

CHAPTER - I

REVIEW OF LITERATURE

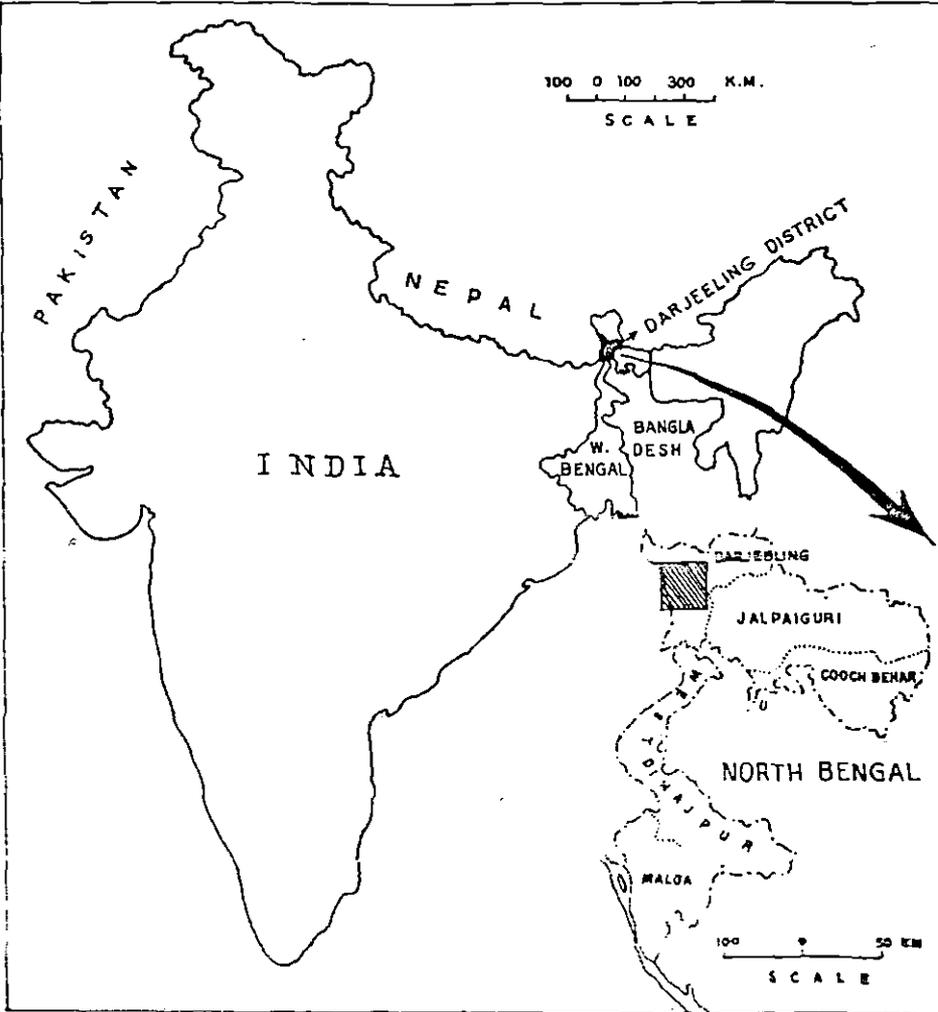
## REVIEW OF LITERATURE

### Position of Balason-Catchment area in the background of the development of forest organisation in North Bengal

The geographical position of Balason-catchment area can be recognised from the Fig. 1. Though the area now a days, is included within Kurseong division of Northern circle it has a long history of forest organisation.

Once the Balason-Catchment area was a part of the kingdom of Raja of Sikkim ( Ray, 1964 ). This area faced a number of wars between East India Company and the Raja of Sikkim. The relation between Sikkim and the British deteriorated and ultimately Terai portion of Sikkim hills including Balason-Catchment area bounded by the Raman and the Great Rangit rivers on the north, by the Tista in the east and Nepal frontier on the west were annexed by the British in 1861. Forest conservancy took its first step when Mr. M.T. Anderson M.D., Superintendent of Botanical Garden, Calcutta was appointed as temporary conservator of forest in 1864. Next year in 1865 three divisions viz., Sikkim, Bhutan and Assam were formed and the said catchment area was controlled within Sikkim division. Later in 1876 it came under the newly formed Darjeeling division. It remained in such a division upto 1905 ( Mullick, 1964 ) when the division was segregated to Darjeeling and Kurseong and the said area came under the last. Since then the position of Balason-Catchment area remains unchanged up to date though at present the state of West Bengal has been divided into Northern circle, Central circle

LOCATION OF MAHANANDA-BALASON-CATCHMENT AREA, DARJEELING DISTRICT, NORTH BENGAL IN INDIA.



LOCATION OF MAHANANDA-BALASON-CATCHMENT, DARJEELING DISTRICT

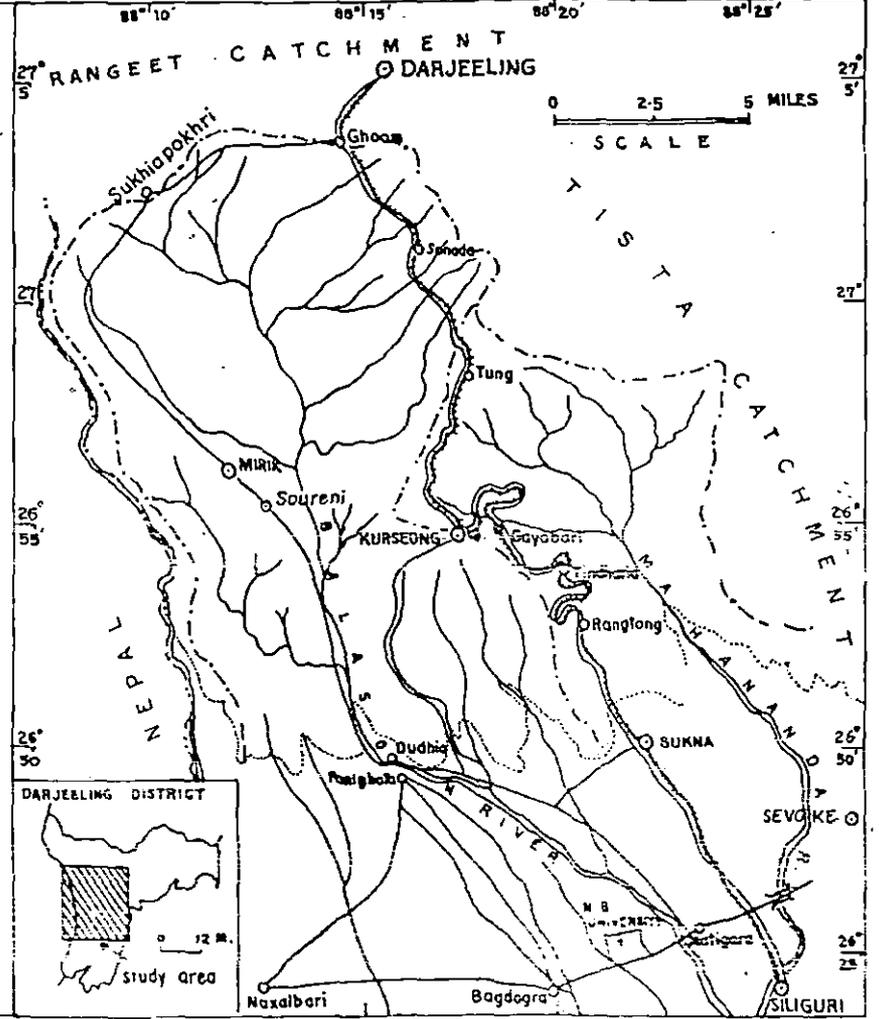


Figure - 1.

and Southern circle, out of which the Northern circle represents the whole of North Bengal including altogether seven divisions and Kurseong is one of them.

### History of forest management in Balason-Catchment area in North Bengal

The history of forest management in North Bengal, spreading over a period covering more than a century, is a fascinating one. Inauguration of forest conservancy in Bengal took place in 1864 and since then the forest management has proceeded gradually from a rudimentary stage to a comparatively intensive system of management now in vogue ( Ray, 1964 ). First regular Working Plan for Balason-Catchment area drawn up by Hatt (1902-1918) prescribed two Working Circles e.g. the plains and hills of the catchment area and provided for selection and improvement fellings with a felling cycle of 15 years. In Gent's Working Scheme (1919-26), it was sought to provide "opportunities for experiments as to the best method of re-generating Sal (Shorea robusta) and other species." Mr. Datta's Plan prepared for 20 years with effect from 1929-30 prescribed clear felling with artificial regeneration for plains sal forests and selection with improvement felling for Hill sal areas. Rotation of Plains sal was fixed at 80 years and yield was prescribed by area-cum-volume method.

The upper hill forest in Balason-Catchment area was managed under various Working Plans of Management, Grieve and Baker prepared

for Darjeeling division upto 1937 ( Ray, 1964 ). The next Working Plan which embraced both the plains and upper hill portions provided for clear felling and artificial regeneration with Sal for the plains Sal areas and prescribed planting with Teak (Tectona grandis) for valley forests. For the upper hill areas separate Working Circles were made for growing timber and fuel with rotation of 125 and 60 years respectively. The system of artificial regeneration by Taungya system proved to be very successful and soon Forest villages were established in different felling series. Besides during Baker's Working Plan for the period 1921-28 the growth of quick growing conifers (Cryptomeria japonica) an alien species was introduced. Disastrous land slide of 1950 and impact of Five Year Plans necessitated revision and accordingly the Working Plan (1954-55 to 1963-64) prescribed clear felling with Sal and set apart large areas for growing Teak. The area at present is being managed under working schemes on year to year basis ( Ray, 1964 ) with the objective to convert, wherever possible, the present irregular and mixed forest into regular, normal and evenaged forest of more valuable species with a view to improve the future yield and thereby to ensure the highest possible monetary return as far as possible.

Another working circle, namely Softwood Working Circle (1967-68 to 1976-77) has been proposed in the plan. This Working Circle which is partly the Cryptomeria Working Circle of the previous plan has been suggested to be intensely worked for the creation of mainly Cryptomeria plantation to meet the current demand of industrial

cellulosic raw material of paper pulp and wood pulp. It is expected that the old doubt raised in 1931 on the value of Cryptomeria will be set at rest during the currency of the present Plan or earlier ( Report, Tenth Working Plan, 1970- ).

### Geology and Soil in Balason-Catchment area in North Bengal

The region is mainly composed of slightly altered sedimentary rocks of Gondwana formation of which sandstone and shale are noteworthy. Small and thin coal seams are found occasionally in sandstone. Altered metamorphic rocks of Daling formation of which Quartzite and Phyllite, Slate and Mica-schist are noteworthy ( Terminal Report, 1985 ).

Soils are acidic in nature ( 4.80-6.35 ). This is not expected because of high rate of leaching as a result of high rainfall during the period from June to September. Mountainous soils are characterised by low clay content ( 1.68 to 2.30% ). The percentage of silica (50%) and rock materials ( 25-38% ) are obviously high compared to clay content. The conductivity rate is reasonable ( 0.12-0.25 m.mohs/cm ) when the soluble electrolytes has been taken into consideration. The percentage of organic matter easily extractable ( 0.37-3.16% ) with caustic soda is fairly high. The data as obtained for inorganic nutrients indicates that except iron and copper the content of potassium, phosphorus and zinc are low ( Report, 1985 ).

Major chemical components of Forest Soil other than that of Balason-Catchment area

The contribution of plant residues and soil humus to plant growth, soil productivity, and genesis is well recognised ( Russel, 1961; Tisdale and Nelson, 1966 ). Energy flow through producers, consumers and decomposers is being stressed in ecosystem research ( ICSU, 1967 ). This plus, the questions concerning the geocycle of nutrients in nature ( Delwiche, 1965 ) and the detoxification of environmental pollutants ( Breth and Stelli, 1966 ), makes it imperative that information be obtained relative to the turn over rate of plant constituents, and recalcitrant humic components in soil. But such informations are lacking from the forests of Balason-Catchment area of North Bengal though these are available in other places.

Carbohydrates :

Glucose constitutes one third of the carbohydrate "C" in mineral horizons and one-half in forest litters which have not undergone extensive degradation ( Paul, 1970 ). The relative concentration of the fucose, ribose, and rhamnose and the presence of 2-9 methylrhamnose and 4-D-methylgalactose in soil indicates their microbial origin ( Whistler and Kirby, 1956; Duff, 1961 ), Except for the forest litter layers and peaty soils, cellulose does not constitute significant portion of the carbohydrate carbon ( Gupta and Sowden, 1964; Schnitzer and Hoffman, 1967 ). Distribution of sugars in hydrolysate of organic matter in different soil profile

such as, podzol, chernozemic and gleysolic have been well reviewed ( Gupta et al., 1963 ). The bacterial polysaccharides and carbohydrate fractions are largely responsible for the stabilization of soil structure ( Harris et al., 1964 ).

### Phosphorus :

The known groups of phosphorus compounds in soil include the inositol phosphates, nucleic acid derivatives, sugar phosphates, phosphoproteins and phospholipids ( Mckercher, 1968 ). There is no evidence to indicate that organic soil phosphate can be utilised to any great extent by plants. It must be mineralized before it is in available form ( Cosgrove, 1967 ). Organisms capable of dephosphorylating all known organic phosphates of plant origin have been isolated from soil ( Greaves et al., 1963 ). According to some authors, some of the agricultural soils containing largest known concentrations of phosphorus ( McKercher and Anderson, 1968 ) are amongst the most deficient in plant available phosphorus ( Rennie and Clayton, 1966 ).

### Nitrogen and humic acids :

Nitrogen is intimately associated with carbon, hydrogen, sulphur and oxygen in skeleton of humic moiety ( Paul, 1970 ). The general distribution of the major forms of nitrogen in a number of soil profile ( Black, Chernozemic, Podzol and Brown Forest ) has been well studied by Ferguson and Sowden (1966). The lignoprotein concept of humic materials postulated that humic acids are formed by a reaction between lignin and protein material and that humic

nitrogen is largely protein in nature ( Paul, 1970 ). The humic materials are relatively low in molecular weight and only one-third of the total nitrogen of most humic preparations can be accounted for as protein nitrogen. This has led to the presently held theory that amino acids formed during either proteolysis or synthesis by microorganisms react with polyphenols from oxydised lignin decomposition products to form soil humic acids ( Laatsch et al., 1951; Flaig, 1960; Kononova, 1966 ).

#### Microbiological Transformations of humus in soil

Although resistant to microbial attack, soil humus is not immune to biodegradation ( Birch and Friend, 1961 ). The organism involved represent a wide range of different types that is, Penicillium, Aspergillus ( Kudrina, 1951 ), Polystictus ( Burges and Latter, 1960 ), Streptomyces, Nocardia ( Schonwalder, 1958 ), Actinomyces ( Ochilova, 1961 a,b ), Pseudomonas ( Nikitin, 1960 ), Corynebacterium, Bacillus ( Schonwalder, 1958 ) and sulphate-reducing bacteria ( Aleksandrova, 1953 ). It has generally been observed that these organisms can utilise the nitrogenous side chains of the humus compounds provided the substracts contains readily utilizable substract. Soil suspensions and mixed cultures of organisms bring about a greater degree of degradation than individual pure cultures ( Pontovich, 1938; Volkova, 1961 ) and sterilization of alkalization of humic substances greatly increases their vulnerability to microbial attack.

A proposed scheme for the degradation of plant residues and the formation of soil organic matter has been represented by Paul (1970). According to him, the carbohydrates and proteinaceous plant constituents serve as a substrate for micro-organisms in turn synthesize amino acids, protein, cell wall constituents and carbohydrates. These products can persist in the soil for extended periods as a portion of the living soil biomass. In addition, plant carbohydrates, proteins, and microbial cell structures can be protected by colloid adsorption ( Ensminger and Giesecking, 1942; Ensterman et al., 1959 ).

Role of leguminous plants in connection with the upliftment of fertility status of soil

Paul Richards (1932) in his classical work "The Tropical Rain Forest" which is based on his study of rain forest in Malayasia, noted a surprising discovery that except for a few tracts with volcanic and alluvial soil, the tropical rain forest soils are of poor quality and deficient in nutrient. John Griffith in "Applied Climatology" lists the characteristics of tropical rain forest soil in tropics. It is the poor quality of soil which express why tropical rain forest, contrary to expectation, generally show poor natural regeneration capacity.

Nitrogenous compounds

Once primeval forest is removed to expose soil to the combined influence of erosion, leaching, insolation and radiation, fertility

decline is rapid, and without some injection of fertility successive crops dwindle quickly until it is not worth while expanding effort to plant further crops. In an effort to postpone fertility drain and improvement of the productive capacity of the soil, legumes usually play an important part ( Skerman, 1977 ). According to Govinda Rajan and Gopala Rao (1978), growth of leguminous crops are said to release nitrogenous compounds into the soil and which can be utilised by non-leguminous crops grown in their association. According to them, beneficial effects have been attributed to such factors as : (1) improvement of the physical condition of the soil, (2) increase in general microbial activity, (3) activation of increased nitrifiability of soil nitrogen, and (4) increase in the organic matter and nitrogen content of the soil.

#### Phosphates, amino acids and organic carbon

Acharya et al (1953) showed after soil analysis that the plots treated with phosphates and Trifolium alexandrinum grown, had 40% higher nitrogen at the end of 10 year period compared to the plots without leguminous plants and according to them, bulk of nitrogen added to the soil came from excretion from the roots where the crop was growing. Biswas and Das (1957) observed that amino acids were excreted into the soil through the roots of legumes and different amino acids were identified with the help of paper chromatography.

The cultivation of leguminous crops and the incorporation of the green matter in the soil is recognised as a means of enriching

the soil in nitrogen ( Skerman, 1977; Govinda Rajan & Gopala Rao, 1978 ). However, there is controversy regarding the contribution of a legume soil nitrogen when it is harvested off leaving the root matter in the soil ( Sen & Viswanath, 1943; Desai and Sen, 1952 ). On the other hand, Acharya et al (1953) observed that there was appreciable increase in soil nitrogen when Trifolium alexandrinum was grown in the soil and harvested for fodder. They believed that the underground roots and nodules of legume would not account for the increase in nitrogen and the indications were that the bulk of the nitrogen added to the soil should have been in the form of nitrogenous excretions from the roots while the crop was growing. These observations were also supported by Rewari et al (1957) in connection with their studies with Cyamopsis psoraloides. Iswaran (1960) observed that where legumes were grown in a crop rotation, larger amounts of C and N were stabilized in the soil compared to the field where non legumes were grown.

### Green manure

The utility of wild legumes as green manures particularly in respect to their rate of nitrification have been studied by many workers. Paul and Sen (1961) studied the nitrification of 12 different legumes and showed that at least upto 12 weeks, the nitrate nitrogen of the soil showed an increase and the correlation between nitrate nitrogen and time was found, highly significant. It was also found that several of the wild legumes including Tephrosia purpurea nitri-

fied at higher rates in soil compared to the other studied ( Idani & Chibber, 1953 ). According to Dauglas et al (1982), fixed nitrogen in the nodule of leguminous plants is liberated when the roots decay thus enriching the soil.

Phytochemical investigation in connection with the identification of plant products - Rotenoids and Non-Rotenoids

Extraction

Over the past decade, the techniques used for extraction and isolation of various plant products emphasized the importance of solvent extraction, purification with column chromatography and crystallisation and this has been well reviewed by Seshadri (1962), Seikel (1962) and Harborne (1982). According to Seshadri (1962), plunging of the plant materials into boiling solvent or by rapid drying there was a possibility of enzyme action to cause breaking down any flavonoid during prior to extraction is very much necessary specially during isolation of rotenone from the fresh leaves ( Bech, 1966; Trim, 1955 ). Different authors have used different solvents for extraction of rotenone from plant parts. Rangaswami and Ramasastri (1956) used chloroform followed by methanol for extraction of rotenone from seeds of T. vogellii Hook. Chloroform extraction has also been done in Derris root ( Cartey et al, 1947, Pagan and White, 1949 ), Tephrosia sp. ( Mutinetta, 1945 ) and T. vogellii ( Pradhan, 1982 ). Methanol was utilised by Stafford (1944) and Pradhan (1982). Extraction by ether has been undertaken by Norton (1944). Petroleum ether extraction of rotenone has also been done

in Piscidia crythrena ( Kapoor et al., 1971 ). Hexane has been utilised by Schwarz (1958) for the purpose. Subba Rao (1945) has utilised cold chloroform for extraction of rotenone from air dried roots of Derris elliptica. Accordingly, solvent was removed and treated with ether. From ether 5% aq. potash was treated to remove resinous matter. It was evaporated and residue was taken in  $CCl_4$ . The complex rotenone- $CCl_4$  was separated in crystalline form which was decomposed with boiling alcohol to yield rotenone. This method of purification was also applied by various authors ( Graham, 1947; Henriet, 1951 ).

Among the non-rotenoid compounds, various phenolic compounds including flavonoids and phenolic acids, alkaloids and phytosterols have been considered. Techniques used for extraction and purification of non-rotenoids have been well reviewed in various literatures ( Harborne, 1973; Harborne et al., 1975 ). Solvents used for extraction are chosen according to the polarity on chemical constituents being studied. Extraction with light petroleum or Hexane is frequently carried out to obtain phytosterols as it is also helpful to get rid of carotenoids, chlorophylls and other fatty substances ( Bhutasu et al., 1969; Clark and Porter, 1972 ). Light petroleum has been noted to be good solvent for methylated flavanone ( Kupchan and Banershmidi, 1971 ). Flavonoid glycosides and the more polar aglycones are generally extracted with acetone, alcohol, water or in combination of them (Cambie and James, 1967; Bhutasu et al., 1969; Markham et al., 1970; Ohta and Tagishita, 1970 ). The use of traces of acid is occasionally

incorporated in the solvent for the extraction of flavonoid glycosides ( Wallace et al., 1969 ).

### Purification

Column chromatography remains the most useful technique for the isolation of large quantity of flavonoids and phenolic acids from crude extract. Phenolic acids occur widely in plants and are second only to the flavonoid in importance. ( Seikel, 1964 ). Technique for separating flavonoids have been discussed in a number of articles ( Seikel, 1962; Mabry & Mabry, 1970, Harborne, 1973 ) and this has been well reviewed by Harborne et al., (1975). Adsorbant commonly used for the separation of flavonoids include Silica gel ( Lebreton and Bouche, 1967 ), Alumina ( Markham & Mabry, 1968; Porter & Markham, 1970; 1970b ), Polyamide ( Anderson & Sewers, 1968; Endres, 1969 ); Cellulose ( Seikel, 1962; Clark & Porter, 1972 ), Sephadex gel ( Gelotte, 1960; Fischer, 1969 ) and ion exchange resin ( Cassidy, 1957; Webster et al., 1967 ).

Column Chromatography remains the most useful technique for isolation of large quantities of rotenone and rotenoids in almost pure form from crude plant extract & various adsorbants have been used during the purification of those compounds. Minoda et al (1977) used silica gel while Harborne (1975) is of the view that silica gel would adsorb the rotenoids and ensure difficulty in isolation. Alumina has been utilised by Norton (1944) during isolation of rotenone from seeds of Paxhyzhizhs crosus.

The techniques used for the separation of phenolic compounds with the help of thin layer chromatography (TLC) has been reviewed in a number of papers ( Truter, 1963; Stahl & Mangold, 1967; Kirchnek, 1967; Stahl, 1969; Harborne, 1973; Harborne et al, 1975; Harborne, 1982 ). This technique is also complementary to paper chromatography in that it provides a new media for the separation of flavonoids on a small scale and persuits the use of wider variety of detecting reagents ( Markham, 1975 ). The absorbents used for the separation are silica gel G, cellulose and polyamide. Solvents of higher polarity are required for the elution as flavonoids are much more strongly held in thin layer ( Harborne, 1973; Markham, 1975 ). Various solvents so far used for the separation of flavonoids and phenolic acids and the spray reagents for detecting these chemicals compounds has been reviewed by Harborne et al (1975). Different aspects in connection with the separation and detection of various sterols have been discussed by Harborne (1975).

Treatment of crude plant extract with charcoal powder is also a useful method for the preliminary purification of flavonoids (Mabry and Mabry, 1970). Phenolic acids are purified after solvent fractionation as they dissolve in dilute bicarbonate and in aqueous sodium acetate. They are soluble in weakly polar solvent such as ether and ethyl acetate and in polar solvent, methanol ( Seikal, 1964 ).

## Identification

For complete identification of various chemicals information of melting point, optical rotation, chromatographic behaviour, microanalysis for various elements, and chemical tests have been applied by various authors ( Rangaswami and Ramasastry, 1956; Acree et al., 1944; Moore and Stanley, 1956; Harborne, 1982 ). During the last few decades, informations on ultraviolet spectra (UV) have been available from the review work of Harborne et al., 1975; Pavia et al., 1979 and Harborne, 1982. Besides, data on IR ( Harborne, 1982, Buchi et al., 1961 ) NMR ( Harborne, 1982; Crombie, 1963; Crombie et al., 1968; Classie et al., 1964 ) and mass spectra ( Pavia et al., 1979; Reed and Wilson, 1963; Harborne et al., 1975; Harborne, 1982 ) are helpful during identification of chemical compounds.

## Earlier report on chemical examination of *Tephrosia candida* DC. and related species

The most important and interesting chemical constituents so far recorded from different species under the genus Tephrosia pers. are grouped as (1) sterols, (2) phenolic acids and (3) flavonoids. The alkaloids occur in seeds in low quantity and their occurrence has been traced in several species. Earle & Quentin (1962) mentioned the presence of an alkaloid in seeds of T. tenelle while Jones and Earle, (1966) reported the presence of alkaloid in T. biocarpa. Basu (1972) has reported an alkaloid (mp. 130°) in T. purpurea (L) pers.

As for steroidal compounds,  $\beta$ -sitosterol has been reported in T. purpurea ( Basu, 1972 ), T. wallichii ( Basu, 1972 ), T. candida DC. ( Pradhan, 1982 ), and T. vogellii ( Pradhan, 1982 ). Different parts of T. vogellii have been reported to contain stigmasterol and Lanosterol ( Pradhan, 1982 ). Caffeic acid was noted in seeds of T. purpurea ( Basu, 1977b ), T. hamiltonii ( Basu & Sircar, 1978 ) and in T. vogellii ( Pradhan, 1982 ). Benzoic acid has been reported from different plant parts of T. vogellii ( Pradhan, 1982 ).

Different flavonoids have also been reported from different species of Tephrosia. Among the flavones, Lanceolatin B,C and A have been reported by Rangaswami and Ramasastry (1956) and Ayenger et al, (1973) isolated them from root and bark of T. lanceolata. The plant has also been known to contain other flavones such as Tephrostachin, Stachyoidin ( Vleggar et al, 1973 ), Tephrocin and Tachrosin ( Small Berger et al, 1971 ). Small Berger et al, (1975) have reported Semiglabinol from the plant parts of T. semiglabra.

Among the flavonols, Kaemferol ( 6, OH Kaemferol-4-Methyl Ether ) has been reported from T. candida ( Sarin et al, 1976 ). Several authors reported the presence of Rutin (Quercetin-3, rutinoside) from leaf of T. purpurea ( Clarke & Banerjee, 1910; Basu, 1977a ), T. vogellii ( Pradhan, 1982 ); T. lanceolata ( Rangaswami & Rao, 1959 ); T. wallichii ( Basu & Ganguli, 1972 ); T. hamiltonii ( Basu, 1977b ). Quercetin has been reported to occur only in T. vogellii leaf ( Pradhan 1982 ) and T. pumilla ( Rangaswami, 1965 ). T. vogellii seed has been reported to contain Vogelatin ( Rangaswami & Rao, 1959 ).

Tephrosia species have been noted to be rich in isoflavone content. Several isoflavones namely, Maxima isoflavone A, B and C have been reported in T. maxima root by different authors ( Ranga-  
swami & Ramasastry, 1956; Kukla and Seshadri, 1962 ). Subha Rao  
(1965), Kukla and Seshadri (1962) reported the presence of purpuramin  
from T. maxima pods.

Only a single anthocyanidin ( Delphinidin ) has so far been  
reported by Basu (1978) from the plant parts of T. purpurea.

Among the Chalcones, ~~Chen~~ Chen et al, (1978) have reported  
obovatin from the plant parts of T. obovata while Lanceolatin C  
has been found to occur in T. lanceolata as reported by Rangaswami  
& Ramasastry (1956).

Different rotenoids have been found to occur in plant parts  
of Tephrosia sp. Among these, the most important one, i.e. Rotenone  
has been reported from T. macropoda ( Roark, 1937 ), T. obovata  
( Yuh-lin and Hong 1958 ), T. candida seed ( Krishna & Ghose, 1938 ),  
root and leaf of T. virginiana ( Delfel et al, 1970 ), T. vogellii  
seed ( Irvine & Freyre, 1959 ) and leaf ( Pradhan, 1982 ), T. maxima  
root ( Ollis, 1961), different vegetative parts of T. densiflora  
( Dalziel, 1937 ) and root of T. falciformis ( Raka and Jain, 1978 ).  
Dehydro rotenone was reported from non-viable seed of T. vogellii  
( Pradhan, 1982 ).

Tephrosin has been reported from leaf and root of T. obovata  
( Yuh-lin & Hong, 1958 ), root and bark of T. candida ( Pradhan,  
1982; Krishna & Ghose, 1937 ), seed of T. vogellii ( Irvine & Freyre,

1959; Pradhan, 1982 ), root of T. maxima ( Ollis, 1961 ), different plant parts of T. densiflora ( Krishna & Ghose, 1937; Dalziel, 1937 ) and T. falciformis root ( Raka and Jain, 1978 ). Isotephrosin has been reported from T. maxima root ( Ollis, 1961 ).

Another important rotenoid Degulin has been reported from the plant parts of T. macropoda ( Roark, 1937 ), T. vogellii ( Irvine & Freyre, 1959; Pradhan, 1982 ); root of T. maxima ( Ollis, 1961 ), T. virginiana ( Delfel et al., 1970 ) and T. falciformis ( Raka and Jain, 1978 ).

Isodegulin has been reported from leaf, root and seed of T. virginiana ( Dalziel, 1937 ). Merz (1932) and Dalziel (1937) have reported the presence of Dehydrodegulin from T. vogellii seed. Toxicarol has been observed to occur in different plant parts of T. obovata ( Yuh-lin & Hong, 1958 ), root and bark of T. hirta ( Rangaswami & Ramasastry, 1956 ) and seeds of T. vogellii ( Merz, 1932; Dalziel, 1937 ). T. wallichii leaf has been reported to contain wallichin ( Basu, 1982 ). Raka and Jain (1978) have reported the presence of Elliptone from T. falciformis root.

#### Quantitative estimation of Rotenoids and related chemicals

For a long time different procedures based on gravimetric, colorimetric, spectrophotometric measurement have been used for quantitative estimation of Rotenoid constituent on micro analytical scale.

Gravimetric estimation of rotenone was performed by Subba Rao (1945). In this method, air dried roots were powdered and extracted with cold chloroform. The solvent was then removed under reduced pressure. The residue was treated with ethanol. Hexagonal plates of rotenone was obtained from the alcoholic solution.

Now a days there has been a rapid change over to the use of microtechnique in the chemistry of natural products. The use of relatively cheap commercial spectrophotometers and spectrocolorimeters becomes the constant companion for a large number of readings ( Milton & Waters, 1949 ).

A colorimetric method of analysis for total content of rotenone was described by Meyer and Rachamad (1947). Barnes & Freyre (1967a) have utilised fresh leaves during the recovery of rotenone from Tephrosia vogellii while other authors isolated the same from dried and powdered plant parts. According to Meyer & Rachamad (1947), 1 gm finely ground Derris root was refluxed with 30 ml acetone for 2 hrs. It was filtered and 0.5 ml of acetone solution was transferred to a test tube and evaporated in boiling water bath. 5 ml of  $\text{NaNO}_2$  solution (1%) and conc.  $\text{H}_2\text{SO}_4$  were added with constant shaking. This was kept in dark for 30 min. and O.D. reading was noted in colorimeter with  $\text{CuSO}_4$  as a filter.

Jones (1945,1946) worked throughly on the development of colour and evaluation of rotenoids with spectrophotometer. Accordingly,  $\text{NaNO}_2$  was prepared in 95% ethanol previously purified in KOH and Zn.

Aq. KOH soln. (40%) was then added. After the addition of  $H_2SO_4$  in the mixture of acetone solution of plant sample and  $NaNO_2$  soln., the colour developed was recorded at 450 nm for the estimation of total rotenoid content. Chloroform was added to the reaction mixture and the absorbance was measured at 560 nm for the estimation of total rotenoid content. Chloroform was added to the reaction mixture and the absorbance was measured at 460 nm for the estimation of rotenone only. Since then, several workers utilized this for the determination of rotenone. Barnes & Freyre (1967) utilized T. vogellii leaves which were punched into small slices and placed in capsules in acetone. The O.D. value of the coloured solution was measured.

Hausler (1946,1947a) proposed a method for determination of the colour test for rotenone with vanillin from Derris root extracts. Accordingly, dried plant sample was heated in small dish with some vanillin and alcohol until the latter was evaporated. A few drops of 30% HCl was added and continued to heat on the waterbath. A strong violet colour soon appeared which changed into a blue. The method is superior than nitrite reaction. Since, color reactions are generally typical for a whole group and not for an individual product, a negative result is definite proof of the absence of rotenone, whereas, a positive result merely indicates the presence of rotenone.

Identification of rotenone by ultraviolet fluorescence has been conducted by Fonseca (1949). The blue fluorescence obtained by rotenone in  $CHCl_3$ , Acetone,  $C_6H_6$  or  $Et_2O$  has been used for the determination of rotenone and absorbance was measured at 450 nm.

During the estimation of rotenone from various plant parts, different authors applied formulas for the purification of the compound. Pucci & Philipp (1953) determined the percentage of rotenone by the application of the formula  $R_o$  (rotenone%) =  $0.719 \frac{SD}{P} \frac{281+C}{D}$  where S is the weight of solute (dried at 40°C), D is the loss of weight of solute (dried at 105°C), C is the correction for solubility of rotenone in  $CCl_4$  (0.28%/100ml) and P is the weight of an aliquot or equivalent. Similarly, Kadylova et al (1974) utilized regression equation for the calculation of the percentage of rotenone from Amorpha fruticosa seed

$$Y = 71.5 + 6.16X_1 + 0.2X_2 + 4.42X_3 + 11.6X_4 + 13.87X_5$$

where,  $X_1$  is the degree of seed grinding,  $X_2$  is temperature of extraction,  $X_3$  is the number of extraction cycles,  $X_4$  is the ratio of volumes of dichloroethane and  $X_5$  is the length of extraction.

Bowman et al (1978) utilised High pressure liquid chromatography (HPLC) in connection with the quantitative estimation of rotenone and related chemicals.

#### Seed Germination behaviour of T. candida & Tephrosia sp.

Sometimes many seeds do not germinate under conditions which are normally regarded as favourable for germination. Such seeds can be shown to be viable, as these can be induced to germinate by various special treatment. These seeds are said to be dormant or in other words they are in a state of dormancy. Besides the dormancy of seed there is another phenomenon in connection with the germination

of seed, is the nonviability. In that state, seeds are unable to germinate inspite of the special treatment. There are various literatures available in connection with these two states of non-viability and dormancy of leguminous seeds.

#### Dormancy of different species of Tephrosia and related plants

The dormancy of seed is determined by various factors which may be exogenous and endogenous in origin ( Nikoleva, 1969 ). The state of dormancy is classified by various authors and is reviewed by Mayer and Poljakoff-Mayber (1978). It is observed that the dormancy of seed is caused by various factors, like nature of seed coat, temperature, light and inhibitors.

#### Nature of seed coat

The dormancy of seed may be attributed to the seed coat which is hard and does not permit water for imbibition and protrusion of radicle. This is found widespread in different plants under Leguminosae ( Mayer & Polijakoff-Mayber, 1978 ). Sometimes the seed coat is found to be a store house for inhibitory substances. Until the leaching of inhibitors is completed germination of seed is not possible ( Black & Naylor, 1959 ). It also restricts the gaseous exchange required for metabolic activities ( Crocker, 1906; Edwards, 1969 ) and gives mechanical resistance to the growth of embryos ( Mayer & Shain, 1974; Pradhan, 1982 ). The role of seed coat on dormancy of seed may be due to impermeability to water and light in Tephrosia vogellii ( Axentjev, 1930; Pradhan, 1982 ).

Physical factors :

Temperature Requirement

Different seeds have different temperature ranges within which they germinate ( Durand et al., 1976; Khokhlova, 1979; Srivastava et al., 1978; Konar et al., 1978; Agrawal et al., 1980; Grey & Steckel, 1977 ). At a very high and at a very low temperature, germination of all seeds is ceased ( Mayer & Poljakoff-Mayber, 1978 ). Germination does not seem to be enhanced when exposed to a series of temperatures but many seeds require some definite temperature before they are placed at favourable temperatures for germination. There are seeds e.g., T. purpurea pers. which can tolerate upto 90°C for prolonged period of time ( Basu, 1972 ). Tolerance of high temperature has been suggested to be due to storage of seed ( Levitt, 1956; Knapp, 1967 ). In some of the seeds the differential temperature i.e. interaction of low and raised temperature, causes change in the permeability and may require for the growth of different parts of embryo ( Mayer et al., 1978; Crocker & Barton, 1953 ). But in some leguminous seeds of desert area germination is possible only due to fluctuation in diurnal temperatures of the area where the day's temperature alternates in light by exceeding cold ( Barton & Schroeder, 1942; Cohen, 1958; Lehmann & Aichaele, 1931 ). T. vogellii seeds have been reported to germinate at a wide range of temperature starting from 0 to 60°C ( Pradhan, 1982 ).

### Light requirement

The importance of light as a factor for germination has been reviewed in a number of papers ( Ciesler, 1883; Gassner, 1915; Lehmann, 1913 ). Light has been reported to have little effect to cultivated plants as they germinate equally in dark and light ( Weinstraub & Johnston, 1944 ). There are wild seeds which can germinate only in light or only in dark ( Haiiao, 1979 ). For the wild seeds, in natural habitat, seeds fall off and enter the soil or is covered by the leaf litter establishing different light conditions ( Kinzel, 1926 ). Visible range of light specially red light has been noted to promote germination ( Fillippe and Litzens, 1980 ). This was also reported in T. vogellii ( Pradhan, 1982 ). That light is supposed to have affected germination interacting with the external factors like temperature in many species including Tephrosia vogellii has been supported by several authors ( Black, 1969; Pradhan, 1982 ). Unlike other legumes, T. candida DC. seeds are reported to germinate in dark ( Pradhan, 1982 ). That photoperiodic effect may be substituted by temperature in many other plants including T. vogellii has been reported by Ishikawa and Ishikawa, 1960; Naguo et al, 1959; Pradhan, 1982.

### Chemical factors

Various chemical factors that can inhibit or stimulate germination includes various hormones and growth regulators ( Mayer et al, 1978 ). These growth hormones enhance seed germination in many other

plants including Tephrosia vogellii at certain optimum concentration ( Grey et al., 1971; Durand et al., 1976; Sawhney et al., 1978; Thomas et al., 1978; Pradhan & Basu, 1981 ). GA was reported to have acceleratory effect on seed germination ( Kahn et al., 1956 and Lona, 1956 ). It has been found to substitute for light enhancing germination in dark reversing the inhibition of temperature and red light ( Evenari et al., 1958 ). Kinetin ( Miller, 1958 ) and ethylene ( Mayer & Poljakoff-Mayber, 1978 ) have also been known to stimulate seed germination. IAA has been found to be concentration and temperature dependent ( Poljakoff-Mayber, 1958; Mayer et al., 1978 ). ABA has been noted to have inhibitory role on seed germination ( Albretcht et al., 1979; Pradhan and Basu, 1982 ).

Sometimes various secondary metabolites like phenolic acids, flavonoids and coumarins which are inhibitory in action have been found to be present in various plant parts ( Stenlid, 1968; Harborne et al., 1971 ). Of these, caffeic acid has been isolated in high quantity from the seed of Tephrosia purpurea and T. hamiltoni and which is supposed to play a role on the inhibition of seed germination ( Basu, 1977b; 1982 ). Other phenolic compounds like Rutin and Quercetin have been found to enhance or inhibit germination of different seeds depending upon their concentration ( Plotnikova et al., 1968; Mestakov et al., 1971; Henry et al., 1979; Pradhan & Basu, 1982 ). Rutin and Quercetin have been noted to enhance seed germination of Tephrosia vogellii ( Pradhan & Basu, 1980, 1981; 1982; Pradhan, 1982 ) and T. purpurea ( Basu, 1972 ).

Besides growth hormones, simple compounds like potassium nitrate and thiourea have been noted to enhance seed germination ( Konar et al., 1978; Esashi et al., 1979; Pradhan, 1982 ). The ionic composition of  $K^+$  and  $Na^+$  salts has been found to play distinct role during germination behaviour in many other species including Tephrosia vogellii ( Mayer et al., 1978; Striver et al., 1980; Pradhan, 1982 ).

#### Breakage of seed dormancy

In order to break the seed coat dormancy, leguminous seed was subjected to several treatments such as scratching of seed coat ( Duarte et al., 1976 ); periodic alteration of temperatures ( Cohen, 1958 ); alcohol or hydrogen peroxide treatments ( Henrothe & Deville, 1976 ), nitric acid treatment and sulphuric acid treatment in Tephrosia vogellii ( Pradhan & Basu, 1980, 1981; 1982; Pradhan, 1982 ) and in other plants ( Waidyanatha et al., 1976; Duarte et al., 1976; Shabany & Rauhani, 1976; Shukurilaev and Khamdamov, 1976; Bulmasova, 1976; Farnandez & Gladys, 1978; Shafiq, 1979 ).

During stratification of seeds, exposure to low temperature under moist condition effects the balance of endogenous growth substances of seed ( Khudairi, 1956 ). This stratification of seed has been found to have an equal effect as that of GA ( Duarte et al., 1976 ). Application of kinetin sometimes overcome dormancy caused by temperature ( Raynold & Thompson, 1973 ). Thiourea and potassium nitrate have been reported to enhance germination substituting the light and temperature requirements in Tephrosia vogellii ( Pradhan, 1982 ). Auxin and

GA are also noted to have substituting the effect of light in Tephrosia vogellii ( Pradhan, 1982 ) and in other plants ( Evenari et al., 1958 ).

### Non-viability of seeds

Non-viability of various seeds collected from very old herbarium specimens have been studied earlier ( Becquerel, 1934; Turner, 1933 ) and has been well reviewed by several authors ( Roberts, 1972; Mayer & Poljakoff-Mayber, 1978 ). In general, viability is retained best under conditions in which the metabolic activity of seeds is greatly reduced i.e., in low temperature and high CO<sub>2</sub> concentration in addition to other factors ( Mayer & Poljakoff-Mayber, 1978 ).

It is known that seeds generally remain viable for longer period if kept in dry condition. Moisture content was observed to be more critical than temperature ( Griffith, 1942 ). It is possible to predict the expected viability and life span of a given seed if the condition of storage, temperature and moisture content of this seed is known ( Roberts, 1972 ). It is noted that damage of cell membrane of seed is the first deteriorative change as evidenced by an increased leaching of solutes and this has always been accompanied by a fall in viability ( Ching et al., 1959 ). That non viable pea seeds with dead tissue leach more solute than normal seeds have been reported by Mathews & Rogerson (1976). Under accelerated aging, decrease of membranous phospholipid and eventually increased leaching was reported ( Koosta & Harrington, 1969 ). Mayer & Poljakoff-Mayber (1978) are

of the opinion that all chemical or histo-chemical methods devised to test viability are only partially satisfactory. The best correlation has been found to be due to the activity of certain oxidizing enzyme reacting the redox dyes, such as tetrazolium ( Mayer & Poljakoff-Mayber, 1978 ). An X-ray contrast technique has also been used quite successfully to predict seed viability ( Kamra, 1964 ). General chemical changes in seed composition during non-viability are discussed by Owen (1956), Abdul-Baki & Anderson (1972). Some of the intrinsic and extrinsic factors responsible for the loss of viability has been discussed by Roberts (1972).

Biochemical changes in the seeds of *Tephrosia candida* caused by various hormones and regulators during germination

The dry seed which has low percentage of water, is characterised by low rate of metabolism and low rate of respiration ( Mayer et al., 1978 ). When the seeds are imbibed in water, the observable metabolism is the higher rate of respiration and rapid breaking down of reserve food materials is initiated by hydration of protein and transport of these materials from one part to another occurs. This is followed by synthesis of new materials including protein from broken down products ( Oota et al., 1953; Ching, 1966; Palmiano and Juliano, 1972 ). The dry weight during germination is steadily decreased in cotyledons and that it is increased in other parts where root starts growing ( Oota et al., 1953 ). The accumulation of insoluble material is observed with the decrease in soluble materials like sugar, nitrogen, protein and nucleic acids ( Oota et al., 1953 ). In leguminous

plants, the reserve material is broken down with the emergence of radicle and total sugar is increased later on ( Reid, 1971 ). The increase in RNA and DNA contents is reported to occur in plants during germination and is transported from one part to another ( McConnell, 1957 ). The breaking down of reserve food matters and synthesis of proteins, nucleic acids, amino acids and sugars are due to rise in the activity of enzymes specific to these materials ( Bevilacqua, 1955; Albaum & Cohen, 1943; Marshall, 1972 ). The enzyme system for leguminous plants, specially the carbohydrate metabolism is studied by Lechevallier (1960). It has been observed that new compound, that are not present in the seeds of leguminous plants, may be synthesized during seedling growth ( Duperon, 1960 ). The enzymes responsible for metabolism are synthesized and activated during germination ( Eldan & Mayer, 1974 ).

It is well established that growth hormones, regulators and inhibitors are also active during germination ( Mayer et al, 1978 ). Gibberellin which induces hydrolysis of starch was first described by Kirsop & Pollock (1957) and identified by Yomo (1960) and Paleg (1960a, b). It has been found to enter at the first stage of short growth and its concentration was found to increase considerably to a maximum level ( Dworstky et al, 1980 ). When  $GA_3$  is applied exogenously it has been found to hydrolyse starch rapidly and form higher amount of reducing sugar and amino acid ( Paleg, 1962 ). The increase in dry weight, elongation of hypocotyl and synthesis of protein are also observed ( Srivastava et al, 1975; Goyal & Baijal, 1981 ).  $GA_3$  is also reported to increase IAA oxidase activity ( Goyal & Baijal,

1981 ). The mechanism of GA<sub>3</sub> is found to control the synthesis of  $\beta$ -amylase that hydrolyses the starch ( Varner et al., 1965 ). This is found to depend on m-RNA synthesis and could be prevented by inhibitors ( Briggs, 1973 ). The increase in the activity of enzymes preceeded the increase of  $\beta$ -amylase and proteolytic activity ( Pollard, 1969 ). The effect of GA<sub>3</sub> is minimised by cyclic AMP ( Pollard, 1971 ) but some of the effects of GA<sub>3</sub> is independent of protein synthesis. GA<sub>3</sub> has been found to reverse the effect of abscisic acid ( Mayer et al., 1978 ).

Endogenous kinetin (6-furfurylamino purine) is reported to be active during early stage of germination ( Van Staden, 1973 ) but in dormant seed it is found to be in low concentration and ultimately is localized in the shoot portion of the seedling ( Dworstky et al., 1980 ) after germination. Kinetin and IAA were noted to elongate cells, increase of cell number and amplify the growth ( Nitsch & Nitsch, 1962b ).

That abscisic acid (ABA) acts as an inhibitor to germination and growth has been established in many higher plants ( Milborrow, 1974; Galson et al., 1974 ) including Tephrosia vogellii ( Pradhan & Basu, 1981; Pradhan, 1982 ). It affects the synthesis of protein ( Chen and Osborne, 1970 ). When ABA is applied exogenously, it was noted to be stored within the seed but it comes out during washing with water and seed behaves normally ( Milborrow, 1974 ). This may account for the inactivation of residual ABA in seed ( Galson et al., 1974 ). ABA may interact with cytokinin and GA<sub>3</sub> ( Ketring, 1973 ). In leguminous seeds, ABA represses the formation of protease activity and amylase formation

( Yomo & Varner, 1973 ).

Though IAA is supposed to be not a controlling factor during seed germination ( Mayer et al., 1978 ) but it is well established that the concentration of endogenous IAA has a great role in the subsequent growth of seedling ( Nitsch & Nitsch, 1962b ). When IAA is exogenously applied in higher concentration, reduction of plant height and accumulation of phenolic acids are observed ( Marigo & Alain, 1979 ). The effect of IAA is found to be controlled through IAA oxidase activity with the cofactors available in plants ( Siegal & Galston, 1967b ) including Tephrosia vogellii ( Pradhan & Basu, 1981; Pradhan, 1982 ).

In the last few decades various chemical substances have been identified to control seed germination and seedling growth. Among these flavonoids, phenolics, coumarins and thiourea are important. Coumarin prevents the falling in quantity of fatty acids during germination while thiourea delays it for a long period ( Poljakoff-Mayber & Mayer, 1955 ) and these two chemicals are reported to effect sugar ( Poljakoff-Mayber, 1952 ), Nitrogen ( Klein, 1955 ) and protein content ( Shain & Mayer, 1965 ) of the seed during germination.

The regulators like phenolic acids and flavonoids have also been reported to show certain role during seed germination and seedling growth ( Mestakov et al., 1971; Stenlid, 1968 ). These phenolics as they are reported to be secondary metabolite ( Harborne, 1971 ) are found to be synthesized from the amino acid phenylalanine in Shikimate pathway ( Casida & Lykkem, 1969 ), resulted to the production of rotenoids ( Crombie et al., 1971 ). According to various

authors ( Steiner, 1972 ) these phenols and flavonoids are not accumulated as end product, rather these are always in a state of continual synthesis, turnover and degradation. This turnover is rapid in floral leaf but slow in the leaves ( Barz et al, 1971 ). Due to dynamic turnover and synthesis of these phenolics and flavonoids, a number of compounds are formed ( Crombie et al, 1971 ) including enzymes ( Koukol & Conn, 1961 ). An inhibition in the synthesis of the phenols and flavonoids involving a chain of reactions will lead to synthesis of another ( McClure, 1968; Galston, 1969 ).

Although, the phenolics have been found to exert effect on growth processes ( Harborne, 1980 ) they may act as protective agents ( Swain, 1977 ). Many phenolics have effect on polar transport of auxins ( Stenlid, 1976a), inhibition of ATP synthesis ( Stenlid, 1970 ) and the control of protoplasmic streaming of root hairs ( Popoviel & Reznik, 1976 ). Rutin has been noted to enhance the germination of seed, antagonize the effect of ABA causing increase of chlorophyll content and dry weight in plant material ( Mestakov et al, 1971 ) and enhance IAA oxidase activity ( Stenlid, 1963, 1968 ). Stenlid and Saddik (1963) evaluated the effect of 20 flavonoids on IAA oxidase preparations from Pisum sativum roots. In every instance, a single 4'-hydroxyl substituent increased enzyme activity while 3'-4'-dihydroxylated flavonoids were inhibitory in functions. A free hydroxyl group at the 7 - position has proved the co-factor nature of the flavonoids. Psenak et al, (1970) reported that simple phenolic glycosides influenced IAA oxidase activity only when glucosidase was present. The reports that flavonoid aglycones are very effective co-factors or inhibitors

of IAA oxidase ( Stenlid and Saddik, 1963 ) support this implication that glycosylation or acylation may be the effective mediators and that glycosylation or acylation may primarily determine the solubility and availability of the flavonoid to the site of IAA oxidase localization within the cell. Monophenols have also been noted by another authors to be acceleratory to IAA oxidase ( Pilet & Gasper, 1968; Tomaszewsky, 1964 ). Pilet & Gasper (1968) also supports that phenols having more than one hydroxy groups are inhibitory to IAA oxidase activity.

The rotenone and rotenoids are restricted in one subfamily papilionaceae of leguminosae ( Harborne, 1980 ). Information in connection with their role in plant metabolism is meagre but the mechanism of rotenone in animal mitochondria is well established. Rotenone inhibits oxidation of glutamate ( Fukami & Tomizawa, 1951, 1956 ), oxidation of pyruvate ( Lindall & Oberg, 1961 ) and activity of NADH oxidase ( Douglas et al., 1968 ). According to Redfearn (1969) rotenoids act in the NADH<sub>2</sub> dehydrogenase of the respiratory chain. It is only because of these activities, rotenoids become potent insecticide and piscicide of natural occurrence.

Ecological significance and biological degradation of phenolic compounds isolated from plant parts of *T. candida* DC.

While it has been difficult to assign a physiological role for the majority of plant phenolics, there is increasing evidence that a considerable number of these substances play an ecological role in plants ( Harborne, 1980 ). The importance of flavonoid pigments con-

tributing to flower and fruit colour, for pollination and seed dispersal was recognised by Charles Darwin and by many naturalists before and since their time. The relationship between flavonoid structure and plant colour has recently been reviewed ( Harborne, 1976 ). There are phenolic chemicals excreted by the plants, which may be autotoxic or affect the growth of other plants in the environment ( Rice, 1974, 1984 ). It has also been found that flavonoids especially tannins have a role on overgrazing by many animal species ( Swain, 1977 ). Phenolics have important function as having with antimicrobial property ( Schonbeck and Schlosser, 1976 ). Phenolic phytoalexins have been recognised and reviewed by Harborne and Ingham (1978).

The phenolic compounds are degraded by bacteria or fungi as sources of energy and carbon ( Barz and Hosel, 1975 ). According to Westlake et al (1961), the species of fungi such as Alternaria, Cephalosporium, Fusarium and Penicillium produced carbon monoxide (CO) after degradation of rutin, quercetrin and quercetin but as the recoveries of CO for the amount of substrate metabolized are in general less than theoretical, more than one pathway may be available to fungi for the degradation of these fungi ( Westlake et al, 1961 ). Pseudomonas although capable for degrading quercetin and its glycoside does not produce CO ( Towers, 1964 ).

Many soil fungi were found to utilise different sources of carbon of hydroxybenzaldehyde, vanillin, syringaldehyde and ferulic acid, benzoic acid, gallic acid, coumaric acid and cinnamic acid and

which are obtained by chemical degradation of lignins ( Henderson and Farmer, 1958 ). Paul (1970) noted that different acids such as coumaric acid, gallic acid, benzoic acid and cinnamic acids are dehydrogenated to produce polymer substances in the humus of soil after degradation by soil microorganisms. Isoflavonoids have less frequently been investigated for microbial assimilation. Most of the results obtained with animals are essentially due to the action of intestinal microflora ( Batterham et al., 1965; 1971; Braden et al., 1967; Shutt and Braden, 1968 ). Bowman et al., (1978) worked on the degradation of rotenone in animal chow. But no detailed work has so far been reported in connection with the degradation of this compound in soil.

#### Detoxification of rotenone and related chemicals

Rapid detoxification of isoflavonoid compound, to reduce their chemical properties has been identified and well reviewed by Dewick (1982) but no information is available in connection with the bio-transformation and detoxification of rotenone and related rotenoids in the soil. The effect of rotenone on isolated mitochondria has been examined in some details ( Wiskich and Day, 1979 ). While its effect on animal mitochondria is well defined, the situation in plant is not so clear. Low concentrations of rotenone ( 1-2 M ) inhibits oxidation of NAD - linked substrates by animal mitochondria completely and specifically ( Gutman et al., 1970; Oberg, 1961 ), its site of action being one of the several non-haem iron centres associated with the internal NADH - dehydrogenase complex ( Ragan & Garland, 1971 ). But

at low concentration no significant effect or malate oxidation by higher plant mitochondria has been observed ( Ikuma & Bonner, 1967; Day and Wiskich, 1974 ). According to Fukami and Nakajima (1971) rotenone and rotenoids have no residual toxicity due to rapid degradation to non-toxic substances.

That the decomposition of soil organic matter is accompanied by microbial action is also well known ( Borner, 1971; McCalla, 1971; Patrick, 1971; Rice, 1974; Wu et al., 1976 ). "Phenolic acids in soil may originate in the decay of lignin by micro-organisms ( Rice, 1974 ). Humic substances are continually incorporated into the soil, and are able to absorb many phenolics, or are transformed into "synthetic humic acid" ( Wang et al., 1971 ). The difficulty of extracting water soluble phytotoxins from the soil is possibly due to the polymerization of phenolic compounds ( Chang, 1983 ). Wang and Li (1977) found that clay minerals can be a catalyst to accelerate the polymerization of proto-catechuic acid, a phenolic acid which is phytotoxic in function. Phytotoxic substances may convert to non-phytotoxic ones when polymerization takes place during the process of humification ( Wang et al., 1971; Chang, 1983 ).

Phytotoxic substances may act in many biological process, i.e. to suppress the mineral uptake by plants, to inhibit cell elongation and cell division, to retard photosynthesis, respiration, and enzymatic activities, resulting in the retardation of plant growth ( Muller et al., 1968, Buchholtoz, 1971; Rice, 1974; Chou and Chiou, 1979 ).

Allelopathy and plant growth with special emphasis on its ecological significance

In 1832, de Candolle pointed out that waste metabolites from higher plants may suppress the growth of the plant itself as well as others. This phenomenon of a detrimental effect of one plant on another mediated by toxic substances is termed allelopathy ( Molisch, 1937 ) and appears ubiquitously in many agricultural and natural vegetations ( Curtis and Cottam, 1950; Muller, 1966, 1969, 1974; McPherson and Muller, 1969; Grodzinsky, 1971; Patrick, 1971; Chou and Muller, 1971; Wittaker and Feeny, 1971; Borner, 1971; Rice, 1974 and Chou & Chung, 1974 ). Since the 1900's allelopathic study has concentrated on crops growing in cultivated lands where reduction of agricultural productivity had been primarily due to a continuous monoculturing system ( Turkey, 1971; Holm, 1971; McCalla, 1971; Wang, et al., 1971; Chou & Young, 1975; Chou and Lin, 1976; Chou and Patrick, 1976 ). In 1966, Muller introduced the allelopathic concept into the field of plant ecology, thereby provided a new approach for plant ecologists to investigate the fundamental process of the dominant vegetation formed. Muller (1970, 1974) also elucidated the allelopathic mechanism for a dominant vegetation. The release of toxic metabolites into the environment takes place by means of volatilization, root exudation, decomposition of plant residues and leaching. The phytotoxic substances released into the environment carry out many important biological activities ( Muller et al., 1968; Muller, 1974; Rice, 1974 ). In the last decade allelopathic research has been intensively conducted in the United States, specially in California and in Oklahoma, where both states

belong to a semi-arid climatic region. Nevertheless, little information regarding allelopathic dominance is known from the semi humid or sub-humid zone areas ( Chang, 1983 ).

According to Chou and Chen (1976), among 25 woody plants studied, six phytotoxic phenolics and five unknown compounds were found to be widely distributed in these plants. They are ferulic, trans-p-coumaric, vanillic, syringic, p-hydroxybenzoic, and o-hydroxy phenylacetic acids. The phytotoxic plants are included in the genera of Sirocalamus, Sinobamboosa, Ficus, Glochidion, and Eucalyptus.