Chapter 3:

MATERIALS & METHODS
The monocultures of such tree species that are extensively used for the purpose in Goa were chosen for the study. The monocultures of teak, cashew and Australian acacia were the natural choices. Preliminary survey was conducted to select appropriate monoculture plantations of chosen tree species. Care was taken such that the selected monoculture sites were approximately of identical sizes in a range of about 5 ha. A stretch of comparable size of a primary forest within the taluka served as control system for comparison.

Initially, the density, height and the girth (diameter at breast height - dbh) of the trees at all the study sites including the primary forest were recorded by analysing all the trees in 5 randomly laid quadrants of 10m x 10m size at each site.

All the subsequent studies pertaining to plant phenology, extent of undergrowth, rate of litter fall, litter decomposition, variations in the nutrient levels in the litter and soil, biodiversity in terms of insects and bird fauna were carried out regularly at monthly intervals. The study was conducted for 2 complete years from March 2001 to February 2003.

Plant phenology

The status and magnitude of phenological events such as leaf fall, sprouting of new leaves, flowering, fruiting, dehiscence, of the trees and the undergrowth plants were studied and recorded at monthly intervals at all the study sites. Qualitative and semiquantitative estimation on a arbitrary 3 point scale as ‘none’, ‘few’ and ‘many’ (Frankie et al. 1974; Guy et al. 1979; Opler et al. 1980; and Balsubramanian and Bole, 1993) was employed for the purpose.

Undergrowth

The variations in diversity and densities of undergrowth were studied by using quadrant method described by Trivedy, et. al. (1987). The species of herbaceous plants and shrubs in the height range of 0.5m to 3m and
number of individuals of each species were enumerated in five randomly laid quadrants at all the study sites at monthly intervals.

**Litterfall**

The rate of litterfall was assessed by following the procedure described by Pande & Sharma (1993). Five permanent litter plots of 1m x 1m were randomly demarcated in each of the study sites. Initially the dead organic material from these plots was swept off. Thereafter the fallen plant litter was collected at monthly interval to estimate rate of litterfall. The litter was weighed in the field and representative sample was carried to laboratory for determination of dry weight. The mean of these trap contents converted to g dry wt/ m² was taken as the quantum of litterfall for the month. The cumulative total of 12 months of the year was considered as the annual litter production.

**Litter decomposition**

Rate of litter decomposition was estimated by litter bag technique (Olson, 1963). Litter bags (35x25 cm) were made of nylon mesh (1mm). The sides of these bags were stitched firmly with synthetic thread at intervals of 2 cm consecutively leaving 2 cm gaps in between. In the month of March during both the years freshly fallen leaf litter was collected and 25g litter was placed in each bag. The litterbags were tied with a rope to the base of the trees and laid on the ground. A total of 75 bags were laid in each site. Five litterbags each were recovered at monthly interval from every site. Immediately after recovery, the litterbags were placed individually in polyethylene bags and transported to the laboratory. The recovered material was subjected to careful washing under running tap water to separate soil particles and any other extraneous material. It was dried at 70°C for 24 hours and weighed to find mass lost. The contents were pulverized using electrical mixer, sieved through 0.5mm mesh screen and stored in plastic airtight bottles for further analysis. Percentage of mass lost and
decomposition constants were calculated as per the formulae employed by earlier workers (Olson, 1963; Maheshwaran and Gunatileke, 1988)

\[
\% \text{ Mass lost} = \frac{(M_1 - M_2)}{M_1}
\]

where, 
\(M_1\) = Initial mass in litterbag 
\(M_2\) = Final mass in litterbag

Decomposition constant: \(\frac{X}{X_0} = e^{-k}\)

Where, \(X_0\) = Original weight of the litter in the bag 
\(X\) = weight of the remaining litter at the end of one year. 
\(e\) = the base of natural log. 
\(k\) = Constant

**Litter nutrients**

The total nitrogen, phosphorous, potassium and organic carbon of the litter remnants were analyzed by Kjeldahl method, spectrophotometry, flame photometry and Walkley Black method respectively as per the procedures outlined by Saxena, (1987) and Trivedy et al, (1987).

**Nitrogen**

To 100g litter sample, 5 ml of concentrated H\(_2\)SO\(_4\) and 100g digestion catalyst (1:8:1 CuSO\(_4\), K\(_2\)SO\(_4\) & SiO\(_2\)) were added. Digestion was continued till the content turned apple green. The digest was allowed to cool, 75 ml distilled water was added, mixed thoroughly and supernatant was transferred to the kjeldahl flask. The content was mixed with 60 ml of 40 % NaOH and boiled. The condensate was collected in 20 ml boric acid containing methyl red and bromocresol green. On absorption of nitrogen the indicator turned blue. This was titrated against 0.01 N HCl until the colour changed to light brown. Blank was run using the same procedure and % nitrogen was calculated using the formula given below.
\[
    \% N = (T_1 - T_2) \times \frac{N \times V_{HCl} \times 1.4}{W}
\]

Where
- \( T_1 = \) vol. of titrant used against sample (ml)
- \( T_2 = \) vol. of titrant used against blank (ml)
- \( N = \) Normality of titrant
- \( W = \) weight of litter sample (g)

**Phosphorous**

For estimation of phosphorous in litter, 100 mg of dried, ground litter sample was taken in a porcelain basin; 5ml of 0.1N magnesium nitrate solution was added. The mixture was heated at low temperature till dryness and later ashed in a muffle furnace at 500 - 550°C for 2 hrs. On cooling 5ml of 50% HCl was added to it. This was filtered and was made to 100 ml. To 10 ml of the filtrate, 0.4ml ammonium molybdate and 1 drop of stannous chloride were added, which gave it blue colour. The optical density (OD) was read at 690 nm after 5 minutes and before 12 minutes. OD was compared with the standard curve of phosphorus and % phosphorous was calculated using the following formula.

\[
    P\% = \frac{\text{mg P/L of ash solution} \times V}{1000 \times S}
\]

where
- \( V = \) total volume of ash solution made (100ml)
- \( S = \) wt of the plant material used (100mg)

**Potassium**

Potassium in litter was estimated by flamephotometric method. 1g litter powder was ignited at 500°C for 2 hours in muffle furnace. It was allowed to cool and later 5 ml of 50% HCl was added. This was heated at low temperature for 15 minutes, and then 1ml of nitric acid was added and was evaporated till dryness. Heating was continued for 1 hour. Residue was dissolved in 1ml of 50% HCl, some water was added and solution was warmed for complete dissolution. The solution was filtered. The filtrate was collected and the total volume was made-up to 100 ml by addition of distilled
water and this was used as sample solution. This sample solution was allowed to aspirate by flamephotometer and optical density was recorded, concentration was determined by using standard curve. Percentage of potassium was calculated using the formula mentioned below.

\[
\%K = \frac{mg\text{ K/L of ash solution} \times V}{10000 \times S}
\]

where, \( V \) = Total volume of ash solution (100 ml)
\( S \) = wt of litter powder in g (1g)

**Carbon**

Carbon in leaf litter was estimated using Walkley & Black method. To 100 mg litter sample, 10 ml of potassium dichromate and 20 ml concentrated sulphuric acid were added. This was allowed to stand for 30 minutes and later it was diluted by addition of 200 ml distilled water. Further 10 ml phosphoric acid, 0.2g of sodium flouride and 1ml of dilphenylamine indicator was added to obtain bluish colour. To measure the unutilized potassium dichromate, the solution was titrated against 0.5 N Ferrous ammonium sulphate, so as to obtain brilliant green colour as the end point. Simultaneously a blank was run using the same procedure and % carbon was calculated.

\[
\%\text{ Carbon} = \frac{6.791 \times \frac{1-T_1}{T_2}}{W \times 1.724}
\]

Where, \( W \) = weight of litter (g)
\( T_1 \) = vol. of titrant used against sample
\( T_2 \) = vol. of titrant used against blank

**Soil nutrients**

At random five sampling sites were selected in each plantation, soil samples were collected with the help of auger to the depth of 15 cm from the top. The samples were dried at 70°C for 24 hrs, ground with mortar and pestle,
sieved through 2 mm screen and preserved in airtight container for analysis of nitrogen, phosphorus, potassium and carbon.

**Nitrogen**

Kjeldahl method was used for estimation of nitrogen in soil. 25ml distilled water was added to 10 gm soil sample and then 20 g digestion catalyst (containing copper sulphate, mercuric oxide, selenium and sodium sulphate) was mixed. To this mixture 35 ml concentrated sulphuric acid was added, the content was heated at low temperature till frothing stopped, heating was continued at high temperature till contents turned to apple green, digestion was continued further for 1 hr. Digest was allowed to cool, 100 ml distilled water was added, mixed thoroughly and supernatant was added to the Kjeldahl flask. Four to five washings with 50 ml distilled water were carried out and washings were transferred in the same flask, leaving behind as much soil as possible. 130 ml of 40 % NaOH was added to the content. The content was mixed thoroughly and distillation was commenced. 150 ml was collected in 25 ml boric acid and mixed indicator, further the blue colour content was titrated against 0.1 N HCl, until the colour changed to light brown. Percentage nitrogen in soil was calculated using formula given earlier in connection with litter nitrogen.

**Phosphorous**

Soil phosphorous was estimated using spectrophotometric method. Suspension of 1 mg soil and 200 ml H₂SO₄ (0.02 N) was stirred for ½ hour on magnetic stirrer. The solution was filtered, 10 ml filtrate was collected which was further processed as per the procedure described for phosphorous analysis of litter. Phosphorus percentage was calculated using the formula given below.

\[
\%P = \frac{mg \ P/L \text{ in soil solution}}{50}
\]
Potassium

Potassium in soil was estimated by using flame photometer. Soil sample of 50 g of soil was stirred on magnetic stirrer with 40% ethyl alcohol. The suspension was filtered after allowing it to stand for 10 minutes. Further the residue was washed with 40% ethyl alcohol and finally with absolute alcohol. To the residue 100 ml ammonium acetate solution was added and was kept overnight. The filtered supernatant was aspirated on flame photometer and the O.D was obtained. The concentration of K was estimated using standard curve

\[
\% K = \frac{mg \text{ K}\text{IL of soil extract} \times V}{1000 \times S}
\]

where, \( V \) = Total volume of solution (100 ml)
\( S \) = wt of soil in g

Carbon

The carbon content in the soil sample was estimated by Walkley and Black method described earlier. For this 0.5g soil sample was used instead of 100mg sample in case of litter analysis.

INSECT FAUNA

Ground level and above ground level insects were collected using pitfall traps and net sweeps (Borror et. al. 1981) respectively. Mid of every month, in randomly chosen 5 quadrats of 1x1m, a pitfall trap each was laid at all the four sites. PET bottles of 11 x 6 cm size constituted the pitfall traps. Formaldehyde (4%) was used as the killing agent. Pitfalls of adequate depth were dug and traps containing 50 ml of 4% formaldehyde each were laid and kept for 48 hrs. Captured insects were transferred to plastic vials and were taken to the laboratory. In the laboratory the insects were identified, enumerated and were preserved in 70% isopropyl alcohol.

Sweep net made up of muslin cloth with 40cm diameter rim, and 1m long handle was used to collect the above the ground level insects. At every study site in a predetermined plot of 20mx20m, 100 sweeps were made
sweeping the net at constant speed covering the whole area. This was done on monthly basis. The insects collected in the net were pushed to the bottom of the net and the part of the net was inserted in the killing bottle, which was covered until the insects were stunned. Chloroform was used as the killing agent. The pieces of vegetation and other debris were separated. The catch was collected in pvc vials and carried to the laboratory. The insects were identified using standard taxonomic keys (Bingham, 1903; Bolton, 1974 & 1975; Borror et al 1981), enumerated and preserved. For the purpose of comparison the total collection of every month at a site by both the type of efforts was treated as the sample and recorded as individuals per monthly cumulative effort (ind /mce).

Avifauna

Initial survey of birds was made in the plantations. The birds were observed with 12 X 25 binoculars and identified using standard field guides (Ali, 1996; Grimmett et. al. 1998). A preliminary checklist was thus prepared and used for further census work. The new species sighted during the course of study were added to the list. The common (english) names and scientific nomenclature of birds has been adopted from (Manakadan & Pittie, 2000)

The avifaunal diversity and abundance was studied by Encounter Rate Method (Bibby et. al. 1992; Javed and Kaul, 2002). At every study site a peripheral 20m wide belt was demarcated just inside the boundary. Two 1km long, predetermined line transects were laid. One was located in the peripheral area by the edge of every plantation and the other was located inside/centre of plantation. The bird census were conducted by walking on both these fixed transects at a speed of 1 km/hr. The morning hours i.e. 7.00 to 9.00 a.m. were utilised for the purpose. The records of the 2 transects were separately maintained to analyse edge effect if any. The pooled data of both the transects at each site was considered for working out the monthly picture at every site. During the year 2001-02 a single set of census data was gathered at every site. With an intention to obtain better accuracy, during the second year of study, i.e. 2002-03, 2 sets of census data were
gathered with a gap of 3 days, in every month at each site. The observed birds were enumerated on field log sheets. The details such as residential status, affiliation to feeding guilds, etc. of the sighted birds for the further analysis were derived from standard literature (Ali & Ripley, 1989)

**Habitat utilization**

During every field visit, whenever a bird or flocks were sighted, records were maintained as to the act in which they were involved at the time of their sightings. Though all major activities of the birds such as resting, roosting foraging and breeding were considered for such a study, emphasis was laid on the latter two activities, they being of primary consideration.

**Breeding**

During every visit, the study sites were carefully scanned for signs of breeding activities of birds such as courtship calls, territorial defense, pairing, ferrying of the nest building material or food, the active/ abandoned nests, and the fledglings.

Whenever a nest was sighted, the species of host plant, its actual location on the host plant, height of the nest from the ground level, nest architecture etc were recorded.

The breeding bird species was identified with relative ease, if the nest sighted was in active state. In such an instance, involvement of single or both the sexes in parental duties was ascertained. In case of abandoned nests, if identification of breeding bird was not possible based on earlier experience, the nest was collected and shifted to laboratory for further investigation based on the available literature (Ali & Ripley, 1989)

**Foraging**

If bird(s) were sighted foraging at the study sites, the details such as host plant, foraging location with reference to the forest canopy, feeding guild composition, foraging actions - gleening, probing, aerial capture and the food material were observed and noted.
Statistical Methods
Species indices

The species diversity, evenness and richness for the populations of avifauna were calculated using the formulae given below.

**Shannon-Wiener species Diversity Index**: (Pielou, 1975)

\[
\text{Species Diversity } (H') = -\sum_{i=1}^{S} P_i \ln P_i
\]

where \( P_i \) is the proportion of individuals belonging to the \( i \)th species and \( S \) is the total number of species.

**Species Evenness or Equatability Index**: (Pielou, 1975)

\[
\text{Species Evenness } (J') = \frac{H'}{\ln S}
\]

where \( H' \) is Shannon-Wiener species Diversity index and \( S \) is the number of species.

**Species Richness Index**: (Margalef, 1968)

\[
\text{Species Richness } (SR) = \frac{S-1}{\ln N}
\]

where, \( S \) is the number of species in the population containing \( N \) number of individuals.

**Sorrenson's similarity coefficient**: (Southwood, 1978)

The similarities in between the sites in terms of avifauna was calculated as follows:

\[
\text{Similarity coefficient } (CS) = \frac{2j}{a+b}
\]

where \( a \) and \( b \) are the number of species at two sites and \( j \) is the number of species common to the two sites.
Presentation and Analysis of Data:

Whenever the data was based on replicate samples or more than one reading over a period of time, it is presented as mean of the sample and the range of dispersion in the form of standard error calculated employing the formulae given below.

\[
\text{Mean} = \frac{\sum_{i=1}^{n} x_i}{N}
\]

Where; \( x_i \) = observation for \( i^{th} \) character \\
\( N \) = no. of observations taken.

\[
\text{Standard Deviation} = \sqrt{\frac{\sum (x-x')^2}{N}}
\]

\[
\text{Standard error} = \frac{\text{Standard deviation}}{\sqrt{N}}
\]

The data was subjected to appropriate statistical tests such as Correlation Coefficient, Unpaired Students t-test, One Way ANOVA, Kruskal Wallis or H test, Mann Whitney U test as per the need.

The correlation between the various parameters within the individual site was analyzed using Correlation coefficient.

The variance in a parameter between the 2 years of study within every site were compared using Unpaired Students t-test.

The variations in different parameters between four sites were analyzed using One Way ANOVA or F test.

The seasonal variance in various parameters was analyzed using the Kruskal Wallis H test.

The variations in the parameter studied, if any, between the identical seasons of the two years were analyzed using Mann Whitney U Test.

The statistical Analysis were carried out using SPSS (version 6.1.3 for windows).