CHAPTER-4

Analysis of Soil Sample
It is well known fact that chemical constituents of the given plant vary depending upon factors like climatic condition and nature of the soil. The composition of soil plays vital role in the biosynthesis of various chemical components of plants. Hence, number of constituents and their relative percentage present in plant, changes from place to place. Therefore, it was thought of to carry out soil analysis of the area from which the roots of *Momordica dioica* Roxb. were collected.

The soil analysis was carried out as detailed below:

1. Sample collection
2. Soil testing
   a) Determination of pH
   b) Determination of available carbon
   c) Determination of available phosphorous
   d) Determination of potassium content

4.1 **Sample Collection:**

Soil samples were collected from different sites, from where the plant material was collected, at Konehosur village, Chordi, Shimoga district (30 Km from Shimoga city). Totally six samples were collected as described below:

Sample 1  – Collected near the plant 1 of *Momordica dioica* Roxb. at 1 foot depth surrounding the tuberous roots.
Sample 2  – Collected 3 mt. away from the plant.
Sample 3  – Collected 15 mt. away from the plant.
Sample 4  – Collected near the plant 2 of *Momordica dioica* Roxb. at 1 foot depth surrounding the tuberous roots.
Sample 5  –  Collected near the plant 3 of *Momordica dioica* Roxb. at 1 foot depth surrounding the tuberous roots.

Sample 6  –  Collected near the plant 4 of *Momordica dioica* Roxb. at 1 foot depth surrounding the tuberous roots.

All the samples were sun dried for 2 days, powdered and mixed in equal quantities to prepare the sample for testing. The mixed sample was heaved and divided into 4 quarters. Opposite quarters were discarded and the remaining quarters were once again mixed and heaved and divided into four quarters. This process was repeated so as to get around 500 g of the sample for soil analysis.

4.2. Soil Testing:

The soil sample was tested for the following parameters:

4.2.1 Determination of pH of the soil:

Soil pH is an indicative measure of chemical and biochemical properties of the soil under consideration. It includes ion exchange, retention of ions by colloids, biological activity and nutrient availability in soil. Thus, the soil pH assists in understanding the fertility status of the soil.

The pH of the soil can be determined either by potentiometric method or conductometric method. In the present investigation, potentiometric method was used to determine the pH of the soil.

Potentiometric Method:

In this method, the required electro chemical cell was constructed by using glass and calomel electrodes, which served as indicator electrode and reference electrode respectively.
Soil sample (10 g, 2 mm sieved) was mixed with distilled water (20 ml) and it was stirred for half an hour. The electrodes were dipped in the suspension and the electrode potential of the cell was recorded after stabilization. The pH was calculated by using the following equation

\[ \text{pH} = \frac{E}{0.0591} \]

Where \( E \) = Electrical potential of the cell

\( 0.0591 \) = Electrode potential of calomel electrode

The potential observed in this case was 0.3605, hence,

\[ \text{pH} = \frac{0.3605}{0.0591} = 6.1 \]

Thus, pH of the soil, under examination, was found to be 6.1 (slightly acidic in character).

4.2.2 Determination of organic carbon in soil:

Carbon and nitrogen exists in a definite ratio of 10:1 in almost all plants. Thus, by estimating the organic carbon, the amount of nitrogen content can be calculated.

Organic matter of the soil is oxidized by potassium dichromate solution in presence of dilute sulphuric acid. Potassium dichromate, being oxidizing agent, oxidizes organic carbon of the soil to carbon dioxide. The corresponding amount of potassium dichromate is reduced to green coloured chromium sulphate. The intensity of green colour depends on concentration of chromium sulphate, which inturn is proportional to the amount of carbon present. Thus, by determining the amount of chromium sulphate by colorimetric method, the amount of carbon content can be determined.
K₂Cr₂O₇ + 4H₂SO₄ → K₂SO₄ + Cr₂(SO₄)₃ + 4H₂O + 3[O],

3C + 6 [O] → 3CO₂

Colorimetric Method:

Standard curve for this experiment was obtained by oxidizing standard solution of sucrose. Accurate volumes of standard sucrose solution i.e., 0, 1, 2, 3, 4, 5, and 6 ml were pipetted out into different conical flasks and solution was made up to 10 ml by adding corresponding volume of potassium dichromate solution. Then, to each conical flask, concentrated sulphuric acid (20 ml) was added slowly. It was allowed to stand for half an hour and distilled water (10 ml) was added and left over night. Absorption was measured next day by using red filter at 660 nm wavelength in colorimeter. Standard curve was obtained by plotting the graph of concentration of sucrose against absorption.

Soil sample (10-15 g) was ground and sieved through 100 mesh sieve, 1 g of the sample was transferred to conical flask (100 ml). To this soil, standard potassium dichromate solution (10 ml) and concentrated sulphuric acid (20 ml) were added. After half an hour this solution was diluted by adding of distilled water (10 ml). It was left over night and next day the reading was taken as described earlier using colorimeter.

The percentage of carbon was measured by using the standard curve. The percentage of organic carbon was calculated from the following equation:

\[ \text{Organic carbon\%} = \text{Graph reading} \times 0.042 \]

\[ = 23.81 \times 0.042 \]

\[ = 1.00 \]
Hence, the percentage of carbon in soil sample was found to be 1% and corresponding nitrogen content was calculated as 0.1%. The result indicated that the soil is highly fertile.

4.2.3 Determination of available phosphorus (kg/acre):

Numerous methods are available for determination of phosphorus in soil sample. However, molybdenum blue method is most sensitive and widely used method for this determination.

The available phosphorous was extracted in solution form. It was estimated by converting phosphorous into phosphomolybdate complex. This complex was reduced by using standard solution of ascorbic acid. The reduced compound has blue colour, which can be measured colorimetrically. The intensity of colour corresponds to phosphorous content in the soil.

Colorimetric method:

Standard curve was obtained as follows.

Standard stock solution of potassium dihydrogen phosphate was prepared and accurate volumes i.e., 0, 2, 4, 6, and 10 ml of the standard solution were pipetted out into different volumetric flasks (50 ml). To each flask, sodium bicarbonate solution (5 ml) was added and the pH was brought down to 5 by adding required amount of 5N sulphuric acid. The volume was made upto 21 ml by adding required quantity of distilled water. Then 4 ml of reagent B was added and allowed to stand for 15 to 20 min. The color developed was measured by using colorimeter. Standard curve was obtained by plotting the graph of concentration of phosphorous pentoxide against absorption.
Reagent A – Standard solution of ammonium molybdate
Reagent B - Standard solution of ascorbic acid in reagent–A

Reaction

\[ \text{H}_3\text{PO}_4 + 12 \text{ H}_2 \text{MoO}_4 \rightarrow \text{H}_3\text{P}[\text{Mo}_3\text{O}_{10}]_4 + 12 \text{ H}_2\text{O} \]

Soil sample (2.5 g) was taken in 100 ml conical flask and activated charcoal (0.5 g) was added, which helps to remove undesired colour. Then Bray’s extractant (50 ml) [Sodium fluoride (22.2 g) was dissolved in distilled water (200 ml) and filtered. The filtrate was added to distilled water (18 l) containing concentrated hydrochloric acid (40 ml). The volume was made with distilled water to 20 l. The above solution has sodium fluoride (0.03 M) and hydrochloric acid (0.025 M)] was added to this solution and shaken mechanically for 30 min and filtered. The above solution (5 ml) was taken in a separate conical flask and to this 5N sulphuric acid (0.5 ml) and distilled water (16 ml) were added. To this solution reagent B (4 ml) was added, stirred well and allowed to stand for 15 to 20 min. The colour developed was measured by using colorimeter.

The amount of available phosphorous was determined by using standard curve.

Average P$_2$O$_5$ kg/acre = A x 3.6

Where A is graph reading = 3.89

Therefore, Average P$_2$O$_5$ kgs/acre = 3.89 x 3.6 = 14

The phosphorous content to the extent of 14 kg/acre is indicative of moderate fertility of the soil with respect to available phosphorous.
4.2.4 Determination of potash (kg/acre):

Mineral soils generally contain potassium in larger amounts than nitrogen and phosphorous. Most of the potassium is present in the inorganic form as part of the crystalline structure of mica, fieldspars and micaceous minerals.

Normally a flame photometer is used to determine the amount of potassium in the soil in terms of available potassium oxide in kg/acre. Ammonium acetate method (Merwin and Peach) is widely used for this purpose.

Standard curve:

Stock solution of potassium—1000 ppm potassium oxide solution was prepared and 10, 20, 30 and 40 ml of stock solution was pipetted out and diluted separately to 1000 ml with neutral ammonium acetate solution. After stabilizing the instrument, readings were recorded for these diluted solutions by using flame photometer. Standard curve was obtained by drawing the graph of concentration in ppm against the instrument reading.

Soil sample (5 g) was ground and neutral ammonium acetate solution (25 ml) was added and the mixture was shaken thoroughly for 5 min. Then the solution was filtered and reading was noted down by using flame photometer. The amount of potassium present in the soil was determined by using standard curve and the following equation:

\[
\text{Available K}_2\text{O (Kg/acre)} = \frac{A}{10^6} \times \frac{\text{Volume of extractant}}{\text{Weight of the soil sample}} \times \frac{2 \times 10^6}{2.2}
\]

\[
= \frac{A}{10^6} \times \frac{25}{5} \times \frac{2 \times 10^6}{2.2}
\]

\[
= A \times 4.5
\]
Where \( A = \) reading of the concentration of \( K_2O \) in ppm

Therefore, Available \( K_2O \) (Kg/acre) = 44.44 x 4.5 = 200

From this value, it can be concluded that a soil sample under consideration is highly fertile with respect to available potash.

**Table-4.1: Summary of the soil analysis**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Examined parameter</th>
<th>Unit</th>
<th>Report</th>
<th>Type of the soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>--</td>
<td>6.1</td>
<td>acidic</td>
</tr>
<tr>
<td>2</td>
<td>Organic carbon</td>
<td>%</td>
<td>1.0</td>
<td>highly fertile</td>
</tr>
<tr>
<td>3</td>
<td>Available phosphorus</td>
<td>Kg/acre</td>
<td>14.0</td>
<td>moderately fertile</td>
</tr>
<tr>
<td>4</td>
<td>Available potassium</td>
<td>Kg/acre</td>
<td>200</td>
<td>highly fertile</td>
</tr>
</tbody>
</table>