MATERIALS AND METHODS

Study area

The study area of plantation *Bambusa bambos* is located at Kallipatty between 11°
28' and 12°E of latitude and 76° 59' and 77° 47’N longitude. Its altitude is 540 m above
MSL. This area has red soil. Soil pH was between 7.4 and 7.8. The maximum soil
nitrogen, phosphorus, potassium, calcium and magnesium were 3800 kg/ha, 360 kg/ha,
3600 kg/ha, 1600 kg/ha and 1800 kg/ha respectively. The mean temperature was 31°C.
Mean annual rainfall was about 600 mm.

Preparation of nursery and planting

Nursery area of 10m x 5m was prepared in the field and filled with a mixture of
soil and sand (3:1). Seedlings, raised by tissue culture techniques were purchased from
Chithode, near Erode, Tamilnadu. When they were about 7 cm in height, seedlings were
removed from their polythene bags, and about 25-30 seedlings were transplanted in one
square metre of raised nursery bed and irrigated 2-3 times a day taking care to avoid
excess saturation. Nursery beds were provided with a coconut thatch to protect the
seedlings from direct sunlight.

Transplantation

The seedlings in the nurseries were carefully uprooted and transplanted to the field
during August (after about 9 months). The seedlings were planted at 6m x 6m spacing in
3 hectare area with a total of 250 seedlings/ha. The transplanted seedlings were irrigated
two hours regularly both in the morning and evening. Weeds were removed from the
plantation area as and when required. After one year, the plantation was adequately
irrigated at 15 days interval. Care was taken to avoid waterlogging and protected against
rodents, grazing and browsing animals.
Production of culms

All transplanted seedlings began to produce rhizomes. From these rhizomes lateral culms were emerging which are the chief causatives for the total biomass yield. The number of culms developed from the rhizome totally constituted to a clump. The productivity of bamboo was assessed by the number of new culms produced annually. At a given site the production of new culms was mostly dependent on the culms of the previous year, the degree of congestion and the clump age.

Growth studies

Counting

The number of clumps and culms presented in the entire study area was counted.

Growth measurement

From each hectare fifteen clumps were selected at random from the sixth year plantation and identified with paint marking. Number of culms presented in these selected fifteen clumps were counted. New culms sprouting from the selected clumps were taken for growth measurements. In each clump, five newly sprouted culms were marked with paint, totalling 150 observations. On alternate days the height of each culm was measured and only after reaching 1.37 m height, the diameter at breast height (DBH) was recorded. All observations were made for the basal area girth, DBH and growth in height on all culms.

Monthly recruitment of culm

From the same study area, another set of fifteen clumps were randomly selected from the sixth year plantation for the assessment of monthly recruitment of culm. Every month, the number of newly sprouted culms and their survival were recorded. The observation continued for full one year.
Pattern of biomass allocation in growing *Bambusa bambos*

The pattern of biomass allocation was studied from the earlier ten selected clumps from which young sprouted bamboo (culm) reached a height of 12 cm. At one week regular interval, these young bamboos were felled and their height was recorded. Each young bamboo after fully differentiated with culm, branch and leaves, the whole unit was felled, and the these components were separated individually. Their fresh weight was determined in the field and all the sub-samples were brought to the laboratory filling in plastic bags. They were oven dried to remove all moisture at 103± 2°C to a constant weight. From moisture free oven-dry weight of these samples, the dry weight of each three component and their total weight was calculated.

**Biomass estimation**

In order to estimate the total biomass in relation to organic productivity fifteen culms were selected randomly from each age groups of plantations. The selected culms were felled to a total of ninety. For economy reasons, rhizome was excavated only from three samples to a total of eighteen. After felling, the total height of each culm, DBH, basal diameter, number of nodes were measured and sub-divided into four main components, leaves, branches, culm (bole) and rhizome. A total of 18 rhizomes 3 from each year plantation were excavated for determination of underground biomass by making a trench around the culm. Fresh weight of the components was estimated in the field and sub-sample from each components was brought to the laboratory in plastic bags. The sub-samples were then oven dried at 103±2°C to a constant weight.

From the oven dry weight of the samples, the total standing biomass (t/ha) of each age group was calculated by multiplying the total number of the bamboos of different ages with the average dry weight of the sample. The annual biomass production (total biomass divided by age) and mean periodic production (the difference between biomass
measurement of two stands in a sequence divided by the age interval) and net primary productivity, was estimated (Kadeba, 1990).

Biochemical analysis

Extraction and estimation of chlorophyll

The chlorophyll was estimated by the method of Arnon 1959. One gram of the fresh sample was homogenized in excess of acetone (100%) in a mortar with pestle. It was filtered through Buchner’s funnel, using Whatman No. 42 filter paper. The extraction was repeated with 80 per cent acetone. The content of mortar was transferred to the Buchner’s funnel and washed with acetone till the brine appeared colourless. The filtrate was pooled and made upto 100 ml in a volumetric flask. From this 5 ml of the solution was transferred to a 50 ml volumetric flask and diluted by making up to the volume with 80 per cent acetone.

Estimation of chlorophyll

The absorbance of 80 per cent acetone extract was measured at 645 and 663 nm using a light path of the one centimeter. The concentration of total chlorophyll, chlorophyll ‘a’ and chlorophyll ‘b’ was calculated using the following formula,

\[
\text{Total chlorophyll mg/g} = \frac{20.2 \ A645 \times 8.02 \ A663}{a \times 1000 \times w} \quad \text{xv}
\]

\[
\text{Chlorophyll ‘a’ (mg/g)} = \frac{12.7 \ A663 - 2.69 \ A645}{a \times 1000 \times w} \quad \text{xv}
\]

\[
\text{Chlorophyll ‘b’ (mg/g)} = \frac{22.9 \ A645 - 4.68 \ A663}{a \times 1000 \times w} \quad \text{xv}
\]

\[a = \text{length of light path in the cell usually 1 cm}\]

\[v = \text{volume of the extract in ml}\]
Carotenoids

The concentration of total carotenoids in the 80 per cent acetone extract was calculated according to MacKinney (1941) using the following formula,

Total carotenoids (µg/ml)

\[ = A_{480} + (0.114 \times A_{663} - 0.638 \times A_{645}) \]

Extraction and estimation of total soluble carbohydrate

Extraction of total soluble carbohydrate

Extraction of total soluble carbohydrate was made by the method of Lee and Toursean (1958).

One gram of plant material was macerated gently with 1.5 of 80 per cent ethanol (w/v) and was extracted over a boiling water for 10 minutes. The ethanol extract was allowed to cool and filtered through whatman No.41 filter paper. The residue was re-extracted with 80 per cent ethanol for four times in succession. The filtrate were combined and washed with petroleum ether until colourless. The pigment free alcohol was evaporated over a boiling water bath and the residue was redissolved in 80 per cent ethanol to a known volume.

Estimation of total soluble carbohydrate

The carbohydrate was measured by the method of Dubois et al. (1951) using known amounts of glucose as standard in spectrophotometer at 625 mm. An aliquot of 1 ml of extract was taken in a test tube to which 8 ml of distilled water and 6 ml of anthrone reagent were added (Anthrone reagent was prepared by dissolving 200 mg of anthrone in 100 ml of concentrated sulfuric acid). A glass marble was kept on the top of each test tube to prevent loss of water by evaporation. The tubes were treated over a boiling water bath for 10 minutes and then cooled to room temperature. The absorbance of a blue green solution was measured at 625 nm using spectrophotometer. From a
standard curve prepared with known amounts of glucose, the amount of total carbohydrates present in each sample was calculated and the results were expressed as mg/g fresh weight of the sample.

**Starch**

Weighed quantity of leaves were cut into small bits, ground vigorously in 80 per cent acetone and centrifuged at 3000 g for 5 minutes. The pellet was reextracted with 80 per cent acetone and centrifuged until the pellet was devoid of green colour. The supernatant residue was taken for starch analysis.

The method of MecReady et al. (1950) was used to determine the soluble starch content. To the residue 6.5 ml perchloric acid and 3.5 ml of water were added. The extract was centrifuged at 3000 x g. The clear pellet was used for the starch estimation. To 0.2 ml of sample, 5 ml of cold anthrone was added and the volume was made upto 10 ml with water. The contents were mixed thoroughly and the absorbance was measured at 600 nm potato starch was used as standard.

**Extraction and estimation of protein**

The protein was estimated by the method of Lowry et al. (1951) using Bouine serum albumin as standard in spectrophotometer at 650 nm. One gram leaf was homogenized in phosphate buffer (0.1 m pH 7.2) and centrifuged at 2000 x g for 30 minutes at 50°C. To the supernatant equal volume of 10 per cent trichloro acetic acid (TCA) was added and allowed to stand for one hour in ice bath. The precipitate obtained was redissolved in phosphate buffer (0.1 m pH 7.2) to estimate the concentration of protein content.

**Reagent A**: 2 per cent sodium carbonate in 0.1 N sodium hydroxide.

**Reagent B**: 0.5 per cent copper sulphate in 1 per cent potassium sodium tartarate

**Reagent C**: Alkaline copper solution, 50 ml of the reagent 'A' was mixed with 1 ml of
the reagent of ‘B’. It was used within a day of preparation. Diluted folin-phenol reagent was prepared by diluting folin-ciocalteu reagent ‘C’ (commercially) with an equal amount of distilled water.

**Estimation of protein**

One ml of the extract and 5 ml of the reagent ‘C’ taken in a test tube and allowed to stand for 10 minutes. Then 1 ml of diluted folin phenol reagent was added and mixed and shaking. The solution was allowed to stand for 30 minutes and the absorbance was determined at 600 nm using spectrophotometer.

**Regression equation**

The regression equation was derived using the method of Whittaker and Woodwell (1968). The data obtained in the productivity study was fitted to the prediction equations. The following linear prediction equations of total yield with one variable (diameter) or with two variables (diameter and height) were computed with their co-efficient of determination ($R^2$).

1. **Regression equation with single independent variable** (simple linear equation):

\[
\begin{align*}
\text{Log } Y &= a + b \text{ log } X_1 \\
\text{Log } Y &= a + b \text{ log } X_2 \\
\text{Log } Y &= a + b \text{ log } X_3 \\
\text{Log } Y &= a + b \text{ log } X_4 \\
\text{Log } Y &= a + b \text{ log } X_5 \\
\text{Log } Y &= a + b \text{ log } X_6
\end{align*}
\]

Where $Y =$ biomass (kg), $X_1 =$ diameter, $X_2 =$ square of diameter and height, $X_3 =$ basal area and height, $X_4 =$ height, $X_5 =$ square of diameter and $X_6 =$ basal area.
2. Regression equation with two independent variables

\[ \log Y = a + b_1 \log X_1 + b_2 \log X_2 \]

Where, \( Y \) = biomass kg, \( X_1 \) = diameter \( X_2 \) = height

3. Multiple regression equation

The following prediction equation of total yield with more independent variables were computed with their co-efficient of determination (R²).

\[ \log y = a + b_1 \log X_1 + b_2 \log X_2 + b_3 \log X_3 + b_4 \log X_4 + b_5 \log X_5 + b_6 \log X_6 \]

Where \( Y \) = biomass (kg) \( X_1 \) = diameter, \( X_2 \) = height \( X_3 \) = square of diameter, \( X_4 \) = diameter and height, \( X_5 \) = basal area and \( X_6 \) = basal area and height.

On the basis of co-efficient of determination (R²) the most suitable prediction equation was selected for the prediction of total above ground yield.

**Litter estimation**

Nine litter plots with a size 1 x 5 cm, were laid down randomly throughout the sample plot. The litter plots were made permanent by enclosing the area with nylon thread, which will last throughout the study period. The contents in the litter plots were taken completely by the end of every month and separated into leaf litter and twig litter. Fresh weight was estimated in the field and sub-samples of each component were taken to the laboratory. The sub-samples were then oven dried at 80°C to a constant weight. From the sample oven dry weight, the total dry weight of the litter was calculated.

**Litter decomposition**

Both freshly fallen and senescent leaves were collected and bagged in polythene bags with 5 mm perforation with a net weight of 50 gram. 50 bags of litter was buried beneath the soil surface by removing one inch surface soil. Five bags of sample was taken every month for a period of one year and the contents were used for nutrient analysis.
Estimation of nutrient elements

I. Nitrogen and phosphorus

Nitrogen and phosphorus was estimated as described by Armstrong et al. (1967) using Technicon Autoanalyzer-II (Gedko International Ltd., U.K.). The sample was digested in a kjeldatherm digestion system KT 408 (Bonn) at 400°C for 3 hours. One gram sample was digested in digestion tube with 2.5 g potassium sulphate and 5 ml. H₂SO₄. After digestion and cooling, digested sample was made upto 50 ml with distilled water and used for analysis of nitrogen and phosphorus.

II. Potassium, calcium and magnesium

The nutrients K, Ca and Mg were analyzed using an Atomic Absorption Spectrophotometer (Perkin Elmer 5000 U.S.A.) after wet digestion of one gram sample with triple acid mixture (10 ml of conc. HNO₃, 4 ml of HClO₄ and 1 ml of conc. HCl). The digested samples were filtered through Whatman No.42 filter paper and made upto 100 ml with distilled water and this solution was stored and used for analysis (Issac and Johnson 1975).

Proximate chemical analysis

The analysis of wood described by W.H. Dore (1931) was followed.

Preparation of the sample

The bamboo is reduced to sawdust by means of electrical saw. The saw dust was sieved initially with 65 meshes and later with 200 meshes per sq.cm. The material retained during the second sieving was called "average wood" and was used for the analysis (the wood used for the analysis was bone-dry).

Procedure

1. Benzene extraction

7.0 gram of sample of average wood was dried in a oven at 100°C. The dried
content was extracted with benzene for 6 hours in Soxhlet, the extract dried at 100°C and weighed.

\[
\text{Benzene extract (\%) = \frac{\text{Extract weight} \times 100}{\text{Bone dry wood weight}}}
\]

2. Alcohol extraction

The residue from the previous operation (Benzene extraction) was extracted for 6 hours with 95 per cent alcohol. The extract dried at a 100°C and weighed.

\[
\text{Alcohol extract (\%) = \frac{\text{Extract weight} \times 100}{\text{Bone dry wood weight}}}
\]

3. Soluble matter in cold water

The residue from the alcohol extraction was dried at 100°C and digested for 24 hours with 400 ml of cold water. It is then filtered through a filtering crucible with fretted disk of coarse porosity G3 and washed. The extract was dried at 100°C for 10-12 hours and weighed.

\[
\text{Cold water extract (\%) = \frac{\text{Extract weight} \times 100}{\text{Bone dry wood weight}}}
\]

4. Soluble matter in 5 per cent NaOH solution

The residue from the soluble matter in cold water is treated with 100 ml. of 5 per cent by weight NaOH solution for 24 hours and the solution filtered through the same filtering crucible with fretted disk of coarse porosity G3. The residue was washed with 100 ml of 10 per cent by vol. acetic acid solution and then with distilled H₂O. The residue at neutral reaction of the filtrate was dried at 100° C for 10 to 12 hours and then weighed.
5. Cross-bevan-cellulose

Two gram of dry material, which has undergone the 4 extractions (1+4), are transferred to the filtering crucible with fretted disk of coarse porosity G3. The filtering crucible was put on vacuum flask with proper ice cooling container. It was washed with warm distilled water, and after having put the ice into the container, chlorine gas flow under slight vacuum was passed through the filtering crucible for 5 minutes (velocity of the flow: 2 bubbles per second). Then the sample was washed 2 to 3 times with a 1 per cent by volume SO₂ solution. The vacuum was then disconnected and the fretted crucible filled up with 1 per cent by volume of soda solution (the material changes color to brown). It was digested with soda during 5 minutes. The vacuum was connected and the chlorine flows again through the filter. This double treatment was repeated until the material contacting the soda remains white. The solid matter was then dried and weighed. Pentosans are determined on the residue.

\[
\text{Extract weight x 100} \\
\text{Alkali extract (\%) = ---------------------} \\
\text{Bone dry wood weight}
\]

\[
\text{Wood theoretic weight} = \frac{\text{wood weight after the four above mentioned extractions x 100}}{100 - \text{sum \% of four extractions}}
\]

\[
\text{Cross Bevan cellulose (\%) =} \frac{\text{Cross Bevan residue weight x100}}{\text{Wood theoretic weight}}
\]

6. Lignin

From the above four extracts fractions one gram sample in each, fraction was placed in four test tubes and treated with 12 ml of concentrated HCl each test tube was closed with a two holed rubber stopper, having an inlet tube going down almost to the bottom and an outlet tube. Through the inlet tube a flow of HCl gas was passed which
was supplied by a Kipp apparatus with NH₄Cl and H₂SO₂. After this stage the test tubes were left closed 24 hours. The contents were mixed with water and filtered off and washed thoroughly until the acid reaction of the filtrate disappeared. Later each sample was dried at 100°C for 8-10 hours and weighed.

\[
\text{Lignin (\%)} = \frac{\text{Extract weight} \times 100}{\text{Wood theoretic weight}}
\]

**Ash**

It was determined on 2 gram of wood

\[
\text{Ash (\%)} = \frac{\text{Ash weight} \times 100}{\% \text{ dry content of wood} \times 2}
\]

**Moisture**

It was determined on 10 gram of wood.

\[
\text{Moisture (\%)} = 100 - \frac{\text{Dry wood weight}}{\text{moist wood weight}} \times 100
\]

**Determination of pentosans**

**Preparation of the sample**

One gram of bone dry wood saw dust is weighed and placed into the distilling flask. Hundred ml of 12 per cent HCl are added through the proper funnel and brought to boiling. After having collected 30 ml of distillate, 30 ml of 12 per cent HCl are added in the distilling flask and thus proceeding until 360 ml of distillate are collected, this amount being certainly sufficient to have all the formed furfural passed into the distillate (generally, all the furfural is contained in the first 300 ml of distillate; however until
furfural is present, a drop of distillate will give a pink color when coming into contact on
filter paper with a drop of aniline reagent). Forty ml of the phloroglucin solution are
added to the distillate, then the volume of the liquid is made up to 400 ml with 12 per
cent HCl. It soon turns greenish-black and furfural-phloroglucide precipitates.
(Hydrochloric phloroglucin solution free from resorting prepared by dissolving 74 g
phloroglucin in 12 per cent by volume HCl, then diluted to 10 liters with 12 per cent HCl.
The possible presence of resorting may be tested as follows: a small quantity of
phloroglucin is dissolved in few drops of acetic anhydride and brought to boiling; at this
moment few drops of concentrated sulfuric acid are added: a violet color indicates the
presence of resorting).

After 16 hours decanting it is tested whether all furfural has precipitated, by
adding a drop of aniline; if pink color is obtained (presence of furfural) more phloroglucin
must be added (Aniline prepared by adding acetic acid or hydrochloric acid drop by drop
to a mixture of equal volumes of aniline and water until a clear solution is obtained).

The precipitate was filtered through a filtering crucible with fretted disk of coarse
porosity 11 G 3 (or 11 G 4) care being taken that the filter is always filled with liquid.
The precipitate is washed with 150 ml of distilled water, dried in oven at 100-150°C for
6 hours, placed into a desiccator until it is cooled and then weighed.

Calculation of the results

The content of pentosans in the sample is calculated from the weight of
furfural-phloroglucide (a) on the basis of the following formula,

When the weight of phloroglucide is under 0.03 g

\[
\text{Furfural} = (a + 0.0052 + 0.0020) \times 0.5170
\]

\[
\text{Pentoses} = (a + 0.0052 + 0.0020) \times 1.0170
\]

\[
\text{Pentosans} = (a + 0.0052 + 0.0020) \times 0.8949
\]
If phloroglucide weight is between 0.03 g and 0.30 g

\[
\text{Furfural} = (a + 0.0052 + 0.0020) \times 0.5185 \\
\text{Pentoses} = (a + 0.0052 + 0.0020) \times 1.0075 \\
\text{Pentosans} = (a + 0.0052 + 0.0020) \times 0.8866 \\
\]

Over 0.30 g

\[
\text{Furfural} = (a + 0.0052 + 0.0020) \times 0.5180 \\
\text{Pentoses} = (a + 0.0052 + 0.0020) \times 1.0026 \\
\text{Pentosans} = (a + 0.0052 + 0.0020) \times 0.8824 \\
\]

The content in percentage is figured from the pentosans content in the sample, by the following formula,

\[
\text{Pentosans (\%)} = \frac{\text{Weight of furfural phloroglycide} + 0.0072 \times 100 \times 0.8866}{\text{Weight of bone dry wood}}
\]

**Note:** The value of pentosans may also be expressed as content of furfural (furfural deriving from hydrolysis). In such a case the possible errors deriving from presence of methyl-pentosans are lowered.

**Pulp and paper characteristics**

To study the pulp and paper characteristics of bamboo, screened chips were collected from summer chipper. They were pulped individually in the laboratory series digester (kamyre design) with 21 per cent of alkali at 172°C for 60 minutes to get a target kappa number (permanganate number) of 27 ± 2. The sulphidity in white liquor was 18 ± 0.5 per cent. The dilution was kept at 1.3. The H factor was maintained at 1300. (The pulping of bamboo can be regulated by the ‘H’ factor which singly represents time and temperature. This concept is useful and should be applied to get more or less the same pulp quality inspite of variations due to unforeseen reasons in temperature or duration of cooking).
The pulps obtained in each case was washed thoroughly. The knots were hand picked, percentage of pulp yield and knots were determined. The pulp was tested for kappa number, Fibre fraction, brightness and bleachability. Bauer McNett classification of fibre of both unbleached and bleached pulps were carried out. Unbleached pulp and bleached pulp were evaluated in ball mill, keeping the ball milling conditions as usual, 3 per cent consistency and 20,000 revolutions. The black liquor obtained in each case was analyzed for total solids, Inorganic-organic ratio, acid insolubles, all sodium salts (Na₂O), free alkali, Swelling Volume Ratio (SVR), Calorific value and viscosity at different concentrations.