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*Short Communication*

# Value addition of essential monoterpene oil(s) in Geranium (*Pelargonium graveolens*) on leaf positions for commercial exploitation

**A. Misra* and N. K. Srivastava**

Central Institute of Medicinal and Aromatic Plants, P.O.CIMAP, Kukrail Picnic Spot Road Lucknow – 226015, India.

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**While culturing geranium in controled glasshouse condition in sand culture techniques for maximum essential monoterpene oil(s), it was found that (0.21%) in young developed 4th position leaf. At 4th position of leaf, net photosynthetic rate, contents of chlorophyll and essential monoterpene oil(s) were affected. The maximum peroxidase activity was obtained at 4th position leaf with the production of biomolecule geraniol. The maximum of monoterpene oil(s) was found at 4th level. However, the relative contents of citronellol, geraniol, linalool, and nerol varied at different leaf positions. As a result of different leaf positions, the contents of Fe, Mn, Zn, and Cu were smaller in shoots of Geranium. Their maximum contents were observed at 4th positions. Thus the added value of essential monoterpene oil(s) seems for commercial exploitation at large scales to be for the collection of leaf position at 4th level and for better quality of total essential oil of Geranium.**

**Key words:** Geranium (*Pelargonium graveolens* L.), chlorophyll, Cu, dry mass, Fe, leaf area, Mn, net photosynthetic rate, plant height, saccharides, Zn.

## INTRODUCTION

Geranium, *Pelargonium graveolens* L. Her. *ex* Ait. (synonym *P. roseum* Willd.) of the family Geraniaceae is the only source of one of the most important essential monoterpene oil(s) called the oil of geranium. It is commonly known as rose geranium. It is distinctly different from the horticultural geranium, which are basically ornamental and have no commercial usage in perfumery industries (Douglas, 1969). *P. graveolens* widely grown cultivars Algerian/uniton, Kelkar/Egyptin, and Bourbon/-Reunion (Rajeshwar and Bhatacharya, 1992) are commonly cultivated in India. Steam distillation of shoot biomass of geranium yields geraniol and citronellol rich monoterpene oil(s), extensively used for perfuming soaps and cosmetics to which they impart a pronounced and lasting rose odour. It is also largely used in flavouring tobacco products, toothpastes, ointments, and other pharmaceutical preparations.

Zn is an essential micronutrient that acts either as a metal component of various enzymes or as a functional, structural, or regulatory cofactor, and is thus associated with saccharide metabolism, photosynthesis, and protein synthesis (Marschner, 1086). Zn-deficiency reduces plant growth and inhibits photosynthesis in many plants including forest trees (Dell and Wilson, 1985), fiber crops (Ohki, 1976), rice (Ajay and Rathore, 1985), and spinach (Randall and Bouma, 1973). Zn retards the activity of carbon metabolism enzymes such as carbonic anhydrase (Ohki, 1976, 1978), ribulose 1,5-bisphosphate carboxylase/oxygenase and fructose-1,6-bisphosphate (Marschner, 1986). Zn, Se, and Cr are antioxidants scavenging free radicals. Zn stimulates the removal of free-radicals (Chakmak and Engles, 1999).

Essential oil biosynthesis in geranium is strongly influenced by Zn-acquisition and the stresses caused by Zn on nutrition and growth. Zn is involved in carbon assimilation, saccharide accumulation, free radical removal, anti-oxidant enzymes, carbon utilization in terpene biosynthesis, and the overall growth of the plants. The requirement of Zn for Japanese mint and its limitations imposed on photosynthetic carbon metabolism and

*Corresponding author. E-mail: amisracimap@yahoo.co.in.

translocation in relation to essential oil accumulation in mint were shown by Misra and Sharma (1991), whereas antioxidants enzymes for free radical quenching in geranium have not been fully documented.

In the present paper we report on the role of Zn as a stimulant for quenching of free-radicals through Zn affected antioxidant enzyme activity. Simultaneously, photosynthetic efficiency in terms of net photosynthetic rate ($P_N$), content of chlorophyll (Chl), leaf fresh and dry mass, leaf area, Zn content in plant shoot biomass and oil yield were also determined.

## MATERIALS AND METHODS

Plant tips (12.5 - 15.0 cm) with 3 - 4 leaves of *P. graveolens* of Bourbon genotype were obtained from the farm nursery of the CIMAP, Lucknow, India. Uniform cuttings were initially planted in 10 000 $cm^3$ earthen pots filled with purified silica sand (Agrawala and Sharma, 1961) for the development of roots. After 15 days, rooted cuttings were transferred to 2 500 $cm^3$ pots. The salts used in nutrient solution of Hoagland and Arnon (1952) were purified for Zn (Hewitt, 1952). The nutrient solution was used in the experiment except Fe which was supplied as Fe-EDTA. Three pots each of Zn treatments ranging from 0.0 to 1.0 g(Zn) $m^{-3}$ were maintained in controlled glasshouse condition at ambient temperature (30±5°C) and irradiance (800 – 1 000 µmol $m^{-2}$ $s^{-1}$). The nutrient solution in each treatment was added at alternate days. With onset of deficiency and toxicity (after 20 days), growth and detailed physiological and biochemical data characteristics were determined. $P_N$ was measured using a computerized portable photosynthesis system Li-COR 6000 (LiCOR, USA) (Misra and Srivastava, 1991). Chl amount in 80% acetone extracts from 3rd leaf was determined spectrophotometrically on Pye Unicam PU8610 according to Arnon (1949). Leaf fresh and shoot dry mass and area (area meter Li-3000) were also recorded. For tissue element analysis 1 g dried leaf samples were digested with 1 M HCl at 60°C for 24 h. Aliquot samples of the clear digest were diluted with water (10 $cm^3$) and analyzed for Zn by atomic absorption spectrophotometer (Pye Unicam SP 2800) (Misra and Sharma, 1991). Antioxidant-reactive peroxidase enzyme activity was estimated as described in Sharon et al. (1996). 2 g of freshly chopped leaves at 3rd position were homogenized with 5 $cm^3$ of 0.1 M phosphate buffer (pH 6.8). Each treatment was replicated 3 times and assayed by SDS-PAGE electrophoresis.

Geranium oil was estimated by steam distillation of 100 g freshly plucked leaves in an apparatus of Clevenger (1928). Geraniol, citronellol, and other associated oil contents were determined by gas liquid chromatography (Perkin-Elmer model 3920 B). The stainless steel column was packed with 10% carbowax (20 mesh) on Chromosorb WNAW. Injector and detector temperature were maintained at 200°C. The flow of $H_2$ was 0.47 cm $s^{-1}$; data processing for area % was done on a Hewlett- Packard integrator model HP-33.

## RESULTS AND DISCUSSION

The fresh and dry biomasses increased with increase in the supply of Zn (Table 1). Maximum fresh and dry biomass and leaf area were observed at $Zn_{0.250}$. Plant height was maximum at $Zn_{0.500}$. $Zn_{1.000}$ was toxic to all growth parameters. The Chl content increased up to $Zn_{0.250}$ and then decreased. The maximum $P_N$ was found at $Zn_{0.250}$; at this Zn supply also the saccharide content was the highest. Zn deficiency and Zn toxicity inhibited $P_N$ in cotton (Ohki, 1976), peppermint (Randall and Bouma, 1973), soybean (Ohki, 1978), and sweet mint (Misra et al., 2003). A decrease in Chl content represents a decline in photochemical capacity of leaf at deficient Zn supply (Ohki, 1976).

Maxima of peroxidase activity were observed at $Zn_{0.250}$. The Zn deficient and toxic cultured plants revealed lesser peroxidase activity with lesser peroxidase isoenzyme band profiles. In Japanese mint similar report was given for Mn nutrition (1996). The maximum of monoterpene oil(s) was found at $Zn_{0.250}$. However, relative contents of citronellol, geraniol, linalool, and nerol varied at different Zn treatments. As a result of different Zn supply the contents of Fe, Mn, Zn, and Cu were smaller in shoots of geranium. Their maximum contents were observed at $Zn_{0.250}$.

Statistical analysis showed a positive significant association between Zn content in leaf and $P_N$ ($\gamma = 0.924 \leq p = 0.5\%$) and between $P_N$ and content of saccharides ($\gamma = 0.879 \leq p = 0.05\%$). However, Zn content in leaf was negatively correlated with Chl a/b ratio. $P_N$ showed a positive significant association with leaf fresh mass ($\gamma = 791 \leq p = 0.05$ %), leaf dry mass ($\gamma = 692 \leq 0.05\%$), leaf area and total monoterpene oil(s) ($\gamma = 0.721 \leq p = 0.01$). A positive significant correlation was also observed between saccharides and total oil ($\gamma = 0.695 \leq p = 0.01\%$). A quadratic trend was observed for all these characters which were comparable in +Zn than in plants grown at Zn deficit or much higher Zn supply.

We found that optimum supply of Zn is $Zn_{0.250}$. Utilization of metabolites from primary photosynthetic process in secondary metabolism regulates monoterpene production (Gershezon and Croteau, 1991). Thus a close relation between photosynthesis, photorespiration, and terpenoid synthesis exists in essential monoterpene oil(s) bearing plants (Maffei and Codignola, 1990). Moreover, the actively growing leaves require a larger supply of an antioxidants stimulator Zn, in association with greater supply of photosynthates. Since essential oil biosynthesis occurs in these rapidly growing leaves, the initial growth period would require a still greater supply of photosynthates and energy.

## Conclusion

Micronutrient contents of leaf tissue concentration suggest that third leaf position serves as a useful guidance in determining the convenience of a better clipping leaf, for better growth, productivity and essential mono-terpene oil(s). The results of this study reveal that the third leaves of the geranium plants which are actively growing leaves, require a larger supply of an antioxidants stimulator Zn, in association with greater supply of photosynthates. Since essential oil biosynthesis occurs in these, rapidly growing leaves, the initial growth period

**Table 1.** Effect of leaf positions on parameters of Geranium.

| Growth attributes | Leaf #1 | Leaf #2 | Leaf#3 | Leaf #4 | Leaf #5 | Leaf # 6 | Leaf #7 | LSD at 5% | LSD at 1% |
|---|---|---|---|---|---|---|---|---|---|
| Plant height (cm) | 57.0 | 58.0 | 61.0* | 62.5** | 63.4** | 64.1** | 59.0 | 2.5 | 4.1 |
| No. of branches | 9 | 10* | 13** | 18** | 10* | 10* | 8 | 1.1 | 3.2 |
| Fresh mass (g plant$^{-1}$) | 218.8 | 238.6* | 224.8 | 252.1** | 282.5** | 215.5** | 196.2 | 11.1 | 16.3 |
| Dry mass (g plant$^{-1}$) | 14.11 | 16.33* | 16.81* | 17.37** | 19.36** | 18.46** | 15.85 | 2.10 | 3.30 |
| Leaf area (cm$^2$) | 8.2 | 12.1* | 25.2** | 39.1** | 40.3** | 37.2** | 11.2 | 3.5 | 6.2 |
| Chl *a* (g kg$^{-1}$(FM)) | 0.68 | 0.79* | 0.94** | 1.35** | 1.48** | 1.01** | 0.82* | 0.11 | 0.15 |
| Chl *b* (g kg$^{-1}$(FM)) | 0.50 | 0.56 | 0.61* | 0.69** | 0.79** | 0.40 | 0.29 | 0.08 | 0.12 |
| Chl *a/b* | 1.36 | 1.41 | 1.54 | 1.96 | 1.87 | 2.53 | 2.83 | - | - |
| $P_N$ (µg($CO_2$) m$^{-2}$ s$^{-1}$) | 0.15 | 0.19* | 0.75** | 0.76** | 0.82** | 0.71** | 0.42** | 0.03 | 0.06 |
| Saccharides (µg ($CH_2O$) m$^{-2}$ s$^{-1}$) | 0.102 | 0.129 | 0.510 | 0.516 | 0.558 | 0.483 | 0.286 | - | - |
| Oil (%) | 0.15 | 0.16 | 0.17* | 0.19 | 0.21** | 0.16 | 0.15 | 0.02 | 0.04 |
| Citronellol | 0.21 | 0.27** | 0.29** | 0.32** | 0.25** | 0.18** | 0.17** | 0.01 | 0.02 |
| Geraniol | 0.09 | 0.09 | 0.10** | 0.11** | 0.07** | 0.12** | 0.10** | 0.01 | 0.01 |
| Linalool | 8.00 | 10.00** | 9.00** | 6.00** | 7.00** | 8.00** | 7.00** | 0.04 | 0.07 |
| Nerol | 1.00 | 1.20** | 1.10 | 1.40** | 1.20** | 0.90** | 0.70** | 0.01 | 0.02 |
| Fe (mg kg$^{-1}$) | 98 | 112 | 142** | 249** | 537** | 419** | 312** | 21 | 42 |
| Mn (mg kg$^{-1}$) | 26 | 37** | 41** | 57** | 98** | 62** | 53** | 9 | 11 |
| Zn (mg kg$^{-1}$) | 12 | 19* | 34** | 45** | 64** | 41** | 36** | 7 | 9 |
| Cu (mg kg$^{-1}$) | 7 | 9 | 11** | 11 | 12** | 7 | 5 | 3 | 5 |

Chl = chlorophyll; $P_N$ = net photosynthetic rate; oil amounts in % of total oil. *, ** Values are significant at p = 0.05 or p = 0.01 levels, respectively.

would require a still greater supply of photosynthates and energy. Therefore, selected micronutrient profile obtained in this study shows that particular 3rd leaves were used as a tool for better essential monoterpene oil(s) and as a value addition for geranium oil.

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