Chapter-III

MATERIALS
AND
METHODS
MATERIALS AND METHODS

An accurate technique of study and careful selection of materials used are of prime importance for obtaining high precision in the result of an experiment. This chapter deals with basic information about the soil and climate conditions which prevailed during the cropping period and details of techniques followed during the course of investigation and the materials used in this work. Therefore, the present investigation was conducted for two consecutive years (2002-03 and 2003-04). The details of the methods followed and materials used in the present investigation are described in this chapter.

Location and Climate:

Geographical situation indicate that Kanpur is located in 25.26° to 26.50° North latitude and 79.31° to 40.34° East longitudes. The climate is subtropical with average annual rainfall of 88.90 cm. The onset of monsoon took place in July and last till October. The post-monsoon showers were received during winter. The summers are extremely hot and dry. The winters are fairly cool. Frost is not common but observed some times during last week of December to the end of January and desiccating hot winds and dust storms during summers. Thus, this part may easily be characterized as a typical semi-arid, subtropical climate zone of Uttar-Pradesh. The Meteorological data at the research station during the period of investigation are presented in table-1 and fig. 1.
Experimental site:

This experiment was carried out as a field trial during 2002-2003 and 2003-04 in the field of Department of Vegetable Science, Chandra Shekhar Azad University of Agriculture and Technology, Kalyanpur, Kanpur.

Table-1 Meteorological data during the period of investigation.

<table>
<thead>
<tr>
<th>Month</th>
<th>Temperature</th>
<th>Relative Humidity (%)</th>
<th>Wind Speed (Km/hr)</th>
<th>Evaporation (mm/day)</th>
<th>Rain fall (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max.</td>
<td>Min.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mar. - 2002</td>
<td>31.5</td>
<td>13.0</td>
<td>63</td>
<td>3.8</td>
<td>4.9</td>
</tr>
<tr>
<td>April - 2002</td>
<td>38.0</td>
<td>21.0</td>
<td>54</td>
<td>4.3</td>
<td>6.8</td>
</tr>
<tr>
<td>May - 2002</td>
<td>39.4</td>
<td>27.9</td>
<td>54</td>
<td>5.2</td>
<td>8.4</td>
</tr>
<tr>
<td>June - 2002</td>
<td>38.7</td>
<td>29.0</td>
<td>56</td>
<td>5.6</td>
<td>9.6</td>
</tr>
<tr>
<td>July - 2002</td>
<td>38.8</td>
<td>29.6</td>
<td>55</td>
<td>7.9</td>
<td>9.9</td>
</tr>
<tr>
<td>Aug. - 2002</td>
<td>32.7</td>
<td>26.3</td>
<td>80</td>
<td>3.6</td>
<td>4.2</td>
</tr>
<tr>
<td>Sept. - 2002</td>
<td>31.3</td>
<td>23.8</td>
<td>80</td>
<td>3.3</td>
<td>3.7</td>
</tr>
<tr>
<td>Oct. - 2002</td>
<td>32.5</td>
<td>20.3</td>
<td>63</td>
<td>1.8</td>
<td>3.7</td>
</tr>
<tr>
<td>Nov. - 2002</td>
<td>28.9</td>
<td>11.9</td>
<td>62</td>
<td>1.4</td>
<td>2.6</td>
</tr>
<tr>
<td>Dec. - 2002</td>
<td>24.4</td>
<td>6.8</td>
<td>63</td>
<td>1.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Jan. - 2003</td>
<td>15.8</td>
<td>3.6</td>
<td>83</td>
<td>2.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Feb. - 2003</td>
<td>24.4</td>
<td>10.0</td>
<td>76</td>
<td>3.4</td>
<td>2.6</td>
</tr>
<tr>
<td>Mar. - 2003</td>
<td>30.3</td>
<td>13.6</td>
<td>60</td>
<td>3.6</td>
<td>5.1</td>
</tr>
<tr>
<td>April - 2003</td>
<td>38.0</td>
<td>20.3</td>
<td>53</td>
<td>4.2</td>
<td>8.0</td>
</tr>
<tr>
<td>May - 2003</td>
<td>40.4</td>
<td>22.2</td>
<td>48</td>
<td>4.9</td>
<td>9.4</td>
</tr>
<tr>
<td>June - 2003</td>
<td>39.1</td>
<td>26.6</td>
<td>59</td>
<td>6.3</td>
<td>8.3</td>
</tr>
<tr>
<td>July - 2003</td>
<td>33.8</td>
<td>24.7</td>
<td>79</td>
<td>5.6</td>
<td>4.4</td>
</tr>
<tr>
<td>Aug. - 2003</td>
<td>32.6</td>
<td>25.0</td>
<td>81</td>
<td>3.0</td>
<td>2.9</td>
</tr>
<tr>
<td>Sept. - 2003</td>
<td>30.9</td>
<td>23.4</td>
<td>86</td>
<td>2.3</td>
<td>2.6</td>
</tr>
<tr>
<td>Oct. - 2003</td>
<td>31.9</td>
<td>16.9</td>
<td>68</td>
<td>1.6</td>
<td>3.3</td>
</tr>
<tr>
<td>Nov. - 2003</td>
<td>28.2</td>
<td>10.2</td>
<td>68</td>
<td>1.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Dec. - 2003</td>
<td>27.6</td>
<td>7.9</td>
<td>77</td>
<td>2.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Jan. - 2004</td>
<td>17.4</td>
<td>8.2</td>
<td>85</td>
<td>4.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Feb. - 2004</td>
<td>25.5</td>
<td>10.7</td>
<td>84</td>
<td>4.8</td>
<td>2.8</td>
</tr>
<tr>
<td>March - 2004</td>
<td>33.5</td>
<td>16.4</td>
<td>55</td>
<td>5.7</td>
<td>5.6</td>
</tr>
</tbody>
</table>
Fig. 1 Meteorological data during the period of investigation
Materials:

The trial was conducted on chilli (*Capsicum annuum* L.) cultivar Azad Mirch-1 having high yielding capacity. Seeds were procured from Department of Vegetable Science, Kalyanpur, Kanpur.

The cropping history of the experimental plot used for the present study is presented below.

**Table 2 (a). Mechanical analysis of experimental soil.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Component</th>
<th>2002</th>
<th>2003</th>
<th>Method of determination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Coarse sand</td>
<td>00.56</td>
<td>00.54</td>
<td>Mechanical dispersion and pipette method pipette sample (Piper, 1950)</td>
</tr>
<tr>
<td>2.</td>
<td>Fine sand</td>
<td>56.19</td>
<td>55.38</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Clay</td>
<td>20.10</td>
<td>19.60</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2 (b). Chemical analysis of the experimental soil.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Item</th>
<th>Results</th>
<th>Category</th>
<th>Method of determination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>pH</td>
<td>7.60</td>
<td>Slightly alkaline</td>
<td>USDA Hand book No 60 (Richard 1954)</td>
</tr>
<tr>
<td>2.</td>
<td>EC (mmhos/cm)</td>
<td>0.47</td>
<td>Low</td>
<td>Method No. 4</td>
</tr>
<tr>
<td>3.</td>
<td>Organic Carbon</td>
<td>0.47</td>
<td>Low</td>
<td>Method No. 24</td>
</tr>
<tr>
<td>4.</td>
<td>Available N kg/ha</td>
<td>205.00</td>
<td>Low</td>
<td>Subbiah &amp; Asija (1956)</td>
</tr>
<tr>
<td>5.</td>
<td>Available P2O5 kg/ha</td>
<td>25.00</td>
<td>Medium</td>
<td>Oslen's method (Oslen et al. 1954)</td>
</tr>
<tr>
<td>6.</td>
<td>Available K2O kg/ha</td>
<td>307.00</td>
<td>Medium</td>
<td>Flame Photometric method (Jackson, 1973)</td>
</tr>
</tbody>
</table>
Table-3. Cropping history of experimental plot:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Year</th>
<th>Kharif</th>
<th>Rabi</th>
<th>Zaid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2001-01</td>
<td>Fallow</td>
<td>Tomato</td>
<td>Fallow</td>
</tr>
<tr>
<td>2.</td>
<td>2001-02</td>
<td>Bhindi</td>
<td>Brinjal</td>
<td>Fallow</td>
</tr>
<tr>
<td>3.</td>
<td>2002-03</td>
<td>Chilli (E)</td>
<td>Chilli (E)</td>
<td>Fallow</td>
</tr>
<tr>
<td>4.</td>
<td>2003-04</td>
<td>Chilli (E)</td>
<td>Chilli (E)</td>
<td>Fallow</td>
</tr>
</tbody>
</table>

Soil Characteristics:

In order to assess the exact nature and composition of the soil experimental field was divided into different plots and soil sample from each plot was collected before application of FYM and planting. All samples were thoroughly mixed and representative sample was analysed in the department of Agril. Chemistry and soil science for physical and chemical components. The analytical details are given in Tab. 2(a) & 2(b).

Experimental details:

The trial laid out in a Single Split Plot design with twenty four treatments replicating thrice presented in figure 2 and other details of given below.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Factors</th>
<th>Treatments</th>
<th>Notations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2 % Di-Ammonium Phosphate</td>
<td>(i) No spray - Control</td>
<td>D1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(ii) One spray - 35 DAT</td>
<td>D2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(iii) One spray - 70 DAT</td>
<td>D2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(iv) Two spray - 35 &amp; 70 DAT</td>
<td>D4</td>
</tr>
</tbody>
</table>
(a) At fruit initiation

Plate I – View of the experimental field

(b) After fruit set
2. NAA  
   (i) 0 ppm  
   (ii) 10 ppm  
   (iii) 20 ppm  

3. Gibberellic acid  
   (i) 0 ppm  
   (ii) 10 ppm  

Treatment Combinations:

1. \( N_1G_1D_1 \)  
2. \( N_1G_1D_2 \)  
3. \( N_1G_1D_3 \)  
4. \( N_1G_1D_4 \)  
5. \( N_1G_2D_1 \)  
6. \( N_1G_2D_2 \)  
7. \( N_1G_2D_3 \)  
8. \( N_1G_2D_4 \)  
9. \( N_2G_1D_1 \)  
10. \( N_2G_1D_2 \)  
11. \( N_2G_1D_3 \)  
12. \( N_2G_1D_4 \)  
13. \( N_2G_2D_1 \)  
14. \( N_2G_2D_2 \)  
15. \( N_2G_2D_3 \)  
16. \( N_2G_2D_4 \)  
17. \( N_3G_1D_1 \)  
18. \( N_3G_1D_2 \)  
19. \( N_3G_1D_3 \)  
20. \( N_3G_1D_4 \)  
21. \( N_3G_2D_1 \)  
22. \( N_3G_2D_2 \)  
23. \( N_3G_2D_3 \)  
24. \( N_3G_2D_4 \)
# Details of design and layout of experiment

1. **Experimental Design**
   - Split plot. NAA (N); GA3 (G) in main plots and DAP (D) in sub-plots.

2. **Replication**
   - 3

3. **Total number of treatments**
   - 24 (4 x 3 x 2)

4. **Total number of plots**
   - 72

5. **Distance from row to row**
   - 0.45 m

6. **Distance from plant to plant**
   - 0.45 m

7. **Field size**
   - 429.3 Sq m
     - **Length of field** 26.5 m
     - **Width of field** 16.2 m

8. **Plot size**
   - 2.43 Sq m
     - **Length of net plot** 1.8 m
     - **Width of net plot** 1.35 m

9. **Field border**
   - 0.5 m

10. **Block partition**
    - 1.0 m

11. **Plot bund**
    - 0.3 m

12. **Irrigation channel**
    - 0.5 m

13. **Number of plants per plot**
    - 12 m
Fig. 2 Layout Plan of Experimental Field
(2002-03 and 2003-04)
Planting Material:

Selection of Variety:

The present investigation was conducted on cultivator Azad Mirch-1, the seeds of which were procured from Department of Vegetable Science, Kalyanpur, Kanpur.

Raising of Seedlings:

The seedlings were raised under the field conditions at vegetable research station farm Kalyanpur, Kanpur. The seeds were sown in the formaldehyde treated, thoroughly prepared and adequately manured (with sieved municipal compost) beds measuring 3.0 x 1.0 m each and having 30 cm wide drainage channel all around. The seeds were sown on 6th July in the first year (2002) and in the second year also on 6th July 2003. The sowing was done in lines nearly 4 cm apart in each bed and the sown seeds were covered with a thin layer of sieved compost. All the beds were sown in each year and just after covering the seeds, water was sprinkled by the watering can. Subsequent watering were done whenever necessary according to weather conditions. "SIRKI" covering was used to protect the seedlings from scorching sun, especially from 11 AM to 3 PM Hand pulling and weeding by 2-5 cm wide Khurpi were done whenever necessary. Hardening of seedlings by reducing the frequency of watering and exposing them to full sunlight was carried out nearly for one week before transplanting.
Preparation of field:

The field was prepared thoroughly by tractor ploughing. The stubbles of previous crop, grasses and pieces of bricks and stones, if any, were removed from the experimental field. It was then demarcated according to the plan of layout showing bunds, path, channels etc. The individual beds were leveled finally to avoid water stagnations during rains and to supply uniform water at the time of irrigation.

Application of fertilizers:

Nitrogen, phosphorus and potash were supplied at the rate of 100 kg, 50 kg & 50 kg per hectare through urea, single super phosphate and muriate of potash respectively. The urea was applied in three equal doses. The first dose of urea was applied before transplanting in prepared beds with full-required amount of single super phosphate and muriate of potash. The second and third dose of urea was applied as top-dressing at 25 and 45 days after transplanting respectively.

Transplanting:

About 6 weeks old seedlings of chilli, having 4 to 6 leaves were transplanted on 19th August during 2002 and on 19th August during 2003. The lifted seedlings were planted on pre-marked spacing 45 cm x 45 cm in the afternoon during both years. The individual seedling was watered immediately after transplanting.
Morphological study of seedlings at transplanting:

Morphological account of the seedlings at transplanting is given below.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Characters Studied</th>
<th>2002</th>
<th>2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Length of seedling from ground level upto growing point (cm)</td>
<td>8.7</td>
<td>9.5</td>
</tr>
<tr>
<td>2.</td>
<td>Number of green leaves per plant</td>
<td>6.8</td>
<td>7.4</td>
</tr>
<tr>
<td>3.</td>
<td>Diameter of stem at the base (mm)</td>
<td>0.35</td>
<td>0.40</td>
</tr>
<tr>
<td>4.</td>
<td>Fresh weight per plant (g)</td>
<td>0.80</td>
<td>0.90</td>
</tr>
<tr>
<td>5.</td>
<td>Dry weight per plant (g)</td>
<td>0.25</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Gap-filling:

In order to maintain uniform stand of the crop, the gaps created due to the death of plants were filled up by new seedling.

Application of NAA:

The aqueous solutions of NAA were prepared concentration wise i.e. 0 ppm (N1), 10 ppm (N2) and 20 ppm (N3) sprayed on the plants at 30 days after transplanting. In case of N1, only the distilled water was sprayed on the plants. The required amount of NAA was calculated on the basis of concentration and required volume of distilled water to spray the plants thoroughly under the individual concentration in all the replications.

Application of GA3:

The required weight of GA3 was calculated on the basis of concentration and required volume of distilled water to spray thoroughly the plants in plots under individual concentration in all
the replications. The GA₃ was dissolved in the distilled water. The solution was prepared just before the spraying at 35 days after transplanting.

**Application of DAP:**

The required amount of DAP was calculated on the basis of 2% concentration and required volume of distilled water to spray thoroughly in plants in each plots. The DAP was dissolved in the distilled water.

For spraying of above hormone and DAP, the solutions were prepared first before the spraying. All possible care was taken to minimise the addition of solution in soil. Spraying was done during the wind silent hours of day, i.e. after 5 PM.

**Aspects of study:**

A. **GROWTH STUDIES:**

The observations on height and number of leaves were recorded on five tagged plants in each net plot at monthly intervals after planting upto harvesting. The observations on fresh and dry weight of plants were registered at final stage, i.e. 140 days after transplanting.

1. **Height of the plant:** The height of five selected plants in each net plot was measured separately in centimeters from ground level up to growing point with the help of a meter scale and the average height per plant was calculated.
2. **Number of leaves**: Number of green leaves of the five already selected plants in each net plot were counted at 50 days after transplanting i.e. the peak of vegetative growth.

3. **Fresh weight of the top**: Five plants were taken (without roots) from the border rows of each plot and weighed on a physical balance and later on, the average fresh weight per plant was determined in gram (g).

4. **Dry weight per plant**: After recording the fresh weight of plant, they were chopped into small pieces. A composite sample of 100 g of chopped material (chilli plant) was taken and put in a perforated paper bag for drying in the sun for 4 to 6 days and then dried in an oven at 60 °C for 24 hours.

B. **DEVELOPMENT STUDIES**:

5. **Days to first flower bud**: The tagged plants in each net plot were observed daily to note the day (date) of appearance of first visible flower bud and then the average number of days required for first flower bud formation after transplanting was calculated.

6. **Days to first flower opening**: Observations on the opening of first flower on each of the five tagged plants in each net plot under all the replications were recorded and average number of days required for opening of first flower after transplanting were found out.
7. **Days to first fruit set**: The initiation of first fruit formation on each of the five tagged plants in each net plot were noted later on, the average number of days after transplanting required for initiation of first fruit formation was worked out.

8. **Days taken for harvest of first red ripe fruit**: Observation on the harvesting of first fruit (Red) from each of the five tagged plants in each net plot under each replication were taken and the average number of days after transplanting required for first harvesting of red fruit was determined treatment wise.

9. **Days taken for harvest of last red ripe fruit**: Observation on the harvesting of last fruit under five tagged plants in each treatment was noted and the average number of days after transplanting required for last harvesting of red fruits was determined treatment wise.

10. **Fruiting Span**: The difference between the first fruit formation and last harvesting in days was determined treatment and replication wise.

C. **YIELD STUDIES**:

11. **Number of fruits per plant**: At each picking, the number of fruits picked from each of the five already selected plants in each net plot were counted. Thus, the average number of picked fruits per plant at different times during the picking period was calculated.
12. **Fresh weight of red ripe fruits per plant**: At each picking, the weight of picked fruits from each of five already selected plants in each plot were weighed and the average weight of picked fruits per plant at different pickings was calculated.

13. **Dry weight of fruits per plant**: The fruits picked from each of tagged plants in each net plot were dried in sunlight at each picking. Thus the dry weight of fruits per plant at each picking was calculated. Later on, the weight of dry fruits per plant was calculated by adding weight of dry fruits per plant of all the pickings.

14. **The yield of fresh red ripe fruits (q/ha)**: The weight of fresh red fruits per net plot (kg) was converted into yield of fresh red-fruits in quintal per hectare.

15. **The yield of dry fruits (q/ha)**: The weight of dry fruits per net plot (kg) was converted into yield of dry fruits in quintal per hectare.

**D. QUALITY OF FRUIT**

16. **Length of red fruit**: Observations on the length of ten randomly selected red fruits was taken from the produce of each net plot. It was recorded from apical to blossom end.

17. **Diameter of red fruit**: At each picking ten fruits were selected randomly from the produce of each net plot and the diameter of each red fruit was measured in middle with the help
Plate II – Recording observation in the field
of vernier callipers and later on the average diameter of fruits was calculated.

18. **Specific gravity of red fruits**: The weight of ten fruits (g) was divided by their volume (ml) ascertained by water displacement and thus, the specific gravity of fruits was determined.

19. **The number of seeds per fruit**: Ten sundried fruits were sampled from the produce of each net plot. The seeds of each fruit were extracted and counted. Thus, the average number of seeds per fruit was worked out.

20. **The weight of fresh red ripe fruits (g)**: The average weight per fruit was calculated at each picking. The average weight of picked fruits per plant was divided by the average number of fruits picked per plant.

21. **The weight of dry fruits (g)**: The average weight per dry fruit was studied at each picking. The average weight of dry fruits per plant was divided by the average number of fruits picked per plant.

**Chemical composition of fruits**:

22. **The protein content**: Protein percentage in chilli fruits was calculated by multiplying the total nitrogen content in fruits with the factor 6.25.

Method: - 0.1 g material was taken in a 50 ml conical flask and to this 2 ml of conc. H₂SO₄ were added. The contents were gently heated on a hot plate. When volume was reduced to half of the original volume, 1.5 ml 30% H₂O₂ were added and heated gently till a clear extract was obtained. The contents were transferred into 100 ml volumetric flask and the volume 100 was made up with distilled water. The nitrogen was estimated in this acid extract of plant material.

Reagent: - Conc. H₂SO₄ (AR), 30% H₂O₂, 10% NaOH, 10% sodium silicate. The 10 g of fine powdered sodium silicate in about 50 ml boiled distilled water was dissolved and after cooling the volume was made up to 100 ml.

Nessler’s reagent: - 35 g of KI (Pot iodine) was dissolved in 100 ml of water and added 10% mercuric chloride (HgCl₂) solution with stirring or shaking until slight red precipitate remained (about 3.25 ml are required). Then introduced with stirring a solution of 120 g NaOH in 250 ml of water and made up to a little more mercuric chloride solution until there is a permanent turbidity. Allow the mixture to stand for one day and decant for sediment and kept the solution in stoppered dark coloured bottles.

Preparation of standard curve:

0.382 g of NH₄Cl was dissolved in water and diluted to 1 litre.

This solution contain 0.1 mg N per ml in the form of Ammonia. If more
dilute solution is required, dilute in 10 ml aliquot to 100 ml. The later solution contains 0.01 mg nitrogen per ml. Took 0,1,2,4,6,8 ml of this solution in separate 50 ml volumetric flask and added to each 10 drops of 10% NaOH and 10 drops of 10% sodium silicate solution and diluted with distilled water. Added 1 ml Nessler's reagent. Shaken the reagent and diluted to 50 ml mark after 15 minute, read the colour intensity with transmittance of 420 MU by colorimeter. Plotted the meter reading against nitrogen concentration and joined the points falling in line.

Development of colour:

(i) Took ml of the prepared extract in a 50 ml volumetric flask
(ii) Added 10 drops of 10% NaOH and 10 drops of 10% sodium silicate.
(iii) Diluted contents up to 40 ml with distilled water added 1 ml of Nessler's reagent.
(iv) The volume was made to 50 ml with distilled water and shaken thoroughly.
(v) Read colour intensity after 15 minutes on the colorimeter with transmittance of 420 using a reagent blank as reference.

24. The Phosphorus content: 1 gm of dried plant material was weighed and transferred into a 50 ml conical flask, 10 ml concentrate HNO₃ was added and conical flask was covered with watch glass. Then kept it for a night or 8 hours. Next day
The contents were heated on a hot plate. When the volume was reduced to half, then the 2 ml of perchloric acid (HClO₄) were added. The content were heated till the acid evaporated, leaving a clear white residue. The contents were transferred to a 100 ml flask and the volume was made upto the mark with distilled water.

**Vanadate - molybdate - yellow method:**

**PRINCIPLE:** Vanadate, molybdate and orthophosphates react to give a yellow complex in acid solutions. In Nitric acid solution the colour does not develop if the acidity is less than 0.2 N and it develops only slowly, if it is greater than 1.6N. The optimum concentration is 0.5 N, although there is a considerable range of Nitric acid concentrations when the colour develops, satisfactorily, 5 ml of 5 NHNO₃ per 50 ml of final volume was sufficient to give the optimum acidity. The acidity in aliquotes of the solution of plant ash is usually not sufficient to affect the density of the yellow colour.

The colour develops in several minutes (usually 30 minutes is allowed for full colour development) and is stable for 2 months at high phosphorus concentrations, but at phosphorus concentrations of 5 ppm it is stable for only 2 weeks.

**Reagents**

(I) **Ammonium molybdate - Ammonium vanadate In Nitric acid:**

22.5 gm of (NH₄)₆M₀₇O₂₄H₂O was dissolved in 400 ml of water
1.25 mg of Ammonium vanadate was dissolved in 300 ml of boiling water. Added the ammonium vanadate solution to the Ammonium molybdate solution and cool to the room temperature. Added 250 ml of concentrated Nitric acid and diluted to one litre.

(II) Phosphate standard solution: Dissolved 0.2195 mg of KH2PO4 (Potassium dyhydrogen phosphate) and diluted to 1 litre. This solution contains 50 ppm phosphorus take 10 ml take up volume 100 ml. This solution containing 5 ppm.

(25) The potassium content: The liquids obtained after wet digestion of P. estimation were diluted to desired level and were analysed for K by a direct reading on flame photometer.

(26) The ash content: The transfer 1 g of chilli powder in a crucible of known weight. Put the crucible containing the dry matter in a muffle furnance. Maintain the temperature of furnance at 400-450°C. Continue the heating till a white or grey coloured ash is formed. Remove the crucible from the furnase and cool it in a desiccater. Weigh the ash along with crucible, Subtract the weight of crucible which will give the weight of ash.

(27) The ascorbic acid content:

REAGENTS AND THEIR PREPARATION:

(I) Preparation of 6 per cent Metaphosphoric acid solution: The metaphosphoric acid solution is prepared by dissolving exactly
60 g of metaphosphoric acid in 160 ml of acetic acid and adding to it 500 ml of distilled water. Finally make 1000 ml with distilled water. Filtered the solution and stored.

(II) Preparation of 3 per cent metaphosphoric acid solution: Three per cent metaphosphoric acid solution is made by dissolving exactly 30 mg of metaphosphoric acid in 80 ml of acetic acid and adding to it 500 ml distilled water and the volume made up to 1000 ml. Filter the solution and store it.

(III) Preparation of dry solution: Weighed 50 mg of 2,6-dichlorophenol indophenol and 42 mg of sodium bicarbonate accurately on a chemical balance. Dissolved both in 150 ml of distilled water. Heated the solution gently on water bath to make it homogenous, and raised volume to 200 ml after cooling at room temperature. Transferred the solution in an air-tight brown container and placed in refrigerator.

(IV) Preparation of standard ascorbic acid solution: Weighed 100 mg of ascorbic acid on a chemical balance and dissolved in 3 per cent metaphosphoric acid and made the volume to 500 ml.

(V) Standardization of dye solution: Took 5 ml of standard ascorbic acid solution in a clear beaker to which 5 ml of 3 per cent metaphosphoric acid solution was added with the help of a pipette. Titrated this solution against the dye till a faint pink colour appeared which persists for not more than 15 seconds.
Estimation of ascorbic acid: weighted 30 gm (w1) of sample and
blended it with equal weight (w2) of six per cent metaphosphoric acid
for 3 to 4 minutes. Took 15g (w3) of this slurry in a 100 ml. (v1)
volumetric flask and made up volume by adding 3% metaphosphoric
acid. Filtered it through a faster filter paper (whatman No.42)

Titration: Filled the burette with standardized 2,6 dichlorophenol
indophenol dye. Took 10 ml (v2) of filtered solution in a conical flask
and titrated immediately against the standard dye solution (v1) till
faint pink colour appeared and persist for 15 seconds.

STATISTICAL ANALYSIS AND PRESENTATION OF DATA

The data recorded on the various characters during both the years of
experimentation were subjected to statistical analysis through
computer as suggested by Panse and Sukhatme (1978). The degrees of
freedom for the replications and factors are given below:

Table: Analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>D.F.</th>
<th>M.S.S</th>
<th>F (Cal)</th>
<th>F Table</th>
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<tbody>
<tr>
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<td>5%</td>
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<tr>
<td>Replication</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
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<td>4.10</td>
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<tr>
<td>G</td>
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<td></td>
<td>4.96</td>
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<tr>
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<td>4.10</td>
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<tr>
<td>Error (A)</td>
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<td></td>
</tr>
<tr>
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<td>2.86</td>
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<tr>
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<td></td>
<td></td>
<td>2.36</td>
</tr>
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<tr>
<td>Error (B)</td>
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</tbody>
</table>

Ea = Error variance of main plots
Eb = Error variance of sub plots
The significance of treatment effect was tested through variance ratio and the significance of difference between any two means of each factor of the present study was judged with the critical difference (C.D.) of 5 per cent level of significance. Which was worked out according to the following formula. The value of standard error was computed by formulas are given below:

\[
\text{S. E.}(d) = \sqrt{\frac{2E_a}{N}}
\]

\[
G = \sqrt{\frac{2E_a}{36}}
\]

\[
N \times G = \sqrt{\frac{2E_a}{12}}
\]

\[
D = \sqrt{\frac{2E_b}{18}}
\]

\[
N \times D
\]

(i) \(D\) at the same level of \(N\) = \(\sqrt{\frac{2E_b}{6}}\)

(ii) Otherwise = \(\sqrt{\frac{2[3E_b+E_a]}{4 \times 6}}\)

\[
G \times D
\]

(i) \(D\) at the same level of \(G\) = \(\sqrt{\frac{2E_b}{9}}\)

(ii) Otherwise = \(\sqrt{\frac{2[3E_b+E_a]}{4 \times 9}}\)
\[ N \times G \times D \]

(i) D at the same level of \( N \times G = \sqrt{\frac{2E_b}{3}} \)

(ii) Otherwise \[ = \sqrt{\frac{2[3E_b+E_a]}{3 \times 4}} \]

C.D. = SE.(d) x corresponding value of \( t \) at 5% p.

\[ t_a = 2.228 \]
\[ t_b = 2.028 \]
\[ t^1 = \frac{3E_b t_b + E_a t_a}{3E_b + E_a} \]