Chapter - V

Materials and Methods
Materials and Methods

The investigatory Experiments were carried out during the months of April to December of 1995 to 1997 to evaluate the effect of some growth regulants on sprouting, early seedling growth and yield of turmeric (*Curcuma longa* L.) under saline stress conditions.

5.1 Materials

5.1.1 Collection and Culture of Test Material
(Turmeric rhizomes)

The healthy mother rhizomes of turmeric (*Curcuma longa* L.), about 4-5 cm long having 6-8 nodes and 2-3 buds (fingers) were collected from the Horticulture and Soil Conservation Department, Government of Manipur (India), located at Sanjenthong, in the month of March 1995, 1996 and 1997 respectively from time to time.

Mother rhizomes of the same age group and uniform size with 2-3 buds were selected and washed with distilled water to remove the soil residues and then soaked in 100 ppm of growth regulants viz., Indole Acetic Acid (IAA) and Phenol for 24 hours at room temperature, separately in glass containers of 5 litre capacity. Sodium chloride (NaCl) and Sodium Sulphate (Na$_2$SO$_4$) were used to prepare different salinity levels by dissolving in 1000 ml of rain water as per their EC - 0, 4, 8, 12 and 16 mmhos/cm (Richard, 1968). The quantity of sodium salts were taken according to Bhumbala’s method (Bhumbala *et al.*, 1968). Soaked mother rhizomes were subjected to polythene bags (black) of same size (30 x 20 cm$^2$) at the rate of three rhizomes per bag and each bag was filled with 2.5 kg of sundried
sandy soils collected from Kongba River bank about 1 km from experimental site. The soils were treated with dilute hydrochloric acid (HCl) for 12 hours and washed in running water and then dried under the sun. The pH of the soil was maintained at 7.0 during the course of experiment by adding the salinity dozes of sodium salts three times from the date of planting the rhizomes at the interval of 90 days. The soil of each bag was uniformly fertilized with murate of potash and urea in the ratio of 1 : 1. The average amount of fertilizer in each pot was 19.60 g (Singh, 1992).

The first category of rhizomes, which were treated with only rain water, considered as control (T₁). The second category of rhizomes, treated with 100 ppm of IAA and Phenol separately were considered as treatments (T₂-T₆). The rhizomes of treatment blocks (T₃-T₆) only were subjected to various experimental concentrations of NaCl and Na₂SO₄ salinity separately (Table 5.1) and 250 cc of rain water was added to the control and treatment blocks at the gap of 15 days from the date of plantation till the maturation of crop. Polythene bags of treatment blocks (T₃-T₄) were maintained at the desired salinity levels of NaCl and Na₂SO₄ till maturity by supplementing respective salt solution three times at 90 days intervals. When the rhizomal sprouting become almost constant, one healthy seedling was maintained per polythene bag for experimental studies.

5.1.2 Geographical Profile of the State

5.1.2.1 Location, Extent and Physiography

Manipur is small hilly state situated on the eastern laps of Himalayan ranges, the Purvanchal (Chatterjee, 1965) in the extreme north-eastern border of India along the Indo-Myanmar (Burma) border with its functional nodal city
Table 5.1
Experimental Salinity Levels

<table>
<thead>
<tr>
<th>Salinity levels* (mmhos/cm)</th>
<th>Quantity of Salts (g) per litre of Solution**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sodium chloride (NaCl)</td>
</tr>
<tr>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>4</td>
<td>0.994</td>
</tr>
<tr>
<td>8</td>
<td>2.047</td>
</tr>
<tr>
<td>12</td>
<td>3.159</td>
</tr>
<tr>
<td>16</td>
<td>4.325</td>
</tr>
</tbody>
</table>

* Soil salinity was measured by determining the Electrical Conductivity (EC) of Saturated Soil Paste Extract (Richard, 1968).

** The sodium salts were added as mentioned in the table to prepared various salinity levels because the soil water contains these salts in this proportion (Bhumbala et al., 1968).

Imphal, the state capital. It is bounded on the north by the state of Nagaland, on the east by Upper Burma, on the south by the Chin Hills of Burma and the State of Mizoram and on the west by Cachar District of Assam (Fig. 5.1).

Manipur is located between latitudes 23.83°N to 25.68°N and longitude 93.03°E to 94.78°E covering a total geographical area of 22,327 sq km (Statistical Hand Book of Manipur, 1981) occupying an area of 0.67 percent of the total geographical area of the country. The state ranks 20th, both in area and population, among the states and union territories in the country. Manipur comprises nine districts. Five in the hills and four including Imphal are in the central valley.

The state can broadly be divided into three land divisions - the Manipur Hills, the Manipur Valley and the Barak basin, which differ to much extent in their
TURMERIC (*Curcuma longa* L.) CULTIVATION IN MANIPUR
DURING THE YEAR (1995-97)

**Fig 5.1**

SOURCE: DIRECTORATE OF HORTICULTURE AND SOIL CONSERVATION, GOVERNMENT OF MANIPUR, INDIA.
physiographical characteristics. The periphery of the state is bounded by a series of parallel hills which covered 9/10th of the total geographical area of the state. Manipur valley at the centre, covers an area of 2,238 sq km The Barak Valley or Jiribam sub-division, covers an area of about 250 sq km and is located beyond the western Manipur hills. The hill ranges are aligned in a series of north-south parallel ridges. The eastern aspects of the hilly terrain are relatively of higher elevation than that of western aspect, where hill ranges are gradually subdued and foot hills merges to Barak valley in the west. The general elevation of the ranges along the eastern aspect of the hilly terrain varies between 1800 to 2500 m above sea level whereas the western ranges gradually gets subdued from 1100 to 800 m above mean sea level towards the West.

Imphal district (East and West) is one of the four valley districts of Manipur, lies between 24°33' to 25°55' North latitude and 93°42' to 94°7' East longitude. The total geographical area of the district is 120100 ha and occupied 5.3 percent of the total geographical area of the state and placed 6th in the aerial spread of the state. Imphal district has two categories of land forms. More than 80 percent of the total geographical area of district constitute valley plains and the remaining is foot hills having moderate slopes.

5.1.2.2 Climate

The climate of the state is pleasant ranging from sub-tropical monsoon to warm temperate in valley and warm to cool temperate in the hills depending on the elevation. The state receives rainfall from monsoon and it varies from 1016 to 1778 mm a year. The rainy season lasts from April to September and the amount of rainfall is more in hills than in the valley. The mean maximum temperature is 31.1°C and the mean minimum daily temperature during the winter recorded as
11.8°C. The driest period is recorded from November to March and the maximum rainfall of the state is recorded in the month of July and August. The average annual rainfall varies from 1000 - 2000 mm feeding the rivers, lakes and ditches of the state.

Imphal district falls under sub-tropical climate with maximum temperature of about 24° - 35°C in summer and minimum temperature of about 0° - 10°C in winter. Annual rainfall as recorded is 1332.4 mm (Source: MSRSC, DSTE, 1990).

5.1.2.3 Soil

Major soils available in the Manipur state (Fig. 5.2) are grouped into two major types: (1) Residual and (2) Transported, which cover the hill areas and the central valley respectively. The residual soils are either laterized or non-laterized. The laterized red soil (Oxisol) covering an area of 1500 km² found in most of the hilly regions. Non-laterized red soils (Ultisol) are also found in major part of the Manipur hills, and sub-humid zones and spread over an area of 17800 km². These soils with loamy texture and granular structure have strong acidity, a fair quality of nitrogen and rich in phosphate and potash.

Transported soils are of two types - alluvial and organic. The alluvial soils (Entisol) cover about 1600 km² agriculturally well developed part of Manipur valley. The alluvial soils have clay loam texture with grey to pale brown colour. They contain a good proportion of potash and phosphate, a fair quantity of nitrogen and organic matter. These soils are less acidic. The organic soils (Histosol) cover the low-lying areas of the central valley along the Loktak Lake, other lakes and marshes. These soils have dark grey colour and clayey loam texture
Fig 5.2

LEGEND

Alluvial soils (Recent)
Lettic soils
Alluvial Red Soils (Old)
Ferruginous Red Soils (non laterite)
Fer. Red Gravely Soils (non laterite)
Peaty and Organic Soils

State Boundary
International Boundary
with high acidity and have a good amount of nitrogen, organic matter and phosphorus but are poor in potash (source: Vedaja Sanjenbam, 1998).

5.2 Experimental Site and Location

The investigation was conducted in the Sainik School campus, located at Pangei about 9 km from Imphal city of Manipur state; at normal temperature in closed room (14.5 m x 10.0 m) situated on second floor/stair about 4.90 m above from the ground level, getting diffused sun light from East side only through windows and ventilators. During experimentation crop plants were not subjected to artificial light during night at all. The school is located at a latitude of 25°N, longitude of 94°E and at an elevation of 780 m from sea level.

5.3 Meteorological Conditions

The meteorological data of Sainik School, Pangei during the period of experimentation were recorded from Tulihal Airport, Imphal and Directorate of Agriculture, Sanjenthong, Manipur respectively, particulary for mean maximum and minimum temperature, mean average relative humidity and percentage and bright sun-shine hours means (Monthly and daily average) which have been shown in Table 5.2 and Fig. 5.3(A to D).

5.4 Layout and Experimental Design

The rhizomes of both the categories - control and treatments were planted in polythene bags by using randomized block design method (Cochran and Cox, 1965) with four replications. A total number of 6 (six) treatments including control were arranged at random in the 190 polythene bags (Fig. 5.4A and 5.4B1 to B4),
MEAN OF METEOROLOGICAL DATA DURING THE CROP SEASONS
(April 1995 to December 1997)

Temperature (°C)

Fig. - 5.3 (A)
- Min. Temperature (°C)
- Max. Temperature (°C)

Relative Humidity (%)
Evening

Bright Sunshine (hrs)

Fig. - 5.3 (B)
- Bright Sun Shine Daily Average
- Bright Sun Shine Monthly Average
MEAN OF METEOROLOGICAL DATA DURING THE CROP SEASONS
(April 1995 to December 1997)

Relative Humidity (%)
Morning

Fig. - 5.3 (C)
- Relative humidity (%) - Low
- Relative humidity (%) - High

Relative Humidity (%)
Evening

Fig. - 5.3 (D)
- Relative humidity (%) - Low
- Relative humidity (%) - High
Fig. 5.4 (A): Randomised Block Design for Statistical Analysis
Fig. 5.4 (B.) : Plan and Layout of Experiment
Plot-II

R_2

P & NaCl  P & Na_2SO_4

T_6

IAA & NaCl  IAA & Na_2SO_4

T_1

P

T_3

IAA

P & NaCl  P & Na_2SO_4

T_3

IAA & NaCl  IAA & Na_2SO_4

P & NaCl  P & Na_2SO_4

T_4

IAA & NaCl  IAA & Na_2SO_4

P & NaCl  P & Na_2SO_4

T_5

IAA & NaCl  IAA & Na_2SO_4

Fig. 5.4(B3): Plan and Layout of Experiment
Plot-III

R3

P & NaCl          P & Na2SO4
     ↑         ↑
     T3       T3

IAA & NaCl       IAA & Na2SO4
     ↓

P & NaCl          P & Na2SO4
     ↑         ↑
     T6       T6

IAA & NaCl       IAA & Na2SO4
     ↓

T1

P
     ↑
     T2

IAA

P & NaCl          P & Na2SO4
     ↑         ↑
     T3       T3

IAA & NaCl       IAA & Na2SO4

P & NaCl          P & Na2SO4
     ↑         ↑
     T4       T4

IAA & NaCl       IAA & Na2SO4

Fig. 5.4(B3) : Plan and Layout of Experiment
Fig. 5.4 (B4) : Plan and Layout of Experiment

Note : Indole Acetic Acid = IAA, Phenol = P, Sodium Chloride = NaCl,
Sodium Sulphate = Na₂SO₄, Control = T₁, Treatments = T₂ - T₆, Replication = R
Table 5.2
Mean of Meteorological Data During the Crop Seasons
(April 1995 to December, 1997)

<table>
<thead>
<tr>
<th>Month</th>
<th>Temperature (°C)</th>
<th>Relative Humidity (%)</th>
<th>Bright-Sunshine hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum</td>
<td>Minimum</td>
<td>Morning (8.30 a.m.)</td>
</tr>
<tr>
<td>April (A)</td>
<td>32.3</td>
<td>11.1</td>
<td>92.6</td>
</tr>
<tr>
<td>May (M)</td>
<td>34.1</td>
<td>15.9</td>
<td>97.3</td>
</tr>
<tr>
<td>June (J)</td>
<td>32.0</td>
<td>18.4</td>
<td>97.3</td>
</tr>
<tr>
<td>July (Ju)</td>
<td>31.6</td>
<td>20.6</td>
<td>97.3</td>
</tr>
<tr>
<td>Aug. (Au)</td>
<td>28.8</td>
<td>19.9</td>
<td>97.3</td>
</tr>
<tr>
<td>Sept. (S)</td>
<td>32.5</td>
<td>19.4</td>
<td>96.0</td>
</tr>
<tr>
<td>Oct. (O)</td>
<td>32.0</td>
<td>13.9</td>
<td>96.3</td>
</tr>
<tr>
<td>Nov. (N)</td>
<td>29.4</td>
<td>06.5</td>
<td>96.0</td>
</tr>
<tr>
<td>Dec. (D)</td>
<td>25.7</td>
<td>03.7</td>
<td>96.3</td>
</tr>
</tbody>
</table>

5.5 Experimental Details

Experimental details are reflected in the Table 5.3(A) and Table 5.3(B) respectively.

5.6 Time and Number of Application

[1] Selected Mother rhizomes of turmeric were soaked once in 100 ppm of growth regulators viz., IAA and Phenol for 24 hours separately in glass containers during morning when weather was free from any natural hazard before subjecting them into the polythene bags for sprouting.
[2] The first dose of experimental salinity levels of NaCl and Na₂SO₄ was added separately to the soils of polythene bags containing rhizomes of treatment categories (T₁-T₆) excluding T₁ and T₂ just after sowing.

[3] The second dose of salinity was administered to plants of treatment categories (T₁-T₆) on 90 days after plantation (DAP).

Table 5.3(A)
Details of Experiment

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Course duration</td>
<td>⇒ 03 (three) years, w.e.f. April to Dec. 1995 to 1997</td>
</tr>
<tr>
<td>Number of treatments</td>
<td>⇒ 06 (six) including control (T₁- T₆)</td>
</tr>
<tr>
<td>Experimental area (gross)</td>
<td>⇒ 14.50 m x 10.0 m</td>
</tr>
<tr>
<td>Crop duration</td>
<td>⇒ 8 to 9 months</td>
</tr>
<tr>
<td>Total number of polythene bags used</td>
<td>⇒ 190</td>
</tr>
<tr>
<td>Experimental design</td>
<td>⇒ Randomized Block Design (RBD)</td>
</tr>
<tr>
<td>Replication</td>
<td>⇒ 04</td>
</tr>
<tr>
<td>Dates of sowing</td>
<td>⇒ 02.04.95, 02.04.96 and 02.04.97</td>
</tr>
<tr>
<td>Fertilizer dose</td>
<td>⇒ Murate of Potash and Urea (1:1), 19.60 g/bag prior to sowing the rhizomes.</td>
</tr>
<tr>
<td>Plantation</td>
<td>⇒ When the sprouting become almost constant, one healthy seedling was maintained per bag for experimental studies till crop maturation.</td>
</tr>
<tr>
<td>Harvesting</td>
<td>⇒ The crop was harvested at maturity after 270 DAP</td>
</tr>
<tr>
<td>Spacing</td>
<td>⇒ a] Experimental area was divided equally into 4 plots (rows) - 03 m</td>
</tr>
<tr>
<td></td>
<td>b] Linear distance, plant to plant - 0.30 m</td>
</tr>
<tr>
<td></td>
<td>c] Distance, row to row - 1.0 m</td>
</tr>
<tr>
<td></td>
<td>d] Spacing between treatment to treatment category 0.5 m</td>
</tr>
</tbody>
</table>
Table 5.3(B)

<table>
<thead>
<tr>
<th>Categories</th>
<th>Experimental salinity levels mmhos/cm</th>
<th>Concentration</th>
<th>Design (RBD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>Growth Regulants (ppm)</td>
<td>NaCl g/l</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td>IAA</td>
<td>Phenol</td>
</tr>
<tr>
<td>T1</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T2</td>
<td>0</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>0.00</td>
</tr>
<tr>
<td>T3</td>
<td>4</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>0.994</td>
</tr>
<tr>
<td>T4</td>
<td>8</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>2.047</td>
</tr>
<tr>
<td>T5</td>
<td>12</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>3.159</td>
</tr>
<tr>
<td>T6</td>
<td>16</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>4.325</td>
</tr>
</tbody>
</table>

[4] The third dose of salinity was provided to the soils of T3-T6 categories after 90 days of second dose.

[5] 250 cc of rain water was added to the plants of control and treatment blocks at the gap of 15 days from the date of sowing (plantation) till the crop attained maturity.

5.7 **Chemicals Used in the Experiment**

5.7.1 **Indole Acetic Acid**

3-Indole Acetic Acid (IAA) is a light pink powder, soluble in ethanol with molecular weight 175.19. It is a plant growth stimulator. Its molecular formula is C10H9NO2. It is marketed by E. Merck (India) Private Limited, Worli, Mumbai - 400 018 bearing Batch No. J 111525.
5.7.2 Phenol

Phenol is a non-volatile organic compound. It is also found in plant cells as a secondary metabolite. Phenol crystals are soluble in water. Its molecular formula is C₆H₅OH. The molecular weight of phenol is 94.11 with freezing point 39.5 - 41°C. It is a product of S.d-Fine-Chem Pvt. Ltd., Boisar - 401 501, bearing batch No. 056/PRS/01/30711.

5.7.3 Sodium Chloride

It is a white crystalline inorganic salt. Its molecular formula is NaCl. The molecular weight of sodium chloride is 58.44. It is marketed by Qualigens Fine Chemicals, A division of Glaxo India Ltd., Mumbai - 400 025, batch No. N007878 57075, Product No. 27605. The density of pure sodium chloride is 2.17 g/cm³ and melting point is 800°C. It is soluble in water but insoluble in alcohol.

5.7.4 Sodium Sulphate Anhydrous

It is a white crystalline inorganic solid. Its molecular formula is Na₂SO₄ with molecular weight 142.04. It is marketed by Apex Chemicals, Post Box No. 1921, Mumbai-1. It is also known as salt cake. It is soluble in water but insoluble in alcohol.

5.7.5 Rainwater

Rainwater collected in plastic containers after first shower to avoid the contamination due to air pollutants by keeping the containers in open. Rain water is the purest form of natural water with molecular weight of 18.
5.7.6 **Hydrochloric Acid**

Sandy soils collected from river bank, first dried in the sun to remove water content and then treated with dilute HCl for 12 hours and washed in running water. HCl treated soils again dried in the sun before experimental use.

5.7.7 **Preparation of Chemicals**

The different chemicals were measured in exact quantity and the water soluble ones like Phenol was diluted in distilled water. However, water insoluble chemical i.e. IAA was prepared by dissolving it in a few drops of ethanol and making up the final volume with distilled water. NaCl and Na₂SO₄ were dissolved in rain water as per their EC- 0,4,8,12 and 16 mmhos/cm (Richard, 1968). The quantity of salts were measured according to Bhumbala’s method (Bhumbala *et al.*, 1968). The salts were added separately to polythene bags (T₁-T₆) by using hand sprayer. However, the T₂ categories were treated with 100 ppm of IAA and phenol respectively.

5.7.8 **Cultural Operations**

The experimental bags were kept weed free by removing the weeds at regular intervals.

5.8 **Methods**

5.8.1 **Plant Sampling**

Observational plants for the study of various experimental parameters were selected at random from the control and treatment categories and the mean values were used for analysing the number of morpho-physio-biochemical aspects.
5.8.2 **Growth Parameters**

5.8.2.1 **Sprouting Rate (SR)**

Sprouting counts were made at 10 days interval upto 40 days after subjecting the rhizomes to the soils of control and treatment categories, when the SR becomes almost constant in each bag. However, sprouting percentage was calculated only at 30 days after sowing. The percent of increase or decrease in rhizomal SR under 100 ppm of IAA and phenol at 0, 4, 8, 12 and 16 EC, over control and mean salinity index were determined at 40 DAP by using the following formulae:

\[
a] \quad \text{Mean Salinity Index of Sprouting in response to growth Regulants} = \frac{\text{Mean of sprouting rate in T7-T8 treatments}}{\text{Sprouting rate value of control (T1)}} \times 100
\]

\[
b] \quad \text{Percent increase or decrease in sprouting rate} = \frac{\text{Mean value of treatment - Control Value}}{\text{Control Value}} \times 100
\]

5.8.2.2 **Plant Height (PH)**

The main shoot was taken to represent the height of the plant. The PH was measured in centimetre at 45, 60, 90, 120, 150, 180, 210 and 240 days after plantation (DAP). The measurement was taken from the soil surface to the shoot apex and the average mean was taken from five plants selected from control and treatment categories randomly. However, the mean salinity index and percent increase or decrease in PH at 180, 210 and 240 DAP in response to growth regulators (IAA and Phenol) at various salinity levels of NaCl and Na\(_2\)SO\(_4\) were calculated by using the formula:
5.8.2.3 **Number of Thin and Tuberous Adventitious Roots**

The counts of thin and tuberous adventitious roots were made at various growth stages (45 DAP - 240 DAP) and average mean was taken from five sample plants selected from control and treatment categories randomly at the interval of 30 days from 45 DAP to 240 DAP. However the mean salinity index in response to growth regulants and percent increase or decrease for this parameter were calculated at 180 DAP, 210 DAP and 240 DAP respectively by using formulae of 5.8.2.2.

5.8.2.4 **Length of Thin and Tuberous Adventitious Roots**

The length of thin as well as tuberous adventitious roots was measured in centimetre from the point of their origin on the rhizome up to the root apex from 45 DAP to 240 DAP at the gap of 30 days by the help of ruler. The average mean of respective root was taken from the samples selected randomly. Each sample was consisting of five plants. However, the mean salinity index and percent increase or decrease in response of growth regulants for root length were calculated at 180, 210 and 240 DAP respectively by repeating the same formulae.

5.8.2.5 **Biomass (B)**

Biomass per plant (dry weight and moisture content) was calculated in percentage at various growth stages (i.e. 120, 180 & 240 DAP) by using the electronic balance with the help of formulae as given below:
a) Dry weight (%) = \frac{\text{Dry Weight (g) of Plant}}{\text{Fresh Weight (g) of Plant}} \times 100

b) Moisture content (%) = \frac{\text{Moisture (g)/Plant}}{\text{Fresh Weight (g)/Plant}} \times 100

The Mean Salinity Index and percent increased or decreased in response of growth regulant per biomass dry weight (g) were calculated at 120, 180 and 240 DAP by using the formulae of 5.8.2.2.

5.8.2.6 Number of Functional Leaves (NFL)

The total number of green leaves per plant of treatments and control categories were counted at various growth stages (45 to 240 DAP). Average mean for NFL was taken from ten plants.

5.9 Physiological and Bio-chemical Aspects

5.9.1 Relative Growth Rate (RGR)

Relative Growth Rate was calculated following the method of Radford (1967) at 180 and 240 DAP respectively by using the formula as given below:

\[
RGR = \frac{1}{w} \times \frac{d_w}{d_t} \, (g/g/day)
\]

where, \(d_w\) = difference between dry weight of two stages; \(d_t\) = difference between time at two stages; \(w\) = initial dry weight.

5.9.2 Net Assimilation Rate (NAR)

Net Assimilation Rate was determined by using the method of Radford (1967) at 240 DAP with the help of formula as mentioned below:
\[ \text{NAR} = \frac{1}{A} \times \frac{d_w}{d_t} \text{ (g/cm}^2\text{/day)} \]

where, \( d_w \) = difference between dry weight at two stages; \( d_t \) = difference between time at two stages and \( A \) = difference between leaf area at two stages.

5.9.3 **Leaf Area (LA)**

Leaf Area was determined by using Portable Area Meter (LI-COR) Model LI-3,000 in cm\(^2\). Average mean of LA was taken from ten plants selected from control and treatment categories respectively at the interval of 30 days from 45 to 240 DAP.

5.9.4 **Leaf Area Index (LAI)**

Leaf Area Index was calculated by using the formula as given below:

\[ \text{LAI} = \frac{\text{Leaf Area (LA) Mean}}{\text{Canopy (C) Mean}} \]

Where \( C \) = length of lamina.

5.9.5 **Leaf Area Duration (LAD)**

Leaf Area Duration was calculated based on average mean of ten plants at 180 and 240 DAP by using the following formula:

\[ \text{LAD} = \frac{L_2 + L_1}{2} \times (T_2 - T_1) \]

where, \( L_2 = \text{LAI at time } T_2; \) \( L_1 = \text{LAI at time } T_1 \)
5.9.6 **Chlorophyll ‘a’ : ‘b’ Ratio**

The chlorophyll ‘a’ : ‘b’ ratio of leaves at different crop growth stages was determined as mentioned below:

\[
\text{Chlorophyll ‘a’ : ‘b’} = \frac{\text{Amount of chlorophyll ‘a’}}{\text{Amount of chlorophyll ‘b’}}
\]

5.9.7 **Photosynthetic Pigments (PP)**

Photosynthetic Pigment in the leaf tissues were determined by Arnon (1949) method at various growth stages and calculated on a fresh weight (F wt) basis employing the following formulae:

1. Total Chlorophyll (mg/g/F wt) = 20.2 \(A_{645}\) + 8.02 \(A_{663}\) x \(\frac{V}{a \times 1000 \times w}\)

2. Chlorophyll-a (mg/g F wt) = 22.9 \(A_{645}\) - 4.68 \(A_{663}\) x \(\frac{V}{a \times 1000 \times w}\)

3. Chlorophyll-b (mg/g F wt) = 22.9 \(A_{645}\) - 4.68 \(A_{663}\) x \(\frac{V}{a \times 1000 \times w}\)

Where, \(a \rightarrow\) Length of path light in the cell (usually 1cm), \(V \rightarrow\) Volume of the extract in ml, \(w \rightarrow\) Fresh weight of the sample taken (g), \(A_{645} \rightarrow\) Absorbance of the extract at 645 nm and \(A_{663} \rightarrow\) Absorbance of the extract at 663 nm
5.9.7.1 **Estimation of Carotenoids**

Carotenoid pigments in methanol extract of the leaf tissues were estimated from absorbance at $A_{450}$ nm in colorimeter by using the formula as given below:

$$\text{mg carotenoid content /g/F wt} = \frac{D \times V \times f \times 10}{2500}$$

Where, $D = \text{Absorbance at 450 nm in a 1.0 cm cell}$, $V = \text{Volume of the Original extract in ml.}$, $f = \text{Dilution factor}$, and $2500 = \text{Average extinction co-efficient of the pigments.}$

5.9.8 **Carbohydrates**

In the plants carbohydrates occur in various forms and are the main source of energy for biological activities. Not only starch but also a number of sugars are available in plant cells in readily assimilable carbon sources for proper growth and metabolism. For the determination of carbohydrates 1g fresh tissues of the leaf samples were extracted with 80% ethanol by using pestle and mortar. The supernatant (alcohol extract of plant material) collected after centrifugation, was then evaporated in a hot water bath maintained at $40 \pm 2^\circ C$ until it was free from alcohol. The extract was then made aqueous with a few ml of water. The aqueous fraction, was taken for the estimation of total available carbohydrates (TAC), total soluble sugars (TSS) and total non-soluble sugars (TNSS). For every estimation a reagent blank was made by adding all the reagent except the plant extract.

5.9.8.1 **Estimation of TAC**

Total Available Carbohydrates were calculated following Murty & Murty (1982). TAC is the total value of the amounts of available starch and sugars in the
leaf tissues extract. The amount of TAC present in the extract was determined by using standard curve prepared from glucose and is expressed in mg TAC per g fresh wt. of the sample.

5.9.8.2 Estimation of TSS

The amount of TSS present in the extracts were estimated by Anthrone method (Dubois et al., 1951) using anthrone reagent. A known volume of the extract of 5.9.8 was heated with anthrone reagent prepared in conc. H₂SO₄ for 10 mins in a boiling water bath and then, allowed to cool at room temperature. The absorbance of the blue-green solution was measured at 625 nm. The amount of TSS present in the extracts was calculated by using a standard curve prepared from glucose.

Anthrone reagent: 2g of anthrone in 1 litre of conc. H₂SO₄.

5.9.8.3 Estimation of TNSS

Total Non-Soluble Sugars are the total value of the amounts of available reducing sugars and non reducing sugars in the leaf tissues of samples. Nelson’s modification of Somogyi’s method (Nelson, 1944) was adopted for the estimation of reducing sugars. In this method copper reagent A,B and arsenomolybdate were used. After 15 mins, the absorbance of the different solutions (blue green) was measured at 500 nm wave length in a spectrophotometer and the amount of reducing sugars was determined by using a standard curve prepared with glucose.

Somogyi’s Reagent:

Reagent A:

i) Sodium-Potassium-Tartarate (Rochelle salt) - 25g
ii) $\text{Na}_2\text{CO}_3$ (anhydrous) - 25g
iii) $\text{NaHCO}_3$ - 20g
iv) $\text{Na}_2\text{SO}_4$ (anhydrous) - 200g
v) Distilled Water - 800ml.

Reagent B:

i) $\text{CuSO}_4\cdot5\text{H}_2\text{O} - 15\%$ (containing 1-2 drops of conc. $\text{H}_2\text{SO}_4$ in 100ml.)
ii) Distilled water - 200 ml.

Reagent A was mixed properly with reagent B in equal volume just before use in Experimentation.

Arsenomolybdate reagent:

Reagent A:

i) Ammonium molybdate ($\text{NH}_4$)$_6$ $\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ - 25 g
ii) Distilled Water - 450 ml.
iii) Conc. $\text{H}_2\text{SO}_4$ - 21 ml.

Reagent B:

i) Sodium Bi-Sulphate Hepta Hydrated ($\text{Na}_2\text{H}_2\text{SO}_4\cdot7\text{H}_2\text{O}$) - 3 g
ii) Distilled Water - 25 ml.

Mixed reagent A & B (equal volume) and stored in a brown glass bottle at 37°C for 24-48 hours.

Formic acid hydrolysis method (Malhotra & Sarkar, 1979) was followed for the estimation of non reducing sugars present in the alcohol free leaf tissues.
extract. Equal volumes of leaf tissues extract and 12M formic acid were mixed. The resulting mixture contained a final concentration of 6M formic acid. The mixture was refluxed for 2 hrs at 40± 2°C to convert non reducing sugars to mono saccharide components (reducing sugars) & estimated the total reducing sugars following Nelson’s method. The difference between the TSS, reducing sugars plus non reducing sugars corresponds to the amount of TNSS mg per g fresh wt of sample.

5.9.9  **Protein and Amino Acids**

The estimation of proteins (PR) and Amino acids (AA) in leaf samples of turmeric at various growth stages was carried out by Lowry’s Technique (1951) and Yemm and Cocking (1955) method respectively.

5.9.9.1  **Estimation of Proteins (PR)**

Lowery *et al* (1951) Introduced a colorimetric method to determine the PR. The method is based on the principle that different PR contain different amounts of aromatic residues which react with folin ciocalteu reagent, giving a blue colour which is read in a colorimeter.

In this, 1g of fresh leaf tissues of samples were homogenised in 1 ml of 1N NaOH (at 100°C for 4 to 5 mins) and than 5 ml of alkaline copper reagent was added, the mixture was allowed to stand at room temperature for minimum 10 mins. After that, 0.5 ml of folin - ciocalteu reagent was mixed rapidly and the absorbance was measured at 750 nm after 30 mins in a colorimeter. The amount of PR in the samples was calculated with a standard curve prepared by using bovine serum albumin and was expressed as mg protein per g fresh wt of the sample.
Reagents:

i) Reagent A :  (2.0% Na$_2$CO$_3$ in 0.1 N NaOH)

20 g of Na$_2$CO$_3$ (anhydrous) was dissolved in 800 ml of distilled water and then 100 ml of 1 N NaOH (4 g of NaOH in 100 ml) was added. The total volume was raised to 1 litre with distilled water.

ii) Reagent B :  (1% CuSO$_4$ .5H$_2$O)

1 g of CuSO$_4$ . 5H$_2$O was dissolved in 100 ml distilled H$_2$O.

iii) Reagent C :  (2% Na-K-tartarate)

i.e. 2 g of Na-K-tartarate dissolved in 100 ml of distilled water.

iv) Reagent D :  (Mixture of A,B and C)

100 ml of reagent A + 0.1 ml of reagent B and 0.1 ml of reagent C.

This mixture is freshly prepared.

v) Reagent E :  Folin-Ciocalteu’s phenol reagent.

This reagent was diluted in 1:2 ratio with distilled water just before use.

vi) Potassium phosphate buffer :

Stock solution (A):
0.2 M solution of monobasic potassium phosphate (KH$_2$PO$_4$
- 27.218 g in 1000 ml distilled water)
Mol. wt. - 136.09.

Stock solution (B):
0.2 M solution of dibasic potassium phosphate (K$_2$HPO$_4$,
- 34.336 g in 1000 ml distilled water).
Mol. wt. -174.18
5.9.9.2 Estimation of Amino Acids (AA)

The estimation of AA was done by modified ninhydrin method (Yemm and Cocking, 1955). In this, 1g of fresh tissues of leaf samples were homogenised in 10 ml of 60% ethanol with a pinch of activated charcoal by using pestle and mortar. The mixture was centrifuged at 1000 rpm for 10 mins and the AA were collected in the form of clear supernatant.

To 1 ml of supernatant, 25 ml of 2% ninhydrin solution (w/v in Isopropyl alcohol) and 2.5 ml of 0.1 M acetate buffer (pH 5.5) were added. The mixture was then heated on boiling water bath at 100°C for 15 mins and was cool under a running tap. After cooling, aqueoue isopropyl alcohol (1:1) was added to the mixture to make up volume to 10 ml. The colour intensity of the violet complex was measured at 570 nm as absorbance. The amount of total AA was calculated with the help of calibration curve prepared from glycine and is expressed as mg AA per g fresh wt of the samples.

5.9.10 Curcumin

Curcumin [1, 7-bis (4-hydroxy-3- methoxy-phenyl)-1, 6-heptadiene - 3, 5-dione] is the principal colouring constituent of the rhizome of the plant. Curcuma longa from which it is readily extracted (Milobendzka et. al., 1910). Beside food additive, it is also used in the dye industry and in medicine as reported to have antiinflammatory and other healing properties. The characteristic chrome orange-yellow colour of curcumin (λ max ~ 430 nm) is due to presence of two olefinic side chains conjugated to the aromatic ring (Fig. 5.5).
Fig. 5.5: Structure of Curcumin: [1,7-bis (4-hydroxy-3-methoxy-phenyle)-1,6-heptadiene-3,5-dione]

- Mol. formula: $C_{21}H_{20}O_6$
- Melting point: 180 - 183°C
- Colour: Orange crystals
- Physical properties: Curcumin is insoluble in water, otherwise soluble in alcohol and glacial acetic acid. It gives brownish red colour with alkali.

5.9.10.1 Extraction of Curcumin

Curcumin extraction from dry rhizomes at 270 DAP was done by using Damayanti et al. (1992) and Sunitibala (1998) techniques.

The dried rhizomes of *Curcuma longa* (1 gm) was homogenised with 10 ml of 60% methanol. The homogenate was centrifuged at 2500 rpm for 20 mins. Then the supernatant was diluted to a solution and absorbance was determined at 420 nm. Pure curcumin was commercially purchased from Sigma Chemicals Co., St. Louis, USA. Stock solution of the "pure" compound was prepared in absolute
methanol and for experimental use was diluted to a final concentration of 20 μM in 60% methanol water. The absorbance spectra of such solution showed a maximum absorbance of 0.9 - 1.0 at 420 nm. The amount of curcumin present in the diluted supernatant was determined by using standard curve prepared from stock solution of pure curcumin sample and was expressed as percent per gram of dry weight of the sample.

5.10 Fresh Yield (FY)

Fresh yield was determined on electronic balance by using conventional method in q/ha from the mean values at the 270 DAP. Mean salinity index and percent increase or decrease for treatments (T2-T6) over the control (T1) were also calculated at 270 DAP.

5.11 Statistical Analysis

The experimental data recorded were analysed statistically by using the methods of Cochran and Cox (1955) and Gomez and Gomez (1976). Significance of the analysis of variance due to treatments and blocks was estimated by calculating the respective ‘F’ values (calculated and table values at 5% and 1% respectively).

The standard error (SE) of difference (d) of mean was calculated by using the formula:

\[ S.E. (d) = \sqrt{\frac{2 \times \text{Error mean square}}{\text{Number of replications}}} \]
To find out the significance of mean difference amongst the treatments, critical difference (CD) was calculated at 5 percent and 1 percent level of significance by multiplying the S.E (d) with appropriate table value of ‘t’ at 5 percent and 1 percent level of probability:

i) \( CD = S.E.(d) \times t \text{ at } 5\% \),

ii) \( CD = S.E.(d) \times t \text{ at } 1\% \).