CHAPTER - V

QUANTITATIVE AND QUALITATIVE STUDY OF DIFFERENT BIOCHEMICAL PARAMETERS IN DIFFERENT PLANT PARTS OF TEPHROSIA CANDIDA DC. AND IN THE SOIL OF THE EXPERIMENTAL CULTURE

INTRODUCTION

In regard to the benefit acquires from mixed cropping, it is generally accepted that during plantation of leguminous plants, much amino acids are excreted into the soil through the roots of the plant (Biswas and Das, 1956; Nutman, 1976). Moreover, nitrogen, protein, carbohydrate and phenolic content are essential constituents of food, forage grasses and lagumes. The content of total nitrogen of leaf is an important criterion for its contribution in green manuring (Stobbs, 1969; Skerman, 1977). The protein content of leaves is important in determining the forage quality besides the forage yield and digestibility (Ahloowalia, 1984; Mishra & Saran, 1987). Thus, attempt has been made to study the role of T. candida DC. on the increase of fertility status of North Bengal with special emphasis on quantitative and qualitative aspects of free amino acids, phenols.

MATERIAL AND METHODS

Materials

Different plant parts of T. candida DC. were collected from the experimental plots of the Centre for Life Sciences, North Bengal University for the purpose. Soil samples were collected from 1" and 6" below the surface of the soil for this part of work.

Methods

Determination of leaf age - Leaf ages (30, 60, 90, 120 and 150 days) were determined after the leaves being tagged during their development.

Methods:

Estimation of total nitrogen according to the method described by Vogel (1961)

Dried and powdered plant parts of T. candida DC. and soil samples (20 mg each) were taken for the estimation. The method is described earlier (page no. 109 Chapter-IV).

Estimation of Protein with Biuret Reagent (Gornall et al. 1948)

Freshly collected different plant parts of T. candida and soil samples (1 gm each) were taken for estimation. These were crushed in 0.05N NaOH and the final volume was made upto 10 ml. Then the extract was centrifused at 5000 rpm for 10 minutes. The supernatent of each sample was taken for estimation. Equal volume of freshly prepared Biuret Reagent was mixed with supernatent and kept at 37°C for incubation (10min.). After cooling to room temperature the bluish violet colour developed was determined in spectrocolorimeter at 540 mm. The protein content (% on fresh weight basis) was measured following the standard curve with egg albumin.

Estimation of total amino acid content by nin-hydrin reaction followed by the method of Moore and Stein (1948)

Extraction

Different parts of T. candida plants and soil samples were collected freshly for the estimation (1 gm each). These were ex-

tracted with aqueous alcohol (80% V/V) immediately after collection. The extracts, after suitable processing to remove extraneous materials present in them were desalted by passage through resin columns (Dowex 50).

Estimation

After extraction, these were centrifused at 2000 rpm (10 min.). The supernatent was taken for estimation. Equal amount of ninhydrin solution (0.3% in 95% ethanol) was added to it. The mixture was heated at 80°C over a boiling water bath for 10 minutes and allowed to cool to room temperature. The violet colour of the solution was determined in specol at 570 mm. Total amino acid content (percentage on fresh weight basis) was measured followed the standard curve of amino acid with DL-Alanine.

Qualitative estimation of free amino acids using liquid ion exchanger following the method proposed by Orme-Johnson and Skinner (1963)

After extraction, the amino acid solutions were subjected to two dimentional paper chromatography with a solvent system prepared by saturating a solution of Amberlite LA-2 liquid ion exchanger in 95 ml Butanol with 100 ml of 0.5 M Phosphate buffer (pH 6). Spots were made visible with ninhydrin reagent (0.2% in acetone). For qualitative analyses, amino acid kit from BDH biochemicals was used.

Estimation of Total carbohydrates content with Dreywood's Anthrone Reagent

The total carbohydrate content was estimated quantitatively following the methods described by Dreywood (1946), Morris (1948), McCready and Ducay (1973) after suitable modifications.

100 mg fresh plant sample was extracted with 10 ml hot ethanol (80%; V/V) at 60°C for 30 minutes. The extract was made colourless with activated charcoal and was filtered. The residual plant sample, after extracted with ethanol, was treated with 10 ml of 70% perchloric acid in order to extract starch. Finally, the ethanolic solutions were made upto 100 ml separately with the addition of distilled water.

1 ml aliquot was taken and 3 ml of 0.1% anthrone reagent was added to it in chilling condition. The grass green colour developed after slight warming the mixture over a boiling water bath was measured at 620 mm. Soluble and insoluble carbohydrate content (percentage on fresh weight basis) was determined following the standard curve prepared with Analar D-glucose (Dextrose).

Estimation of total phenol following the method outlined by Swain & Hillis (1959)

Dried plant parts and soil samples were used for the purpose (100 mg each). The extraction of phenolic acids in all the cases were carried out by the method of Ibrahim and Towers (1960). The

blue colour developed with Folin-Phenol reagent (Folin-Phenol reagent (Folin-Ciscalteau:Water; 1:1) was determined in specol at 760 nm. Total phenol content (percentage on dry wt. basis) was measured following the standard curve prepared with Caffeic acid.

Qualitative analysis of phenolic acids followed by the method described by Ibrahim & Towers (1960)

For separation of individual phenolic acids, the final alcoholic extract was subjected to paper chromalographic analysis using two dimensional ascending technique. In this connection, Butanol:Acetic acid:Water (4:1:5; V/V/V) and Benzene:Acetic acid: Water (6:7:3; V/V/V) were used as the solvents in the first direction while, Isopropanol:Ammonia:Water (10:1:1; V/V/V) and Sodium formate:Formic acid:Water (10:1:200; W/V/V) were used in the second direction. Phenolic acids were identified by spraying with diazotized sulfanilic acid and p-nitroaniline.

Quantitative estimation of total nitrogen from leaf and root of *T. candida* DC.

Oven dried plant parts of *T. candida* were used for the estimation. 20 mg were required for the purpose.

Quantitative estimation of total nitrogen from soils under control and *T. candida* vegetation

Soils were collected and dried in oven for 2 days at 60°C 20 mg soil sample was required in each case for the estimation.

Quantitative estimation of protein from leaf and root of *T. candida* DC.

Freshly collected leaves of different ages (15, 30, 60, 90, 120 and 150 days old) were used for the purpose. 1 gm was required for the estimation in each case.

Quantitative estimation of protein from soils under *T. candida* vegetation and control

Freshly collected and air dried soil samples (1 gm each) were used for the purpose.

Quantitative estimation of total amino acid content from leaf and root of *T. candida* DC.

Freshly collected leaves and roots of different ages (1 gm each) were used for the purpose.

Quantitative estimation of amino acid content from soils under *T. candida* vegetation and control

Air dried soil samples (1 gm each) were used for the estimation.

Qualitative estimation of free amino acids from leaf and root of *T. candida* DC.

Freshly collected leaves of different ages were used for the purpose. 10 gms were taken in case for the estimation.

Qualitative estimation of free amino acids from soils under *T. candida* and control.

Freshly collected and air dried soil samples were used for the estimation. 50 gms were required for the purpose.

Quantitative estimation of soluble and insoluble carbohydrate content from soils under *T. candida* vegetation and control

Freshly collected and air dried soil samples (50 gm each) were used for the estimation.

Qualitative analysis of phenolic acids from leaf and root of *T. candida* DC.

Leaves were collected according to the age (15, 30, 60, 90, 120 and 150 days old). Young and mature roots (30 and 180 days old) were also collected for the purpose. Soon after collection, these were kept in hot air oven at 60°C for 2 days. The materials were powdered. 30 gms were taken for 15, 30 and 60 days old leaf. 90 days old leaf required 40 gms for the estimation while 50 gms were required for 120 and 150 days old leaf for the purpose.

Qualitative analysis of phenolic acids from soils under control and *T. candida* vegetation

Soils were collected and dried in oven for 2 days at 60°C. 200 gms soil samples were required in each case for chromatographic analysis.

Table - 21

Distribution of free amino acid content in leaf and root of *Tephrosia candida* DC.

Age (days) / Aminoacids	LEAF						ROOT	
	15	30	60	90	120	150	30	180
1. L-Glycine	+	+	-	-	-	-	-	-
2. DL-Threonine	+	+	-	-	-	-	+	+
3. DL-Methionine	+	+	+	+	-	-	+	+
4. L-Hydroxyproline	+	+	+	-	-	+	-	-
5. L-Leucine	+	+	+	+	-	-	-	-
6. L-Proline	+	+	+	-	+	-	-	-
7. DL-β-Phenylalanine	-	-	+	+	+	+	-	-
8. DL-nor-Leucine	-	+	+	+	+	-	-	-
9. DL-3:4-Dihydroxy phenylalanine	-	-	+	+	+	+	-	-
10. DL-Ornithine	-	+	+	+	+	+	-	-
11. DL-Tryptophan	-	-	+	+	+	-	-	-
12. L-Cysteine	-	-	-	-	+	+	-	-
13. DL-Valine	-	+	+	-	-	-	-	-
14. Butyric acid	-	-	+	-	-	-	-	-
15. L-Histidine	-	-	+	+	+	+	+	+
16. DL-Aspertic acid	-	-	+	+	+	+	-	+
17. DL-Isoleucine	-	-	-	-	+	+	-	-
18. L-Tyrosine	-	-	-	+	-	+	-	+
19. L-Lysine	-	-	-	-	-	-	+	+
Total no. of amino acids	6	9	13	10	10	9	4	6

+denotes presence of amino acids.

- denotes absence of amino acids.

Table - 22

Nitrogen, protein, amino acids, carbohydrates and phenol contents in young and mature root of *T. candida* DC.

Age (days)	Total nitrogen (%)	Protein (%)	Total amino acids (%)	Carbohydrates		Total phenol (%)
				Soluble (%)	Insoluble (%)	
30	1.12	3.54	0.06	1.46	2.20	1.00
180	1.78	4.24	0.08	1.48	2.30	0.60
S.E.(±)	0.46	0.49	0.01	0.01	0.07	0.28
C.D. at 5%	1.41	1.45	0.21	0.21	0.55	1.09

Table - 23

Distribution of phenolic inhibitors in leaf root and soil under the cultivation of T-candida DC.

Phenolic acid	Leaf(age in days)						Root(age in days)		T.candida soil		Control soil	
	15	30	60	90	120	150	30	180	1" below the surface	6" below the surface	1" below the surface	6" below the surface
1. 3,4 dihydroxy benzoic acid	+	+	-	-	-	-	-	-	-	-	-	-
2. o-coumaric acid	+	+	+	-	-	-	-	-	-	-	-	-
3. Gallic acid	+	+	+	+	+	+	+	+	+	-	-	-
4. 4-hydroxy cinnamic acid	-	-	+	+	+	-	-	-	-	-	-	-
5. 4-hydroxy, 3-methoxy cinnamic	+	+	+	+	+	+	+	+	-	+	-	-
											+*	+**
Total no of phenolic acid	4	4	4	3	3	2	2	2	1	1	1	1

\+ denotes presence of phenolic acid

\- denotes absence of phenolic acid

+* denotes presence of one unidentified phenolic acid

+** denotes presence of another unidentified phenolic acid.

Table - 24

Availability of total nitrogen, protein, amino acid, carbohydrate and phenol contents in soils influenced by the cultivations of *T. candida* DC. in different edaphic condition

	Total Nitrogen (%)		Protein (%)		Amino acid (%)		Carbohydrate				Total Phenol (%)	
							Soluble (%)		Insoluble			
	1" below the surface	6" below the surface	1" below the surface	6" below the surface	1" below the surface	6" below the surface	1" below the surface	6" below the surface	1" below the surface	6" below the surface	1" below the surface	6" below the surface
T. candida (A)	0.35	0.34	1.8	1.3	0.03	0.003	0.63	0.45	1.2	1.0	0.30	0.01
(B)	0.24	0.22	1.9	1.5	0.04	0.005	0.62	0.51	1.3	1.0	0.35	0.023
(C)	0.20	0.19	1.6	1.2	0.04	0.004	0.68	0.36	1.2	1.0	0.20	0.03
(D)	0.22	0.23	1.3	1.0	0.025	0.004	0.67	0.32	1.0	0.7	0.21	0.015
T. candida (Old)												
(I)	0.30	0.22	2.2	1.8	0.04	0.01	0.68	0.59	1.7	1.2	0.38	0.026
(II)	0.32	0.16	2.2	1.8	0.05	0.01	0.69	0.59	1.8	1.3	0.39	0.025
Without *T. candida* (Control)	0.16	0.14	1.7	1.1	0.012	0.001	0.68	0.41	1.3	0.8	0.27	0.02
S. E. (±)	0.07	0.07	0.32	0.33	0.01	3.45	0.03	0.12	0.30	0.21	0.08	6.87
C. D. at 5%	0.22	0.23	0.46	0.47	0.08	1.53	0.14	0.28	0.45	0.37	0.23	2.15

I & II : Two separate plots.

Table - 25

Chromatographic determination of qualitative increase in free amino acids in soils influenced by the cultivation of *T. candida* DC.

Amino acids	T. candida soil		Control soil	
	1" below the surface	6" below the surface	1" below the surface	6" below the surface
1. DL-Alanine	-	-	+	+
2. L-Cysteine	+	-	-	-
3. DL-Threonine	+	+	-	-
4. DL-Valine	+	-	-	-
5. DL-nor-Leucine	+	-	-	-
6. L-Proline	+	-	-	-
7. DL-Isolencine	+	-	+	-
8. DL-Methionine	-	+	-	-
9. L-Leucine	+	+	-	-
10. L-Lysine	-	-	+	-
11. L-Histidine	+	+	-	-
12. L-Hydroxyproline	+	+	-	-
13. DL-Ornithine	+	-	-	-
14. DL-Tryptophan	+	-	-	-
15. L-Tyrosine	+	-	-	+
16. DL-3:4-Dehydroxyphenyl alanine	+	-	-	-
17. DL-Aspartic acid	+	+	-	-
Total No. of amino acids	14	6	3	2

+denotes presence of amino acids.
-denotes absence of amino acids.

RESULTS AND DISCUSSION

Earlier workers have reported the capacity of wild legumes to fix large amounts of nitrogen from the atmosphere like the cultivated ones (Virtanen and Meitinen, 1950; Stewart, 1966). Tephrosia foliage is considered to be one of the best known organic fertilizers (Idani and Chibber, 1953). In this connection, a high content of total nitrogen (2.6%) has been estimated from the mature leaf of T. candida DC. which shows that the leaves are rich in the major plant nutrient-nitrogen (Fig.34). As the value exceeds 2%, the maximum over which nitrogenous materials render nitrogen available for plant growth (Idani and Chibber, 1953), T. candida may help in nitrogen supply to the soil.

Figure 34 also shows that T. candida leaf is rich in protein content. A high content of crude protein (17.5%) has been estimated from nature leaf of 60 days while young leaves contain 11-14% in this respect (Fig.34).

A high content of soluble and insoluble carbohydrates have been estimated from mature leaf (Fig.34). As complex carbohydrates play an important role in storage of energy (Conn and Stumpf, 1976), the high content of carbohydrates in T. candida leaf may be of some importance in this respect.

T. candida leaves have been found to contain a very high content of free amino acids (Fig.34). In this respect, 3.3% total amino acid content have been estimated from mature leaf (Fig.34).

Chromatographic analysis shows that a maximum number of 13 amino acids are available in mature leaf compared to young leaf (Table-21). Several early workers have used paper chromatography for the identification of free amino acids from different plants (Andreeva and Osipova, 1962; Maria, 1963; Stewart, 1963).

Total Phenol content in leaf of different age shows higher values in young leaves than the older one (Fig.34). In this respect, young leaf (15 days old) shows 7.35% of total phenol as compared to 4.5% obtained from mature leaf (Fig.34). As phenols are regarded as plant growth inhibitors (Pridham, 1965) and gallic, o-coumaric and cinnamic acids isolated from T. candida leaf (Table-2) already known as strong inhibitors at higher concentrations (Zenk and Muller, 1963; Chou and Patrick, 1976; Zinsmeister and Hollmuller, 1964), T. candida leaf may have some role in allelopathy which has been discussed a separate chapter VII.

The deteriorative processes in the leaf which lead to its senescence begin as soon as the leaf reached its full size (Rabinowitch, 1951). That there is a gradual decline in photosynthesis with leaf age and the loss in synthetic capacity can proceed so far that there is an actual decline in dry weight of the leaf has been known for many years (Rabinowitch, 1951; Yemm, 1956; Woolhouse, 1967; Carr and Pate, 1967; Das, 1968). The view that there is a rapid loss in synthetic capacity as senescence develops can be supported by the rapid decrease in total nitrogen, protein, amino acid, carbohydrate and phenol contents in 90 days old senescent leaf (Fig.34).

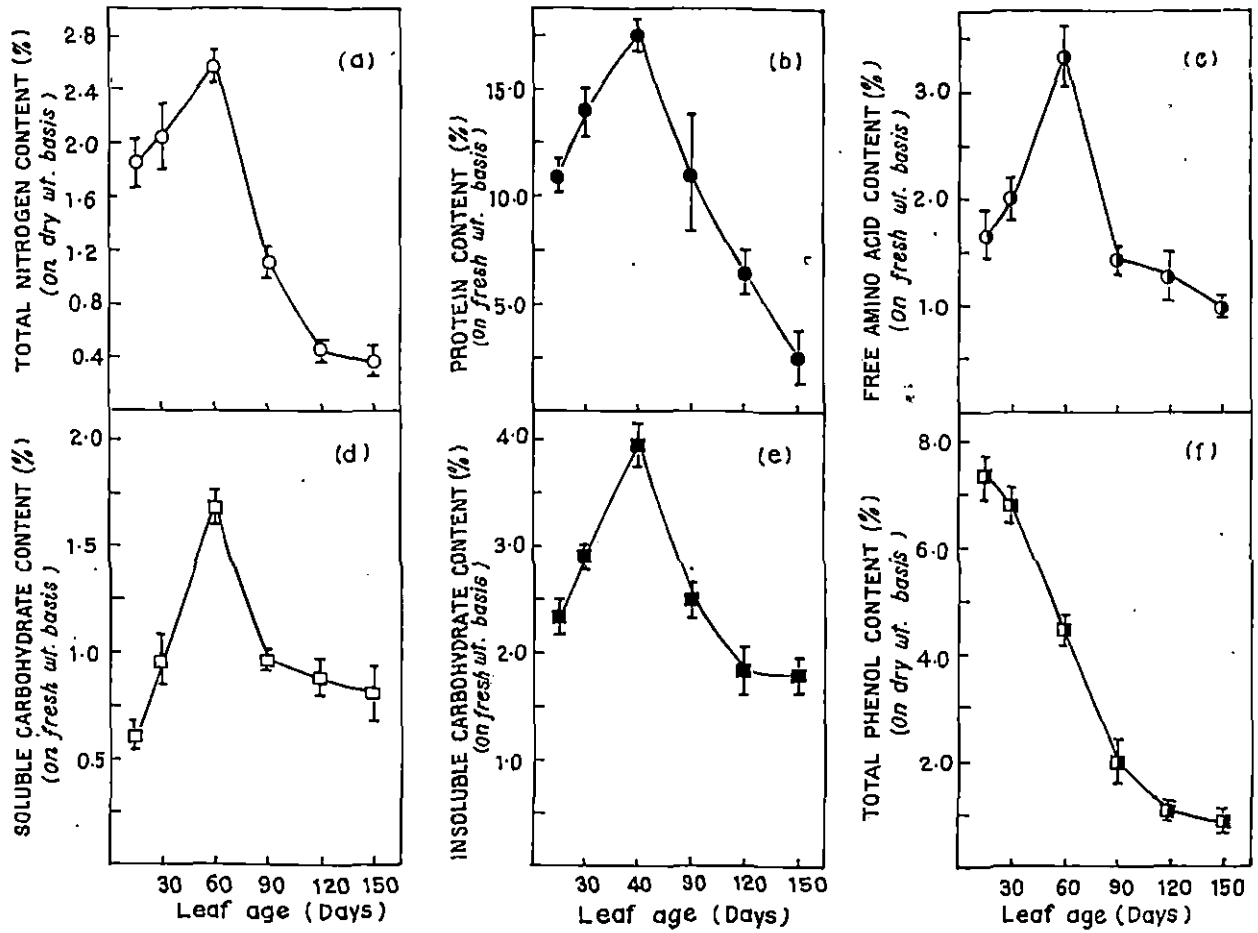

Fig. 34. Nitrogen (o—o), protein (●—●), amino acid (o—o); soluble (□—□ and insoluble (■—■) carbohydrate and phenol (▯—▯) contents in *T. candida* leaf during the developmental stages. Vertical bars indicate mean of 5 replicates ± S.D. at P = 0·05 .

However, Raizada and Srivastava (1987) have reported 14 amino acids in senescent leaf of Populus deltoides in comparison to 11 of them present in green leaf. The decline in all biochemical parameters continues till the decomposition of leaf within the soil (Fig.34). Partly decomposed leaf has been found to contain decreased amount of nitrogen, protein, amino acids and carbohydrates (Fig.34). In this respect, phenol content which was found to be very high in green leaf, has been noted to decrease considerably to 0.9% (Fig.34) after its decomposition in the soil and which may further help in reducing the allelopathic effect of the plant due to the presence of highly inhibitory phenolic content. This has been discussed in Chapter-VII.

The contents of total nitrogen, protein, amino acid, carbohydrate and phenols have been noted to be low in T. candida root than those obtained from leaf (Table-22). The values are found to be high in mature root than those obtained from young root (Table-22). However, total phenol content shows higher value in young root (1.0%) compared to the mature one (0.6%) (Table-22). Qualitative analyses show that root contains 6 amino acids (Table-21) and 2 phenolic acids (Table-23).

Small scale field experiments of T. candida in different edaphic conditions show that all the cultural conditions show increased values of total nitrogen content in soils both at 1 and 6" below the surface over control (Table-24). Several workers showed that the leaves of Tephrosia candida and T. purpurea nitrify

easily and commence to make available adequate nitrogen to feed crops, from just the 2nd week of their incorporation in soil (Idani and Chibber, 1953). Accordingly, Tephrosia released 42% of its nitrogen in available forms as it contains more than 2% total nitrogen. Protein, amino acid and carbohydrate contents have also been increased in most of the cases compared to the control soil (Table-24). Moderately high land culture shows the heightest values in this respect (Table-24).

Results on quantitative and qualitative estimations show a significant decrease in phenol content in soil compared to leaf (Table-23-24). In this connection, the total number of phenolic acids has been found to be reduced to only 2 within 6" soil surface (Table-23) and in most of the cases, the total phenol content under T.candia vegetation shows no significant increase or decrease over control (Table-24). In connection with qualitative estimation T. candida has been noted to show tremendous effect in increasing the free amino acids content in soil. While only 4 free amino acids were available in control soil within 6" below the surface, T. candida has been found to increase the number as many as 15 (Table-25). Accumulation of free amino acids in soils is reported to obtain from the decaying leaves and root system of leguminous plants (Biswas and Das, 1956). As T. candida leaves are very rich in free amino acids (table-21), it is expected that major part of total amino acids in soil are coming from leaf decaying process and some amount may be donated by root system. Increase in soil amino acid enhances the activity of various soil degrading bacteria and fungi which again help in degra-

ding the complex molecules to simpler molecules necessary for growth and development of seedlings of other plants (Daubenmire, 1974; Daumans *et al*, 1984). Thus, *T. candida* may have some role in increasing the soil fertility in this region.

S U M M A R Y

T. candida leaf has been found to be very rich in nitrogen, protein, amino acid, carbohydrate and phenol contents. A high content of total nitrogen (2.6%) in green leaf may help in nitrogen supply to the soil. A considerable number (13) of free amino acids have been identified from green leaf by the application of paper chromatography.

Green leaf of 60 days show highest value in total nitrogen, protein, amino acid and carbohydrate as compared to other developing leaves. Phenols on the other hand show the highest value in younger leaves in comparison to the older one.

All the values gradually decline during senescence. Partly decomposed leaves show much decrease in this respect. The high content of total phenol in green leaf (4.5 - 7.35%) has become only 0.9% after decomposition.

Root shows lower amount of nitrogen, protein, amino acids, carbohydrate and phenol contents in comparison to leaf. 6 amino acids and 2 phenolic acids have been identified from *T. candida* root.

Field experiment of *T. candida* shows increased values of total nitrogen, protein, carbohydrates in soil over control in most of the cases. Culture of moderately high land shows the highest values in this respect.

Total phenol content was found to be very low in soil (0.01 - 0.39%) than that of leaf. Only 2 phenolic acids have been identified qualitatively from soils under T. candida vegetation.

As T. candida has been noted to be very much effective to increase the free amino acid contents in soils both quantitative and qualitative point of view, the importance of T. Candida in connection with the increased fertility status of the soil in the region has been stressed.