 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 [6]. Therefore, it has a direct effect on the stimulation of essential oil biosynthesis, which directly benefits essential oil yield and quality.

According to Seni *et al.* [11], ABA and jasmonic acid are classified as primary plant-stress relief hormones due to their special function in alleviating plant stress, which subsequently facilitates plant development and tissue repair, as well as improving the primary and secondary metabolite content of plants. The application of ABA and jasmonic acid in the form of methyl jasmonate (MeJA) has previously been reported to improve the biosynthesis of secondary metabolites such as essential oils under normal growing conditions and adverse conditions. For example, Muthuramalingam *et al.* [12] reported that ABA improved the secondary metabolites of wounded mango (*Mangifera indica* L.), while Glauser *et al.* [13] reported an increase in jasmonic acid content in wounded leaves of *Arabidopsis thaliana* (L.). Moreover, Erb *et al.* [14] reported an increase in ABA and jasmonic acid upon wounding on maize (*Zea mays* L.). The application variation of ABA and MeJA has been reported to vary between 0.001 mM to 1.5 mM per plant, as well as 0.01 mM to 20 mM solution per plant [15].

Since ABA and MeJA have been proven to improve the yield and quality of plants under adverse conditions, this study seeks to determine the best plant stress hormone to improve the essential oil biosynthesis of simulated hail-damaged rose geranium plants. It is hypothesized that low concentrations of MeJA, applied daily for seven days will improve the essential oil yield and quality attributes of rose geranium compared to ABA applied for a short and prolonged period of application of 14 days.

MATERIALS AND METHODS

Study site and general crop management practices

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), South Africa. The site is situated in a semi-arid region, at ca. 1390 m above sea level. The greenhouse temperature was maintained at a constant 26°C. This experiment was conducted during the 2017/18 crop-growing season (September 1st, 2017 - March 10th, 2018). In South Africa, 'Bourbon' is the most common cultivar [16]; therefore, the rooted rose geranium cuttings 'Bourbon-type' (ca. 10 cm tall) were sourced and obtained from a reputable commercial grower (Siyakholwa Development Foundation, South Africa). These rooted cuttings were transplanted into 5 L potting bags on 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. As tabulated in Table 1, water analysis was conducted, and taken into account during the formulation of the nutrient solution The feeding water had an initial pH (25°C), electrical conductivity (25°C), and alkalinity ($CaCO_3$) of 7.9, 15.7(mS/m), and 19.2 (mg/L), respectively (Table 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 as illustrated in Table 2 [9]. No phytophagous pests were documented during the experiment, however, Malasol (an organophosphate) was preventively sprayed at 1.75 ml/L throughout the cropping season. These applications were repeated for 3-6 days, at 4-week intervals.

Hail damage simulation

Hail damage simulations were conducted on December 25, 2017, 116 days after transplantation of the rooted cuttings. In South Africa, thunderstorms occur mostly during the afternoon [17]; therefore, hail damage simulation was performed at ca. 2:00 p.m. 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. 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.

Experimental layout and treatment design

Experimental design and layout

This study had a 4 x 2 factorial treatment design. Plants were treated with four different concentrations of elicitors: 75 µM ABA, 150 µM ABA, 10 mM MeJA, and 20 mM MeJA after the hail damage simulations. The elicitors were applied for a duration of either 7 or 14 days, a day after simulated hail damage. Treatment combinations were arranged in a randomized 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.

Application of abscisic acid and methyl jasmonate

The application of the two elicitors (Sigma-Aldrich, RSA) took place between December 25, 2017, and January 7, 2018. The ABA and MeJA treatments were applied daily at ca. 6:00 a.m., using a calibrated precision sprayer (0.3 MPa pressure) (Ferndale, UK). A treatment solution of each of the elicitors (50 mL) was applied per plant in each treatment unit. To prevent the spray mixture from 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 to compare the two elicitors: 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 [18]. For the MeJA treatment solutions, 2.1 mL and 4.2 mL of the stock solution were diluted in 1 L distilled water to obtain final concentrations of 10 mM and 20 mM, respectively [19]. Triton X-100 at 0.1% (v/v) was added to the stock solution as a wetting agent. According to Kim *et al.* [20], stress and defense-related phytohormones are significantly downregulated and become irregular after stress; thus, prolonged application (7 and 14 days) was followed in this study to improve the plant's recovery chances.

Data collection

Data for essential oil yield, composition, and quality parameters were collected between the 5^{th} and 10^{th} of March 2018, 75 days after the hail damage simulations [21]. Plant foliar fresh mass (FFM) was determined by weighing foliar fresh plant material with a PGL 2002 Adam scale (USA) to determine the essential oil content. Rose geranium essential oil was extracted from three plants using a custom-built steam distillation unit. Approximately 2 - 5 kg of fresh plant material was distilled at ca. 98°C for one hour. Essential oil mass (yield) was determined by weighing the oil volume using a PGL 2002 Adam scale

(USA) immediately following extraction, as described by Khetsha *et al.* [22]. The foliar essential oil was determined by multiplying the essential oil mass by the net herbage yield (FFM). The extracted essential oil was analyzed using gas chromatography (GC) (Agilent 7890B) [22]. The ISO standard (ISO 4731 [E]) [2] was used to characterize rose geranium ('Bourbon-type' cv.) essential oil quality parameters (as used in the perfumery industry).

Statistical analysis

The FFM, essential oil yield, and essential oil quality parameters were statistically analyzed 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. The Shapiro-Wilks test of variance was performed on standardized residuals to test for any deviations from normality. Essential oil yield and the essential oil quality parameters were subjected to multivariate data analysis, using principal component analysis (PCA) function of PCA-XLSTAT (2015) to identify and evaluate the groupings between the variables. As described by Sedibe [23], PCA was performed by adjusting the data matrix, *X*, which consists of *n* observations (rows) on *p* variables (columns). The basic equation of PCA is, in matrix notation, given by:

$$Y = WX$$

where W is a matrix of coefficients that is determined by PCA.
These equations are also written as:

$Yij =$ W1i X1j+W2iX2j+…+WpiXpj

As seen, the components are a weighted average of the original variables. The weights, W, are constructed so that the variance of *y1, Var(y1)*, is maximized. Also, so that *Var(y2)* is maximized and that the correlation between *y1* and *y2* is zero. The remaining *yi*'s are calculated so that their variances are maximized, subject to the constraint that the covariance between *yi* and *yj*, for all *i* and *j* (*i* not equal to *j*), is zero.

RESULTS AND DISCUSSION

Selected essential oil yield attributes

The FFM and essential oil content were not improved at any level by the interactions between the elicitor treatment, regardless of the length of the period of application (Table 3). However, a significant interaction was observed between the elicitors treatment, and the length of the period of application on the essential oil yield (mass), where the highest yield was observed when MeJA (10 mM) was applied for 7 consecutive days compared to the 14-day treatment. In this study, increasing the length of the period of application of MeJA from 7 to 14 days reduced the essential oil significantly, and this could be 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 [24]. MeJA concentrations as low as 0.1 mM resulted 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.) [25]. Nonetheless, this significantly high essential oil yield due to the MeJA (10 mM) applied was not significantly different from the ABA and MeJA applied at 75 µM and MeJA (20 mM), regardless of the length of the period of application. From this observation in this study, it could be asserted that the essential oil yield of hail-damaged rose geranium may be negatively affected by increasing the two elicitors, regardless of the length of period of application.

Essential oil compounds and quality parameters

As shown in Table 4a citronellyl formate, guaia-6,9-diene, and isomenthone compounds were not significantly affected by either the main factors treatments or the interactions between plant stress hormones treatment and the length of period of application. However, in this study, all three geraniol esters (geranyl formate, geranyl butyrate, and geranyl tiglate) were recorded as significantly higher where MeJA (10 mM) was applied (Table 4a). Geraniol usually undergoes biotransformation into other terpenoids in aromatic plants, which influences the quality of the essential oil [26]. Geranyl formate, geranyl butyrate, geranyl tiglate, and geranyl acetate are some of the acyclic monoterpenes derived from geraniol, which are regarded as geraniol esters [27]. In this study, it was recorded that the geranyl formate content declined marginally when the MeJA concentration was increased to 20 mM, yet this was not significantly different compared to the MeJA (10 mM). The content of geranyl butyrate and geranyl tiglate was not statistically different from where ABA (150 µM) was applied at a higher level. There were no significant interactions recorded in all the geraniol esters (Table 4a). Results in this study may be due to the prolonged application of a low concentration of MeJA, which may have increased the content of geraniol esters. According to Zhang *et al.* [28], jasmonate is upregulated by wounding stress and is directly involved in the biosynthesis of these terpenes. Therefore, the accumulation of geraniol esters in this study could be attributed to simulated hail damage, followed by the subsequent daily application of MeJA. In addition, Liu *et al.* [27] reported that the accumulation of geraniol esters may be attributed to the biosynthesis of geraniol, and the effects of subsequent daily use of MeJA, which corroborated the findings reported by Zhang *et al.* [28]. 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. Interestingly, it was also observed that exogenous applications of ABA at higher levels tended to decrease these geraniol esters. Our observation indicate that costs associated with the use of plant stress hormones as biostimulants may be reduced by applying MeJA at a lower level, 10 mM to improve the geraniol esters contents to meet the International Standard ISO 4731(E) [2] for perfumery and aromatic industry.

As illustrated in Table 4b, no significant interactions between the elicitor treatment and the length of the period of application were recorded on the *cis*-rose oxide, *trans*-rose oxide, linalool, citronellol, and geraniol, and C:G ratio, the essential oil quality determining parameter. In addition, the two rose oxide compounds (*cis*- and *trans*-) were not significantly affected by the elicitors or the length of period of application treatments. However, when the two elicitors were compared, the linalool, citronellol, and geraniol content, and the C:G ratio tended to differ significantly.

A significantly high content of linalool was observed where ABA was applied at any level; however, this was not significantly different from when MeJA was applied at a higher level following severe simulated hail damage (Table 4b). Findings in this study could be attributed to abiotic stress, the 100% defoliation as a way of simulating the severe hail damage, which tends to trigger the accumulation of plant stress hormones like ABA as described by Jin *et al.* [29]. Ye *et al.* [30] corroborated this outcome, as ABA signal and linalool have strong crosstalk, in which cooperation and antagonism co-exist. However, contrary to this study, Huang *et al.* [31] reported that linalool significantly declined in ABA-treated grape (*Vitis vinifera* L.) fruit. On the other hand, jasmonate is directly involved in the mevalonic acid pathway, through the enzyme mevalonate-5-diphosphate carboxylase, which directly affects linalool biosynthesis [32]. 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, Van Schie *et al.* [33] demonstrated that applications over 18 μM (MeJA) may significantly affect the accumulation of linalool content.

The citronellol was significantly higher where ABA was applied at a lower level (75 μM), and this was within the threshold determining the citronellol content as described in the International Standard ISO 4731(E) [2] (Table 4b). Although MeJA (20 mM) was similar to the ABA (75 μM), it should be noted that the 26.09% was higher than the threshold of 26% as per the International Standard ISO 4731(E) [2]; thus, ABA (75 μM) was better compared to MeJA administration. It remains unclear as to whether increasing MeJA to 20 mM induces genes responsible for citronellol and geraniol biosynthesis since they are inseparable physically and chemically [34]; therefore, as suggested by Yue *et al.* [35], the upregulation of *VviGT14, VviGT15 and VviUGT85A1L1* transcript levels by MeJA could have been hyperinduced, thereby subsequently increasing the citronellol content. In addition, findings in this study were further supported by Lv *et al.* [36], who reported that the combination of ABA and brassinosteroids significantly promoted the citronellol content in grape plants. However, this was not the case in this study, as geraniol content was significantly lower where ABA was administered, irrespective of the levels. In plants, geraniol and ABA biosynthesis share a similar pathway [37]: 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. ABA and geraniol biosynthesis occur at this stage through the isopentenyl diphosphate source [38]. Croteau *et al.* [39] produced geranyl pyrophosphate synthase activity localised in the leaf epidermal glands of sage (*Salvia officinalis* L.), where monoterpenes are biosynthesised, suggesting that geranyl pyrophosphate synthase supplies the C10 precursor to synthesize monoterpenes. In this study 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 [38]. On the other hand, both levels of the MeJA were shown to increase the geraniol content, yet the lower level was not significantly different compared to the ABA treatment levels; thus, MeJA at 20 mM was favoured for increasing the geraniol content. The increased geraniol could be attributed to the two transcripts of *BmG10H* (*BmG10H-1and BmG10H-2*) following simulated-hail damage and characterisation by MeJA, a similar report by Jeena *et al.* [40] for waterhyssop (*Bacopa monnieri* (L.) Pennell.) after three hours of MeJA administration following wounding treatment in the shoot.

The C:G ratio was affected by the biosynthesis of citronellol and geraniol (Table 4b). The C:G ratio is used by the perfumery industry and International Standard ISO 4731(E) [2] 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 [40]. The biosynthesis pathways of citronellol and geraniol compounds are similar, which involve the conversion of mevalonic acid into isopentenyl pyrophosphate, for which the ABA is directly engaged through geranyl pyrophosphate [37]. 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. Interestingly, the best essential oil quality was recorded where MeJA was applied at 10 mM; however, this was not significantly different when compared to the 20 mM (MeJA) administration.

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For this study, the ANOVA results from the essential oil quality data were further subjected to multivariate data analysis, using PCA to determine the association between essential oil quality variables and elicitors treatment and application time. The score plot and loading matrix accounted for 54.5% of the total variance (Table 5). In PC1 (35.9%), linalool, guaia_6,9_diene, geranyl butyrate, and geranyl tiglate highly corresponded to the elicitor treatment and application time with the largest squared cosine. As illustrated in Figure 1, PC1, geraniol esters; geranyl butyrate, and geranyl tiglate were positively correlated by the use of 150 µM ABA (7 days) and 10 mM MeJA (14 days). On the other hand, linalool and guaia-6,9-diene were negatively correlated by the application of 150 µM ABA for 14 days: thus, indicating a decline with the prolonged application of a higher level. In PC2 (18.5%), this was not the case, as none of the essential oil compounds and quality variables correspond to the elicitor treatment and application time factors (Table 5). The results from the PCA corroborated the observations from the ANOVA results, clearly indicating that the application of MeJA effectively improved the essential oil quality compared to where ABA was applied following severe hail damage as prescribed by the International Standard (ISO 4731 (E)). Interestingly, it was also observed that the geraniol esters were partially improved by the application of MeJA at 10 mM.

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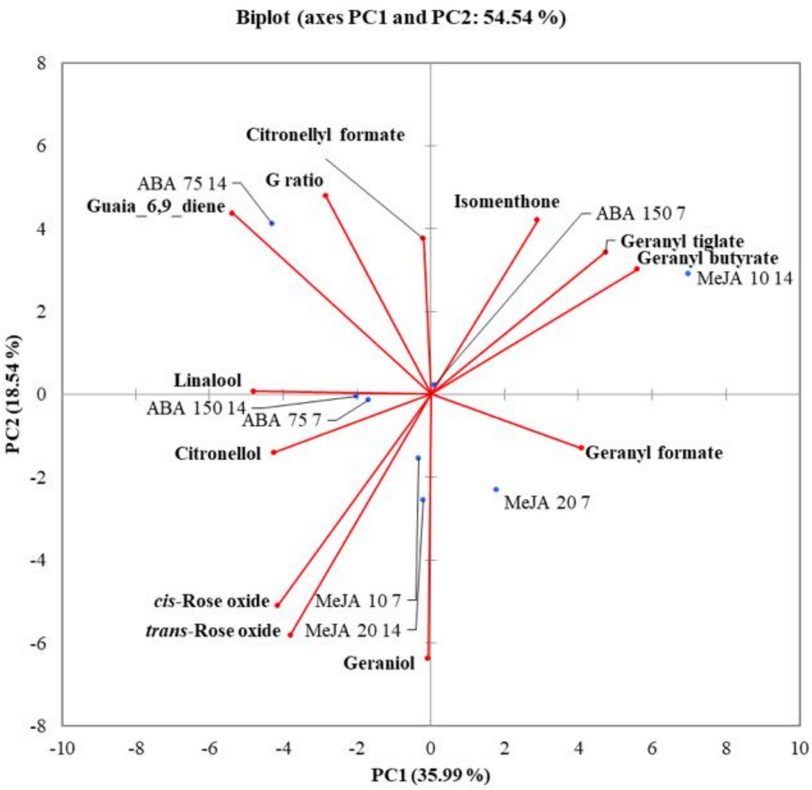

Figure 1: Principal component biplot illustrating the variations between simulated hail-damaged rose geranium essential oil compound and quality parameters, as affected by the application of the two elicitors and the application period

CONCLUSION, AND RECOMMENDATIONS FOR DEVELOPMENT

In this study, MeJA applied at 10 mM for a shorter period (7 days) improved the essential oil yield when compared to ABA application. Some of the key essential oil quality parameters such as linalool and citronellol were better when ABA was administered; however, it was interesting to note that the essential oil quality determining parameter, C:G ratio was better when MeJA was applied. Therefore, it could be concluded that the application of MeJA at 10 mM may improve the quality and most essential oil compounds biosynthesis of hail-damaged rose geranium plants when compared to ABA (at any level). We suggest that MeJA treatment could be adopted as part of the production system by emerging farmers and growers in South Africa to improve the post-hail damage biosynthesis capacity of rose geranium. On the other hand, where growers are interested in higher contents of linalool and citronellol, ABA may be used at any level.

This study, as a sequel to the pilot study only recommends the post-hail damage use of MeJA as an alternative when compared to ABA; however, future studies need to establish the natural source of MeJA from plants or any cheaper option other than the expensive inorganic source available. Therefore, the authors concluded that this study warrants the development of a biostimulant containing MeJA as a key ingredient for hail-damaged essential soil plants such as rose geranium.

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Table 1: Mineral water analysis for the experimental site during the 2017 and 2018 growing season (Testit-labs, RSA)

Minerals	Quantity
Ammonium (mg/L)	1.6
Calcium (mg/L)	16.8
Nitrate (mg/L)	<1
Phosphate (mg/L)	0.1
Potassium (mg/L)	2.3
Sulphate (mg/L)	8.6
Magnesium (mg/L)	3.2
Copper (µg/L)	<500
Manganese (µg/L)	<200
Zinc (µg/L)	0.07
Molybdenum (µg/L)	<20
Boron (µg/L)	<40
Chloride (mg/L)	9.4
Sodium (mg/L)	6

Table 2: Macro- and micronutrient compositions used in the nutrient solution

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

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Table 3: The effects of abscisic acid (ABA) and methyl jasmonate (MeJA) concentrations on the productivity of simulated hail-damaged rose geranium

Factors	Essential oil yield		
	FFM (g/plant)	Oil mass (g/plant)	Oil content (%)
Elicitor			
ABA (75 µM)	373.47[a]	0.25[a]	0.07[a]
ABA (150 µM)	309.72[a]	0.19[a]	0.09[a]
MeJA (10 MM)	378.05[a]	0.22[a]	0.05[a]
MeJA (20 mM)	438.88[a]	0.27[a]	0.06[a]
F-value	1.85	1.02	0.87
P-value	0.16[ns]	0.42[ns]	0.90[ns]
Application period			
7 days	397.77[a]	0.24[a]	0.06[a]
14 days	347.55[a]	0.22[a]	0.07[a]
F-value	2.76	0.22	1.36
P-value	0.15[ns]	0.64[ns]	0.40[ns]
Elicitor x Application period			
ABA (75 µM) x 7 days	346.11[a]	0.26[a,b]	0.07[a]
ABA (75 µM) x 14 days	458.33[a]	0.25[a,b]	0.07[a]
ABA (150 µM) x 7 days	419.44[a]	0.15[c,d]	0.04[a]
ABA (150 µM) x 14 days	383.05[a]	0.22[b,c]	0.15[a]
MeJA (10 mM) x 7 days	368.05[a]	0.37[a]	0.09[a]
MeJA (10 mM) x 14 days	378.89[a]	0.07[d]	0.02[a]
MeJA (20 mM) x 7 days	409.16[a]	0.30[a,b]	0.07[a]
MeJA (20 mM) x 14 days	210.27[a]	0.24[a,b]	0.06[a]
F-value	1.87	4.55	3.53
P-value	0.15[ns]	*0.01	0.45[ns]

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

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Table 4a: The effects of abscisic acid (ABA) and methyl jasmonate (MeJA) concentrations on essential oil compounds of simulated hail-damaged rose geranium

Factors	Essential oil compounds					
	Citronellyl formate	Guaia-6,9-diene	Isomenthone	Geranyl formate	Geranyl butyrate	Geranyl tiglate
Elicitor						
ABA (75 µM)	17.37^{a}	9.05^{a}	3.28^{a}	6.06^{b}	1.09^{b}	1.61^{b}
ABA (150 µM)	16.03^{a}	8.57^{a}	3.27^{a}	6.21^{b}	1.28^{a,b}	1.98^{a,b}
MeJA (10 MM)	16.21^{a}	8.05^{a}	3.48^{a}	7.27^{a}	1.42^{a}	2.27^{a}
MeJA (20 mM)	16.90^{a}	7.95^{a}	2.73^{a}	6.52^{a,b}	1.16^{b}	1.69^{b}
F-value	1.00	0.78	0.42	3.96	4.24	3.72
P-value	0.43^{ns}	0.55^{ns}	0.79^{ns}	*0.01	*0.01	*0.02
Application period						
7 days	16.36^{a}	8.18^{a}	2.88^{a}	6.41^{a}	1.23^{a}	1.87^{a}
14 days	16.76^{a}	8.58^{a}	3.34^{a}	6.72^{a}	1.23^{a}	1.90^{a}
F-value	0.70	0.90	0.85	1.09	0.03	0.01
P-value	0.41^{ns}	0.35^{ns}	0.36^{ns}	0.31^{ns}	0.85^{ns}	0.97^{ns}
Elicitor x Application period						
ABA (75 µM) x 7 days	17.28^{a}	8.26^{a}	3.66^{a}	6.11^{a}	1.07^{a}	1.49^{a}
ABA (75 µM) x 14 days	17.46^{a}	9.84^{a}	2.91^{a}	6.00^{a}	1.11^{a}	1.73^{a}
ABA (150 µM) x 7 days	15.84^{a}	8.57^{a}	3.34^{a}	6.09^{a}	1.31^{a}	2.07^{a}
ABA (150 µM) x 14 days	16.23^{a}	8.56^{a}	3.19^{a}	6.33^{a}	1.24^{a}	1.90^{a}
MeJA (10 mM) x 7 days	15.02^{a}	8.64^{a}	2.61^{a}	7.17^{a}	1.24^{a}	2.04^{a}
MeJA (10 mM) x 14 days	17.01^{a}	7.65^{a}	4.06^{a}	7.33^{a}	1.54^{a}	2.43^{a}
MeJA (20 mM) x 7 days	16.63^{a}	7.67^{a}	2.57^{a}	6.41^{a}	1.21^{a}	1.77^{a}
MeJA (20 mM) x 14 days	17.17^{a}	8.32^{a}	2.90^{a}	6.62^{a}	1.12^{a}	1.60^{a}
F-value	0.64	0.77	0.93	0.48	2.43	1.01
P-value	0.64^{ns}	0.55^{ns}	0.46^{ns}	0.75^{ns}	0.08^{ns}	0.43^{ns}

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

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Table 4b: The effects of abscisic acid (ABA) and methyl jasmonate (MeJA) concentrations on essential oil compounds of simulated hail-damaged rose geranium

Factors	Essential oil compounds					
	cis-Rose oxide	*trans*-Rose oxide	Linalool	Citronellol	Geraniol	C:G ratio
Elicitor						
ABA (75 µM)	0.29[a]	0.38[a]	1.90[a]	25.66[a]	7.42[b,c]	3.48[a]
ABA (150 µM)	0.48[a]	0.46[a]	1.69[a]	23.15[a,b]	7.33[c]	3.16[a,b]
MeJA (10 MM)	0.27[a]	0.28[a]	1.11[b]	21.32[b]	7.99[a,b,c]	2.72[c]
MeJA (20 mM)	0.96[a]	0.44[a]	1.43[a,b]	26.09[a]	8.96[a]	2.96[b,c]
F-value	1.44	1.22	3.38	3.43	3.43	4.01
P-value	0.26[ns]	0.33[ns]	*0.03	*0.03	*0.05	*0.03
Application period						
7 days	0.42[a]	0.93[a]	1.49[a]	23.10[a]	7.88[a]	3.06[a]
14 days	0.35[a]	0.77[a]	1.49[a]	24.32[a]	7.83[a]	3.13[a]
F-value	1.33	1.01	0.05	1.90	0.01	0.38
P-value	0.26[ns]	0.32[ns]	0.82[ns]	0.18[ns]	0.92[ns]	0.54[ns]
Elicitor x Application period						
ABA (75 µM) x 7 days	0.95[a]	0.41[a]	2.10[a]	24.60[a]	7.42[a]	3.33[a]
ABA (75 µM) x 14 days	0.82[a]	0.35[a]	1.69[a]	26.73[a]	7.43[a]	3.64[a]
ABA (150 µM) x 7 days	1.09[a]	0.49[a]	1.76[a]	22.18[a]	6.86[a]	3.23[a]
ABA (150 µM) x 14 days	0.96[a]	0.44[a]	1.62[a]	24.13[a]	7.80[a]	3.10[a]
MeJA (10 mM) x 7 days	0.88[a]	0.41[a]	1.25[a]	22.18[a]	9.33[a]	2.38[a]
MeJA (10 mM) x 14 days	0.40[a]	0.19[a]	1.02[a]	20.75[a]	7.10[a]	2.94[a]
MeJA (20 mM) x 7 days	1.02[a]	0.47[a]	1.21[a]	26.82[a]	9.69[a]	2.31[a]
MeJA (20 mM) x 14 days	0.90[a]	0.41[a]	1.64[a]	25.36[a]	8.23[a]	3.31[a]
F-value	0.57	0.63	1.27	0.35	2.64	2.86
P-value	0.68[ns]	0.64[ns]	0.32[ns]	0.83[ns]	0.07[ns]	0.06[ns]

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

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Table 5: Principal component analyses for the essential oil compounds and quality attributes of rose geranium

Traits	PC1	PC2
Eigenvalue	8.63	4.45
Variability %	35.99	18.54
Cumulative %	35.99	54.53
Linalool	**0.51**	0.00
cis-rose oxide	0.38	0.29
trans-rose oxide	0.32	0.38
Isomenthone	0.18	0.20
Citronellyl formate	0.00	0.16
Geranyl formate	0.37	0.01
Guaia_6,9_diene	**0.63**	0.21
Geranyl butyrate	**0.69**	0.10
Geranyl tiglate	**0.50**	0.13
Citronellol	0.39	0.02
Geraniol	0.00	0.46
C:G ratio	0.18	0.26

Values in bold correspond for each variable to the factor for which the squared cosine is the largest. PC = Principal Component; C:G ratio = Citronellol: Geraniol ratio

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