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RESEARCH PAPER

Sulphur Requirement of Petrocrop *Pedilanthus tithimaloides* Poit. *var*. *cuculatus* in Sand Culture

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ABSTRACT

A sand pot-culture study, related to graded S-nutrition of a petro crop Pedilanthus tithimaloides Poit. var. cuculatus, reveated that the S requirement for biomass, being initially similar to most plants (2 mM), increased (4 mM) at plants maturity. A higher magnitude of depression in the biocrude yield than in biomass by S deficiency suggested for a role of S in the biosynthetic metabolism of biocrude secondary metabolite components. An increased magnitude of biomass as well as biocrude per unit tissue S under conditions of S deficiency indicated plant's adaptation against stress. Critical values of deficiency, sufficiency and/or excess, both supply and young leaf tissue S for the biomass as well as biocrude productivity of the petro crude have been established.
Key words: Biocrude, Biomass, Critical Sulphur, Petro crop, Pedilanthus tithimaloides, and Sulphur requirement.

INTRODUCTION

Deficiency of S is very frequent in coarse textured and highly weathered vertisols, oxisols, alfisols and inceptisols of the arid and semi-arid tropics which also abound in waste-lands, now being considered for large scale petro crop farming to augment fuel energy resources (Frick, 1938, Calvin, 1974 and Pachauri and Dhawan, 1987). With little information available on the nutritional requirement including of S, of the petro crops, a sand pot-culture study was undertaken on the S nutrition of an important Brazilian Laticiferous petro crop, *Pedilanthus tithimaloides* Poit. *Var*. *cuculatus* (Family Euphorbiaceae). Its biomass production potential under Indian conditions is 25.6 tons ha^{-1} yr^{-1} which, with about 8% biocrude (Acetone + benzene extr.) content, is equivalent to 11-12 barrels of the hydrocarbon fuel ha^{-1} yr^{-1} (Srivastava, 1986).

MATERIAL AND METHODS

35 day old cuttings of *Pedilanthus tithimaloides* Poit. initially raised in purified sand irrigated with de ionized water *var. cuculatus* were translated and raised in refined sand culture (Agarwala and Sharma, 1976) at 6 graded levels of S, <0.001 (through cultural contaminations), 0.2, 0.5, 2, 4 and 10 mM, supplied in form and concentrations as described earlier (Mehrotra et al, 1991), except that in the nutrient solution, Mg was supplied as Cl^{-2} and NaCl was omitted. Other experimental details were the same as described earlier (Mehrotra et al, 1991). Biomass of roots and above-ground (top) plant parts was determined separately at two stages viz. 17 and 33 weeks after transplantation (atp). Tissue-S was estimated in the nitric-perchloric acid (1:15:1) wet-digests (Piper, 1942) of the oven-dried material of thoroughly cleaned young leaves at both of these stages as Ca-oxalate, turbidimetrically (Chesnin and Yien, 1951). Chlorophyll a and b, extractable in 85% acetone, in the top two fully expanded young leaves was estimated (Arnon, 1949) at week 12 atp. Biocrude (sequential hexane, methanol and acetone extractives) was estimated in air-dried then oven-dried and Willey mill ground above ground plant material of <0.001, 2, 4 and 10 mM S supply sampled at week 33 atp only, as described earlier (Mehrotra et al, 1991). Critical values of S, both for supply and tissue concentration in young leaves indicative of its optimum, thresholds of deficiency and excess, deficiency and excess for the top biomass as well as biocrude yield were worked out by the method of Agarwala and Sharma (Agarwala and Sharma, 1979). Biomass and biocrude productivity of plants per unit of tissue S in young leaves was also computed from the yield and tissue-S data. All the data were statistically analyzed and tested for significance at $p<0.05$ (Pance and Sukhatme, 1961).

RESULTS AND DISCUSSION

Visible effects

Visual effects of S deficiency, first discemible by week 3 atp at <0.001 mM S and upto 0.5 mM S a little later, were chlorosis, more intense in younger leaves reddish pigmentation on abaxial surface of chlorotic leaves and reduced size of lamina. The S excess effect, initially discernble as growth reduction at 10 mM S got pronounced and extended upto 4 mM by week 15 atp. In the marginally chlorotic old leaves of such S excess plants, irregular white-turning-brown necrotic spots developed in the chlorotic areas, the affected leaves got inwardly cupped, and the effects gradually proceeded to younger leaves. By week 25 atp, the visual effects of S-excess disappeared from 4 mM plants. This indicated an increase in the s requirement of plants at later stages of growth.

Growth and biomass

Both deficiency and excess of S depressed height, branching, canopy cover and number and average area of leaves. The effect of deficiency was more pronounced than that of excess particularly on height and leaf area. Maximum growth of tops, up to week 17 atp, was found at 2 mM S, 4 mM level being almost at par.

However, later at week 33 atp, maximum growth of plants was found at 4 mM S. the effect of variation in S supply on various growth parameters was also reflected in the biomass which was found maximum at 2 mM S at week 17 and at 4 mM S at week 33 atp (Table 1). A higher requirement of S for the biomass productivity of the petrocrop, than most other plants was thus indicated. The depression in biomass due to excess s was found at $\geq$ 4 mM upto week 17 but only at 10 mM at week 33 atp. Depression in biomass at 1/10th and 5 times the optimal S supply was around 60% at week 17 and 44% by deficiency and 21% by excess at week 33 atp. Both serve deficiency and excess of S depressed dry matter % in tissues, particularly in tops (Table 1).

Biocrude

An increase in S supply from deficiency to optimum (< 0.001 to 4 mM) increased the concentration as well as yield of total extractable biocrude (Table 2). A higher magnitude of depression in the total biocrude yield of tops as compared to that in the top biomass suggested for a role of S in the biosynthesis of secondary metabolite constituents of hydrocarbon-rich biocrude of the petro crop. A lower magnitude of increase in the biocrude viz a viz the biomass per unit tissue S at deficient levels of S supply and a higher magnitude of decrease in the bicrude than in the biomass per unit tissue S at excess S (Table 2) also led to the same conclusion. The increase in biomass as well as biocrude per unit tissue S under conditions of S deficiency could be adaptive in nature. Such biosynthetic role of S could be through S containing acetyl-COA, (CH_3 CO-COA), aceto-acetyl-COA and hydroxyl-methyl-glutaryl-COA, known to be involved in the biosynthesis of isopentenyl pyrophosphate, dimethyl allyl-pyrophosphate and geranyl-pyrophosphate, the common precursors of many plant secondary metabolites including terpenes and polyisoprenes (Goodvin and Merser, 1972 and Suttie, 1977).

Critical S values

The critical values of S for biomass indicative of its deficiency were 0.42 to 0.95 mM (at week 17) and 2.0 and 2.8 mM (at week 33) for supply and 0.086 to 0.093% (at week 17) and 0.195 to 0.210% (at week 33). A higher optimal value of S, both for supply as well as tissue S at week 33 than at week 17 atp confirmed the plant's increased S requirement for biomass at maturity. The critical values of S for the biocrude productivity were almost similar to that for biomass.

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Table 1. Effect of graded S-supply on certain growth, chlorophyll, and biomass and tissue concentration attributes of *Pedilanthus tithimaloides* Poit. *var. cuculatus.*

Observations	Growth stage (week atp	S supply (mM)						LSD P=0
		Deficient			Optimum	Supra-optimum		
		<0.001	0.2	0.5	2	4	10	
GROWTH								
Height-tops (cm)	4	17	22.8	24	26.5	28	21.3	4.3
	16	15.4	59.5	61	72.6	70	59.3	15
Branches								
no.plant-1	4	3.5	4.8	6	5	5	4	3.5
Total	16	9	8.8	9	10	11.8	8.5	5.8
leaf (no.plant-1)	4	27.5	34.3	43.6	49.3	49.8	25.5	16
Av.leaf area (cm2)	4	8.6	9.2	10.9	13	11.5	9.4	9.5
CHLOROPHYL-Y.L.	16	14.8	18.2	22.4	25.3	24.2	20.1	13
chl-a(mg 100g-1 F.M.)	12	26.6	36.2	44.1	56.5	70.6	58.3	22
chl-b(mg 100g-1 F.M.)	12	10.8	15.7	19.6	23.1	25.4	23.7	8.5
total chl.(a+b)(mg 100-1 F.M.)	12	37.4	51.9	63.7	79.6	96	88	32
chl-a/chl-b	12	2.4	2.3	2.2	2.4	2.8	2.5	
Tissue S (% dry matter)								
young leaves	17	0.0078	0.085	0.086	0.13	0.18	0.185	0
	33	0.063	-	0.182	0.189	0.218	0.304	0
g s plant-top-1	17	0.018	0.021	0.039	0.072	0.0617	0.04	0
	33	0.048	-	0.154	0.17	0.327	0.36	0
Biomass (g D.M. plant-1)								
Tops	17	23.1	24.4	45.3	55.6	34.3	21.6	13
	33	75.5	-	84.7	90	149.9	118.4	52
Root	17	2.4	2.4	3.9	5.5	4.5	2.3	1.5
	33	8.3	-	9.5	9.6	16.5	10.4	7
Total	17	25.4	26.8	49.2	61.1	37.8	23.8	15
	33	83.8	-	94.2	99.6	166.4	128.7	61
Top /Root ratio	17	9.8	10	11.8	10.2	9.8	9.8	-
	33	7.1	-	8.9	9.4	9.1	11.4	-
(g g-1 tissue-S)	17	1281.9	1176.4	1162.7	769.2	555.6	540.5	-
	33	1587.1	-	549.5	529.1	458.7	328.9	-
Dry matter (%)								
Top	17	8.2	8.6	10.3	10.1	8	6.1	6
	33	10.3	-	13	14.7	14.3	13.8	5
Root	17	13.3	14.8	14.6	14.4	14.3	12.6	10
	33	13.8	-	14.4	18.8	17.2	15.5	8

atp= after transplantation

Table 2. Biocrude (hexane, methanol and acetone extraction) concentration and yield of *Pedilanthus tithimalodies Poit. var. cuculatus* grown at graded levels of S supply in sand cultures week atp.

Observations	S supply (mM)				LSD
	Deficient		Optimum	Supra-optimum	P=0.
	<0.001	2	4	10	
BIOCRUDE (% D.M.-tops)					
Hexane extrr.	1.51	1.91	2.19	1.62	0.52
Methanol extr.	2.66	3	3.17	2.68	1.16
Acetone extr.	4.74	6.1	9.88	6.5	2.35
Total (H+M+A) extr.	8.91	11.01	15.24	10.8	4.96
BIOCRUDE (g plants-1-tops)					
Hexane extrr.	1.14	1.72	3.28	1.92	1.01
Methanol extr.	2.01	2.7	4.75	3.17	1.75
Acetone extr.	3.58	5.49	14.81	7.7	2.68
Total (H+M+A) extr.	6.73	9.91	22.84	12.79	5.65
BIOCRUDE (g g-1 tissue S)					
Hexane extrr.	23.8	10.1	10.1	5.33	-
Methanol extr.	42.2	15.9	14.5	8.8	-
Acetone extr.	75.2	32.3	40.3	21.4	-
Total (H+M+A) extr.	141.4	58.3	69.9	35.5	-

atp: after transplantation, H: Hexane, M: Methanol and A: Acetone

Table 3. Critical values of S indicative of threshold of deficiency, optimal growth, threshold of excess and excess of S the biomass and biocrude productivity of *Pedilanthus tithimalodies Poit. var. cuculatus* grown at graded levels of S supply in sand cultures.

Observations	Critical S levels				
	Optimum	Deficiency		Excess	
		Threshold	Deficiency	Threshold	Excess
BIOMASS		**S supply (mM)**			
Top (week 17 atp)	2	0.95	0.42	3.35	3.98
(week 33 atp)	4	2.8	2	7.9	-
		Tissue S-young Leaves (% D.M)			
Top (week 17 atp)	0.130	0.093	0.086	0.157	0.179
(week 33 atp)	0.22	0.21	0.195	0.27	-
BIOCRUDE TOPS (WEEK 33 atp)					
		S supply (mM)			
Hexane extr.	4	2.75	2	5	9.55
Methanol extr.	4	2.63	2.29	6.61	-
Acetone extr.	4	2.63	2.4	5	7.94
Total (H+M+A) extr.	4	2.5	2	5.75	8.71
		Tissue S-young Leaves (% D.M)			
Hexane extr.	0.22	0.21	0.19	0.26	0.29
Methanol extr.	0.22	0.21	0.19	0.26	-
Acetone extr.	0.22	0.21	0.2	0.25	0.28
Total (H+M+A) extr.	0.22	0.21	0.2	0.26	0.29

atp= After transplantation

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