

Response of Micronutrients Application to Biomass, Candellila Wax and Biocrude of *Euphorbia antisiphilitica* zucc. Grown on Gomti Upland Alluvium

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Abstract A pot-culture study was made to assess the effects of micronutrients application to soils on growth, biomass and yield of candellila wax and extractive biocrudes of *Euphorbia antisiphilitica* zucc. grown on natural, loamy sand soils of Gomti upland plain. Application of Zn, Cu and Mn besides increasing biomass, increased the plant concentrations of epi-cuticular wax (Alkanes) and acetone extractive resinous hydrocarbons including terpenoids. The best response was in the treatment of Zn + Mn + Cu which increased the biomass by 40% and the concentration of wax and acetone extractives by 232 and 83% respectively. An involvement of micronutrients in the biosynthesis of secondary metabolites of the fuel range, particularly of Cu, Mn and Zn in that order of waxes (alkanes) and the acetone extractive resinous hydrocarbons has been indicated. The dry phylloclade tissue nutrient responsive values were found to be 28, 10 and 9 ppm for Zn, Mn and Cu respectively.

Key words Biocrude, Biomass, Candellila wax, Critical micronutrient values, *Euphorbia antisiphilitica*, Micronutrient response, Petro crop.

In the context of fast depleting natural fuel resources in the world, prospects of petrocrops as an alternate renewable source of fuel energy has been highlighted, specially on waste and under utilised lands (Calvin 1987, Dayal 1986, Pachauri & Dhawan 1987). Such lands include highly arid, calcareous, halomorphic, leached, acidic and eroded soils, which are known to suffer from multiple nutritional problems including micronutrient deficiencies (Mehrotra 1991). As very little information is available on the nutritional aspects, specially involving micro-nutrients, on the biomass and biocrude productivity of petrocrops, the present investigation was undertaken to study the effect of micro-nutrients on *Euphorbia antisiphilitica* Zucc., a highly potential petrocrop in the Indian context (Dayal 1986). The plant is reported to produce 12.5 tons biomass ha⁻¹ yr⁻¹ under Indian conditions equivalent to about 5-6 barrels of hydrocarbon fuel ha⁻¹ yr⁻¹ (Srivastava 1986). The plant is also commercially important for its epi-cuticular candellila wax. The low molecular

weight, non-polar extractives of the plant are hydrocrackable into compounds of gasoline fuel range (Bhatia 1987).

Materials and Methods

A loamy sand (coarse sand, 0.5%; fine sand, 71%; silt, 24.5%; and clay, 4%) from Gomti upland alluvial soil was collected in bulk from Badshahbagh, Lucknow, India. The soil had pH (1:2.5) 6.86; CaCO₃ equivalence 0.8%; organic matter 1%; EC₂ at 25°C, 0.25 dSm⁻¹ and DTPA extractable Zn, Fe, Mn and Cu, 0.45, 12.6, 9.5 and 0.88 ppm, respectively. The soil was made sufficient in N, P and K by basal application of 50, 22.2 and 40.5 ppm (w/w) and top-dressing of additional 50 ppm N, through their salt solutions purified against of micronutrients by phosphate adsorption and dithizone extraction techniques (Hewitt 1966). The soil was then divided into 4 parts. One part served as the control. To the other three lots, AR grade sulphate salts of Zn, Mn and/ or Cu at the

normal doses of 5, 10 and 2.5 ppm w/w, respectively were mixed so as to obtain three micro-nutrient treatments-(i) Zn, (ii) Zn + Mn and (iii) Zn + Mn + Cu. Soils of these four treatments were separately filled in 25 cm diameter bitumen painted and alkali lined clay flower-pots. There were 6 pots for each treatment, arranged in 2 blocks in a glass-top green house. Three 45 day old plants of initially raised from 15 cm stem cuttings in pure sand irrigated with deionized water, were transplanted in each pot. The experiment was continued upto 53 weeks. Top dressing of N in split doses (12.5, 12.5 and 25 ppm) was done at 20, 35 and 48 weeks after transplantation (atp), respectively. The plants were irrigated with deionised water (100-250 ml day⁻¹), around moisture equivalence at the time of irrigation. Observations of height, cover and branching were made at weeks 18, 35 and 53 atp. The growth rate, in terms of height (um day⁻¹) between 18 to 35 and 35 to 53 weeks atp, was computed by the formula : growth rate = $10^{\log_e H_1/H_0/t}$ where, 8 is the base of the natural logarithm, H₁ is the final height, H₀ is the initial height, and t is the period of growth in days between the two observations.

At 53 week, the above ground shoot parts (phylloclades) of plants were harvested. The epicuticular wax was extracted by repeated dippings and brushing of a known weight of fresh phylloclades in petroleum ether (60-80°C) for a few minutes, drying the ether extract, redissolving the residue in n-pentane, and drying the n-pentane soluble residue (Radin et al. 1982). Rest of the fresh phylloclades was utilized for estimating biomass and tissue analyses for micronutrients and biocrude. For biomass, the initially sun-dried plant material was dried in a forced draught oven at 70°C for 48 h and weighed.

A portion of the fresh plant material was successively washed with a detergent solution, 0.01 N HCl de-ionised water and glass-distilled water and then oven-dried at 70°C. A known amount of this material, was wet-digested in nitric and perchloric acids (1:15:1) and estimated for the concentrations of Fe, Mn, Cu and Zn on Atomic Absorption

Spectrophotometer. For biocrude, the dried plant material was ground in a Wiley Mill and a portion utilized for the sequential soxhlet extraction and estimating of acetone and hexane extractable biocrude components, Erdman and Erdman (1981). The entire dated have been statistically analysed and tested for significance at P 0.05.

Results and Discussion

Growth and biomass : Plants responded differently to the three micronutrient treatments. These responses became evident by week 18 atp and got particularly marked by week 53 atp. The plants recorded maximum increase in the height and biomass in the number of primary branches (nearly double) and in the number of secondary branches and over all canopy cover (Table) in the treatment of Zn + Mn + Cu. The rate of growth in height was thrice that of control during the 35-53 weeks atp period. As compared to Zn alone, which also caused an increase in all the growth attributes, the second best treatment Zn + Mn, increased the height, overall canopy cover and biomass by 14%, overall branching by 31% and the growth rate during the later period by 10%. The micronutrient amendment, particularly of Mn + Cu, and Mn, in that order along with Zn also increased the dry matter percentage of phylloclades indicating an increase in the biosynthetic assimilates.

Wax and biocrude : The best responsive treatment of Zn + Mn + Cu, vis a vis control, than trebled the concentration of candellila wax and nearly doubled that of acetone extractive biocrude (Table 1). While Zn alone also increased the concentration of wax and acetone extractives, the increase with Zn + Mn over the Zn treatment was 29 and 10% for the two secondary metabolites respectively. The increase with of Zn + Mn + Cu + over Zn + Mn was 136% in the concentration of candellila wax and 25% for the acetone extractives. The results, thus, indicate a role of micronutrients, in the biosynthesis of secondary metabolites, particularly waxes (alkanes), the alcoholic esters of fatty acids, and acesstone extractive resinous hydrocarbons including terpenoids. The effect of

Table 1 Effect of micronutrient application on the growth, biomass, candellila wax and biocrude of *Euphorbia* plants

Plant estimate	Soil micronutrient amendment ($\mu\text{g g}^{-1}$ soil)				LSD 5%
	Control	Zn ₅	Zn ₅ Mn ₁₀	Zn ₅ Mn ₁₀ Cu _{2.5}	
Growth					
Height of tops (cm)	91.5	102.5	116.5	125.5	14.0
Growth rate (height $\mu\text{m day}^{-1}$)					
18-35 weeks	0.50	0.71	0.86	0.66	
35-53 weeks	2.91	2.76	5.79	8.98	
Overall canopy cover (cm)	24	42	48	67	3.0
Phylloclade branches (no. plant ⁻¹)					
Primary	7.8	10.0	14.0	16.0	2.7
Secondary	10.0	15.5	20.0	26.0	2.9
Above-ground Biomass					
(g plant ⁻¹)	96.6	109.5	124.8	132.5	10.5
Epi-cuticular Wax					
Candellila wax (% dry matter)	3.62	3.98	5.08	12.01	1.73
Biocrude					
Acetone extractives (% dry matter)	3.37	4.51	4.96	6.18	0.34
Hexane extractives (% dry matter)	1.74	1.57	1.35	1.11	0.19

Cu and Mn, was most marked. Similar observations were made earlier for the resinous hydrocarbons in *Calotropis procera* (Ansari & Mehrotra 1990) and *Pedilanthus tithimaloides* (Mehrotra et al. 1991) and for epi-cuticular wax in the former (Mehrotra & Ansari 1988).

Deficiency of both Cu (Baszynski et al. 1978) and Mn (Copper & Girton 1963) are known to decrease the lipid content of plants. The resins, like other lipid derivatives including wax, are synthesised by a complex process initiated by the carboxylation of Acetyl-CoA, with IPP (Isopentenyl pyro-phosphate) and GPP (Granyl pyro-phosphate) as the intermediates. The role of Cu in the lipid biosynthesis is probably during the biosynthesis of IPP and GPP (Harwood & Russel 1984) while that of Mn through the Shikimic acid pathway for the biosynthesis of secondary metabolites requiring Mn as a co-factor at its several biosynthetic steps (Burnell 1988). The probable roles of these micronutrients could also be indirect through their effects on photosynthesising capacity affecting the availability of C-skeleton for the

biosynthesis of fatty acids (Marschner 1986). However, more work is required to elucidate the exact role of Mn and Cu in the biosynthesis of secondary plant metabolites, specially of the fuel-range hydrocarbons.

Tissue micronutrients and their ratios : The soil samendments of different micro-nutrients raised the concentrations of respective micronutrients in phylloclades (Table 2). The phylloclade concentrations of Zn, Mn and Cu as 28, 10 and 9 ppm in the dry matter respectively. The control plants had the highest tissue ratios of Fe/Mn and Fe/Zn which got ameliorated to the maximum by the treatment of Zn + Mn + Cu (Table 2). These results are also in accordance with those obtained earlier for *Calotropis* and *Pedilanthus* grown on a similar alluvial soil (Mehrotra & Ansari 1988; Mehrotra et al. 1991). The present observation of a marked reduction in the tissue concentration of Fe, unaccountable by the dilution effect, by Zn and Cu amendmets are also in line with the known antagonistic interactions of these nutrients.

Table 2. Effect of micronutrient application on their contents in the phylloclades of *Euphorbia*.

Micronutrient application (µg g ⁻¹)	Nutrient Content in phylloclades (µg g ⁻¹)					
	Fe	Mn	Cu	Zn	Fe/Zn	Fe/Mn
Control	193.7	9.6	8.6	20.3	6.87	20.3
Zn ₅	86.1	12.4	10.4	48.3	1.78	7.0
Zn ₅ Mn ₁₀	71.8	15.3	14.7	54.7	1.33	4.7
Zn ₅ Mn ₁₀ Cu _{2.5}	43.1	19.1	17.8	70.2	0.61	2.3
LSD 5%	7.9	1.1	0.8	6.4	-	-

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