Chapter 3
Experimental Site
&MATERIALS AND METHODS
Experimental Site and Materials and Methods

The proposed investigations were carried out on morphological, ecological and physiological behaviour on *Leucaena* variants (S24, S22, S14, S10 and K8) to understand their behaviour contributing to the biomass production and energetics. These variants were from the final evaluation trial where K-8 was used as control.

3.1 The Experimental Site

*i) Geographical situation:* Jhansi lies in part of Uttar Pradesh state and forms a significant district in the Bundelkhand region. Jhansi is situated at 75.35 east longitude and 25.27 north latitude at about 275 m above the mean sea level. Due to its position in the south-West corner of the state it bulges in the Madhya Pradesh which surrounds it from three sides (Fig. 3.1)

*ii) Geology:* The Vindhyan high lands are formed by sedimentary of cuddapah and Vindhyan systems i.e., sand stone, lime stone, shales, conglomerate, quartzite, gneiss and igneous rocks like granite, dolomite and diorite. The upper Vindhyans have massive sand stone with small conglomerate at the base and rest directly on the genesis. The lower Vindhyans intervene between *Bijawar* and upper Vindhyans and consists of sandstone and shale in small out crops. The Bijawar series which succeeds the genesis occupy a narrow strip on south and consist of sandstone limestone and slates.

*iii) Surface relief:* The whole region of Bundelkhand is covered with Vindhyan ranges presenting undulating plains with rocky hills at places or ravines of the river beds running east to west. The extreme southern part is Vindhyan plateau rising in two escarpments, one of which rises to a height of about 90 to 160 m above the plains and is not well defined. The second one rises to an average height of about 300 m and is
- Fig. 3.1: Location of the study site
more clearly defined. The principal rivers that drain the southern tract are Shahjad, Sajnam and Jamini flowing towards north. The north part is drained by Betwa, Pahuj and Yamuna having deep and long stretched ravines. The slope is from south towards north with sharp gradient in the south and gentle in the north (Singh 1971).

iv) Climate: The central situation of Bundelkhand region shows the feature of transitional climate between maritime climate of east coast and tropical continental dry climate of west. The mean rainfall based on 65 years average is 950.4 mm with almost all the months with some rain. The ombrothermic diagram based on 65 year data of rainfall and mean temperature drawn on a scale of P=2T shows four months as wet and the remaining months dry (Fig. 3.2 A). The months of June to September being wet show the peak rainfall exceeding 300 mm during July followed by August with more than 250 mm. September also had rainfall of about 170 mm. This feature of long term average with peak mean temperature of May and minimum of January shows that the area is sub-humid. In one of the studies Hazra (1981) found the area as semi-arid with moisture index from -40 to -60.

During the study period (1995 - 1996) the climatic features show a very erratic situation whereby during both the years four months were totally dry without any rainfall (Fig. 3.2 B and C). During 1995 the total rainfall was 829.9 mm with 42 rainy days while during 1996 it was 952.7 mm on 52 days. Thus there was a net increase of 122.8 mm on the 10 days. The month of July received maximum precipitation during both the years with maximum during 1995. the rain during July was more spread over in 1996 compared to 1995 (18 compared to 15 days). As the trend is, August in the second rainiest month. During 1996 there was 316.6 mm rain during August compared to 211 mm in 1995. The situation was just like July showing higher intensity with higher rainfall in 1996 compared to 1995 with low rainfall and longer spread over. The most interesting feature is 110.57 mm rain on two days during October 1996 compared to no rain during 1995. The following two months were rainless up to December. Rain during
Fig. 3.2: Ombrothermic diagram and climatic parameters during the study period at Jhansi
September was quite low during 1996 compared to 1995. The year 1996 showed five months from June to October with more than 50 mm rain while in 1995 only four months from June to September were rainy.

Jhansi receives very high summer temperature with peak mean maximum during May (43.1 °C during 1995 and 41.8 °C during 1996). The mean minimum temperature was lowest during January (4.8 °C in 1995 compared to 6.8 °C in 1996). The figures show very low variation between maximum and the minimum temperature during July and August during both the years while it was maximum during March - May and November - December. The narrow range of variation during July and August is conducive to fast growth and leaf expansion while the broader range is responsible for reproductive growth and seed setting.

The evaporation demands are maximum in May (13.9 mm/day) and the minimum in January (2.1 mm/day). The evaporation demands in October, November, February, April and May are more than the precipitation (Fig. 3.3). The months with high evaporation demands are also associated with longer duration of bright sunshine hours (8-10.6 hours) and higher wind velocity (4-9.6 km/hour). These factors affect relative humidity and all these factors contribute to the soil moisture, temperature, plant growth and anthesis. A noteworthy feature of Jhansi is also daily peak temperatures during summer months when for a few days to a week the peak temperature may touch beyond 48 °C (during the last week of May or first week of June).

v) Soil: In this tract there are two major soil groups viz, red and black. The red soils are normally coarse, grained upland soil and the black soils are heavy and distributed in low lying areas. These soils are residual in nature and are formed from parent material in situ. The red soils originated from gneiss and granite and some time even from sand stone, while the black soils are formed from lime stone. On the basis of their colour and texture the two soil types are further grouped in to Rakar and Parwa for red
soils and Kabar and Mar for black soil. The experiments were carried out on red soils.

v.i) **Soil characteristics of the site:** The soil characteristics were studied as per the method of Piper (1957) and Jackson (1962). The results are presented in table 3.1.

Table 3.1: Edaphic parameters of the plantation site (pH 6.6 - 6.9, water holding capacity 27 - 29 %, conductivity 31.3 - 51 m mho/cm²).

<table>
<thead>
<tr>
<th>Month</th>
<th>Depth (cm)</th>
<th>Organic carbon (kg/ha)</th>
<th>Available nitrogen (kg/ha)</th>
<th>Available phosphorus (kg/ha)</th>
<th>Available potassium (kg/ha)</th>
<th>Calcium (%)</th>
<th>Magnesium (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept. 95</td>
<td>0 - 15</td>
<td>0.28</td>
<td>265</td>
<td>32.1</td>
<td>224</td>
<td>0.017</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>15 - 30</td>
<td>0.22</td>
<td>207</td>
<td>26.6</td>
<td>196</td>
<td>0.017</td>
<td>0.012</td>
</tr>
<tr>
<td>March 96</td>
<td>0 - 15</td>
<td>0.37</td>
<td>274</td>
<td>32.1</td>
<td>210</td>
<td>0.014</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>15 - 30</td>
<td>0.26</td>
<td>207</td>
<td>27.8</td>
<td>196</td>
<td>0.017</td>
<td>0.015</td>
</tr>
</tbody>
</table>

a) **Soil colour:** The soil of the experimental site was reddish

b) **Soil pH:** The soil pH was taken from the experimental site at 15 cm and 30 cm depths. At 15 cm depth it was 6.7 while at 30 cm depth it was 6.6 indicating its neutral nature.

c) **Soil conductivity:** The conductivity varied from 51.0 to 31.5 m moles /cm³ at 15 and 30 cm.

d) **Water holding capacity:** The water holding capacity was in the range of 27-30% with low values at 30 cm depth.

e) **Soil moisture:** Soil moisture percentage at 15 cm depth was found to be maximum in the month of August while minimum in the month of May. At 30 cm depth maximum soil moisture percentage was found in the month of August and minimum
Fig. 3.3: Rainfall and evaporation pattern at experimental site, Jhansi (1995-96)

Fig. 3.4: Soil moisture at two levels in the *Leucaena leucocephala* plantation (1995-96)
in the months of March and October (Fig. 3.4).

f) **Soil organic carbon**: The soil organic carbon content was found to be maximum in the month of March at both depths. This behaviour indicates utilization and build up of carbon in the soil during cold dry months when the growth activities are dormant.

g) **Soil nitrogen**: Available nitrogen content was high in the month of March at 15 cm depth while at 30 cm depth it was found to be same during both the observation months (Table 3.1).

h) **Soil phosphorus**: Soil phosphorus content was found to be same in both the months while at 30 cm depth it was high in March.

i) **Soil potassium**: Soil potassium content was found to be high in September at 15 cm depth while at 30 cm depth it was found to be same in both months.

j) **Soil calcium**: Calcium was found to be almost same at both the depths in both the months although at 15 cm depth it was less during March.

k) **Soil magnesium**: The magnesium percentage was found to be high in the month of March at both the depths compared to September.

### 3.2 Experimental Materials

The growth and biomass production of selected varieties of *Leucaena leucocephala* has been studied at the Military padao area of the IGFRI experimental farm. It was a sloppy calcareous degraded grassland area where calcium carbonate nodules were quite apparent even on the surface and it was very dry during most parts of the year. The plantations raised during 1989 and coppiced in 1994 were used for the
Plate 2: A general view of *Leucaena leucocephala* plantation.

A) A view of different rows.
B) A view of different blocks.
study of growth, phenology, biomass production, litter production and seed production studies. Three plants of each variety were marked for measuring the growth of height, collar diameter and diameter at breast height (dbh). Number of branches, canopy structure and clean bole height were also recorded. Three plants of each variety were uprooted for recording the biomass production data at one and two year growth. The components were separated into bole, branch, leaf and pod fractions, weighed and finally dried in the hot air oven at 80 °C.

For early growth, morphological and physiological characters of these varieties the pot culture experiments were conducted at nursery/net house of the Division of Plant Physiology & Biochemistry of IGFRI, Jhansi.

3.3 Research Methodology

3.3.1 Litter Collection: Seasonal pattern of leaf fall, twig fall and shedding of other plant parts was studied by collecting the material under the tree every 15 days. Litter traps (1 X 1 m) were placed under the tree in each replication and the variety. The samples were collected, separated manually into leaf, twig, pod flower and other miscellaneous parts, weighed and kept in the oven for recording the dry matter at 80°C.

3.3.2 Seed Collection: Healthy and viable seeds of Leucaena leucocephala variants in uniform shape and size were collected from the plantations. Seeds from individual trees were collected and stored separately.

3.3.3 Raising of Seedlings: The seeds of uniform size of Leucaena were scarified and sown in polythene bags containing garden soil and sand (1:1) in the nursery/pot culture house of Plant Physiology and Biochemistry Division during last week of May. The uniform seedlings of 3-4 leaf stage were transplanted in the porcelain pots (29.5 x 20.5 cm) filled with garden soil, farm yard manure and sand (2:1:1 ratio) Each pot contained
only one plant. The plants were reared regularly as per the standard agricultural practices.

3.3.4 Growth Behaviour and Morphological Studies: To study the growth behaviour, biomass production, physiological and biochemical processes in relation to seasonal growth and age during one year of growth, the uniform seedlings were selected. The pots were arranged randomly in rows and distance between two adjacent rows was maintained at 90 cm to allow better light penetration and also to facilitate inter-cultural operations.

3.3.5 Recording of Morphological Data: For recording the morphological and growth parameters three plants of each variety were uprooted at monthly intervals and the observations on plant height, stem diameter, number of branches, number of leaves, root length, number of nodules, leaf area, fresh and dry weight were recorded. For taking fresh weight the plants were washed properly in running water and blotted to remove extra surface water before weighing. The leaf area of fresh leaf was measured by automatic portable leaf area meter (model LI - 3000, USA) before weighing. The samples of each plant parts were dried in electric oven at 80 °C for 48 hours and then the dry weight was recorded.

3.3.6 Analysis of Growth Data: The different parameters of growth were calculated by using the formulae of Evans (1972) as follows:

i. Relative Growth Rate (RGR):

\[
RGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1} \quad \text{g/g/day}
\]

(Where \(W_2\) and \(W_1\) are dry weights in g at harvest times \(t_2\) and \(t_1\) respectively).
ii. **Root : Shoot Ratio :**

\[
\text{Root: Shoot ratio} = \frac{\text{Mean root dry weight}}{\text{Mean shoot dry weight}}
\]

iii. **Partitioning of Dry Matter :**

The partitioning of dry matter in different plant parts was calculated as:

\[
\% \text{ Dry weight} = \frac{\text{Dry weight of individual part} \times 100}{\text{Total dry weight}}
\]

iv. **Specific Leaf Area (SLA) :**

The specific leaf area is calculated as

\[
\text{SLA} = \frac{\text{Leaf area}}{\text{Leaf weight}} \quad (\text{cm}^2/\text{g})
\]

v. **Specific Leaf Weight (SLW) :**

The specific leaf weight is calculated as:

\[
\text{SLW} = \frac{\text{Leaf dry weight}}{\text{Leaf Area}} \quad (\text{mg/cm}^2)
\]

vi. **Leaf Area Ratio (LAR) :**

The leaf area ratio is calculated as:

\[
\text{LAR} = \frac{\text{Total leaf area of plant}}{\text{Total plant dry weight}} \quad (\text{cm}^2/\text{g})
\]
vii. *Leaf Weight Ratio (LWR)*:

The leaf weight ratio is calculated as:

\[
LWR = \frac{\text{Dry weight of leaf}}{\text{Total plant dry weight}}
\]

3.3.7 *Growth and Biomass Data from the Plantations*: The coppice growth data of plant height and diameter (Collar diameter and diameter at breast height (1.37 m)) were recorded quarterly from the three replications of the *Leucaena leucocephala* varieties in the field. At the end of one year and two years, selected trees were felled from each replication for recording the biomass data (Newbould 1967). Trees were cut in to one meter segments. From each segment the biomass was separated in to bole, branch, leaf, flower and fruit segments and weighed. From the one meter bole segments, further 5 cm disc was cut by saw, the bark and wood was separated, weighed and kept in the oven at 80 °C temperature for the determination of the dry weight. Small samples from other parts viz., branch, leaf, pod were kept for the dry matter determination. The below ground parts were also excavated up to a depth of 1 m. Roots were carefully collected, washed and weighed. Sample was also drawn for the determination of the dry matter. The third year biomass was worked out from the basic growth data through the available production models (Pathak et al. 1987).

3.3.8 *Physiological Observations*: Measurements of photosynthetically active radiation (PAR), air temperature (AT), relative humidity (RH), net photosynthesis (PN), stomatal conductance (CS), intercellular CO\textsubscript{2} concentration (CINT) and transpiration rate (TR) were made in the leaves by using the LI-6250 portable photosynthesis system (LICOR, USA). In each replication fully expanded healthy second and third leaves were used to record the observations. All the measurements were made at ambient CO\textsubscript{2} between 1100 and 1200 hours on a clear sky day. The ratio of PN/TR, PN/CINT were also calculated.
3.3.9 Biochemical Estimations

i. Photosynthetic pigments: Photosynthetic pigments in fresh leaves were estimated by the method of Duxbury & Yantsch (1956) and Comer (1962). One gram leaves were homogenized in 80% acetone and the supernatant was filtered through funnel using filter paper in 50 ml volumetric flask and made volume with acetone. A suitable amount of aliquot was taken in 25 ml volumetric flask and made up to 25 ml with acetone. Concentration was measured by measuring the absorbance of the solution using UVS-119 spectrophotometer at 660 & 640 mu wave length for total chlorophyll, chlorophyll 'a' and chlorophyll 'b' whereas carotene content was measured at 510 & 480 mu. The fractions of chlorophyll were calculated from the absorption values using equation given in A.O.A.C. (1970) and expressed in mg/g of the fresh weight.

Chlorophyll 'a' = 9.93 x O.D. (660 mu) - 0.777 x O.D. (640 mu)

Chlorophyll 'b' = 17.3 x O.D. (640 mu) - 2.8 x O.D. (660 mu)

Total chlorophyll = Chl a + Chl b

Carotene = 7.0 (O.D. at 480 mu) - 1.47 (O.D. at 510 mu)

ii. Nitrate reductase activity: Nitrate reductase activity in fresh leaves was estimated by using methods of Bar-Akiva and Sturbaum (1965) and modified by Kleepet al. (1971). Weighed 0.3 g of fresh leaves and cut into small pieces and placed in 5 ml tubes added 3 ml of 0.2 M KNO3 and 3 ml of 0.1 m phosphate buffer (7.5 pH) and incubated for 2 hours at 30°-33 °C. After that, tubes were removed and immersed in boiling water bath for 4 minutes to stop the reaction for effective removal of nitrate accumulation in plant tissues. Cooled the tubes and added 1 ml of 1% sulphonilamide + 1 ml of 0.01% N (1-Nepthalenadiamine hydrochloride) and mixed thoroughly and kept
for 25 minutes for colour development. Subsequently 0.2 ml was taken in separate tube and made the volume up to 6 ml with distilled water. Optical density was measured at 540 mu on UV - VIS-119 spectrophotometer and the nitrate reductase activity was calculated from the optical density and expressed in m mole NO$_2$/ g fresh weight /h of the fresh weight.

\[
10 \times \text{O.D.} \times \text{Total Volume} \\
0.09 \times \text{Volume of aliquot taken}
\]

iii. **Crude Protein**: Crude protein of the samples was determined by multiplying the value of total nitrogen by 6.25. Total nitrogen was determined by micro-Kjeldahl’s method (A.O.A.C. 1960). 100 mg oven dried samples were taken in a dry micro-Kjeldahl flask containing 5 ml. H$_2$SO$_4$ (Nitrogen free) and catalytic mixture and kept for digestion. The desired material was transferred into volumetric flask and the volume was made up to 50 ml. A suitable aliquot (about 5 ml) was taken in to the distillation apparatus along with 5 - 10 ml 40% NaOH. Ammonia collected in 21% boric acid containing 2 - 3 drops of mixed indicator for 7-10 minutes was titrated with N/50 standard sulphuric acid solution.

\[
\text{% of } N = \frac{\text{Sample titrated} - \text{Blank taken}}{\text{Sample weighed in mg}} \times 100 \times \text{normality of H}_2\text{SO}_4 \times 100
\]

Crude Protein = 6.25 X % of Nitrogen

iv. **Determination of Sugar and Starch**: Sugar and starch content of samples was estimated by anthrone method (Morris 1948). 100 mg ground dried sample was taken into a 15 ml centrifuge tube and 10 ml of 80% ethanol was added to it. These tubes were kept on water bath at 80-85°C for 30 minutes. It was centrifuged and decanted into a 25 ml beaker. Ethanol was evaporated on a water bath at 80 - 85°C until most of the alcohol was removed. This was made up to 25 ml with distilled water. 5 ml of this sugar extract was taken in a 100 ml volumetric flask and the volume was made up with distilled water. 5 ml of this solution was taken in a test tube in ice bath. To
each tube 10 ml of 0.2% anthrone reagent was added. These tubes were kept into a boiling water bath for exactly 7.5 minutes cooled in ice and absorbance was measured at 630 mu on Spectronic-70.

The residue left in the centrifuge tube was dried at 80 °C in an oven for starch extraction. After adding 2 ml of distilled water, tubes were kept on boiling water bath for 15 minutes. After cooling it 2 ml of 9.2 N perchloric acid was added to it and then made up to about 10 ml and centrifuged. After collecting the supernatant, 2 ml of 4.6 N perchloric acid was added to the residue and made up to 10 ml with distilled water. It was centrifuged and the supernatant mixed with the previous one. This was made up to 50 ml with distilled water. 5 ml of it was taken in a 50 ml volumetric flask and the volume was made up with distilled water. Now 5 ml of it was taken in a tube, kept in a ice bath and 0.2% anthrone was added to it. After keeping it for 7.5 minutes on boiling water bath and cooling in ice absorbance was taken at 630 mu on Spectronic-70. The values for sugar and starch content were obtained by putting the values of O.D. in the standard curve.

v. Mimosine : Mimosine content in leaves of *L. leucocephala* varieties was determined by calorimetric method described by Brewbaker and Kaye (1981). One gram fresh leaf sample was macerated with 9 ml 0.1 N HCl in paste and mortar and centrifuged for 5 minutes at 2000 R.P.M. 1 ml of supernatant liquid was mixed with 2 ml of 0.1NHCl with charcoal, boiled for 15 minutes on water bath and filtered through Whitman paper No. 42. Two ml of aliquot was taken mixed with 5 ml of EDTA solution (Na₂ EDTA 2H₂O 1g in 4 liter distilled water) and 1 ml solution of ferric chloride (2 g Fe Cl₃ 6H₂O dissolved in 500 ml of 0.1N HCl) and mixed well. The absorbance was measured at 535 mu on Spectronic-20 along with the control sample. The values for mimosine content were obtained from the standard curve.

vi. *Determination of energy value in plant samples* : The energy in terms of calorie
value was estimated in the dry material of leaf, stem and root. The dried material was powdered in a sample grinder (20 mesh screen) and 1 g pellets were prepared from the fine powder. These pellets were dried and kept in a desiccator for the determination of calorie values using an oxygen Bomb Calorimeter (model -RSB 3). The calorie value was presented in kcal/g dry wt.

vii. *Energy flow and ecological efficiency*: The dry matter values of the plant biomass were multiplied by their energy value to obtain the energy allocation pattern to different components of the biomass. From these data the energy flow diagrams were prepared for the year. The ecological efficiency was calculated by dividing the total energy produced by the utilisable solar energy received per unit area multiplied by 100.

viii. *Statistical analysis and presentation of data*: The data were analyzed statistically and significant responses at 5% level have been compiled using the methods of Fisher and Yates (1963) and Panse and Sukhatme (1967). The critical difference (CD) has also been worked out for comparing the mean values of the treatments and their effects on the stages and their interactions.

Correlation coefficients among various important morphological, physiological and their interdependence of the characters. Graphical representations have been made to facilitate easy understanding of the table and responses to the treatments.