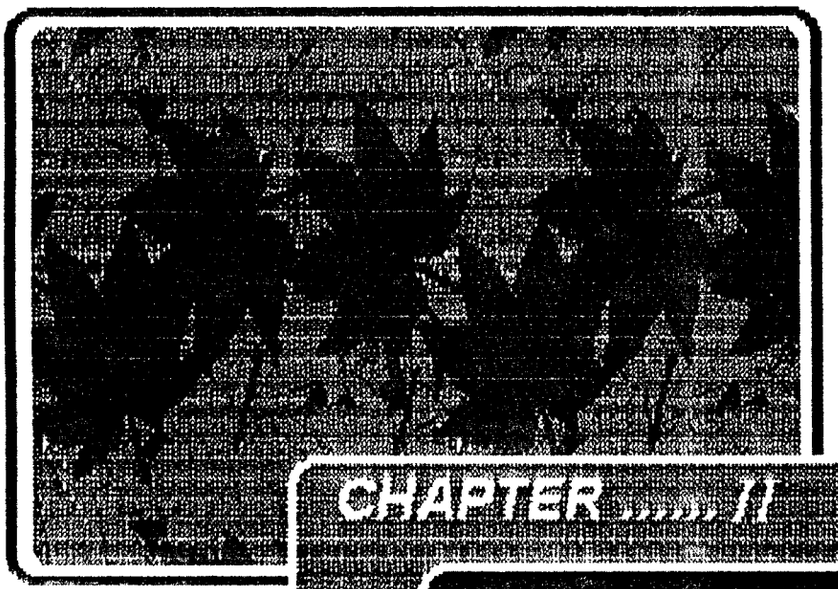

CHAPTER II

Materials and Methods.

1) MATERIALS

The coastal area of Konkan is rich in mangrove vegetation. Ratnagiri is situated at 17° Ø′ north and 73° 3′ east. The river Kajali meets the Arabian sea at Bhatye. The Bhatye creek upto 1Ø km from the sea shore is surrounded by patches of mangroves vegetation on both the sides. Hence Bhatye creek was surveyed for the presence of Caesalpinia crista L. The spots from Pomendi-Someshwar and Kolambe villages 9 and 6 kms away from sea-shore respectively were selected as the study sites rich in Caesalpinia crista fig. 3a. At both the study sites along with Caesalpinia crista other mangroves and mangrove associates like Rhizophora mucronata, Sonneratia acida, Acanthus ilicifolius Fig. 2a, Avicennia marina, Aegiceras corniculatum, Derris trifoliata Fig.2b, Pongamia pinnata, Clerodendrum inerme, Acrosticum aureum etc. were prominently noticed.

The identification of Caesalpinia crista L. was confirmed with the help of key adopted by Hattink [1974] and the taxonomist Dr. M.K.Vasudeva Rao. of Botanical Survey of India Pune. The study sites were visited repeatedly during different seasons to observe the phenological behavior of C.crista such as flowering, pod development, seed production, seed germination, leaf senescence & association with other plants.

For the ecophysiological studies of C. crista collections were made periodically during the year, first

Fig. 1a - Caesalpinia crista L.
Flowering and fruiting twig.

Fig. 1b - Caesalpinia crista L.
Habitat.

Fig. 2b - Caesalpinia crista L.
Plants in association with Derris trifoliata.

Fig. 2a - Caesalpinia crista L.
Plants in association with Acanthus ilicifolius.

during monsoon [August] when the salinity of substratum was least, second during winter [December] and third during summer [May] when the salinity was highest. The water and soil samples were collected for habitat analysis. The seeds of Caesalpinia crista were collected during December - January and in May for germination studies and for seed analysis. The plant material collected was used for organic and inorganic quantitative estimation.

2) **Methods**

A> **Habitat analysis-**

a) **Soil analysis** - The soil samples were collected from the root zones of C. crista seasonally during monsoon, winter and summer season. Soil samples from five different sites were collected and pooled to obtain the soil sample for analysis. The soil samples were air dried and then oven dried for analysis.

i>Electrical Conductivity and pH

For electrical conductivity and pH determination oven dried soil was sieved through 72 number sieve. 5g soil was added in 25 ml distilled water and kept overnight. On second day after filtering the soil extract, the electrical conductivity and pH, were recorded on Elico MHOS pH meter PE-133 and Elico pH meter LI-1ØT respectively.

ii>Inorganic Constituents from soil samples

Soil extracts were prepared for ion determination

as follows.

Soil sample was leached for exchangeable cation and anions by placing 5 g of dry powdered soil sample on a filter paper. The sample was flushed 4 times with 25 ml of normal neutral ammonium acetate. The soil sample was allowed to drain before the next flush was applied. The normal neutral ammonium acetate was prepared by adding 57.5 ml of glacial acetic acid to 8ØØ ml of distilled water and 65 ml of conc. NH_4OH was added. The pH was adjusted to 7.Ø with NH_4OH.

The soil leachate was evaporated to dryness and the dry residue was dissolved in dil. HCl. It was then filtered into a 1ØØ ml volumetric flask and made upto the mark.

This acid digest was used as a source of Na^+ and K^+ estimation, flame photometerically. The elements Mn^{2+}, Co^{2+}, Zn^{2+} and Ca^{2+} from the acid digest were estimated with the help of atomic absorption Spectrophotometer (Perkin-Elmer 3Ø3Ø).

P^{5+} was estimated colorimetrically following the method of Sekine et al (1965). Mg^{2+} was estimated according to the method of Drosdoff and Nearpass (1948) colorimetrically. Fe^{2+} was estimated according to the method of Durie et al (1965). The details of these methods are given in the section C a of this chapter. Chloride from the same soil sample was determined from the extract prepared in

distilled water by titrating against $AgNO_3$ (USDA Book No.60).

b) Water Analysis

Creek water flowing over the soil around C.crista at high tides and draining afterwards was collected in plastic bottles during monsoon, winter and summer season and was used for analysis. pH and electrical conductivity of the water samples were recorded using pH meter Elico MHOS pH meter PE-133 and Elico pH meter LI - 10 T respectively.

Inorganic constituents from water samples.

Na+ and K+ contents from the water samples were estimated using flame photometer. The elements Mn^{2+}, Co^{2+}, Zn^{2+} and Ca^{2+} were estimated with the help of atomic absorption spectrophotometer. P was estimeted following the method of Sekine et al (1965), Mg^{2+} was estimated according to the colorimatric method of Drosdoff and Nearpass (1948). Fe^{2+} was estimated according to the method of Durie et al. (1965). The details of these methods are given in the section C a of this chapter. Chloride from the water sample was estimated by titrating against AgNO (USDA Book No. 60)

B) Germination studies.-

While conducting germination studies, characters of the disseminules and seeds were studied first. Buoyancy test of pods and germinability of the seeds were conducted. Germination under saline conditions was also studied. For all

these studies naturally dried pods were collected from the plants during December-January and in May. They were air dried for one month before the experiments were set up.

a) **Characters of Disseminules.**

Disseminules of Caesalpinia crista are the pods. Density, buoyancy of the pods and germinability of the seeds were examined according to the method of Nakanishi (1988). To estimate density dry weight and volume of a single pod were measured using single pan balance and standard hydrometer respectively. Average density of pod was determined by taking mean of density values of 25 pods.

Buoyancy was determined as follows -

100 pods were placed on the surface of natural sea water in a plastic container. The number of pods that sank to the bottom of the container was counted every 10 days.

For germinability determination pods were kept in separate plastic containers containing sea water for one month and two months respectively. After one month and two months period, 10 pods were taken out from each container and throughly washed with fresh water. 10 dry pods were taken separately as control. Seeds were removed and sown in plastic containers containing acid free silica sand. The sand was watered with tap water frequently. The number of seedlings emerging was counted every fifth day.

b) Seed Characters and Seedling Growth.

Under natural conditions, seeds of U. orista germinate within the pods and the seedling emerge after decaying of the pod wall. This takes about 2 to 6 months after shedding of the pods depending upon the time of pod maturation. To save the time required for the decaying of pod wall, the seeds were separated from the pods manually and were used for germination studies whenever needed.

Seeds were removed from the dry pods and seed characters were studied. Their maximum lengths and breadths were measured in cm. Their maximum thickness was measured with micrometer. Weight of seeds was determined to calculate 100 seed weight. Then the seeds were kept in oven at 60°c for drying till constant weight was obtained and then the weight of dry seeds was recorded to calculate moisture percentage.

On the basis ofseed characters, two types of seeds were separated and sown separately in the plastic pots containing acid free silica sand. The pots were supplied with half- dilute Hoagland nutrient solution and tap water alternately at one day interval. Number of seeds germinated was recorded and germination percentage was calculated.

The seedlings were allowed to grow for four months in the same pot and then uprooted for the study of various growth parameters. Their root length, shoot length, number of leaves and total number of leaflets were recorded. Biomass

was recorded in terms of fresh weight of the seedling in grams. The above experiments were performed in triplicate.

C) **Seed Germination under Saline Condition.**

Large seeds were sown in the pots containing acid free silica sand. Different salinities like Ø% control, Ø.25%, Ø.5Ø%, Ø.75%, 1.Ø%, 2.Ø% etc upto 5%, NaCl were supplied for separate pots along with half strength Hoagland nutrient solution. Salinity treatment was altered with one litre fresh water at one day interval so as to avoid excessive accumulation of salt, due to evaporation of water. There were two replicates of each salinity treatment. Germination percentage and the time required for germination was recorded.

C) **PLANT ANALYSIS**

a) Analysis of Ingorganc Constituents.

The plant material collected during monsoon, winter and summer seasons was immediately brought to the laboratory.

The different plant parts viz. leaves, rachis, stem and roots were separated, cut into small pieces and kept in an oven at 6Ø°C for drying, till constant dry weight was obtained. Seeds, green and senescent leaves collected during summer season were also used for their inorganic constituent analysis. The dried plant parts were finely powdered and used for further analysis.

Different inorganic constituents were estimeted from the acid digest of the powdered plant material.

Preparation of acid digest - It was carried out according to the method of Toth et al., (1948).

500 mg of oven dried powdered material was transferred to 150 ml capacity beaker to which 20 ml concentrated HNO_3 was added. The beaker was covered with watch glass and was kept till primary reactions subsided. It was then heated slowly on hot plate to dissolve solid particles. After cooling to room temperature, 10 ml of perchloric acid (60%) was added to it and mixed throughly.

It was then heated strongly until about 2-3 ml clear and colourless solution was obtained. It was then cooled and transferred to 100 ml capacity volumetric flask, diluted to 100 ml with distilled water and kept overnight. Next day it was filtered through dry Whatman No. 44 filter paper and the filtrate was used as the source of different inorganic constituents.

i) Sodium and Potassium

Inorganic constituents like sodium and potassium were estimated flame photometrically.

ii) Magnesium

Quantitative microdetermination of magnesium was carried out according to the method of Drosdoff and Nearpass (1948).

5 ml acid digest was taken into a 50 ml volumetric flask. To it 1 ml hydroxylamine hydrochloride solution was added which was followed by 5 ml starch compensating solution and 1 ml thiazole yellow. Finally after through mixing 5 ml of 2.5 N NaOH was added. Volume was then made up to 50 ml by adding distilled water and allowed to stand for 30 min. The colour intensity was read on Spectronic -20 at 525 nm.

Reagent blank and standard magnesium curve were prepared in the same manner by using different concentrations of standard magnesium soution (100 ppm standard magnesium solution was made of $MgSO_4$). Calibration curve was prepared by taking different concentrations. With the help of standard curve the amount of magnesium in the plant material was calculated.

The reagents used in this method were prepared as follows.-

1) Hydroxylamine hydrochloride solution - 25g hydroxylamine hydrochloride dissolved in 250 ml distilled water and made the final volume 500 ml in volumetric flask.

2) starch compensating solution - It was prepared by mixing starch solution and compensating solution in equal proportion. For the preparation of starch solution 2g soluble starch was made to a thin paste with distilled water. The paste was poured in 100 ml boling water with stirring, cooled and filtered. This solution was prepared fresh. Compensating

solution was made by dissolving 3.7g calcium chloride, Ø.74g aluminium sulphate, Ø.36g sodium phospate in 5ØØ ml distilled water containing 1Ø ml concentrated HCl in a litre volumetric flask and the volume was made 1 litre.

3) Thiazole yellow- Ø.1 g thiazole yellow was dissolved in 5Ø ml distilled water and made the volume upto 1ØØ ml finally.

iii) **Phosphorus**

Phosphorus content was estimated colorimetrically according to Sekine et al., (1965).

To 1 ml of acid digest in a test tube 2 ml of 2 N HNO3 was added followed by 1 ml of molybdate-vanadate reagent(1.25g of ammonium vanadate in 5ØØ ml 1 N HNO3 and 25g ammonium molybdate in 5ØØ ml distilled water mixed in equal volume at the time of experiment.). Volume was made to 1Ø ml with distilled water. The ingradients were mixed well and allowed to react for 2Ø minutes. After 2Ø mins. colour intensity was measured at 42Ø nm using a reaction blank containing no phosophorus on Spectronic -2Ø.

Calibration curve of standard phosphorus was prepared from standard phosphorus solution (Ø.11Øg KH_2PO_4/lit.= Ø.ØØ25 mg P^{5+}/ml) taking different concentrations Ø.ØØ25, Ø.Ø5, Ø.Ø75,Ø.1 and Ø.15 mg P^{5+}. All other chemicals added were similar as described above. With the help of standard curve the amount of phosphorus in the plant material

was calculated.

iv) **Calcium, Manganese , Zinc and Copper**

Calcium, manganese, zinc, copper were estimated using atomic absorption spectrophotometer (Perkin-Elmer 3Ø3Ø)

v) **Iron**

Iron from the plant material was estimated according to Durie et al., (1965) from acid digest .

In the separate 5Øml Nesseler's tubes 5 ml acid digest, 5 ml distilled water for blank and different concentrations of standard Iron solution were taken. 5 ml hydroxylamine hydrochoride 1Ø % w/v was added in each tube followed by a small square of Cango red paper. Acetate buffer was then added dropwise until the indicator paper just changed from blue to red. (14Ø g sodium acetate was dissolved in water and 6Ø ml glacial acetic acid was added to it. The total volume was made 1 lit. with distilled water). Lastly 4 ml o - phenanthrolin solution (Ø.25 % w/v) was added to the tubes and mixed well. The volume was made to 5Ø ml with distilled water and was allowed to stand for 2 hrs. The absorbance was read at 51Ø nm against reagent blank.

vi) **Chloride.**

Analysis of chloride in plant parts was carried out according to the method of Matsumaru (1991) by hot water extraction method.

Dry and finely powdered plant samples were used for analysis. Ø.5 g sample was kept in a beaker and to it was added 2Ø ml distilled water. It was stirred well and the beaker was kept on hot plate, untill the material inside was boiled for 2 to 3 min. by continuous stirring. It was cooled and filtered through ashless filter paper and volume was made upto 1ØØ ml.

Different concentrations of standard Cl solution and 1Ø ml filtrates were taken in separate test tubes. To each test tube 2 ml of $Hg(SCN)_2$ solution and 4ml $(NH_4)Fe(SO_4)_2$, 12 H_2O solution were added. Then the volume was made upto 25 ml, with distilled water. The reaction mixtures were vigorously shaken for 1Ø minutes to facilitate the reactions. After 1Ø minutes absorbance was read at 48Ø nm. From the standard Cl curve the values of chloride contents from each part of the plant were calculated.

The reagents were prepared as follows.-

1). $Hg(SCN)_2$ solution -

a) 5g $Hg(NO_3)$, H_2O was dissolved in 2ØØ ml of Ø.5 N HNO_3

b) $(NH_4)Fe(SO_4)_2.12\ H_2O$ was dissolved in 1 N.HNO_3 until saturation.

c) KSCN solution - 4g of KSCN dissolved in 1ØØ ml distiled water. 2ØØ ml of solution a, 3 ml of solution b and a drop of solution was mixed together so as to precipitate Hg - $(SCN)_2$. Then the precipitate was filtered and washed with

distilled water. It was then dried in open air. Ø.3g Hg - $(SCN)_2$ powder prepared was dissolved in 1ØØ ml 95% ethyl alcohol.

2) $(NH_4)Fe(SO_4)_2$. 12 H_2O solution - 6g of $(NH_4)Fe(SO_4)_2$. 12 H_2O was dissolved in 1ØØ ml of 6 N HNO_3.

3) Standard Cl (Ø.Ø5Ø mg / ml.) solution. KCl was heated at 11Ø°C for 2 hrs. 2.1Ø3g dried KCl was dissolved in distilled water and volume was made upto 1 litre. This solution was the stock solution (Cl- 1ØØØ mg/ lit.) 5Ø ml stock solution was diluted to 1 lit. to prepare standard Cl solution. (Ø.Ø5Ø mg/ml)

The quantity of the mineral contents is expressed in g/1ØØ g dry weight basis, but whenever needed for comparision these values are converted and expressed in equi./ m^3 plant water basis. The conversion is done using the following formulae.

$$\text{Value in equi./m}^3 \text{ plant water basis} = \frac{1ØØØ(1ØØ-\text{moisture}\%) \times \text{g/1ØØg value} \times \text{Valency}}{\text{Moisture \%} \times \text{Atomic weight or molecular weight.}}$$

b) **Analysis of Organic Constituents :**

Fresh plant material collected was immediately analysed for different organic constituents.

i) **Moisture Percentage**

Moisture percentage in various parts was determined from the difference between fresh weight and dry weight seasonally. (Weight taken after drying the plant material in oven at 6Ø°c till a constant weight was obtained.)

ii) **Chlorophylls**

Chlorophylls were estimated according to the method of Arnon (1949). Fresh leaves were washed with distilled water and blotted to dryness. Ø.5 g leaves were extracted in 8Ø % acetone. The extract was filtered through the Buchner's funnel using Whatman No.1 filter paper. The residue was washed repeatedly with 8Ø% acetone and washings were collected in the same container. Volume of the filtrate was made 1ØØ ml with 8Ø% acetone. The absorbance was read at 663 nm and 645 nm for chlorophyll a and chlorophyll b respectively.

Chlorophylls (mg/1ØØ g fresh tissue) were calculated using the following formulae.

$$\text{Chlorophyll a} = (12.7 \times A_{663}) - (2.69 \times A_{645}) \text{ ----- } x$$

$$\text{Chlorophyll b} = (22.9 \times A_{645}) - (4.68 \times A_{663}) \text{ ----- } Y$$

$$\text{Chlorophylls a/b mg/1ØØg fresh tissue} = \frac{X/Y \times (\text{Volume of extract}) \times 1ØØ}{1ØØØ \times \text{Weight of material(g)}}$$

iii) Organic acids

Titratable acid number (the number of ml. of decinormal NaOH required to neutralise the acid content in 1ØØg of fresh tissue) was estimated according to the method of Thomas and Beevers (1949). Freshly collected leaves were washed with distilled water and blotted to dryness. The leaves were cut into small pieces. 1 g leaf pieces were immersed in boiling water and boiled for half an hour. It was then allowed to cool and filtered through muslin cloth. The volume of the filtrate was made upto 5Ø ml with distilled water. 1Ø ml filtrate was then titrated against N/4Ø NaOH using phenolpthelin indicator which was standradised previously against N/4Ø oxalic acid.

Titratable acid number was calculated by using the following formala.

$$TAN = \frac{\text{ml of oxalic acid taken for titration}}{\text{Oxalic acid titration reading}} \times \frac{\text{Extract titration Reading}}{\text{ml of extract taken for titration}} \times \frac{\text{Volume of extract}}{\text{weight of plant material}} \times \frac{1ØØ}{4}$$

iv) Carbohydrates

Carbohydrates were estimated according to method of Nelson (1944).1 g plant material (leaf, stem, rachis, root.) was extracted with 8Ø % ethanol. The extract was filtered through Buchnner's funnel using Whatman No.1 filter paper. The filtrate was collected and used for the estimation

of sugars while the residue was used for the estimation of strach.

The filtrate was condensed to 3.5 ml on water bath in porcelain dish. 1g potassium oxalate and 1g lead acetate was added then to decolorize the contents. 15 ml distilled water were added to it and filtered through Whatman NO. 1 filter paper. The residue was washed three times with distilled water and final volume of the filtrate was noted. Then used this filtrate for the estimation of redusing sugars. A known volume of this extract was then used for estimation of total sugars(reducing + nonreducing). 2Ø ml of this extract was hydrolysed with 2 ml conc. HCl in 15Ø ml conical flask with a bored cork kept overnight and on second day it was heated on water bath for 1Ø min. It was then cooled, neutralized with sodium carbonate, filtered and used the filtrate was used for total sugar estimation.

The residue left after the alcohlic extraction was transferred to a conical flask with 5Ø ml distilled water and 5 ml conc HCl. Then it was hydrolysed at 15 lbs pressure for half an hour, cooled to room temperature, neutralized with sodinum carbonate and filtered. This filtrate contains reducing sugars produced as a result of hydrolysis of starch. The sugars so available were estimated for the starch present in the tissue.

The requisite quantities of the above filtrates, Ø.5 ml of the extracts prepared for reducing and total sugars

and Ø.1 ml for starch were taken separately in 1Ø ml marked test tube. In another set of test tubes different concentractions (Ø.1 to Ø.5 ml) of standard glucose solutions (Ø.1 mg/ml) were taken. 1ml Somogyi's alkaline copper tartarate reagent (4g $CuSO_4$ $5H_2O$, 24g unhydrous Na_2 Co_3, 12 Na-Ktartarate, 16g $NaHCO_3$ and 18Øg andydrous Na_2SO_4 dissolved in 1ØØØ ml distilled water.) was added to each tube. All the test tubes were then heated on water bath for 1Ø mins and cooled to room temperature. 1ml of arsenomolybate reagent (25g ammonium molyabdate in 45Ø ml distilled water, to which 21 ml concentrated H_2SO_4 was added. To this then added 3g sodium arsenate dissolved in 25 ml distilled water. All ingradients were mixed well and solution was placed in an incubator at 37°C for 48 hr. before use) was added to each reaction mixture. The contents of each test tube were then diluted to 1Ø ml with distilled water. A blank was prepared by the same way but without sugar solution. After 1Ø min. the absorbance of each reaction mixture was read at 56Ø nm.

With the help of standard curve of glucose, the amounts of the carbohydrate fractions were determined.

v) **Total Nitrogen.**

The amount of nitrogen from the plant material was estimated according to the method of Hawk <u>et al.</u> (1948) colorimetrically. Ø.5g oven dried powdered plant material was digested in a Kjeldahl flask with 1:1 diluted sulphuric acid and a pinch of microsalt (a mixture of anhydrous copper

sulphate and potassium sulphate in 1:40 proportion) till the colourless liquid was obtained at the bottom of the flask. Then it was cooled to room temperature and transfered quantitatively to volumetric flask and volume was adjusted to 100 ml with distilled water. It was filtered through dry filter paper next day and the filtrate was used for nitrogen estimation.

In the Nessler's tubes different concentrations of standard ammonium sulphate (0.05mg N_2/ml) like 0.05, 0.1, 0.15, 0.2 and 0.25 are taken. The 2ml filtrate was taken in seprate Nessler's tube. To these test tubes a drop of 8% potassium bisulphate was added and the volume was adjusted to 35ml with distilled water. Then 15 ml of Neseler's reagent was added to each tube (Nesseler's reagent is a mixture of reagent A - 7 g potassum iodide and 10 g mercuric iodide dissolved in 40ml distilled water and reagent B - 10 g sodium hydroxide in 50 ml distilled water, mixed in 4:5 proportion) The colour intensity of the orange brown product, ammonium murcuric iodide produced by the reaction between ammonia liberated free from the sample and the reagent, was measured at 520 nm on Spectronic - 20.

vi) **Free Proline**

The free proline content was estimated colorimetrically from oven dried powdered plant material (leaves, rachis, stem and roots) according to the method of Bates et al.(1973)

Ø.5 g ovendried powdered plant material was homogenised in 1Øml of 3% sulfosalicylic acid. After complete homogenisation, it was filtered through Whatman No. 1 filter paper the volume of the filtrate was recorded. 2 ml acid ninhydrin reagent (prepared by warming 1.25 g ninhydrin in 3Ø ml glacial acetic acid and 2Øml 6 M phosphoric acid with agitation, cooled and stored at 4°c) and 2 ml glacial acetic acid were added to this filtrate. The reaction was allowed to continue for 1 hr. on boiling water bath.

After 1 hr., reaction was terminated in ice bath. 4 ml toluene was added in each test tube and shaked vigorously for 15-2Ø sec. The reaction mixtures were brought to room temperature and the absorbance of toluene chromatophore was read at 52Ø nm using toluene blank on spectronic - 2Ø.

At the same time reaction mixtures for standard curve of proline using different concentration of proline solution (Ø.1 mg / ml proline in 3% sulfosalicylic acid) were prepared in spearate test tubes. With the help of standrd proline curve the amount of free proline from the samples were determined.

vii) **Polyphenols**

Polyphenol centents were estimated by method of Folin and Denis (1915) from the freshly collected leaves, seeds and roots. Leaves, seeds and roots were washed with

distilled water. 1g material was then crushed in 80% acetone, and filtered through Buchnner's funnel under suction using Whatman No.1 filter paper. Residue was washed repeatedly with acetone and volume was adjusted to 50ml. This filtrate was used for estimation of pholyphenols.

In the 50ml. marked Nesseler's tubes, different concentrctions of standard polyphenol solution (tannic acid 0.1mg/ml) 0.5, 1, 2,3, and 4ml etc. in different test tubes and 1ml. filtrate in different test tubes was taken for assay. 10 ml. 20% sodium carbonate soluation, to make the medium alkaline and 2ml. Folin Denis reagent (100g sodium tungstate and 20g phosphomolydic acid dissolved in 200ml distilled water were mixed with 200ml of 25% phosphoric acid and refluxed for about 3 hrs., cooled to room temperature and volume was made 1000ml with distilled water) was added to the test tubes. Finally volume of each test tube was adjusted to 50ml with the help of distilled water and the ingradients were allowed to mix throughly. Absorbance was read at 660 nm on spectronic 20. With the help of standard polyphenol curve the amounts of polyphenol from the samples were calculated.

viii) **Crude lipids.**

The crude lipid content was determined from ovendried seed material. Two clean Corning beakers (150ml) were taken and their weights (W_1) were recorded. Exactly 5g seeds were crushed throughly in mortar with pestle in petroleum ether. The extract was filtered through Buchnner's

funnel using Whatman No.1 filter paper. Then filtrate was kept in preweighed beaker and condensed on water bath till all petroleum ether evaporated and weight of cooled beaker (W_2) was taken. Lipid content was calculated by using following formula.

$$\text{Lipids present in 100g seed material} = \frac{W_2 - W_1}{\text{Weight of the seed material}} \times 100$$

where W_1 = Weight of empty beaker

W_2 = Weight of beaker + extracted lipids.