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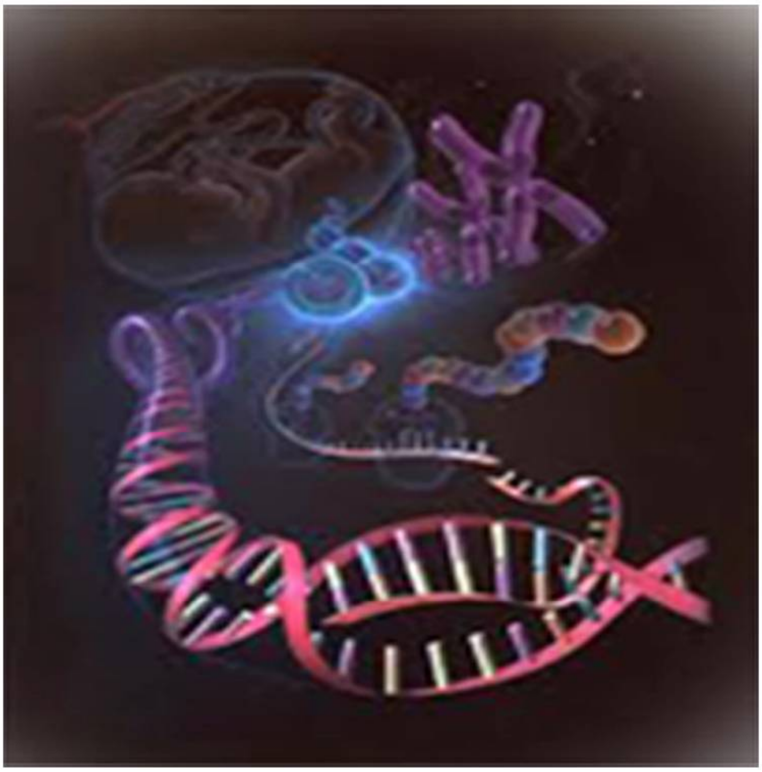

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Research Paper

INFLUENCE OF ZN STRESSES ON GROWTH AND PHYSIOLOGY IN KHUS-KHUS (VETIVERIA ZIZANOIDES NASH.) AND ITS ESSENTIAL SESQUITERPENE OIL(S), IN RELATION TO ROOTS DIAMETER CIRCUMFERENTIAL POSITIONS

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Khus-Khus (*Vetiveria zizanoides* Nash.) plants slips of 12.5-15 cms. length, were raised in controlled glass house conditions in different 0-0. 5 and 1.0 Zn mg/l applications. Total monoterpene oil(s) maximum (0.21%) was obtained at 0.250 Zn mg/l application at 4-5 inner one circumferential position of roots. At middle 4th position of leaf, net photosynthetic rate, 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 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. For commercial exploitation of the total monoterpene oil(s), the midle circumferential position roots are more important for hydro distilation of Khu-Khus oil.

Keywords: Chlorophyll, Dry mass, Sesquiterpene, Saccharides, Net photosynthetic rate.

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 (Douglas1969). *Vetiveria zizanoides Nash is*

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widely grown cultivars and improved variety 'Gulabi' is widely used in northern plains of India (Rajeshwar Rao and Bhatacharya, 1992). Steam distillation of roots of Khus-Khus yields Khusimol and Khusinol rich monoterpene Vetiver oil(s), which is extensively used for total oil contents % , Khusimol and Khusinol. Further the oil contents are widely used in perfuming the soaps and cosmetics and also in aromatherapy. Because it imparts a pronounced and lasting peculiar odour of Khus oil. 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. Phytochelating properties are more pronounced in organometalic formation of heavy metals like Zn-chelation. Therefore, the plant *Vetiveria zizanoides* Nash is more useful in phytoremediation of heavy metals extraction, from the heavily polluted soils.

Zn is an essential micronutrient and acts as a phytochelating 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 (Del and Wilson, 1985), fiber crops (Ohki, 1976), rice (Ajay and Rathore,1995), and spinach (Randall and Bouma, 1973). Zn retards the activity of carbon metabolism enzymes such as carbonic anhydrase (Ohki, 1976, Sharon et al., 1966), ribulose 1,5-bisphosphate carboxy-lase/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 Khus-khus 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 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 of quenching of free-radicals through Zn affected peroxidase and iso-enzymes of peroxidase antioxidant enzyme activity. Simultaneously, photosynthetic efficiency in terms of net photosynthetic rate (P_N), content of chlorophyll, leaf fresh and dry mass, leaf area, Zn content in plant shoot biomass, and oil yield were also determined.

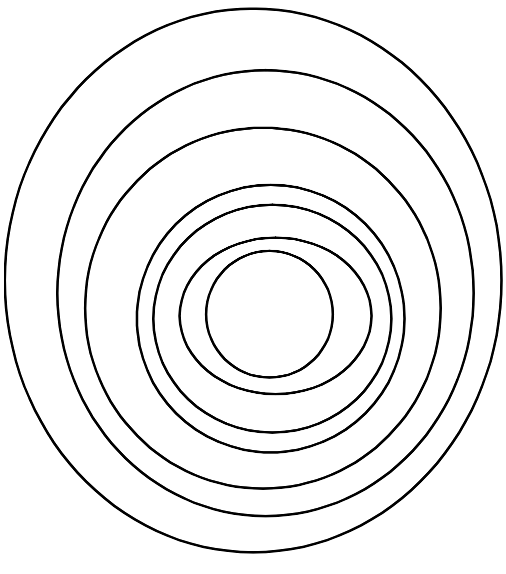
MATERIALS AND METHODS

Plants: Tips (12.5-15.0 cm) with 3-4 leaves of *Vetiver 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 2500 cm^3 pots. The salts used in nutrient solution of Hoagland and Arnon (Hoagland and Arnon1952) and were further 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

Figure 1: *Vetiveria zizanoides* Nash plants, Left hand side full cultivated plant with), 05 mg Zn/L, middle one full developed root after the harvesting and the right side is young root

Figure 2: Diagrammatic representation of *Vetiveria zizanoides* root circumferential distances from central circle as # 1 followed by 2 to 7 in ascending order, i.e., the outer one is as # 7



from 0.0 to 0.5 and 1.5 mg (Zn) m^{-3} as Zn-sulphate were maintained in controlled glasshouse condition at ambient temperature (30±5 °C) and irradiance (800-1000 µmol m^{-2} s^{-1})The experiment was organised in complete randomised design (CRD), with 7 treatments and three replicates. 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 charac-teristics were determined. P_N was measured using a computerized portable photosynthesis system *Li-COR 6000* (LiCOR, USA) (Srivastava and Misra, 1991). Chlorophyll amount in 80 % acetone extracts from 3rd leaf was determined spectrophotometrically on *Pye Unicam PU8610* according to Arnon (Arnon, 1949). Leaf fresh and shoot dry mass and area (area meter *Li-3000*) were also recorded. For

tissue concentration for micronutrients 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*. (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 (Figure 1) area from circumferances (Root area #1 to 7 i.e., Outer area (Area.# 7 is from 1-1.5 cms, than the following Area # 6 to 1 in decending orders) (Figure 2), 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 *Chromo-sorb WNAW*. Injector and detector temperature was 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*.

Statistical analysis was done on growth attributes, total oil contents and oil contents with khusimol and khusinol contents and Zn uptake were subjected to critical difference test (CD) using the lay-out of a completely randomized design (CRD). The correlation coefficient were compound to determine the linear relationship among the charecters. The quadratic equation $Y= a+bX+cX^2$ was used to detect the effect of Zn on the characters under study.

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 Chlorophyll 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 Chlorophyll content represents a decline in photochemical capacity of leaf at deficient Zn supply (Ohki, 1978). Heavy metals accumulation also reported high in the middle portion of the roots (Table 1).

Maxima of peroxidase activity i.e., 46 % increase over control was observed at $Zn_{0.250}$ (Figure 3). The Zn deficient and toxic cultured plants revealed lesser peroxidase activity (-10% decrease), with lesser peroxidase iso-enzyme band profiles.

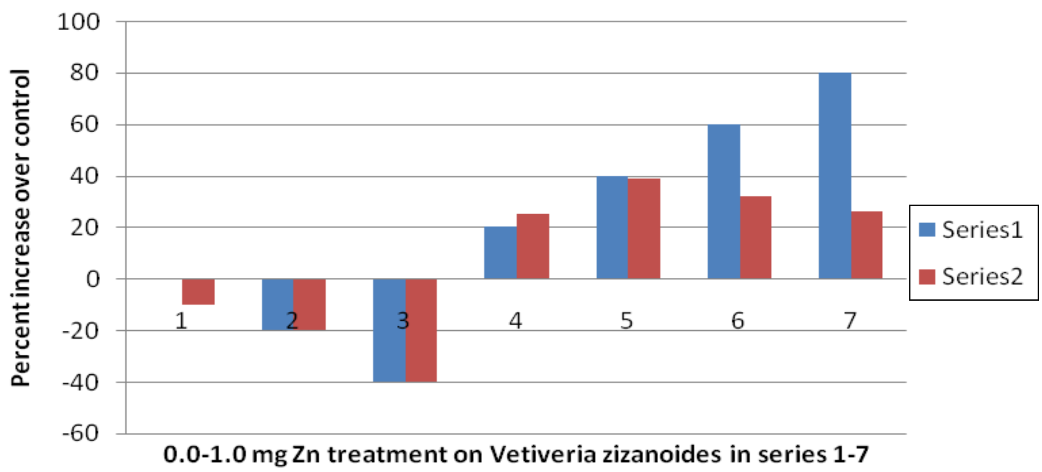
The graph revealed that 0.0 to 1.0 mg Zn/l application showed 40% decrease over contol, where as 4-7 application with increase Zn application increases, % increase in peroxidase activity on *Vetiveria zizanoides* plants. The same trend was reported in Japanese mint in Mn nutrition (Misra, 1996). The maximum of monoterpene oil(s) was found at $Zn_{0.250}$. However, relative contents of Khusimol and Khusinol were varied at different Zn treatments. As a result of different Zn supply the concentrations of heavy metals accumulation of Fe, Mn, Zn, and Cu were smaller in shoots of Khus-Khus (Table 2). The maximum contents were observed at $Zn_{0.250}$ mg Zn/l treatments. The

Table 1: Effect of Root Positions on Parameters of *Vetiver zizanoides*

Growth attributes	Root ar. #1 0-0.5	Root ar. #2 0.5-1.0	Root ar. #3 1.0-1.5 Distance in	Root ar. #4 1.5-2.0 Cms.	Root ar. #5 2.0-2.5	Root ar. #6 2.5-3.0	Root ar. #7 3.0-3.5	CD at 5 %	CD 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 [ìg(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

Note: Chl = chlorophyll; P_N = net photosynthetic rate; oil amounts in % of total oil at 0.250 mg Zn / ml treated plants. *, ** Values are significant at P==0.05 or P=0.01 levels, respectively.

Figure 3: Peroxidase Activity



higher vegitative growth attributes suggests the maximum photosynthesis activity and saccharides formation.

Statistical analysis showed a positive significant asso-ciation between Zn content in leaf and P_N (α = 0.924 d" *p* = 0.5 %) and between P_N and content of saccharides (α = 0.879 d" *p* = 0.05 %). However, Zn content in leaf was negatively correlated with Chlorophyll *a/b* ratio. P_N showed a positive significant association with leaf fresh mass (α =O.791 d" *p* = 0.05 %), leaf dry mass (ã =0.692 d" 0.05%), leaf area and total monoterpene oil(s) (α = 0.721 d" *p*=0.01). A positive significant correlation was also observed between saccharides and total oil (α = 0.695 d" *p*=0.01%) (Table 2). A quadratic trend was observed for all these characters which were comparable in Zn at 0.250 mg Zn/l, than in plants grown at Zn deficit or at much higher Zn supply. Most positive significant quadiratic regression analysis was obtained Zn con-centration, inbetween total oil and leaf area (Table 3).

We found that optimum supply of Zn is Zn_{250}. Utilization of metabolites from primary photosynthetic

Table 2: Effect of Root Positions on Concentrations of Heavy Metals in Roots of *Vetiver zizanoides* at 0.250 mg Zn/ml Treated Plants

Growth attributes	Root ar. #1 0-0.5	Root ar. #2 0.5-1.0	Root ar. #3 1.0-1.5 Distance in	Root ar. #4 1.5-2.0 Cms.	Root ar. #5 2.0-2.5	Root ar. #6 2.5-3.0	Root ar. #7 3.0-3.5	CD at 5 %	CD at 1 %
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

Note: *, ** Values are significant at P==0.05 or P=0.01 levels, respectively.

Table 3: Correlation Among Growth , Oil Quality Parameters, and Zn Uptake and Their Non Linear Regression With Zn Concentration in Khus-Khus

Correlation Coefficient Y	(1)	(2)	(3)	(4)	(5)	(6)	Quadratic regression with Zn concentration X
Fresh Wt.	0.692*	0.924**	0.791*	-0.719	0.692	0.687	
Dry Wt. (1)		0.911**	0.771*	–0.701	0.617	0.627	
Zn Content(2)			0.762*	-0.621	0.618	0.671	
Photosynthesis(3)				-0.671	0.682	0.719	
Chlorophyl l a/b(4)					0.614	0.812*	
Leaf area(5)						0.829**	
Total oil (6)							-Y= 75.669+4.595X 0.1961X^2; R^2=0.995

Note: Significantly at *p=0.05, **p=0.01.

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 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 photo-synthates and energy.

CONCLUSION

Actively growing outer ring circumference and most inner area roots are having lesser essential sesquiterpene oils than the 3-4 the inner older area of roots.

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