Plant Testing and Rapid Plant Tissue Tests

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Soil fertility evaluation can be carried out not only by soil tests but also by characterization of growth and nutrient composition of plants. Some plant-based soil fertility evaluation techniques are available i.e. visual symptoms of nutrient deficiency of the plants at field and plant tissue analysis by different chemical methods at laboratory.

1. Visual Nutrient Deficiency Symptoms :-

When soil is not able to supply adequate amounts of one or more plant nutrients, plants start showing starvation signs called deficiency symptoms. These symptoms are nutrients specific and show different patterns in different plants. For example, P deficiency typically causes stunted growth and N deficiency an overall yellowing of leaves and stunted growth. Potassium deficiency shows as marginal 'spotting' that develops into complete necrosis of the leaf margins. It is a good tool to detect deficiencies of nutrients in the soil. Some other nutrients deficiency symptoms are shown by the crop plants are given below :

- Complete crop failure at the seedling stage
- Severe stunting of plants
- Specific leaf symptoms appearing at varying times during the season
- International abnormalities such as clogged conductive tissue
- Delayed crop maturity
- Reduces crop productivity
- Poor quality of crops including difference in protein, oil or starch

However, one must develop diagnostic proficiency through practice and close observation to identify nutrient deficiencies in different plants. The deficiency symptoms in many cases are not always clearly defined and in cases, the symptoms can be common to other causes or may be masked by other nutrients. Some typical examples are given as under :

- N deficiency can be confused with S deficiency.
- Calcium deficiency can be confused with B deficiency.
- Fe deficiency can be confused with Mn deficiency.
- Effect of virus, little leaf etc. can be confused with Zn and/or B deficiency.
- Brown streak disease of rice can be confused with Zn deficiency.

Deficiency symptoms always indicate severe starvation and therefore the crop may have suffered before the deficiency symptoms appear. Many crops start losing yields well before deficiency signs start showing. This yield limiting condition is called hidden hunger which refers to a situation in which a crop needs more of a given nutrient and yet has not shown any deficiency symptom. The nutrient content is above the deficiency symptom zone but still considerably below that needed for optimum crop production. In this case, significant responses can be obtained with application of nutrients even though no recognizable symptoms have appeared. To overcome such conditions, it is always recommended to confirm deficiency problem with other diagnostic techniques and the most common is soil testing.

2. Plant Analysis :-

The plant root absorbs the nutrient from the soil and these nutrients are transported to other plant parts where they are needed. The concentration of the nutrients in the cell sap is usually a good indication of how the plant is supply at the time of testing. It is possible to anticipate certain problems of deficiency of nutrients. Composition of plants or a portion of the plant with respect to elements essential for growth as worked out by plant analysis can be interpreted by two widely used methods: (i) critical nutrient concentration and (ii) the Diagnosis and Recommendation Integrated System (DRIS), which deals primarily with nutrient ratios.

As with soil testing, critical nutrient concentration approach consists of correlation and calibration studies, because a significant relationship exists between the nutrient concentration in a plant and plant response to addition of the nutrient *(*Figure 3*)*. At the critical concentration plant performance changes from suboptimum to optimum. Below the critical concentration an increase in the supply of the nutrient leads to an increase in yield. Plant analysis is useful in identifying hidden hunger in plants and in locating soil areas where deficiencies of one or more nutrients occur. Critical nutrient concentration depends upon growth stage and plant part that is sampled. To be of use in deficiency diagnosis, critical concentrations must be determined for individual crops, when no other nutrient limits growth. Under these conditions the critical value should be independent of soil type. Plant analysis is particularly useful for fruit crops because of their perennial nature and having extensive root systems. The optimum values of nutrient content in plants are pre-standardized so as to make recommendation after sample analysis.

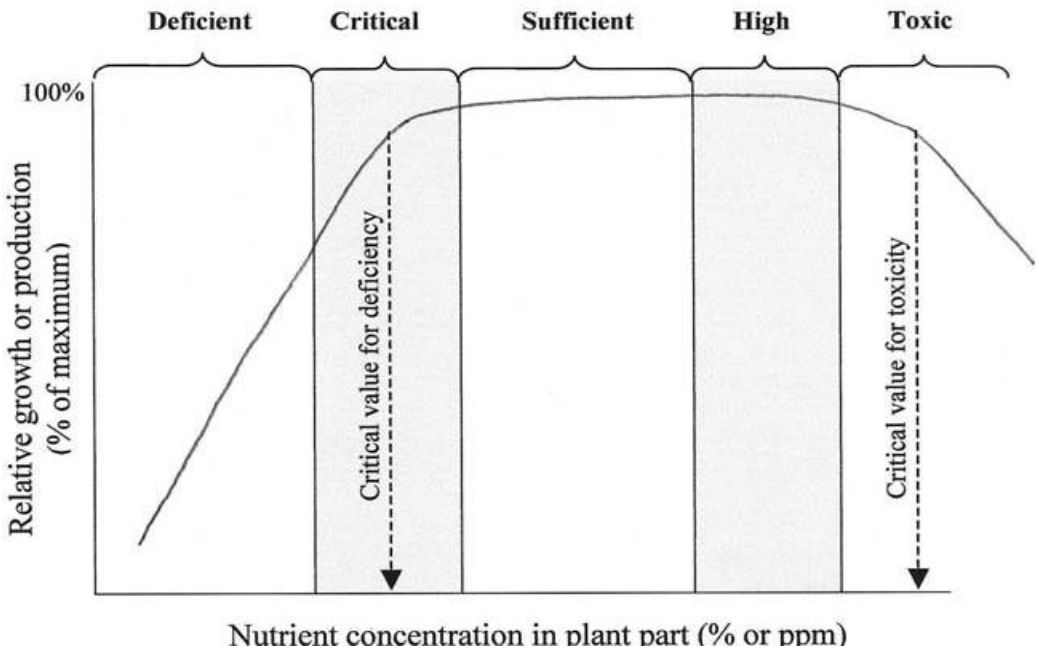

Figure 3 : Critical nutrient range approach for plant analysis interpretation

The crop growth and productivity is conditioned by many factors of which, the nutrient status (contents) of plant parts such as leaf, stem etc. play a critical role. Moreover, the leaf and stem are considered as the indicator parts of plants for assessing the nutrient content of plant. Each crop plant requires the essential element at a specific concentration at different growth stages and it is known as 'Critical Level'.

Critical levels and foliar concentration of nutrients in plants :

Element	**General range**	**Critical level**	**Concentration for Foliar Spray**
Nitrogen (N)	2.0 - 4.0 %	< 2 %	1.0 – 3.0 %
Phosphorous (P)	0.2 - 0.5 %	< 0.1 %	0.05 – 1.0 %
Potassium (K)	1.5 - 3.0 %	< 1.0 %	0.8 – 1.2 %
Calcium (Ca)	0.5 - 3.0 %	< 0.1 %	0.3 – 0.6 %
Magnesium (Mg)	0.2 - 0.5 %	< 0.2 %	0.2 – 0.4 %
Sulphur (S)	0.2 - 0.5 %	< 0.15 %	0.2 – 0.3 %
Iron (Fe)	50 – 150 ppm	< 5.0 ppm	20 – 400 ppm
Copper (Cu)	5 – 20 ppm	< 4.0 ppm	10 – 20 ppm
Zinc (Zn)	20 – 100 ppm	< 15.0 ppm	15 – 50 ppm
Manganese (Mn)	20 – 500 ppm	< 20.0 ppm	2.0 – 10 ppm
Nickel (Ni)	0.05 to 5 ppm	< 10.0 ppm	0.03 – 0.06 ppm
Boron (B)	2 – 100 ppm	< 20.0 ppm	5.0 15 ppm
Molybdenum (Mo)	1.0 – 2.0 ppm	< 0.1 ppm	0.5 – 5.0 ppm
Chloride (Cl)	0.2 - 2.0 ppm	-	-

When the nutrients content of plant depletes below the critical level the plants may exhibit some symptoms. Based on the plant or soil test, the required nutrients can be applied for crops to sustain the growth and rectify the deficiency disorders. The rapid tissue test would way to rectifying the nutritional problems for quick recovery. However, the quantitative estimation of both plant and soil for nutrients concentration will be more useful and economic for applying fertilizers either as basal or foliar and would be the long term strategy to reduced nutritional problems.

On dry weight basis, the normal healthy cultivated crop plant will have the foliar concentration of essential nutrients. It will vary depends upon the variety, soil types, growth stages and other environmental and cultural operations.

Purpose or advantage of plant analysis **-** The general purposes of plant analysis are as follows :

- To confirm diagnosis of visible symptoms.
- To identify hidden hunger.
- To locate areas of incipient deficiencies.
- To indicate whether applied nutrients entered the plants.
- To understand the interactions among nutrients.
- To aid the understanding of internal plant functioning.

Plant Sampling –

The sample must be a true representative of the crop under field conditions. The best way to collect a representative plant sample is to traverse the field diagonally and collect the samples from the deficient and normal plants. These samples should be composited separately representing poor growth and normal growth. Plants which are infected with disease or attack of insects, which are under stress due to excess water or drought and those which are heavily coated with dust should not form a part of the sample. Extra ordinary care must be taken to avoid contamination resulting from dust, soil, fertilizer and spray residues etc. The samples so collected should be transferred to paper bags indicating the sample number and/or the field number or other details. In case the sampling site is far away from laboratory and requires longer travel period, the sample may be placed in polyethylene bag and transported in an ice chest. Low temperature will minimize the physiological activity and also check the spoilage of sample due to higher temperature and high humidity.

Processing and storage of plant samples -

Washing :

The fresh plant samples brought to the laboratory should be immediately washed in order to make them free from dust or any other adhering substance. Samples should first be washed under the running tap water and if necessary. Subsequently, these samples should be washed with acidified distilled water (one ml concentrated HCl/liter) followed by thorough rinsing twice with deionized water or distilled water.

Drying :

After washing, the excess water in the sample may be soaked by placing them between the folds of filter paper sheets. All the samples should dry as rapidly as possible so as to reduce chemical and biological degradation if any. Sample should be dried in a hot air oven at 70^{o}C. The material to be dried should be loosely packed so as to allow the free movement of moisture laiden air. Oven made of stainless steel shelves should be used. Plant samples have been dried generally for 24 to 36 hours. Care should be taken to insure that the plant sample is not bunched together in isolated area of the oven, as large losses of N may occur. Plant samples may also be dried in a microwave oven. This procedure is rapid and can dry individual samples to a brittle state within five minutes.

Grinding and storage : The oven dried samples should be group in a grinder, fitted with stainless steel blades so as to pass the samples through a 40- mesh sieve. After grinding, the plant sample should be mixed thoroughly and transferred to polyethylene or paper bags, labelled clearly and then stored in the room meant for plant samples. Avoid regular type of grinder as it normally provided contamination. The sample grinder consisting of hard plastic can be used for grinding.

Precautions :

1. Avoid sampling the plants which are infected with disease and/or insects or those suffering from effects of excess or scarcity of water.
2. Before subjecting the samples for testing, decontaminate the collected samples by thorough washing, and rinsing.
3. Washed samples should be dried as rapidly as possible. The samples should not be packed tightly during drying in an oven and the temperature should not exceed 70^{o}C.
4. The grinder to be used for grinding the samples should have stainless steel blades or hard plastic to avoid contamination and the ground samples must be passed through a 40- mesh sieve to ensure uniform fineness. For iron determination, it is advisable to grind the sample by hand in an agate or porcelain mortar or in a mill made of non-ferrous alloy if hard plastic grinder is not available.

5. The processed samples should be kept in polyethylene or paper bags and stored in room free of dust soil and smoke etc.
6. Care should be taken not to rub the plant tissue acid mixture of any samples.

Methods of digestion or ashing of plant tissues -

Different types of acids are required for the estimation of different nutrient contents in plant samples. Phosphorous, potassium, calcium, magnesium, sulphur, micronutrients (Zn, Fe, Mn & Cu) etc are estimated by diacid mixture (1 :2.5 $HClO_4$: HNO_3) solution. While, Conc. H_2SO_4 is used for nitrogen estimation in plant samples.

Wet ashing is accomplished by digestion with HNO_3, $HClO_4$ and H_2SO_4 or else with HNO_3 and $HClO_4$ especially for S determination. The method is unsuited for B determination as the element is volatilized during wet ashing. While, dry ashing is done in muffle furnace at temperature varying from 400 to 500^oC for 2-8 hours. The plant sample taken in platinum or vitreous silica crucible is treated with $Mg(NO_3)_2$ or Na_2CO_3 prior to placing in a muffle furnace. Dry ashing at 500^oC is considered satisfactory for the determination of Fe, Mn, Cu in biological materials. The use of stainless steel lined muffle furnaces eliminates contamination from Al and Zn if any during ashing. Both wet and dry ashing procedures are widely practiced. The choice of ashing procedure depends mainly upon the availability of equipment and reagents and personal considerations of the worker. Some merits and demerits of the two procedures are given below :

Wet ashing	**Dry ashing**
• Boron completely and halides to a great extent are lost.	• Unless bases such as $MgNO_3$, or Mg-acetate are employed P and S are lost.
• Silica formed dose not absorb micronutrients and thus, the extract after filtering off silica is fit for most analyses.	• Silica in ash absorbs micronutrients and thus determining of Cu, Fe, Mn & Zn silica is to be removed by HF treatment. HF treatment is time consuming and requires platinum dishes.
• Requires substantial amount of reagents and more time per sample. Furthermore, arrangements are needed for the disposal of acid fumes	• Plant material is directly ashed and requires only the porcelain or silica dishes and muffle furnace.

Wet digestion of plant sample with diacid mixture :

The plant sample is diacid with mineral acid mixture [$HClO_4$ (60%) and Conc. HNO_3] and heated for more rapid decomposition. The volatile constituents disappear and non-volatile mineral elements enter into solution. The heating is continued until digest is reduced to few ml of clear white residue. The residue is dissolved in HCl. The test solution is prepared by dilution and filtration.

- **Diacid mixture (1 : 2.5 $HClO_4$: HNO_3) :** Take one liter of 60% hydrochloric acid and 2.5 liter conc. nitric acid in five liter reagent bottle and mixed properly.

Procedure

- Add 5 ml of diacid mixture into the beaker in which 1.0 g plant sample has been transferred in the beaker and place on a hot plate for brisk heating.
- Keep a portion of the beaker mouth open to permit the gases to escape.
- Continue heating until evolution of copious dense white fumes subsides leaving about 3 ml of colourless solution in the beaker which on cooling gives a whitish residue. If the residue is not white and signs of beginning of charring are seen, remove the beaker from the hot plate and let it cool.
- Then add 2 ml of diacid mixture and place the beaker again on hot plate.
- Continue heating until the contents is reduced to about 2-3 ml of colourless clear solution. In order to avoid excessive heating, a layer of sand may be spread over the hot plate.
- If white residue is not obtained, the residue is treated again with 2ml of the diacid mixture and the heating be continued till the contents is reduced to about 2-3ml.
- Thereafter, the beaker to be removed from the hot plate or sand bath.
- Allow the beaker to cool.

Preparation of plant test solution :

- Add 5 ml of glass distilled water into the beaker containing digested residue.
- Heat the contents gently on a hot plate until white fumes appear.
- Remove the beaker and let it cool.
- Then, add 10 ml of glass distilled water and heat the contents to dissolve the residue.
- Take a 100 ml volumetric flask fitted with a funnel lined with Whatman No. 1 paper.
- Transfer the contents of the beaker into the funnel using a glass rod collecting the filtrate in the volumetric flask.
- Rinse the beaker with about 15 ml portions of glass distilled water and transfer each rinsing into the funnel in order to transfer the digested residue quantitatively.
- Collect the filtrate from each washing into the same volumetric flask.
- Then wash the residue on filter paper with small portions of glass distilled water and collect the washing until the volume of filtrate reaches 100 ml mark.
- Stopper the flask, label it and then preserve it for the elemental analysis other than Ca, Mg and S.
- Similarly run a blank digestion and subsequently prepare the blank test solution following the above mentioned procedure.

If Ca and Mg is to be determined, add 5 ml of conc. HCl instead of glass distilled water into the beaker containing the plant digested residue. Swirl the contents and heat it to warm. Take a clean 100 ml volumetric flask fitted with a funnel lined with Whatman No. 1 filter paper. Transfer the contents of the beaker into the funnel and collect the filtrate in the volumetric flask. Then, add another 5 ml of conc. HCl into the beaker to dissolve the remaining residue in the beaker. Transfer the solution into the same funnel, collecting the filtrate in the same volumetric flask. Repeat subsequent washing of the contents of the beaker with small portions of 6N HCl and transfer the washing into the same funnel until the filtrate reaches 100 ml mark. Similarly run a blank digestion and then prepare test solution using conc. And 6N HCl instead of glass distilled water as described above.

Method of plant analysis :-

Parameters	**Digested by**	**Name of Instruments**
Nitrogen	Conc. H_2SO_4	Kjeldahl flask or Nitrogen analyser
Phosphorous	Diacid Mixture	Spectrophotometer
Potassium	Diacid Mixture	Flame-photometer
Sulphur	Diacid Mixture	Spectrophotometer
Ca & Mg	Diacid Mixture	Volumetrically
Micronutrients (Cations)	Diacid Mixture	Atomic Absorption Spectrophotometer
Boron / Molybdenum	Diacid Mixture	Spectrophotometer

3. Diagnosis and Recommendation Integrated System (DRIS) :-

The Diagnosis and Recommendation Integrated System (DRIS) was developed by Beaufils in 1973, this method consist in dual relation between a pair of nutrients (N/P, P/N, N/K, K/N...) instead of the use of sufficiency range or critical level that are called univariate methods, because only the individual concentration of the nutrients in leaf tissue is taken into consideration while no information about the nutritional balance is provided. DRIS enables the evaluation of the nutritional balance of a plant, ranking nutrient levels in relative order, from the most deficient to the most excessive.

With the use of dual relation on DRIS, the problem with the effect of concentration or dilution on the nutrients in plants is solved, because, according to Beaufils (1973); Walworth & Sumner (1987) with the growth of leaf tissue, on one hand the concentration of nitrogen, phosphorus, potassium and sulphur decrease in older plants and the concentration of calcium and magnesium increase in older plants on the other hand. When it is used the DRIS method, where the dual ratio is used, the values

remain constant, minimizing the effect of biomass accumulation, that is one of the major problem with sufficiency range and critical level method.

The DRIS approach focuses on nutrient ratios because within the plant these are more important than the concentration of any individual nutrient. As nutrient ratios in plants tend to be more constant throughout the growing season, use of DRIS allows greater flexibility in the time of plant sample collection. The DRIS system first establishes norms for all nutrient ratios associated with maximum crop yield either by widespread sampling of a crop in a given region or from scientific literature. All factors that affect crop yields are also measured at the time of plant sample collection so as to establish an integrated relationship between the DRIS norms, crop yield and other growth limiting factors. Interpretation of plant analysis by the DRIS system can be done graphically or mathematically by the calculation of DRIS indices. Computer programmes are now available for rapidly calculating DRIS indices for wider use of DRIS by plant analysis laboratories.

Advantages : There are four advantages of DRIS -

- The scale of interpretation is continuous numeric scale, and easy to use,
- Put the nutrients in order of the most deficiency to the most excessive,
- Identify cases where the yield of plant is being limited by into factor as nutritional status and
- The Nutritional Balance Index (NBI) give a result of combined effects of nutrients.

4. Rapid Plant Tissue Tests :-

It is a qualitative or semi quantitative method. Fresh plant tissue or sap from ruptured cells is using for N, P, K and other nutrients testing. In the cell sap, added certain reagents to develop colour. Based on intensity of colour low, medium and high colour is categorized which indicates the deficiency, adequate and high nutrients in the plants respectively. It is mainly used for predicting deficiencies of nutrients and it is possible to forecast certain production problems.

Rapid plant tissue test for NPK (Cook and Wheeler, 1978) :-

Rapid plant tissue tests are performed in the standing crops on above ground portions to find out deficiency of particular element before the plant become acute deficient and help to correct the nutritional level by applying deficient elements through fertilizers. Plants accumulate in their tissues nitrate, phosphate and potassium as ions which can be determined by chemical tests. These tests are essentially rapid qualitative

tests in which the nutrients are extracted from leaf parts with the help of chemical reagents and the concentration of a particular nutrient is estimated by the difference in intensity of colour, the results are classified as low, medium, high and very high.

1. Tests for nitrates :

In most of the crops like wheat, corn, sorghum, potato etc. except paddy nitrogen accumulates in nitrate form that represents the chief inorganic nitrogen reserve of the plant tissues hence plant supply of nitrogen can be related to the nitrate content of the plant tissue. These tests are useful from the early growth period to the grand growth period. As nitrogenous fertilizers can be applied to standing crops, the plant tissue test for nitrate nitrogen is of practical importance.

Diphenyl amine test : This is the most common field test used for determining the relative abundance of nitrates in plant tissues.

Reagent - Diphenyl amine (1%) : Dissolve 1 g of diphenyl amine in 100 ml of concentrated sulphuric acid. This solution is very corrosive hence handle it with care.

Procedure :

- In case of thick stalk plants like maize, sorghum etc. nitrate test is made at the base of the leaf midrib without destroying the entire plant. Cut a thin vertical section at the plant node and add a drop of the diphenyl amine reagent. In about 30 seconds blue colour develops if plant sap contains nitrate nitrogen.
- In case of thin stalk plants like wheat, barley, pearl millet etc. Uproot the plant and cut the stem near the lower node in slanting manner.
- Add 2-3 drops of diphenyl amine reagent. A dark blue colour will develop if plant sap contains abundance of nitrates.

Observation and interpretation :

The intensity of blue colour indicates the concentration of nitrate nitrogen in the sap. The inference of colour is interpreted in the following manner.

- No colour - Plant is severely deficient in nitrogen (urgent need to apply nitrogenous fertilizers).
- Slightly blue colour - Plant is deficient in nitrogen (need to apply nitrogenous fertilizers).
- Medium blue colour - Plant is slightly deficient in nitrogen (application of nitrogenous fertilizers will give a slight increase in crop yield, but will increase the protein in cereal grains).

- Dark blue colour - Plant is adequately supplied with nitrogen (no need to apply nitrogen fertilizers).

2. **Test for phosphate :** Inorganic phosphate ($H_2PO_4^{2-}$, HPO_4^{2-}) are present in the cell sap hence the test is carried out with the leaf tissue

Reagent :

- Dissolve 8 g of ammonium molybdate in 200 ml of distilled water in a beaker. In another beaker take 74 ml of distilled water and add 126 ml of concentrated hydrochloric acid and shake well. Now add this dilute solution of HCl to the solution of ammonium molybdate slowly with constant stirring. The concentrated phosphate reagent should be diluted four times with distilled water just before use. The diluted reagent become unsuitable for use after a few weeks hence fresh working solution should be prepared while determining phosphate in plant tissue.
- Dry stannous oxalate or stannous chloride.

Procedure :

- Cut leaf blade into fine pieces after removing thick mid rib.
- Place a tea spoonful of the finely cut tissue in 50 ml graduated beaker.
- Now, add phosphate reagent 1 up to the 10 ml mark and shake the contents vigorously for a minute to remove most readily soluble phosphates.
- After shaking, add a small amount of stannous oxalate (approximately the size of a pin head).
- Mix the contents and observe the colour.

The amount of inorganic phosphate present in the plant tissue is indicated by the intensity of blue colour, which may range from light blue to dark blue.

Observation and interpretation :

The intensity of colour noted from the test is interpreted as follows:

- No colour or yellow colour - Plant is highly deficient in phosphorus (need to apply phosphatic fertilizers for increasing crop yield)
- Green or bluish green - Plant is deficient in phosphorus (need to apply phosphatic fertilizers).
- Light blue colour - Plant has medium phosphorus supply (Slight increase in yield is expected with application of phosphatic fertilizer).
- Medium blue colour - Plant is adequately supplied with phosphorus (no need to apply phosphatic fertilizers).

- Dark blue colour - Plant is abundantly supplied with phosphorus (no need to apply phosphatic fertilizers).

When the test indicates need to apply phosphatic fertilizers, a phosphatic fertilizer like super phosphate which contains water soluble P_2O_5 should be applied as to enable the growing plants to utilize the phosphorus immediately. During the growth period, foliar application of phosphatic fertilizers of suitable concentration is more effective than soil application.

3. Test for potassium :

Potassium is not known to be a definite part of any plant structure or tissue, but it exists in soluble form in the cell sap

Reagent :

- Dissolve 5 g of sodium cobaltinitrite and 30 g sodium nitrite in 50 to 70 ml distilled water, add 5 ml of glacial acetic acid, make it to 100 ml in a volumetric flask and allow to stand for several days.
- Add 5 ml of this solution to a solution of 15 g of sodium nitrite in 100 ml of distilled water and adjust the pH 5.0 with acetic acid. Since the sodium cobaltinitrite concentration is an important factor in determining the sensitivity of the test, use this chemical in pure form.
- Ethyl alcohol (95%)

Procedure :

- Cut leaf tissue into fine pieces with scissor
- Place 1/4th tea spoon of the finely cut leaf tissue in a glass beaker and add 10 ml of potassium reagent 1 at 21 ^oC.
- Maintain the temperature 21 ^oC with the use of ice water.
- Shake the contents of beaker vigorously for a minute carefully.
- Then add 5 ml of 95% ethyl alcohol (potassium reagent) and mix.
- After 2-3 minutes, observe the turbidity formed.

Observation and interpretation :

- Only a trace of turbidity –Plant is deficient in potassium supply (need to apply potassic fertilizers as top dressing or in the form of foliar spray).
- Medium turbidity- Doubtful potassium supply in plant (no need to apply potassic fertilizer).

- Very high turbidity-Plant is adequate in potassium supply (no need to apply potassic fertilizer).

Precautions :

- For rapid plant tissue test the stage of growth and plant part to be taken should be considered first.
- Intensity of turbidity developed by various reagents for an element in a non deficient plant should be compared with a deficient plant for comparison.
- The knife and other equipments used for plant tissue test should be cleaned and washed with distilled water.
- Do not use reagents which has been kept for longer period.
- Do not use oxidized stannous oxalate powder.
- Use AR grade sodium cobalti-nitrite for the rapid tissue test of K.

5. Biological Test :-

The use of growing plants or microorganism understandably has much appeal in the study of fertilizer requirements and serves as a tool for measuring the fertility status of the soils. This includes field test, laboratory and greenhouse tests, microbiological methods.

5.1 Field Test –

The field-plot method is one of the oldest and best-known of the biological tests. The series of treatments selected depends on the particular question the experimenter wishes to have answered. The treatments are then randomly assigned to an area of land, known as a replication, which is representative of the conditions. Several such replications are used to obtain more reliable results and to account for variations in soil and management.

These experiments are helpful in the formulation of general recommendations. When large numbers of tests are conducted on soils that are well characterized, recommendations based on such studies can be extrapolated to other soils with similar characteristics. Field tests are expensive and time consuming, and one is unable to control climatic conditions and other limiting factors. They are valuable tools, however, and are widely used by experiment stations, although they are not well adapted for use in determining the nutrient status of large numbers of soils. Rather, they are used in conjunction with laboratory and greenhouse studies as a final proving ground and in the calibration of soil and plant tests.

5.2 Laboratory and Greenhouse Tests –

Simpler and more rapid biological techniques which still involve higher plants but utilize small quantities of soil have been developed. These methods have met with wide acceptance on the European continent and have frequently been employed in the United States.

5.3 Mitscherlich Pot Culture:

In this method oats are grown to maturity in pots containing 6 lb of soil. A total of 10 pots are used. The yields of the N-P and N=K treatments are expressed as a percentage of the yield from the complete N-P-K treatment. For example, if the N-P-K treatment yielded 80 g and the N-K treatment, 60 g, the yield would be 75%.

With these percentage yields, the plant nutrient reserve in the unfertilized soil can be read in pounds per acre from yield tables prepared by Mitscherlich, and from these same tables predictions of the percentage increased in yield expected from the addition of given amounts of nutrients can be obtained.

5.4 Neubauer Seedling Method:

The Neubauer technique is based on the uptake of nutrients by a large number of plants grown on a small amount of soil. The roots thoroughly penetrate the soil, exhausting the available nutrient supply within a short time. The nutrients removed are usually determined quantitatively by chemical analysis of the entire plant.

In some procedures, however, the tops and the roots are harvested and analyzed separately. Tables have been set up to give the minimum values for satisfactory yields of various crops. The Neubauer method has been used for the availability of several nutrients including P, K, Ca, micro-nutrients, or fertilizer materials.

5.5 Jenny's pot culture tests:

This method using lettuce crop with NPK nutrients. He grouped the plant into 3 categories i.e.

(i) Definite deficiency,

(ii) Probable deficiency and

(iii) Uncertain deficiency based on the percentage of yields.

6. Microbiological Methods –

Winogradsky was one of the first to observe that in the absence of mineral elements certain micro-organisms exhibited a behavior similar to that of higher plants. It was shown that the growth of *Azotobacter* served to indicate the limiting mineral nutrients in the soil, especially calcium, phosphorus, and potassium, with greater

sensitivity than chemical methods. Microorganisms are sensitive to deficiency of nutrients and could be used to detect the deficiency of any nutrient. A soil is treated with suitable nutrient solutions and cultures of various microbial species (bacteria, fungi) and incubated for a few days. Then observing the growth and development of organisms in terms of weight or diameter of the mycelia pad, the amount of nutrient present in the soil is estimated. For example – (a.) *Azotobactor* method for Ca, P and K; (b.) *Aspergillus Niger* test for P and K

***Aspergillus Niger* test for P and K :**

To determine phosphorus and potassium small amounts of soil are incubated for a period of four days in flasks containing the appropriate nutrient solution. The weight of the mycelial pad or the amount of potassium absorbed by these pads is used as a measure of the nutrient deficiency. Mehlich devised a more refined technique in which the mycelial pad is also analyzed for potassium. An example of the criteria used is tabulated.

Weight of four pods (g)	K absorbed by Aspergillus Niger per 100 g of soil (mg)	Degree of K-deficiency
< 1.4	< 12.5	Very deficient
1.4 – 2.0	12.5 – 16.6	Moderate to slight deficiency
> 2.0	> 16.6	Not deficient