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*Full Length Research Paper*

# Essential sesquiterpene oil(s) in Khus-Khus (*Vetiveria zizanoides* Nash.) on roots diameter circumference positions for commercial usage

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

Central Institute of Medicinal and Aromatic Plants, P. O. CIMAP, Lucknow-226015, India.

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**The culturing Khus in controled glasshouse condition in sand cultures for maximum essential monoterpene oil(s) was found (0.21%) in young developed middle position circumferences of roots. At middle position of leaf, net photosynthetic and contents of chlorophyll were affected. The maximum peroxidase activity was obtained at middle position of leaf and roots circumferances area, with the maximum production of biomolecule of khusimol and khusinol at 250 mg Zn/L. The maximum of monoterpene oil(s) (0.21%) was also found at middle position of developed roots. However, the relative contents of khusimol and Khusinol, which varied at different circumferanceal area of positions. As a result of different root developmental positions, the contents of Fe, Mn, Zn, and Cu were smaller in quantity in *Vetiver*. Their maximum contents were observed at middle positions of developed root's. Thus, the value addition of essential monoterpene oil(s) seems for commercial exploitation at large scales to be for the collection of developed roots position at middle levels (roots area of 5th positions developments) for better quality of total essential oil of Khus.**

**Key words:** Chlorophyll, dry mass, leaf area, net photosynthetic rate, plant height, saccharides, Zn.

## INTRODUCTION

Khus-Khus (*Vetiveria zizanoides* Nash.) is an aromatic grass of the family Poaceae and it is the only source of one of the most important essential monoterpene oil(s) called the oil of Khus. It is commonly known as Khus oil. It is distinctly different from the horticultural khus, which are basically in uses of soil errosion and have no commercial usage in perfumery industries (Douglas, 1969). *V. zizanoides Nash* widely grown cultivars improved varity' Gulabi' (Rajeshwar and Bhatacharya, 1992) are commonly cultivated in India. Steam distillation of root biomass of Khus-Khus yields Khusimol and Khusinol rich monoterpene Vetiver oil (s), extensively used for perfuming soaps and cosmetics and in aromatherapy to which they impart a pronounced and lasting Vetiver oil peculiar odour. It is also largely used in flavouring soft drinks products and other pharmaceutical preparations and also the plant is used in soil erosion as the roots are having soil binding properties. Apart from this, it is widely used in phytochelatin properties and in phytoremediation of heavy metals extraction from the heavily polluted environment.

Zn is an essential micronutrient and acts as a phytochelatin 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, 1986). Zn-deficiency reduces plant growth and inhibits photosynthesis in many plants including forest trees (Dell and Wilson, 1985), fiber crops (Ohki, 1976), rice (Ajay, 1995), and spinach (Randall and Bouma, 1973). Zn retards the activity of carbon metabolism enzymes such as carbonic anhydrase (Ohki, 1976,

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

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

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

## MATERIAL METHODS

Plant tips (12.5 to 15.0 cm) with 3 to 4 leaves of *V. zizanoides* L. genotype of diploid-Gulabi were obtained from the farm nursery of the CIMAP, Lucknow, India. Uniform slips 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 to 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 day), 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) (Srivastava and Misra, 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 spectro-photometer (*Pye Unicam SP 2800*) (Misra and Sharma, 1991). Antioxidant-reactive peroxidase enzyme activity was estimated as described in Sharon et al. (1966). 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. Vetiver oil was estimated by steam distillation of 100 g freshly plucked roots area from circumferences (Root area #1 to 7, that is, outer area (ar.#1:1.5 cms, then the following ar.2 to 7 in decending orders), in an apparatus of Clevenger (1928). Khusimol and Khusinol 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 (Srivastava et al., 1997), 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 (Misra, 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 (Gershenzon 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

**Table 1.** Effect of root positions on parameters of *V. zizanoides*.

| Growth attribute | Root ar. #1 | Root ar. #2 | Root ar.#3 | Root ar. #4 | Root ar.#5 | Root ar. # 6 | Root ar. #7 | LSD | LSD |
|---|---|---|---|---|---|---|---|---|---|
| | 0.00 | 0.01 | 0.100 | 0.200 | 0.250 | 1.0 | 1.5 mg Zn/L | at 5 % | 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* | .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 |
| Khusimol (% of total oil) | 0.21 | 0.27** | 0.29** | 0.32** | 0.25** | 0.18** | 0.17** | 0.01 | 0.02 |
| Khusinol (% of total oil) | 0.09 | 0.09 | 0.10** | 0.11** | 0.07** | 0.12** | 0.10** | 0.01 | 0.01 |
| **Roots tissue concentrations:** | | | | | | | | | |
| 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 and P = 0.01 levels, ar= area respectively.

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.

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