Rose-scented Geranium (*Pelargonium* spp.) Plant Growth, and Essential Oil Yield and Composition as Affected by Paclobutrazol Application

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Abstract. Rose-scented geranium oil is extracted from the shoots (mostly the leaves) of the *Pelargonium* spp. through steam- or hydro-distillation. To extract less than 0.2% oil, farmers must transport and distil bulky herbage. This makes geranium oil production costly, and high time- and energy-consuming process. To investigate the effect of different paclobutrazol (PBZ) concentrations (0, 100, 200, 300 mg/L of water) on vegetative growth, and oil yield and composition of rose-scented geranium, three pot experiments were conducted. The experiments were conducted in a glasshouse of the University of Fort Hare, Alice, South Africa (located at 25°45' S and 28°16' E, an altitude of 520 m above sea level), between Oct. 2011 and May 2013. The treatments were arranged in a randomized complete block design (RCBD) in four replications. The PBZ was sprayed on the plants at 1 month of regrowth stage. Chlorophyll content increased with concentration of PBZ. The reduction of plant height in all PBZ-treated plants was significant, ranged between 18% and 33%. Plant canopy also reduced by 5% to 23%, and the differences were more noticeable in the plants grown between January and May (summer/autumn season), producing compact plants. Leaf area and internode length reduced as PBZ concentration increased. Paclobutrazol had no significant effect on leaf number, and essential oil yield and composition. This implies that, through applying PBZ, compacted (less bulky) rose-scented geranium could be produced without significant change in essential oil yield per plant and essential oil composition.

Rose-scented geranium (*Pelargonium* spp.) is a perennial herb that belongs to the Geraniaceae family (Eiasu et al., 2007; Rajeswara Rao et al., 1996). It is cultivated mainly for its high-value essential oil that is used in the perfumery, aromatherapy, pharmaceutical, and food processing industries (Prins et al., 2010; Singh, 1999). Rose-scented geranium oil, widely known as “geranium oil,” can be extracted from fresh leaves and, to very small amount, from tender stems and flowers, through steam-distillation, hydro-distillation, or both techniques. Motsa et al. (2006) describes geranium oil has fine rose-, citrus-, and mint-like odors.

Market value of geranium oil is governed by the quality and quantity supplied. The major constituents of geranium oil are citronellol, geraniol, iso-menthone, citronellyl formate, geraniol formate (Peterson et al., 2006; Weiss, 1997). Department of Agriculture, Forestry and Fisheries (2012) claims

that the rose-scented geranium produced in South Africa meets the quality standard of the Bourbon-type geranium oil (with a citronellol to geraniol ratio around 1:1). Report of Eiasu (2009), however, indicates that the citronellol to geraniol (C:G) ratio was high (most of the time more than 3:1) in Pretoria (in the Gauteng Province of South Africa). Even in quantity wise, South Africa has not made a significant contribution of geranium oil to the world essential oil market (Bhan et al., 2005).

Bulky herbage yield contributes to low income from rose-scented geranium because it results in high labor, transport, and distillation costs. Geranium oil content is usually less than 0.2% of the herbage fresh weight (Shawl et al., 2006). Therefore, to extract the reasonable amount of geranium oil, a large distillation steel is needed. In addition, a large quantity of herbage needs to be transported to the distillation site (Eiasu, 2009). The leaves are the main source of essential oil of geranium oil; the contribution of stems and petiole is negligible (Mallavarapu et al., 1997). Finding a means of reducing bulkiness of the shoots of rose-scented geranium harvested for distillation without reducing the number of leaves, maintaining the oil yield per plant, would improve profits from rose-scented geranium cultivation.

Some exogenous plant growth regulators are known to reduce cell elongation, often by interfering with the synthesis of naturally occurring hormones, gibberellins (Berova and Zlatev, 2000; Polk and Walker, 2004). According to Hickman (1986), commercial growers achieve production of compacted ornamental plants through applying growth regulators (retardant). Povh and Ono (2006) observed that 100 mg/L of gibberellic acid increased essential oil yield of sage (*Salvia officinalis*). The authors attributed the higher essential oil yield attained to an increase in leaf number. Treating *Thimus mastichina* with benziladenine (at concentration of 0.1 mg/L) resulted in higher essential oil yield through increased trichome density (Fraternali et al., 2003). Similarly, work of Sudriá et al. (2004) showed that benziladenine delays disruption of essential oil glands and leaf senescence in lavender (*Lavandula dentata*), which helped the plants to retain their essential oil for a longer time.

Paclobutrazol, also available in chemical stores by the name “cultural,” is a vegetative growth retardant. It counteracts the biosynthesis, activity, or both of endogenous gibberellins in plants (Costa et al., 2012). Several studies have demonstrated numerous beneficial effects of PBZ (as growth regulator) including reduced plant vegetative growth to produce dwarf, sturdy plants (Davis and Curry, 1991; Lolaei et al., 2013), improved fruit quality (Quinlan 1981; Wolstenholme et al., 1988), and increase potato yield (Tsegaw, 2005). In addition, Esmaelpour et al. (2011) reported that PBZ increased leaf yield in potatoes. Therefore, application of PBZ on rose-scented geranium could result in reduced plant size, improving or without severely reducing the number of leaves which are the major source of the essential oil produced (Eiasu et al., 2012; Mallavarapu et al., 1997). Producing compacted geranium plants (Lolaei et al., 2013) could also allow farmers to increase rose-scented geranium oil through increasing the number of plants per area. Hence, the main objective of these experiments was to determine the effect of different PBZ concentrations on rose-scented geranium vegetative growth, and essential oil yield and composition.

Materials and Methods

Growing system and planting culture. The experiments were conducted between Oct. 2011 and May 2013 in a greenhouse at the University of Fort Hare, Alice Campus, South Africa. Experiment 1 was harvested in May 2012, Experiment 2 in Dec. 2012, and Experiment 3 in May 2013. The site is located at latitude and longitude of 25°45' S and 28°16' E, respectively, and an altitude of 535 m above sea level. Light penetration through the polycarbonate roofing sheet of the greenhouse was in the range between 80% and 90%. A computer-controlled cooling system was set to regulate the temperature when it arises to temperatures higher

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than 25 °C. Sometimes the temperature may surge up to 35 °C for few hours during the hottest days. Rose-scented geranium (*Pelargonium* spp.) was used as plant material. For each experiment, healthy stem cuttings of rose-scented geranium, taken from healthy growing plants, were raised in seedling trays. Hygromix® (Hygrotech, Pretoria, South Africa) was used as rooting media, and the rooting process and development of the plantlets were conducted on a mist bed for about 45 d. Plantlets were transplanted to 15-L pots, filled with sandy loam soil (56.2% sand, 21.8% clay, and 22% silt content) from the University of Fort Hare Research Farm.

Treatments and experimental designs. Before applying treatments, the plants were allowed to grow for 2 months. Then, they were cut back to start the experiment on plants with more or less uniform growth. Thus, each experiment had duration of 6 months after transplanting (2 months before cutting back and 4 months of regrowth until harvesting). Four PBZ concentrations [T1 (control): 0 mg/L; T2: 100 mg/L; T3: 200 mg/L; and T4: 300 mg/L] were applied as treatments. Experiments were laid out as a RCBD replicated four times. In each block, each treatment was represented by 10 plants. The space between adjacent rows on the tables was 1 m, and the plants within a row were 0.30-m apart. In each experiment, plants were sprayed with PBZ (until shoot surfaces get wet) 2 months after cutting back and harvested 2 months after treatment application.

Agricultural practices. Following Eiasu et al. (2012) fertilizer application rates, during transplanting and after cutting back for regrowth, each plant received 3 g nitrogen (N), 4.5 g phosphorus (P), and 3 g potassium (K) [in the form of 2:3:2 (22) as a split in Week 1 and 7 of each regrowth cycle]. In addition, 1 g N (as limestone ammonium nitrate) and 1 g K (as potassium chloride) were applied to each pot in Week 9 of the regrowth cycles. Pest (usually white flies and aphides) control measures were taken when necessary.

Computer-controlled spaghetti-type drip irrigation system was used for irrigation. On average, the spaghetti drippers were emitting about 4.5 L/h. Plants were well watered on well-drained soil (sandy clay loam). The irrigation system was set to water the plants three times a day: around 1000, 1400, and 1700 hr. Irrigation duration ranged between 2 and 8 min/irrigation event (i.e., 150–600 mL), depends on growth stage and temperature of the growing system. Municipality water (with 7.4 pH and 38.5 mS/m electrical conductivity) was used as source of irrigation water.

Data collected and statistical analysis. Starting from Week 3 after treatment applications, chlorophyll readings were taken on a weekly basis (Minolta Chlorophyll Meter, SPAD-502DL; Konica Minolta, Osaka, Japan). Immediately before harvesting (4 months after cutting back), canopy diameter, plant height (vertical distance

Fig. 1. Plant height, internode length, and canopy diameter of rose-scented geranium plants treated with different paclobutrazol concentration: vertical bars are least significantly different at 5% probability levels for the same parameter and the same harvesting.

from the soil surface to the tops of the plants), and internode length (from shoot top between the fifth and sixth fully open leaves) were recorded.

Five plants per “plot” were cut to a height of about 10–15 cm aboveground (leaving at least two nodes on each branch as potential sites for next regrowths). Then leaves and stems were separated and data related to leaf area [using an LI 3100 belt-driven leaf area meter (LI-COR, Lincoln, NE)], leaf number, and fresh weights recorded.

The rest five plants were harvested and leaves separated from the stem. After thoroughly mixing, leaves of about 500 g were sampled from each replication of the treatment, and essential oils were separated (extracted) through hydro-distillation method (using 10-L reacting flask mounted on heating mantle, and Inland Revenue condenser and Clevenger separator). Distillation duration (starting from the boiling of the water) was 90 min.

Essential oil composition was analyzed at the SABS (South African Bureau of Standards) using the gas chromatography (GC) analysis technique, where an Agilent GC (FID) model 6890N (Agilent Technologies, Inc., Santa Clara, CA) fitted to a 30 m × 0.25 mm fused silica capillary column and a film thickness of 0.25 µm was used. Helium gas was employed as carrier. The GC-analysis temperature was set to progress from 50 to 200 °C with ramp amount of 5 °C·min⁻¹. A temperature of 220 °C was used for the detector and injector. Essential oil constituents were identified by comparing their retention time and retention indices to standard values (Adams, 2004).

Data analysis. Data collected were subjected to analysis of variance using MSTAT-C,

Fig. 2. (A) Plant canopy sizes of rose-scented geranium plant treated 0 and 300 mg/L paclobutrazol concentrations and (B) effect of PBZ on growth of secondary branches (indicated by arrows) and leaves.

a data analyzing microcomputer program (MSTAT-C, 1991). Where applicable, means were compared using the least significant difference test (at $\alpha = 0.05$).

Results and Discussion

Effect PBZ on plant size. Plant size parameters (plant height, canopy diameter, and internode length) are presented in Fig. 1. All of these variables showed inverse relationships with PBZ concentration: they showed progressive declining trends as the PBZ concentration increased. Plant height and internode length were more sensitive

to the treatments (significantly responded to the 100 mg/L) unlike the canopy diameter, which started to significantly decline at PBZ concentration of 200 mg/L, particularly in Experiment 1.

There was no noticeable difference among 100, 200, and 300 mg/L PBZ concentrations on canopy diameter. Plant height and internode length of plants treated with 100 mg/L were significantly higher than those treated with 200 and 300 mg/L PBZ concentrations. Effect of the two lowest PBZ concentrations on these parameters was the same. The reduced plant height and canopy diameter resulted in compact plants that would need smaller space (Figs. 1 and 2A).

These results agree with reports on the effect of PBZ applied on different crops as growth regulator (Lolaei et al., 2013). According to Tekalign and Hammes (2004), PBZ reduced vegetative growth in potato, shifted the sink strength to tuber, and maximized yield in potatoes. Esmailpour et al. (2011) also agree with the current results that overall vegetative growth of potatoes treated with PBZ reduced as a result of reduction in stem length. Bañón et al. (2002) also recommended PBZ application on *Dianthus caryophyllus* cv. Mondriaan (an ornamental plant) to control growth to improve commercial quality of the plant. In addition, PBZ suppressed plant height and canopy size in sage (*Salvia sclarea* L.) (Singh et al., 2008). Such a result indicates that treating geranium plants with PBZ would reduce bulkiness of herbage that is distilled; thus, distillation steels can accommodate shoot of more plant at each distillation event. In addition, such a reduction in plant size would potentially enable farmers to plant more plants per given area.

Stem and leaf mass. In all harvests, stem mass was significantly reduced in those plants that were sprayed with PBZ (Table 1). The declining in stem fresh weight was in the ranges between 14% to 16% in the 100 mg/L, 26% to 28% in the 200 mg/L, and 22% to 36% in the 300 mg/L PBZ concentrations. These results support the reports of Esmailpour et al. (2011); Lolaei et al., (2013) and Tsegaw (2005), implying that overall stem growth retards as PBZ concentration increases, resulting in low mass. Such a decrease in the mass of stem rose-scented geranium would minimize labor and transport costs.

The response of leaf fresh weight to the different PBZ concentrations was not consistent. In Experiment 1, no significant differences were observed among the treatments including the Control. However, the highest PBZ concentration significantly reduced leaf mass in Experiments 2 and 3. The possible reason for the relatively lower reduction of leaf mass in the 100 and 200 mg/L is that the mass of individual leaves reduced, but at least to certain degree, PBZ promoted development of secondary branches that slightly increased the number of leaves per plant (Fig. 2B). In agreement with the current results, Hashemabadi et al. (2012) observed

Table 1. Fresh leaf and stem masses of rose-scented (*Pelargonium* spp.) geranium plants treated with different paclobutrazol levels.

Paclobutrazol concn	Expt. 1: Fresh wt (g/plant)		Expt. 2: Fresh wt (g/plant)		Expt. 3: Fresh wt (g/plant)	
	Stem	Leaf	Stem	Leaf	Stem	Leaf
0 mg/L	136.3 a ²	246.4 a	274.9 a	450.0 a	218.3 a	363.6 a
100 mg/L	113.7 c	189.9 a	235.2 b	395.1 ab	187.4 b	352.1 ab
200 mg/L	100.2 b	189.8 a	197.1 c	352.2 ab	153.2 c	328.0 bc
300 mg/L	105.3 b	200.0 a	175.4 c	313.2 b	140.9 c	310.0 c
Grand mean	113.9	212.5	220.7	386.1	174.0	338.4
CV (%)	6.06	21.4	22.1	22.4	8.1	6.9
LSD (5%)	12.06	72.0	36.6	108.6	19.3	32.2

²Mean values in each column with the same letter are not significantly different at 5% probability level. LSD = least significant difference.

Table 2. Leaf area and leaf number of rose-scented geranium (*Pelargonium* spp.) plants treated with different paclobutrazol levels for Experiment 3.

Paclobutrazol concn	Leaf area (m ² /plant)		Number of leaves/plant	
	Expt. 1	Expt. 3	Expt. 1	Expt. 3
0 mg/L	1.90 a ²	1.26 a	311.1 a	278.08 a
100 mg/L	1.50 b	0.97 b	301.4 a	296.97 a
200 mg/L	1.14 bc	0.84 b	320.4 a	301.79 a
300 mg/L	1.07 c	0.80 b	305.6 a	269.06 a
Grand mean	1.40	0.97	309.6	288.98
CV (%)	17.38	14.90	14.6	19.30
LSD (5%)	0.38	0.21	35.2	46.30

²Mean values in each column with the same letter are not significantly different at 5% probability level. LSD = least significant difference.

an increased number of leaves in *Calendula officinalis* treated with growth retardant (daminozide). These results also highlight that PBZ inhibits apical dominance of the primary shoots. Otherwise, these results do not agree with Esmailpour et al. (2011) who claimed that PBZ increases leaf yield (in terms of mass). Probably different plant species respond to PBZ differently as observed in turfgrass seedlings (Shahrokhi et al., 2010).

Leaf area and leaf number. Leaf area and leaf number of the rose-scented geranium treated with PBZ in Experiments 1 and 3 are presented in Table 2. These two parameters are important because most of the essential oils are secreted, stored, or both in the oil glands (trichomes) which are situated on the surface of the leaves (Werker, 2000). In both Experiments, mean leaf area/plant was significantly reduced in the plants treated with PBZ. Such results indicate that, although not as severe as in stem length (Ghosh et al., 2010; Ozgur, 2011), PBZ reduces leaf size. These results agree with the findings of other research's reports in other plant species. Lolaei et al. (2012), for example, reported that PBZ reduced leaf area in strawberry (*Fragaria ×ananassa* Duch. cv. Selva).

The results of the current experiments reveal that PBZ application did not significantly affect leaf number per plant. Although in consistent, in some PBZ rates, the number of leaves tended to be slightly higher than in the control, which could be as a result of enhanced development of secondary branches and inhibition of apical dominance of the primary branches observed (Fig. 2B). The overall observation, however, confirms the findings of Wolstenholme et al. (1988) on avocados that indicated that leaf number was not affected by PBZ application. Lolaei

Fig. 3. Leaf chlorophyll contents of rose-scented geranium plants treated with different paclobutrazol concentration: vertical bars are least significantly different at 5% probability levels for the chlorophyll content measured in the same week.

Fig. 4. Essential oil content (% on leaf fresh weight basis) of rose-scented geranium plants treated with different paclobutrazol concentration: vertical bars are least significantly different at 5% probability level.

et al. (2012), on the other hand, reported that PBZ reduced leaf number in strawberry.

Effect of PBZ on leaf chlorophyll content. In general, chlorophyll concentration was the

Fig. 5. Essential oil yield of plants treated with different paclobutrazol concentrations; vertical bars are least significantly different at 5% probability level.

Fig. 6. Composition of rose-scented geranium treated with different paclobutrazol concentrations.

lowest in the control (0 mg/L PBZ) in all the sampling events (Fig. 3). A significantly higher concentration was recorded only for the highest PBZ concentration applied (300 mg/L). In agreement with the current results, an extensive review by Lolaei et al. (2013) confirms that PBZ increases total chlorophyll content in plants. Tekalign and Hammes (2004) also reported increases in chlorophylls a and b in potatoes treated with PBZ. Such an increasing chlorophyll concentration in the reduced leaf area may enable the leaves in the higher PBZ concentration to produce as much photosynthate as those large leaves in the control treatment.

Data recorded on Weeks 13 and 14 (Fig. 3) show that, although leaves under all PBZ treatments tend to lose their chlorophyll as plant/leaf age advances, the declining rate was the fastest in the control. As a result, all leaves sprayed with PBZ retained significantly higher leaf chlorophyll compared with that of the control. Such results could be explained by possible leaf anatomical changes induced by PBZ, as reported by Tsegaw (2005). The author investigated effect of PBZ on leaf morphology of potato and discovered that PBZ induced changes in leaf anatomy. The leaf anatomical change may delay senescence. This delayed senescence could have positive contribution to essential oil yield of rose-scented geranium and other aromatic and medicinal plants where there could be a positive relationship between leaf longevity and biosynthesis of this biochemical (Ibrahim et al., 2014; Weiss 1997; White et al., 1987). Similarly, work of Sudriá et al. (2004) showed that benziladenine increased

green color of an essential oil plant, lavender (*Lavandula dentate*). According to the authors, persistence of the green color was accompanied by delayed leaf senescence and low disruption of essential oil glands (trichomes).

Miller and Armitage (2002) and Steinke and Stier (2003), cited in Gliózeris et al. (2007), agree that chlorophyll content per unit leaf area increases; However, they are of the opinion that the increase in chlorophyll concentration as a response to plant growth regulator application to be as a result of reduction of leaf expansion accompanied with unchanged chlorophyll biosynthesis rate per leaf.

Essential oil content and yield. In all experiments, essential oil content (% on fresh weight basis) showed an increased tendency with PBZ concentrations (Fig. 4). The two higher PBZ concentrations (200 and 300 mg/L) significantly increased oil content compared with that of the control. The lower concentration (100 mg/L) was not significantly higher than that of the control. The increasing trends of oil content with the concentration of PBZ could be attributed to the slight decreases in leaf size (as reflected in mean leaf mass and leaf area in Tables 1 and 2), whereas the essential oil per leaf remains unaffected. These results support the reports of Eiasu et al. (2012). The authors highlighted that the number of oil glands and oil yield per leaf are determined at the leaf initiation (primordial) stage (no new oil glands are produced when the leaves continue to expand). Such facts were also established by White et al. (1987) in an experiment that evaluated the contribution of leaf number and size to essential oil yield of pepper mint. The authors concluded that leaf number was more important than leaf size. Sudriá et al. (2004), on the other hand, reported trichome number and trichomes retention trade-off in lavender plants treated with growth regulators: growth retardant hormones reduced trichome number but delayed trichome disruption.

Singh et al. (2008) claimed that PBZ increases oil yield of sage (*Salvia sclarea*). Similarly, Keramati et al. (2016) observed increase of essential oil yield in basil (*Ocimum basilicum* L.) treated with PBZ. The current results, however, show that different PBZ concentrations did not significantly affect rose-scented geranium oil yield (Fig. 5). Several factors could have contributed to such a result, including less effect on the number of leaves if any, promoting secondary branches (Fig. 2B), and keeping the leaves afresh/green for a relatively longer period of time thus delaying senescence (Fig. 3). This results indicate that canopy size (stem length in other words) may not affect essential oil yield provided that the treatment retard stems, which have negligible contribution to essential oil yield, without significant effect on the number of leaves as stated by Mallavarapu et al. (1997) and Eiasu et al. (2012). In addition, it proves that leaf number to be more important than leaf size as the essential oil yield did not

follow the reduction in leaf area (Table 2). Therefore, by applying PBZ, volume of herbage distilled can be reduced without significantly reducing the oil yield. Such a practice could also reduce transport cost and energy (power) used for distillation. In addition, the current results show that big (untreated) plants give the same amount. These results indicate that essential oil yield is a function of number of plants per hectare.

Essential oil composition. Rose-scented geranium essential oil is composed of several compounds that belong to different groups of organic chemicals, including alcohols, esters, and acids (Deans, 2002; Kayser et al., 1998). However, in the perfumery and aromatherapy industries, the major quality determinant compounds are geraniol, citrenellol, geraniol formate, citronellyl formate, linalool, isomenthone and guaia-6,9-diene (Motsa et al., 2006; Shawl et al., 2006; Verma et al., 2010). The major essential oil component of the PBZ-treated rose-scented geranium plants did not show significant differences (Fig. 6). Citronell and its fomate remained dominant, and the citronellol to geraniol ratio was more or less the same (within the range between 3.3. and 4), which was not significantly different. In agreement with the current results, Keramati et al. (2016) reported that PBZ had no noticeable effect on oil composition in basil (*O. basilicum*). This indicates that PBZ does not alter the biosynthesis of the major rose geranium essential oil constituents. Lin et al. (2008), on the other hand, reported that secondary metabolite (enzymes and antioxidants) concentrations changed in sweetpotatoes (*Ipomoea batatas*) treated with PBZ. Oudin et al. (2007) also reported an increase in essential oil yield and alteration in essential oil composition of *L. dentate* treated with cytokinin (0.1 mg/L). Singh et al. (2008) noticed an increase in linalool-linalyl acetate content by about 12% in sage (*S. sclarea*).

The current experiments consistently proved that PBZ reduces rose-scented geranium plant size (canopy diameter), leaf area without significant effect on leaf number. Reduction in leaf size brought in relatively higher essential oil content and chlorophyll content. Essential oil yield per plant was not significantly declined. In addition, it did not significantly interfere with the biosynthesis of the major components of the essential oil. Such a discovery will improve the profitability of rose-scented geranium producer by reducing bulkiness of the plant materials that are harvested for distillation. Reduced vegetative growth also reduce transport costs, distillation time (for harvested per hectare), and the expenses for labor and energy (electricity and fuel). Therefore, farmers are advised to retard geranium plants by applying PBZ (preferably 200 mg/L a.i. of PBZ) when the plants start to regrow at a faster rate (2 months after cutting back).

The current result implies that, as PBZ reduces plant size, through application of PBZ, the number rose-scented geranium plants per area can be increased, which would result in increased essential oil yield. Therefore, research

needs to be done to determine optimum density of PBZ-treated plants that maximizes essential oil yield at field level.

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