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Biomass and Biocrude Reponse of *Pedilanthus tithymalodies* var. Cuculatus to Fe in Calcareous Gomti Entisol

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Abstract In a calcareous Gomti Entisol of Marion, Lucknow, maximum biomass and biocrude response of *Pedilanthus tithymaloides* Poit. var. cuculatus was obtained at 50 μg Fe g^{-1} soil. A 6-9 folds higher magnitude of Fe response in biocrude than in biomass reaffirmed the indicated role of Fe in the biosynthesis of biocrude hydrocarbons, specially the resinous methanol and acetone extractives. DTPA-NH_4–HCO_3 (pH 7.6) extractable available and tissue active-Fe were better indicators of plant response to Fe. Critical Fe-deficiency values associated with 66% yield depression, both for soil available and tissue active-Fe, were higher for biocrude than biomas. A decline in the magnitude of biomass response to Fe at maturity, being associated with decreased growth rate in Fe-amended soils *vis a vis* control indicated for the existence of some adaptive physiological adjustment mechanism to tolerate the Fe-deficiency stress in the rooting medium.

Key words Biomass/biocrude response to Fe, Calcareous Entisol, Critical-Fe, *Pedilanthus tithymaloides*, Petrocrop

Fe-deficiency is a wide spread nutritional problem associated with edaphic stress features of shallowness and aridity (Dudal 1976). Such problem soils abound in wastelands where large scale energy plantations, including petrocrops, are being envisaged for their environment management and for providing alternate non- conventional renewable sources of fuel energy (Pachauri & Dhawan 1987, Bhatia 1988). This paper deals with the biomass and biocrude response to Fe and establishment of critical limits of soil available and tissue Fe for a high Fe-requiring petrocrop, *P. tithymaloides* var. cuculatus in calcareous Entisol.

Materials and Methods

Pedilanthus tithymaloides Poit. var. cuculatus plants, initially raised through cuttings in purified sand and than were grown in calcareous ($CaCO_3$ 1.65%) Entisol collected from village Marion Lucknow, having 5, 10, 25 and 50 μg Fe g^{-1} soil applied through $FeSO_4$. $7H_2O$. The level of S applied through $FeSO_4$ was equated at each Fe level through required application of $CaSO_4$. $2H_2O$. The soil, before planting, was made adequate in other nutrients through basal application of purified salt solutions supplying N 25, P 22.5, K 40.5, Zn 2.5, Mn 5, Cu 2, B 0.5 and Mo 0.5 μg g^{-1} soil (w/w) and aditional top dressing of N in 3 splits, 12.5 μg g^{-1} each time, at week 13, 18 and 43 after transplanting (atp). Initially, there were 8 plants at each Fe-amendment level, 2 in each 25 cm high pot arranged in two blocks. At week 18 atp, after sacrificing above ground portion of one plant from each pot for yield, only one plant was left in each pot. The experiment was continued upto week 63 atp. Soil was maintained at near field capacity with deionised water throughout the experimental period.

Besides a periodical record of growth (height, branching, lateral spread and leaf area), growth rate in terms of biomass was calculated for the week 18-63 atp. At week 63 atp, above ground plant parts of the remaining plants were harvested from the ground level, air dried and ground in a wiley mill. Sequential extraction and estimation of hexane, methanol and acetone extractive biocrude was done in oven dried (45° C) samples, as described by Mehrotra & Ansari (1991). A portion of the fresh material, meant for nutrient analysis, dry matter percentage determinations and computing the biomass data, was throughly cleaned of surface contaminations and oven dried at 80°C for 48-72 h. It's young leaf (YL) fraction was analysed for total and active Fe, as described by Mehrotra & Ansari (1991) and P by the method of Nicholas as described by Wallace (1961). Young leaf samples

Table 1 *Soil Fe-amendment response on soil available Fe, growth attributes biomass, tissue-Fe, P and their ratios, and extractive biocrude in above-ground parts of pedilanthus tithymaloides var, cuculatus grown on a calcareous Gomti Entisol in pot-culture: week 63 atp.*

Observation	Soil applied Fe ($\mu g\ g^{-1}$)				
	O (T_0) control	5 (T_1)	10 (T_2)	25 (T_3)	50 (T_4)
	Absolute value	% increase, + or decrease, − over control			
Soil Available Fe ($\mu g\ g^{-1}$)					
(a) DTPA-TEA-$CaCl_2$, pH7.3	6.7^{a}	$+10^{a}$ (86)*	$+40^{b}$ (73)	$+70^{c}$ (81)	$+109^{d}$ (85)
(b) DTPA-NH_4-HCO_3, pH 7.6	3.1^{a}	$+81^{b}$ (50)	$+116^{bc}$ (64)	$+145^{bc}$ (82)	$+177^{c}$ (89)
(c) N NH_4OAC, pH 4.8	2.0^{a}	$+60^{ab}$ (76)	$+100^{b}$ (80)	$+140^{bc}$ (90)	$+275^{d}$ (89)
Plant growth attributes					
Height tops (cm)	54^{a}	$+1^{a}$	$+14^{b}$	$+29^{c}$	$+51^{d}$
Branch (no. $Plant^{-1}$) − primary	6.5	+15	+8	0	−15
− secondary	17^{a}	$+12^{a}$	$+35^{ab}$	$+50^{b}$	$+150^{c}$
Lateral spread NSxEW (cm)	$21x26^{a}$	$+2^{a}x+6^{ab}$	$+5^{a}x+31^{ab}$	$+14^{a}x+41^{ab}$	$+23^{a}x+69^{b}$
Leaf area (cm^2) − young	6.9^{a}	$+14^{ab}$	$+16^{ab}$	$+39^{ab}$	$+57^{b}$
− middle	13.6^{a}	$+10^{a}$	$+59^{b}$	$+79^{b}$	$+124^{b}$
− old	9.4^{a}	$+15^{a}$	$+17^{a}$	$+15^{a}$	$+23^{a}$
Growth rate 18−63 W atp, above − ground Part (mg $plant^{-1}$ day^{-1})	22.8	−40	−34	−43	−66
Dry matter − tops (%)	8.2^{a}	$+2^{a}$	$+11^{ab}$	$+24^{b}$	$+9^{a}$
Tissue nutrients/ratios					
Total − Fe ($\mu g\ g^{-1}$ DM)	111^{a}	$+84^{a}$	$+226^{c}$	$+347^{d}$	$+429^{e}$
Active − Fe ($\mu g\ g^{-1}$ DM)	27^{a}	$+50^{b}$	$+424^{c}$	$+538^{d}$	$+580^{e}$
Plant active- Fe/total − Fe (%)	24.5	-18^{a}	$+61^{c}$	$+43^{b}$	$+29^{b}$
Tissue − P (mg g^{-1} DM)	1.3^{a}	$+69^{b}$	$+146^{c}$	$+277^{d}$	$+138^{c}$
Plant P/total − Fe	12^{a}	-8^{a}	-25^{b}	-16^{a}	-55^{c}
Plant P/active − Fe	48	$+20^{b}$	-53^{d}	-41^{c}	-65^{e}
Biocrude (%) DM					
Hexane Extr − H	0.99^{a}	$+40^{b}$	$+144^{c}$	$+172^{cd}$	$+199^{d}$
Methanol Extr − M	1.49^{a}	$+61^{b}$	$+105^{c}$	$+240^{d}$	$+231^{d}$
Acetone Extr − A	0.56^{a}	$+79^{b}$	$+189^{c}$	$+288^{d}$	$+261^{d}$
Total Extr − T	3.04^{a}	$+58^{b}$	+154	$+227^{d}$	$+226^{d}$

* Figures in parentheses denote the percentage of applied Fe fixed in soil.

** Figures indicating Fe amendment response effect in each horizontal row superscribed by the same letter, a,b,c,d & e, do not differ significantly (P = 0.05)

NS = north-south, EW = east-west, DM = Dry matter, W atp = Week after transplanting, Extr = Extractable

drawn at week 12 atp were also analysed for total and active Fe and P.

Alongwith plant harvest, soil samples were simultaneously drawn from each pot with the help of a stainless steel augar, composited Fe-level wise, and analysed for available Fe, extractable in (a) DTPA-TEA-$CaCl_2$, pH 7.3 (Lindsay & Norvell 1978), (b) DTPA-NH_4-HCO_3, pH 7.6 (Havlin & Soltanpour 1981), and (c) N NH4OAc pH 4.8 (Olsen & Carlson 1949). Fe in all the extract was estimated on atomic absorption spectrophoto meter.

Fe-utilization quotient for biomass and biocrude were computed as unit yield attribute

Table 2 *Soil Fe-amendment response on Fe-utilization quotient (g attribute mg^{-1} plant-top^{-1}) in respect of biomass and extractive biocrude in above-ground parts of pedilanthus tithymaloides var. cuculatus grown on a calcareous Gomti Entisol in pot-culture : week 63 atp.*

Attribute	Soil applied Fe (μg g^{-1})				
	0 (T_0) control	5 (T_1)	10 (T_2)	25 (T_3)	50 (T_4)
	actual value	% increase , + or decrease, over control			
		Fe-utilization quotient based on total-Fe			
Above – ground biomass	9^a	–46^b	–69^c	–78^d	–81^c
Biocrude, hexane extr – H	0.090^a	–24^b	–25^b	–39^{bc}	–44^c
– methanol extr – M	0.134^a	–12^b	–37^c	–24^{bc}	–37^c
– acetone extr – A	0.051^a	–3a^b	–11^{ab}	–13^b	–32^c
– total extr – T	0.274^a	–14^b	–28^c	–27^c	–38^d
		Fe-utilization quotient based on active-Fe			
Above – ground biomass	37^a	–34^b	–81^c	–84^c	–85^c
Biocrude–hexane Extr – H	0.368^a	– 7^a	–54^b	–58^b	–56^b
methanol Extr – M	0.553^a	+ 6^a	–61^b	–47^b	–52^b
acetone Extr – A	0.207^a	+18^b	–45^c	–40^c	–47^c
total Extr – T	1.128^a	+ 4^b	–56^c	–51^c	–52^d

unit^{-1} tissue Fe plant^{-1} (Grundon 1972). Critical Fe was worked out from the data of soil available Fe, tissue active-Fe, biomass and biocrude yields through extrapolation by the method of Agarwala and Sharma as described by Mehrotra & Ansari (1991).

Results and Discussion

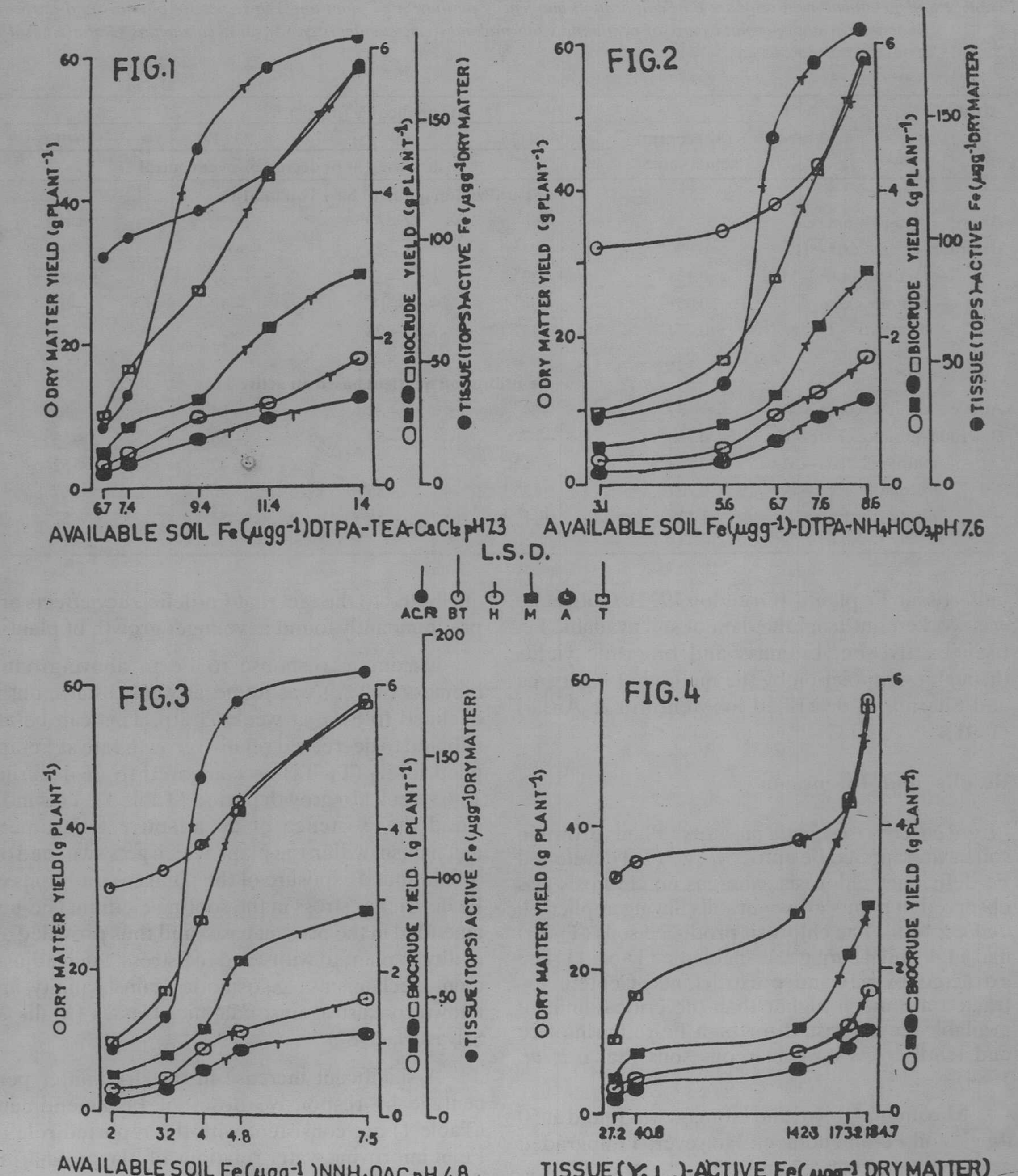
Visual effects, growth and biomass : Plants grown in soil having applied Fe upto 25 μg g^{-1} (T_3) developed Fe deficiency chlorosis, whereas no chlorosis was observed in plants grown in soils having applied 50 μg Fe g^{-1} soil. The chlorosis produced soils (T_1-T_3) had 11.4 and 4.8 μg g^{-1} available Fe (Table 1). The extracted by a,b and c extractants. Content extracted are much higher than the critical limit of available Fe suggested for high Fe-requiring rice and lentil crops on calcareous soils (Sakal *et al.* 1984).

Maximum growth and biomass was found at 50 μg g^{-1} soil Fe-amendment. However, T_1 upgraded available Fe to 9.4, 6.7 and 4 μg g^{-1} extracted by a, b and C extractents, respectively. This may have brought a significant increase in various growth attributes (Table 1 Fig1–3). Higher magnitude of response to applied Fe in leaf area of young and middle leaves as compared to old leaves could be attributed to the fact that Fe-deficiency effects are predominantly found in younger growth of plants.

Maximum response to Fe in above-ground biomass (429%) was found at week 18 atp, but it declined to 81% at week 63 atp. This can be attributed to decreased relative growth rate at Fe-applied levels (T_1–T_4) as compared to (T_0) during 18-63 week atp growth period (Table 1). This indicated the existence of an adaptive adjustiment mechanism within the plant, which gets switched on by continued exposure of the plant to conditions of Fe deficiency stress in the rooting medium (Fe-unamended in the present case) and thus provided an ability to plant to withstand the stress. Such adjustment mechanisms e.g., osmotic against salinity, are known to exist against edaphic stresses (Malik & Srivastava 1982).

A significant increase in the dry matter percentage by responsive doses of Fe amendment (Table 1) are consistent with the reported role of Fe in improving water relations of plants (Sharma & Sharma 1986).

Biocrude : Besides increasing biomass, Fe-amendment, also increased the concentration of biocrude (Table 1) and because of this, the magnitude of response to Fe was much higher for the biocrude yield (6-9 folds) than for the biomass (Table 2). A role of Fe in the biosynthetic secondary metabolism



Figs 1—4 Relationship of soil availabe Fe, DTPA–TEA–$CaCl_2$, P^H 7.3 (Fig 1), DTPA–NH_4HCO_3, pH 7.6 (Fig 2) and N NH_4OAc, pH 4.8 (Fig 3) and of young phylloclade active–Fe (Fig 4) with biomass (BT), active-Fe (AC Fe) and yields of hexane (H), methanol (M) acetone (A) and total (T) extractive biocrude in above ground parts of *Pedilanthus tithymaloides* var. cuculatus grown at graded Fe-amendment in a calcareous Gomti Entisol in pot-culture: Week 63 atp. Cut points on each curve are at 90% (threshold of deficiency) and 66% (deficiency) levels of the maximal yield.

of biocrude components, more so of the resinous acetone and methanol extractives was thus imperative. It is consistent with our earlier observations for the plant under controlled sand culture conditions (Mehrotra & Ansari 1991).

Tissue Fe and nutrient ratios : Soil Fe-amendment in the entire range, increased the young leaf concentration of Fe and P (Table 1). At all Fe levels, the active Fe values at 12 week atp were 20, 58, 145, 168 and 188 and those at 63 week atp (harvest) 27, 41, 142, 173 and 185 $\mu g\ g^{-1}$, respectively. This indicated that active Fe values remained fairly stable during such a long growth period.

At the Fe responsive levels (10 $\mu g\ g^{-1}$), Fe amendment also increased the active fraction of total-Fe and decreased P/Fe ratio in young leaves (in accord with Bekock 1981), more markedly when expressed on active Fe basis (in accord with Mehotra 1991). Fe-amendment related increase in tissue Fe, specially active-Fe, corresponded well with the increase in biocrude yield, especially with it's methanol and acetone extractive resinous components (Fig 4).

Fe utilization Quotient : At responsive Fe-amendment levels (10 $\mu g\ g^{-1}$), Fe utilization quotient, both for biomass and biocrude, decreased as compared to control. The magnitude of decrease was relatively more for biomass than biocrude, was gradual in relation to increasing levels of Fe amendment when expressed on total-Fe basis and fairly stable when expressed on active-Fe basis (Table 2). This indicated the linkage of metabolically linked active Fe fraction (Mehrotra 1991) with the biosynthesis of secondary metabolite components of the biocrude.

Critical soil and tissue active-Fe : In the range of Fe-amendment levels, maximum biomass and biocrude yield was found at the highest level of Fe-amendment (T_5). Hence, the critical Fe values indicative of optimum, threshold of excess and excess of Fe could not be accurately determined. However, from the maximal yields obtained the values corresponding to 66% of the maximum were regarded as critical Fe values indicative of definite Fe- deficiency. These were found to be higher for biocrude than for biomass, both for soil available and young leaf active Fe, e.g., 9.6 and 10.8 $\mu g\ g^{-1}$ for a, 6.6 and 7.4 for b, 4.1 and 4.6 $\mu g\ g^{-1}$ for c extractants extractable soil Fe and 142 and 168 $\mu g\ g^{-1}$ for young leaf active Fe for the biomass and biocrude, respectively. This reaffirmed our earlier observations from controlled sand culture studies (Mehrotra & Ansari 1991) regarding the probable role of Fe in the biosynthetic metabolism of biocrude hydrocarbons.

Conclusion

Adequate Fe amendment to calcareous Entisol, raised the soil available, specially DTPA NH_4-HCO_3, pH 7.6 extractable and tissue active Fe which is required for maximising biomass and specially biocrude productivity of *Pedilanthus tithymaloides* var. cuculatus plants.

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