NAVSARI AGRICULTURAL UNIVERSITY

As per the Fifth Dean Committee Recommendations for the
B. Sc. (Hons.) Agri. Course Curriculum

ICAR

Practical Manual

Ag. Chem. 2.2 (2 + 1)

Manures, Fertilizers and Soil Fertility Management

Second Semester B.Sc. (Hons.) Agriculture

PREPARED BY

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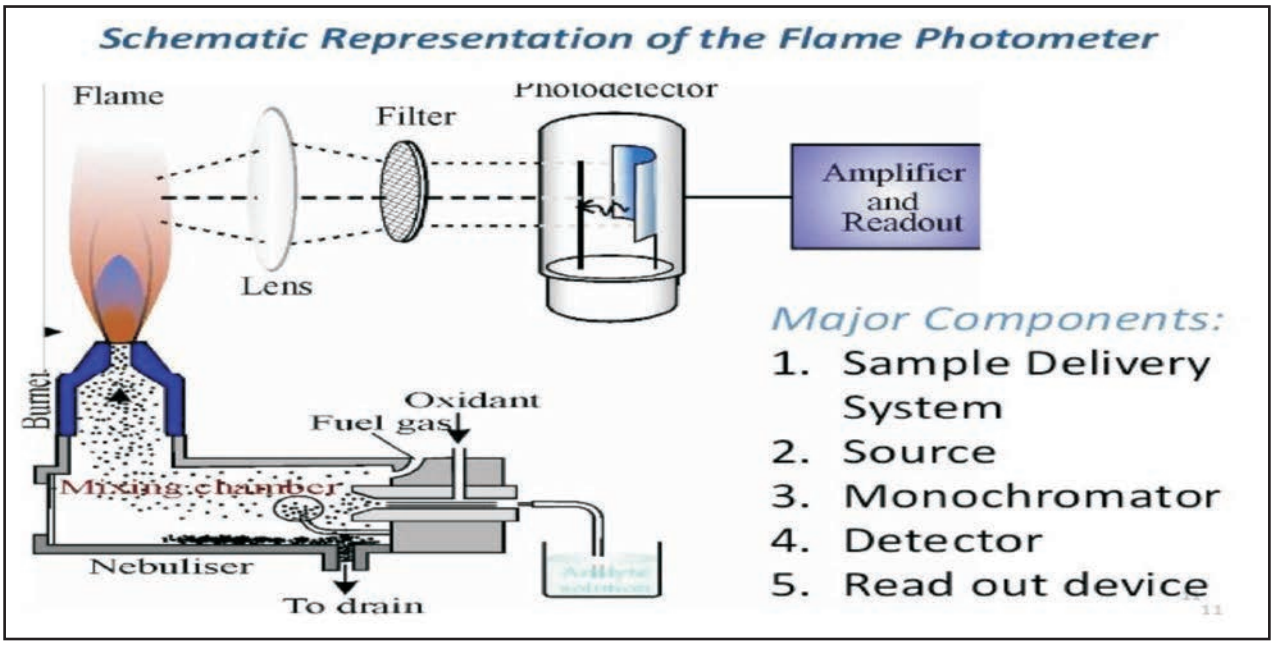
Khjeldahl Unit

Spectrophotometer

Flame Photometer



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FOREWORD

The course entitled "Manures, Fertilizers and Soil Fertility Management" is offered as a mandatory course in the curriculum of Second Semester B.Sc. (Hons.) Agriculture through the Department of Agricultural Chemistry and Soil Science, under College of Agriculture, Navsari Agricultural University, Waghai (Dangs), Gujarat. The manual contains very basic and practically useful information on estimation on nutrient from organic manure, fertilizer and soil.

A new course on Manures, Fertilizers and Soil Fertility management has been designed in agricultural universities at undergraduate as per syllabus laid out by the 5th Deans Committee recommendations of ICAR. The 36th academic council meeting of NAU, held on 25th April 2017, with item note 36.05 and approved V Dean recommendation from the year 2017-18 along with the detail distribution of courses.

An attempt is being made in this manual to compile the available up-to-date information on the subject in the most easily understandable manner and to make the information user-friendly. Board of studies of Soil Science and Agricultural Chemistry, Navsari Agricultural University, has already decided to prepare practical manual of various courses. So, keeping in view the requirement as per ICAR and necessity of students, the manual has been published.

I am sure that this manual will clear the basic concepts of manures, fertilizers and soil fertility management and it will be a useful ready reference material for all the students of second semester B.Sc.(Hons.)Agriculture.I shall feel more than satisfied, if this manual would serve the purpose of students in pursuit of their academic goals. I convey my hearty congratulations to Prof. H. P. Dholariya, Dr. Navneetkumar and Prof. R. P. Bambharolia for their commendable efforts in bringing out this practical manual.

January, 2019

(Z. P. Patel)

Reg. No. : _____ **Batch No.: _____**

Roll No. : _____ **Uni Seat No.: _____**

CERTIFICATE

This is to certify that the practical work has been satisfactory carried out by Shri/Kumari _____, in the course No. **Ag Chem. 2.2,** Course Title **"Manures, Fertilizers and Soil Fertility Management" (2+1)** of Second semester B.Sc. (Agri.) in the laboratory of Department of Agricultural Chemistry and Soil Science during the academic year _____.

He/She has certified_____ practical exercise out of _____ in the subject of **"Manures, Fertilizers and Soil Fertility Management".**

External Examiner **Course teacher**

Place :

Date :

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:Syllabus:

Course No.: Ag. Chem. 2.2

Title:: Manures, Fertilizer and Soil Fertility Management

Credit Hours: (2+1) = 3

Theory:

Classification and importance of organic manures, properties and methods of preparation of bulky manures. Green/leaf manuring. Transformation reactions of organic manures in soil and importance of C:N ratio in rate of decomposition. Integrated nutrient management. Chemical fertilizers: classification, composition and properties of major nitrogenous, phosphatic, potassic fertilizers, secondary & micronutrient fertilizers, complex fertilizers, nano-fertilizers, Soil amendments, fertilizer storage, fertilizer control order. History of soil fertility and plant nutrition. Criteria of essentiality. Role, deficiency and toxicity sympotams of essential plant nutritions, Mechanisms of nutrient transport to plants, factors affecting nutrient availability to plants. Chemistry of soil nitrogen, phosphorus, potassium, calcium, magnesium, sulphur and micronutrients. Soil fertility evaluation, soil testing. Critical levels of different nutrients in soil. Forms of nutrients in soil, plant analysis, rapid plant tissue tests. Indicator plants. Methods of fertilizer recommendations to crops. Factor influencing nutrient use efficiency (NUE), Methods of application under rainfed and irrigated conditions.

Practical:

Determination of moisture and organic matter content from manures samples. Estimation of N, P, K and S from manure sample. Determination of N from urea fertilizer. Determination of NH_4-N, NO_3-N from nitrogenous fertilizers. Determination of P from phosphatic fertilizer. Determination of K from Potassic fertilizer. Determination of S from sulphur fertilizer, Estimation of available N, P, K, S and Micronutrient (Fe, Mn, Zn, Cu) from Soil sample.

1. DETERMINATION OF MOISTURE AND ORGANIC MATTER CONTENT FROM MANURE SAMPLE

Moisture content of the organic manure is a very important property as it influences nutrient transformation, nutrient losses through volatilization and microbial population during storage. Moisture is also important to express nutrient content on dry weight basis. As per **FCO-1985, moisture content of the organic manure must be 15-25 percent by weight.** Organic matter is determined by Ignitation method and organic matter content of manure is also a very important quality property as it directly influences soil health. As per **FCO-1985, organic matter content of the organic manure must be minimum 20.69 percent by weight.**

Objective:

1. To know the moisture content in organic manure
2. To know the organic matter content in organic manure

(i) Determination of moisture content from Manure:

Principle: Weighed manure sample is placed in an oven at 105^{o}C and it is dried upto constant weight. The weight difference is considered to be moisture content in manure sample.

Apparatus:

1. Metallic Crucible 2. Balance 3. Oven 4. Desiccator

Procedure :

1. Weigh the empty Metallic Crucible.
2. Weigh accurately 1 g of manure sample adjusting with empty Metallic Crucible and put it immediately in the Desiccator and close it to prevent loss of moisture by evaporation.
3. Place the Metallic Crucible containing manure sample in oven at 105^{o}C for 24 hrs. Weight the Metallic Crucible containing manure sample and again put it for 2 to 3 hours till constant weight
4. After drying, allow the sample to cool for some time in oven. Then close the Metallic Crucible and put it in the desiccator for further cooling. After cooling at room temperature, note the final weight of Metallic Crucible with dried sample.
5. Complete the observation sheet and work out the moisture percentage from manure sample.

Result:

Sample	Moisture %
Manure	

(i) Determination of moisture content from manure sample:

Observations:

1. Wt. of empty Metallic Crucible (A) =_____

2. Wt. of Metallic Crucible + manure sample (B) =_____

3. Wt. of organic sample (B-A) =_____

4. Wt. of Metallic Crucible + oven dry sample (C) =_____

5. Moisture content in organic manure sample (B-C) =_____

Calculations:

$$\text{Percent moisture content in organic manure sample} \quad \frac{B - C}{B - A} \times 100$$

= _____

(ii) Determination of organic matter from manure:

Observations:

1. Wt. of empty Metallic/Silica Crucible (D) =_____

2. Wt. of Metallic/Silica Crucible + manure sample (E) =_____

3. Wt. of organic manure sample (E-D) =_____

4. Wt. of Metallic/Silica Crucible + Ignited sample (F) =_____

5. Organic matter content in manure sample (E-F) =_____

Calculations:

$$\text{Percent organic matter content in manure sample} \quad \frac{E - F}{E - D} \times 100$$

= _____

(ii) Determination of organic matter from Manure:

Principle: Weighed manure sample is placed in Muffle Furnace at 650^{o}C ± 700^{o}C and weight difference due to ignition of organic matter shows the organic matter content in manure sample.

Apparatus:

1. Metallic/Silica Crucible, 2. Balance, 3. Muffle Furnace and 4. Desiccator

Procedure :

6. Weigh the empty metallic/silica crucible.
7. Weigh accurately 1 g of organic manure sample adjusting with empty metallic/silica crucible and put in the desiccator.
8. Place the metallic/silica crucible containing organic manure sample in Muffle Furnace at 650^{o}C ± 700^{o}C for 6-8 hrs till there will be no black particles seen in manure sample.
9. After ignition, allow the crucible to cool for some time in Muffle Furnace. Then put the Metallic/Silica Crucible in the desiccator for further cooling. After cooling at room temperature, note the final weight of Metallic/Silica Crucible with ignited sample.
10. Complete the observation sheet and work out the organic matter percentage of organic manure sample.

Result:

Sample	Organic matter %
Organic Manure	

Answer the following question based on performed practical:

1. What is the FCO standard for moisture and organic matter content in organic manure?
2. Which method is used for determination of organic matter from organic manure?
3. At what temperature sample is placed in oven for the determination of moisture content from manure?
4. Why manure is sample placed in desiccator immediately after oven drying?
5. At what temperature manure sample is placed in muffle furnace for the determination of organic matter content from manure**?**

Observations:

1. Weight of manure sample taken =_____
2. Volume of 0.01N H_2SO_4 used for sample titration (S) =_____
3. Volume of 0.01N H_2SO_4 used for blank titration (B) =_____
4. Volume of 0.01N H_2SO_4 actually used (S-B) =_____

Calculations:

1000ml	1N H_2SO_4	14g N
1ml	1N H_2SO_4	0.014g N
1ml	0.01N H_2SO_4	0.00014g N
(S-B)ml	0.011N H_2SO_4	(S-B) x 0.00014g N

0.5g plant sample : (S-B) x 0.00014g N

Therefore, 100g plant sample : $\frac{\text{(S-B) x 0.00014g N x 100}}{\text{Weight of manure sample}}$

$$\text{Per cent N in manure} = \frac{\text{(S-B) x N of } H_2SO_4 \text{ x 0.014 x 100}}{\text{Weight of manure sample}}$$

$$= \_\_\_\_\_$$

2. ESTIMATION OF NITROGEN FROM MANURE SAMPLE

Total Nitrogen content in the organic manure is a very important property as Nitrogen is a major plant nutrient, responsible for green colour of plant, rapid plant growth, protein content and yield of crops. As per **FCO-1985, minimum Total N content of the organic manure must be 0.80 percent by weight.** Total Nitrogen content from organic manures is determined by **KJELDAHL METHOD**

Objective:

1. To know the nitrogen content in organic manure.

Principle:

Organic manure contain Nitrogen in form of organic-N and NO_3-N hence, organic manure sample is digested with H_2SO_4 and salicylic acid in the presence of K_2SO_4, $CuSO_4$ and $Na_2S_2O_3$ (catalyst). In this process K_2SO_4 raises the boiling point of H_2SO_4 and $CuSO_4$ is act as catalyst. Salicylic acid forms nitro compound which is reduced by $Na_2S_2O_3$ to amino compounds which are converted into ammonium sulphate and estimated by Kjeldhal method. Addition of 40% NaOH make media alkaline and Ammonia will be liberated which is absorbed in 4% boric acid and titrated with 0.1or 0.05 or 0.01 N H_2SO_4 or HCl.

$2(R\text{-}\underline{\mathbf{COONH_2)}} + 2H_2SO_4 + 5H_2 \rightarrow \underline{\mathbf{2(NH_2)_2SO_4}} + 2(R\text{-}COOH)$

$2(NH_4)_2SO_4 + 4NaOH \rightarrow 2Na_2SO_4 + 4H_2O + \underline{\mathbf{4NH_3}}$

$4NH_3 + 4H_2O \rightarrow \underline{\mathbf{4NH_4OH}}$

$NH_4OH + H_3BO_3 \rightarrow \underline{\mathbf{NH_4[B(OH)_4]}}$

$2NH_4[B(OH)_4] + 2H_2SO_4 \rightarrow (NH_4)_2SO_4 + 2H_3BO_3 + 2H_2O$ **(Titration)**

Apparatus:

1. Weight box **2**. Balance **3.** Kjeldahl flask **4.** Burette **5**. Glass rod **6.** 500 ml Beaker **7.** Class beads **8.** Distillation Set

Reagents:

1. **Sulphuric salicylic acid :**Dissolve 1 g salicylic acid in 30 ml concentrated sulphuric acid
2. **Catalyst mixture:** Mix 10 parts potassium sulphate or sodium sulphate, 1 parts copper sulphate and grind the mixture to pass a 40 mesh sieve.
3. **Sodium thiosulphate:** 24 mesh dried powder
4. **Sodium hydroxide (40%) :** Dissolve 400g NaOH in 1 litre water
5. **Mixed indicator:** Dissolve 0.5 g bromocresol green 0.1 g methyl and red in 100 ml of 0.95% ethanol. Adjust the pH 4.5 using 0.1N NaOH or HCl
6. **Boric acid (4%):** Place 40g H_3BO_3 in 1 litre volumetric flask, add about 900ml distilled water and heat and swirl the flask until the H_3BO_3 is dissolve. Cool the solution and add 5ml of mixed indicator (0.5 g of bromocresol green and 0.1 g of methyl red in 100 ml of 95% ethanol: adjust pH 4.5 with dilute NaOH of HCl).

7. **Standard sulphuric acid (0.01N):** Dissolve 2.8ml concentrated H_2SO_4 in distilled water and diluted to 1 litre. This will give 0.1N H_2SO_4. Now take 100ml of this 0.1N H_2SO_4 and make it to 1 litre to get 0.01N H_2SO_4. Standardize with 0.01N Na_2CO_3 using methyl orange indicator.

Procedure:

(A) Digestion:

1. Transfer 0.5g powdered manure sample wrapped in piece of filter paper to 100ml Kjeldahl flask.
2. Add 50 ml of the sulphuric- salicylic acid mixture and shake to get intimate contact of the sample with the reagent.
3. Add 5 g sodium thiosulphate and heat on heating coil, taking care to avoid frothing.
4. Cool, add 10 g catalyst mixture and then digest in Kjeldahl flask at full heat till the solution become clear. Cool and add 100 ml distilled water
5. Now distillation is carried out as follows

(B) Distillation

1. 25ml of 4% boric acid solution containing mix indicator is taken in a conical flask and flask is placed in such a way that condenser outlet of distillation apparatus is dipped into this boric acid solution
2. Transfer the content of Kjeldahl flask to distillation flask. Wash 2 to 3 times with distilled water to ensure that whole content of Kjeldahl flask is transferred
3. Add 100 ml 40% sodium hydroxide along the sides of the flask and 5 to 10 glass beads (5 mm in diameter).
4. Connect to distillation head, and distil off 150 ml into 25 of 4% boric acid solution.
5. Titrate it to the first faint pink colour with standard (0.1N) sulphuric acid solution.
6. Blank should be run and the titration carried to the same end point in exactly the same manner.

Result

Sample	Total N (%)
Manure	

Answer the following question based on performed practical:

1. What is the FCO standard for the total nitrogen content in manure?
2. Which method is used for determination of total N from manure?
3. Named the form of N in manure?
4. Give the role of K_2SO_4 in determination total N from organic manure.
5. Give the role of H_2SO_4 and $CuSO_4$ in determination total N from organic manure.
6. Why 40% NaOH is added in determination total N?
7. Name the indicator that is used in determination total N?
8. Name the chemicals used in preparation of mixed indicator.
9. Why glass beads added in distillation flask during estimation of total N?

(i) Observations for Total P

(A) For standard Curve

No.	Aliquot taken from working standard (50mg P/l) ml	Conc. of P in 50 ml diluted solution (mg/l)	% T	O.D.	Net O.D.
1	0.0	0.0			
2.	1.0	1.0			
3	1.5	1.5			
4	2.0	2.0			
5	2.5	2.5			
6	3.0	3.0			
7	3.5	3.5			
8	4.0	4.0			
9	4.5	4.5			
10	5.0	5.0			
		Total (A)			**Total (B)**

Graph factor $= \frac{A}{B} =$ _____

Construct a standard curve between net optical density and the concentration of P

3. ESTIMATION OF PHOSPHORUS, POTASSIUM AND SULPHUR FROM MANURE SAMPLE

Total Phosphorus, Potassium and Sulphur content are very important quality parameter of organic manure as Phosphorus and Potassium are major plant nutrient and Sulphur is a secondary plant nutrient and played a vital role in plant nutrition as well as crop production. As per **FCO-1985, minimum total P_2O_5 and K_2O content of the organic manure must be 0.80 percent by weight.**

Objective:

1. To know the phosphorus, potassium and sulphur content in organic manure

Wet Digestion

To convert organic P, K and S in to mineral form, manure sample is digested with di-acid mixture. This procedure is preferable than dry ignition, because of the possibility of loss of mineral constituents at high temperature during dry ignition.

Apparatus:

1. Weight box, 2. Balance, 3. 150ml conical flask, 4. Hot plate, 5. Filter paper, 6. Funnel, 7. 100-ml Volumetric flask

Reagents:

(A) Diacid mixture : 10 HNO_3 : 4 $HClO_4$

(B) Triacid mixture : 10 HNO_3 : 4 HClO4 :1 H_2SO_4

Procedure:

1. Transfer 0.5 g dried manure sample in a 150 ml conical flask
2. Add 10 ml of di-acid or tri-acid mixture , place a funnel on the flask and allow to stand for overnight
3. Heat gently at first, then heat more vigorously until a clear, colourless solution results.
4. Do not take it to dryness as phosphorus and sulphur are lost if the solution goes to dryness, discontinue heating the volume is reduce to approximately 3 to 5 ml
5. Cool and transfer quantitatively to a 100 ml volumetric flask, make it to volume, mix, allow to stand overnight and filter through Whatman No. 1 filter paper
6. Retain this solution and use for analysis of P, K and S.

(i) Total Phosphorus Estimation:

Principle:

The organic manure P is converted to orthophosphates during digestion. These orthophosphates react with molybdate and vanadate and give yellow coloured unreduced vanado-molybdo-phosphoric heteropoly complex in acid medium. The yellow colour is attributed to a substitution of oxyvanadium and oxymolybdenum redicals for oxygen of phosphate. The colour develops in about 10 minutes and stable for 2 to 8 weeks.

$$H_3PO_3 + 12H_2MoO_4 \rightarrow H_3P(Mo_3O_{10})_4 + 12H_2O$$

(B) Observations for manure sample

1. Weight of manure sample taken =_____g
2. Volume of acid digest made =_____ml
3. Aliquot taken from acid digest =_____ml
4. Volume of aliquot made =_____ml
5. Spectrophotometer reading for blank (OD) = _____
6. Spectrophotometer reading for sample(OD) = _____
7. Net O.D. =Sample O.D. –Blank O.D. = _____
8. Graph factor (GF) =_____

Calculation:

$$\text{P content (mg/kg)} = \text{Net OD} \times GF \times \frac{\text{Volume of acid extract}}{\text{Weight of manure sample}} \times \frac{\text{Final volume made}}{\text{Aliquot taken}}$$

$$\text{P content (\%)} = \text{Net OD} \times GF \times \frac{100}{0.5} \times \frac{50}{10} \times \frac{1}{10{,}000} = \_\_\_\_$$

P_2O_5 content (%) = P content (%) x 2.29 =_____

The intensity of this colour is directly proportional to the concentration of phosphorus present in the sample which can be read on spectrophotometer setting suitable wavelength.

Apparatus:

1. 50-ml volumetric flask 2. Pipette 3. Spectrophotometer

Reagents:

1. **Vanadate-molybdate solution:** Prepared solution **(A)** by dissolution of 25g of ammonium molybdate in 400 ml water. Prepared solution **(B)** by dissolving 1.25g ammonium metavanadate in 300 ml of boiling water cool it and add 250 ml concentrated HNO_3. Cool again at room temperature. Now add solution A to solution B and dilute to one litre.
2. **Standard P solution**: Prepare solution containing 50 mg P/Lby dissolve 0.2195g potassium dihydrogen phosphate (KH_2PO_4) in distilled water and making the volume to 1 litre

Procedure:

For standard curve:

1. Take 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5 ml of 50 mg P/l solution in a 50 ml volumetric flasks.
2. Add 10 ml of vanadate-molybadate regent.
3. Dilute the solution to 50 ml with distilled water and mix well
4. Read colour intensity on the spectrophotometer after10 minutes at 470 nm wavelength.
5. Prepare standard curve by plotting P concentrations on X-axis and optical density on Y-axis.

For Manure :

1. Pipette 10 ml aliquot (of the manure sample digested) in a 50 ml volumetric flask
2. Add 10 ml vanadate-molybadate solution and diluted to 50 ml with distilled water
3. Read colour intensity at 470 nm on spectrophotometer
4. From the standard curve or using given formula calculate the per cent P_2O_5 in the manure sample.

Result:

Sample	Total P_2O_5 %
Organic Manure	

(ii) Observations for Total K

(A) Standard Curve

No.	Aliquot taken from working standard (50mg K/l) ml	Conc. of K in 50 ml diluted solution (mg/l)	Flame photometer reading
1	0.0	0.0	
2.	1.0	1.0	
3	1.5	1.5	
4	2.0	2.0	
5	2.5	2.5	
6	3.0	3.0	
7	3.5	3.5	
8	4.0	4.0	
9	4.5	4.5	
10	5.0	5.0	
		Total (A)	**Total (A)**

Graph factor $= \frac{A}{B} = \frac{\quad}{\quad}$

Plot a standard curve between concentration K and Flame photometer reading.

(B) Manure sample

1. Weight of manure sample taken =_____g
2. Volume of acid digest made =_____ml
3. Aliquot from acid digest =_____ml
4. Flame photometer reading (R) =_____
5. Graph factor (GF) =_____

Calculation:

$$\text{K content (mg/kg)} = R \times GF \times \frac{\text{Volume of acid extract}}{\text{Weight of manure sample}} = \_\_\_\_\_$$

$$\text{K content (\%)} = \text{Net OD} \times GF \times \frac{100}{0.5} \times \frac{1}{10{,}000} = \_\_\_\_\_$$

K_2O content (%) = K content (%) x 1.2 = _____

(ii) Estimation of Total Potassium:

Principle:

The determination of potassium is based on measurement of spectral line intensities of K atoms exited when filtrate is sprayed on a flame of flame photometer. Atoms of K present in aliquot absorbed energy from flame and get excited and jumped to the higher energy orbit. Being a stable atom, they return to original orbit releasing energy and give spectral lines which are proportional to the concentration of atoms of that feed aliquot.

Apparatus/Instruments:

1. Flame photometer 2. Beaker

Reagent:

Reagents:

(i) **Standard K solution (1000 mg K/l):** Dissolve 1.907g pure KCl in distilled water and make the volume 1 litre.

(ii) **Working standard:** 25, 50, 75 and 100 mg K/l.

Procedure for Stand Curve:

1. Standard the flame photometer using 25, 50 and 100 mg/L
2. Now feed different standard K solutions one by one in increasing order of concentration and note down the reading of each.
3. Plot a standard curve between concentration and reading of standard K standard solutions and flame photometer readings.

Procedure for Manure:

1. Standard the flame photometer using 25, 50 and 100 mg/L
2. Atomize the digested filtrate in the flame and record flame photometer reading
3. From a standard curve of known potassium concentrations, find out the amount of potassium in the given acid extract.

Result:

Sample	Total K_2O (%)
Manure	

(ii) Observations for Total S

(A) Observations for standard curve:

No.	Aliquot taken from working standard (50 mg S/l) ml	Conc. of S in 50 ml diluted solution (mg /l)	% T	O.D.	Net O.D.
1	0	0			
2.	1	1			
3	2	2			
4	3	3			
5	4	4			
6	5	5			
7	6	6			
8	7	7			
9	8	8			
10	9	9			
11	10	10			
		Total (A)			**Total (B)**

Graph factor $= \frac{A}{B} =$ _____

Construct a standard curve between net optical density and the concentration of S

(B) Observations for organic Manure:

1. Weight of manure sample = _____ g
2. Final Volume made of manure acid extract = _____ ml
3. Volume of the manure acid extract taken = _____ ml
4. Final Volume = _____ ml
5. Spectrophotometer reading for blank (OD) = _____
6. Spectrophotometer reading for sample (OD) = _____
7. Net O.D. =Sample O.D. –Blank O.D = _____
8. Graph factor (**GF)** = _____

Calculations:

$$\text{Total S in manure (\%)} = \text{Net OD} \times GF \times \frac{\text{Final volume acid extract}}{\text{Weight of manure sample}} \times \frac{\text{Final volume}}{\text{Aliquot Taken}} \times \frac{1}{10000}$$

=_____

(iii) Estimation of Total Sulphur:

Principle:

Sulphur from the acid extract can be estimated turbidimetrically using spectrophotometer. The method involves two parts in S analysis: (1) decomposition of organic components of the sample and conversion of the entire inorganic S to the sulfate (SO_4) form by di-acid digestion method and (2) quantification of the SO_4^{2-} in the extract can be estimated turbidimetrically using spectrophotometer. Addition of $BaCl_2$ react with sulphur and forms white precipates in form of $BaSO_4$. Intensity of this white turbidity is measured in form of OD (absorption) on spectrophotometer at suitable wavelength.

Apparatus/Instruments/Equipments:

1. Spectrophotometer, 2. 50 ml volumetric flasks, 3. 10 ml pipette, 4. 10ml graduated pipette, 5. Funnel, 6. Mechanical shaker 7. Balance

Reagents:

1. **Morgan's reagent:** Dissolved 100g sodium acetate in 800 ml distilled water. Adjust the pH 4.8 by adding glacial acetic acid and make volume 1 litre.
2. **Gum acacia solution:** Dissolve 0.5g pure gum acacia in 200ml distilled water. Filter the solution with Whatman No. 42 filter paper to get clear filtrate. Check the clarity of solution before use
3. **Barium chloride:** Ground the barium chloride crystal with mortar and pestle to get fine powder.
4. **Standard S solution (50 mg S/l):** Dissolve 0.2717g pure potassium sulphate in distilled water and make the volume 1 litre

For standard Curve:

Procedure:

1. Take 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10ml of 50 ppm S solution in 50 ml volumetric flasks.
2. To this Add 20 ml o f Morgan's reagent .
3. Then add 2 ml of Gum acacia solution and one spoon barium chloride and mix well and make the volume with distilled water.
4. After waiting for 20 minute read the turbidity on spectrophotometer at 410 nm wavelength.

For Manure:

Procedure:

1. Take 20 ml Acid Extract in 50 ml volumetric flask.
2. Add 20 ml Morgan's reagent, 2 ml gum acacia and one spoon barium chloride.
3. After 20 minute, measure the turbidity in spectrophotometer at 410 nm wavelength.

Result:

Sample	Total S (%)
Manure	

Answer the following question based on performed practical:

1. What is the FCO standard for the total P_2O_5 and K_2O content in manure?
2. Why di or tri acid mixture is preferred in digestion of manure for P, K and S estimation from manure?
3. Name the acids are used in preparation of di-acid or tri-acid mixture?
4. Which wavelength is selected for determination of total P on spectrophotometer?
5. Which instrument is used for determination of total P?
6. Name the instrument that is used for determination of K?
7. Calculate the weight of KCl to prepared 1000 ppm K solution?
8. Which wavelength is selected for determination of total S on spectrophotometer?
9. Why gum acacia is added during the estimation of S?
10. Give the conversion factor that is used to convert K to K_2O and P to P_2O_5.

Observations:

1	Weight of fertilizer taken		_____g
2	Final volume made up		_____ml
3	Aliquot taken for digestion		_____ml
2	Burette reading with 0.1 N H_2SO_4 for the sample	**S**	_____ml
3	Burette reading with 0.1 N H_2SO_4 for the blank	**B**	_____ml
4	Net 0.1 N H_2SO_4 required to react with NH_3 liberated from 10 ml fertilizer solution	**(S-B)**	_____ml

Calculation:

1000 ml 1 N H_2SO_4 = 14 g N

1000 ml 0.1 N H_2SO_4 = 1.4 g N

1 ml 0.1 N H_2SO_4 = 0.0014 g N

$$\% N = \frac{(S-B) \times 0.0014 \times \text{final volume} \times 100}{\text{Weight of fertilizer} \times \text{Aliquot taken}}$$

$$\% N = \frac{(S-B) \times 0.0014 \times 250 \times 100}{1 \times 10}$$

% N = (S - B) x 3.5

% N =_____(X)

Purity of fertilizer:

Percent N in Urea [NH_2 CO NH_2]=28 (N)+4 (H)+12 (C)+16 (O) = 60 is molecular weight

60 g Urea contains 28 g of N

100 g Urea contains (?)

$\frac{100 \times 28}{60}$ = 46.7 % N

If 46.7 % N 100 % pure

X % N (?)

$\frac{100 \times X}{46.7}$ = _____% purity

Where X = % N in ammonium nitrate (Total nitrogen)

4. DETERMINATION OF NITROGEN FROM UREA FERTILIZER: [NH_2-CO-NH_2]

Nitrogen is a first major plant nutrient and is supplied to the crop by addition of nitrogenous fertilizers. Among nitrogenous fertilizers, Urea is the most familiar nitrogenous fertilizer containing 46% Nitrogen in form of Amide (NH_2).

Objective:

1. To know the purity of urea fertilizer.

Principle:

In Urea, N is present in form of Amide (NH_2). When this organic nitrogenous material is digested with H_2SO_4 gets oxidized and inorganic N is released. During digestion, part of H_2SO_4 is reduced to SO_2 which in turn reduces nitrogenous materials to ammonia. This NH_3 combines with H_2SO_4 and converted into $(NH_4)_2SO_4$ at the end of digestion. The NH_3 is distillated in alkaline medium and absorbed in boric acid which is back titrated with standard H_2SO_4 using mixed indicator. The K_2SO_4 and $CuSO_4$ are added during digestion to raise the boiling point of the acid and rate of oxidation. During digestion the $CuSO_4$ acts as a catalyst which hastens the process. Among various catalysts used to hasten the digestion process are $CuSO_4$, Hg, HgO etc. $CuSO_4$ being cheap though significantly less effective is normally used. The ideal temperature for digestion should be between 360^OC and 410^OC. At lower temperature, the digestion may not be completed, while above 410^OC the loss of NH_3 may occur.

Reaction:

$$NH_2\,CO\,NH_2 \xrightarrow[H_2SO_4,\ K_2SO_4,\ CuSO_4]{\text{Digestion}} (NH_4)_2SO_4$$

(Urea)

$$(NH_4)_2SO_4 + 2NaOH \xrightarrow{\text{Distillation}} 2H_2O + Na_2SO_4 + 2NH_3\uparrow$$

$$3NH_3 + H_3BO_3 \xrightarrow{\text{Absorption}} (NH_4)_3BO_3 \text{ (Ammonium borate)}$$

$$2(NH_4)_3BO_3 + 3H_2SO_4 \xrightarrow{\text{Titration}} 3(NH_4)_2SO_4 + 2H_3BO_3 \text{ (Boric acid)}$$

Reagents:

(i) **Concentrated H_2SO_4:**

(ii) **Digestion mixture:** K_2SO_4 : $CuSO_4$= 10 : 1 ratio

(iii) **4 % Boric acid**: Dissolve 40 g H_3BO_3 in one litre volumetric flask, add about 900 ml distilled water and heat and swirl the flask until the H_3BO_3 is dissolve.

(iv) **40 % NaOH**: Dissolve 400 g loose alkali (NaOH) in water and dilute to one litre volume.

(v) **0.1 N H_2SO_4:** Dissolve 2.8 ml concentrated H_2SO_4 in distilled water and diluted to one litre. Standardize with 0.1N Na_2CO_3 using methyl orange indicator.

(vi) **Mixed indicator: Dissolve** 0.5 g Bromocresol green and 0.1 g methyl red in 100 ml of 95 % ethanol: Adjust the pH 4.5 with dilute NaOH or HCl.

Procedure:

(i) Weight accurately 1.0 g of urea on clean dry watch glass, transfer it to a clean beaker by washing the watch glass 3 to 4 times with distilled water. Dissolve it in water completely by stirring with glass rod.

(ii) Transfer the solution to 250 ml volumetric flask by washing the beaker several times with distilled water. Make the final volume up to the mark, stopper it and shake well.

(iii) Pipette 10 ml solution into Kjeldahl's flask. Add 25 ml concentrated H_2SO_4 and about 10 g digestion mixture and rotate the flask.

(iv) Heat the flask at first gently over low flame in the digestion chamber (gas heater/electric heater), when flask has white fumes of SO_2, raise the flame.

(v) Continue the digestion over full flame for an hour, remove the flask and cool it.

(vi) Dilute the content of Kjeldahl's flask with about 50 ml water and transfer to distillation flask. Wash the flask several times and transfer all the washings to the solution carefully to a distillation flask, wash the Kjeldahl's flask 3to4 times and transfer all the washings to the distillation flask, The content in the distillation flask should be about 300 ml.

(vii) Take 25 ml, 4 % boric acid solution in clean 250 ml beaker and add 3 to 4 drops of mixed indicator and place the beaker under the condenser in such a way that the tip of condenser should be dipped into the boric acid solution.

(viii) Add 2 to 3 glass beads in the distillation flask.

(ix) Take about 100 ml or more of 40 % NaOH solution and add into the distillation flask in such a way that it runs down from neck to the bottom without mixing. The content of the flask must become distinctly alkaline.

(x) Connect the splash head immediately and circulate the water in the condenser.

(xi) Light the burner (electric heater) and start heating at once to avoid the danger of sucking back.

(xii) Continue distillation till about 150 ml of distillate is collected in the beaker. Test for NH_3 using litmus paper.

(xiii) Remove the beaker by washing the tip of condenser with distilled water and then put-off the burner to prevent sucking back.

(xiv) Run a blank using same procedure without addition of digested solution of urea.

(xv) Titrate the distillate with standard 0.1 N H_2SO_4 till the wine red colour is obtained and calculate the percentage of N and purity of Urea sample.

Result:

Sample	Total N (%)	Purity (%)
Urea		

Answer the following question based on performed practical:

1. Give form of N present in urea.
2. What is the ideal temperature for digestion during determination of N from urea?
3. Give the content of nitrogen in urea.
4. Name the flask that is used in digestion of Urea?
5. Give the chemical formula of urea.

Observations:

1	Weight of fertilizer sample taken		1.0 g
2	Volume made up		250 ml
3	Aliquot taken for distillation		25 ml
4	Burette reading with 0.1 N H_2SO_4 for the sample	**S**	_____ml
5	Burette reading with 0.1 N H_2SO_4 for the blank	**B**	_____ml
6	Net 0.1 N H_2SO_4 required to react with NH_3 liberated from 25 ml of fertilizer solution	**(S-B)**	_____ml

Calculation:

1000 ml 1 N H_2SO_4 = 14 g N

1000 ml 0.1 N H_2SO_4 = 1.4 g N

1 ml 0.1 N H_2SO_4 = 0.0014 g N

$$\% \ N = \frac{(S-B) \text{ x } 0.0014 \ x \ 100 \ x \ 250}{1 \ x \ 25}$$

% N = (S - B) x 1.4

% N = X = _____

Purity of fertilizer:

Percent N in $(NH_4)_2SO_4$ = 28 + 8 + 32 + 64 = 132 (is molecular weight)

132 g AS contains 28 g of N

100 g AS contains (?)

$$\frac{100 \text{ x } 28}{132} \quad 21.21\% \ N$$

If 21.21 % N 100 % pure

X % N (?)

$$\frac{100 \text{ x } X \ (\% \ N)}{21.21} \quad \_\_\_\_\_ \ \% \ purity$$

Where X = % N obtained from Ammonical Nitrogenous Fertilizer analysis

5. DETERMINATION OF NH_4-N FROM NITROGENOUS FERTILIZERS: $[(NH_4)_2SO_4]$

Most popular inorganic nitrogenous fertilizers are Ammonium Sulphate, Calcium Ammonium Nitrate, Potassium Nitrate, Sodium Nitrate, Ammonium Nitrate etc. Form of N present is: Ammonium Sulphate (20.6% NH_4-N), Calcium Ammonium Nitrate (13% NH_4-N and 13% NO_3-N), Potassium Nitrate (13.9% N, 61.4% NO_3-N), Sodium Nitrate (16% NO_3-N), Ammonium Nitrate (33% NH_4-N)

Objective:

1. To know the purity of ammonium sulphate fertilizer.

(i) DETERMINATION OF NH_4-N FROM AMMONICAL FERTILIZERS :

Principle:

Nitrogen in any ammonical fertilizers can be determined by its distillation with alkali (NaOH or MgO). The solution containing a known amount of fertilizer is distilled with 40 % NaOH solution and the NH_3 liberated is absorbed in known volume of 4 % boric acid solution. The amount of NH_3 absorbed is determined by titrating it with standard 0.1N sulphuric acid solution using mixed indicator. The end point is indicated by change of colour from sky blue to pink.

Reactions:

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow 2H_2O + Na_2SO_4 + NH_3\uparrow$$

$$3NH_3 + H_3BO_3 \longrightarrow (NH_4)_3BO_3 \text{ (Ammonium borate)}$$

$$2(NH_4)_3BO_3 + 3H_2SO_4 \longrightarrow 3(NH_4)_2SO_4 + 2H_3BO_3 \text{ (Boric acid)}$$

Reagents:

(i) **4% Boric acid**: Dissolve 20 g H_3BO_3 in one litre volumetric flask, add about 900 ml distilled water and heat and swirl the flask until the H_3BO_3 is dissolved and dilute to one litre volume.

(ii) **40 % NaOH**: Dissolve 400 g loose alkali (NaOH) in water and dilute to one litre volume.

(iii) **0.1 N H_2SO_4:** Dissolve 2.8 ml concentrated H_2SO_4 in distilled water and dilute to one litre. Standardize with 0.1N Na_2CO_3 using methyl orange indicator.

(iv) **Mixed indicator :** Dissolve 0.5 g Bromocresol green and 0.1 g methyl red in 100 ml of 95 % ethanol. Adjust the pH 4.5 with dilute NaOH or HCl.

Procedure:

(i) Weigh accurately 1 g of the given sample (AS) on a clean dry watch glass.

(ii) Dissolve it in distilled water and transfer to the 250 ml of volumetric flask. Wash the watch glass and beaker thoroughly and transfer all the washings into the flask. Make the final volume up to the mark. Stopper and shake well.

(iii) Pipette 25 ml of aliquot from it and transfer it to 1 litre of distillation flask.

(iv) Add about 300 ml distilled water in distillation flask.

(v) Take 25 ml boric acid solution in a 250 ml beaker and add 3 to 4 drops of mixed indicator.

(vi) Place the beaker containing boric acid and mixed indicator under condenser in such a way that tip of condenser should be dipped into the boric acid solution.

(vii) Add 2 to 3 glass beads in the distillation flask.

(viii) Take about 25 ml of 40 % NaOH solution and add into the distillation flask in such a way that it runs down from neck to the bottom without mixing. Connect the splash head immediately and circulate the water in the condenser.

(ix) Light the burner and start heating at once to avoid the danger of sucking back.

(x) Continue distillation till about 150 ml of distillate is collected in the beaker. Test for NH_3 using litmus paper. Wash the tip with distilled water.

(xi) Remove the beaker first and then put-off the burner to prevent sucking back.

(xii) Run a blank using same procedure without addition of $(NH_4)_2SO_4$ solution.

(xiii) Titrate the distillate with standard 0.1 N H_2SO_4 till the wine red colour is observed.

(xiv) Calculate the per cent of N and purity percentage of the given sample of fertilizer.

Result:

Sample	**NH_4-N (%)**
$(NH_4)_2SO_4$	

Answer the following question based on performed practical:

1. List out the most popular inorganic nitrogenous fertilizers.
2. Which compound is formed during the absorption of ammonia in boric acid?
3. Give the pH of mixed indicator and Why?
4. What is the chemical formula of ammonium sulphate?

Observations:

1	Weight of sample taken		1.0 g
2	Volume made up		250 ml
3	Aliquot taken for distillation		25 ml
4	Burette reading with 0.1 N H_2SO_4 for the sample	**S**	_____ml
5	Burette reading with 0.1 N H_2SO_4 for the blank	**B**	_____ml
6	Net 0.1 N H_2SO_4 required to react with NH_3 liberated from 25 ml of fertilizer solution	**(S-B)**	_____ml

Calculation:

1000 ml 1 N H_2SO_4 = 14 g N

1000 ml 0.1 N H_2SO_4 = 1.4 g N

1 ml 0.1 N H_2SO_4 = 0.0014 g N

$$\% \ N = \frac{(S - B) \times 0.0014 \ x \ 100 \ x \ 250}{1 \ x \ 25}$$

% N = (S - B) x 1.4

Per cent N = X = _____

Purity of fertilizer:

Purity of sodium nitrate	**Purity of potassium nitrate**
Per cent N in $NaNO_3$=23+14+48=85 Molecular weight = 85 85 g $NaNO_3$ contains 14 g of N 100 g $NaNO_3$ contains (?) $\frac{100 \times 14}{85} = 16.47$ If 16.47 % N 100 % pure X % N (?) $\frac{100 \times X}{16.47}$ = _____% purity Where X = % N obtained by analysis	Per cent N in KNO_3=39+14+48=101 Molecular weight = 101 101 g KNO_3 contains 14 g of N 100 g KNO_3 contains (?) $\frac{100 \times 14}{101} = 13.86$ If 13.86 % N 100 % pure X % N (?) $\frac{100 \times X}{13.86}$ = _____% purity Where X = % N obtained by analysis

6. DETERMINATION OF NO_3-N FROM NITROGENOUS FERTILIZERS: [KNO_3, $NaNO_3$]

Objective:

1. To know the purity of potassium nitrate and sodium nitrate fertilizer.

Principle:

The **NO_3-N** is reduced to NH_3 by Devarda's alloy (Cu : Al : Zn = 50 : 45 : 5) in alkaline solution. The ammonia liberated is absorbed in known volume of 4 % boric acid solution. The amount of NH_3 absorbed is determined by titrating it with standard 0.1 N sulphuric acid solution using mixed indicator. The end point is indicated by change of colour from sky blue to pink.

Reactions:

$$Zn + 2NaOH \longrightarrow Na_2ZnO_2 \text{ (Sodium zincate)} + 2H^+$$

$$Al + 2NaOH \longrightarrow Na_2AlO_2 \text{ (Sodium aluminate)} + 2H^+$$

$$NO_3 + 9H^+ \longrightarrow NH_3 + 3H_2O$$

$$3NH_3 + H_3BO_3 \longrightarrow (NH_4)_3BO_3 \text{ (Ammonium borate)}$$

$$2(NH_4)_3BO_3 + 3H_2SO_4 \longrightarrow 3(NH_4)_2SO_4 + 2H_3BO_3 \text{ (Boric acid)}$$

Reagents:

(i) **Devarda's alloy** : Mixture of metals in the ratio of Cu : Al : Zn :: 50 : 45 : 5 .

(v) **2 % Boric acid**: Dissolve 20 g H_3BO_3 in one litre volumetric flask, add about 900 ml distilled water and heat and swirl the flask until the H_3BO_3 is dissolved and dilute to one litre volume.

(ii) **2.5 % NaOH**: Dissolve 25 g loose alkali (NaOH) in water and dilute to one litre volume.

(iii) **0.1 N H_2SO_4:** Dissolve 2.8 ml concentrated H_2SO_4 in distilled water and dilutete one litre. Standardize with 0.1N Na_2CO_3 using methyl orange indicator.

(iv) **Mixed indicator:** Dissolve 0.5 g Bromocresol green and 0.1 g methyl red dissolved in 100 ml of 95 % ethanol. Adjust the pH 4.5 with dilute NaOH or HCl.

Procedure:

(i) Weigh accurately 1 g of the given fertilizer sample (KNO_3/$NaNO_3$) on a clean dry watch glass.

(ii) Dissolve it in distilled water and transfer to the 250 ml of volumetric flask. Wash the watch glass and beaker thoroughly and transfer all the washings into the flask. Make the final volume up to the mark. Stopper and shake well.

(iii) Pipette 25 ml of aliquot from it and transfer it to 1 litre of distillation flask.

(iv) Add about 300-400 ml distilled water in distillation flask.

(v) Take 25 ml boric acid solution in a 250 ml beaker and add 4 to 5 drops of mixed indicator.

(vi) Place the beaker containing boric acid and mixed indicator under condenser in such a way that tip of condenser should be dipped into the boric acid solution.

(vii) Add 2 to 3 glass beads in the distillation flask.

(viii) Add 2 gram of Devarda's Alloy in distillation flask.

(ix) Take about 25 ml of 2.5 % NaOH solution and add into the distillation flask in such a way that it runs down from neck to the bottom without mixing. Connect the splash head immediately and circulate the water in the condenser.

(x) Light the burner and start heating at once to avoid the danger of sucking back.

(xi) Continue distillation till about 150 ml of distillate is collected in the beaker. Test for NH_3 using litmus paper. Wash the tip with distilled water.

(xii) Remove the beaker first and then put-off the burner to prevent sucking back.

(xiii) Run a blank using same procedure without addition of fertilizer solution.

(xiv) Titrate the distillate with std. 0.1 N H_2SO_4 till the wine red colour is observed .

(xv) Calculate the percent of N and purity percentage of the given sample of fertilizer.

Result:

Sample	**NO_3-N(%)**
KNO_3	

Answer the following question based on performed practical:

1. Give the role of devarda's alloy in estimation of NO_3-N from nitrogenous fertilizer
2. What is the composition of devarda's alloy?
3. How to prepared 2 % boric acid?
4. What is the molecular weight of potassium nitrate?

Observations

(A) For standard curve

No.	Aliquot taken from working standard (50mg P/l) ml	Conc. of P in 50 ml diluted solution (mg/l)	O.D.	Net O.D.
1	0.0	0.0		
2.	1.0	1.0		
3	1.5	1.5		
4	2.0	2.0		
5	2.5	2.5		
6	3.0	3.0		
7	3.5	3.5		
8	4.0	4.0		
9	4.5	4.5		
10	5.0	5.0		
		Total (A)		**Total (B)**

Graph factor $= \frac{A}{B} =$ _____

Construct a standard curve between net optical density and the concentration of P

(B) For Fertilizer sample

1. Weight of Phosphatic fertilizer (DAP) sample taken = _____ g
2. Volume made up =_____ ml
3. Aliquot taken =_____ ml
4. Volume of aliquot made = _____ ml
5. Spectrophotometer reading for blank (OD) = _____
6. Spectrophotometer reading for sample (OD) = _____
7. Net O.D. =Sample O.D. –Blank O.D. = _____
8. Graph factor (GF) =_____

Calculation:

$$\text{P content (mg/kg)} = \text{Net OD} \times \text{GF} \times \frac{250}{1} \times \frac{50}{10} = \_\_\_\_$$

$$\text{P content (\%)} = \text{Net OD} \times \text{GF} \times \frac{250}{1} \times \frac{50}{10} \times \frac{1}{10{,}000} = \_\_\_\_$$

P_2O_5 content (%) = P content (%) x 2.29 =_____

7. DETERMINATION OF PHOSPHORUS FROM PHOSPHATIC FERTILIZER: [DAP]

Phosphorus is a second major plant nutrient and P is supplied to the crop by addition of phosphatic fertilizers. Most popular inorganic Phosphatic fertilizers are Diammonium phosphate (46% P_2O_5), Single Super Phosphate (16% P_2O_5), Monoammonium phosphate (20% P_2O_5) etc.

Objective:

1. To know the purity of diammonium phosphate fertilizer.

Principle:

The orthophosphates of water soluble fertilizer react with molybdate and vanadate and give yellow coloured unreduced vanado molybdo phosphoric heteropoly complex in acid medium. The yellow colour is attributed to a substitution of oxyvanadium and oxymolybdenum redicals for oxygen of phosphate.

$$H_3PO_3 + 12H_2MoO_4 \rightarrow H_3P(Mo_3O_{10})_4 + 12H_2O$$

The intensity of this colour is directly proportional to the concentration of phosphate present in the sample which can be read on spectrophotometer. The colour develops in about 10 minutes and stable for 2 to 8 weeks.

The advantages of method:

- Extreme simple.
- Stability of colour for long period.
- Free from interference of range of ionic species in concentration upto 1000 ppm
- Adaptability to HNO_3, HCl, H_2SO_4, $HClO_4$ system

Reagents:

1. **Vanadate-molybdate solution:** Prepare solution A by dissolving 25g of ammonium molybdate in 400 ml distilled water. Prepare solution B by dissolving 1.25g ammonium metavanadate in 300 ml of boiling water Cool it and add 250 ml concentrated HNO_3. Cool again at room temperature. Now, add solution A to solution B and dilute to one litre.
2. **Standard P solution**: Prepare solution containing 50 mg P/L by dissolve 0.2195g potassium dihydrogen phosphate (KH_2PO_4) in distilled water and making the volume to 1 litre

Procedure:

For standard curve:

1. Take 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5 ml of 50 mg P/l solution in a 50 ml volumetric flask.
2. Add 10 ml of vanad molybadate regent.
3. Dilute the solution to 50 ml with distilled water and mix well.
4. Read the colour intensity in the spectrophotometer 10 minutes after addition of the vand-molybdate solution using a 470 nm wavelength.
5. Prepare standard curve by plotting P concentrations on X-axis and optical density on Y-axis.

Purity of fertilizer:

Percent P_2O_5 in DAP $[(NH_4)_2HPO_4]$=28 (N) + 9 (H) + 31(P)+64(O)=132 is molecular weight

If 46.0 % P_2O_5 100 % pure

X % P_2O_5 (?)

$$\frac{100 \times X}{46.0} = \text{\_\_\_\_\_\% purity}$$

Where X = P_2O_5 in DAP obtained by analysis.

Purity of fertilizer:

Percent P_2O_5 in SSP $[Ca(H_2PO_4)_2.H_2O + CaSO_4.2H_2O]$ = 80 (Ca) + 10 (H) + 62 (P) + 240 (O) + 32 (S) = 424 is molecular weight

If 16.0 % P_2O_5 100 % pure

X % P_2O_5 (?)

$$\frac{100 \times X}{16.0} = \text{\_\_\_\_\_\% purity}$$

Where X = % P_2O_5 obtained by analysis

For Fertilizer:

1. Weigh accurately 1 gm of Phosphatic fertilizer (DAP) sample in clean dry watch glass.
2. Transfer the fertilizer in beaker by washing watch glass with hot distilled water.
3. Dissolve the fertilizer by stirring well.
4. Filter the solution in 250 ml of volumetric flask by washing residues with hot distilled water and make the final volume up to the mark, stopper the flask and shake well.
5. Pipette out 10 ml of the aliquot in 50 ml volumetric flask.
6. Add 10 ml vanadate-molybadate solution.
7. Make the volume up to the mark with distilled water and mix well.
8. The yellow colour will develop rapidly but take reading after 10 minutes to ensure full strength.
9. Take reading for yellow colour intensity at 470 nm on spectrophotometer.
10. Run a blank using same procedure without fertilizer sample.
11. Calculate the per cent P_2O_5 in the Phosphatic fertilizer (DAP) sample.

Result:

Sample	**P_2O_5 (%)**
DAP	

Answer the following question based on performed practical:

1. List out the most popular inorganic phosphatic fertilizers.
2. Which compound is formed when vanado-molybadate solution is added in P containing solution?
3. Which colour is appears when we add vanado-molybadate solution in P containing solution?
4. How to prepared vanadate-molybadate solution?

Observations:

(A) For standard curve

Sr. No.	Volume taken from 100 mgK/L in 100ml volumetric flask	Concentration of K in solution (mg/l)	Reading of flame photometer
1	00	00	
2.	10	10	
3	20	20	
4	30	30	
5	40	40	
6	50	50	
7	60	60	
8	70	70	
9	80	80	
10	90	90	
11	100	100	
		Total (A)	**Total (B)**

Graph factor $= \frac{A}{B} =$_____

Plot a standard curve between concentration K in diluted solution and flame photometer readingreadings.

(B) For Fertilizer sample

(i) Weight of Potassic fertilizer (KCl) sample taken =_____ g

(ii) Volume made up =_____ ml

(iii) Aliquot taken =_____ ml

(iv) Volume of aliquot made = _____ ml

(v) Flame photometer reading (R) = _____

(vi) Graph factor (GF) = _____

Calculation:

$$(\%)K \text{ content} = \text{K Concentration in graph against FPM reading} \times \text{Dilution} \times \frac{1}{10,000} = \_\_\_\_\_$$

K_2O content (%) = K content (%) x 1.2 =_____

EXERCISE 8: DETERMINATION OF POTASSIUM FROM POTASSIC FERTILIZER: [KCl]

Potash is a third major plant nutrient and K is supplied to the crop by addition of Potassic fertilizers. Most popular inorganic Potassic fertilizers are Murate of Potash- KCl (58-60% K_2O) and Sulphate of Potash-K_2SO_4 (48-50% K_2O).

Objective:

1. To know the purity of potassium chloride fertilizer.

Principle:

The determination of potassium is based on measurement of spectral line intensities of K atoms exited when filtrate is sprayed on a flame of flamephotometer. Atoms of K present in aliquot absorbed energy from flame and get excited and jumped to the higher energy orbit. Being a stable atom, they return to original orbit releasing energy and give spectral lines which are proportional to the concentration of K atoms of that feed aliquot.

Reagents:

(iii) **Standard K solution (1000 mg K/l):** Dissolve 1.907g pure KCl in distilled water and make the volume 1 litre.

(iv) **Working standard:** 25, 50, 75 and 100 mg K/l.

Procedure for Stand Curve:

(i) Standard the flame photometer using 25, 50 and 100 mg/L

(ii) Now feed different standard K solutions one by one in increasing order of concentration and note down the reading of each.

(iii) Plot a standard curve between concentration and reading of standard K standard solutions and flame photometer readings.

Procedure for fertilizer:

4. Weigh accurately 0.5 g of K fertilizer and transfer it to beaker by washing the watch glass with distilled water and dissolve the fertilizer by stirring with glass road.

5. Transfer the solution in 250 ml volumetric flask by washing the beaker several times with distilled water and make final volume upto the mark, stopper the flask and shake well.

6. Pipette out 1 ml of solution and transfer it into another 25 ml volumetric flask, make final volume upto the mark, stopper the flask and shake well.

7. Atomize the solution through the flame and record flame photometer reading.

8. From a standard curve of known potassium concentrations, find out the amount of potassium in the given diluted solution.

Purity of fertilizer:

Percent K_2O in KCl (Muriate of potash)= 39 (K) + 35.5(Cl) = 74.5 is molecular weight

74.5 g KCl contains 39 g of K
100 g KCl contains (?)

$$\frac{100 \times 39}{74.5} \times 1.2 = 62.82\ \%\ K_2O$$

If 62.82 % K_2O 100 % pure
X % K_2O (?)

$$\frac{100 \times X}{62.82} = \text{\_\_\_\_\_\% purity}$$

Where X = % K_2O in KCl obtained by analysis

Result:

Sample	**K_2O (%)**
MOP	

Answer the following question based on performed practical:

1. List out the most popular inorganic potassic fertilizers.
2. Calculate the percent K_2O in KCl.
3. How to prepared 25, 50, 75 and 100 mg K/l solution from 1000 mg K/l solution?

Observations for standard curve:

No.	Aliquot taken from working standard (50 mg S/l) ml	Conc. of S in 50 ml diluted solution (mg /l)	O.D.	Net O.D.
1	0	0		
2.	1	1		
3	2	2		
4	3	3		
5	4	4		
6	5	5		
7	6	6		
8	7	7		
9	8	8		
10	9	9		
11	10	10		
		Total (A)		**Total (B)**

Graph factor $= \frac{A}{B} =$ _____

Observations for S containing fertilizer:

1. Weight of fertilizer sample taken = 1.0 g
2. Volume made up = 250 ml
3. Volume of the fertilizer solution taken = 10 ml
4. Final Volume made up = 50 ml
5. Spectrophotometer reading for blank (OD) = _____
6. Spectrophotometer reading for sample (OD) = _____
7. Net O.D. =Sample O.D. –Blank O.D = _____
8. Graph factor GF) = _____

Calculations:

$$\% S = \text{Net OD} \times GF \times \frac{250}{1} \times \frac{50}{10} = \_\_\_\_\_$$

$$\% SO_4 = \% S \times 3 = \_\_\_\_\_$$

9. DETERMINATION OF SULPHUR FROM SULPHATE FERTILIZERS: [$(NH_4)_2SO_4$)]

Sulphur is a third secondary plant nutrient and S is supplied to the crop by addition of elemental sulphur or by addition of S containing inorganic fertilizers like: Ammonium sulphate (24% S), Sulphate of Potash (17.6 % S) and Micronutrient containing fertilizers ($FeSO_4$ 18.8%, $MnSO_4$ 21.2%, $ZnSO_4$17.8%, $CuSO_4$12.8%).

Objective:

1. To know the purity of ammonium sulphate fertilizer.

Principle:

Sulphur in any sulphate fertilizers can be determined by turbidimetrically using spectrophotometer. The solution containing a known amount of fertilizer in volumetric flask and reacted with $BaCl_2$ form the insoluble precipitations of barium sulphate. The intensity of precipitations is then measured in spectrophotometer at 430 nm wavelength.

Reagents:

(i) **Standard S solution (50 mg S/l):** Dissolve 0.2717g pure potassium sulphate (K_2SO_4) in distilled water and make the volume 1 litre

(ii) **Morgan's reagent:** Dissolve 100g sodium acetate in 800ml distilled water. Adjust the pH 4.8 by adding glacial acetic acid and make the volume 1 litre.

(iii) **Gum acacia solution:** Dissolve 0.5g pure gum acacia in 200 ml distilled water. Filter the solution with Whatman No. 42 filter paper to get clear filtrate. Check the clarity of solution before use

(iv) **Barium chloride:** Ground the barium chloride crystal (AR grade) with mortar and pestle to get fine powder

(A) Procedure for Standard Curve:

1. Take 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10ml of 100 ppm S solution in 50 ml vol. flasks.
2. To this add 20 ml of Morgan's reagent and 20 ml of 0.15% $CaCl_2$ are solution.
3. Then add 2 ml of Gum acacia solution and one spoon barium chloride and mix well. Then make the volume with distilled water.
4. After waiting for 20 minutes read the turbidity on spectrophotometer at 430 nm wavelength.
5. Construct a standard curve between net optical density against concentration of S.

Purity of fertilizer:

Percent S in $(NH_4)_2SO_4$ = = 28 + 8 + 32 + 64 = 132 (is molecular weight)

132 g **$(NH_4)_2SO_4$** contains 32 g of S

100 g **$(NH_4)_2SO_4$** contains (?)

$$\frac{100 \times 32}{132} \times 24.24\ \%\ S$$

If 24.24 % S 100 % pure

X % K_2O (?)

$$\frac{100 \times X}{24.24} = \_\_\_\_\_\%\ \text{purity}$$

Where X = % S in **$(NH_4)_2SO_4$** obtained by analysis

(B) Procedure for fertilizer sample:

(i) Weigh accurately 1 g of the S fertilizer on a clean dry watch glass.

(ii) Dissolve it in distilled water and transfer to the 250 ml of volumetric flask. Wash the watch glass and beaker thoroughly and transfer all the washings into the flask. Make the final volume up to the mark. Stopper and shake well.

(iii) Pipette 10 ml of aliquot and transfer it to 50 ml volumetric flask.

(iv) Add 20 ml Morgan's reagent, 2 ml gum acacia and 0.5 g barium chloride powder in each flask including for blank. Mix well and make the volume with distilled water.

(v) After 20 minute, measure the turbidity in spectrophotometer at 430 nm wavelength.

(vi) Calculate percentage of S and purity percentage of the given fertilizer samples using standard curve.

Result:

Sample	**S (%)**
$(NH_4)_2SO_4$)	

Answer the following question based on performed practical:

1. List out the S containing inorganic fertilizers.
2. Which factor is used to convert S to SO_4?
3. Name the chemicals used in preparation of Morgan’s reagent?

Observations

1. Weight of soil taken = _____ g
2. Volume of _____N H_2SO_4 used for sample titration (S) = _____ ml
3. Volume of _____N H_2SO_4 used for blank titration (B) = _____ ml
4. Volume of _____N H_2SO_4 actually used (S-B) = _____ ml

Calculations

1000 ml 1N H_2SO_4 = 14g N

1ml 1N H_2SO_4 = 0.014g N

$$\textbf{Available N (kg/ha)} = \frac{\text{(S-B) x 0.014 x N of } H_2SO_4 \text{ x 22,40,000}}{\text{Weight of Soil}}$$

OR

$$\textbf{Available N (kg/ha)} : \frac{\text{(S-B) x 0.014 x N of } H_2SO_4 \text{ x 100 x 10000 x 2.24}}{\text{Weight of Soil}}$$

10. ESTIMATION OF AVAILABLE NITROGEN FROM SOIL

(By Alkaline $KMnO_4$ Method)

Objective:

1. To know the available nitrogen content in soil.

Principle: A known weight of sample is the mixed with excess alkaline $KMnO_4$ solution. Potassium permanganate is oxidized the part of organic matter and alkaline media convert the organic N into ammonia gas and it absorbed in boric acid. The content is then titrated with standard sulphuric acid using a mixed indicator.

$$2KMnO_4 + H_2O \xrightarrow{NaOH} 2KOH + 2MnO_2 + 3(O)$$

$$R\text{-}CH\ NH_2COOH + \tfrac{1}{2}O_2 \xrightarrow{Oxidation} R\text{-}CO\text{-}COOH + NH_3$$

$$NH_3 + H_3BO_3 \longrightarrow (NH_4)_2BO_3$$

$$(NH_4)_2BO_3 + 2H_2SO_4 \longrightarrow (NH_4)_2SO_4 + 2H_3BO_3$$

Apparatus:

1. Distillation apparatus, 2. Glass beads, 3. 250 ml beakers, 4. Burette 5. Chemical balance

Reagents:

1. **0.1N H_2SO_4:** Dissolve 2.8ml concentrated H_2SO_4 in distilled water and diluted to 1 litre. Standardize with 0.1N Na_2CO_3 using methyl orange indicator.
2. **0.32% $KMnO_4$ solution:** Dissolve 3.2 g pure $KMnO_4$ in water and dilute to one litre volume.
3. **2.5% NaOH solution:** Dissolve 25 g loose alkali (NaOH) in water and dilute to one litre volume.
4. **Mixed indicator:** Dissolve 0.5 g bromocresol green 0.1 g methyl red in 100 ml of 95% ethanol. Adjust the pH 5.0 using 0.1N NaOH or HCl
5. **Boric acid (4%) :** Place 40g H_3BO_3 in 1 litre volumetric flask, add about 900ml distilled water and heat and swirl the flask until the H_3BO_3 is dissolve. Cool the solution and add 5ml of mixed indicator (0.5 g of bromocresol green and 0.1 g of methyl red in 100 ml of 95% ethanol: adjust pH 4.5 with dilute NaOH of HCl).
6. **Paraffin liquid**

Procedure:

6. Transfer 20g soil in a 800ml distillation flask.
7. Moisten the soil with water, wash down the soil adhering to the neck of the flask, if any.
8. Add 100ml of 0.32% $KMnO_4$ solution
9. Now add a few glass beads and 2-3ml paraffin liquid.
10. Measure 25ml 4% boric acid containing mixed indicator in a 250ml beaker and place it under the receiver tube. Dip the receiver tube end in the boric acid.
11. Add 100 ml of 2.5 % NaOH solution and immediately fit it up in the distillation apparatus.

12. Switch on the heater on and continue distillation until about 150ml of distillate is collected.
13. First remove the beaker containing distillate and then swich off the heater to avoid back suction.
14. Run a blank without soil
15. Titrate the distillate with std. 0.1 N H_2SO_4 up to pink coloured end point.

Result:

Available Nitrogen (kg/ha)= _____

Advise to Farmer:

Based on the amount of available N, soil been classified as follows:

Low : < 250 kg/ha
Medium : 250-500 kg N/ha
High : > 500 kg N/ha

Answer the following question based on performed practical:

1. Why $KMnO_4$ added in estimation of available nitrogen from Soil?
2. Which method is used for determination of available N from Soil?
3. Calculate the weight of KMnO4 to prepare 0.32% $KMnO_4$ solution.
4. Why paraffin liquid is added during estimation of available N from Soil?

(A) Observations standard curve:

No.	Aliquot taken from working standard (2mg P/l) ml	Conc. of P in 50 ml diluted solution (mg/l))	% T	O.D.	Net O.D.
1	0	0.00			
2.	1	0.04			
3	2	0.08			
4	3	0.12			
5	4	0.16			
6	5	0.20			
7	6	0.24			
8	7	0.28			
9	8	0.32			
10	9	0.36			
11	10	0.40			
		Total (A)			Total (B)

Graph factor	=	A / B	=	_____

Construct a standard curve between net optical density and the concentration of P

(B) Observations for soil analysis:

1. Weight of soil taken = _____
2. Volume of extractant added = _____
3. Volume of the filtrate taken = _____
4. Final Volume = _____
5. Spectrophotometer reading for blank(OD) = _____
6. Spectrophotometer reading for sample (OD) = _____
7. Optical density for blank = 2-log%T = _____
8. Optical density for sample = 2-log%T = _____
9. Net O.D. =Sample O.D. –Blank O.D. = _____
10. Graph factor **(GF)** = _____

11. DETERMINATION OF AVAILABLE PHOSPHORUS FROM SOIL (Olsen method)

Objective:

1. To know the available phosphorus content in soil.

Principle of Spectrophotometer:

Lambert's law: The intensity of emitted light decreased exponentially as the thickness of absorbing medium increases arithmetically

$$T = \frac{P}{P_0} = 10^{-kb} = \log T = \log \frac{P}{P_0} = -kb$$

k = Constant b = Path thickness

Beer's law: The intensity of beam of monochromatic light decreases exponentially as the concentration of the absorbing substances increase arithmetically

$$T = \frac{P}{P_0} = 10^{-k'c} = \log T = \log \frac{P}{P_0} = -k'c$$

k' = Constant c = Concentration

If we combine Lambert's and Beer's law

$$T = \frac{P}{P_0} = 10^{-abc} = \log T = \log \frac{P}{P_0} = -abc$$

a = Combine constant b = Path thickness c = Concentration

Absorbance is reverse of transmittance

$$A = -\text{Log } T = \log \frac{1}{T} = \log \frac{P_0}{P} = abc$$

Since, it is expressed in percentage

$$A = \log \frac{1}{T} \times 100 = \text{Log } 100 - \text{Log } T = 2 - \text{Log } T$$

Note: The absorbance is also known as optical density

Calculations:

$$\text{P in soil (mg/kg)} = \text{Net OD} \times \text{GF} \times \frac{100}{5} \times \frac{25}{5} = \_\_\_\_\_$$

$$\text{P}_2\text{O}_5 \text{ in soil (mg/kg)} = \text{Net OD} \times \text{GF} \times \frac{100}{5} \times \frac{25}{5} \times 2.29 = \_\_\_\_\_$$

P_2O_5 in soil(kg ha) = ppm P_2O_5 x 2.24= _____

$$\text{P}_2\text{O}_5 \text{ in soil (kg/ha)} = \text{Net OD} \times \text{GF} \times \frac{100}{5} \times \frac{25}{5} \times 2.29 \times 2.24$$

Apparatus/Instruments:

1. Spectrophotometer, 2. Volumetric flask, 3. Pipette, 4. Graduated pipette
5. Plastic bottle

Reagents:

1. **0.5 M sodium bicarbonate:** Weight accurately 42g $NaHCO_3$ dissolve and dilute to one litre with distilled water. Adjust the pH of this solution to 8.5 with 1 N NaOH.
2. **1.5 % Ammonium molybdate:** Dissolve 15 g Ammonium molybdate in 300 of warm distilled water. Filter the mixture if necessary and allow it to cool. Then add 410 ml concentrated HCl gradually with mixing and dilute to 1 litre with distilled water.
3. **Stannous chloride:** Dissolve 10 g $SnCl_2$ in 25 ml concentrated HCl. This solution is highly unstable if exposed to light because Sn^+ gets oxidized to Sn^{2+}. Keep in amber coloured bottle. Take 0.5 ml of this stock solution of $SnCl_2$ and diluted to 66 ml with distilled water at a time of analysis. Use this dilute solution for analysis.
4. **Phosphorus free activated charcoal**
5. **Standard P solution (50 mg P/l):** Dissolve 0.2195g pure KH_2PO_4 in distilled water and make the volume 1 litre.
6. **Working standard (2 mg P/l):** Take 40ml of 50 mg P/l standard solution and diluted to 1 litre with distilled water.

(A) Procedure for standard curve:

1. Take 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10ml of 2 mg P/l solution in 50 ml volumetric flasks.
2. To this 10 ml of 0.5M $NaHCO_3$ and 10 ml of 1.5% Ammonium molybdate are added and mix well.
3. Then add 1 ml of working stannous chloride and make the volume with distilled water. One blank is also prepared.
4. Within 15 minutes, read the intensity of the blue colour on spectrophotometer at 660 nm.
5. Construct a standard curve between net optical density against the concentration of P and draw a graph factor.

(B) Soil analysis

Principle:

The 0.5M $NaHCO_3$ pH 8.5 solution is most suitable for neutral to alkaline soils and is design to control the ionic activity of calcium through solubility product of $CaCO_3$, thus extracting the most reactive forms of P from Al-, Fe- and Ca-P. Phosphate in extract is measured by reaction of phosphate with ammonium molybdate in an acid medium to form molybdophosphoric acid. Molybdophosphoric acid is then reduced to blue coloured complex (reduced phosphomolybdenum blue) through reaction with stannous chloride. Absorbance reading are taken at a 660nm wavelength using spectrophotometer.

Procedure:

1. Weight 5 g of 2 mm sieved soil into 250 ml plastic bottle. Add one teaspoon of activated charcoal and 100 ml 0.5 M $NaHCO_3$ solution.
2. Shake the bottle for 30 minute on mechanical shaker
3. Filter the suspension through a Whatman No.42 Filter paper.
4. Take 5 ml aliquot in a 25 ml volumetric flask
5. Add 5 ml ammonium molybdate solution and add little quantity of distilled water and shake well
6. Add 1 ml working $SnCl_2$ solution in each 25 ml volumetric flask and make the volume up to 25ml with distilled water and shake well.
7. Measure the transmittance of the solution at 660 nm wavelength in spectrophotometer during the time of 5 minutes after and 20 minutes before the addition of $SnCl_2$ solution
8. Determine P_2O_5 concentration in the given soil samples using standard curve

Result: Available P_2O_5 (kg/ha)=_____

Advise for farmer:

Based on the amount of available P_2O_5, soil been classified as follows:

Low : < 28 kg/ha
Medium : 28-56 kg/ha
High : > 56 kg/ha

Answer the following question based on performed practical:

1. Which extractant is used for determination of available phosphorus from Soil?
2. Name the method used in determination of available phosphorus from Soil?
3. Name the reductant used in determination of available phosphorus from soil.
4. Give the Lambert's and Beer's law.
5. Why 0.5 M NaHCO3 solutions are most suitable for P determination in neutral to alkaline soils?

Observations

(A) For standard curve

Sr. No.	Volume taken from 100 mgK/L in 100ml volumetric flask	Concentration of K in solution (mg/l)	Reading of flame photometer
1	00	00	
2.	10	10	
3	20	20	
4	30	30	
5	40	40	
6	50	50	
7	60	60	
8	70	70	
9	80	80	
10	90	90	
11	100	100	
		Total (A)	Total (B)

Graph factor $= \frac{A}{B} =$ _____

Plot a standard curve between concentration K in diluted solution and flame photometer readingreadings.

(B) Observations for soil sample:

1. Weight of soil taken = ----------
2. Volume of N ammonium acetate added = ----------
3. Sample reading on flame photometer (R) = ----------
4. Graph factor (GF) = ----------
5. Dilution factor if any: =_____

Calculation

$$K_2O \text{ in soil (kg/ha)} = R \times GF \times \frac{25}{5} \times 1.20 \times 2.24$$

= _____

12. DETERMINATION OF AVAILABLE POTASSIUM FROM SOIL (Flame photometric Method)

Objective:

1. To know the available potassium content in soil.

Principle of instrument:

Atomic emission is caused by the excitement of electron which jump from lower energy level to higher energy level. These excited electrons come back to their original energy level. While returning to their original energy level, electrons give out the absorbed heat energy in the form of characteristic radiation. By measuring the intensity of emitted radiation, we can measure the amount of element present in the sample. The basic equation governing the phenomena is:

$$\text{ÄE} = E_1 - E_0 = hv = \frac{hc}{ë}$$

Where;

ÄE : Difference in energy of two electronic state of energies E_1 and E_0

E_1 : Energy at excited state of electrons

E_0 : Energy at ground state of electrons

h : Plank's constant (6.626×10^{-27} erg)

v : Frequency of radiation

c : Velocity of light (2.998×10^{10} cm/sec)

ë : Wavelength

Apparatus/Instruments:

1. Flame photometer, 2. Volumetric flasks, 3. Ggraduated pipette

Reagents:

1. **Neutral normal ammonium acetate solution:** Dissolve 77.0 g ammonium acetate (CH_3COONH_4) in 1 liter distilled water. Adjust the pH to 7.0 by adding NH_4OH or CH_3COOH

2. Standard K solution (1000 mg K/l): Dissolve 1.907g oven dried pure KCl in distilled water and make the volume 1 litre.

3. Working standard: 25, 50, 75 and 100 mg K/L

(A) Procedure for standard curve:

6. Take 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100ml of 100 mg K/L solution in 100ml volumetric flasks.
7. Feed these diluted solution directly to Flame photometer and note the reading
8. Construct a standard curve between Flame photometer reading against the concentration of K and draw a graph factor.

(B) Soil analysis

Principle:

Potassium in soil exists as water soluble, exchangeable, non exchangeable (fixed) and lattice-K. The first two forms constitute only a small part, normally not more than one per cent of the total content and are considered to be easily available to the plant. Only when these two forms are depleted, part of non exchangeable K moves to exchange sites and soil solution. Therefore, most of the methods suggested are based on the determination of easily available fraction i.e. water soluble and exchangeable K. Being the almost similar ionic radii, K^+ is more effectively replaced by NH_4^+. Soil is shaken with neutral normal ammonium acetate. During the extraction ammonium ions replace potassium ions absorbed on the soil colloids (Colloid) $K^+ + CH_3COONH_4 \rightarrow$ (Colloid) $NH^+ + CH_3COOK$). The extract is then filtered and potassium is determined with flame photometer.

Procedure:

1. Take 5 g soil in 150 ml conical flask or plastic bottle
2. Add 25 ml of neutral N ammonium acetate solution and shake for 30 minutes on an mechanical shaker
3. Filter the content through a Whatman No. 1 filter paper
4. Set up flame photometer to 0 scale reading by atomizing distilled water, and to 100 by atomizing 100 mg K/l solution
5. Feed the filtrate to the flame photometer and note the reading.

Result: Available K_2O /ha=_____

Advise for Farmer:

Based on the amount of available potash, soil been classified as follows:

Low : < 140 kg K_2O /ha
Medium : 140-280 kg K_2O /ha
High : >280 kg K_2O /ha

Answer the following question based on performed practical:

1. Which extractant is used in determination of available potassium from Soil?
2. Which method is used for determination of available potassium from Soil?
3. Give the principle flame photometer.
4. Calculate the weight of CH_3COONH_4 to prepare 1N Ammonium Acetate solution.
5. Why the extractant Ammonium Acetate used in estimation of available potassium is called Neutral Normal?

(A) Observations for standard curve:

No.	Aliquot taken from working standard (50 mg S/l) ml	Conc. of S in 50 ml diluted solution (mg /l)	O.D.	Net O.D.
1	0	0		
2.	1	1		
3	2	2		
4	3	3		
5	4	4		
6	5	5		
7	6	6		
8	7	7		
9	8	8		
10	9	9		
11	10	10		
		Total (A)		**Total (B)**

Graph factor $= \frac{A}{B} =$ _____

Construct a standard curve between net optical density and the concentration of S

(B) Observations for soil sample:

17. Weight of soil taken = 10g
18. Volume of extractant added = 50 ml
19. Volume of the filtrate taken = 20 ml
20. Final Volume = 50 ml
21. Spectrophotometer reading for blank (OD) = _____
22. Spectrophotometer reading for sample OD) = _____
23. Net O.D. =Sample O.D. –Blank O.D = _____
24. Graph factor (**GF)** = _____

Calculations:

S in soil (mg/kg) = Net OD x GF x $\frac{50}{10}$ x $\frac{50}{20}$ = _____

13. DETERMINATION OF AVAILABLE SULPHUR FROM SOIL (Turbidity method)

Objective:

1. To know the available sulphur content in soil.

Apparatus:

1. Spectrophotometer, 2. Volumetric flasks, 3. Pipette, 4. Graduated pipette, 5. Plastic bottle

Reagents:

i. **0.15% $CaCl_2$ $2H_2O$:** 1.5 g of $CaCl_2$ $2H_2O$ dissolve in distilled water and make volume one litre
ii. **Morgan's reagent:** Dissolved 100g sodium acetate in 800ml distilled water. Adjust the pH 4.8 by adding glacial acetic acid and make volume 1 litre.
iii. **Gum acacia solution:** Dissolve 0.5g pure gum acacia in 200ml distilled water. Filter the solution with Whatman No. 42 filter paper to get clear filtrate. Check the clarity of solution before use
iv. **Barium chloride:** Ground the barium chloride crystal with mortar and pestle to get fine powder
v. **Standard S solution (50 mg S/l):** Dissolve 0.2717g pure potassium sulphate in distilled water and make the volume 1 litre

(A) Standard curve for S:

Procedure:

6. Take 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10ml of 100 ppm S solution in 50 ml volumetric flasks.
7. To this 20 ml of Morgan's reagent and 20 ml of 0.15% $CaCl_2$ are added.
8. Then add 2 ml of Gum acacia solution and one spoon barium chloride and mix well and make the volume with distilled water.
9. After waiting for 20 minute read the turbidity absorption on spectrophotometer at 410 nm wavelength.
10. Construct a standard curve between absorption (OD) against the concentration of S and draw a graph factor.

(B) Soil analysis

Principle:

Besides some amount in soil solution, available S in mineral soils occurs mainly as adsorbed SO_4^{2-} ions. Both $CaCl_2$ and phosphate solutions are generally used for replacement of SO_4^{2-} ions. Uses of Ca salts have a distinct advantage over those of Na and K, as Ca prevents deflocculation in heavy textured soils and leads to easy filtration. SO_4^{2-} in the extract can be estimated turbidimetrically using spectrophotometer.

Procedure:

1. Transfer 10 g soil into a 100 ml capacity plastic bottle
2. Add 50 ml 0.15% $CaCl_2$ to it
3. Shake it for 30 minute on mechanical shaker
4. Filter the suspension and pipette 20 ml aliquot into 50 ml volumetric flasks
5. Add 20 ml Morgan's reagent, 2 ml gum acacia and one spoon barium chloride.
6. After 20 minute, measure the turbidity in spectrophotometer at 410 nm wavelength
7. Determine S concentration in the given soil samples using standard curve

Result: Available S (mg/kg) = _____

Advise to farmer:

Based on the amount of available S, soil been classified as follows:

Low < 10 mg/kg
Medium 10-20 mg/kg
High > 20 mg/kg

Answer the following question based on performed practical:

1. Which extractant is used for determination of available sulphur from Soil?
2. Name the method used in estimation of available sulphur from Soil?
3. Give the principle of Spectrophotometer?
4. Name the metods that is used in determination of available sulphur from Soil?
5. How to prepared 50 mg S/l from potassium sulphate?
6. Which wavelength is selected on spectrophotometer for determination of available sulphur from soil.
7. Give the rating for available sulphur in soil.

Observations:

1. Weight of soil sample taken =_____ g
2. Volume of DTPA solution added in soil =_____ ml
3. Reading of AAS (R) =_____ ml

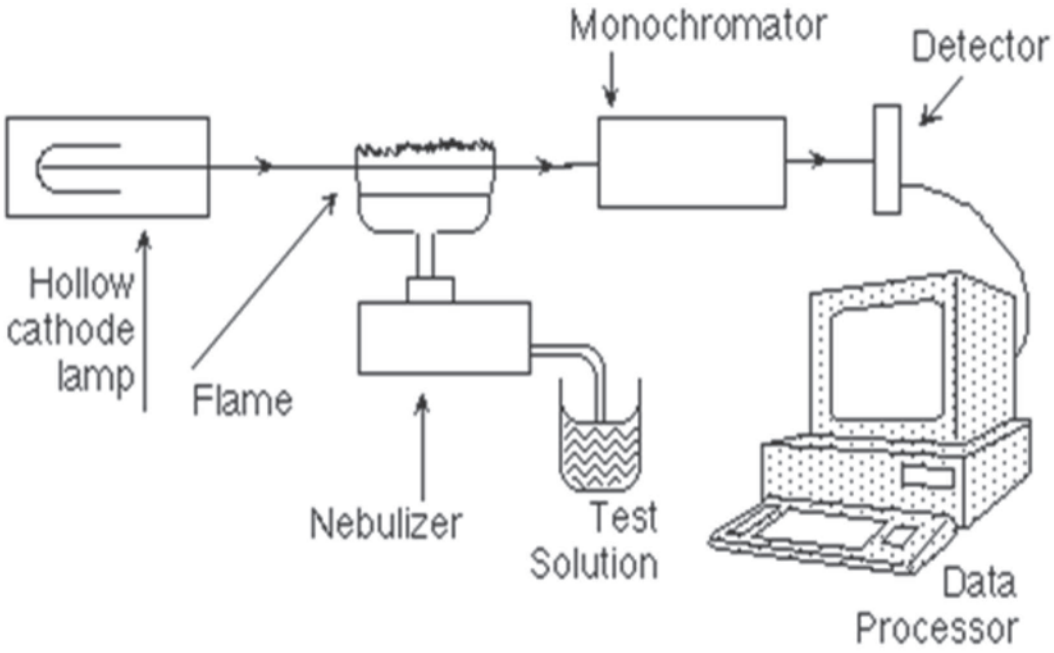
Calculation:

$$\text{DTPA extractable micronutrient (mg/kg)} : \frac{R \quad X \quad \text{Vol. of DTPA}}{\text{Wt of soil}}$$

=_____

14. DETERMINATION OF DTPA EXTREACTABLE MICRONUTRIENTS FROM SOIL USING (Atomic Absorption Spectrophotometer method)

Principle of instrument:



Many gaseous metal atoms normally remain at unexcited state (in ground state) but these atoms are capable of absorbing radiant energy of their own specific resonance wavelength, which in general, is a wavelength of radiation that the atom would emit, if they are excited from ground state. Hence, if the light of resonance wavelength is passed through a flame containing the atoms, the part of light will be absorbed and the extent of light absorption will be proportional to the number of ground state atom present in the flame. The process can be summarized as follows

$$M + hv \rightarrow M^*$$

(Ground state atom) (Excited atom)

Objective:

1. To know the available micronutrients content in soil.

Soil analysis:

Principle: Diethylene Triamine Penta Acetic acid (DTPA), a chelating agent, combines with free metal ions in solution and forms soluble complexes. Due to the reduced ionic activity in solution desorption takes place, bringing some more ions in solid phase. DTPA offers the most favourable combination of stabil ity constant for simultaneous complexing of Fe, Mn, Zn, and Cu. DTPA. Suitability of this method has been proved through excellent relationships between the test value and plant utilizable nutrients under pot and field studies conducted world over.

Apparatus/Instruments/Equipments:

1. Mechanical shaker, **2.** AAS, **3.** Plastic bottle, **4.** Whatman No. 42 filter paper **5.** Funnel, **6.** Chemical balance **7.** Beaker

Reagents:

1. **DTPA extractant** (0.005M DTPA, 0.1M TEA and 0.01M $CaCl_2.2H_2O$): Dissolve 1.967 g of AR grade diethylene triamine penta acetic acid (DTPA) and 1.47 g of $CaCl_2.2H_2O$ in about 25 ml of double distilled water by adding 13.3 ml of triethanolamine (TEA), followed by 100 ml more of DDW. Transfer the solution to one litre volumetric flask giving 4 to 5 washing. Just before making the volume, adjust pH to 7.3 with dilute HCl.

2. **Standards::** To calibrate the AAS for each element

Fe: 1.0, 2.0, 3.0 and 4.0 ppm
Mn: 0.5, 1.0, 2.0 and 3.0 ppm
Zn: 0.3, 0.6, 0.9 and 1.2 ppm
Cu: 0.4, 0.8, 1.2 and 1.6 ppm

Soil analysis:

(A) Extraction

1. Weigh 10 g of soil sample in 100 ml of plastic bottles.
2. Add 20 ml of the DTPA extractant (keep soil: DTPA ratio - 1:2) and shake for 2 hours on a mechanical shaker at 120 rpm.
3. Filter through Whatman No. 42 filter paper in a 50 ml beaker.
4. Use this filtrate for micro nutrient measurement on AAS.

(B) Analysis

1. Calibrate the AAS through standard procedure
2. Then feed the DTPA extracts and record the concentration of the element.
3. Repeat the above steps for every element.
4. If the concentration of element in the sample is above the standardize range, then make dilution and feed the sample again and record the concentration.

Results:

(1) Concentration of DTPA extractable Fe = _____ mg/kg

(2) Concentration of DTPA extractable Mn = _____ mg/kg

(3) Concentration of DTPA extractable Zn = _____ mg/kg

(4) Concentration of DTPA extractable Cu = _____ mg/kg

Advise to farmer:

(1)

(2)

(3)

(4)

Based on the amount of available Micro nutrients, soil has been classified as follows:

Elements	Low	Medium	High
	(mg/kg or ppm)		
DTPA-Fe	< 5.0	5.0-10.0	>10.0
DTPA-Mn	< 5.0	5.0-10.0	>10.0
DTPA-Zn	< 0.50	0.50-1.00	>1.00
DTPA-Cu	< 0.20	0.20-0.40	>0.40

a. If DTPA-Fe is in low range, advise the farmer to add 50 kg ferrous sulphate per hectare (once in three years).
b. If DTPA-Mn is in low range, advise the farmer to add 10 kg manganese sulphate per hectare(once in three years).
c. If DTPA-Zn is in low range, advise the farmer to add 50 kg zinc sulphate per hectare (once in three years).
d. If DTPA-Cu is in low range, advise the farmer to add 5 kg copper sulphate per hectare (once in three years).

Answer the following question based on performed practical:

1. Which extractant is used for determination of cationic micronutrient from Soil?
2. Name the method that is used for determination of cationic micronutrient from Soil.
3. Give the principle Atomic Absorption Spectrophotometer.
4. Give the full form of DTPA.
5. Why DTPA is used as an extractant for determination of micronutrient from soils?
6. Name the essential cationic and anionic micronutrients for plant.
7. Name the chemicals used in preparation of DTPA extractant.
8. Suggest the names of cationic micronutrient fertilizers for soil application.

Important Conversions

Milli equivalent weight (m.e.):

Equivalent weight expressed in mg is known as milli equivalent. i.e. It is the thousand part of gram Eq.wt.

m.e. = Vol. of solution (ml) x N

mg = Vol. of solution (ml) x N x Eq.wt. (OR)

= me x Eq.wt.

Parts per million (ppm):

Parts per million (ppm) is the parts of unit weight present in one million unit weight or volume.

1 million = 10, 00,000

ppm = mg/liter = mg/kg = g/ton = mg % x 10

= me/liter x Eq.wt. or me/100g x 10 x Eq.wt

= % x 10,000

(mg = me x eq. wt),

kg /ha = ppm x 2.24,

10,000 sq.mt = 1 ha = 2.471 acre = 100 gunthas

43560 sq.ft. = 1 acre = 0.447ha = 40 gunthas

Lbs/acre x 1.12 = kg/ha (1 lbs = 0.4536 kg)

Angstron unit(A^0) = 10^{-8} cm = 10^{-9}mm = 10^{-10} mt.

1 meter = 100cm = 1000mm

micron = 10^{-3}mm = 10^{-6}m = 10^{-4}cm

Nano meter (nm) = 10^{-7}cm = 10^{-9}m

desi (d) = 10^{-1} (1/10)

centi (C) = 10^{-2} (1/100)

milli (m) = 10^{-3} (1/1000)

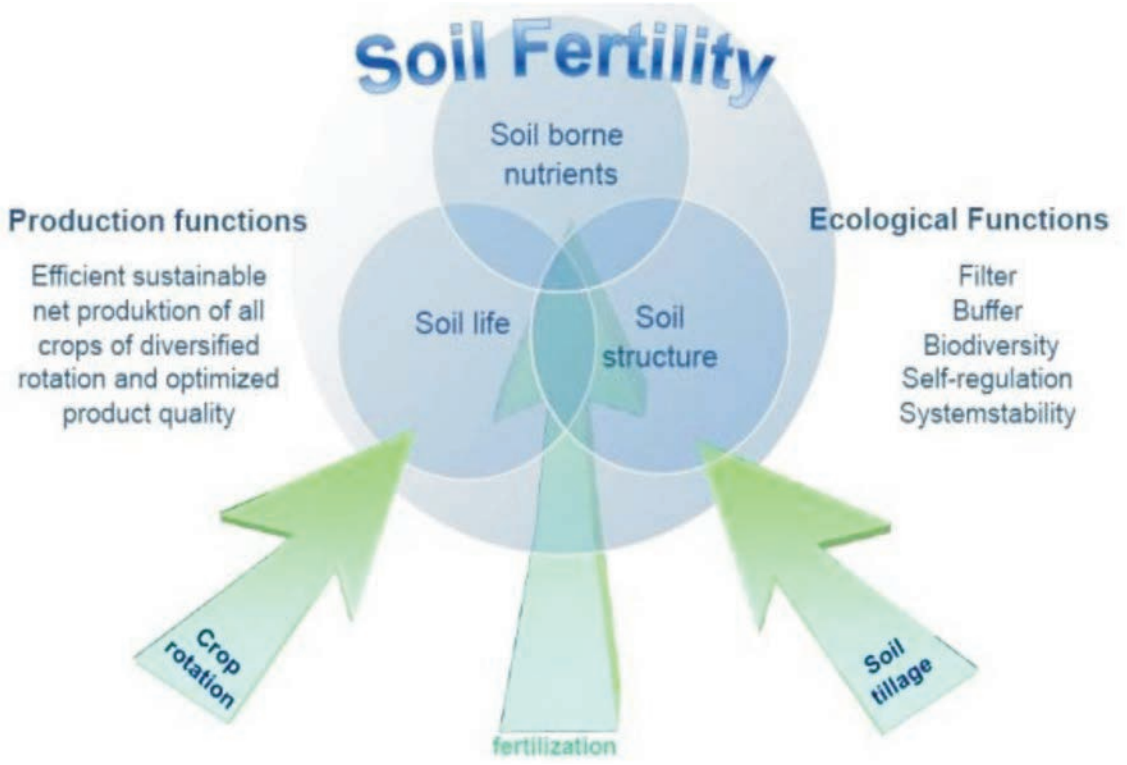
micro (d) = 10^{-6} (1/1000000)

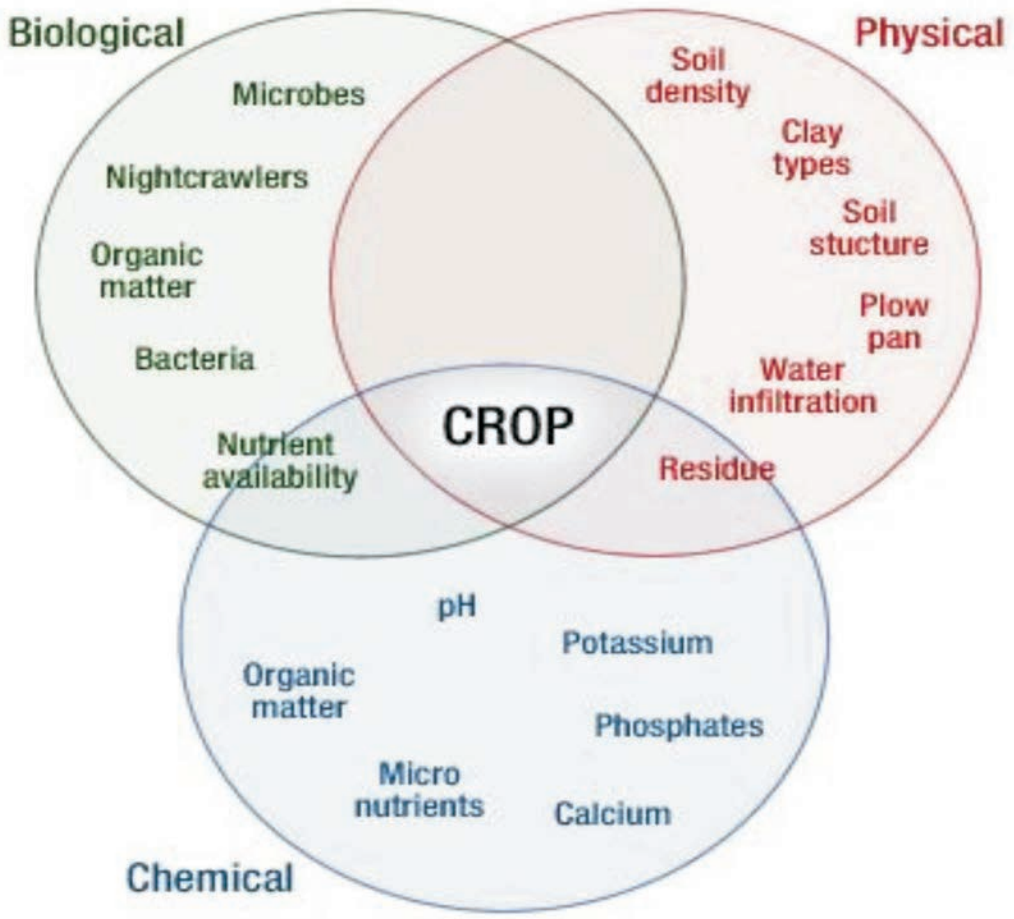
nano (n) = 10^{-9} (1/1000000000)

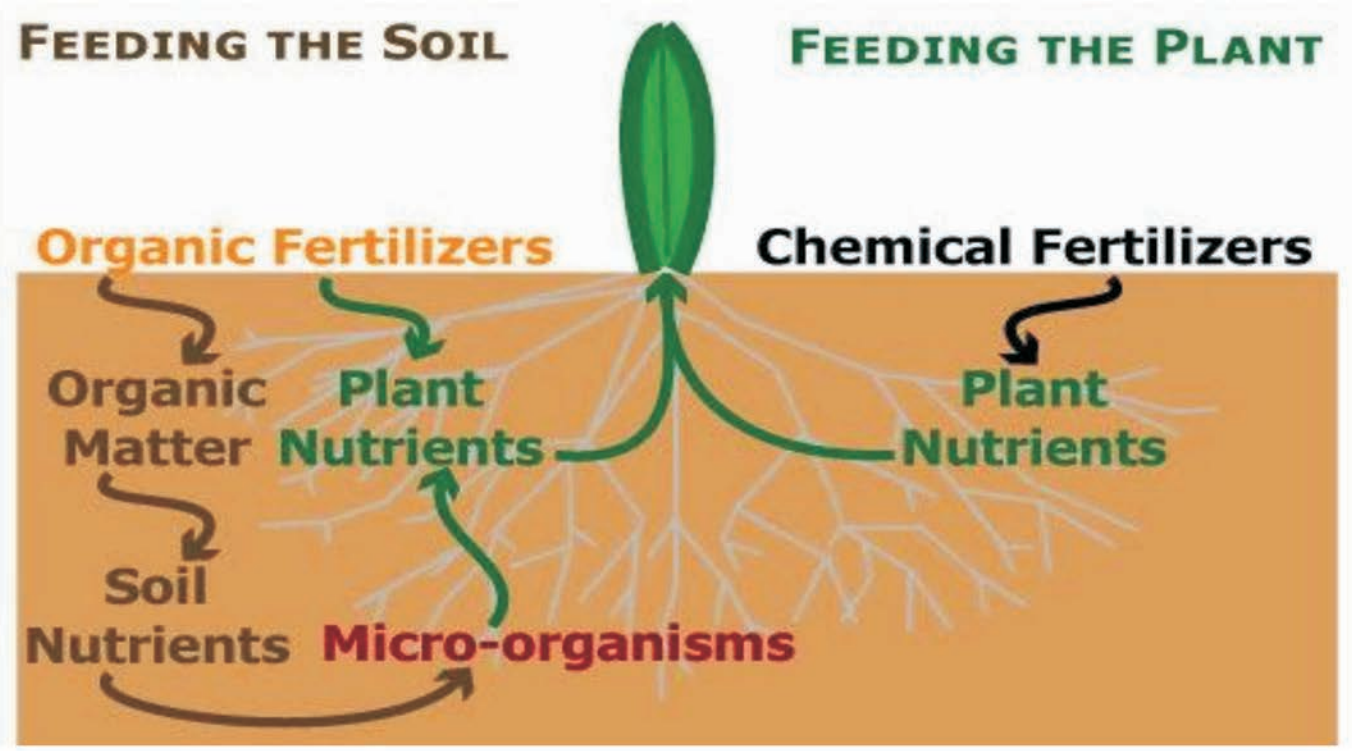
pico (p) = 10^{-12} (1/1000000000000)

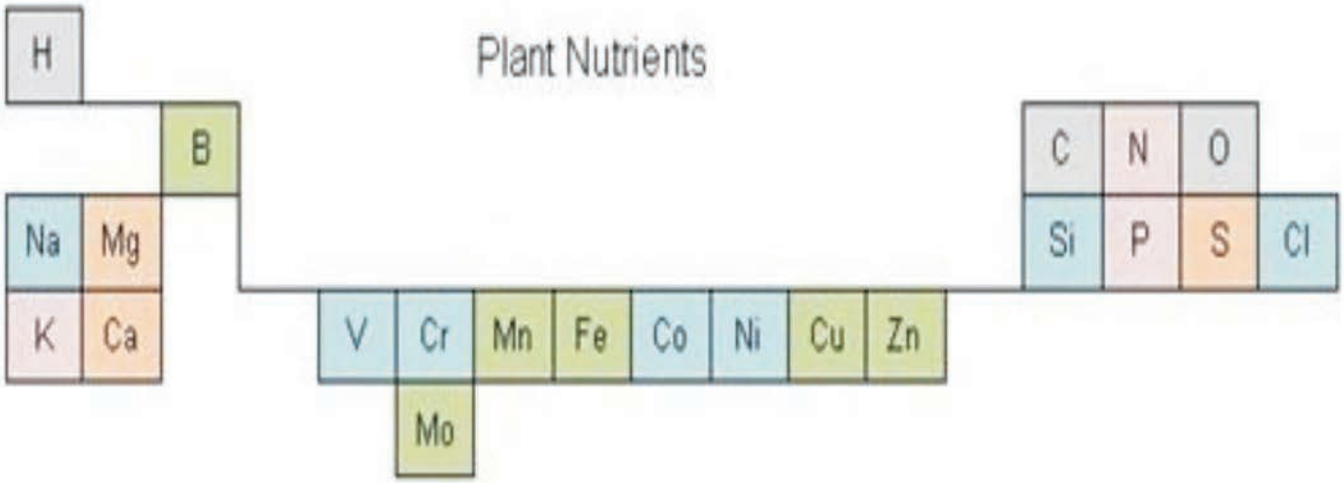
femto (f) = 10^{-15} (1/1000000000000000)

atto (a) = 10^{-18} (1/1000000000000000000)


Soil Fertility
Soil borne nutrients
Production functions
Efficient sustainable net produktion of all crops of diversified rotation and optimized product quality
Ecological Functions
Filter
Buffer
Biodiversity
Self-regulation
Systemstability
Soil life
Soil structure
Crop rotation
fertilization
Soil tillage


Biological
Microbes
Nightcrawlers
Organic matter
Bacteria
Nutrient availability
Physical
Soil density
Clay types
Soil stucture
Plow pan
Water infiltration
Residue
CROP
pH
Potassium
Organic matter
Phosphates
Micro nutrients
Calcium
Chemical





Organic		Major		Secondary		Micronutrients		Functional	
C	Carbon	N	Nitrogen	Mg	Magnesium	B	Boron	Na	Sodium
H	Hydrogen	P	Phosphorus	Ca	Calcium	Cu	Copper	V	Vanadium
O	Oxygen	K	Potassium	S	Sulphur	Fe	Iron	Co	Cobalt
						Mn	Manganese	Si	Silicon
						Mo	Molybdenum	Cl	Chlorine
						Zn	Zinc		

Farm Yard Manure (FYM)

Caster Cake

Compost

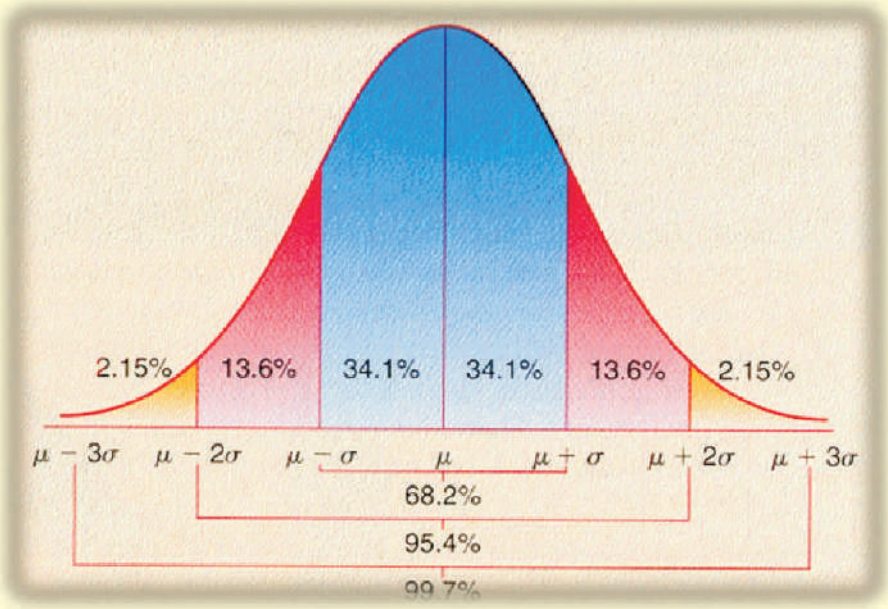
Vermicompost

Micronutrients Fertilizer

Practical Manual

Agricultural Statistics

Ag.Stat. 2.2



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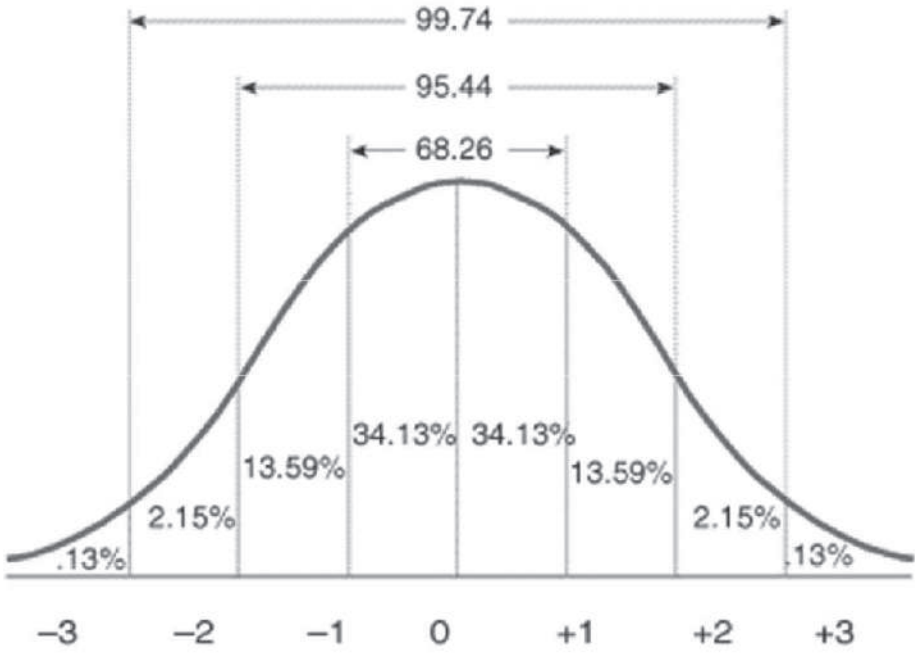
I can prove
anything
by statistics
except
the truth.

- George Canning

Practical Manual

Agricultural Statistics

Ag.Stat. 2.2



Name of Student : _____

Uni. Seat No.: _____ **Registration No. :** _____

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CERTIFICATE

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Registration No.: _____

This is to certify that Mr./Miss. _____ of the Second semester B.Sc. (Hons.) in Agriculture student has satisfactorily carried out essential exercises as shown in the practical manual of **Ag.Stat.2.2** (Agricultural Statistics) in the Department of Agricultural Statistics during the year 20_____.

(Course Teacher)

Head of Department

Signature of External Examiner

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Exercise No. 1 **Date: / /**

Introduction to Statistics

In the modern world of computers and information technology, the importance of statistics is very well renowned by all the disciplines. Statistics has originated as a science of statehood and found applications in Agriculture, Economics, Biology, Medicine, Industry, planning, education and so on. Now days, there is no other part of life, where statistics cannot be applied.

The word 'Statistics' is derived from the Latin word '**Status**', means a '**Political state**'. The theory of statistics as a distinct branch of scientific method is of comparatively recent growth. Statistics can be used either as **plural** or **singular**. When it is used as plural, it is a systematic presentation of facts and figures. It is in this context that majority of people use the word statistics. **E.g.** 1. Production of food grains in different years, 2. Area under cereal crops in different years, 3. Per capita income in a particular state at different times etc. These are generally published in trade journals, economics and statistics bulletins, newspapers, etc.

When statistics is used as singular, it is a science, which deals with collection, classification, tabulation, analysis and interpretation of data. It involves the methods of analysis used in the analysis and interpretation of data and It is known as statistical methods.

1.1 Definition: The following are some important definition of statistics.

1. **Sir Ronald Aylmer Fisher (R. A. Fisher - 1890-1962) - Father of Statistics**- 'The science of statistics is essentially a branch of applied mathematics and is concerned with observational data'. It is a study of population, variation and the methods for reduction of the data.
2. **Lovitt-** Statistics is the branch of science which deals with the collection, classification and tabulation of numerical facts as the basis for explanations, description and comparison of phenomenon
3. **Corxton & Cowden-** The science, which deals with the collection, analysis and interpretation of numerical data
4. **Bowley-** Statistics may be called the science of counting or science of averages or statistics is the science of the measurement of social organism, regarded as whole in all its manifestations
5. **Boddington-** Statistics is a science of estimates and probabilities
6. **Wallis and Roberts-** Statistics is a branch of science, which provides tools (techniques) for decision making in the face of uncertainty (probability)

1.2 Functions/Aim of Statistics:

1. **Condensation:** the word 'to condense', we mean to reduce or to lessen. Statistical measures help to reduce the complexity of the data and consequently to understand any huge mass of data.
2. **Comparison:** Classification and tabulation are the two methods that are used to condense the data. They help us to compare data collected.
3. **Forecasting:** we mean to predict or to estimate before hand from different sources. Eg. The rainfall data of last twenty years of a particular state in India, it is possible to predict or forecast the rainfall for the near future. In business, also forecasting plays a dominant role in connection with production, sales, profits etc. The time series analysis and regression analysis plays an important role in forecasting.
4. **Estimation:** The main objective of statistics is drawn inference about a population from the analysis for the sample drawn from that population. The four major branches of statistical inference are
   1. Estimation theory
   2. Tests of Hypothesis
   3. Non-Parametric tests
   4. Sequential analysis

   In estimation theory, we estimate the unknown value of the population parameter based on the sample observations.
5. **Tests of Hypothesis:** A statistical hypothesis is some statement about the probability distribution, characterizing a population based on the information available from the sample observations. In the formulation and testing of hypothesis, statistical methods are extremely useful. Whether Crop yield has increased because of the use of new fertilizer example of statements of hypothesis

1.3 Uses of Statistics:

1. Statistics are indispensable in planning and in taking decisions regarding export, import, and production etc., Statistics serves as foundation of the super structure of planning.
2. Statistics helps the businessperson in the formulation of polices with regard to business. Statistical methods are applied in market and production research, quality control of manufactured products.
3. Statistics is indispensable in economics. Any branch of economics that require comparison, correlation requires statistical data for salvation of problems
4. Administration is largely depends on facts and figures thud it needs statistics. In collecting the information about population, military strength etc.,
5. Bankers, stock exchange brokers, insurance companies all make extensive use of statistical data.

6. Problems relating to poverty, unemployment, food storage, deaths due to diseases, due to shortage of food etc., it comes in to focus due to the statistical balance. Thus, statistics is helpful in promoting human welfare.
7. In **agricultural research,** Statistical tools have played a significant role in the analysis and interpretation of data. Statistics Play important role in different divisions of agriculture such as Irrigation, Fertilizer requirement, Soil chemistry, Entomology, Agricultural economics, Animal science, Agril. Engineering etc.
    a. It helps to understand nature of variability or differences.
    b. To arrive at the meaningful conclusion on the basis of sample study in the field.
    c. Express the data/result of the field experiment in summary form.
    d. Sampling used in state agricultural survey, for estimation of areas and yield of crops.
    e. In price fixation policy of various agricultural commodities.
    f. In agricultural extension survey, to study the impact of programs.
    g. In agricultural economics survey, to study the demand-supply policy, the growth rate of population and cost of production of various crops.
    h. In agricultural meteorology for weather forecasting and to correlate weather parameters with crop production

1.4 Limitations of statistics:

Statistics has wide application in every area of all human activities with some of the limitations are given below.

1. **Statistics is not suitable to the study of qualitative phenomenon:** Since statistics is a science and deals with a set of numerical data, it is applicable to the study of only these subjects of enquiry, which can be expressed in terms of quantitative measurements. In fact, qualitative phenomenon like honesty, poverty, beauty, intelligence etc., cannot be expressed numerically and any statistical analysis can not be applied directly on these qualitative phenomenons. However, statistical techniques may be applied indirectly by converting the qualitative data to quantitative forms. E.g., Quality of fruits can be categorized by giving values of nutrients availability or no. of days perishable life.
2. **Statistics does not study individuals:** Statistics does not give importance to the individual items, in fact it deals with an aggregate (group) of objects. Individual items, does not constitute as a statistical data and do not serve any purpose for any statistical enquiry.
3. **Statistical laws are not exact and Statistical conclusions are valid only on average base:** Statistical laws are not exact. These are only approximations. Statistical conclusions are not

universally true. They are true only on an average. This is because statistics as a science less exact as compared to natural sciences. Eg., Average consumption of milk per head in a certain locality is half liter but it does not give any idea of the shortage of milk faced by the poor.

4. **Statistics is liable to be misused:** Only experts must use Statistics; otherwise, statistical methods are the most dangerous tools on the hands of the inexpert. The use of statistical tools by the inexperienced and untraced persons might lead to wrong conclusions. Thus the phrase has come- **'Statistics are like clay of which one can make a God or Devil as one pleases'.**
5. **Statistics does not reveal the entire information:** Statistics is only, one of the methods of studying a problem. Statistical method do not provide complete solution of the problems because problems are to be studied taking the background of the society, philosophy or religion into consideration. Therefore, the statistical study depends on supplemented by other evidences.

Exercise No. 2 **Date:**

Frequency Distribution and Frequency curve

2.1 Frequency and Frequency Distribution

The word 'frequency' is defined as how frequently a variable occurs *i.e.* how many times a particular value of a variable is repeated. A tabular arrangement of the data which gives the frequency corresponding to each successive value of the variable is called 'frequency distribution'.

A frequency distribution is constructed for following reasons:

1. To facilitate the analysis of data.
2. To estimate frequencies of the unknown population distribution from the distribution of sample data and
3. To facilitate the computation of various statistical measures

2.2 Types of Frequency Distribution

a) Simple or Ungrouped frequency distribution

In this form of distribution, the data are presented in a way that exact measurements of units are clearly indicated. Each class is distinct and separate from the other class. Non-continuity from one class to another class exists. For example: the number of students in a class, the number of universities in a state, the number of children in a family etc.

The process of preparing this type of distribution is very simple. We have to just count the number of times a particular value is repeated, which is called the frequency of that class. In order to facilitate counting

- Arrange the data of individual item in ascending order of magnitude and put it in a column.
- A bar (|) called tally mark is put against the number when is occurs. When we have four tallies then the fifth occurrence is represented by putting a cross tally (\) on the first four tallies. We finally count the number of bars and get frequency.

Example 1

In a survey of 40 families in a village, the number of children per family was recorded and the following data obtained.

1	0	3	2	1	5	6	2
2	1	0	3	4	2	1	6
3	2	1	5	3	3	2	4
2	2	3	0	2	1	4	5
3	3	4	4	1	2	4	5

Represent the data in the form of a discrete frequency distribution.

Solution:

Frequency distribution of the number of children

No. of Children	Tally marks	Frequency
0	|||	3
1	𝍸 ||	7
2	𝍸 𝍸	10
3	𝍸 𝍸	8
4	𝍸 |	6
5	||||	4
6	||	2
	Total	**40**

b) Grouped frequency distribution

The simple frequency distribution does not reduce the bulk of data much and in such situation, the frequency table remains too lengthy. So it is necessary to condense the data by putting them into smaller groups or classes called 'class intervals'. The number of values belonging to each class interval is called its frequency.

Example: 2 Score obtained out of 100 marks in Subject of statistics of 100 students

Score obtained	No. of Students
0-20	5
20-40	12
40-60	26
60-80	40
80-100	17
Total	100

2.3 Basic terminologies: The following are some basic technical terms when a continuous frequency distribution is formed

a) Class limits

The class limits are the lowest and the highest values that can be included in the class. E.g. 20-40, the lowest value of the class is 20 and highest class is 40. The two boundaries of class are known as the lower limits and the upper limit of the class. In statistical calculations, lower class limit is denoted by **L** and upper class limit by **U**.

b) Class Interval

The class interval may be defined as the size of each grouping of data. For example, 50-75, 75-100, 100-125… are class intervals.

c) Width or size of the class interval

The difference between the upper and lower class limits is called width or size of class interval and is denoted by 'C'.

d) Range

The difference between largest and smallest value of the particular frequency distribution is called the Range and is denoted by 'R' i.e.

$$R = \text{Largest value} - \text{Smallest value}$$

$$R = L - S$$

e) Mid-value or mid-point

The central point of a class interval is called the mid value or mid-point. It is calculated by adding the upper and lower limits of a class and dividing the sum by 2 (i.e.)

$$\text{Mid value} = (L + U)/2$$

f) Frequency

Number of observations falling within a particular class interval is called frequency of that class. In the above **example 2**, the class frequency is 5, 12, 26, 40 and 17. The total frequency is equal to 100. The total frequency indicates the total number of observations considered in a frequency distribution.

g) Number of classes

If the number of classes and width of the class intervals of a data are not given then the number of classes and approximate width of class interval can be determined by the **Sturges** formula for the preparation of frequency distribution.

$$k = 1 + 3.322 \log_{10} N$$

where, N = Total number of observation

k = Number of classes.

E.g. Thus if the number of observation is 100, then the number of classes will be

$$k = 1 + 3.322 \log_{10} 100 = 7.644 \approx 8 \qquad (\log_{10}100 = 2)$$

Approximate width of class interval is

$$i = \frac{L - S}{1 + 3.322 \log_{10} N} = \frac{L - S}{k}$$

Where, L= Largest observation in the data

S = Smallest observation in the data

The number of classes can be determined by the **Yules** formula,

$$\text{No. of classess} = 2.5 \times n^{1/4}$$

If 10 observations are being studied, the number of class interval is

$$\text{No. of classess} = 2.5 \times (10)^{1/4} = 4.445 \approx 5$$

h) Size of the class interval : The size of the class interval is inversely proportional to the number of class interval in a given distribution. The approximate value of the size (or width or magnitude) of the class interval '**C**' is obtained by using **Sturges rule** as

Size of class interval = C = Range/ Number of class interval

2.6 Types of class intervals:

There are three methods of classifying the data according to class intervals namely

a) Exclusive method

b) Inclusive method

c) Open-end classes

Points while deciding class interval/classes:

1. It should be of uniform width, which facilitates the statistical computation.
2. Range of the class should cover the data and should be continuous.
3. It should be convenient to make the mid-point of a class.
4. It should not be over lapping.

a) Exclusive method: When the class intervals are so fixed that the upper limit of one class is the lower limit of the next class; it is known as the exclusive method of classification.

Wages (Rs/day)	Frequency
0-100	40
100-200	38
200-300	20
300-400	20
400-500	12
Total	**130**

b) Inclusive method: In this method, the overlapping of the class intervals is avoided. Both the lower and upper limits are included in the class interval. This type of classification may be used for a grouped frequency distribution for discrete variable like members in a family, number of workers in a factory etc., where the variable may take only integral values. It cannot be used with fractional values like age, height, weight etc.

This method may be illustrated as follows:

No. of Worker	Frequency
0-100	40
101-200	38
201-300	20
301-400	20
401-500	12
Total	130

*In case of continuous variables, the **exclusive method** must be used. The **inclusive method** should be used in case of discrete variable.

c) Open end classes: A class limit is missing, either at the lower end of the first class interval or at the upper end of the last class interval or both are not specified. The necessity of open end classes arises in a number of practical situations, particularly relating to economic and medical data when there are few very high values or few very low values which are far apart from the majority of observations.

Wages (Rs/day)	Frequency
below-100	40
100-200	38
200-300	20
300-400	20
400 and above	12
Total	130

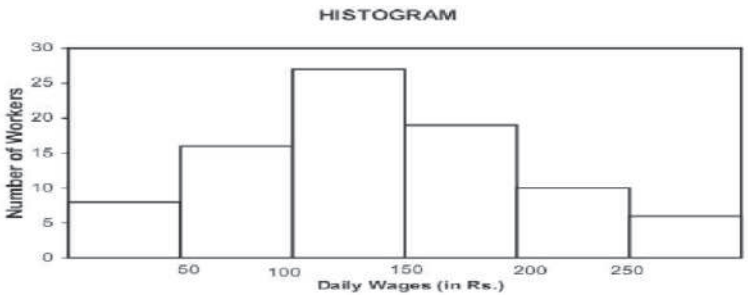
2.8 Histogram:

A histogram is a bar chart or graph showing the frequency of occurrence of each value of the variable being analysed. In histogram, data are plotted as a series of rectangles. Class intervals are shown on the 'X-axis' and the frequencies on the 'Y-axis'. The height of each rectangle represents the frequency of the class interval. Each rectangle is formed with the other to give a continuous picture. Such a graph is also called staircase or block diagram. However, we cannot construct a histogram for distribution with **open-end classes**. It is also quite misleading if the distribution has unequal intervals and suitable adjustments in frequencies are not made.

Example 3: Draw a histogram for the following data

Daily Wages	Number of Workers
0-50	8
50-100	16
100-150	27
150-200	19
200-250	10
250-300	6

Solution:



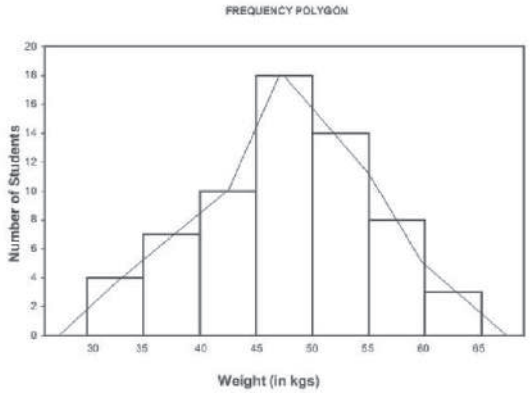
2.9 Frequency Polygon:

If we mark the midpoints of the top horizontal sides of the rectangles in a histogram and join them by **a straight line,** the figure so formed is called a Frequency Polygon. This is done under the assumption that the frequencies in a class interval are evenly distributed throughout the class. The area of the polygon is equal to the area of the histogram, because the area left outside is just equal to the area included in it.

Example 4: Draw a frequency polygon for the following data.

Weight (in kg)	Number of Students
30-35	4
35-40	7
40-45	10
45-50	18
50-55	14
55-60	8
60-65	3

Solution:

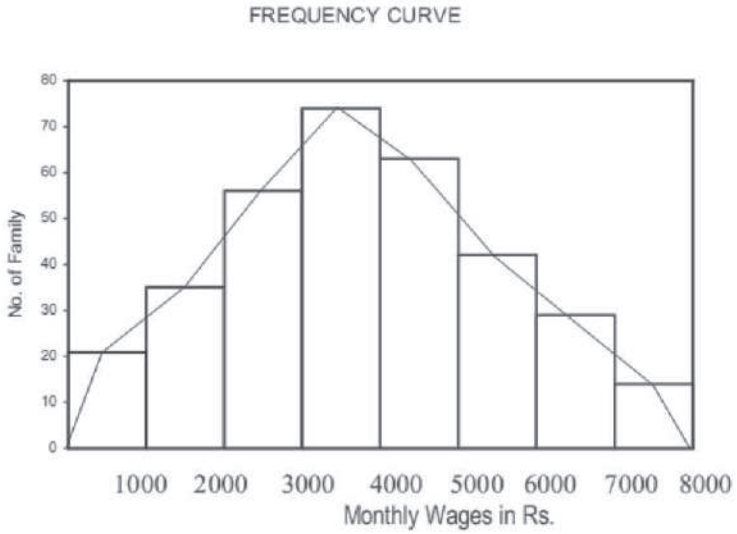


2.10 Frequency Curve:

If the middle point of the upper boundaries of the rectangles of a histogram is corrected by a **smooth freehand curve,** then that diagram is called frequency curve. The curve should begin and end at the base line.

Example 5: Draw a frequency curve for the following data.

Monthly Wages (in Rs.)	No. of family
0-1000	21
1000-2000	35
2000-3000	56
3000-4000	74
4000-5000	63
5000-6000	40
6000-7000	29
7000-8000	14



2.11 Ogives:

For a set of observations, we know how to construct a frequency distribution. In some cases, we may require the number of observations less than a given value or more than a given value. This is obtained by an accumulating (adding) the frequencies up to Monthly Wages in Rs. (or above) the give value. This accumulated frequency is called cumulative frequency. These cumulative frequencies are then listed in a table is called cumulative frequency table. The curve table is obtained by plotting cumulative frequencies is called a **cumulative frequency curve** or an **ogive**. There are two methods of constructing ogive namely:

1. The **'less than ogive'** method: we start with the upper limits of the classes and go adding the frequencies. When these frequencies are plotted, we get a rising curve.

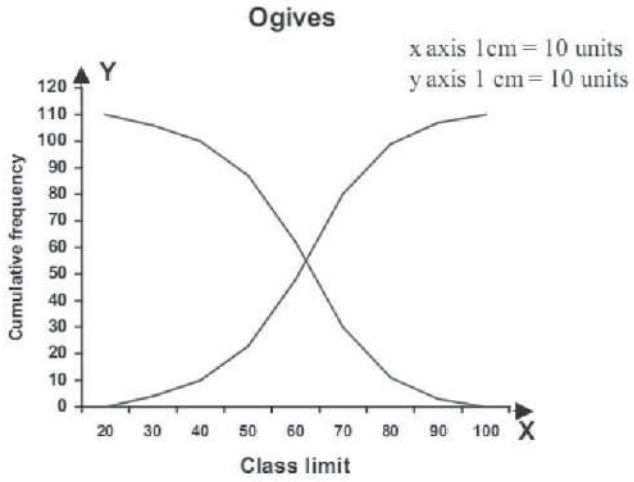
2. The **'more than ogive'** method: we start with the lower limits of the classes and from the total frequencies we subtract the frequency of each class. When these frequencies are plotted we get a declining curve.

Example 6: Draw the Ogives for the following data

Class interval	Frequency
20-30	4
30-40	6
40-50	13
50-60	25
60-70	32
70-80	19
80-90	8
90-100	3

Class Limit	Less than ogive	More than ogive
20	0	110
30	4	106
40	10	100
50	23	87
60	48	62
70	80	30
80	99	11
90	107	3
100	110	0

Solution:



Solve following problem:

Q. 1. The following table, give the age-wise distribution of the students in a certain school. Calculate more than and lower than frequency table.

Age in years	**No of students**	**Less than (ogive) cumulative frequency**	**More than (ogive) cumulative frequency**
14-15	12		
15-16	35		
16-17	40		
17-18	65		
18-20	68		
20-25	156		
25-35	412		
35-45	587		
45-55	336		
55-60	214		
60-65	75		
Total			

Q. 2. The following table gives the yield (kg/plot) of the wheat crop for each of 40 plots:

69.5	68.5	69.5	65	68.5	67.5	73	71
65.5	67	67.5	67	71	65.5	66	64.5
69	68.5	65	65.5	66.5	68	65	64.5
68.5	71	68	69	67.5	71	68.5	66.5
69.5	70	69.5	67.5	68.5	70	70.5	68.5

Prepare a suitable frequency distribution table.

Q. 3 The following table gives the weights of the 55 student of the class. Prepare suitable frequency table by method of Inclusive and Exclusive. Find Class interval and No. of Classes.

42	53	68	66	72	74	90	69	49	50	40
76	95	77	79	60	84	80	90	52	88	54
79	84	92	41	65	54	42	51	62	78	73
64	86	75	77	59	69	78	83	56	81	56
70	94	63	100	95	71	101	67	79	76	96

Exercise No. 3 **Date: / /**

Measures of Central Tendency

3.1 Definition:

1. Any mathematical measure which is intended to represent the center or central value of a set of observations is known as **measure of central tendency**
2. The single value, which represents the group of values, is termed as **measure of central tendency** or a measure of location or an average.

3.2 Types of average:

1. Arithmetic Mean (A.M.)
2. Geometric Mean (G.M.) } Algebraic average
3. Harmonic Mean (H.M.)
4. Median (Md)
5. Mode (Mo) } Positional average

3.3 Arithmetic mean:

Arithmetic mean (A.M.) or simply the mean of a variable is defined as the sum of the observations divided by the number of observations. If the variable 'x' assumes n values $x_1, x_2, \ldots x_n$ then the mean, $\bar{x}$, is given by

$$\bar{x} = \frac{x_1 + x_2 + \ldots + x_n}{n}$$

$$= \frac{1}{n}\sum_{i=1}^{n} x_i$$

Deviation method (Assumed mean): Under this method an assumed or an arbitrary average (indicated by A) is used as the basis of calculation of deviations from individual values. The formula is

$$\bar{x} = A + \frac{\sum d}{n}$$

where, A = the assumed mean or any value in x

d = the deviation of each value from the assumed mean $d = x - A$

Example 1: A student's marks in 5 subjects are 75, 68, 80, 92, 56. Find average mark of students.

Solution:

	X	d=x-A
	75	7
A	68	0
	80	12
	92	24
	56	-12
	Total	31

$$\bar{x} = A + \frac{\sum d}{n}$$

$$= 68 + \frac{31}{5}$$

$$= 68 + 6.2$$

$$= 74.2$$

Grouped Data:

The mean for grouped data is obtained from the following formula:

$$\bar{x} = \frac{\sum f x}{N} \qquad \because N = \sum f_i$$

where, x = the mid-point of individual class

f = the frequency of individual class

N = the sum of the frequencies or total frequencies.

Deviation method (Assumed mean):

$$\bar{x} = A + \frac{\sum f d}{N} \times c$$

$$d = \frac{x - A}{n}$$

where, A = any value (assumed mean) in x

N = total frequency

c = width of the class interval

Example 2: Given the following frequency distribution, calculate the arithmetic mean

Marks:	64	63	62	61	60	59
Number of Students:	8	18	12	9	7	6

Solution:

X	F	fx	d=x-A	fd
64	8	512	2	16
63	18	1134	1	18
62	12	744	0	0
61	9	549	–1	–9
60	7	420	–2	–14
59	6	354	–3	–18
	60	3713		- 7

$$\bar{x} = \frac{\sum fx}{N} = \frac{3713}{60} = 61.88$$

Deviation method:

$$\bar{x} = A + \frac{\sum fd}{N} = 62 - \frac{7}{60} = 61.88$$

Example: 3 Calculate A.M.

Income C.I	Number of Persons (f)	Mid X	$d = \frac{x - A}{c}$	Fd
0-10	6	5	-3	-18
10-20	8	15	-2	-16
20-30	10	25	-1	-10
30-40	12	A 35	0	0
40-50	7	45	1	7
50-60	4	55	2	8
60-70	3	65	3	9
	50			-20

$$\bar{x} = A + \frac{\sum fd}{N} \times c$$

$$= 35 - \frac{20}{50} \times 10$$

$$= 35 - 4$$

$$= 31$$

3.4 Weighted Arithmetic mean:

The average whose component items are being multiplied by certain values known as "weights" and the aggregate of the multiplied results are being divided by the total sum of their "weight". If x_1, x_2…n be the values of a variable x with respective weights of w_1, w_2… w_n assigned to them, then weighted A.M.

$$\overline{x_w} = \frac{w_1x_1 + w_2x_2 + \ldots + w_nx_n}{w_1 + w_2 + \ldots + w_n} = \frac{\sum w_ix_i}{\sum w_i}$$

3.5 Geometric mean:

The geometric mean of a series containing 'n' observations is the n^{th} root of the product of the values. If $x_1, x_2 \ldots, x_n$ are observations then,

$$G.M = \sqrt[n]{x_1 . x_2 \ldots x_n}$$

$$= (x_1 . x_2 \ldots x_n)^{1/n}$$

$$\log GM = \frac{1}{n} \log(x_1 . x_2 \ldots x_n)$$

$$= \frac{1}{n} (\log x_1 + \log x_2 + \ldots + \log x_n$$

$$= \frac{\sum \log x_i}{n}$$

$$GM = \text{Antilog} \frac{\sum \log x_i}{n}$$

For grouped data:

$$GM = \text{Antilog} \left[\frac{\sum f \log x_i}{N} \right]$$

3.6 Harmonic mean (H.M):

Harmonic mean of a set of observations is defined as the reciprocal of the arithmetic average of the reciprocal of the given values. If $x_1, x_2 \ldots, x_n$ are n observations,

For ungrouped data $H.M. = \dfrac{1}{\frac{1}{n} \sum_{i=1}^{n} \left(\frac{1}{x_i} \right)}$

For grouped data; $H.M. = \dfrac{1}{\frac{1}{N} \sum_{i=1}^{n} f_i \left(\frac{1}{x_i} \right)}$

3.7 Median:

The median is that value of the variate, which divides the group into two equal parts, one part comprising all values greater, and the other, all values less than median.

Ungrouped data:

Arrange the given values in the increasing or decreasing order. If the numbers of values are odd, median is the middle value. If the numbers of values are even, median is the mean of middle two values.

By formula,

$$Median\ (Md) = \left(\frac{n+1}{2} \right)^{th} item$$

Grouped Data:

In a grouped distribution, values are associated with frequencies. Grouping can be in the form of a discrete frequency distribution or a continuous frequency distribution. Whatever may be the type of distribution, cumulative frequencies have to be calculated to know the total number of items.

Cumulative frequency: (cf)

Cumulative frequency of each class is the sum of the frequency of the class and the frequencies of the pervious classes, i.e. adding the frequencies successively, so that the last cumulative frequency gives the total number of items.

Discrete Series:

Example 4: Find the median of the following frequency distribution;

x	1	2	3	4	5	6	7
f	8	11	14	18	11	9	5

Solution:

x	f	cf
1	8	8
2	11	8+11=19
3	14	19+14=33
4	**18**	33+18=**51**
5	11	51+11=62
6	9	62+9=71
7	5	71+5=76
Total	76	

$$Median\ (Md) = \left(\frac{n+1}{2}\right)^{th} item$$

$$= \left(\frac{76+1}{2}\right)$$

$$= 38.5$$

The cumulative frequencies just greater than 38.5 is 51. and the value of x corresponding to 51 is 4. Hence the median size is 4.

Continuous Series:

The steps given below are followed for the calculation of median in continuous series.

Step1: Find cumulative frequencies.

Step2: Find

Step3: See in the cumulative frequency the value first greater than, Then the corresponding class interval is called the Median class.

Then apply the formula

$$Median = Md = l + \frac{\frac{N}{2} - m}{f} \times c$$

Where, l = Lower limit of the median class
m = cumulative frequency preceding the median
c = width of the median class
f = frequency in the median class.
N = Total frequency.

If the class intervals are given in inclusive type convert them into exclusive type and call it as true class interval and consider lower limit in this

3.8 Mode:

The mode refers to that value in a distribution, which occur most frequently. It is an actual value, which has the highest concentration of items in and around it. Its importance is very great in marketing studies where a manager is interested in knowing about the size, which has the highest concentration of items. For example, in placing an order for shoes or ready-made garments the modal size helps because these sizes and other sizes around in common demand.

Grouped Data:

The highest frequency then the corresponding value of class interval is called the modal class. Then apply the formula.

$$Mode = M_0 = l + \frac{f_0 - f_1}{2f_0 - f_1 - f_2} \times c$$

where, f_1 = frequency of the class preceding the modal class

f_0 = frequency of the modal class

f_2 = frequency of the class succeeding the modal class

l = lower limit of modal class

c = class interval

Solve following problems:

Q. 1 Calculate mean and median for the following data:

100, 150, 200, 250, 360, 490, 500, 600, 671

Q. 2. Find the arithmetic mean for following data series by direct method and deviation method.

x	8	13	14	18	11	9	5

Q. 3. Calculate the arithmetic mean for following group data by direct method and deviation method.

Marks (x)	: 64	63	62	61	60	59
No. of students (f)	: 8	18	12	9	7	6

Q. 4. The following are the 405 soybean plant heights collected from a particular plot. Find the arithmetic mean of the plants by direct and indirect method.

Plant height (Cms)	8-12	13-17	18-22	23-27	28-32	33-37	38-42	43-47	48-52	53-57
No. of plants(f_i)	6	17	25	86	125	77	55	9	4	1

Q.5 Calculate weighted mean for following data.

Designation	Monthly salary (in Rs)	Strength of the cadre
Class 1 officers	1500	10
Class 2 officers	800	20
Subordinate staff	500	70
Clerical staff	250	100
Lower staff	100	150

Q.6 The marks secured by some students of a class are given below. Calculate the harmonic mean.

Marks X	No of students f
20	4
21	2
22	7
23	1
24	3
25	1
	18

Q.7 Calculate G.M. for the following data

Class of people	Number of families	Monthly income per head (Rs)
Landlords	2	5000
Cultivators	100	400
Landless – labours	50	200
Money – lenders	4	3750
Office Assistants	6	3000
Shop keepers	8	750
Carpenters	6	600
Weavers	10	300

Q.8 The following table gives the frequency distribution of 325 workers of a factory, according to their average monthly income in a certain year. Calculate Median and Mode for following table.

Income group (in Rs)	Number of workers
Below 100	1
100-150	20
150-200	42
200-250	55
250-300	62
300-350	45
350-400	30
400-450	25
450-500	15
500-550	18
550-600	10
600 and above	2
	325

Exercise No. 4 **Date: / /**

Measures of Dispersion

The measures of central tendency serve to locate the center of the distribution, but they do not reveal how the items are spread out on either side of the center. The degree of variation is evaluated by various measures of dispersion. **Small dispersion** indicates **high uniformity** of the items, while **large dispersion** indicates **less uniformity**.

4.1 Measure of dispersion:

1. Range
2. Mean Deviation
3. Standard deviation
4. Variance

4.2 Range:

This is the simplest possible measure of dispersion and is defined as the difference between the largest and smallest values of the variable.

In symbols, **Range = L – S**. Where, L = Largest value and S = Smallest value

In continuous series, the following two methods are followed.

Method 1:

L = Upper boundary of the highest class

S = Lower boundary of the lowest class.

Method 2:

L = Mid value of the highest class.

S = Mid value of the lowest class

Co-efficient of Range :

$$= \frac{L - S}{L + S}$$

4.3 Mean Deviation (M.D.):

Mean deviation is the arithmetic mean of the absolute deviations of a series computed from any measure of central tendency; i.e., **the mean, median or mode;** it is given by the formula,

Ungrouped Data:

$$M.D. = \frac{\sum |x_i - \bar{x}|}{n} = \frac{\sum |D_i|}{n}$$ mean deviation about mean

$$M.D. = \frac{\sum |x_i - Median|}{n}$$ mean deviation about median

$$M.D. = \frac{\sum |x_i - Mode|}{n}$$ mean deviation about mode

Grouped Data:

$$M.D. = \frac{\sum f_i |x_i - \bar{x}|}{n} = \frac{\sum f_i |D_i|}{n}$$

Coefficient of mean deviation:

$$Coeff.\ of\ M.D. = \frac{M.D.}{Mean\ or\ Median\ or\ Mode} \times 100$$

4.4 Standard Deviation (S.D.) σ:

Karl Pearson introduced the concept of standard deviation in 1893. It is the most important measure of dispersion and is widely used in many statistical formulae. Standard deviation is also called Root-Mean Square Deviation. The standard deviation is denoted by the **Greek letter σ (sigma).** It is based on deviation from arithmetic mean and is denoted by σ,

σ = Standard deviation for population.

Definition: It is defined as the positive square-root of the arithmetic mean of the Square of the deviations of the given observation from their arithmetic mean. It is given by the formula,

Standard deviation (SD) for Population

	Ungrouped Data:	Grouped Data:
By Actual Mean (Direct) method	$\sigma = \sqrt{\frac{1}{n}\sum (x_i - \bar{x})^2}$	$\sigma = \sqrt{\frac{1}{N}\sum f_i (x_i - \bar{x})^2}$ *where*, $\sum f_i = N$
by simplification	$\sigma = \sqrt{\frac{1}{n}\left[\sum x_i^2 - \frac{(\sum x_i)^2}{n}\right]}$	$\sigma = \sqrt{\frac{1}{N}\left[\sum f_i x_i^2 - \frac{(\sum f_i x_i)^2}{N}\right]}$
by deviation (Assumed mean) method	$\sigma = \sqrt{\frac{1}{n}\left[\sum d_i^2 - \frac{(\sum d_i)^2}{n}\right]}$ $d_i = x_i - A$ A = Assumed mean	$\sigma = \sqrt{\frac{1}{N}\left[\sum f_i d_i^2 - \frac{(\sum f_i d_i)^2}{N}\right]} \times c$ $d_i = \frac{x_i - A}{c}$ A = Assumed mean, c = Class interval

4.5 Variance:

Variance is the square of standard deviation. It is also called the "**Mean square deviation**". It is being used very extensively in analysis of variance of results from field experiment. Symbolically denoted by,

σ^2 = Population variance

Variance (V) for Population

	Ungrouped Data:	Grouped Data:
By Actual Mean (Direct) method	$\sigma^2 = \frac{1}{n}\sum (x_i - \bar{x})^2$	$\sigma^2 = \frac{1}{N}\sum f_i (x_i - \bar{x})^2$ *where*, $\sum f_i = N$
by simplification	$\sigma^2 = \frac{1}{n}\left[\sum x_i^2 - \frac{(\sum x_i)^2}{n}\right]$	$\sigma^2 = \frac{1}{N}\left[\sum f_i x_i^2 - \frac{(\sum f_i x_i)^2}{N}\right]$
by deviation (Assumed mean) method	$\sigma^2 = \frac{1}{n}\left[\sum d_i^2 - \frac{(\sum d_i)^2}{n}\right]$ $d_i = x_i - A$ A = Assumed mean	$\sigma^2 = \frac{1}{N}\left[\sum f_i d_i^2 - \frac{(\sum f_i d_i)^2}{N}\right] \times c$ $d_i = \frac{x_i - A}{c}$ A = Assumed mean, c = Class interval

Coefficient of Variation (C.V.)

Coefficient of variation is the percentage ratio of standard deviation and the arithmetic mean. It is usually expressed in percentage. The formula for C.V. is,

$$Coefficien\ t\ of\ Variation\ \ (C.V.) = \frac{S.D.}{Mean} \times 100$$

4.6 Standard error of mean (S.E.$_m$)

The standard deviation is the standard error of a single variate where as standard error of mean is the standard deviation of sampling distribution of the sample mean or it refers to the average magnitude of difference between the sample estimate and population parameter taken over all possible samples from the population.

Definition : It is defined as square root of the ratio of the variance to the total no. of observations in a given set of data.

Symbolically, it is written as $S_{\bar{X}}$ for sample and $\sigma_{\bar{X}}$ for population.

$S_{\bar{x}} = \frac{S}{\sqrt{n}}$ where, S = Standard deviation; n = No. of observations

For statistical analysis work, the use of $S_{\bar{x}}$ is common. It is also used to provide confidence limit on population mean and for test of significance.

$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$ where, σ = Standard deviation; n = No. of observations

Example: 1 Find out the mean deviation from mean and median from the following series.

Age in years	No.of persons
0-10	20
10-20	25
20-30	32
30-40	40
40-50	42
50-60	35
60-70	10
70-80	8

Solution:

X	m	f	$d = \frac{m-A}{c}$ (A=35,C=10)	fd	$\|D\| = \|m-\bar{x}\|$	f|D|
0-10	5	20	-3	-60	31.5	630.0
10-20	15	25	-2	-50	21.5	537.5
20-30	25	32	-1	-32	11.5	368.0
30-40	**35**	40	0	0	1.5	60.0
40-50	45	42	1	42	8.5	357.0
50-60	55	35	2	70	18.5	647.5
60-70	65	10	3	30	28.5	285.0
70-80	75	8	4	32	38.5	308.0
		212		32		3193.0

$$\bar{x} = A + \frac{\sum fd}{N} \times c$$

$$= 35 + \frac{32}{212} \times 10 \quad = 35 + \frac{320}{212} = 35 + 1.5 = 36.5$$

$$M.D. = \frac{\sum f|D|}{N} = \frac{3193}{212} = 15.06$$

Example: 2 Calculation of Mean Deviation from Median

X	m	f	c.f	|D| = |m-Md|	f|D|
0-10	5	20	20	32.25	645.00
10-20	15	25	45	22.25	556.25
20-30	25	32	77	12.25	392.00
30-40	35	40	117	2.25	90.00
40-50	45	42	159	7.75	325.50
50-60	55	35	194	17.75	621.25
60-70	65	10	204	27.75	277.50
70-80	75	8	212	37.75	302.00
				Total	3209.50

$$\frac{N}{2} = \frac{212}{2} = 106$$

l = 30, m = 77, f = 40, c = 10

$$\text{Median} = l + \frac{\frac{N}{2} - m}{f} \times c$$

$$= 30 + \frac{106 - 77}{40} \times 10$$

$$= 30 + \frac{29}{4}$$

$$= 30 + 7.25 \quad = 37.25$$

$$\text{M. D.} = \frac{\sum f|D|}{N}$$

$$= \frac{3209.5}{212} = 15.14$$

$$\text{Coefficient of M.D} = \frac{\text{M.D}}{\text{Median}}$$

$$= \frac{15.14}{37.25} \quad = 0.41$$

Example: 3 The table below gives the marks obtained by 10 students in statistics. Calculate standard deviation by actual mean and deviation method.

Student Nos :	1	2	3	4	5	6	7	8	9	10
Marks :	43	48	65	57	31	60	37	48	78	59

Solution:

Student No.	Marks (x)	$x_i - \bar{x}$	$(x_i - \bar{x})^2$	$d_i = x_i$-A (A=57)	d^2
1	43	-9.6	92.16	-14	196
2	48	-4.6	21.16	-9	81
3	65	12.4	153.76	8	64
4	57	4.4	19.36	0	0
5	31	-21.6	466.56	-26	676
6	60	7.4	54.76	3	9
7	37	-15.6	243.36	-20	400
8	48	-4.6	21.16	-9	81
9	78	25.4	645.16	21	441
10	59	6.4	40.96	2	4
***n*=10**			**1758.40**	**-44**	**1952**

$$S.D. = \sqrt{\frac{1}{n}\sum (x_i - \bar{x})^2}$$

by actual mean

$$S.D. = \sqrt{\frac{1}{10}(1758.4)}$$

$$S.D. = \sqrt{175.84}$$

$$S.D. = 13.26$$

$$S.D. = \sqrt{\frac{1}{n}\left[\sum d_i^2 - \frac{(\sum d_i)^2}{n}\right]}$$ by deviation (Assumed mean) method

$$S.D. = \sqrt{\frac{1}{10}\left[1952 - \frac{(-44)^2}{10}\right]}$$

$$S.D. = \sqrt{175.84}$$

$$S.D. = 13.26$$

Example: 4 The following are the 381soybean plant heights in cms collected from a particular plot. Find the Standard deviation of the plants by direct and deviation method.

Plant heights (Cms)	No. of Plants (f_i)
6.8 -7.2	9
7.3 -7.7	10
7.8-8.2	11
8.3-8.7	32
8.8-9.2	42
9.3-9.7	58
9.8-10.2	65
10.3-10.7	55
10.8-11.2	37
11.3-11.7	31
11.8-12.2	24
12.3-12.7	7

Solution:

C.I.	f_i	x_i	f_ix_i	$f_ix_i^2$	$d_i = \frac{x_i - A}{C}$	f_id_i	$f_id_i^2$
6.8-7.2	9	7.0	63	441	-5	-45	225
7.3-7.7	10	7.5	75	562.5	-4	-40	160
7.8-8.2	11	8.0	88	704	-3	-33	99
8.3-8.7	32	8.5	272	2312	-2	-64	128
8.8-9.2	42	9.0	378	3402	-1	-42	42
9.3-9.7	58	9.5	551	5234.5	0	0	0
9.8-10.2	65	10	650	6500	1	65	65

10.3-10.7	55	10.5	577.5	6063.75	2	110	220
10.8-11.2	37	11.0	407	4477	3	111	333
11.3-11.7	31	11.5	356.5	4099.75	4	124	496
11.8-12.2	24	12.0	288	3456	5	120	600
12.3-12.7	7	12.5	87.5	1093.75	6	42	252
	N =381		$\Sigma f_i x_i$ =3793.5	$\Sigma f_i x_i^2$ =38346.25		$\Sigma f_i d_i$ = 348	$\Sigma f_i d_i^2$ = 2620

By Actual Mean Method:

$$\text{A.M.} = = \frac{\sum_{i=1}^{n} f_i x_i}{N} \; ; \quad \text{where N} = \sum_{i=1}^{n} f_i$$

$$\text{S.D.} = \sqrt{\frac{1}{N}\left[\sum_{i=1}^{n} f_i x_i^{\,2} - \frac{\left(\sum_{i=1}^{n} f_i x_i\right)^2}{N}\right]}$$

$$\text{A.M.} = \frac{3793.5}{381} = 9.96 \text{ Cms}$$

$$\text{S.D.} = \sqrt{\frac{1}{381}\left[38346.25 - \frac{(3793.5)^2}{381}\right]}$$

$$= \sqrt{\frac{1}{381}[38346.25 - 37770.71]}$$

$$= \sqrt{1.5106} = 1.23 \text{ Cms.}$$

Deviation (Assumed mean) method:

$$\text{A.M.} = A + \left(\frac{\sum_{i=1}^{n} f_i d_i}{N}\right) C$$

$$\text{S.D.} = C\sqrt{\frac{1}{N}\left[\sum_{i=1}^{n} f_i d_i^{\,2} - \frac{\left(\sum_{i=1}^{n} f_i d_i\right)^2}{N}\right]}$$

$$\text{A.M.} = 9.5 + \left(\frac{348}{381}\right)0.5 = 9.96 \text{ Cms}$$

$$\text{S.D.} = 0.5\sqrt{\frac{1}{381}\left[2620 - \frac{(348)^2}{381}\right]}$$

$$= 0.5 \text{x} 47.98 = 1.23 \text{ Cms.}$$

Solve following problems:

Q. 1 Calculate the value of Range and Coefficient of range, Mean deviation and SD for following series data

24	28	21	18	16	11	29	15	3	8

Q. 2 The following table gives the marks obtained by 50 students in midterm examination. Find the value of range and coefficient of range.

Marks	No. of student
0-10	8
10-20	6
20-30	16
30-40	9
40-50	11

Also calculate mean deviation, coefficient of mean deviation and standard deviation for above distribution table

Q. 3 Obtain the variance and coefficient of variance for the following table which gives the age distribution of 700 members

Age group	No. of members
20-30	51
30-40	184
40-50	203
50-60	128
60-70	112
70-80	16
80-90	4
90-100	2

Q. 4 Calculate mean deviation from mean and median for the following data:

100, 150, 200, 250, 360, 490, 500, 600, 671

also calculate co-efficients of M.D.

Q. 5 Calculate mean deviation from mean and median for the following data and also calculate co-efficients of M.D.

Height in cms	158	159	160	161	162	163	164	165	166
No. of persons	15	20	32	35	33	22	20	10	8

Q. 6 Calculate mean deviation from mean and median for the following data and also calculate co-efficients of M.D

Age in years	No.of persons
0-10	20
10-20	25
20-30	32
30-40	40
40-50	42
50-60	35
60-70	10
70-80	8

Q. 7 In two factories A and B located in the same industrial area, the average weekly wages (in rupees) and the standard deviations are as follows:

Factory	Average	Standard Deviation	No. of workers
A	34.5	5	476
B	28.5	4.5	524

1. Which factory A or B pays out a larger amount as weekly wages?
2. Which factory A or B has greater variability in individual wages?

Exercise No. 5 **Date: / /**

Tests of Significance

Some of the important terminologies used to in test of significance are describe hereunder:

5.1. Parameter: A characteristics of population values is known as parameter. For example populations mean (μ) and population variance (σ^2). It also called as statistical constants of the population. In practice, if parameter values are not known and the estimates based on the sample values are generally used.

5.2. Statistic: A Characteristic of sample values is called a statistic. It is statistical measures computed based on sample observations alone.

For example, sample mean ($\bar{x}$), $\bar{x} = \frac{x_1 + x_2 + ... + x_n}{n}$ and

Sample variance (s^2) $s^2 = \frac{1}{n}\left[\sum x_i^2 - \frac{(\sum x_i)^2}{n}\right]$

5.3. Estimate: Specific numerical valueof an estimator calculated from sample is called the Estimate.

5.4. Estimator: Statistic ($\hat{\theta}$) proposed for estimating a parameter (θ) is called an estimator of θ

5.5. Sampling distribution: The distribution of a statistic computed from all possible samples is known as sampling distribution of that statistic.

5.6. Standard error: The standard deviation of the sampling distribution of a statistic is known as its standard error, abbreviated as S.E.

$S.E.(\bar{x}) = \sigma/\sqrt{n}$; where, σ = population standard deviation and n = sample size

5.7. Sample: A finite subset of statistical objects in a population is called a sample and the number of objects in a sample is called the **sample size**.

5.8. Random sampling: If the sampling units in a population are drawn independently with equal chance, to be included in the sample then the sampling will be called **random sampling**.

5.9. Why it needs of test of significance:

A very important aspect of the sampling theory is the study of the 'tests of significance', which enable us to decide on the basis of the sample results, if (i) the deviation between sample statistic and hypothetical parameter value or (ii) the deviation between independent sample statistic; is significance or might be attributed to chance or the fluctuations of sampling.

5.10. Null Hypothesis (H_0): we first set up a hypothesis – a definite statement about the population parameter. Such a hypothesis, which is usually a hypothesis of no difference, is called **null hypothesis** and

is usually denoted by H_0. According to Prof. R.A. Fisher, null hypothesis is the hypothesis which is tested for possible rejection under the assumption that it is true.

If **test is significant** which means Null hypothesis is rejected and If **test is non-significant** which means Null hypothesis is accepted.

5.11. Alternative Hypothesis (H_1): Any hypothesis which is complementary to the null hypothesis is called an alternative hypothesis, usually denoted by H_1.

If null hypothesis is $H_0: \mu = \mu_0$ the alternate hypothesis become,

$H_1: \mu \neq \mu_0$ Two tailed alternative

$H_1: \mu > \mu_0$ Single tailed or right tailed alternative

$H_1: \mu < \mu_0$ Single tailed or left tailed alternative

5.12. Simple Hypothesis: A hypothesis is said to be simple if it completely specifies the distribution of the population. For instance, in case of normal population with mean μ and standard deviation σ, a simple null hypothesis is of the form

$H_0: \mu = \mu_0$

Such a test, the probability of committing the type-1 error is expressed as **exactly α**

5.13. Composite Hypothesis: If the hypothesis does not specify the distribution of the population completely, it is said to be a composite hypothesis. Following are some examples;

$H_0: \mu > \mu_0$ and σ is known

$H_0: \mu < \mu_0$ and σ is known

All these are composite because none of them specifies the distribution completely. Hence, for such a test the (level of Significance) LOS is specified not as **α,** but as 'at most **α'.**

5.14. Types of error or decision error:

The main objective in sampling theory is to draw valid inferences about the population parameters on the basis, of the sample results. In practice we decide to accept or reject the lot after examining a sample from it. As such we are liable to commit the following two types of errors:

5.14.1 Type-I error: Rejecting H_0, when H_0 is true. The probabilities of type-I errors is denoted by α. i.e. Prob. (Rejecting H_0, when it is true) = α (it is also known as Producer risk).

5.14.2 Type-II error: Accepting H_0, when H_0 is false. The probabilities of type-II errors is denoted by β. i.e. Prob. (Rejecting H_0, when it is false) = β (it is also known as Consumer risk).

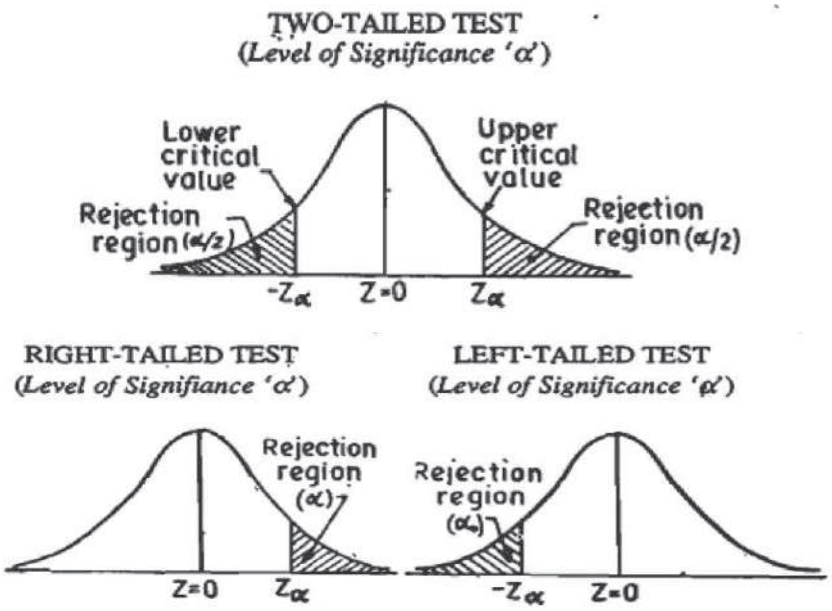
5.15. Level of Significance: The maximum probability at which we would be willing to risk a type-I error is known as level of significance or the **size of Type-I error** is level of significance. The levels of significance usually employed in testing of hypothesis are 5% and 1%. The Level of significance is always

fixed in advance before collecting the sample information. LOS 5% means the results obtained will be true is 95% out of 100 cases and the results may be wrong is 5 out of 100 cases.

5.16. Degrees of freedom: It is the number of observation that free to vary after certain restrictions have been place in data. It is also defined as the difference between the total number of items and the total number of constraints. If 'n' is the total number of items and 'k' the total number of constraints then the degrees of freedom (d.f.) is given by d.f. = n-k.

5.17. Critical region: a region where null hypothesis (H_0) is rejected is called critical region of rejection.

5.17.1 Critical Values or Significant Values : The value of test statistic which separates the critical (or rejection) region and acceptance region is called the critical value or significant value. It is depends on (i) level of significance used and (ii) the alternative hypothesis whether it is two tailed or one tailed.



Critical values (Z_α) of Z		
Critical values	1%	5%
Two tailed test	$\|Z_\alpha\| = 2.58$	$\|Z_\alpha\| = 1.96$
Right tailed test	$Z_\alpha = 2{\cdot}33$	$Z_\alpha = 1{\cdot}645$
Left tailed test	$Z_\alpha = -2{\cdot}33$	$Z_\alpha = -1{\cdot}645$

5.18. Procedure (steps involved in) for Testing of Hypothesis:

1. **Null hypothesis:** set up the null hypothesis H_0.
2. **Alternative Hypothesis:** Set up the Alternative Hypothesis H_1. This will enable us to decide whether we have to use a single-tailed (right or left) test or two-tailed test.

3. **Level of Significance:** Choose the appropriate level of Significance 'α'. The Level of significance is always fixed in advance before collecting the sample information. Most of the problem it is 5% LOS and 1% LOS.
4. **Test Statistic** (or Test Criterion): Compute the test statistic under the null hypothesis,

$$Z = \frac{t - E(t)}{S.E.(t)}$$

5. **Conclusion:** We compare 'z' the computed value of 'Z' in step 4 with the significant value (tabulated value) Zα, at the given level of significance, 'α'.
   - If |Zα| > Zα; i.e. if the computed value of test statistic is greater than the critical or Significant value, then we say that it is significant and the null hypothesis is rejected at level of significance 'α'.
   - If |Zα| < Zα; i.e. if the computed value of test statistic is less than the critical or Significant value, then we say that it is not significant and the null hypothesis is accepted at level of significance 'α'.

Confidence limit or Fiducial limit:

If the population is normal and its mean is unknown the 95% confidence limit for mean, *E(t)* is given by,

$$|Z| \le 1.96 \text{ i.e. } \left|\frac{t - E(t)}{S.E.(t)}\right| \le 1.96$$

$$\bar{x} - 1.96 \times S.E.(t) \le \mu \le \bar{x} + 1.96 \times S.E.(t)$$

Thus 95% confidence limits for parameter μ are, $\bar{x} \pm 1.96 \times S.E.(t)$

$$\text{or } \left\{\bar{x} - 1.96 \times S.E.(t),\ \bar{x} + 1.96 \times S.E.(t)\right\}$$

Similarly,

Thus 99% confidence limits for parameter μ are, $\bar{x} \pm 2.58 \times S.E.(t)$

$$\text{or } \left\{\bar{x} - 2.58 \times S.E.(t),\ \bar{x} + 2.58 \times S.E.(t)\right\}$$

Exercise No. 6 **Date: / /**

Large Sample Tests - Z-test

If the sample size '*n'* is > 30 then it is considered as **large sample**.

The Range of Z - distribution is $-\infty$ *to* $+\infty$

6.1 SND Test or One Sample (Z-test)

6.1.1. Case-I: Population standard deviation (σ) is known

Assumptions:

1. Population is normally distributed
2. The sample is drawn at random

Conditions:

1. Population standard deviation σ is known
2. Size of the sample is large (say n > 30)

Procedure: Let x_1, x_2, ……x_n be a random sample size of n from a normal population with mean μ and variance σ^2.

Let $\bar{x}$ be the sample mean of sample of size 'n'

Null hypothesis (H_0): population mean (μ) is equal to a specified value μ_0

i.e. $H_0 : \mu = \mu_0$

Under H_0, the test statistic is,

$$Z = \frac{\left|\bar{x} - \mu_0\right|}{\frac{\sigma}{\sqrt{n}}} \sim N(0,1)$$

i.e the above statistic follows Normal Distribution with mean '0' and variance '1'.

If the calculated value of |Z| > table value of Z at 5% level of significance, H_0 is rejected and hence we conclude that there is significant difference between the population mean and the one specified in H_0 as μ_0.

If the calculated value of |Z| < table value of Z at 5% level of significance, H_0 is accepted and hence we conclude that there is no significant difference between the population mean and the one specified in H_0 as μ_0

Confidence limit or Fiducial limit:

If we take large sample from normal population with mean μ and Standard deviation σ, then,

$$Z = \frac{|\bar{x} - \mu_0|}{\frac{\sigma}{\sqrt{n}}} \sim N(0,1)$$

$$P(-1.96 \le Z \le 1.96) = 0.95 \text{ i.e. } P\left(-1.96 \le \frac{|\bar{x} - \mu_0|}{\frac{\sigma}{\sqrt{n}}} \le 1.96\right) = 0.95$$

$$P\left(\bar{x} - 1.96\frac{\sigma}{\sqrt{n}} \le \mu \le \bar{x} + 1.96\frac{\sigma}{\sqrt{n}}\right) = 0.95$$

Thus 95% confidence limits for unknown parameter μ are, $\bar{x} \pm 1.96\frac{\sigma}{\sqrt{n}}$

And 95% confidence interval $\left\{\bar{x} - 1.96\frac{\sigma}{\sqrt{n}} \ , \ \bar{x} + 1.96\frac{\sigma}{\sqrt{n}}\right\}$

Similarly, Thus 99% confidence limits for parameter μ are, $\bar{x} \pm 2.58\frac{\sigma}{\sqrt{n}}$

And 99% confidence interval $\left\{\bar{x} - 2.58\frac{\sigma}{\sqrt{n}} \ , \ \bar{x} + 2.58\frac{\sigma}{\sqrt{n}}\right\}$

6.1.2. Case-II: If σ is not known

Assumptions:

1. Population is normally distributed
2. Sample is drawn from the population should be random
3. We should know the population mean

Conditions:

1. Population standard deviation σ is not known
2. Size of the sample is large (say n > 30)

Null hypothesis, $H_0 : \mu = \mu_0$

Under H_0, the test statistic is,

$$Z = \frac{|\bar{x} - \mu_0|}{\frac{s}{\sqrt{n}}} \sim N(0,1) \qquad \text{where, } s = \sqrt{\frac{1}{n}\left[\sum x_i^2 - \frac{(\sum x_i)^2}{n}\right]}$$

$\bar{x}$ be the sample mean of sample of size 'n'

If the calculated value of |Z| > table value of Z at 5% level of significance, H_0 is rejected and hence we conclude that there is significant difference between the population mean and the one specified in H_0 as μ_0.

If the calculated value of |Z| < table value of Z at 5% level of significance, H_0 is accepted and hence we conclude that there is no significant difference between the population mean and the one specified in H_0 as μ_0

The table value of Z at 5% level of significance = *1.96* and table value of Z at 1% level of significance = *2.58*.

6.2. Two sample Z-Test or Test of significant for difference of means

6.2.1. Case-I: when σ is known

Assumptions:

1. Populations are distributed normally
2. Samples are drawn independently and at random

Conditions:

1. Populations standard deviation σ is known
2. Size of samples is large

Procedure: Let $\bar{x}_1$ be the sample mean of random sample of size 'n_1' from the population with mean μ_1 and variance σ_1^2.

Let $\bar{x}_2$ be the sample mean of random sample of size 'n_2' from the population with mean μ_2 and variance σ_2^2.

Null hypothesis, $H_0 : \mu_1 = \mu_2$

Alternative hypothesis, $H_1 : \mu_1 \neq \mu_2$

i.e. The null hypothesis states that the population means of the two samples are identical.

Under the null hypothesis the test statistic becomes,

$$Z = \frac{\left|\bar{x}_1 - \bar{x}_2\right|}{\sqrt{\left(\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}\right)}} \sim N(0,1) \qquad (1)$$

If $\sigma_1^2 = \sigma_2^2 = \sigma^2$ (say) i.e both samples have the same standard deviation then the test statistic becomes,

$$Z = \frac{\left|\bar{x}_1 - \bar{x}_2\right|}{\sigma\sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}} \sim N(0,1) \qquad (2)$$

If the calculated value of |Z| > table value of Z at 5% level of significance, H_0 is rejected.

If the calculated value of |Z| < table value of Z at 5% level of significance, H_0 is accepted and which shows that there is no significant difference between two population means of the two samples are identical.

6.2.2. Case-II: when σ is not known

Assumptions:

1. Populations are distributed normally
2. Samples are drawn independently and at random

Conditions:

1. Populations standard deviation σ is not known
2. Size of samples is large (n > 30)

Procedure: Let $\bar{x}_1$ be the sample mean of random sample of size 'n_1' from the population with mean μ_1 and sample variance s_1.

Let $\bar{x}_2$ be the sample mean of random sample of size 'n_2' from the population with mean μ_2 and sample variance s_2.

Null hypothesis, $H_0 : \mu_1 = \mu_2$

Alternative hypothesis, $H_1 : \mu_1 \neq \mu_2$

i.e. The null hypothesis states that the population means of the two samples are identical.

Under the null hypothesis the test statistic becomes,

$$Z = \frac{\left|\bar{x}_1 - \bar{x}_2\right|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}} \sim N(0,1) \qquad (1)$$

If $s_1^2 = s_2^2 = s^2$ (say) i.e both samples have the same sample variance then the test statistic becomes,

$$Z = \frac{\left|\bar{x}_1 - \bar{x}_2\right|}{s\sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}} \sim N(0,1) \qquad (2)$$

If the calculated value of |Z| > table value of Z at 5% level of significance, H_0 is rejected otherwise accepted.

Solve following problems

Q.1. The data relate to the sample of 900 members consisting of mean 3.5 cm and standard deviation 2.61 cms. Test the single mean 3.5 which is conformable to population mean 3.25?

If the population is normal and its mean is unknown, find the 95% and 99% confidence limit for mean.

Q.2. The means of two single large samples of 1000 and 2000 members are 67.5 inches and 68.0 inches respectively. Can the samples be regarded as drawn from the same population of standard deviation 2.5 inches? (test at 5% level of significance)

Q.3. The average panicle length of 60 paddy plants in field no. 1 is 18.5 cms and that of 70 paddy plants in field no.2 is 20.3 cms. With common S.D. 1.15 cms. Test whether there is significant difference between two paddy fields w. r. t. panicle length.

Q.4. A breeder claims that the number of filled grains per panicle is more in new variety of paddy GR-11 compared to that of an old variety GR-28. To verify his claim a random sample of 50 plants of GR-11 and 60 plants of GR-28were selected from the experimental fields. The following results were obtained:

GR-11	**GR-28**
$\overline{x_1} = 139.4$-grains/panicle	$\overline{x_2} = 112.9$ grains/panicle
$s_1 = 26.864$	$s_2 = 20.1096$
$n_1 = 50$	$n_2 = 60$

Test whether the claim of the breeder is correct.

Q.5. The average hourly wages of a sample of 150 workers in plant 'A' was Rs. 2.56 with standard deviation of Rs. 1.08. The average hourly wages of a sample of 200 workers in plant 'B' was Rs. 2.87 with standard deviation of Rs. 1.28.

Exercise No.7 **Date: / /**

Small Sample Tests - t-test

If the sample size n < 30 then it is considered as **small sample**

The entire large sample theory was based on the application of 'normal test'. However, if the sample size 'n' is small, the distribution of the various statistics, are far-away from normality and as such 'normal test' cannot be applied if 'n' is small.

In such cases exact sample tests, we use t-test pioneered (1st discovered) by **W.S. Gosset (1908)** who wrote under the pen name of student, thus it is also called '**Student's t- test**'. The test later on developed and extended by Prof. R.A. Fisher.

7.1. Student's t-test: Let $x_1, x_2, \ldots\ldots x_n$ be a random sample size of 'n' from a normal population with mean μ and variance σ^2 then student's t is defined by the statistic,

$$t = \frac{|\bar{x} - \mu_0|}{\frac{s}{\sqrt{n}}} \sim N(0,1) \qquad \text{where, } s = \sqrt{\frac{1}{n-1}\left[\sum x_i^2 - \frac{(\sum x_i)^2}{n}\right]}$$

This test statistic follows t-distribution with (n-1) degrees of freedom (d.f.). To get the critical value of 't', we have to refer the table for t-distribution against (n-1) d.f. and the specific level of significance. Comparing the calculated value of 't' with critical value, we can accept or reject the null hypothesis.

The Range of t - distribution is $-\infty \; to \; +\infty$

7.2. One Sample t-test

In one sample t-test, we know the population mean. We draw a random sample from the population and then compare the sample mean with population mean and make a statistical decision as to whether or not the sample mean is different from the population mean. In one sample t-test, sample size should be less than 30.

Assumptions:

1. Population is normally distributed
2. Sample is drawn from the population and it should be random
3. We should know the population mean

Conditions:

1. Population S.D. σ is not known
2. Size of the sample is small (<30)

Procedure: Let x_1, x_2, ……x_n be a random sample size of 'n' from a normal population with mean μ and variance σ^2.

Null hypothesis (H_0): population mean (μ) is equal to a specified value μ0

i.e. H_0: $\mu = \mu_0$

Under H_0, the test statistic is,

$$t = \frac{|\bar{x} - \mu_0|}{\frac{s}{\sqrt{n}}} \sim N(0,1) \qquad \text{where, } s = \sqrt{\frac{1}{n-1}\left[\sum x_i^2 - \frac{(\sum x_i)^2}{n}\right]}$$

and follows student's t – distribution with (n-1) degrees of freedom

If the calculated value of | t | > table value of t at 5% level of significance, H_0 is rejected.

If the calculated value of | t | < table value of t at 5% level of significance, H_0 is accepted and which shows that there is no significant difference between the population mean and the one specified in H_0 as μ_0.

Confidence limit or Fiducial limit:

95% confidence limits for unknown parameter μ are, $\bar{x} \pm t_{0.05}\frac{s}{\sqrt{n}}$

And 95% confidence interval $\left\{\bar{x} - t_{0.05}\frac{s}{\sqrt{n}} \;,\; \bar{x} + t_{0.05}\frac{s}{\sqrt{n}}\right\}$

Similarly, Thus 99% confidence limits for parameter μ are, $\bar{x} \pm t_{0.01}\frac{s}{\sqrt{n}}$

And 99% confidence interval $\left\{\bar{x} - t_{0.01}\frac{s}{\sqrt{n}} \;,\; \bar{x} + t_{0.01}\frac{s}{\sqrt{n}}\right\}$

7.3. Two Samples t-test

Assumptions:

1. Populations are distributed normally
2. Samples are drawn independently and at random

Conditions:

1. Standard deviations in the populations are same and not known
2. Size of the sample is small

Procedure: If two independent samples x_i (i = 1,2,….,n_1) and y_j (j = 1,2, …..,n_2) of sizes $n_1$and n_2 have been drawn from two normal populations with means μ_1 and μ_2 respectively.

Null hypothesis (H_0): $\mu_1 = \mu_2$

The null hypothesis states that the population means of the two groups are identical, so their difference is zero.

Under H_0, the test statistic is,

$$t = \frac{\left|\bar{x} - \bar{y}\right|}{S\sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

where, $S^2 = \frac{1}{n_1 + n_2 - 2}\left[\sum x_i^2 - \frac{(\sum x_i)^2}{n} + \sum y_i^2 - \frac{(\sum y_i)^2}{n}\right]$

or $S^2 = Pooled \quad \text{var}\, iance = \frac{(n_1 - 1)s^2{}_1 + (n_2 - 1)s^2{}_2}{n_1 + n_2 - 2}$

where s_1 and s_2 are the variances of the first and second samples respectively where $\bar{x}$ and $\bar{y}$ are the two sample means, which follows student's t – distribution with $(n_1+n_2 - 2)$ degrees of freedom.

If calculated value of $|t|$ > table value of t with (n_1+n_2-2) d.f. at specified level of significance, then the null hypothesis is rejected otherwise accepted.

7.4. Paired t – test

The paired t-test is generally used when measurements are taken from the same subject before and after some manipulation such as injection of a drug. For example, you can use a paired t test to determine the significance of a difference in blood pressure before and after administration of an experimental presser substance.

Assumptions:

1. Populations are distributed normally
2. Samples are drawn independently and at random

Conditions:

1. Samples are related with each other
2. Sizes of the samples are small and equal
3. Standard deviations in the populations are equal and not known

Null hypothesis (H_0): $\mu_d = 0$

The null hypothesis states that the population means of the two groups are identical, so their difference is zero.

Under H_0, the test statistic is,

$$t = \frac{|\bar{d}|}{\frac{S}{\sqrt{n}}} \sim \text{t(n - 1)d.f.}$$

where, $\bar{d} = \frac{\sum d_i}{n}$ $\qquad$ $S^2 = \frac{1}{n-1}\left[\sum d_i^2 - \frac{(\sum d_i)^2}{n}\right]$

where, S^2 is variance of the deviations, n = sample size; where $d_i = x_i - y_i$ (i = 1,2,……,n).

If calculated value of | t | > table value of t with (n_1-1) d.f. at specified level of significance, then the null hypothesis is rejected otherwise accepted. If H_0 is accepted, we conclude that the two samples may belong to the same population.

Solve following problems

Q.1. The mean weekly sales of soap bars in departmental stores was 16.3 bars per store. After an advertising campaign the mean weekly sales in 22 stores for typical week increased to 153.7 and showed a standard deviation of 17.2. was the advertising campaign successful?

Q.2. A random sample of 10 boys had the following I.Q.'s:

70, 120, 110, 101, 88, 83, 95, 98, 107, 100

Do these data support the assumption of a population mean I.Q.'s of 100? Find 95% confidence interval in which most of mean I.Q.'s values of samples of 10 boys lie.

Q.3. Below are given the gain in weights (imn lbs.) of pigs fed on two diets A and B Gain in weight

Diet A: 25, 32, 30, 34, 24, 14, 32, 24, 30, 31, 35, 25

Diet B: 44, 34, 22, 10, 47, 31, 40, 30, 32, 35, 18, 21, 35, 29, 22

Test, if two diets differ significantly as regards their effect on increase in weight.

Q.4. Samples of two types of electric light bulbs were tested for length of life and following data were obtained:

	Type I	Type II
Sample No.	8	7
Sample Mean	1234 hrs	1036 hrs
Sample SD	36 hrs	40 hrs

Is the difference in the means sufficient to warrant that type I is superior to type II regarding length of life?

Q.5. Eleven school boys were given a test in statistics. They were given a months tuition and second test was held at the end of it. Do the marks give evidence that the students have benefited by extra coaching?

Boys:	1	2	3	4	5	6	7	8	9	10	11
Marks in 1^{st} test:	23	20	19	21	18	20	18	17	23	16	19
Marks in 2^{nd} test:	24	19	22	18	20	22	20	20	23	20	18

Q.6. in a certain experiment to compare two types of pig food A and food B, the following results of increase in weights were observed in pigs:

Pig no.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
Increase in wt.	**Food A**	49	53	51	52	47	50	52	53	407
	Food B	52	55	52	53	50	54	54	53	423

(i) Assuming that the two samples of pigs are independent, can we conclude that food B is better than food A?

(ii) Also examine the case when the same sets of eight pigs were used in both the foods.

Exercise No. 8 **Date: / /**

F-test: Test for Variances

In agricultural experiments the performance of a treatment is assessed not only by its mean but also by its variability. Hence, it is of interest to us to compare the variability of two populations. In testing of hypothesis the equality of variances, the greater variance is always placed in the Numerator and smaller variance is placed in the denominator.

8.1 Where F-test does is used?

1. To test the equality of two population variances,
2. To test equality of several regression coefficients
3. Analysis of Variance, (ANOVA).

F- test was discovered by **G.W. Snedecor.**

The range of F: 0 to ∞.

Let x_i (i = 1,2,….,n_1) and y_j (j = 1,2, …..,n_2) be the two independent random samples of sizes n_1 and n_2 drawn from two normal populations with means μ_1 & μ_2 and variance σ^2_1& σ^2_2 respectively. $S^2_1$and S^2_2 are the sample variances of the two samples.

Null hypothesis H_0: $\sigma^2_1 = \sigma^2_2$

Under H_0, the test statistic is,

$$S = \frac{S_1^2}{S_2^2} \qquad \text{where, } S_1^2 > S_2^2$$

which follows F-distribution with (n_1-1, n_2-1) d.f

If calculated value of F > table value of F with (n_1-1, n_2-1) d.f at specified level of significance, then the null hypothesis is rejected and hence we conclude that the variances of the populations are heterogeneous otherwise homogeneous.

$$\text{where, } S_1^2 = \frac{1}{n_1 - 1}\left[\sum x_i^2 - \frac{(\sum x_i)^2}{n}\right] \text{ and } S_2^2 = \frac{1}{n_2 - 1}\left[\sum y_i^2 - \frac{(\sum y_i)^2}{n_2}\right]$$

$$S = \frac{S_2^2}{S_1^2} \qquad \text{where, } S_2^2 > S_1^2$$

Which follows F-distribution with (n_2-1, n_1-1) d.f

If calculated value of F < table value of F with (n_2-1, n_1-1)d.f at specified level of significance, then the null hypothesis is accepted and hence we conclude that the variances of the populations are homogeneous otherwise heterogeneous.

Solve the following problems:

Q.1. If $S_1 = 1.2$, $S_2 = 1.5$, $n_1 = 15$ and $n_2 = 16$ then calculated F-test and draw your conclusion or write your result.

Q.2. The heights in meters of Rice crop plants with two level of nitrogen fertilizer in two field are as follows:

Nitrogen 1 (x)	3.5	4.2	2.8	5.2	1.7	2.6	3.5	4.2	5.0	5.2
Nitrogen 2 (y)	1.9	2.6	2.3	4.3	4.0	4.2	3.8	2.9	3.7	

Test whether the variances of the two level of nitrogen fertilizer are homogeneous.

Q.3. Pumpkins were grown under two experimental conditions. Two random samples of 11 and 9 pumpkins show the sample standard deviations of their weights as 0.8 and 0.6 respectively. Assuming that the weight distribution are normal test hypothesis that the true variances are equal against the alternative that they are not at the 5 per cent [Assume that $P(F_{10,8} \geq 3.35)=0.05$ and $P(F_{8,10} \geq 3.07)=0.05$].

Q.4. In one sample of 8 observations the sum of the squares of deviations of the sample values from the sample mean was 84.4 and in the other sample of 10 observation it was 102.6. test whether this difference is significant at 5 per cent level, given that the 5 per cent point of F for $n_1=7$ and $n_2=9$ degrees of freedom is 3.29.

Exercise No. 9 **Date: / /**

Chi-square (χ^2) test

The various tests of significance studied earlier such that as Z-test, t-test, F-test was based on the assumption that the samples were drawn from normal population. Under this assumption the various statistics were normally distributed. Since the procedure of testing the significance requires the knowledge about the type of population or parameters of population from which random samples have been drawn, these tests are known as **parametric tests**.

But there are many practical situations in which the assumption of any kind about the distribution of population or its parameter is not possible to make. The alternative technique where no assumption about the distribution or about parameters of population is made are known as **non-parametric tests**.

Chi-square test is an example of the non parametric test. Chi-square distribution is a **distribution free test**.

Chi-square distribution was first discovered by **Helmert in 1876** and later independently by Karl Pearson in 1900. The range of chi-square distribution is **0 to** ∞.

If $X \sim N(0,1)$ then $\sum x_i^2 \sim \chi_n^2$ (follows chi-square with n d.f.)

9.1. Applications (Uses) of Chi-square Test:

1. Testing the independence of attributes for 2 x 2, 2 x c, r x 2 and r x c contingency table.
2. To test the goodness of fit
3. Testing of linkage in genetic problems
4. Comparison of sample variance with population variance
5. Testing the homogeneity of variances
6. Testing the homogeneity of correlation coefficient

9.2. Properties of Chi-Square distribution

1) Chi-square distribution is **not exact distribution as "t"** distribution.
2) It is not symmetrical distribution but it is **positively skewed** distribution.
3) The value of its varies from **0 to** ∞**.** When there is a perfect agreement of observed frequency distribution with hypothetical frequency distribution, the value of chi-square will be **zero**, while the value of its increases as there is a departure from the agreement and will increased up to infinity.
4) The different central moments are $\mu_2 = 2n$; $\mu_3 = 8n$, $\mu_4 = 48n + 12n^2$.

5) As the number of observation tends to **infinity**, the chi-square distribution tends to **normality**.

6) The table chi-square value depends upon degrees of freedom. The table chi-square values can be obtained for 1 to 30 d.f., then it is not available from the table. As the number of degrees of freedom exceeds 30, it is found that $\sqrt{\chi^2}$ will be distributed approximately normal about the mean $\sqrt{2n-1}$ with a unit standard deviation. Therefore, the **Z value** can be worked out by using the following formula and it should be compared with table Z value at 5 per cent or 1 per cent level of significance.

$$Z = \sqrt{2\chi^2} - \sqrt{2n-1}$$

9.3. Chi-square test for population variance:

Suppose we want to test if a random sample x_i (i=1,2,….n) has been drawn from a normal population with a specified variance $\sigma^2 = \sigma^2_0$.

$H_0: \sigma^2 = \sigma^2_0$

Under the null hypothesis, test statistic, $\chi^2 = \sum \left[\frac{(x_i - \bar{x})^2}{\sigma^2_0} \right]$

$$= \frac{ns^2}{\sigma^2_0} \sim \chi^2_{(n-1)} \text{ d.f.}$$

9.3.1 Condition:

Apply above test only if the population from which sample is drawn is normal. If n > 30 then, we can used fisher's approximation i.e.

$$Z = \sqrt{2x^2} - \sqrt{2n-1} \quad \sim N(0,1)$$

Then apply normal test i.e. compare with Zα table value and finally draw conclusion.

9.4. Chi-square (χ^2) test for Goodness of Fit

A very powerful test for testing the significance of the discrepancy between theory and experiment was given by Prof. Karl Pearson in 1900 and is known as "Chi-square test of goodness of fit". It enables us to find if the deviation of the experiment from theory is just by chance or is it really due to the inadequacy of the theory to fit the observed data.

If Oi (i= 1,2, …, n) is a set of observed (experimental) frequencies (values) and Ei (i= 1,2, …, n) is the corresponding set of expected (theoretical or hypothetical) frequencies (values), then Karl Pearson's chi-square, given by

$$\chi^2 = \sum \left[\frac{(O_i - E_i)^2}{E_i} \right] \qquad \sum O_i = \sum E_i$$

where Oi = observed frequencies

Ei = expected frequencies

n = number of classes

follows chi-square distribution with (n-1) d.f.

The null hypothesis H_0 = the observed frequencies are equal with the expected frequencies

If calculated value of χ^2 > table value of χ^2 with (n_1-1) d.f. at specified level of significance, then the null hypothesis is rejected otherwise accepted.

9.4.1 Conditions for the validity of χ^2 - test:

i) The sample observations should be independent

ii) Constraints on the cell frequencies, if any, should be linear $\sum O_i = \sum E_i$

iii) N, the total frequency should be reasonably large, say greater than 50

iv) If any theoretical (expected) cell frequency is < 5, then for the application of chi-square test it is pooled with the preceding or succeeding frequency so that the pooled frequency is more than 5 and finally adjusts for the d.f. lost in pooling.

9.5. Chi-square (χ^2) test for Independence of Attribute

A characteristic which can not be measured but can only be classified to one of the different levels of the character under consideration is called an **attribute**.

9.5.1 2x2 Contingency table: When the individuals (objects) are classified into two categories with respect to each of the two attributes then the table showing frequencies distributed over 2x2 classes is called 2x2 contingency table.

Suppose the individuals are classified according to two attributes say intelligence (A) and colour (B). The distribution of frequencies over cells is shown in the following table.

A / B	A1	A2	Row totals
B1	a	b	R1 = (a+b)
B2	c	d	R2 = (c+d)
Column Total	C1 = (a+c)	C2 = (b+d)	N = (a+b+c+d)

The null hypothesis H_0: the two attributes are independent (if the color is not dependent on intelligent)

Based on above H_0, the expected frequencies are calculated as follows.

$$E(a) = \frac{R1 \times C1}{N},\ E(b) = \frac{R1 \times C2}{N},\ E(c) = \frac{R2 \times C1}{N},\ E(a) = \frac{R2 \times C2}{N}$$

To test this hypothesis we use the test statistic,

$$\chi^2 = \sum \left[\frac{(O_i - E_i)^2}{E_i} \right]$$

If the degrees of freedom for mxn contingency table is (m-1) x (n-1)

If the degrees of freedom for 2x2 contingency table is (2-1) x (2-1) = 1

This method is applied for all rxc contingency tables to get the expected frequencies.

The degree of freedom for rxc contingency table is (r-1) x (c-1)

If calculated value of χ^2 > table value of χ^2 with (2-1) x (2-1) = 1 d.f. at specified level of significance, then the null hypothesis is rejected otherwise accepted.

9.5.2 The alternative formula for calculating χ^2 in 2x2 contingency table is

$$\chi^2 = \frac{(ad - bc)^2 N}{R1 \times R2 \times C1 \times C2}$$

9.6. Yates correction for continuity in a 2x2 contingency table:

In a 2x2 contingency table, the number of d.f. is (2-1)(2-1) = 1. If any one of Expected cell frequency is less than 5, then we use of pooling method for χ^2 - test results with '0' d.f. (since 1 d.f. is lost in pooling) which is meaningless.

In this case we apply a correction due to Yates, which is usually known a Yates correction for continuity. Yates correction consists of the following steps;

(1) Add 0.5 to the cell frequency which is the least,

(2) Adjust the remaining cell frequencies in such a way that the row and column totals are not changed.

It can be shown that this correction will result in the formula,

$$\chi^2 (corrected\quad) = \frac{N\left[|ad - bc| - \frac{N}{2} \right]^2}{R1 \times R2 \times C1 \times C2}$$

If calculated value of χ^2 > table value of χ^2 with (2-1) x (2-1) = 1 d.f. at specified level of significance, then the null hypothesis is rejected otherwise accepted.

Solve the following problems:

Q.1. The following figures show the distribution of digits in numbers chosen at random from a telephonic directory:

Digits:	0	1	2	3	4	5	6	7	8	9	Total
Frequency:	1026	1107	997	966	1075	933	1107	972	964	853	10000

Test whether the digits may taken to occurs equally frequently in the directory.

Q.2. It is believed that the precision of an instrument is no more than 0.16. write down the null hypothesis and carry out the chi sq. test at 1% level, Given 11 measurement of the same subject on the instrument: 2.5, 2.3, 2.4, 2.3, 2.5, 2.7, 2.5, 2.6, 2.6, 2.7, 2.5

Q.3. The theory predict the proportion of beans in the four groups A, B, C and D should be 9 : 3 : 3 : 1. In an experiment among 1600 beans, the numbers in the four groups were 882, 313, 287 and 118. Does the experimental result support the theory?

Q.4. A bird watcher sitting in a park has spotted a number of birds belonging to 6 categories. The e xact classification is given below:

Category:	1	2	3	4	5	6
Frequency:	6	7	13	17	6	5

Test at 5% level of significance whether or not the data is compatible with assumption that this particular park visited by birds belonging to these six categories in the proportion 1 : 1 : 2 : 3 : 1 : 1

Q.5. The following table gives the number of aircraft accidents that occurred during the seven days of the week. Find whether the accidents are uniformly distributed over the week.

Days:	Mon.	Tue.	Wed.	Thu.	Fri.	Sat.	Total
No. of accidents:	14	18	12	11	15	14	84

Q.6. Two sample polls of votes for two candidates A and B for public offices are taken one from among the residents of rural areas. The results are given in the table. Examine whether the nature of the area is related to voting preference in this election.

Votes for Area	**A**	**B**	**Total**
Rural	620	380	1000
Urban	550	450	1000
Total	1170	830	2000

Q.7. Examine the following table showing the number of plants certain characters; test the hypothesis that the flower color is independent of shape of leaf.

Flower color	**Shape of leaf**		**Totals**
	Flat leaves	**Curled leaf**	
White flower	99	36	135
Red flower	20	4	24
Total	119	40	160

Exercise No.10 **Date: / /**

Correlation

The study related to the characteristics of only variable such as height, weight, ages, marks, wages, etc., is known as **Univariate analysis**.

The statistical Analysis related to the study of the relationship between two variables is known as **Bivariate analysis**. Sometimes the variables may be inter-related. In health sciences we study the relationship between blood pressure and age, consumption level of some nutrient and weight gain, total income and medical expenditure, etc.

10.1 Definitions:

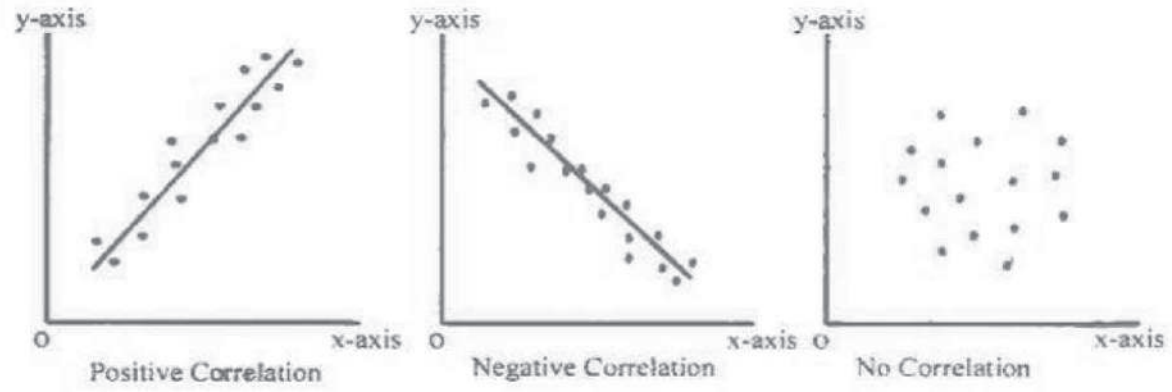
1. Correlation Analysis attempts to determine the degree of relationship between variables - Ya-Kun-Chou.
2. Correlation is an analysis of the co-variation between two or more variables.- A.M.Tuttle

10.2 Scatter Diagram:

It is the simplest method of studying the relationship between two variables diagrammatically. One variable is represented along the horizontal axis and the second variable along the vertical axis. For each pair of observations of two variables, we put a dot in the plane. There are as many dots in the plane as the number of paired observations of two variables. The direction of dots shows the scatter or concentration of various points. This will show the type of correlation.

1. If all the plotted points form a straight line from lower left hand corner to the upper right hand corner then there is Perfect positive correlation.
   We denote this as r = +1
2. If all the plotted dots lie on a straight line falling from upper left hand corner to lower right hand corner, there is a perfect negative correlation between the two variables. In this case the coefficient of correlation takes the value r = - 1.
3. If the plotted points in the plane form a band and they show a rising trend from the lower left hand corner to the upper right hand corner the two variables are highly positively correlated.
4. If the points fall in a narrow band from the upper left hand corner to the lower right hand corner, there will be a high degree of negative correlation.
5. If the plotted points in the plane are spread all over the diagram there is no correlation between the two variables. We denote this as r = 0

Figures:



Merits:

1. It is a simplest and attractive method of finding the nature of correlation between the two variables.
2. It is a non-mathematical method of studying correlation. It is easy to understand.
3. It is not affected by extreme items.
4. It is the first step in finding out the relation between the two variables.
5. We can have a rough idea at a glance whether it is a positive correlation or negative correlation.

Demerits:

By this method we cannot get the exact degree or correlation between the two variables.

10.3 Types of Correlation:

i) Positive and negative.

ii) Linear and non-linear.

iii) Partial and total.

iv) Simple and Multiple

10.3.1 Positive and Negative Correlation:

It depends upon the direction of change of the variables. If the two variables tend to move together in the same direction (ie) an increase in the value of one variable is accompanied by an increase in the value of the other, (or) a decrease in the value of one variable is accompanied by a decrease in the value of other, then the correlation is called positive or direct correlation. Price and supply, height and weight, yield and rainfall, are some examples of positive correlation.

If the two variables tend to move together in opposite directions so that increase (or) decrease in the value of one variable is accompanied by a decrease or increase in the value of the other variable, then the correlation is called negative (or) inverse correlation. Price and demand, yield of crop and price, are examples of negative correlation.

10.3.2 Linear and Non-linear correlation:

If the ratio of change between the two variables is a constant then there will be linear correlation between them. Consider the following.

X	2	4	6	8	10	12
Y	3	6	9	12	15	18

Here the ratio of change between the two variables is the same. If we plot these points on a graph we get a straight line.

If the amount of change in the one variable does not shows a constant ratio of the amount of change in the other. Then the relation is called Curvi-linear (or) non-linear correlation. **The graph will be a curve**.

10.3.3 Simple and Multiple Correlation:

When we study only two variables, the relationship is simple correlation. For example, quantity of money and price level, demand and price.

But in a multiple correlation we study more than two variables simultaneously. The relationship of price, demand and supply of a commodity are an example for multiple correlation.

10.3.4 Partial and total correlation:

The study of two variables excluding some other variable is called Partial correlation. For example, we study price and demand eliminating supply side. In total correlation all facts are taken into account.

10.4 Methods of computation of correlation:

1. Scatter diagram method/Graphic method
2. Algebraic method: Karl Pearson's coefficient of correlation
3. Spearman's Rank method

10.4.1 Scatter diagram method/

Discussed in above 9.2

Graphic method:

This method is used the individual values of the two variables are plotted on the graph paper. We thus obtain two curves, one for X-variable and another for Y variable. By examining the direction and closeness of the two curves so drawn we can infer whether or not the variables are related. If both the curves drawn on the graph is moving in the same direction (either upper or downward) correlation is said to be positive. On the other hand, if the curves are moving in the opposite directions correlation is said to be negative.

10.4.2. Algebraic method: Karl Pearson's coefficient of correlation

Karl pearson, a great biometrician and statistician, suggested a mathematical method for measuring the magnitude of linear relationship between the two variables. It is most widely used method in practice and it is known as pearsonian coefficient of correlation. It is denoted by 'r'.

The formula for calculating 'r' is

$$r_{xy} = \frac{Cov\ (x, y)}{\sqrt{V(x)\ V(y)}}$$

$$r_{xy} = \frac{\frac{1}{n}\sum (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\frac{1}{n}\sum (x_i - \bar{x})^2}\sqrt{\frac{1}{n}\sum (y_i - \bar{y})^2}}$$

After simplification, $$r_{xy} = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum (x_i - \bar{x})^2}\sqrt{\sum (y_i - \bar{y})^2}}$$

Or

$$r_{xy} = \frac{\sum xy - \frac{(\sum x)(\sum y)}{n}}{\sqrt{\sum x^2 - \frac{(\sum x)^2}{n}}\sqrt{\sum y^2 - \frac{(\sum y)^2}{n}}}$$

10.4.3. Spearman's Rank method

It is studied when no assumption about the parameters of the population is made. This method is based on ranks. It is useful to study the qualitative measure of attributes like honesty, colour, beauty, intelligence, character, morality etc. The individuals in the group can be arranged in order and there on, obtaining for each individual a number showing his/her rank in the group. This method was developed by **Edward Spearman in 1904**. It is defined as by the formula;

$$\text{Rank Correlation, } r_{xy} = 1 - \frac{6\sum D^2}{n(n^2 - 1)}$$

where, $\sum D^2$ = sum of squares of differences between the pairs of ranks.

n = number of pairs of observations.

It is also denoted by 'ρ'. The value of 'r' lies between -1 and +1. If r = +1, there is complete agreement in order of ranks and the direction of ranks is also same. If r = -1, then there is complete disagreement in order of ranks and they are in opposite directions.

10.5 Properties of Correlation:

1. Correlation coefficient lies between –1 and +1
2. r = +1 perfect positive correlation and r = -1 perfect negative correlation between the variables.

3. 'r' is independent of change of origin and scale.
4. It is a pure number independent of units of measurement.
5. Independent variables are uncorrelated but the converse is not true.
6. Correlation coefficient is the geometric mean of two regression coefficients. $r = \sqrt{b_{xy} \times b_{yx}}$
7. The correlation coefficient of x and y is symmetric. $r_{xy} = r_{yx}$

10.6 Interpretation:

The following rules helps in interpreting the value of 'r'.

1. When r = 1, there is perfect +ve relationship between the variables.
2. When r = -1, there is perfect –ve relationship between the variables.
3. When r = 0, there is no relationship between the variables.
4. If the correlation is +1 or –1, it signifies that there is a high degree of correlation (Positive or Negative) between the two variables. If 'r' is near to zero, then there is less correlation.

Solve the following problems:

Q.1. The following are the heights and weights of 15 students of a class. Draw a graph to indicate whether the correlation is negative or positive. Determine the coefficient of correlation for this set of data.

Heights (cms)	170	172	181	157	150	168	166	175	177	165	163	152	161	173	175
Weights (kgs)	65	66	69	55	51	63	61	75	72	64	61	52	60	70	72

Q.2. A study was conducted to find whether there is any relationship between the weight and blood pressure of an individual. Determine the coefficient of correlation for this set of data.

Weight	78	86	72	82	80	86	84	89	68	71
Blood Pressure	140	160	134	144	180	176	174	178	128	132

Q.3. The data given below are obtained from student records. Calculate the rank correlation coefficient 'R' for the data.

Subject	Grade Point Average (x)	Graduate Record exam score (y)
1	8.3	2300
2	8.6	2250
3	9.2	2380
4	9.8	2400
5	8	2000
6	7.8	2100
7	9.4	2360
8	9	2350
9	7.2	2000
10	8.6	2260

Q.4. A horse owner is investigating the relationship between weight carried and the finish position of several horses in his stable. Calculate r and R for the data given

Weight Carried	110	113	120	115	110	115	117	123	106	108	110	110
Position Finished	2	6	3	4	6	5	4	2	1	4	1	3

Exercise No. 11 **Date: / /**

Regression

After knowing the relationship between two variables we may be interested in estimating (predicting) the value of one variable given the value of another. The variable predicted on the basis of other variables is called the '**dependent**' or the '**explained**' variable or '**regressed**' and the other the '**independent**' or the '**predicting**' variable or '**regressor**'. The prediction is based on average relationship derived statistically by regression analysis. The equation, linear or otherwise, is called the regression equation or the explaining equation.

The relationship between two variables can be considered between, say, rainfall and agricultural production, price of an input and the overall cost of product, consumer expenditure and disposable income. Thus, regression analysis reveals average relationship between two variables and this makes possible estimation or prediction.

11.1 Definition:

Regression is the measure of the average relationship between two or more variables in terms of the original units of the data. It is functional relationship between a dependent variable, Y with one or more independent variables, X is called **Regression equation**.

11.2 Types of Regression:

The regression analysis can be classified into:

a) Simple and Multiple

b) Linear and Non –Linear

c) Total and Partial

a) Simple and Multiple:

In case of simple relationship only two variables are considered, for example, the influence of advertising expenditure on sales turnover. In the case of multiple relationships, more than two variables are involved. On this while one variable is a dependent variable the remaining variables are independent ones. For example, the turnover (y) may depend on advertising expenditure (x) and the income of the people (z). Then the functional relationship can be expressed as $y = f(x,z)$

b) Linear and Non-linear:

The linear relationships are based on straight-line trend, the equation of which has no-power higher than one. But, remember a linear relationship can be both simple and multiple. Normally a linear relationship is taken into account because besides its simplicity, it has a better predictive value; a linear

trend can be easily projected into the future. In the case of non-linear relationship curved trend lines are derived. The equations of these are parabolic.

c) Total and Partial:

In the case of total relationships all the important variables are considered. Normally, they take the form of a multiple relationships because most economic and business phenomena are affected by multiplicity of cases. In the case of partial relationship one or more variables are considered, but not all, thus excluding the influence of those not found relevant for a given purpose.

11.3 Linear Regression Equation:

If two variables have linear relationship then as the independent variable (X) changes, the dependent variable (Y) also changes. If the different values of X and Y are plotted, then the two straight lines of best fit can be made to pass through the plotted points. These two lines are known as regression lines. Again, these regression lines are based on two equations known as regression equations. These equations show best estimate of one variable for the known value of the other. The equations are linear. Linear regression equation of Y on X is

$$Y = a + b_{yx}X \ldots\ldots (1)$$

And X on Y is

$$X = a + b_{xy}Y \ldots\ldots (2)$$

Where, a, b are constants. b_{yx} & b_{xy} are coefficient of regression (or slop of line)

11.4 Principle of 'Least Squares':

Regression shows an average relationship between two variables, which is expressed by a line of regression drawn by the method of 'least squares'. In above equation (1) & (2), the constants 'a' and 'b' can be estimated with by applying the `least squares method'.

This involves minimizing $\sum e_i = \sum (Y - a - b_{yx} X)^2$

This gives $b_{yx} = b = \dfrac{Cov\ (X, Y)}{V\ (X)}$

$$b_{yx} = \frac{\sum XY - \dfrac{(\sum X)(\sum Y)}{n}}{\sum X^2 - \dfrac{(\sum X)^2}{n}}$$

and $a = \overline{Y} - b_{yx}\,\overline{X}$

where, b_{yx} is called the estimate of regression coefficient of Y on X and it measures the change in Y for a unit change in X.

Similarly, the regression equation of X on Y is given as, $X = a + b_{xy}Y$

This gives $b_{xy} = b = \frac{Cov\ (X, Y)}{V\ (Y)}$

$$b_{xy} = \frac{\sum XY - \frac{(\sum X)(\sum Y)}{n}}{\sum Y^2 - \frac{(\sum Y)^2}{n}}$$

and $a = \overline{X} - b_{xy}\overline{Y}$

where, b_{xy} is called the estimate of regression coefficient of X on Y and it measures the change in X for a unit change in Y.

11.5 Assumptions Made in the Linear Regression Analysis

1. The X's are non random or fixed constants. For example, it may apply 40, 60, 80 and 100 kg of N_2 per ha to 10 plots each. Then it may observe the crop yield (Y) corresponding to each of these selected level (X).
2. At each fixed value of X, the corresponding values of Y have a **Normal distribution** about theoretical mean.
3. For any given x, the variance of Y is the same or **homoscedastic.**
4. The y's observed at different value of X are completely independent.

11.6 Properties of Regression Co-efficient:

1. Both regression coefficients must have the same sign, i.e. either it will be positive or negative.
2. Correlation coefficient is the geometric mean of the regression coefficients i.e. $r = \sqrt{b_{xy} \times b_{yx}}$
3. The correlation coefficient will have the same sign as that of the regression coefficients.
4. If one regression coefficient is greater than unity, then other regression coefficient must be less than unity.
5. Regression coefficients are independent of origin but not of scale.
6. Arithmetic mean of b_{xy} and b_{yx} is equal to or greater than the coefficient of correlation. Symbolically, $\frac{b_{xy} + b_{yx}}{2} \geq r$
7. If r=0, the variables are uncorrelated, the lines of regression become perpendicular to each other.
8. If r= +1, the two lines of regression either coincide or parallel to each other
9. Angle between the two regression lines is $\theta = \tan^{-1}\left[\frac{m_1 - m_2}{1 + m_1 m_2}\right]$. Where, m_1 and, m_2 are the slopes of the regression lines X on Y and Y on X respectively.
10. The angle between the regression lines indicates the degree of dependence between the variables.

11.7 Uses of Regression Analysis:

1. Regression analysis helps in establishing a functional relationship between two or more variables.
2. Since most of the problems of economic analysis are based on cause and effect relationships, the regression analysis is a highly valuable tool in economic and business research.
3. Regression analysis predicts the values of dependent variables from the values of independent variables.
4. We can calculate coefficient of correlation (r) and coefficient of determination (r^2) with the help of regression coefficients.
5. In statistical analysis of demand curves, supply curves, production function, cost function, consumption function etc., regression analysis is widely used.

11.8 Difference between Correlation and Regression

S. No.	Correlation	Regression
1	Correlation is the relationship between two or more variables, which vary in consideration with the other in the same or the opposite direction.	Regression means going back and it is a mathematical measure showing the average relationship between two variables
2	Both the variables X and Y are random variables	Here X is a random variable and Y is a fixed variable. Sometimes both the variables may be random variables
3	It finds out the degree of relationship between two variables and not the cause and effect of the variables.	It indicates the causes and effect relationship between the variables and establishes functional relationship
4	It is used for testing and verifying the relation between two variables and gives limited information.	Besides verification it is used for the prediction of one value, in relationship to the other given value.
5	The coefficient of correlation is a relative measure.	Regression coefficient is an absolute figure. If we know the value of the independent variable, we can find the value of the dependent variable.
6	The range of relationship lies between –1 and +1	The range of relationship lies between $-\infty$ and $+\infty$

7	There may be false correlation between two variables	In regression there is no such false regression
8	It has limited application, because it is confined only to linear relationship between the variables	It has wider application, as it studies linear and non- linear relationship between the variables
9	It is not very useful for further mathematical treatment.	It is widely used for further mathematical treatment
10	If the coefficient of correlation is positive, then the two variables are positively correlated and vice-versa	The regression coefficient explains that the decrease in one variable is associated with the increase in the other variable

Solve the following problems:

Q.1. The data given below are obtained from student records. Calculate the regression equation and compute the estimated GRE scores for GPA = 7.5 and 8.5

Subject	Grade Point Average (x)	Graduate Record exam score (y)
11	8.3	2300
12	8.6	2250
13	9.2	2380
14	9.8	2400
15	8	2000
16	7.8	2100
17	9.4	2360
18	9	2350
19	7.2	2000
20	8.6	2260

Q.2. Develop a regression equation which may be used to predict final examination scores from the mid – term score. Find final examination scores, if mid-term score is 72 and Find mid-term scores, if final examination score is 82.

Student	1	2	3	4	5	6	7	8	9	10
Mid – term	98	66	100	96	88	45	76	60	74	82
Final	90	74	98	88	80	62	78	74	86	80

Q.3. A horse was subject to the test of how many minutes it takes to reach a point from the starting point. The horse was made to carry luggage of various weights on 10 trials.

Trial No.	Weight (in Kg)	Time taken (in min)
1	11	13
2	23	22
3	16	16
4	32	47
5	12	13
6	28	39
7	29	43
8	19	21
9	25	32
10	20	22

Fit the regression equation between the load and the time taken to reach the goal. Estimate the time taken for the loads of 35 Kg, 23 Kg, and 9 Kg.

Q.4. From a paddy field, 15 plants were selected at random. The length of panicles (cm) and the no. of grains per panicle were recorded, given in the table. Fit the regression line for this set of data and compute the estimated no. of grains per panicle if length of panicle is 25.2 cm.

Length of Panicle (cm)	22.4	23.3	24.1	24.3	23.5	23.1	21.0	20.6	26.4	25.4	23.4	21.4	23.6	24.5	22.5
No. of Grains per panicle	95	109	133	132	136	116	94	85	143	138	129	88	127	142	110

Exercise No. 12 **Date: / /**

Experimental Design- terms and definitions

In order to verify a hypothesis pertaining to some scientific phenomena we have to collect data. Such data are obtained by either observation or by experimentation. The main topics connected with data collection are Theory of Sample Surveys and Experimental Designs. In sample survey, a researcher makes observations on existing population and records data without interfering with the process that is being observed.

Modern concepts of experimental design are due primarily to R.A. Fisher. He developed them in the planning of agricultural field experiments. They are now used in many fields of science.

Design of Experiment- It is defined as 'the logical construction of the experiment in which the degree of uncertainty with which the inferences is drawn may well define.'

Design of experiment includes- (i) planning of experiment, (ii) Obtaining information from statistical hypothesis under study, (iii) Making statistical analysis of data

12.1 Basic Terminology & Definition

1. **Experiment-** It is device or means of getting answer to the problem under consideration.

   a) Comparative experiment- Compare effect of two or more objects on population characteristics. E.g. comparison of different manures and fertilizers, comparison of different variety of crops

   b) Absolute experiment- determine absolute value of all population characteristics. E.g. Correlation coefficient of bi-variate distribution, Average of I.Q. of group of people.

2. **Treatment -** the objects of comparison in an experiment are defined as treatments. For example:
   i) Suppose an Agronomist wishes to know the effect of different spacing on the yield of a crop, different spacing will be treatments. Each spacing will called a treatment.
   ii) If different of fertilizer are tried in an experiment to test the responses of a crop to the fertilizer doses, the different doses will be treatments and each dose will be a treatment.
   ii) A teacher practices different teaching methods on different groups in his class to see which yields the best results.
   iii) A doctor treats a patient with a skin condition with different creams to see which is most effective.

3. **Experimental unit-** Smallest division of experimental material to which applies treatment & makes observation on the variable under study is termed as experimental unit. For example

i) In laboratory insects may be kept in groups of five or six. To each group, different insecticides will be applied to know the efficacy of the insecticides. In this study different groups of insects will be the experimental unit.

ii) If treatments are different varieties, then the objects to which treatments are applied to make observations will be different plot of land. The plots will be called experimental units.

4. Block- In agricultural experiments, most of the times we divide the whole experimental unit (field) into relatively homogeneous sub-groups or strata. These strata, which are more uniform amongst themselves than the field as a whole, are known as blocks.

5. Yield- The measurement of the variable under study on different experimental unit (plots)

6. Experimental error- In agricultural experiment, field experiment is divided in small plots with homogeneous condition. Then apply dose of fertilizer to all plots and measure the yield. Experiment will give different yield from different plots; it will be due to treatment effect or uncontrollable (chance) factor. Suppose, we apply same treatment on all plots still there is variation in yield, it is due to soil fertility, such variation are plot to plot which beyond the control of human control is called experimental error.

7. Precision- The reciprocal of the variance of the mean is termed as the precision. If experiment is replicated in 'r' times, the precision is given by,

$$\frac{1}{Var\ (\bar{x})} = \frac{r}{\sigma^2}$$

8. Efficiency of Design- let it be D_1 and D_2 are experimental design, with σ_1 and σ_2 per unit and replication r_1 and r_2 respectively. The variance of the difference between two treatment means is given by $\frac{2\sigma_1^2}{r_1}$ and $\frac{2\sigma_2^2}{r_2}$ for D_1 and D_2 respectively. Then the ratio, $E= \frac{2\sigma_2^2}{r_2} \times \frac{r_1}{2\sigma_1^2} = \frac{r_1}{\sigma_1^2} \div \frac{r_2}{\sigma_2^2}$ is termed as efficiency of design D_1 w.r.t. D_2.

If E=1, then both the designs D_1 and D_2 is said to be equally efficient.

If E>1 (E<1), then D_1 is said to be more (less) efficient than D_2.

9. Uniformity trials- The fertility of the soil does not increase or decrease uniformly in any direction but is distributed over the entire field in erratic manner. **Uniformity trials** enable us to have an idea about the fertility variation of the field. In which field (experimental material) is divided into small units (plots) and same treatment is applied on each units and their yield are recorded. From these yields, we can draw a 'fertility contour map' which gives us a graphic picture of the variation of the

soil fertility and enables us to form a good idea about the nature of the soil fertility variation. The fertility contour map is obtained by joining the points of equal fertility through lines.

12.2 Principles of an experimental design

The purpose of designing an experiment is to increase the precision of the experiment. In order to increase the precision, we try to reduce the experimental error. For reducing the experimental error, we adopt some techniques. These techniques form the basic principles of experimental designs. The basic principles of the experimental designs are replication, randomization and local control.

1. Replication: Repetition of treatment to different experimental units is known as Replication. In other words, the repetition of treatments under investigation is known as replication. We have no means of knowing about the variations in the results of a treatment. Only when we repeat the treatment several times we can estimate the experimental error.

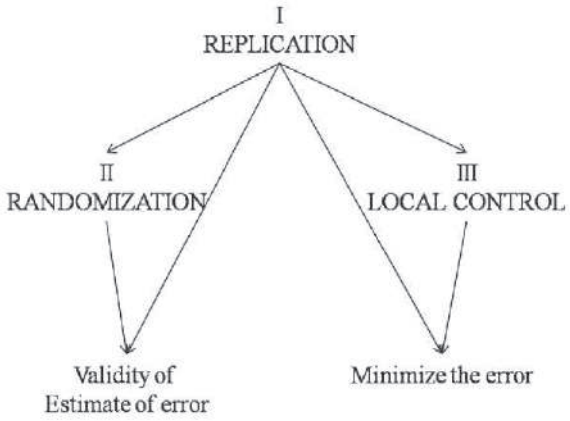
A replication is used *(i)* to secure more accurate estimate of the experimental error, a term which represents the differences that would be observed if the same treatments were applied several times to the same experimental units; *(ii)* to reduce the experimental error and thereby to increase precision, which is a measure of the variability of the experimental error.

The standard error of treatment mean is $\frac{\sigma}{\sqrt{r}}$. Where, σ is S.D. of treatment in the population and `r' is the number of replications. As the `r' increases, the standard error of mean will decreases. Also in the analysis of variance the replication of treatments provides estimate of experimental error which is essential for the application of F-test.

2. Randomization: when all the treatments have equal chances of being allocated to different experimental units it is known as randomization **or**

Random allocation of treatments to different experimental units is known as randomization.

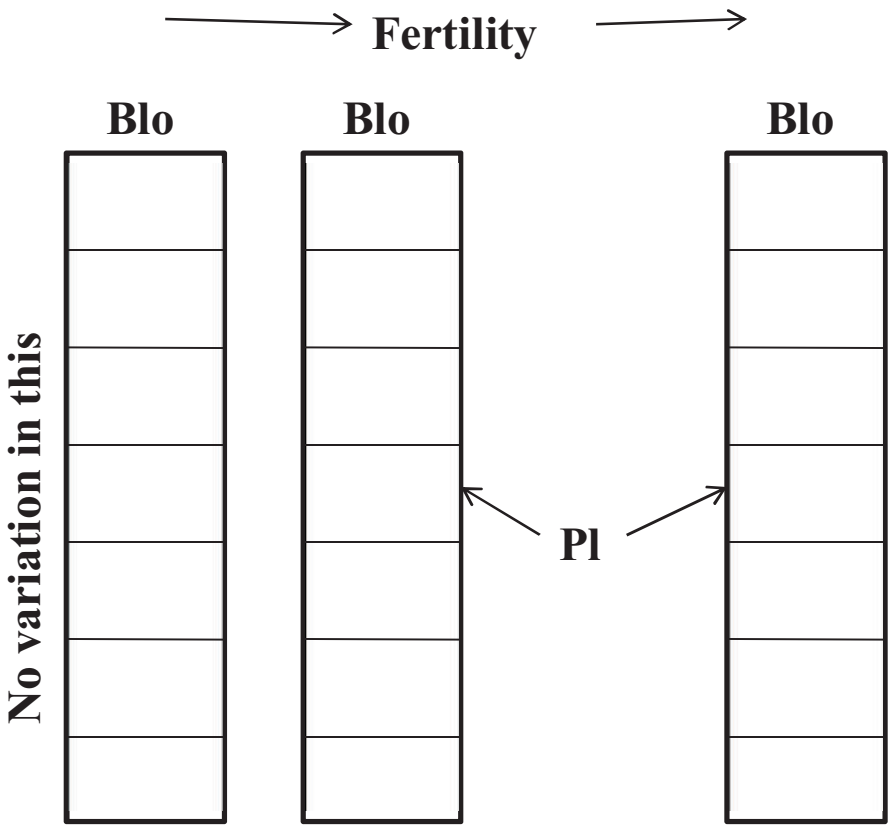
The **purpose of randomization** is to remove bias and other sources of extraneous variation which are not controllable. Another advantage of randomization (accompanied by replication) is that it forms the basis of any valid statistical test. Hence the treatments must be assigned at random to the experimental units. Randomization is usually done by using tables of random numbers.



Fisher's Diagram

3. Local control: It has been observed that all extraneous sources of variation are not removed by randomization and replication. This necessitates a refinement in the experimental technique. For this purpose, we make use of local control, a term referring to the grouping of homogeneous experimental units. The process of reducing the experimental error by dividing the relatively heterogeneous experimental area (field) in to homogeneous blocks (due to physical contiguity as for as field experiments are concerned) is known as **Local control.** The local control by reducing the experimental error increases the efficiency of the design.

- The precision of the experiment increases with an increase in the plot size.



Shape of blocks and plots: the shape and size of the blocks will usually depend up on the shape and size of the plots. In order to control the experimental error it is desirable to divide the whole experimental area into different subgroups (blocks) such that within each block there is as much homogeneity as possible but between blocks there is maximum variation. Further each block is to be divided into as many plots as the number of treatments. For maximum precision the plots should be rectangular in shape with their long sides parallel to the direction of the fertility gradient and the blocks should be arranged one after the other along the fertility gradient as shown in the figure.

Exercise No. 13 **Date: / /**

Completely Randomized Design (C.R.D.)

The CRD is the simplest of all the designs, based on principles of randomization and replication. In this design, treatments are allocated at random to the experimental units over the entire experimental material. In case of field experiments, the whole field is divided into a required number of plots equal size and then the treatments are randomized in these plots.

13.1 Layout of C.R.D.: The placement of the treatments on the experimental units along with the arrangement of experimental units is known as the **layout of an experiment**.

Suppose, that we have 't' treatment, the i^{th} treatment being replicated r_i times. Then whole experimental material divided in to $n = \sum r_i$ experimental units and treatments are distributed completely at random over the units subject to condition that the i^{th} treatment occurs r_i times.

If each treatment is repeated an equal number of times r, then $n = r \times t$

For example, there are 3 treatments A, B and C and it is desired to replicate (repeat) them 4 times. The experimental area is divided into 12 equal plots and the treatments are allotted to these plots at random. There is no restriction of assigning the treatments to plots.

Completely Randomized Design with 3 treatments and 4 replications

B 1	A 5	C 9
A 2	B 6	A 10
C 3	C 7	C 11
A 4	B 8	B 12

13.2 Advantages of C.R.D.

1. It results in the maximum use of the experimental units since all the experimental material can be used
2. The design is very flexible. Any number of treatment can be used and different treatment can be used unequal number of times.
3. The statistical analysis remain simple if some or all the observation for any treatment are rejected or lost or missing.
4. It provides maximum number of degrees of freedom for the estimation of the error variance.

13.3 Disadvantages of C.R.D.

1. When the experimental material is heterogeneous, the experimental error would be inflated and consequently the treatments are less precisely compared. The only way to keep the experimental

error under control is to increase the number of replications thereby increasing the degrees of freedom for error.

2. In field experiments there is generally large variation among experimental plots due to soil heterogeneity. Hence, CRD is not preferred in field experiments.

13.4 Applications of CRD

1. CRD is most useful in laboratory techniques and methodological studies, such as in Agril Chemistry, Plant pathology and Animal experiments, where the experimental material is expected to be homogeneous.
2. In laboratory experiments and green house studies, it is easy to achieve homogeneity of experimental materials. Therefore, CRD is most useful in such experiments.
3. CRD is also recommended in situations where an appreciable fraction of units is likely to be destroyed or fail to respond

13.5 Statistical analysis:

Let us suppose that there are '*t*' treatments and applied to *'r'* plots. These can be represented by the symbols as follows:

Treatment	1….2……j……n	Total	Mean
t_1	$y_{11}, y_{12}, \ldots\ldots y_{1j}\ldots\ldots y_{1r}$	T_1	$\overline{T_1}$
t_2	$y_{21}, y_{22}, \ldots\ldots y_{2j}\ldots\ldots y_{2r}$	T_2	$\overline{T_2}$
t_i	$y_{i1}, y_{i2}, \ldots\ldots y_{ij}\ldots\ldots y_{ir}$	T_i	$\overline{T_i}$
t_t	$y_{t1}, y_{t2}, \ldots\ldots y_{tj}\ldots\ldots y_{tr}$	T_t	$\overline{T_t}$
		GT	

Mathematical model:

$y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$ (i= 1,2,….t; j= 1,2,….,r)

Where, y_{ij} is the response of the j^{th} block and i^{th} treatment

μ= general mean effect

α_i= effect due to i^{th} treatment

ε_{ij}= error term

Null hypothesis, H_0: There is no significant difference between the treatment effects.

$A_1 = \alpha_2 = \ldots\ldots\ldots\ldots = \alpha_t = 0$ Where, (i= 1,2,…..t)

The null hypothesis can be verified by applying the ANOVA procedure. The steps involved in this procedure are as follows:

1. Correction factor, $= C.F. = \frac{(GT)^2}{N}$, since $N = \sum r_i$

2. Treatment Sum of Square (T.S.S.) $= \left[\frac{T_1^2}{r_1} + \frac{T_2^2}{r_2} + ... + \frac{T_t^2}{r_i}\right] - C.F.$

$= \frac{\sum T_i^2}{r} - C.F.$ (if all replication are equal)

3. Total Sum of Square (Total S.S.) $= \left[y_{11}^2 + y_{12}^2 + y_{13}^2 + ... + y_{tr}^2\right] - C.F.$

$= \sum y_{ij}^2 - C.F.$

4. Error Sum of Square (E.S.S.) = Total S.S. - T.S.S.

ANOVA Table:

Source of Variation (S.V.)	Degrees of Freedom (d.f)	Sum of Square (S.S.)	Mean Square (M.S.)	F-Calculated value	F-tabulated value
Treatment (t)	t-1	T.S.S	T.M.S. = T.S.S./t-1	T.M.S./M.S.E.	$F_{(t-1, n-t)}$ at 0.05 or 0.01 level of significance
Error	n-t	E.S.S	M.S.E. = E.S.S./n-t		
Total (n)	**n-1**	**Total S.S.**			

If the calculated value of F > table value of F, H_0 is rejected and treatment means are significantly different.

Then the problem is to know which of the treatment means are significantly different. For this, we calculate critical difference (CD).

CD = SE(d) x $t_{(0.05)}$

Where, SE(d) = Standard Error of difference between the Treatments.

$t_{(0.05)}$ table value for **error d.f**. at 5% level of significance.

Equal no. of replications i.e. '*r*' , then

$$SE(d) = \sqrt{\frac{2M.S.E.}{r}}$$

$$C.D. = t_{0.05,(n-t)} \times \sqrt{\frac{2\,M.S.E.}{r}}$$

Unequal no. replications, then

$$SE(d) = \sqrt{M.S.E.\left(\frac{1}{r_i} + \frac{1}{r_j}\right)} \qquad \text{where i = 1,2,…..,t and j = 1,2,…..,t}$$

$$C.D. = t_{0.05,(n-t)} \times \sqrt{M.S.E.\left(\frac{1}{r_i} + \frac{1}{r_j}\right)}$$

The treatment means are arranged first in descending order of magnitude. If the difference between the two treatment means is less than CD value, it will declared as non significant otherwise significant.

Coefficient Variation (C.V.) = $\frac{\sqrt{M.S.E.}}{\bar{y}} \times 100$ Where, $\bar{y}$ = Grand mean

Solve the following problems

Q.1. The data related to a varietal trial on green gram that conducted using CRD having five variety V1, V2, V3, V4 and V5 with 3, 4, 5, 4 and 4 replications respectively. The results are given below:

V1	1.6 1.2 1.5
V2	2.5 2.2 2.4 1.9
V3	1.3 0.9 0.8 1.1 1.0
V4	2.0 1.5 1.6 1.4
V5	1.6 1.0 0.8 0.9

Q.2 The data relate to the five varieties of seas sum using CRD conducted in a greenhouse with four pots per variety.

		Seed yield of sea sum (g/pot)
Variety	V1	25, 21, 21, 18
	V2	25, 28, 24, 25
	V3	24, 24, 16, 16
	V4	20, 17, 16, 19
	V5	14, 15, 13, 11

Q.3 Analyze the following data using CRD and draw your conclusion.

Assignment 1: 14, 15, 9, 10, 12, 15, 16, 18

Assignment 2: 10, 12, 9, 7, 11, 8, 12, 9, 10, 14, 16

Assignment 3: 11, 5, 19, 10, 6, 8, 8, 7, 3

Exercise No. 14 **Date: / /**

Randomized Block Design (R.B.D.)

In field experimentation, if the whole of the experimental area is not homogeneous and the fertility **gradient is only in one direction** then a simple method of controlling the variability of the experimental material consists in grouping the whole area in to relatively homogeneous sub-group called 'block' or 'replicates' **perpendicular to direction of fertility gradient.** Now if the treatments are applied at random to homogeneous block and replicated over all the blocks, the design is a Randomized Block Design (R.B.D.).

In a Completely Randomized Design no local control measure was adopted excepting that the experimental units should be homogeneous. Usually, when experiments require a large number of experimental units, completely randomized designs cannot ensure precision of the estimates of treatment effects. But in R.B.D. treatments are allocated at random within the units of each block i.e. randomization is restricted. Also variation among blocks is removed from variation due to error. Hence, if it is desired to control one source of variation by sub grouping/blocking. **Thus experimenter should select the R.B.D. rather than C.R.D**.

14.1 Layout of R.B.D.: Let us consider 5 treatments A, B, C, D & E and each treatment is replicated 4 times. Thus, whole experimental area divided into 4 relatively homogeneous blocks and each block into 5 plots. Treatments are then allocated at random to the plots of a block, **fresh randomization being done for each block**. A particular layout as follows,

Block I	Block II	Block III	Block IV
B	A	C	D
A	C	D	E
C	D	E	B
D	E	B	A
E	B	A	C

For randomization, it may use **Tippet's** random number tables. Let us select one digited number in order of their occurrence in the table leaving zero and number greater than 5. Suppose we get a random number from 1 to 5 as : 2, 1, 3, 4, 5. So first block allocate treatment A to second plot, B to first plot, C to

third plot, D to 4th and E th the 5th plot. Similarly fresh randomization for each of the three blocks and allocates the treatment accordingly.

14.2 Statistical Analysis: The results from R.B.D. can be arranged in two way table according to the replications (blocks) '*r*'and treatments '*t*'; there will be '***rt***' observations in total. The data can be arranged in the following table,

Treatment	**Block** $b_1 \ldots b_2 \ldots b_j \ldots \ldots b_r$	**Tratment Total**	**Mean**
t_1	$y_{11}, y_{12}, \ldots y_{1j} \ldots y_{1r}$	T_1	$\overline{T_1}$
t_2	$y_{21}, y_{22}, \ldots y_{2j} \ldots y_{2r}$	T_2	$\overline{T_2}$
t_i	$y_{i1}, y_{i2}, \ldots y_{ij} \ldots y_{ir}$	T_i	$\overline{T_i}$
t_t	$y_{t1}, y_{t2}, \ldots y_{tj} \ldots y_{tr}$	T_t	$\overline{T_t}$
Block Total	$B_1, B_2 \ldots B_j \ldots B_t$	GT	
Mean	$\overline{B_1}\ \overline{B_2} \ldots \overline{B_j} \ldots \overline{B_r}$		

14.2.1 Mathematical model:

$y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$ (i= 1,2,….t; j= 1,2,….,r)

Where, y_{ij} is the response of the j^{th} block and i^{th} treatment

μ= general mean effect

α_i= effect due to i^{th} treatment

β_j = effect due to j^{th} block/replication

ε_{ij}= error term

Null hypothesis, H_0: There is no significant difference between the treatment effects.

$\alpha_1 = \alpha_2 = \ldots\ldots\ldots\ldots = \alpha_t = 0$ Where, (i= 1,2,…..t)

H_0: There is no significant difference between the block effects.

$\beta_1 = \beta_2 = \ldots\ldots\ldots\ldots = \beta_r = 0$ Where, (i= 1,2,…..r)

The null hypothesis can be verified by applying the ANOVA procedure. The steps involved in this procedure are as follows:

1. Correction factor, $= C.F. = \frac{(GT)^2}{N}$, since $N = rt$

2. Treatment Sum of Square (T.S.S.) $= \frac{1}{r}\left[T_1^2 + T_2^2 + \ldots\ldots\ldots T_t^2\right] - C.F.$

$$= \frac{\sum T_i^2}{r} - C.F.$$

3. Replication (Block) Sum of Square (R.S.S.) = $\frac{1}{t}\left[B_1^{\,2} + B_2^{\,2} + \ldots\ldots B_r^{\,2}\right] - C.F.$

$$= \frac{\sum B_j^2}{t} - C.F.$$

4. Total Sum of Square (Total S.S.) = $\left[y_{11}^2 + y_{12}^2 + y_{13}^2 + \ldots + y_{tr}^2\right] - C.F.$

$$= \sum y_{ij}^2 - C.F.$$

5. Error Sum of Square (E.S.S.) = Total S.S. - T.S.S. – R.S.S.

ANOVA Table:

Source of Variation (S.V.)	Degrees of Freedom (d.f)	Sum of Square (S.S.)	Mean Square (M.S.)	F-Calculated value	F-tabulated value
Replication /Block	r-1	R.S.S.	R.M.S. = R.S.S./r-1	R.M.S./M.S.E.	$F_{[r-1,\,(r-1)(t-1)]}$ at 0.05 level of significance
Treatment (t)	t-1	T.S.S	T.M.S. = T.S.S./t-1	T.M.S./M.S.E.	$F_{[t-1,\,(r-1)(t-1)]}$ at 0.05 level of significance
Error	(r-1)(t-1)	E.S.S	M.S.E. = E.S.S./(r-1)(t-1)		
Total (n)	rt-1	Total S.S.			

If calculated value of F (Treatments) > table value of F, we reject H_0 and hence we may conclude that there is significant difference between the treatment means. If the treatments are significantly different, the comparison of the treatments is carried out on the basis of Critical Difference (C.D.).

CD = SE(d) x $t_{(0.05)}$ – table value for **error d.f**. at 5% level of significance.

Where, SE(d) = Standard Error of difference between the Treatments.

$$SE\ (d) = \sqrt{\frac{2\,M.S.E.}{r}}$$

$$C.D. = t_{0.05\,,[(r-1)(t-1)]} \times \sqrt{\frac{2\,M.S.E.}{r}}$$

Coefficient Variation (C.V.) = $\frac{\sqrt{M.S.E.}}{\overline{y}} \times 100$ Where, $\overline{y}$ = Grand mean

14.3 Advantages of R.B.D.:

1. **Accuracy**: The principle advantage of R.B.D. is that it increases the precision of the experiment. This is due to the reduction of experimental error by adoption of local control.

2. **Efficient:** The amount of information obtained in R.B.D. is more as compared to CRD. Hence, R.B.D. is more efficient than C.R.D.
3. **Flexibility** is another advantage of R.B.D. Any number of replications can be included in R.B.D. If large numbers of homogeneous units are available, large number of treatments can be included in this design.
4. **Easy to Analyze**: Since the layout of R.B.D. involves equal replication of treatments, statistical analysis is simple. Even when some observations are missing of certain treatments, the data can be analyzed by the use of missing plot technique.

14.4 Disadvantages of R.B.D.:

1. When the number of treatments is increased, the block size will increase. If the block size is large it may be difficult to maintain homogeneity within blocks. Consequently, the experimental error will be increased. Hence, R.B.D. may not be suitable for large number of treatments. But for this disadvantage, the R.B.D. is a **versatile design**. It is the most frequently used design in agricultural experiments.
2. The optimum blocks size in field experiments is 21 plots i.e. we cannot compare treatments which are > 21 in R.B.D. to preserve homogeneity of plots, within a block.

Solve the following problems

Q1. An experiment was conducted in RBD to study the effect of seed treatment in controlling white tip nematodes in paddy seeds. Data recorded for Nematodes per 100 paddy seeds after treatment, are given in the table.

Treatment	**Replication**		
	I	**II**	III
T1	220	242	231
T2	253	264	242
T3	253	253	253
T4	275	275	275
T5	242	242	231
T6	275	275	286
T7	275	275	275

Test whether seed treatment has significant effect in disinfestations of white tip nematodes in paddy seeds? Find most effective treatment to disinfestations of nematodes.

Q.2. The data relate to the yields of 6 wheat varieties in an experiment in 4 randomized block.

		Yield of wheat varieties					
		V1	**V2**	**V3**	**V4**	**V5**	**V6**
Block	**1**	27.8	30.6	27.7	16.2	16.2	24.9
	2	27.3	28.8	22.7	15.0	17.0	22.5
	3	28.5	31.0	34.9	14.1	17.7	22.7
	4	38.5	39.5	36.8	19.6	15.4	26.3

Test whether varietal effect are equal?

Q.3. An experiment was designed to study the performance of four different detergents. Reading recorded over three different models of washing machines.

		Machine		
		I	**II**	**III**
Detergent	**A**	45	43	51
	B	47	46	52
	C	48	50	55
	D	42	37	49
	E	39	45	35

Test the detergents as treatment and machine as blocks. Use 5% level of significance.

Exercise No. 15 **Date: / /**

Latin Square Design (L.S.D.)

In R.B.D. whole of the experimental area is divided in to relatively homogeneous groups (blocks) and treatment are allocated at random to units within each block i.e. randomization was subjected to one restriction i.e. within blocks. But in field experimental, it may be that experimental area (field) show fertility gradient in strips, it is not in any single direction, it may two directions. Therefore, experimental material is divided into rows and columns and the treatments are allocated such that **each treatment occurs only once in a row** and **once in a column, the design is known as Latin Square Design**. In this design eliminating fertility variations consists in an experimental layout which will control variation in two **perpendicular** directions.

Latin square designs are normally used in experiments where it is required to remove the heterogeneity of experimental material in **two directions**. This design requires that the number of replications (rows) equal the number of treatments. In LSD the number of rows and number of columns are equal. Hence the arrangement will form a **square**.

15.1 Layout of L.S.D.:

In this design the number of rows is equal to the number of columns and it is equal to the number of treatments. Thus if there are 'm' treatments, there have to be m×m = m^2 experimental units (plots) arranged in a square so that each row as well as each column contain 'm' plots. The 'm' treatments are then allocated at random to these rows and columns in such a way that every treatment occurs once and only once in each row and each column such a layout is known as **m×m Latin Square Design** and is extensively used in agricultural experiments. The minimum and maximum number of treatments required for layout of LSD is **5 to 12**.

In LSD the treatments are usually denoted by alphabets like A,B,C…etc. For a Latin Square with five treatments the arrangement may be as follows:

A	B	C	D	E
B	C	D	E	A
C	D	E	A	B
D	E	A	B	C
E	A	B	C	D

A	B	C	D	E
B	A	D	E	C
C	E	A	B	D
D	C	E	A	B
E	D	B	C	A

A	B	C	D	E
B	A	E	C	D
C	D	A	E	B
D	E	B	A	C
E	C	D	B	A

These squares are said to be **Standard Squares.** A standard square is one in which the first row and first column are order alphabetically.

Two standard squares are said to be **Conjugate Squares** when rows of one square are the column of the other square.

15.1.1 Randomization procedure for 5 x 5 LSD

1. The layout of LSD starts with dividing the experimental material in to row and columns depending on the number of treatments.
2. Suppose a *t x t* standard square LSD selected. The standard square may be selected from Fisher and Yates statistical tables.
3. For 5 x 5 latin square, In **Fisher and Yates tables** given as 25 pairs of standard square plus 6 separate standard squares, there are 56 possible standard square.
4. The first 25 squares are assigned pairs of numbers like (1,2), (3,4) and so on. First number indicates the Standard Square and second number indicate the conjugate of that square.
5. As a **first step** a random number between 1 and 56 using random number table. If the selected ranom number is 50 or below then see whether it is odd or even. If it is odd, the standard square with that number taken as such. If number is even, the conjugate of the standard square with that number is written. If the number is between 51 and 56 the corresponding standard square is selected.
6. Let it be the selected random number is 48, the standard square corresponding to this number is

A	B	C	D	E
B	E	A	C	D
C	D	B	E	A
D	C	E	A	B
E	A	D	B	C

7. Conjugates of this square is

A	B	C	D	E
B	E	D	C	A
C	A	B	E	D
D	C	E	A	B
E	D	A	B	C

8. In the **second step** the column of the square are randomized. For this purpose a set of five single digit random numbers from 1 to 5 are selected. The random number may be 5, 1, 3, 2, 4. It represents column number and their random order, i.e. the original column 5 will be column 1, column 1 will be column 2 and so on. The randomization will result in square below,

E	A	C	B	D
A	B	D	E	C
D	C	B	A	E
B	D	E	C	A
C	E	A	D	B

9. In the next step the rows of the square are randomized keeping first row as such. For this purpose a set of four single digit random numbers from 2 to 5 are selected. The random number may be 4, 2, 5, 3. This indicates that row 4 should be placed after row 1, row 2 next and so on. Thus we have square shown below,

E	A	C	B	D
B	D	E	C	A
A	B	D	E	C
C	E	A	D	B
D	C	B	A	E

10. This final layout of 5 x5 LSD.

15.2.1 Mathematical model:

$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \varepsilon_{ij}$ $(i = j = k = 1,2,\ldots.m)$

Where, y_{ijk} is the response from the unit (plot) in i^{th} row, j^{th} column and receiving the k^{th} treatment

μ= general mean effect

α_i= effect due to i^{th} row

β_j = effect due to j^{th} column

γ_k = effect due to k^{th} treatment

ε_{ij}= error term

Null hypothesis, H_0: There is no significant difference between the row, column and treatment effects.

$H_0 : \alpha_1 = \alpha_2 = \ldots\ldots\ldots\ldots = \alpha_t = 0$

$H_0 : \beta_1 = \beta_2 = \ldots\ldots\ldots\ldots = \beta_r = 0$

$H_0 : \gamma_1 = \gamma_2 = \ldots\ldots\ldots\ldots = \gamma_r = 0$

The null hypothesis can be verified by applying the ANOVA procedure. The steps involved in this procedure are as follows:

1. Correction factor, $= C.F. = \frac{(GT)^2}{N}$, since $N = m^2$

2. Row Sum of Square (RowS.S.) $= \frac{1}{m}\left[R_1^2 + R_2^2 + \ldots\ldots\ldots R_i^2\right] - C.F.$

$$= \frac{\sum R_i^2}{m} - C.F.$$

3. Column Sum of Square (Column S.S.) $= \frac{1}{m}\left[C_1^2 + C_2^2 + \ldots\ldots\ldots C_j^2\right] - C.F.$

$$= \frac{\sum C_j^2}{m} - C.F.$$

4. Treatment Sum of Square (T.S.S.) = $\frac{1}{m}\left[T_1^{\,2} + T_2^{\,2} + \ldots\ldots T_k^{\,2}\right] - C.F.$

$$= \frac{\sum T_k^{\,2}}{m} - C.F.$$

5. Total Sum of Square (Total S.S.) = $\left[y_{11}^2 + y_{12}^2 + y_{13}^2 + \ldots + y_{ijk}^2\right] - C.F.$

$$= \sum y_{ijk}^{\,2} - C.F.$$

6. Error Sum of Square (E.S.S.) = Total S.S. – Row S.S. – Column S.S. – T.S.S.

ANOVA Table:

Source of Variation (S.V.)	Degrees of Freedom (d.f)	Sum of Square (S.S.)	Mean Square (M.S.)	F-Calculated value	F-tabulated value
Row	m-1	Row S.S.	R.M.S. = Row S.S./m-1	F_r = R.M.S./M.S.E.	$F_{[(m-1),\,(m-1)(m-2)}$ at 0.05 level of significance
Column	m-1	Column S.S.	C.M.S. = Column S.S./m-1	F_c = C.M.S./M.S.E.	-do-
Treatment	m-1	T.S.S	T.M.S. = T.S.S./t-1	F_t = T.M.S./M.S.E.	-do-
Error	(m-1)(m-2)	E.S.S	M.S.E. = E.S.S./(m-1)(m-2)		
Total (n)	m^2-1	Total S.S.			

If calculate value of F_t < table value of F at 5% LOS, H0 is accepted and hence we may conclude that there is **no significance** difference between treatment effects.

If calculate value of F_t > table value of F at 5%LOS, H0 is rejected and hence we may conclude that there is **significance** difference between treatments effects.

If the treatments are significantly different, the comparison of the treatments is carried out on the basis of Critical Difference (C.D.).

CD = SE(d) x $t_{(0.05)}$ – table value for **error d.f**. at 5% level of significance.

Where, SE(d) = Standard Error of difference between the Treatments.

$$SE\ (d) = \sqrt{\frac{2\,M.S.E.}{m}}$$

$$C.D. = t_{0.05,[(m-1)(m-2)]} \times \sqrt{\frac{2\,M.S.E.}{m}}$$

Coefficient Variation (C.V.) = $\frac{\sqrt{M.S.E.}}{\bar{y}} \times 100$ Where, $\bar{y}$ = Grand mean

If F is significant, the significance of any treatment contrast can be tested by using the C.D. value.

15.3 Advantages of L.S.D.:

1) With two way grouping or stratification LSD controls more of the variation than C.R.D. or R.B.D
2) L.S.D. is an incomplete 3-way layout. It's advantage over complete 3-way layout is that instead of m^3 experimental units only m^2 units are needed. Therefore, 4x4 L.S.D. results in saving of 64-16 = 48 observations over a complete 3-way layout.
3) The statistical analysis is simple though slightly complicated than for R.B.D. Even with missing data the analysis remains relatively simple.
4) More than one factor can be investigated simultaneously.
5) The missing observations can be analyzed by using missing plot technique.

15.4 Disadvantages of L.S.D.:

1) The fundamental assumption that there is no interaction between different factors may not be true in general
2) L.S.D. is suitable for number of treatments between 5 and 10. For more than 10 to 12 treatment the design is not often used since in that case the square becomes too large and does not remains homogeneous.
3) In case of missing plots, when several units are missing the statistical analysis become quite complex.
4) In the field layout it is not so easy to manage than R.B.D.

Solve the following problems:

Q.1. An experiment on cotton was conducted to study the effect of foliar application of urea in combination with insecticidal sprays in the cotton yield. Five treatments were tried in a 6 x 6 Latin Square Design. The layout plan and yield is given below:

T_2 4.9	T_4 6.4	T_5 3.3	T_1 9.5	T_3 11.8
T_3 9.3	T_1 4.0	T_2 6.2	T_5 5.1	T_4 5.4
T_4 7.0	T_3 15.4	T_1 6.5	T_2 6.0	T_5 4.6
T_5 5.3	T_2 7.6	T_3 13.2	T_4 8.6	T_1 4.9
T_1 9.3	T_5 6.3	T_4 11.8	T_3 15.9	T_2 7.6

Analyzed the data and draw your conclusion.

Q.2. The yield of five varieties of wheat, tried in a Latin square design, along with the plan have been given below. Analyzed the data and interpret the result.

E 68	B 78	D 80	C 122	A 100
D 72	E 73	A 70	B 58	C 129
A 78	C 99	E 57	D 75	B 72
C 113	A 69	B 60	E 73	D 64
B 48	D 70	C 76	A 82	E 73

TABLE I. THE NORMAL DISTRIBUTION

P	·00	·01	·02	·03	·04	·05	·06	·07	·08	·09
·0	∞	2·575829	2·326348	2·170090	2·053749	1·959964	1·880794	1·811911	1·750686	1·695398
·1	1·644854	1·598193	1·554774	1·514102	1·475791	1·439531	1·405072	1·372204	1·340755	1·310579
·2	1·281552	1·253565	1·226528	1·200359	1·174987	1·150349	1·126391	1·103063	1·080319	1·058122
·3	1·036433	1·015222	·994458	·974114	·954165	·934589	·915365	·896473	·877896	·859617
·4	·841621	·823894	·806421	·789192	·772193	·755415	·738847	·722479	·706303	·690309
·5	·674490	·658838	·643345	·628006	·612813	·597760	·582842	·568051	·553385	·538836
·6	·524401	·510073	·495850	·481727	·467699	·453762	·439913	·426148	·412463	·398855
·7	·385320	·371856	·358459	·345126	·331853	·318639	·305481	·292375	·279319	·266311
·8	·253347	·240426	·227545	·214702	·201893	·189118	·176374	·163658	·150969	·138304
·9	·125661	·113039	·100434	·087845	·075270	·062707	·050154	·037608	·025069	·012533

P	·002	·001	·000,1	·000,01	·000,001	·000,000,1	·000,000,01	·000,000,001
x	3·090232	3·29053	3·89059	4·41717	4·89164	5·32672	5·73073	6·10941

The value of P for each entry is found by adding the column heading to the value in the left-hand margin. The corresponding value of x is the deviation such that the probability of an observation falling outside the range from $-x$ to $+x$ is P. For example, P = ·03 for x = 2·170090; so that 3 per cent. of normally distributed values will have positive or negative deviations exceeding the standard deviation in the ratio 2·170090 at least.

The table of probits (Table IX) provides a more extensive (P, x) table. The probit table refers to a single tail of the distribution, and the P's derived from that table must therefore be multiplied by 2 to bring them into line with the P's of Table I.

TABLE II. ORDINATES OF THE NORMAL DISTRIBUTION

x	·00	·01	·02	·03	·04	·05	·06	·07	·08	·09	1	2	3	4	5
0·0	·3989	·3989	·3989	·3988	·3986	·3984	·3982	·3980	·3977	·3973	0	0	−1	−1	−1
0·1	·3970	·3965	·3961	·3956	·3951	·3945	·3939	·3932	·3925	·3918	−1	−1	−2	−2	−3
0·2	·3910	·3902	·3894	·3885	·3876	·3867	·3857	·3847	·3836	·3825	−1	−2	−3	−4	−5
0·3	·3814	·3802	·3790	·3778	·3765	·3752	·3739	·3725	·3712	·3697	−1	−3	−4	−5	−6
0·4	·3683	·3668	·3653	·3637	·3621	·3605	·3589	·3572	·3555	·3538	−2	−3	−5	−6	−8
0·5	·3521	·3503	·3485	·3467	·3448	·3429	·3410	·3391	·3372	·3352	−2	−4	−6	−8	−9
0·6	·3332	·3312	·3292	·3271	·3251	·3230	·3209	·3187	·3166	·3144	−2	−4	−6	−8	−10
0·7	·3123	·3101	·3079	·3056	·3034	·3011	·2989	·2966	·2943	·2920	−2	−5	−7	−9	−11
0·8	·2897	·2874	·2850	·2827	·2803	·2780	·2756	·2732	·2709	·2685	−2	−5	−7	−9	−12
0·9	·2661	·2637	·2613	·2589	·2565	·2541	·2516	·2492	·2468	·2444	−2	−5	−7	−10	−12
1·0	·2420	·2396	·2371	·2347	·2323	·2299	·2275	·2251	·2227	·2203	−2	−5	−7	−10	−12
1·1	·2179	·2155	·2131	·2107	·2083	·2059	·2036	·2012	·1989	·1965	−2	−5	−7	−10	−12
1·2	·1942	·1919	·1895	·1872	·1849	·1826	·1804	·1781	·1758	·1736	−2	−5	−7	−9	−11
1·3	·1714	·1691	·1669	·1647	·1626	·1604	·1582	·1561	·1539	·1518	−2	−4	−7	−9	−11
1·4	·1497	·1476	·1456	·1435	·1415	·1394	·1374	·1354	·1334	·1315	−2	−4	−6	−8	−10
1·5	·1295	·1276	·1257	·1238	·1219	·1200	·1182	·1163	·1145	·1127	−2	−4	−6	−7	−9
1·6	·1109	·1092	·1074	·1057	·1040	·1023	·1006	·0989	·0973	·0957	−2	−3	−5	−7	−8
1·7	·0940	·0925	·0909	·0893	·0878	·0863	·0848	·0833	·0818	·0804	−2	−3	−5	−6	−8
1·8	·0790	·0775	·0761	·0748	·0734	·0721	·0707	·0694	·0681	·0669	−1	−3	−4	−5	−7
1·9	·0656	·0644	·0632	·0620	·0608	·0596	·0584	·0573	·0562	·0551	−1	−2	−4	−5	−6
2·0	·0540	·0529	·0519	·0508	·0498	·0488	·0478	·0468	·0459	·0449	−1	−2	−3	−4	−5
2·1	·0440	·0431	·0422	·0413	·0404	·0396	·0387	·0379	·0371	·0363	−1	−2	−3	−3	−4
2·2	·0355	·0347	·0339	·0332	·0325	·0317	·0310	·0303	·0297	·0290	−1	−1	−2	−3	−4
2·3	·0283	·0277	·0270	·0264	·0258	·0252	·0246	·0241	·0235	·0229	−1	−1	−2	−2	−3
2·4	·0224	·0219	·0213	·0208	·0203	·0198	·0194	·0189	·0184	·0180	0	−1	−1	−2	−2
2·5	·0175	·0171	·0167	·0163	·0158	·0154	·0151	·0147	·0143	·0139	0	−1	−1	−2	−2
2·6	·0136	·0132	·0129	·0126	·0122	·0119	·0116	·0113	·0110	·0107	0	−1	−1	−1	−2
2·7	·0104	·0101	·0099	·0096	·0093	·0091	·0088	·0086	·0084	·0081	0	−1	−1	−1	−1
2·8	·0079	·0077	·0075	·0073	·0071	·0069	·0067	·0065	·0063	·0061	0	0	−1	−1	−1
2·9	·0060	·0058	·0056	·0055	·0053	·0051	·0050	·0048	·0047	·0046	0	0	0	−1	−1

	·0	·1	·2	·3	·4	·5	·6	·7	·8	·9
3·0	·0044	·0033	·0024	·0017	·0012	·0009	·0006	·0004	·0003	·0002

TABLE III. DISTRIBUTION OF t

n	Probability. ·9	·8	·7	·6	·5	·4	·3	·2	·1	·05	·02	·01	·001
1	·158	·325	·510	·727	1·000	1·376	1·963	3·078	6·314	12·706	31·821	63·657	636·619
2	·142	·289	·445	·617	·816	1·061	1·386	1·886	2·920	4·303	6·965	9·925	31·598
3	·137	·277	·424	·584	·765	·978	1·250	1·638	2·353	3·182	4·541	5·841	12·924
4	·134	·271	·414	·569	·741	·941	1·190	1·533	2·132	2·776	3·747	4·604	8·610
5	·132	·267	·408	·559	·727	·920	1·156	1·476	2·015	2·571	3·365	4·032	6·869
6	·131	·265	·404	·553	·718	·906	1·134	1·440	1·943	2·447	3·143	3·707	5·959
7	·130	·263	·402	·549	·711	·896	1·119	1·415	1·895	2·365	2·998	3·499	5·408
8	·130	·262	·399	·546	·706	·889	1·108	1·397	1·860	2·306	2·896	3·355	5·041
9	·129	·261	·398	·543	·703	·883	1·100	1·383	1·833	2·262	2·821	3·250	4·781
10	·129	·260	·397	·542	·700	·879	1·093	1·372	1·812	2·228	2·764	3·169	4·587
11	·129	·260	·396	·540	·697	·876	1·088	1·363	1·796	2·201	2·718	3·106	4·437
12	·128	·259	·395	·539	·695	·873	1·083	1·356	1·782	2·179	2·681	3·055	4·318
13	·128	·259	·394	·538	·694	·870	1·079	1·350	1·771	2·160	2·650	3·012	4·221
14	·128	·258	·393	·537	·692	·868	1·076	1·345	1·761	2·145	2·624	2·977	4·140
15	·128	·258	·393	·536	·691	·866	1·074	1·341	1·753	2·131	2·602	2·947	4·073
16	·128	·258	·392	·535	·690	·865	1·071	1·337	1·746	2·120	2·583	2·921	4·015
17	·128	·257	·392	·534	·689	·863	1·069	1·333	1·740	2·110	2·567	2·898	3·965
18	·127	·257	·392	·534	·688	·862	1·067	1·330	1·734	2·101	2·552	2·878	3·922
19	·127	·257	·391	·533	·688	·861	1·066	1·328	1·729	2·093	2·539	2·861	3·883
20	·127	·257	·391	·533	·687	·860	1·064	1·325	1·725	2·086	2·528	2·845	3·850
21	·127	·257	·391	·532	·686	·859	1·063	1·323	1·721	2·080	2·518	2·831	3·819
22	·127	·256	·390	·532	·686	·858	1·061	1·321	1·717	2·074	2·508	2·819	3·792
23	·127	·256	·390	·532	·685	·858	1·060	1·319	1·714	2·069	2·500	2·807	3·767
24	·127	·256	·390	·531	·685	·857	1·059	1·318	1·711	2·064	2·492	2·797	3·745
25	·127	·256	·390	·531	·684	·856	1·058	1·316	1·708	2·060	2·485	2·787	3·725
26	·127	·256	·390	·531	·684	·856	1·058	1·315	1·706	2·056	2·479	2·779	3·707
27	·127	·256	·389	·531	·684	·855	1·057	1·314	1·703	2·052	2·473	2·771	3·690
28	·127	·256	·389	·530	·683	·855	1·056	1·313	1·701	2·048	2·467	2·763	3·674
29	·127	·256	·389	·530	·683	·854	1·055	1·311	1·699	2·045	2·462	2·756	3·659
30	·127	·256	·389	·530	·683	·854	1·055	1·310	1·697	2·042	2·457	2·750	3·646
40	·126	·255	·388	·529	·681	·851	1·050	1·303	1·684	2·021	2·423	2·704	3·551
60	·126	·254	·387	·527	·679	·848	1·046	1·296	1·671	2·000	2·390	2·660	3·460
120	·126	·254	·386	·526	·677	·845	1·041	1·289	1·658	1·980	2·358	2·617	3·373
∞	·126	·253	·385	·524	·674	·842	1·036	1·282	1·645	1·960	2·326	2·576	3·291

TABLE IV. DISTRIBUTION OF χ^2

	Probability.												
n	·99	·98	·95	·90	·80	·70	·50	·30	·20	·10	·05	·02	·01	·001
1	$\cdot 0^3157$	$\cdot 0^3628$	·00393	·0158	·0642	·148	·455	1·074	1·642	2·706	3·841	5·412	6·635	10·827
2	·0201	·0404	·103	·211	·446	·713	1·386	2·408	3·219	4·605	5·991	7·824	9·210	13·815
3	·115	·185	·352	·584	1·005	1·424	2·366	3·665	4·642	6·251	7·815	9·837	11·345	16·266
4	·297	·429	·711	1·064	1·649	2·195	3·357	4·878	5·989	7·779	9·488	11·668	13·277	18·467
5	·554	·752	1·145	1·610	2·343	3·000	4·351	6·064	7·289	9·236	11·070	13·388	15·086	20·515
6	·872	1·134	1·635	2·204	3·070	3·828	5·348	7·231	8·558	10·645	12·592	15·033	16·812	22·457
7	1·239	1·564	2·167	2·833	3·822	4·671	6·346	8·383	9·803	12·017	14·067	16·622	18·475	24·322
8	1·646	2·032	2·733	3·490	4·594	5·527	7·344	9·524	11·030	13·362	15·507	18·168	20·090	26·125
9	2·088	2·532	3·325	4·168	5·380	6·393	8·343	10·656	12·242	14·684	16·919	19·679	21·666	27·877
10	2·558	3·059	3·940	4·865	6·179	7·267	9·342	11·781	13·442	15·987	18·307	21·161	23·209	29·588
11	3·053	3·609	4·575	5·578	6·989	8·148	10·341	12·899	14·631	17·275	19·675	22·618	24·725	31·264
12	3·571	4·178	5·226	6·304	7·807	9·034	11·340	14·011	15·812	18·549	21·026	24·054	26·217	32·909
13	4·107	4·765	5·892	7·042	8·634	9·926	12·340	15·119	16·985	19·812	22·362	25·472	27·688	34·528
14	4·660	5·368	6·571	7·790	9·467	10·821	13·339	16·222	18·151	21·064	23·685	26·873	29·141	36·123
15	5·229	5·985	7·261	8·547	10·307	11·721	14·339	17·322	19·311	22·307	24·996	28·259	30·578	37·697
16	5·812	6·614	7·962	9·312	11·152	12·624	15·338	18·418	20·465	23·542	26·296	29·633	32·000	39·252
17	6·408	7·255	8·672	10·085	12·002	13·531	16·338	19·511	21·615	24·769	27·587	30·995	33·409	40·790
18	7·015	7·906	9·390	10·865	12·857	14·440	17·338	20·601	22·760	25·989	28·869	32·346	34·805	42·312
19	7·633	8·567	10·117	11·651	13·716	15·352	18·338	21·689	23·900	27·204	30·144	33·687	36·191	43·820
20	8·260	9·237	10·851	12·443	14·578	16·266	19·337	22·775	25·038	28·412	31·410	35·020	37·566	45·315
21	8·897	9·915	11·591	13·240	15·445	17·182	20·337	23·858	26·171	29·615	32·671	36·343	38·932	46·797
22	9·542	10·600	12·338	14·041	16·314	18·101	21·337	24·939	27·301	30·813	33·924	37·659	40·289	48·268
23	10·196	11·293	13·091	14·848	17·187	19·021	22·337	26·018	28·429	32·007	35·172	38·968	41·638	49·728
24	10·856	11·992	13·848	15·659	18·062	19·943	23·337	27·096	29·553	33·196	36·415	40·270	42·980	51·179
25	11·524	12·697	14·611	16·473	18·940	20·867	24·337	28·172	30·675	34·382	37·652	41·566	44·314	52·620
26	12·198	13·409	15·379	17·292	19·820	21·792	25·336	29·246	31·795	35·563	38·885	42·856	45·642	54·052
27	12·879	14·125	16·151	18·114	20·703	22·719	26·336	30·319	32·912	36·741	40·113	44·140	46·963	55·476
28	13·565	14·847	16·928	18·939	21·588	23·647	27·336	31·391	34·027	37·916	41·337	45·419	48·278	56·893
29	14·256	15·574	17·708	19·768	22·475	24·577	28·336	32·461	35·139	39·087	42·557	46·693	49·588	58·302
30	14·953	16·306	18·493	20·599	23·364	25·508	29·336	33·530	36·250	40·256	43·773	47·962	50·892	59·703
32	16·362	17·783	20·072	22·271	25·148	27·373	31·336	35·665	38·466	42·585	46·194	50·487	53·486	62·487
34	17·789	19·275	21·664	23·952	26·938	29·242	33·336	37·795	40·676	44·903	48·602	52·995	56·061	65·247
36	19·233	20·783	23·269	25·643	28·735	31·115	35·336	39·922	42·879	47·212	50·999	55·489	58·619	67·985
38	20·691	22·304	24·884	27·343	30·537	32·992	37·335	42·045	45·076	49·513	53·384	57·969	61·162	70·703
40	22·164	23·838	26·509	29·051	32·345	34·872	39·335	44·165	47·269	51·805	55·759	60·436	63·691	73·402
42	23·650	25·383	28·144	30·765	34·157	36·755	41·335	46·282	49·456	54·090	58·124	62·892	66·206	76·084
44	25·148	26·939	29·787	32·487	35·974	38·641	43·335	48·396	51·639	56·369	60·481	65·337	68·710	78·750
46	26·657	28·504	31·439	34·215	37·795	40·529	45·335	50·507	53·818	58·641	62·830	67·771	71·201	81·400
48	28·177	30·080	33·098	35·949	39·621	42·420	47·335	52·616	55·993	60·907	65·171	70·197	73·683	84·037
50	29·707	31·664	34·764	37·689	41·449	44·313	49·335	54·723	58·164	63·167	67·505	72·613	76·154	86·661
52	31·246	33·256	36·437	39·433	43·281	46·209	51·335	56·827	60·332	65·422	69·832	75·021	78·616	89·272
54	32·793	34·856	38·116	41·183	45·117	48·106	53·335	58·930	62·496	67·673	72·153	77·422	81·069	91·872
56	34·350	36·464	39·801	42·937	46·955	50·005	55·335	61·031	64·658	69·919	74·468	79·815	83·513	94·461
58	35·913	38·078	41·492	44·696	48·797	51·906	57·335	63·129	66·816	72·160	76·778	82·201	85·950	97·039
60	37·485	39·699	43·188	46·459	50·641	53·809	59·335	65·227	68·972	74·397	79·082	84·580	88·379	99·607
62	39·063	41·327	44·889	48·226	52·487	55·714	61·335	67·322	71·125	76·630	81·381	86·953	90·802	102·166
64	40·649	42·960	46·595	49·996	54·336	57·620	63·335	69·416	73·276	78·860	83·675	89·320	93·217	104·716
66	42·240	44·599	48·305	51·770	56·188	59·527	65·335	71·508	75·424	81·085	85·965	91·681	95·626	107·258
68	43·838	46·244	50·020	53·548	58·042	61·436	67·335	73·600	77·571	83·308	88·250	94·037	98·028	109·791
70	45·442	47·893	51·739	55·329	59·898	63·346	69·334	75·689	79·715	85·527	90·531	96·388	100·425	112·317

For odd values of n between 30 and 70 the mean of the tabular values for $n-1$ and $n+1$ may be taken. For larger values of n, the expression $\sqrt{2\chi^2}-\sqrt{2n-1}$ may be used as a normal deviate with unit variance, remembering that the probability for χ^2 corresponds with that of a single tail of the normal curve. (For fuller formulæ see Introduction.)

TABLE V. VARIANCE RATIO—*continued*

5 Per Cent. Points of e^{2z}

n_2 \ n_1	1	2	3	4	5	6	8	12	24	∞
1	161·4	199·5	215·7	224·6	230·2	234·0	238·9	243·9	249·0	254·3
2	18·51	19·00	19·16	19·25	19·30	19·33	19·37	19·41	19·45	19·50
3	10·13	9·55	9·28	9·12	9·01	8·94	8·84	8·74	8·64	8·53
4	7·71	6·94	6·59	6·39	6·26	6·16	6·04	5·91	5·77	5·63
5	6·61	5·79	5·41	5·19	5·05	4·95	4·82	4·68	4·53	4·36
6	5·99	5·14	4·76	4·53	4·39	4·28	4·15	4·00	3·84	3·67
7	5·59	4·74	4·35	4·12	3·97	3·87	3·73	3·57	3·41	3·23
8	5·32	4·46	4·07	3·84	3·69	3·58	3·44	3·28	3·12	2·93
9	5·12	4·26	3·86	3·63	3·48	3·37	3·23	3·07	2·90	2·71
10	4·96	4·10	3·71	3·48	3·33	3·22	3·07	2·91	2·74	2·54
11	4·84	3·98	3·59	3·36	3·20	3·09	2·95	2·79	2·61	2·40
12	4·75	3·88	3·49	3·26	3·11	3·00	2·85	2·69	2·50	2·30
13	4·67	3·80	3·41	3·18	3·02	2·92	2·77	2·60	2·42	2·21
14	4·60	3·74	3·34	3·11	2·96	2·85	2·70	2·53	2·35	2·13
15	4·54	3·68	3·29	3·06	2·90	2·79	2·64	2·48	2·29	2·07
16	4·49	3·63	3·24	3·01	2·85	2·74	2·59	2·42	2·24	2·01
17	4·45	3·59	3·20	2·96	2·81	2·70	2·55	2·38	2·19	1·96
18	4·41	3·55	3·16	2·93	2·77	2·66	2·51	2·34	2·15	1·92
19	4·38	3·52	3·13	2·90	2·74	2·63	2·48	2·31	2·11	1·88
20	4·35	3·49	3·10	2·87	2·71	2·60	2·45	2·28	2·08	1·84
21	4·32	3·47	3·07	2·84	2·68	2·57	2·42	2·25	2·05	1·81
22	4·30	3·44	3·05	2·82	2·66	2·55	2·40	2·23	2·03	1·78
23	4·28	3·42	3·03	2·80	2·64	2·53	2·38	2·20	2·00	1·76
24	4·26	3·40	3·01	2·78	2·62	2·51	2·36	2·18	1·98	1·73
25	4·24	3·38	2·99	2·76	2·60	2·49	2·34	2·16	1·96	1·71
26	4·22	3·37	2·98	2·74	2·59	2·47	2·32	2·15	1·95	1·69
27	4·21	3·35	2·96	2·73	2·57	2·46	2·30	2·13	1·93	1·67
28	4·20	3·34	2·95	2·71	2·56	2·44	2·29	2·12	1·91	1·65
29	4·18	3·33	2·93	2·70	2·54	2·43	2·28	2·10	1·90	1·64
30	4·17	3·32	2·92	2·69	2·53	2·42	2·27	2·09	1·89	1·62
40	4·08	3·23	2·84	2·61	2·45	2·34	2·18	2·00	1·79	1·51
60	4·00	3·15	2·76	2·52	2·37	2·25	2·10	1·92	1·70	1·39
120	3·92	3·07	2·68	2·45	2·29	2·17	2·02	1·83	1·61	1·25
∞	3·84	2·99	2·60	2·37	2·21	2·10	1·94	1·75	1·52	1·00

Lower 5 per cent. points are found by interchange of n_1 and n_2, *i.e.* n_1 must always correspond with the greater mean square.

TABLE V. VARIANCE RATIO—*continued*

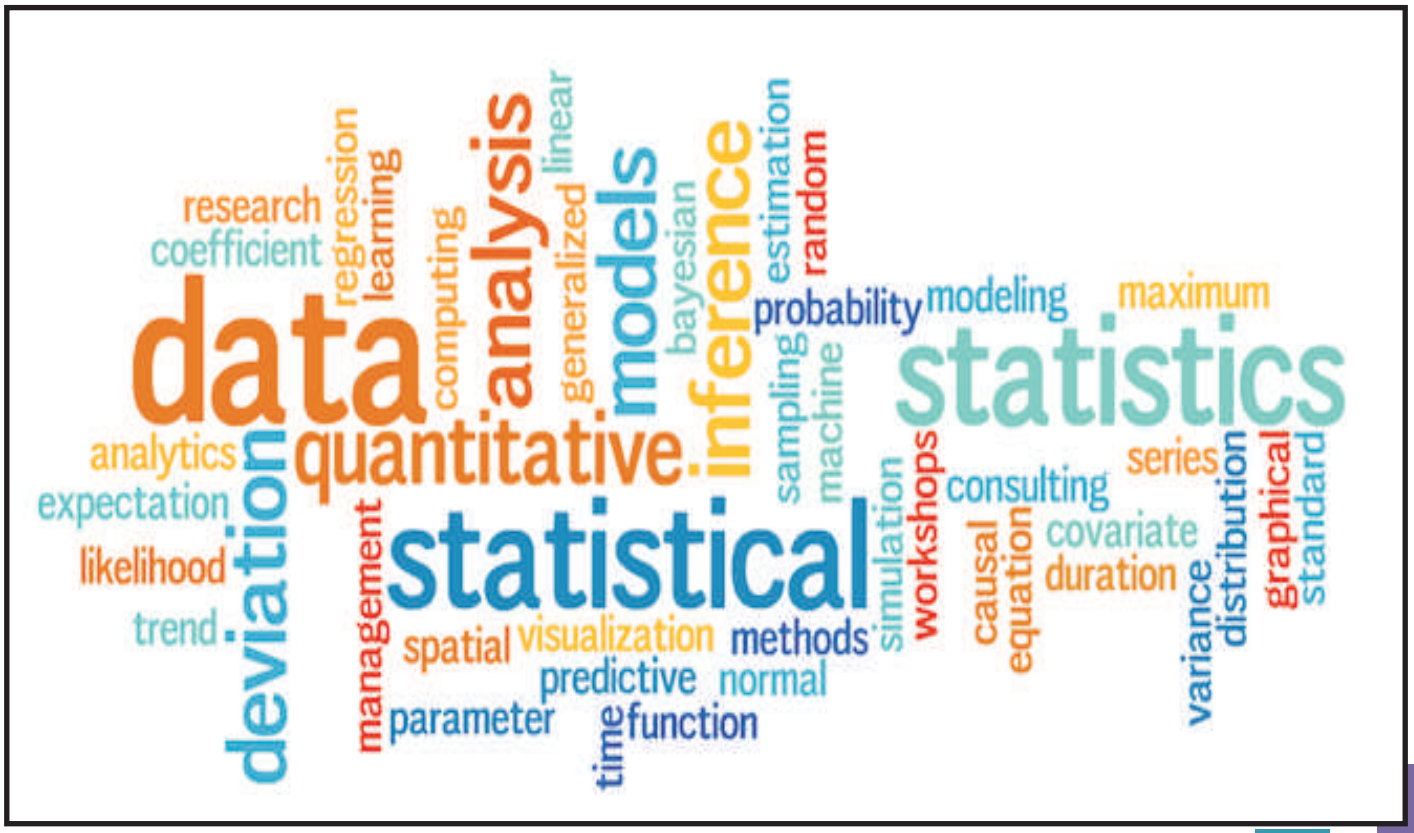
1 Per Cent. Points of e^{2z}

n_2 \ n_1	1	2	3	4	5	6	8	12	24	∞
1	4052	4999	5403	5625	5764	5859	5982	6106	6234	6366
2	98·50	99·00	99·17	99·25	99·30	99·33	99·37	99·42	99·46	99·50
3	34·12	30·82	29·46	28·71	28·24	27·91	27·49	27·05	26·60	26·12
4	21·20	18·00	16·69	15·98	15·52	15·21	14·80	14·37	13·93	13·46
5	16·26	13·27	12·06	11·39	10·97	10·67	10·29	9·89	9·47	9·02
6	13·74	10·92	9·78	9·15	8·75	8·47	8·10	7·72	7·31	6·88
7	12·25	9·55	8·45	7·85	7·46	7·19	6·84	6·47	6·07	5·65
8	11·26	8·65	7·59	7·01	6·63	6·37	6·03	5·67	5·28	4·86
9	10·56	8·02	6·99	6·42	6·06	5·80	5·47	5·11	4·73	4·31
10	10·04	7·56	6·55	5·99	5·64	5·39	5·06	4·71	4·33	3·91
11	9·65	7·20	6·22	5·67	5·32	5·07	4·74	4·40	4·02	3·60
12	9·33	6·93	5·95	5·41	5·06	4·82	4·50	4·16	3·78	3·36
13	9·07	6·70	5·74	5·20	4·86	4·62	4·30	3·96	3·59	3·16
14	8·86	6·51	5·56	5·03	4·69	4·46	4·14	3·80	3·43	3·00
15	8·68	6·36	5·42	4·89	4·56	4·32	4·00	3·67	3·29	2·87
16	8·53	6·23	5·29	4·77	4·44	4·20	3·89	3·55	3·18	2·75
17	8·40	6·11	5·18	4·67	4·34	4·10	3·79	3·45	3·08	2·65
18	8·28	6·01	5·09	4·58	4·25	4·01	3·71	3·37	3·00	2·57
19	8·18	5·93	5·01	4·50	4·17	3·94	3·63	3·30	2·92	2·49
20	8·10	5·85	4·94	4·43	4·10	3·87	3·56	3·23	2·86	2·42
21	8·02	5·78	4·87	4·37	4·04	3·81	3·51	3·17	2·80	2·36
22	7·94	5·72	4·82	4·31	3·99	3·76	3·45	3·12	2·75	2·31
23	7·88	5·66	4·76	4·26	3·94	3·71	3·41	3·07	2·70	2·26
24	7·82	5·61	4·72	4·22	3·90	3·67	3·36	3·03	2·66	2·21
25	7·77	5·57	4·68	4·18	3·86	3·63	3·32	2·99	2·62	2·17
26	7·72	5·53	4·64	4·14	3·82	3·59	3·29	2·96	2·58	2·13
27	7·68	5·49	4·60	4·11	3·78	3·56	3·26	2·93	2·55	2·10
28	7·64	5·45	4·57	4·07	3·75	3·53	3·23	2·90	2·52	2·06
29	7·60	5·42	4·54	4·04	3·73	3·50	3·20	2·87	2·49	2·03
30	7·56	5·39	4·51	4·02	3·70	3·47	3·17	2·84	2·47	2·01
40	7·31	5·18	4·31	3·83	3·51	3·29	2·99	2·66	2·29	1·80
60	7·08	4·98	4·13	3·65	3·34	3·12	2·82	2·50	2·12	1·60
120	6·85	4·79	3·95	3·48	3·17	2·96	2·66	2·34	1·95	1·38
∞	6·64	4·60	3·78	3·32	3·02	2·80	2·51	2·18	1·79	1·00

Lower 1 per cent. points are found by interchange of n_1 and n_2, *i.e.* n_1 must always correspond with the greater mean square.

TABLE XXXIII. RANDOM NUMBERS (I)

03	47	43	73	86	36	96	47	36	61	46	98	63	71	62	33	26	16	80	45	60	11	14	10	95
97	74	24	67	62	42	81	14	57	20	42	53	32	37	32	27	07	36	07	51	24	51	79	89	73
16	76	62	27	66	56	50	26	71	07	32	90	79	78	53	13	55	38	58	59	88	97	54	14	10
12	56	85	99	26	96	96	68	27	31	05	03	72	93	15	57	12	10	14	21	88	26	49	81	76
55	59	56	35	64	38	54	82	46	22	31	62	43	09	90	06	18	44	32	53	23	83	01	30	30
16	22	77	94	39	49	54	43	54	82	17	37	93	23	78	87	35	20	96	43	84	26	34	91	64
84	42	17	53	31	57	24	55	06	88	77	04	74	47	67	21	76	33	50	25	83	92	12	06	76
63	01	63	78	59	16	95	55	67	19	98	10	50	71	75	12	86	73	58	07	44	39	52	38	79
33	21	12	34	29	78	64	56	07	82	52	42	07	44	38	15	51	00	13	42	99	66	02	79	54
57	60	86	32	44	09	47	27	96	54	49	17	46	09	62	90	52	84	77	27	08	02	73	43	28
18	18	07	92	46	44	17	16	58	09	79	83	86	19	62	06	76	50	03	10	55	23	64	05	05
26	62	38	97	75	84	16	07	44	99	83	11	46	32	24	20	14	85	88	45	10	93	72	88	71
23	42	40	64	74	82	97	77	77	81	07	45	32	14	08	32	98	94	07	72	93	85	79	10	75
52	36	28	19	95	50	92	26	11	97	00	56	76	31	38	80	22	02	53	53	86	60	42	04	53
37	85	94	35	12	83	39	50	08	30	42	34	07	96	88	54	42	06	87	98	35	85	29	48	39
70	29	17	12	13	40	33	20	38	26	13	89	51	03	74	17	76	37	13	04	07	74	21	19	30
56	62	18	37	35	96	83	50	87	75	97	12	25	93	47	70	33	24	03	54	97	77	46	44	80
99	49	57	22	77	88	42	95	45	72	16	64	36	16	00	04	43	18	66	79	94	77	24	21	90
16	08	15	04	72	33	27	14	34	09	45	59	34	68	49	12	72	07	34	45	99	27	72	95	14
31	16	93	32	43	50	27	89	87	19	20	15	37	00	49	52	85	66	60	44	38	68	88	11	80
68	34	30	13	70	55	74	30	77	40	44	22	78	84	26	04	33	46	09	52	68	07	97	06	57
74	57	25	65	76	59	29	97	68	60	71	91	38	67	54	13	58	18	24	76	15	54	55	95	52
27	42	37	86	53	48	55	90	65	72	96	57	69	36	10	96	46	92	42	45	97	60	49	04	91
00	39	68	29	61	66	37	32	20	30	77	84	57	03	29	10	45	65	04	26	11	04	96	67	24
29	94	98	94	24	68	49	69	10	82	53	75	91	93	30	34	25	20	57	27	40	48	73	51	92
16	90	82	66	59	83	62	64	11	12	67	19	00	71	74	60	47	21	29	68	02	02	37	03	31
11	27	94	75	06	06	09	19	74	66	02	94	37	34	02	76	70	90	30	86	38	45	94	30	38
35	24	10	16	20	33	32	51	26	38	79	78	45	04	91	16	92	53	56	16	02	75	50	95	98
38	23	16	86	38	42	38	97	01	50	87	75	66	81	41	40	01	74	91	62	48	51	84	08	32
31	96	25	91	47	96	44	33	49	13	34	86	82	53	91	00	52	43	48	85	27	55	26	89	62
56	67	40	67	14	64	05	71	95	86	11	05	65	09	68	76	83	20	37	90	57	16	00	11	66
14	90	84	45	11	75	73	88	05	90	52	27	41	14	86	22	98	12	22	08	07	52	74	95	80
68	05	51	18	00	33	96	02	75	19	07	60	62	93	55	59	33	82	43	90	49	37	38	44	59
20	46	78	73	90	97	51	40	14	02	04	02	33	31	08	39	54	16	49	36	47	95	93	13	30
64	19	58	97	79	15	06	15	93	20	01	90	10	75	06	40	78	78	89	62	02	67	74	17	33
05	26	93	70	60	22	35	85	15	13	92	03	51	59	77	59	56	78	06	83	52	91	05	70	74
07	97	10	88	23	09	98	42	99	64	61	71	62	99	15	06	51	29	16	93	58	05	77	09	51
68	71	86	85	85	54	87	66	47	54	73	32	08	11	12	44	95	92	63	16	29	56	24	29	48
26	99	61	65	53	58	37	78	80	70	42	10	50	67	42	32	17	55	85	74	94	44	67	16	94
14	65	52	68	75	87	59	36	22	41	26	78	63	06	55	13	08	27	01	50	15	29	39	39	43
17	53	77	58	71	71	41	61	50	72	12	41	94	96	26	44	95	27	36	99	02	96	74	30	83
90	26	59	21	19	23	52	23	33	12	96	93	02	18	39	07	02	18	36	07	25	99	32	70	23
41	23	52	55	99	31	04	49	69	96	10	47	48	45	88	13	41	43	89	20	97	17	14	49	17
60	20	50	81	69	31	99	73	68	68	35	81	33	03	76	24	30	12	48	60	18	99	10	72	34
91	25	38	05	90	94	58	28	41	36	45	37	59	03	09	90	35	57	29	12	82	62	54	65	60
34	50	57	74	37	98	80	33	00	91	09	77	93	19	82	74	94	80	04	04	45	07	31	66	49
85	22	04	39	43	73	81	53	94	79	33	62	46	86	28	08	31	54	46	31	53	94	13	38	47
09	79	13	77	48	73	82	97	22	21	05	03	27	24	83	72	89	44	05	60	35	80	39	94	88
88	75	80	18	14	22	95	75	42	49	39	32	82	22	49	02	48	07	70	37	16	04	61	67	87
90	96	23	70	00	39	00	03	06	90	55	85	78	38	36	94	37	30	69	32	90	89	00	76	33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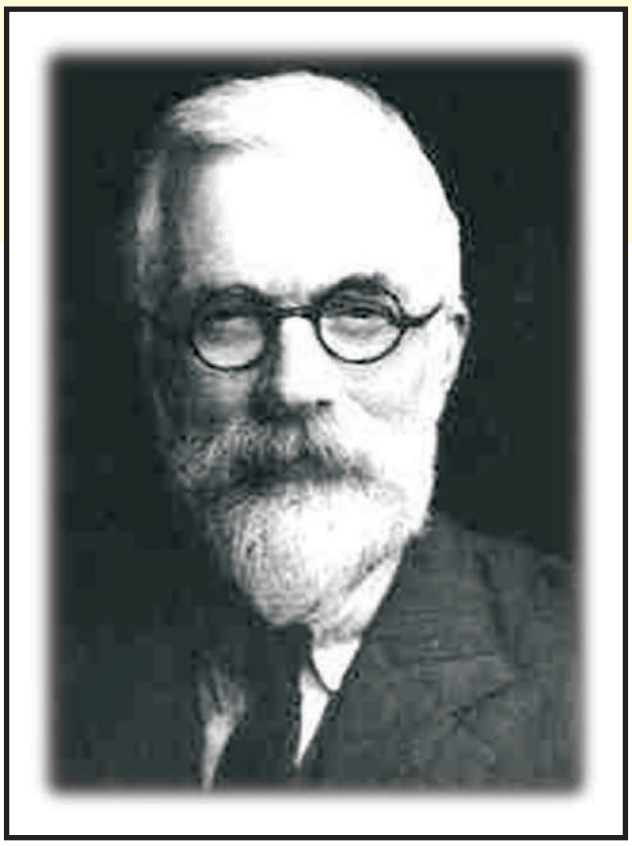

Sir Ronald Aylmer Fisher
Father of Statistics

P. C. Mahalanobis
Indian Statistician

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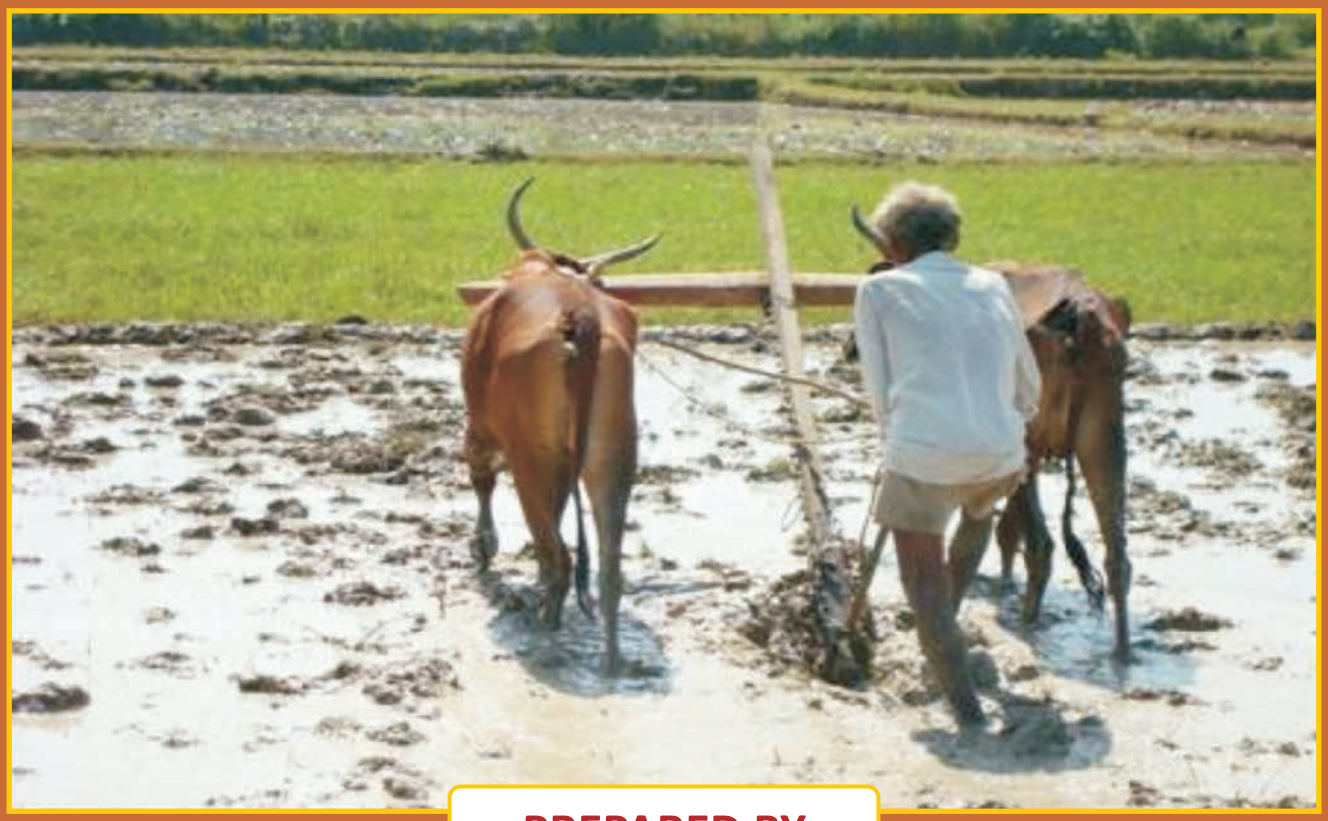
PRACTICAL MANUAL
AGRON 2.2
FUNDAMENTALS OF AGRONOMY (3 + 1)



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CERTIFICATE

Reg. No. : _____

Uni. Seat No.: _____

This is to certify that Mr./Miss_____studying in Second semester B. Sc. (Hons.) Agriculture has satisfactorily carried out _____ practical exercises in the subject ofAgron. 2.2 (**Fundamentals of Agronomy**) (3 + 1) on the College Agronomy Farm, College of Agriculture, NAU., Waghaiduring the _____.

Professor and Head **Course Teacher**

External Examiner

EXERCISE - 1

Identification of Crops, Seeds, Fertilizers, Pesticides and Tillage implements

❖ Identification of Crops and Seeds

PRACTICAL SIGNIFICANCE

1. Familiarity with seeds of different crops helps student to identify the crops in the field.

OBJECTIVES

1. To acquaint with different crops and crop seeds.

INTRODUCTION

Crops and seeds are too often confuses a student, particularly different varieties of a crop, if he/she does not have proper acquaintance previously. Similarities in morphological characters like plant stature, size of the ear head, colour and size of the seed leads to lot of confusion especially for those not having farming back ground or lack of exposure to such crops and seeds. Therefore maintenance of the crop cafeteria (crop museum) is a mandatory for each college farm.

In crop cafeteria different crops are sown. In each instructional farm (college farm/ students farm) a piece of land is meant for raising crop cafeteria which includes different crops with recommended package of practices for student instructional purpose (identification).

Different types of field crops like cereals, pulses, oil seeds, commercial crops, fibers etc. are grown in limited area considering the season of growing at a time with the purpose of studying detail characteristics and demonstration is called crop cafeteria. This crop cafeteria serves as an important guide to the students and farmers to know the important characteristics of different crops and offering an opportunity to choose suitable crop or crops.

Objectives :

(1) To know the crop growth stages of different crop viz. germination stage, tillering stage, flowering stage, maturity stage etc.

(2) To know the life period of different crops.

(3) To know the periodical growth habit of the crops viz. height and width of crop plants.

(4) To know the growing season of the crops viz. *Rabi*, *Kharif* and summer season.

(5) To know the germination period, growth period, flowering and fruiting period of different crops.

(6) To know the different stages at which the crops are affected by insects, pests and diseases and nature of damage.

(7) To know the climatic effect on different crops.

(8) To know the time of sowing, method of the sowing, seed rate, fertilizer and irrigation requirement, maturity and time of harvesting.

(11) To identify two similar crops at initial stage of the crop growth e.g. sorghum and pearlmillet; wheat, oat and barley; Lucerne and fenugreek.

(12) To know insecticides, pesticides, herbicides and fungicides used in different crops.

The study of crop cafeteria is therefore most important for the students of agriculture to acquaint with the different field crops and their varieties.

MATERIAL REQUIRED

Crop museum (crop cafeteria), seed museum.

PROCEDURE

Visit the students' farm/college farm; observe the existing crops, their morphological characters for easy identification.

No.	Crop	Family	Botanical name	Variety
1.	**Rice**	*Gramineae (Poaceae)*	*Oryza sativa*	Masuri, Jaya, Gurjari, Gujarat Anand Rice 1 (GAR 1), GAR-2,GAR-3
2.	**Wheat**	*Gramineae (Poaceae)*	*Triticumaestivum*	GW-496, GW-451, GADW-3
3.	**Barley**	*Gramineae (Poaceae)*	*Hordeumvulgare*	Rekha, RD -2503, RD -2552, Priti, Sindhu, Narmada, DWR-28
4.	**Maize**	*Gramineae(Poaceae)*	*Zea mays*	Vijay, Vikram, Amber, Jawahar, Shakti, Ganga Safed-2 (Hy.), Ganga -5 (Hy.), Ganga -11, Gujarat maize-1,2,3,4

No.	Crop	Family	Botanical	Variety
5.	**Sorghum**	*Gramineae (Poaceae)*	*Sorghum bicolor*	CSH-1, CSH -5, CSH -6, CSH-13, GJ -8, GJ -9, GJ -35, GJ -36, GJ -37, GJ -38, GJ-39, GJ -40, GJ -41, GJ -42,
6.	**Pearlmillet**	*Gramineae (Poaceae)*	*Pennisetumglaucum*	GHB-30, GHB -32, GHB -27, MH -169, MH -179, GHB-181,GHB-183, GHB -215, GHB-229, GHB -235,GHB-316
7.	**Chickpea**	*Leguminosae*	*Cicerarietinum*	GG-1,GG-2,GG-3,GG-4, GG-5,
8.	**Pigeonpea**	*Leguminosae*	*Cajanuscajan*	T-15-15, BDN -2, GT -100, ICPH-8, GT-1, C-11, Pusa-33, Pusa-74, C-11, ANDT-3 (AVT-1), AGT 2
9.	**Greengram**	*Leguminosae*	*Phaseolusaureus*	Gujarat moong -1,2,3,4, K-851, Pusabaisakhi, CO -4, Meha, GAM-5
10.	**Blackgram**	*Leguminosae*	*Phaseolusmung*	Zandewal, G -75, T -9, TPU-4, Pusa-1
11.	**Cowpea**	*Leguminosae*	*Vignaunguiculata*	Pusafalguni, Gujarat Cowpea-1,2,4, V-16,Pusa-152, AVCP-1
12.	**Lentil**	*Leguminosae /Fabaceae*	*Lens culinaris*	IPL 406, DLP 62, DLP 15, Lens 4076, PL 81 -7, Sapana, JLS
13.	**Groundnut**	*Leguminosae*	*Arachishypogaea*	GAUG-10, GG-11, GG-12, GG-13, GG -20, J -11, GG -2, JL-24, GG-4, GG-5, GG-6
14.	**Mustard**	*Cruciferae*	*Brassica juncea*	GM 3, Ragini, NRCYS 05 -02, YSH 0401
15.	**Sesame**	*Pedaliaceae*	*Sesamum indium*	Mrug -1, Patan -64, GS.-1 (Gujarat Till -1). GS. -2 (Gujarat Till-2) Purva till-1, Tajpur-5

No.	Crop	Family	Botanical	Variety
16.	**Sunflower**	*Asteraceae*	*Helianthus annus*	Co.1, Modern, EC -68413, EC-68414, EC 68415, APSH-11, KSFH -1, TNAU SUF-7, Gujarat Sunflower-1,
17.	**Safflower**	*Asteraceae*	*Carthamustinctorius*	ParbhaniKusum, PhuleKusum, Nari -NH-1, Nari-H-15,
18.	**Soybean**	*Leguminosae or Fabaceae*	*Glycine max*	Gujarat Soybean -1,2,3, J-202, J-231,
19.	**Linseed**		*Linumusitatissimum*	Pusa-2,Pusa-3, Garima, Sweta, Kiran, Gaurav, Jivan, KL-31, LC- 8528
20.	**Castor**	*Euphorbiaceae*	*Ricinuscommunis*	GCH-3, GAUCH-1, GCH-2, GCH-4, GCH-5, GCH-6
21.	**Sugarcane**	*Poaceae*	*Saccharumofficinarum*	Co N 5071, Co N 5072, Co N 7071, Co N 7072, CO-C-671, CO-419, Gujarat Sugarcane-1,2
22.	**Cotton**	*Malvaceae*	*Gossypiumhirsutum*	Guj.Cot.-10 (SRT -1), Guj.Cot.100, 101,11,12,13,14,15,16,17,19,21,18,23,AnandDeshi Cotton-1 (ADC-1), GADC-2, Deshi Hy.Co t.-7,Deshi Hy.Cot.-9, Hy.Cot.-4, Hy.Cot.-6, H y.Cot.-8, Hy.Cot.-10
23.	**Tobacco**	*Solanaceae*	*Nicotianatabacum*	Anand-2, Anand-119, GT-4, GT-5, GT-6, GT-7,GT-9,Gujarat AnandBidiTobacco – 11, Anand-145,GT-8, Rustica-9, GC-1, GC-2

Identification of *Khaif* season crop plant and seeds

					
Plants	Seed	Plants	Seed	Plants	Seed
Rice / Paddy		**Maize**		**Sorghum**	
					
Plants	Seed	Plants	Seed	Plants	Seed
Bajra		**Fingermillet**		**Kodomillet**	
					
Plants	Seed	Plants	Seed	Plants	Seed
Pigeonpea		**Greengram/Mungbean**		**Urbean/Blackgram**	
					
Plants	Seed	Plants	Seed	Plants	Seed
Clusterbean		**Cowpea**		**Groundnut**	

					
Plants	Seed	Plants	Seed	Plants	Seed
Castor		**Sunflower**		**Sesamum**	
					
Plants	Seed	Plants	Seed	Plants	Seed
Fennel		**Cotton**		**Sunhemp**	
					
Plants	Seed	Plants	Seed		
Lucerne		**Oat**			

Identification of *Rabi* season crop plant and seeds

					
Plants	Seed	Plants	Seed	Plants	Seed
Wheat		**Chickpea**		**Indian bean**	

					
Plants	Seed	Plants	Seed	Plants	Seed
Lentil		**Peas**		**Mustard**	
					
Plants	Seed	Plants	Seed	Plants	Seed
Safflower		**Linseed**		**Cumin**	
					
Plants	Seed	Plants	Seed	Plants	Seed
Fenugreek		**Coriander**		**Dil seed**	
					
Plants	Seed	Plants	Seed	Plants	Seed
Ajvain		**Sugarcane**		**Isabgol**	

					
Plants	Seed	Plants	Seed	Plants	Seed
Calcutti Tobacco		**Chicory**		**Potato**	
					
Plants	Seed	Plants	Seed		
Lucerne		**Oat**			

❖ Identification of manures and fertilizers

A fertilizer is any material, organic or inorganic, natural or synthetic, that supplies necessary plant nutrients for plant growth and optimum yield. Organic fertilizers are natural materials of either plant or animal origin, including livestock manure, green manures, crop residues, household waste, compost etc. Plant absorbed a large number of elements from the soil and other sources during their growth and development. There are sixteen mineral elements are found to be essential for plant nutrients. The growing plant have three sources from which they can get necessary nutrients are listed below

Macro Nutrients	**Source**	**Macro Nutrients**	**Source**
Carbon	Air	Sulpher	Soil
Oxygen	Air	Iron	Soil
Hydrogen	Water	Managenese	Soil
Nitrogen	Soil	Boron	Soil
Phosphorus	Soil	Zinc	Soil
Potassium	Soil	Copper	Soil
Calcium	Soil	Molybdenum	Soil
Magnesium	Soil	Chlorine	Soil

From the above given list of plant nutrients it is seen that most of the nutrients are absorbed by plant from soil. The absorption of macro nutrients is relatively high from the soil. If these nutrients are not added in to the soil, the fertility status of soil goes down hence, these elements must be added to the soil through manures and fertilizers.

What is manures :

Any organic material of animal or plant origin, added to the soil in order to improve the physical condition of soil and supply one or more plant nutrients to the soil is known as manure, These can be two types of manures.

(1) Bulky Organic manure :These organic manures are bulky in nature because they contain small quantities of plant nutrients, Such manure are farm Yard manure (FYM), farm compost, night soil, sewage and sludge, green manure etc. Among these, FYM, farm compost and green manure are most important and widely used.

(2) Concentrated organic manure: Concentrated organic manure contain higher percentage of major plant nutrients like nitrogen. Phosphorus, and potash compared to bulky organic manures. These manures are made from raw materials of animal and plant origin. Such manures are oilcake, blood meal, fish manure, meat meal, horn and hoof meal.

❖ **FERTILIZER :**

Any chemically manufactured material added to the soil in order to supply one or more plant nutrients is known as **fertilizer**. The term in generally applied to inorganic material.

Classification of various fertilizers is given below

(1) Straight Fertilizer: When fertilizer contain only one primary plant nutrient, it is known as straight fertilizer.

They are also termed as incomplete or special fertilizer

A. Nitrogenous Fertilizer

B. Phosphates' fertilizer

C. Potassic fertilizer

(A) Nitrogenous Fertilizer : Nitrogenous fertilizer may be classified into four groups on the basis of chemical form in which nitrogen is combined with other elements within fertilizer.

(i) Nitrate fertilizer : Nitrogen is combined as NO_3 (nitrate) with other elements. E.g sodium nitrate or Chilean nitrate (16 % N), Calcium nitrate (15.50 % N)

(ii) Ammonium containing : In these fertilizers nitrogen is combined in ammonium (NH_4) form with other elements. E.g. Ammonium sulphate (20 % N), Ammonium phosphate (20 % N), Ammonium chloride (24 to 26 % N), Anhydrous Ammonia (82 % N), Ammonium solution (20 to 25 % N).

(iii) Nitrate and Ammonium containing : These fertilizers contain nitrogen in the form of nitrate as well as ammonium. E.g ammonium nitrate (34 % N), Calcium ammonium nitrate (26 % N), Ammonium sulphate nitrate (26 % N).

(iv) Amide containing : These fertilizer contain nitrogen in amide form e.g Urea (46 % N) and Calcium cynamide (21 % N).

(B) Phosphaticfertilizer : Phosphatic fertilizers can be classified into three groups depending on the form in which phosphoric acid is combined with calcium.

(i) Phosphatic fertilizers containing water soluble phosphoric acid or monocalcium phosphate Ca $(H_2PO_4)_2$. They contain water soluble phosphoric acid which can be absorbed quickly by the plant roots. These fertilizers are suitable on neutral to alkaline soils. E.g Single super phosphate (16 % P_2O_5), Double super phosphate (32 % P_2O_5), Triple super phosphate (48 % P_2O_5) and ammonium phosphate (20 % P_2O_5).

(ii) Phosphatic fertilizers containing citric acid soluble phosphate or dicalcium phosphate Ca_2H_2 $(PO_4)_2$ or $CaHPO_4$. These fertilizer are suitable for acidic soils because at low pH citrate soluble phosphoric acids gets converted in monocalcium phosphate and there are less chances of fixation as iron and aluminium phosphate e.g basic slag (14 to 18 % P_2O_5), dicalcium phosphate (34 to 39 % P_2O_5), raw and stream bone meal (part of P_2O_5 soluble in citric acid)

(iii) Phosphatic fertilizers containing phosphoric acid which is not soluble in water or citric acid or containing insoluble phosphoric acid or tricalcium phosphate Ca_3 $(PO_4)_2$. These fertilizer are well suitable for strongly acidic soils e.g rock phosphate (20 to 40 % P_2O_5), bone meal (20 to 25 % P_2O_5), steamed bone meal 22% P_2O_5).

(A) Potasicfertilizer Potassic fertilizers can be classified into two groups depending upon the from of K in fertilizer

(i) Fertilizers having K in chloride form : Muriate of potash (60 % K_2O)

(ii) Fertilizers having K in non chloride form :sulphate of potash (48 to 52 % K_2O)

(1) Complex Fertilizers :

The commercial fertilizers containing at least two or more of the primary essential

nutrients, when such fertilizers contain only two of the primary nutrients. They are designated as incomplete complex fertilizers. While those containing all the three primary nutrients are called as complete complex fertilizers.

	Name of fertilizer	Content (N-P-K)
Incomplete Fertilizer	Ammonium phosphate	20-20-0
	Diammonium phosphate	18-46-0
	Urea ammonium phosphate	28-28-0
complete Fertilizer	Sulphala	15-15-15
	Suphala	18-18-9
	NPK	12-32-16

(1) Mixed fertilizers

A mechanical mixture of two or more straight fertilizer materials is referred as mixed fertilizer or fertilizer mixture. Sometimes complex fertilizers containing two plant nutrients are also used in formulation fertilizer mixture. In different state of India different fertilizer mixtures have been introduced and approved by Department of Agriculture of respective state. For Gujarat state the following mixture fertilizer grades of NPK have been approved.

5-10-5	10-10-0	15-05-05	10-05-00
10-10-10	15-10-10	10-5-5	10-15-15
5-15-15	20-10-10	10-15-15	5-15-15

Average percentage of N, P and K in the different manure and farm waste materials.

Sr.No	Manures	Percentage		
		N	P_2O_5	K_2O
Farm waste material				
1	Cow and Buffalo Dung	0.40	0.20	0.10
	Cow and Buffalo Urine	1.00	Trace	1.35
2	Sheep and Goat Dung	0.75	0.50	0.45
	Sheep and Goat Urine	1.35	0.05	2.10

Sr.No	Manures	Percentage		
		N	P_2O_5	K_2O
3	Horse Dung	0.55	0.30	0.40
	Horse urine	1.35	Trace	1.25
4	Pig Dung	0.55	0.50	0.40
	Pig Urine	0.40	0.10	0.45
Straw of crops				
1	Paddy	0.36	0.08	0.71
2	Wheat	0.53	0.10	1.10
3	Sorghum	0.40	0.23	2.17
4	Maize	0.42	1.57	1.65
Bulky organic manure				
1	Farm Yard Manure	0.50	0.20	0.50
2	Farm compost	0.50	0.15	0.50
3	Town compost	1.40	1.00	1.40
4	Night Soil	5.50	4.00	2.00
5	Sludge	1.5-3.5	0.78-4.0	0.3-0.6
6	Green manures	0.3-1.1	-	-
7	Poultry manure	3.03	2.63	1.40
8	Vermicompost	3.00	1.00	1.50

Concentrated Organic manure				
1	Castor cake	4.30	1.80	1.30
2	Cotton seed cake (undecorticated)	3.90	1.80	1.60
3	Cotton seed cake (decorticated)	6.40	2.90	2.20
4	Karanj cake	3.90	0.90	1.20
5	Mahuva cake	2.50	0.80	1.20

6	Neem cake	5.20	1.00	1.40
7	Safflower cake (undecorticated)	4.90	1.40	1.20
8	Safflower cake (decorticated)	7.90	2.20	1.90
9	Ground nut cake	7.30	1.50	1.30
10	Coconut cake	3.00	1.90	1.80
11	Linseed cake	4.90	1.40	1.30
12	Niger cake	4.70	1.80	1.30
13	Rape seed cake	5.20	1.80	1.20
14	Sesamum cake	6.20	2.00	1.20
15	Blood meal	10-12	1-2	1.00
16	Meat meal	10.50	2.50	0.50
17	Fish manure	4-10	3-9	0.3-1.5

Q.1. Give the student an exercise regarding identification of different crops and note down the observations in a record book.

Sr.No.	Crop	Identification mark

Q.2. Nitrogen is applied in split doses where as P and K is applied before sowing. Why ?

Q.3. Write down difference between bulky and concentrated organic manure.

EXERCISE - 2

LAY OUT AND TYPES OF SEED BED PREPARATION

Practical Significance :

1. Knowledge of perfect area of the field is important for
   - ➢ Calculating the input requirement.
   - ➢ Utilizing the field to its fullest extent.
   - ➢ Making yield prediction.
2. The layout is also paramount to control error component as well as to help the students to layout individual experiments at post-graduation level.

Objective :

1. To impart practical knowledge about measurement of the area of a field.
2. To impart the practical knowledge about the layout preparation of the plots.

Introduction :

Land is a very scarce and precious resource. Judicious use of inputs helps to produce economic yield and sustainability of soil. For this purpose one should know about the right quantity of inputs to be supplied and right quantity can be calculated on the basis of area of the field.

Proper layout not only helps in utilization of the field to its fullest extent by minimizing the wastage area, but also improves efficiency of cultural operations and inputs.

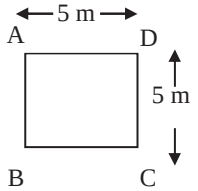
Tools Required :

1. Meter scale
2. Measuring tape, plastic rope
3. Iron / wooden / bamboo pegs
4. Spades etc.

Procedure :

1. Measure the all four boundaries of the field with measure tape.
2. Note the measurement in your field book.
3. Calculate the area with following calculations as per the shape of the plot.

Calculation :

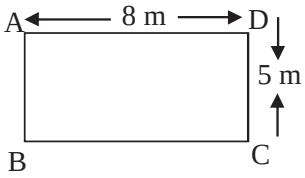


1. For Square plot :

   Area = length x breadth

Here, length (l)= breadth (b)

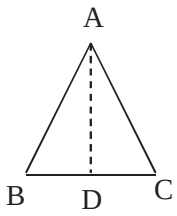
Area = (Length of any side)^2



2. For Rectangular Plot :

Area = length (l) x breadth (b)

Area = l x b



3. For Triangle Plot :

Area = ½ (Base x Perpendicular)

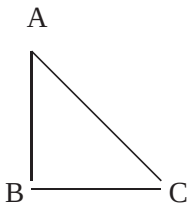
= ½ (BC x AD)

4. For Right angle Plot

As per the pythogarous model….

Hypotenuse^2 = side^2 + side^2

ac^2 = ab^2 + bc^2

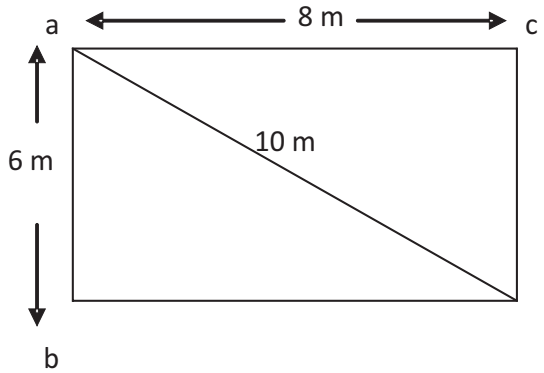


Precautions to be made while calculating area :

- As far as possible, try to divide the field in square or rectangular plots.
- Make long and narrow plots for sowing
- Always keep space for irrigation / drainage channels while preparing the field for planting/sowing.

Field Layout :

After the perfect land preparation and leveling the gross plot should be laid out. Care should be taken while laying out the plot to overcome the unequal lengths and breadths which results in more error and precision of the results will be vitiated while computing the per hectare yields due to disproportionate plant stand than the optimum required per unit area. The schematic layout of a plot admeasuring two cents (48 Sq.m.) is shown below.



Make straight bunds of 6 m width wise (ab) and 8 m length wise (bc) with the help of the plastic rope, meter scale and spade.

As it is shown in the figure the opposite sides of the rectangular should be equal as the shape of the plot assumes to be a rectangular with the given dimensions.

i.e. ab = cd and bc = ad.

Under field conditions it is not possible due to variation in topography of the land. Therefore to ensure equal sides the correct length of the hypotenuse should be fixed.

As shown above for right angle plot use the pythogarous model....

$$\text{Hypotenuse}^2 = \text{side}^2 + \text{side}^2$$

$$ad^2 = ab^2 + bd^2$$

Therefore, $ad^2 = 6^2 + 8^2$

$$ad = \sqrt{36+ 64} = \sqrt{100} = 10.0$$

After fixing the length of hypotenuse the opposite side bunds should be constructed i.e. make 8 m straight bund from a to c. Join cd with straight bund. It will be exactly 6 m. Then only the gross plot size of 48 Sq.m. is correct.

Table 1. Field Layout Plan

Space for diagram of the field

Location	:	Name of the Student	:
Block	:	I.D.No.	:
Field No.	:	Batch	:
Plot No.	:	Date	:

Type of Soil

Season :

Gross plot size :

SEED BED PREPARATION

The steps to properly preparing a seedbed will make more sense when you understand the overall needs for seed germination. A key to plant growth is that there must be good seed to soil contact. Good seed to soil contact helps the seed to utilize the moisture in the soil and later the emerging plant can utilize the nutrients in the soil. Proper seed bed preparation will reduce the weeds, facilitate planting/sowing, and provide a suitable bed for seed germination.

Characteristics of a good seedbed

1. Uniformly firm soil to depth of 5 inches
2. Adequate soil moisture
3. A firm seedbed is essential.
4. A field should be smooth and not cloddy.
5. Free from weeds and stubble of previous crop.

Each of these characteristics helps the seed to have the best chance to germinate and flourish. A seedbed that is weed free allows the desired crop to grow without the competition for nutrients, space, and sunlight. Adequate soil moisture triggers the enzymatic changes needed to grow.

Different methods of seedbed Preparation are :

1. **Flat beds**

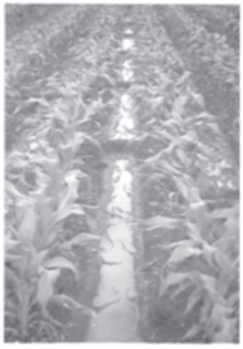
Flat beds are prepared easily in sandy loam type of soil because sandy soil is better workable. The length of the beds should be kept according to the slop of nursery. For easy working the beds should be prepared about 3.60 meter in length and 1.20 meter in width for easy and convenient operations.

2. Raised beds

Raised beds are mostly preferred in heavy black soil having maximum water holding capacity and poor drainage capacity. The basal measurement of raised beds should be 10.0 meter x 1.0 meter. Dig the soil near to the boundary of the beds and raised the soil on top.

3. Ridges and furrows

The field must be formed into ridges and furrows. Furrows of 30-45 cm width and 15-20 cm height are formed across the slope. The furrows guide runoff water safely when rainfall intensity is high and avoid water stagnation. They collect and store water when rainfall intensity is less. It is suitable for medium deep to deep black soils and deep red soils. It can be practiced in wide row spaced crops like cotton, maize, chillies, tomato etc. It is not suitable for broadcast sown crops and for crops

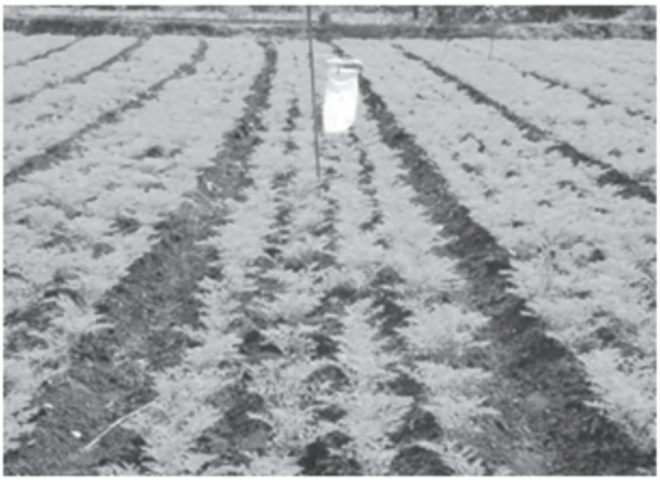


2. Tied ridging

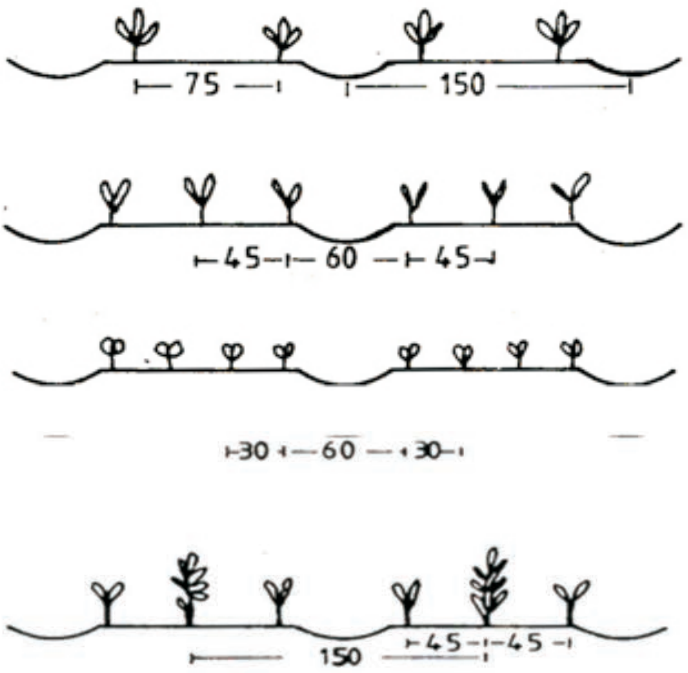
It is a modification of the above system of ridges and furrows wherein the ridges are connected or tied by a small bund at 2-3 m interval along the furrows to allow the rain water collection in the furrows which slowly percolated in to the soil profile.

4. Broad bed furrows (BBF)

This practice has been recommended by ICRISAT for vertisols or black soils in high rainfall areas (> 750 mm). Here beds of 90-120cm width, 15 cm height and convenient length are formed, separated by furrows of 60 cm width and 15 cm depth. When runoff occurs, its velocity will be reduced by beds and infiltration opportunity time is increased. The furrows have a gradient of 0.6%. Crops are sown on the broad beds and excess water is drained through number of small furrows which may be connected to farm ponds. It can be formed by bullock drawn or tractor



drawn implements. Bed former cum seed drill enables BBF formation and sowing simultaneously, thus reducing the delay between receipt rainfall and sowing.



Broad beds and furrows are adapted to many row spacings
A maize crop

A sorghum or millet crop

A groundnut or chickpea crop

A pigeonpea/sorghum intercrop or a pigeonpea/maize intercrop

Br

- It helps in moisture storage
- Safely dispose of surplus surface runoff without causing erosion
- Provide better drainage facilities
- Facilitate dry seeding
- It can accommodate a wide range of crop geometry i.e. close as well as wide row spacing.
- It is suitable for both sole cropping and intercropping systems.
- Sowing can be done with seed drills.

5. Dead furrows

At the time of sowing or immediately after sowing, deep furrows of 20 cm depth are formed at intervals of 6 to 8 rows of crops. No crop is raised in the furrow. The dead furrows can also be formed between two rows of the crop, before the start of heavy rains (Sep – Oct). It can be done with wooden plough mostly in red soils. The dead furrows increase the infiltration opportunity time.

6. Scooping

Scooping the soil surface to form small depressions or basins help in retaining rain water on the surface for longer periods. They also reduce erosion by trapping eroding sediment. Studies have shown that runoff under this practice can be reduced by 50 % and soil loss by 3 to 8 t /ha

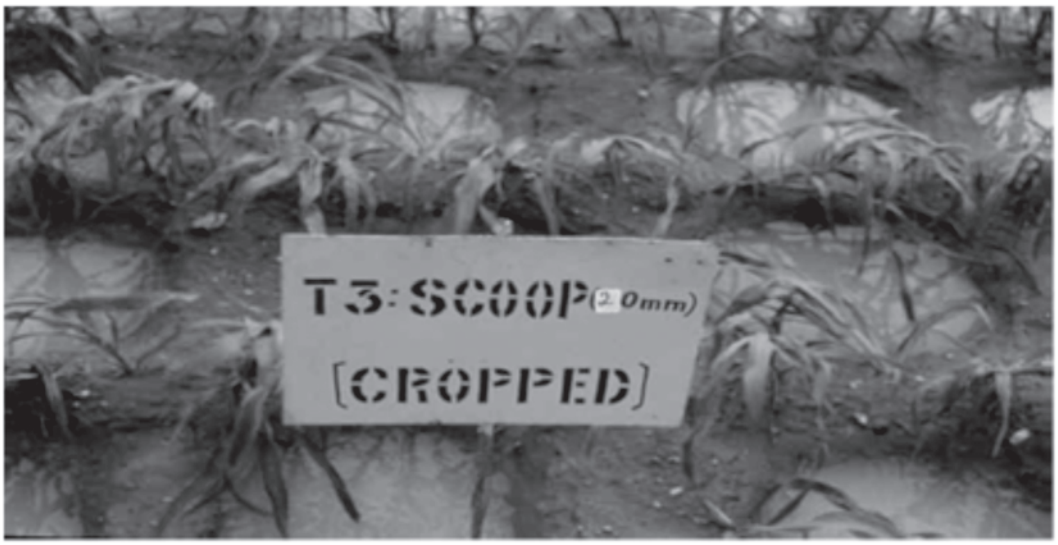
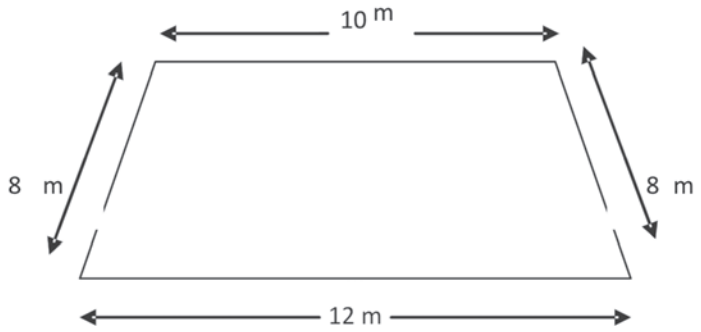


Fig. : Scoops for insitu moisture conservation

Study Questions

Q.1. Calculate the area of the field depicted below.



Ans.

EXERCISE - 3

PRACTICES OF DIFFERENT *METHODS OF SOWING*

Sowing is an operation for putting the seeds in the soil at particular distance and depth for raising the crop after proper preparation of a land.

Seeds are sown either directly on the field (seed bed) or in nursery where seedlings are raised and transplanted later.

Points to be consider at the time of sowing :

- Prepare firm and fine seed bed for sowing by various tillage operations.
- There should be proper degree of moisture in the soil for germination.
- Place the seed at proper depth, so it gets all necessary environments for germination.
- Uniform sowing of seeds should be done.
- Sowing should be done at uniform seed rate.

(1) Broadcasting : - This is an oldest method. This method is suitable for close planted crop which do not require a specific geographic area. Crop plants which do not require special type of cultural practices e.g. earthingup or Inter culturing etc. may be sown by broadcasting. This method is following in the crop having short life period, seeds are spread or scatter by hands over the field and covered with the help of wooden rake of light plank.

Advantage : -

(i) This method is cheap.

(ii) It is easy and quick.

Disadvantage : -

(i) Require more seed rate.

(ii) Uneven distribution of seed is possible.

(iii) Uneven depth of sowing.

(iv) Inter culturing is not possible.

(v) Weeding become difficult.

(vi) Selection of seeds are not possible.

(vii) Covering seeds with the help of rake is necessary.

e.g. cumin isabul, lucerne, coriander, Rajgira, berseem etc. and is mixed cropping situation.

(2) Drilling : - Drilling is a practice of dropping the seeds in furrows by a mechanical device at a specified distance between two row. Seeds are drilled in parallel line, distribution of seeds is regulated by realizing seeds in to the bowl by the hand. For covering the seeds light planking done by samar.

Advantage : -

(i) Uniform distance between two rows can be maintained.

(ii) Less seed is required as compared to broadcasting.

(iii) Inter culturing is possible between two rows.

(iv) Seeds are placed at uniform depth and covered and compacted uniformly.

Disadvantage :-

(i) Distance between two plant within the row is not maintained.

(ii) Thinning and gap filling operation are necessary.

(iii) Selection of seed is not possible.

e.g. upland rice, wheat, bajra, barley, mustared, green gram, cowpea etc and in intercropping situation.

(3) Dibbling : - Putting the seed or few seeds in a hole or pocket made at predetermine spacing and depth with a dibbler or very often by hand. This method is suitable for wide space crops requiring a specific geometric area for their canopy development or cultural practices. First all lines are marked vertically and horizontally with the help of marker at a particular distance.

At each cross seed are dibbled with the help of dibber manually. The seeds are covered with soil.

Advantage :-

(i) Spacing is maintained between two rows and between two plants within the rows.

(ii) Requirement of seed rate is less as compared to broadcasting and drilling.

(iii) Depth of sowing is maintained.

(iv) Selection of good seed is possible.

(v) Give rapid and uniform germination with good seedling vigour.

Disadvantage : -

(i) More laborious and time consuming method.

(ii) It is costly.

(iii) E.g. Cotton, castor, Indian bean, pigon pea etc.

(4) Planting :- Placing of plant part (vegetative propagules) in soil called planting. The vegetative propagules are planted directly on the field should be good in health, vigour, age, stage of growth and desirable number of readily sprouting buds.

Advantages :-

(i) Proper distance can be maintained between two rows and between two plants within the row.

(ii) Providing opportunity for selection of planting material.

(iii) Depth of sowing can be maintained.

Disadvantages :- (i) It requires more labour.

(ii) It is costly and time consuming method

e.g.

Tuber	-	Potato	**Rhizomes**	-	Turmeric
Bulb	-	Onion	**Cloves**	-	Garlic
Vine set	-	Sweet potato	**Sets**	-	Sugarcane
Root cutting	-	Pointed gourd	**Rooted Slips**	-	Napier grass, Blue panic grass

(5) Transplanting :- Transplanting is the removal of an actively growing plant from one place and planting it in another for further growth and production. It this method seed are not directly sown in the field but seeds are sown first in nursery with proper care. After proper growth (generally four weeks) seedlings are uprooted and transplanted in well prepared main field. This method is useful for raising the crops which have small size seeds and require more care in the initial stage.

Advantages :-

(i) Economy of costly seeds.

(ii) Maintaining of desire plant density with healthy and pure seedlings.

(iii) Available sufficient time for preparing seedbed.

(iv) Provide better chances for better care in small area during seedling stage.

Disadvantages :-

(i) Total duration of crop may be more.

(ii) It increase the labour and power requirement in a peak period.

(iii) It increase the cost of land preparation, uprooting and transplanting of seedling.

e.g. Seedling = Rice, Tabacco, Tomato, Brinjal, Chilly, Onion, Cabbage, Cauliflower etc.

Exercise

Q – 1 Broadcasting is not a scientific method of sowing .Why ?

Ans. _____

_____

_____

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Q – 2 Write the suitability of dibbling method of sowing.

Ans. _____

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Q – 3 What do you mean by planting and transplanting methods ?

Ans.

Q – 4 Enlist the methods of sowing and describe broad casting and dibbling methods.

Ans.

Q – 5 **Define sowing. Enlist the points to be considered at the time of sowing.**

Ans.

Q – 6 **Differentiate between planting and transplanting.**

Ans.

EXERCISE - 4
STUDY OF YIELD CONTRIBUTING CHARACTERS AND YIELD ESTIMATION OF MAJOR CROPS

Practical significance

Knowledge of yield contributing characteristics of different crops is essential to predict the yield of concerned crops.

Introduction

It is necessary to record the periodical observation of different growth and yield attributes from sowing to harvest of the crop which will gives us over all idea of yield of the crop. The growth and yield attributes for important different field crop are as under.

1. Rice

1. Initial plant stand
2. Number of tillers per plant
3. Effective tillers per plant
4. Periodically plant height (cm) at 15 days interval
5. Date of 50 % flowering
6. Length of panicle (cm)
7. No. of grains/panicle
8. No. of spike lets per panicle
9. Days to maturity
10. Grain and straw yields (kg/ha)
11. Grain to straw ratio
12. Test weight (g) 1000 grains

2. Wheat

1. Initial plant stand per m^2 area
2. No. of tillers per plant
3. Effective tillers per plant
4. Periodical plant height at 15-20 days

5. Periodical plant height at 15-20 days interval (cm)
6. Average plant height at the maturity stage
7. days of 50% flowering
8. Length of spikelet (cm)
9. No. of grains per spikelet
10. Taste weight of grain(1000 seeds)
11. Grain and straw yield(kg/ha)

3. Maize

1. Periodically plant height (cm)
2. Days taken for tasseling and silking
3. Days to maturity
4. Stem thickness (cm)
5. No. of cobs per plant
6. Cob girth and length (cm)
7. No. of grains/cob
8. No. of grain row/cob
9. Grain and stalk yield (kg/ha)
10. Test weight (g)
11. Shelling percentage (%)

4. Sorghum

1. Initial plant population
2. Periodical plant height (cm)
3. Total no. of functional leaves per plant
4. Leaf size (cm)
5. No. of ear head per plant
6. Days to 50 % flowering
7. Days to maturity

8. Grain and stalk yield (kg/ha)
9. Grain to stalk ratio (%)
10. Test weight (g)

5. Bajara

1. Initial plant population
2. Periodical plant height (cm)
3. Number of tillers/plant
4. Number of effective tillers/plant
5. Days to 50 % flowering
6. Length of ear head (cm)
7. Days to maturity
8. Grain and stalk yield (kg/ha)
9. Grain to stalk ratio (%)
10. Test weight (g)

6. Pulses

1. Initial plant population
2. Periodical plant height (cm)
3. No. of branches per plant
4. Days to 50 % flowering
5. Days to maturity
6. No. of pods per plant
7. No. of seeds per pod
8. Length of pods (cm)
9. Seeds and stover yield (kg/ha)
10. Test weight (g)
11. Protein percentage
12. No. of root nodules per plant

13. Fresh and dry weight of root nodules (g)

7. Groundnut

1. Plant population
2. No. of branches per plant
3. Periodical plant height (cm)
4. No. of pods per plant
5. No. of kernels per pod
6. Pod and haulm yield (kg/ha)
7. Test weight (g)
8. Shelling percentage
9. Oil percentage

8. Castor

1. Initial and final plant population
2. Plant height up to main spike (cm)
3. Days to 50 % flowering
4. Length of main spike (cm)
5. No. of capsules per spike
6. No. of spikes per plant
7. Seed yield (kg/ha)
8. Test weight (g)
9. Oil percentage

9. Sesamum

1. Final plant height (cm)
2. No. of branches per plant
3. No. of capsules per plant
4. Length of capsules
5. Test weight (g)

6. Seed yield (kg/ha)
7. Oil percentage

10. Fennel

1. Plant population
2. Periodical plant height (cm)
3. No. of branches per plant
4. Days to 50 % flowering
5. No. of umbel per plant
6. Days to maturity
7. Seed and stalk yield (kg/ha)
8. Test weight (g)
9. Protein percentage

11. Cotton

1. Plant population
2. Periodical plant height (cm)
3. No. of branches per plant
4. Days to 50 % squaring
5. Days to 50 % flowering
6. No. of bolls per plant
7. Weight of 5 bolls (g)
8. Seed cotton yield (kg/ha)
9. Stalk yield (kg/ha)
10. Lint to seed ration
11. Oil percentage

❖ **Yield estimation :-**

Crop estimation is mainly concerned with yield and production. “Yield” is defined as production of economic plant parts per unit land area. Since the area may change from year

to year, production can be standardized by dividing it by area. Yield unit varies with different countries, but basically it involve the weight or number of the economic plant per unit land area.

Objects of estimation

1. To estimate the average yield/ha and annual production of crops for individual district and the state as a whole.
2. To provide material for obtaining accurate and timely yield forecast of crops.
3. To determine crop-wise standard yield and normal yield at district level every year.
4. To estimate highly precise yield rates/ha of crops and production at state level and reasonable precise estimation of yield rates and production of crops at district level too.
5. To know the estimate/ expected yield of certain field crops well in advance / prior to crop harvest to actual harvest of crop for better and sound planning of the market as well as for "Anawary System" by revenue department or to get the idea about expected yields.
6. To study the previous year crop estimated position / condition.

Estimation of yield be partitioned into three main methods

1. Judgment of experts, at the smallest level possible, without sampling and thereafter aggregating and evaluating the data to a national level.
2. Judgments of experts as previously, but sampling representative areas of the national or regional levels.
3. Direct measurement of yields from samples collected from the field.

There are two sources of error related to item (1) and (2). They are **:**

(i) The error in the evaluation of yield which cannot be computed because it is an error of judgment.

(ii) The error related to the field sampling. This error is not only very important but also is unknown if no consistent sampling procedure is followed.

(iii) The third procedure has the advantage of being more reliable than the other two methods. Unfortunately, this method is very difficult to apply on some crop species, as measurable representative samples and difficult to collect as for example, the case for hay and perennial plants and is extremely time consuming one.

Methods of estimation :

Broadly speaking, in general there are two popular methods of crop yield estimation, these are:

- Eye estimation: (i.e. by own experienced only)
- Crop cutting experiments: (Through experimental research base)

1) Eye estimation

This is the most common and practical method among the farming community. Experienced person can estimate the yield same as the actual yield obtained after harvesting the crop (i.e. through his practical long experience). There are chances of getting error if the method employed by un experienced person. For getting precise results of crop yield, following points must be exercised**:** To know .....

i. The actual positions of the even plant stand.

ii. Uniform growth of plant; i.e. nature of development of crop plant through out the field.

iii. Even maturity of the crop for the purpose for which it is grown.

iv. General crop condition i.e. attack of diseases / pests / weed population / crop lodging etc.

2) Crop cutting experiments

Under this, there are two sub-methods have prescribed viz.,

i. Method of crop cutting experiment and location of the plot.

ii. Test harvest for estimating the yield of crops.

In these methods, the produce can be estimated by harvesting the plant samples and weighing the produce as the crop gets matured. These methods are entirely objective and (sample) will be influenced by the experimenter or by sampler. There should be sufficient number of samples taken from the representative areas of the field in random manner. These are the essential features of the crop cutting experiments. Such estimates lead to unbiased estimates of the normal yield which will be reliable of the total produce.

Method of crop cutting experiment and location of the plot

In this method, length (L) and breadth (B) of the selected field is measured first, and from that areas 10 m x 5 m plot size is selected. The selected plot should be fixed at any corner of the field. The side of the rectangle considered as 'X' and 'Y' for breadth and length, respectively. The next (IInd selected) plot be choosen in such a manner as it is shown in the diagram (Fig.1)

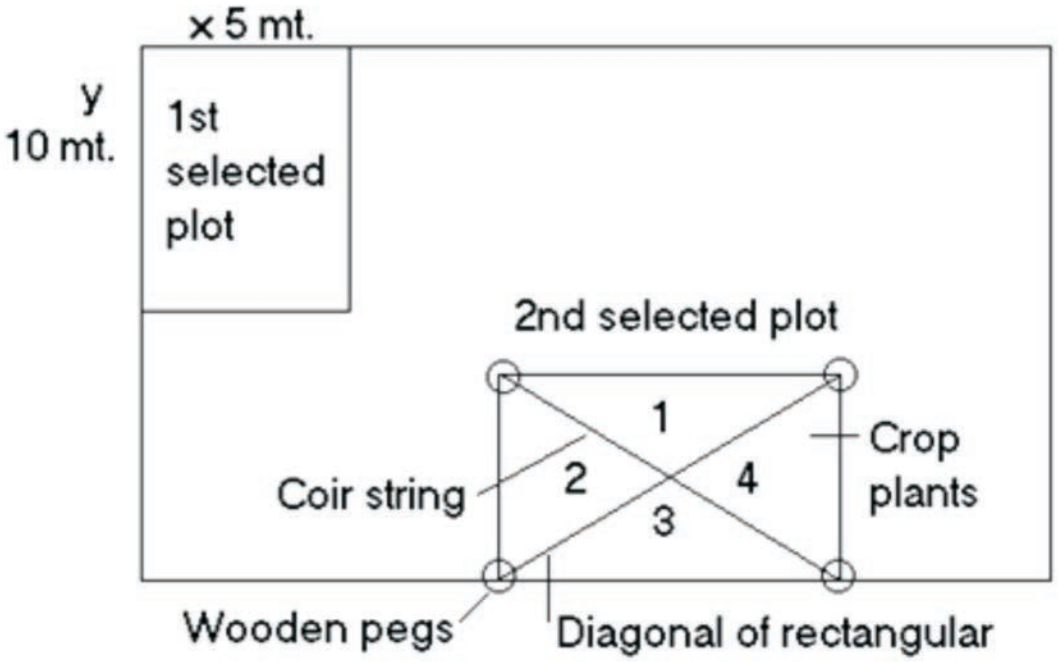


Fig - 1

The four corners of rectangle are marked with four wooden pegs. The accuracy of the rectangular, sub plots are checked by measuring the diagonal of the rectangle. The four sides are lined out with thin coir string. The plants falling inside and out side of the row are separated, having done this, harvesting of the crop should be carried out.

Carry out threshing, winnowing, cleaning and weight. Record the crop yield data in the proforma as shown below:

Proforma - 1 Statement showing the sample estimated yield

Crop: ..... Variety: .....

Sample No.	Weight of the harvested produce		Area of the sample taken in m^2
	Main produce (grain)	Sub-produce (Straw)	
1.			
2.			
3.			
4.			
5.			
Total			
Average			

ii. Test harvest for estimating the yield of crops

This is very quick method of cutting experiments. In this method circular ring of iron wire is used whose inner diameter should be exactly one meter, In most of the estimates instead of iron ring, a square quadrangular of iron rod is to be used having a length and breath 1 m (1 m x 1 m), as it is shown in Figure 2.

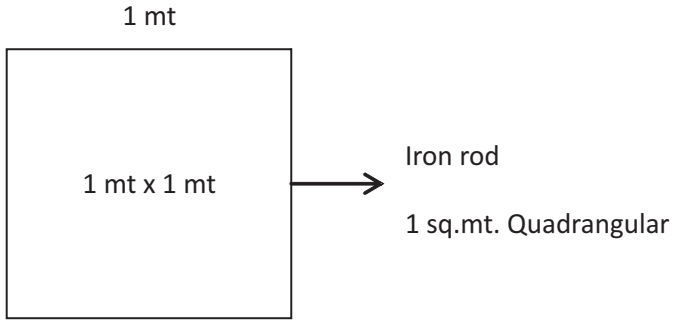


Fig. - 2

The accuracy of the test of harvest is mainly depends on size of the ring of square and uniformity of the crop. The selection of place in the plot should be done in the plot should be done in the random manner. The 1 x 1 m square iron rod is thrown in the field, which is to be estimated. The plant coming within the square should be harvested. Likewise, five to ten such samples are taken from the field. The samples are thereafter composited and plants are weighed and calculated on the hectare basis.

Random Sample Harvesting

This is common practice used for many field crops. This method of random sampling some what resemble to eye estimation. In this method first take round of the entire field. Take samples of the suitable size which varies from 1 to 5 sq. m depending upon the type of crops. Samples are selected at random without any bias. The number of samples selected varies from 5-10 depending upon the variation existing in the field. Record the yield of individual sample and make one composite sample. Calculate the yield in kg/ha.

The above stated method varies under different conditions such as crops, purpose of crop grown and method of sowing/planting etc. As such, keeping the basic principles in view; following sub-groups are made for easy estimation of crop yields of different classes/ group of crop as well as type of crops.

Following are the sub-groups for estimation

(A) Estimation of the crops which are drilled or planted in lines and are sown as a 'sole' crop whose life period ends at one harvest, can be entered in this group and their estimation would be carried out according to the procedure discussed above e.g. pearl millet, great millet, sugarcane, paddy, kodo millet etc.

(B) Estimation of the mixture crops like bajra mixture. In this case, crops are mixed together, but are sown in separate line e.g. bajra mixture (bajra +Tur + cluster bean). For such, the samples of each crop should be taken separately. Follow the procedure as stated above. The mixed crops in which lines for the different crops are not separated, but mixture of all the crops (bajra + till +cluster bean + green gram + red gram) are observed in a single row. Then samples of suitable size should be taken and from the samples the crop wise estimation should worked out.

(C) For the estimation of the transplanted crops of which whose harvesting is to be completed at one time can be placed in this group. 10-25 samples should be taken for estimation e.g. cabbage, cauliflower and isolated crops such as banana, papaya etc.

(D) Estimation of the planted crops in lines of which whose harvesting can be completed at a time e.g. potato, sweet potato, onion, garlic, carrot, radish elephant foot etc. In this, group take a suitable size sample at random from the entire field. The samples must be representative of the area, estimation must be carried out for the marketable produce only i. e. in potato tuber yield is important not the yield of leaves.

(E) Estimation of planted or transplanted crops of which, whose harvest is not completed in one time e.g. bottle gourd, egg plant, chilies, tomato etc. In this group selection of the sample should be taken up either in row or in square. Take at least 5 or more such samples at random. Harvest the marketable produce only. Considering the number of harvest and yield of each harvest for the crop, estimate the yield on hectare basis. Generally for this group, yield of first picking is equal to the yield of last picking, while middle picking or harvest would be equal in yield or equally double to the first and last yield. Finally, the estimation should be carried out for the whole season.

(F) Estimation of broadcasted crops of which whose harvesting can not be completed in one time (in single phase). e.g. Alfalfa, Egyptian clover etc. for such crops take enough representative samples of the size 2 m x 2 m or 1.5 m x 1.5 m (5 to 10 samples) weigh the each sample individually and calculate the yield until life span of the crop. For that following points should be taken into consideration.

- Total No. of cutting/ ha,
- Percentage of yield/ cutting
- Nco. of cutting during life span of the crop etc.

EXERCISE - 5

SEED GERMINATION AND VIABILITY TEST

❖ **Seed**

Seed is a fertilized ovule consisting of intact embryo, store food and seed coat which is viable and has got the capacity to germinate.

❖ **Agronomic definition** :

A seed or seed material is the living organ/s of the crop in rudimentary form which is used for propagation or any part of the crop from which a new crop will grow.

The number of plants/unit area is an important factor in crop production. If the initial plant stand is more, extra plants should be removed after sowing and before it resumes growth which is known as Thinning,whereas the initial plant stand is poor, extra plants or seeds should be planted or shown after sowing which is called Gap filling. To obtained high yield at the low cost, selection of seed for sowing is most important.

OBJECTIVES :

- To check weather seed is germited or not
- To estimate the planting value of seed lot
- To issue seed certificate, tag and label
- To enforce the seed act and its rules
- To determine maximum germination potential of seed

GENERAL PRINCIPLES :

- To know the planting value of the seed lot.
- Germination test is conducted with seeds from the pure seed fraction of a purity test.
- At least four hundred seeds are required to conduct germination test.
- No pre-treatment is to be given to the seed except those recommended for breaking the hard seed coat or dormancy.

❖ **There are two types of germination:**

- **Epigeal Germination:** In this type of germination, the hypocotyl elongates rapidly and arches upwards pulling the cotyledons which move above the soil.Example : bean and onion seeds.

- **Hypogeal Germination:** In this type of germination, the epicotyl elongates and the cotyledons remain below the soil.Example : pea and gram seeds, all grasses and many other species.

GENERAL REQUIREMENTS FOR SEED GERMINATION :

- **Substrata :** The substrata serve as the moisture reservoir and surface or medium on which the seeds can germinate and the seedling grow. The commonly used substrata are paper, sand and soil.
- **Adequate water or moisture :** Moisture is supplied to seeds through the substratum. Water should be free from organic or inorganic impurities. Its pH should be 6.0 to 7.5.
- **Favourable Temperature :** Germination of seeds occurs under different ranges of temperature provided the seed is given adequate moisture. For seeds of various crop species, temperature requirement for germination is different.
- **Light :** Some seeds requires light during germination test. Example : such as lettuce, tobacco
- **Seeds :** Seeds of a crop variety under germination test.

APPARATUS USED FOR GERMINATION TEST :

a. Working sample
b. Germination paper
c. Butter paper
d. Seed counting board
e. Germination chamber etc.

Methods for testing germination :

Following methods are used for germination test.

1) Petri-dish method :

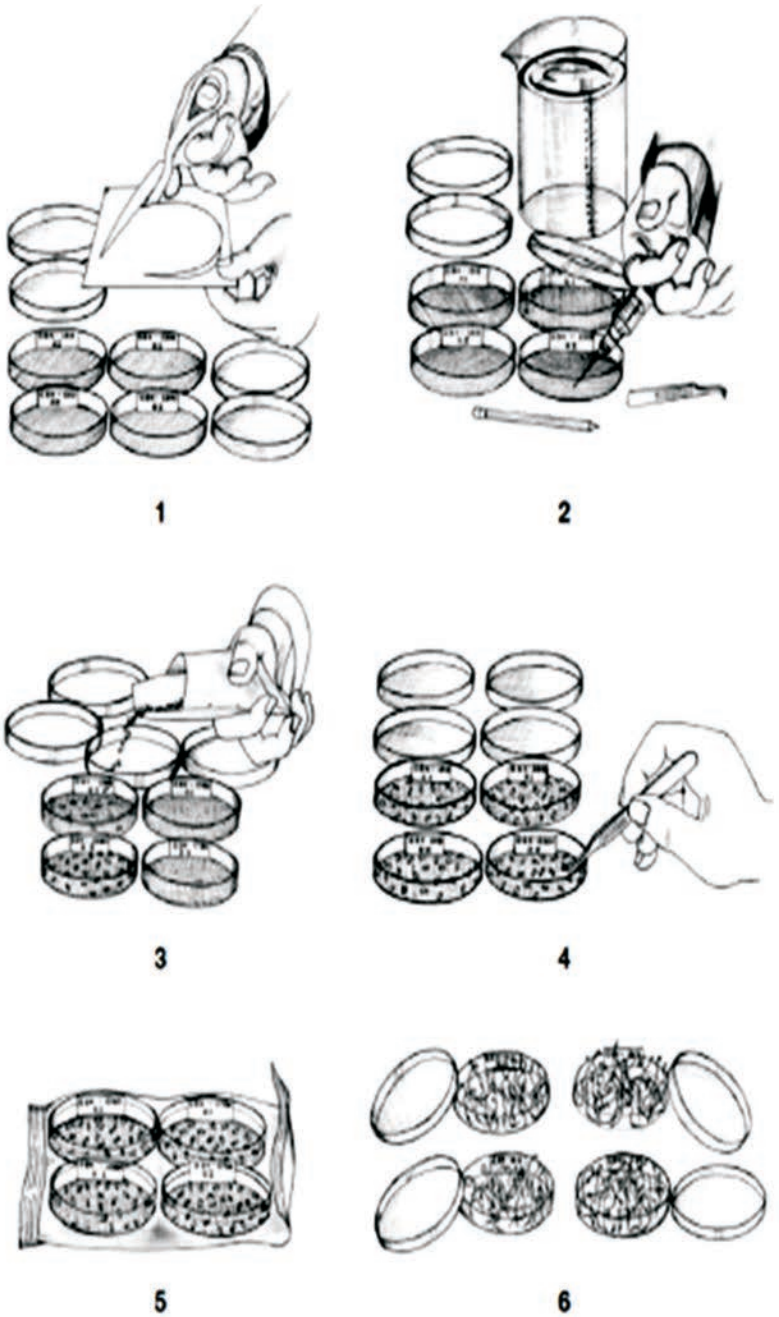
1) Two blotters or filter papers are placed on the bottom of Petridish and they are soaked with water.
2) Number of blotters can be increased according to the need of water by seed during the germination and size of the seed.
3) A convenient number of seeds ranging from 10-20, depending upon their size are placed on the surface of water soaked blotters in the petridish.
4) The kind of seed, date and time of seed soaking are written on the tea glass cover of petri dish with the help of a grass marking pencil.

5) The size of petridish and number of replicates depend upon the size of seed to be tested.

6) Usually the germination percentage is calculated and reported on the basis of the results of germination of about 100 to 200 seeds.

7) Generally two counts of the germinates seeds are made for calculated and valid report.

8) The petri dish methods is more suitable for small seeds like tobacco, tomato, cabbage, cauliflowers, mustard, lettuce, brijal under the blotters or filter papers to increase the water content in side the petri dish.



2) Rolled towel test

- In this method two wet towels are placed on a smooth table top.
- The appropriate number of seeds are placed on the upper surface of the towels and are covered by two wet towels.
- A fold is made on the bottom of the towel to prevent the seeds from falling out.
- The towel are then rolled from right to left.
- The full information regarding the test i.e the kind of seed, lot number, date and time of seed socking are noted on the roll with the help of an indelible pencil.
- The rolls of one type of seed are grouped together and fastened with a rubber band.
- The rolls are the put in a rack.
- These rolled towel are placed in a single layer to avoid the incidence of moulds and save emerging seeding from charring due to excessive heat evolved during the germination of the seeds.
- The towels should rolled loosely to allow normal expansion of the seedling during the test period.
- This method is suitable for comparatively large size seeds like maize, wheat, pea and gram.

3) Folded paper towel method

- Two wet paper towels (big bamboo sheets) specially made for germination test, are placed on the working table top.
- The surface of the paper towel is marked into two halves.
- The right half of towel surface is planted with seeds and the left side half folded cover the right half and information regarding the seed sample, date, time of seed soaking are write on the fold with an indelible pencil.
- Water is frequently sprinkled on these towels and observed on germination seedlings and their numbers are taken periodically.
- These paper towels like rolled towels, can be kept in racks, singly a required temperature for germination is maintained.
- This method of germination test is convenient for relatively large sized seeds.

1

2

3

4

5

6

4) Sand method

- In this method aluminium pans of 23 cm X 23 cm and 8 cm size taken as containers and river sand is used after washing, as germination medium.
- About 75 ml of water is poured in for 5 gm of sand and two are mixed well.
- The pans are filled with moistened sand to three forth of their depth.
- The seed are plant on the surface of sand and are then covered with a thin layer sand.
- The thickness of the sand cover ranges from 1.25 to 2.5 cm depending upon the type of seeds used for testing.
- The pans are then label for the name of crop seed, lot number, planting date and time.
- The pans are sprinkled with water as and when they need and germination count seedling vigour and time taken for germination are recorded.
- This method is suitable for all types of seeds to be tested for germination but it gives generally low germination percentage as compared to other methods described earlier.

- However, data obtained from this method are of much practical utility because the conditions here are quite comparable to that of filed.

5) Rag doll method :

- Rags or gunny sacs are used in this method. The seeds to tested are simply wrapped in a moistened rag or gunny sac, which is then rolled and tied loosely in the form of a bundle.
- This is kept at a proper temperature on a rack or convenient place for germination.
- The rolls are opened after fixed period of Time and the number of germinated seeds is counted.
- The germination percentage is calculated on the basis of a total of two counts.

6) Germination test through germinators :

- There are many types of apparatuses which are used for testing the germination of seeds.
- Cabinets of the incubator type with thermostatically controlled temperature may be used for the purpose.
- In these cabinets the seeds may be placed evenly on moist filter paper in petri dishes or between filter papers kept moist by folds on moistened panel or large seeds may be sown in dishes containing sand or fine soil.
- Adequate water is applied when needed by the help of a wash bottle or sprayer.
- Some time box type of germinators used for testing germination of small samples.
- The box type germinators have perforated plates made of tins and the seeds are placed over them on special filter papers which are moistened with water.
- Hot air if needed, is released from below the perforated tin plate through a thermostatically controlled device and required temperature in maintained during the germination periods of seeds through regulator and the temperature inside the box mat be found out by the help at thermometer fitted therein.
- Another type of germinator is Roadewald which consists of a try of wet sand on which unglazed porcelain dishes of blocks are bedded.
- The tray filts above a tray of water, the temperature of which is controlled thermostatically.
- The seeds are arranged on porous dishes or blocks.

- Which absorb water from the wet sand.
- The germinated seeds are counted periodically to find out the germination percentage.

POINT SHOULD BE REMEMBERED FOR GERMINATION TEST OF SEEDS

There are some of the important points which must be taken into account while proceeding for germination test of seeds.

- Only healthy seeds, which are separated out during purity test should be taken for the test.
- The media used for germination must be sterilized so that the seeds may not be affected by harmful pathogens during the process of germination.
- The ideal germination requirements viz substratum, moisture, temperature, light and air must be provided to the germinating seeds for getting a satisfactory test data.

REAL VALUE OF SEED

The real value of seed represents its seed quality, in terms of purity and germination. It can be evaluated by multiplying the purity % and germination % of a seed sample and dividing the product by 100.

$$\text{Real Value} = \frac{\text{Purity \% x Germination \%}}{100}$$

Example 1 : Find out the real value of sample, if the seed sample-A has 85 percent purity and 95 percent germination as compared to seed sample B which has 98 percent purity and 80 percent germination

Thus, the real value of sample

$$A = \frac{85 \times 95}{100} = 80.75\ \%$$

$$B = \frac{98 \times 80}{100} = 78.40\ \%$$

It is therefore, more economically and better to buy the seed from seed lot of The seeds having a real value of lower than 70 are usually not preferred for sowing purpose because of poor germination and purity value.

Viability :

A **viable seed** is one which is capable of **germination** under suitable conditions.

Methods for determining seed viability :

1. Tetrazolium test
2. Germination test
3. Biochemical test
4. Conductivity test
5. Excised embryo test
6. X-Ray test
7. Free fatty acid test

❖ **The Tetrazolium Chloride (TZ) test**

TZ test was developed by George lakon. (1940's) which is now used for estimation of germination potential in a short time. This test is very useful in processing, handling, storing, marketing, vigour rating of seed lot, supplementary germination test results and diagnosing caused of seed deterioration.

❖ **OBJECTIVES :**

- ➢ To obtain a quick indication of viability of seed samples.
- ➢ To determine rapidly viability of seeds of dormant seeds.

MATERIALS REQUIRED :

- 2,3,5,Tryphenyl Tetrazolium Chloride
- Distilled water
- Electronic balance
- Pre-conditioned seed
- Beakers, Petri dishes and other glassware
- Needles
- Forceps
- Magnifying lens
- Oven or incubator

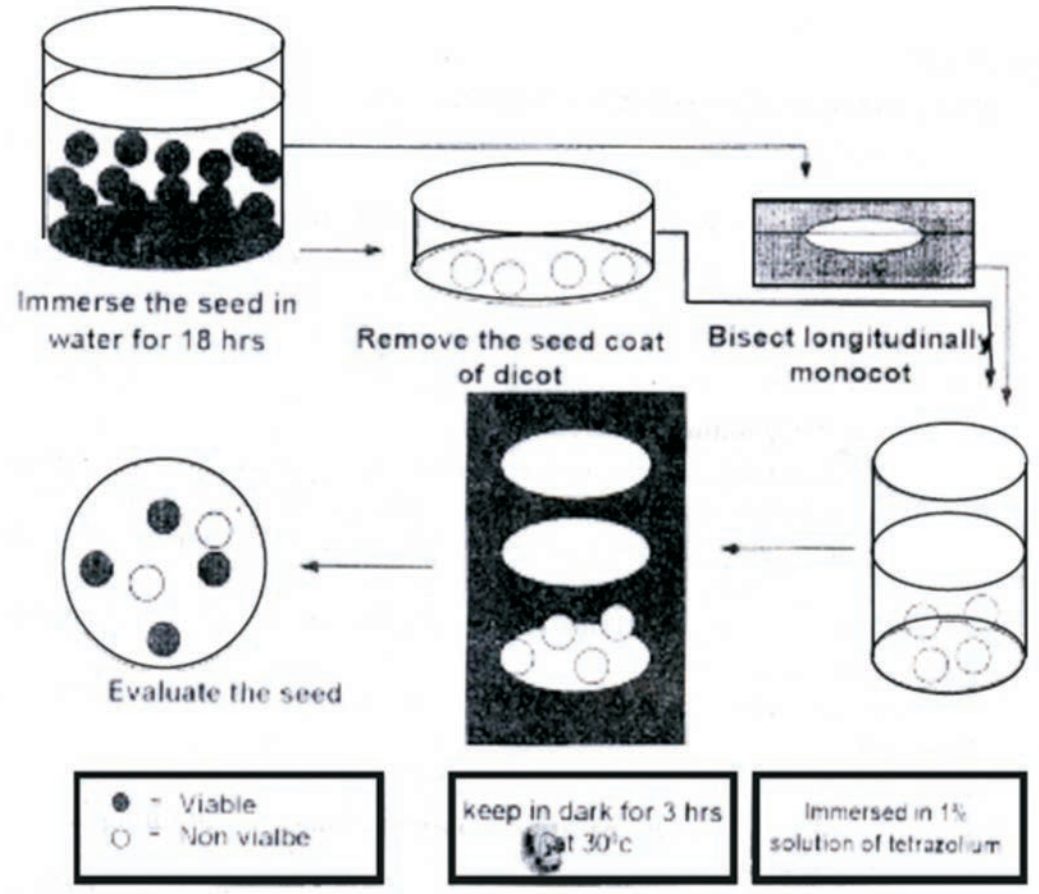


Fig. 5.1 : Tetrazolium test for seed viability

PROCEDURE :

(A) Conditioning and preparing the seed :

i. At least 200 randomly pure seeds should be tested in replicate of 100 seeds or less.

ii. Seeds should be soaked in water overnight at room temperature.

iii. The water soaked monocot seeds are then cut longitudinally (e.g. wheat, maize) or laterally (e.g. small seeded grasses) to express the embryo. Seed coats of dicot should be removed to facilitate the quick penetration of tetrazolium (e.g. gram).

(B) Staining in tetrazolium solution :

i. After preparing the desired number of seeds, they should be soaked in one per cent tetrazolium solution (pH 6 to 7) at above 30°C for 3-4 hours.

ii. Solution with high pH value develops darker stain, while with low pH value develops weaker stain.

iii. If the acidity of the tetrazolium solution is higher, the colour will not develop even with a viable embryo.

ADVANTAGE OF TZ TEST :

a. Quick and fairly accurate

b. Can also determine the viability of a dormant seed lot in short time.

c. Seeds are not damaged (in dicot) and can be germinated.

DISADVANTAGES OF TZ TEST :

a. Distinct between normal and abnormal seedlings is difficult.

b. Cannot differentiate between dormant and non-dormant seeds.

c. Correct evaluation is possible only after prolonged experience.

d. Microorganisms harmful for seedling emergence remain undetected.

e. No sophisticated equipment is required.

Exercise

Q.1. List out the methods for selection of good seeds.

Q.2. Define seed. Write down the characteristics of good seed.

Q.3. Enlist the different methods of seed germination test.

Q.4. What is real value of seed ? How it is useful in selection of good seed.

EXERCISE - 6

NUMERICAL EXERCISES ON PLANT POPULATION AND SEED RATE

PRACTICAL SIGNIFICANCE

1. It enables to calculate the correct quantity of seed required per unit area as per crop variety and
2. It helps the personnel engaged in promotion, extension and usage of seed.

OBJECTIVES

1. To estimate correct quantity of seed required for sowing per unit area
2. To estimate plant stand per unit area

INTRODUCTION

- Seed is a matured ovule.
- Seeds are the source for spreading a plant from place to place and means of propagation of particular variety of a crop.
- Improved varieties or hybrids have greater potential for yield and quality compared to traditional varieties.
- The cost of the improved varieties and hybrids is high.
- Correct quantity of seed with good germination percentage (usually 95-98 %) and optimum plant population should be used to getting the higher yields.
- The quantity of seed required per unit area (seed rate) differs from crop to crop and it depends upon the spacing, test weight (1000 seed weight for small seeds and 100 seed weight for large seeds), no. of seedlings per hill, germination percentage, variety, cropping system risk factors and soil fertility etc.

❖ Plant population

Number of plants in a particular area depends on the canopy coverage of the individual plants. If vigour of the plant is less, canopy coverage is less and requirement of plants per unit area will be more. Thus, spacing is maintained in such a way that its canopy mathematically covers the entire area to intercept maximum sunshine without interfering the neighbouring crop plants.

$$\text{Plant population} = \frac{10{,}000}{\text{Row to row distance (m)} \times \text{Plant to plant distance (m)}}$$

***Example* 1 :**

Calculation the plant population/ha, if the spacings of two crop species A and B are (i) 20 cm x 20 cm, (ii) 15 cm x 15 cm, respectively.

i) Plant population/m^2 of species A = 20 x 20 cm^2

= 400 cm^2

$$= m^2 \ \frac{400}{100 \times 100}$$

= 0.04 m^2

i.e. 0.04 m^2 is covered by = 1 plant

$$1\ m^2 \text{ is covered by } = \frac{}{0.04} \text{ Plants} = 25 \text{ plants}$$

Plant population/ha = 25 x 10,000

= 2,50,000

OR

$$\text{Plant population} = \frac{10{,}000}{\text{Row to row distance (m)} \times \text{Plant to plant distance (m)}}$$

(i) Plant population/ha of species B = $\frac{10{,}000}{0.15 \times 0.15}$

= 4,44,444.44

Calculate the seed rate per hectare with the information given. Then the quantity of seed required for any extent of cultivable area can be arrived at as mentioned below.

***Example* 1 :**

Solution :

Calculate the seed rate for rice crop to transplant two hectares with the following information.

(i) Spacing - 20 cm x 10 cm

(ii) Test weight - 30 grams (1000-seed weight)

(iii) No.of seedlings / hill- Two

(iv) Germination % - 90

(v) Risk factor - 10%

***Method* - I**

Step-I : Calculate the number of hills per sq.m. with help of the spacing i.e.

20 x 10 cm - one hill

10^8 cm^2 (1 Ha) - ?

$$= \frac{10000 \times 10000}{20 \times 10} = 5,00,000 \text{ hills}$$

Number of hills per Sq.m. = $\frac{500000}{10000} = 50$; Since 1 ha = 10,000 sq. m.

Therefore no. of seedlings per sq. m. = 50 x 2 = 100

Step-II : Calculate the seed required / Sq. m. with help of the test weight: i.e,

$$= \frac{100 \times 30}{1000} = 3 \text{ g/m}^2$$

per hectare = 3 x 10000 = 30,000 grams or 30 Kilograms.

Step-III : Calculate the seed rate / ha with 90% germination

$$= \frac{30 \times 100}{90} = 33.3 \text{ kg}$$

add 10% additional seed since risk factor is 10% i.e.

$$= 33.3 \text{ kg} + \frac{(33.3 \times 10)}{100}$$

Therefore total seed rate/ha will be 33.3 + 3.33 kg = 36.63 Kg.

For transplanting two hectares the seed rate

= 36.63 x 2 = 73.26 Kg

***Method* - II**

Seed rate (kg/ha) = $\frac{\text{1000 grain weight in grams x No.of seedlings per hill}}{\text{Spacing in Sq.cm.}} \times 100$

= $\frac{30 \times 2 \times 100}{200}$ = 30 kg

Seed rate after correction for germination = $\frac{30 \times 100}{90}$ = 33.3 kg

Seed rate after allowance for risk = $33.3 + \frac{33.3 \times 10}{100}$

= 33.3 + 3.33 = 36.63 Kg

For two hectares = 36.63 x 2 = 73.26 Kg.

Problem.1.

Calculate the plant population/ha, if maize is dibbled at a spacing of (i) 75 cm x 25 cm (ii) 60 cm x 25 cm, respectively.

Problem.2.

Calculate the seed rate of greengram per hectare with the help of the following data.

1. Test weight – 27 g. /1000 seeds
2. No. of seeds/ hill – 1
3. Spacing – 30 x 10 cm
4. Germination – 90 %
5. Risk factor – 5 %

EXERCISE - 7
STUDY OF TILLAGE IMPLEMENTS AND USE OF REVERSIBLE PLOUGH, ONE WAY PLOUGH, HARROW AND LEVELER

Tillage implements are broadly categorized into several groups depending on the purpose for which they are use:

Primary Tillage implements

Implements used for opening and loosening of the soil are known as ploughs. Ploughs are used for primary tillage. Ploughs are of three types: wooden ploughs, iron or inversion ploughs and special purpose ploughs.

Wooden plough or Indigenous plough

Indigenous plough is an implement which is made of wood with an iron share point. It consists of body, shaft pole, share and handle. It is drawn with bullocks. It cuts a V shaped furrow and opens the soil but there is no inversion. Ploughing operation is also not perfect because some unploughed strip is always left between furrows. This is reduced by cross ploughing, but even then small squares remain unploughed.

Soil Turning Ploughs

Soil turning ploughs are made of iron and drawn by a pair of bullocks or two depending on the type of soil. These are also drawn by tractors.

Mouldboard Plough

The parts of mouldboard plough are frog or body, mouldboard or wing, share, landside, connecting, rod, bracket and handle. This type of plough leaves no unploughed land as the furrow slices are cut clean and inverted to one side resulting in better pulverisation. The animal drawn mouldboard plough is small, ploughs to a depth of 15 cm, while two mouldboard ploughs which are bigger in size are attached to the tractor and ploughed to a depth of 25 to 30 cm. Mouldboard ploughs are used where soil inversion is necessary. Victory plough is an animal drawn mouldboard plough with a short shaft.

Disc Plough

The disc plough bears little resemblance to the common mouldboardplough. A large, revolving, concave steel disc replaces the share and the mouldboard. The disc turns the furrow slice to one side with a scooping action. The usual size of the disc is 60 cm in diameter and this turns a 35 to 30 cm furrow slice. The disc plough is more suitable for land in which there is much fibrous growth of weeds as the disc cuts and incorporates the weeds. The disc plough works well in soils free from stones. No harrowing is necessary to break the clods of the upturned soil as in a mouldboard plough.

Turn-wrest or Reversible or One-way Plough

The plough bottom in this plough is hinged to the beam such that the mouldboard and the share can be reversed to the left or to the right side of the beam. This adjustment saves the trouble of turning the plough in hilly tracts, but yet facilitates inversion of the furrow slice to one side only.

SPECIALPLOUGHS :-

Subsoil Plough :

Subsoil plough is designed to break up hard layers or pans without bringing them to the surface. The body of the subsoil plough is wedge shaped and narrow while the share is wide so as to shatter the hard pan and making only a slot on the top layers.

Chisel Plough :

Chisel plough is used for breaking hard pans and for deep ploughing (60-70 cm) with less disturbance to the top layers. Its body is thin with replaceable cutting edge so as to have minimum disturbance to the top layers. It contains a replaceable share to shatter the lower layers.

Ridge Plough :

Ridge plough has two mould boards, one for turning the soil to the right and another to the left. The share is common for both the mould boards i.e. double winged. These mould boards are mounted on a common body. The, ridge plough is used to split the field into ridges and furrows and for earthing up of crops. Ridge ploughs are used to make broad bed and furrows by attaching two ridge ploughs on a frame at 150em spacing between them.

Rotary Plough or Rotary Hoes:

Rotary plough cuts the soil and pulverizes it. The cutting of soil is done by either blades or tynes. The blade types are widely used. The depth of cut is up to 12 to 15 cm. It is suitable for light soils.

Basin Lister :

Basin lister is a heavy implement with one or two mouldboards or shovels. These shovels are mounted on a special type of frame on which they act alternately. This implement is used to form listed furrows (broken furrows with small dams and basins) to prevent free runoff of rainfall and blowing off the soil in low rainfall areas.

Secondary Tillage Implements

Different types of implements like cultivators, harrows, planks and rollers are used for secondary tillage.

Tractor Drawn Cultivator :

Cultivator is an implement used for finer operations like breaking clods and working the soil to a fine tilth in the preparation of seedbed. Cultivator is also known as tiller or tooth harrow. It is used to further loosen the previously ploughed land before sowing. It is also used to destroy weeds that germinate after ploughing. Cultivator has two rows of tynes attached to its frame in staggered form. The main object of providing two rows and staggering the position of tynes is to provide clearance between tynes so that clods and plant residues can freely pass through without blocking. Provision is also made in the frame by drilling holes so that tynes can be set close or apart as desirect. The number of tynes ranges from 7 to 13. The shares of the tynes can be replaced when they are worn out.

Sweep Cultivator

In stubble-mulch farming, it is difficult to prepare the land with ordinary implements due to clogging. Sweep cultivator is the implements useful under this condition. It consists of large inverted V shaped blades attached to a cultivator frame. These blades run parallel to soil surface at a depth of 10 to IS cm. They are armged in two rows and staggered. Sweep cultivator is used to cut up to 12 to 15cm depth of soil during first operation after harvest and shallower during subsequent operations. It is worked frequently to control weeds. It can also be used for harvesting groundnut.

Harrows

Harrows are used for shallow cultivation in operations such as preparation of seedbed, covering seeds and destroying weed seedlings. Harrows a~ of two types: disc harrow and blade harrow. '

Disc Harrow

The disc harrow consists of a number of concave discs of 45 to 55 cm in diameter. These discs are smaller in size than disc plough, but more number of discs are arranged on a frame. These discs are fitted 15cm apart on axles. Two sets of discs are mounted on two axles. All the discs revolve together with axles. The discs cut through the soil and effectively pulverise the clods.

Blade Harrow

Blade harrows are used for different purposes like removal of weeds and stubbles, crushing of clods working of soil to shallow depth, covering the seeds, intercutivation and harvesting of groundnut etc. The blade harrows useful for intercultivation are discussed later. Blade harrows are two types viz. indigenous and improved.

Indigenous Blade Harrows

The general design of an indigenous blade harrow which is known as *guntaka*consists of a beam to which two pegs are attached at the ends. A blade is attached to these two pegs. Two shaft poles and' a handle are the other parts of *guntaka.* Depending on the beam length and weight, the are known by different names and used for- different purposes.

Plank and Roller

Plank is a very simple implement and consists *of* a heavy wooden beam *of* 2 m in length. In addition, shafts and handle are fixed to the beams. When it is worked most *of* the clods are crushed due to its weight. It also helps in micro levelling and slight compaction necessary after sowing. Rollers are used mainly, to crush the hard clods and to compact the soil in seed rows.

Implements for sowing

Plough

The seeds are dropped by hand in the furrow formed by the country plough. The seeds fall at uneven depths due to falling at random in furrow slice. To avoid this problem *Akkadi*is used. *Akkadi*is a hollow bamboo tube which is sharpened at one end and with wide hopper at another end. It is tied to country plough with the help of a rope and seeds dropped in the *akkadi' s*hopper. Seeds pass through the tube and fall in the furrow opened by the plough.

Seed Drill

Seed drill consists of a wooden beam to which 3 to 6 tynes are fixed. These tynes open the furrows into which the seeds are dropped. Holes are made into these tynes and into these holes, the bottom ends of bamboo or metal seed tubes are fitted. These seed tubes are connected at the top to a wooden seed receptacle called hopper. The seeds are fed at a uniform rate 'into this hopper by skilled labour walking behind the seed drill.

Ferti-cum-Seed Drill

Fertilisers are placed at a depth of 5 cm and 5 cm away from seed rows for effective utilisation of fertilisers. Both operations *viz*. drilling seeds and fertilizers are done simultaneously by ferti-cum-seed drill. It is similar to seed drill, but with extra tynes and hopper for drilling fertilizers.

Mechanical Seed Drill

The seed drill consists of a seed drum with holes in the bottom plate corresponding to the number of seed tubes for passing the seed into the seed tubes. A rotating disc has holes in a circular path and it is kept over a bottom plate. When the holes of rotating disc and bottom plate coincide, seed falls into the tube on its way into the soil. The distance between two holes in rotating disc is proportional to the inter-row spacing of crop. For sowing seeds of different sizes, rotating discs with different sized holes are used. There is provision for altering the distance between the rows by changing distance between the tynes. Inter-row spacing can be changed by using rotating discs with more space between the holes. Seed drills with different mechanisms for automatic drilling of seed are also available.

Q.1. What are the different uses of indigenous plough ?

Q.2. Write uses of mouldboard plough and disc plough.

Q.3. Write the specific operations which are carried out by various harrows.

EXERCISE - 8

STUDY OF SOWING IMPLEMENTS / EQUIPMENTS

There are different methods of sowing viz..broadcasting. Dibbing.Line sowing by drill. Planting and transplanting of seedlings etc. Out of these methods. Sowing in lines by seed drill is the most common and popular method of sowing in Gujarat. The crops are sown in lines parallel to one another, therefore weeding and interculturing can be easily done between two lines of the crop. Regulation of seed rate and adjustment of depth of sowing is also possible by this method.

Seed drill

Seed drill is a machine for placing the seed in a continuous flow in furrows at uniform rate and at controlled depth with or without the arrangement of covering them with soil.

Function of seed drill: Seed drill perforforms the following functions.

(i) To carry the seeds

(ii) To open furrow to an uniform depth

(iii) To meter the seed

(iv) To place the seed in furrows in an acceptable pattern

(v) To cover the seed and compact the soil around the seed

Classification of seed drill :

(A) Indigenous Seed Drill :

(1) SuratFaidko

(2) Charotartarfan

(3) Amadavadchavad

(4) Dantal of Saurashtra

(5) Bharuch seed drill or Vankhedu

(6) Panchmahal seed drill or Panchotiyo

(7) Bhal seed drill

(B) Improved seed drill :

(1) Seed- Cum-fertilizer drill

(2) Cultivator with seeding attachment

(3) Planter

(1) Seed- Cum-fertilizer drill :

Seed drills, fitted with fertilizer dropping attachment, distribute the fertilizer uniformly on the ground. It is called seed cum fertilizer drill. Such a drill has a large seed box which is divided length wise into two compartments. One for seeds and antother for fertilizer, seed drill may be classified as: (i) Bullock drawn (ii) Tractor drawn

Depending upon the method of metering the seed. Bullock drawn seed drill can be further divided into two groups viz; those in which seeds are dropped (a) by hand, (b) or mechanically. There are a number of bullock drawn implements which are used for sowing seeds in which seeds are dropped by hand. In different sizes and shapes.

Components of tractor drawn seed drill :

A seed drill with mechanical seed metering device mainly consists of: (i) Frame (ii) Seed box (iii) Seed metering mechanism (iv) Furrow openers (v) Covering device (vi) Transport wheels.

(i) Frame: The frame is usually made of angle iron with suitable braces and brackets. The frame is strong enough to withstand all types of loads in working condition.

(ii) Seed box: It may be made of mild steel sheet or galvanized iron with a suitable cover. A small agitator is sometimes provided to prevent clogging of seed.

(iii) Covering device: It is a device to refill a furrow with soil after the seed has been placed in it. Covering the seeds are usually done by pattas, chains, drags, packers, rollers or press wheels, designed in various sizes and shapes.

(iv) Transport wheel: There are two wheels fitted on each main axle. Some seed drills have got pneumatic wheels aiso. The wheels have suitable attachments to transmit power to operate seed dropping mechanism.

(v) Seed metering mechanism : The mechanism of a seed drill or fertilizer distributor which delivers form the hopper at selected rates is called seed metering mechanism.

(vi) Furrow openers: The furrow openers are provided in a seed drill for opening the furrows before dropping the seeds. It may be called a part of the seed drill for opening boot from where they faill into the furrow.

Components of animal drawn seed drill :

(i) Head piece : It is made up of wood and all other parts are fixed on it.

(ii) Coulters whith tines: Coultersalong with tines are fixed into the Head piece.

(iii) Seed tubes : Through seed tubes seeds pass from seed bowl into the soil.

(iv) Seed bowl : It is funnelstaped and fixed on a handle. Seeds are poured into it.

(v) Beam: It is used to connect seed drill with yoke.

(vi) Handle with grip: Used for support and turn around the seed drill.

(vii) Hitching Peg: Used to adjust the depth of sowing.

Advantages :

1. Seed and fertilizer can be sown at a time.
2. Seeds of different size can be sown.
3. The required quantity of seed and fertilizer can be sown.
4. The fertilizer can be sown below the seeds.
5. Inter row spacing can be adjusted accoriding to our requirement.
6. It is made up of iron. Hence its durability is more.

(2) Cultivator with seeding attachment

A bullock drawn cultivator with seeding attachment is the country. It may be three rows cultivator. Three tynedcultivator with seeding attachment is used for sowing seeds in three rows at a time. The rate of seed dropping is controlled by hand. The main parts of the cultivator are the frame, three tynes fitted with reversible shovels, handle with seeding attachment and the beam. This Implement is drawn by a pari of average bullocks.

(3) Planter:

Planter is normally for those seeds which are larger in size and can not be sown by usual seed drill.

Function of Planter :

(i) To open the furrow

(ii) To meter the seed

(iii) To deposit the seed in the furrow

(iv) To cover the seeds and compact the soil over it

Components: A Planter consists of following parts :

(i) Hopper

(ii) Feed metering device

(iii) Knock out arrangement

(iv) Cut-off mechanism

(v) Furrow opener and

(vi) Other accessories

A Planter has seed hopper for each for. Hopper is usually made of mild steal or any other suitable material.

The different types of Planter are:

1. Potato Planters
2. Sugarcane Planter (Semi-automatic)
3. Manual rice transplanter
4. Japanese rice transplanter
5. Fertilizer distributor (Tractor mounted)

Figure : Ferti-cum-Seed Drill

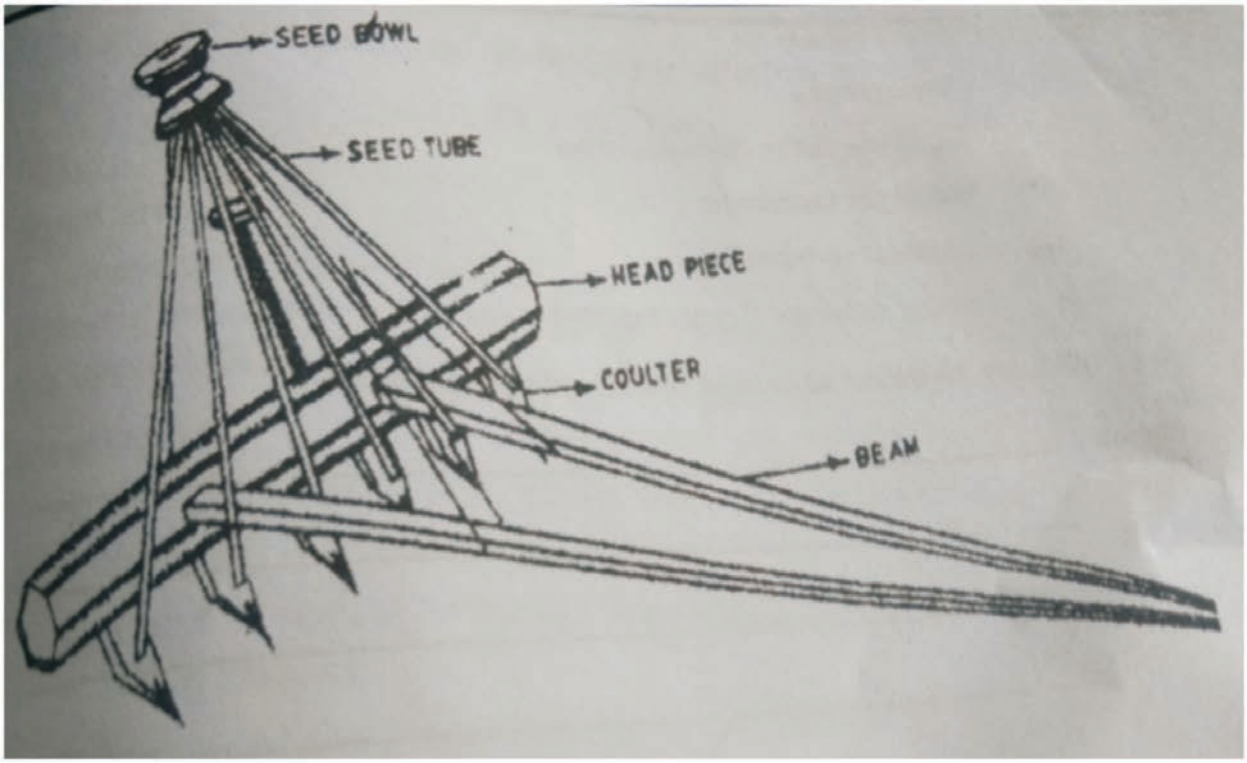


Figure : Animal drawn seed-drill

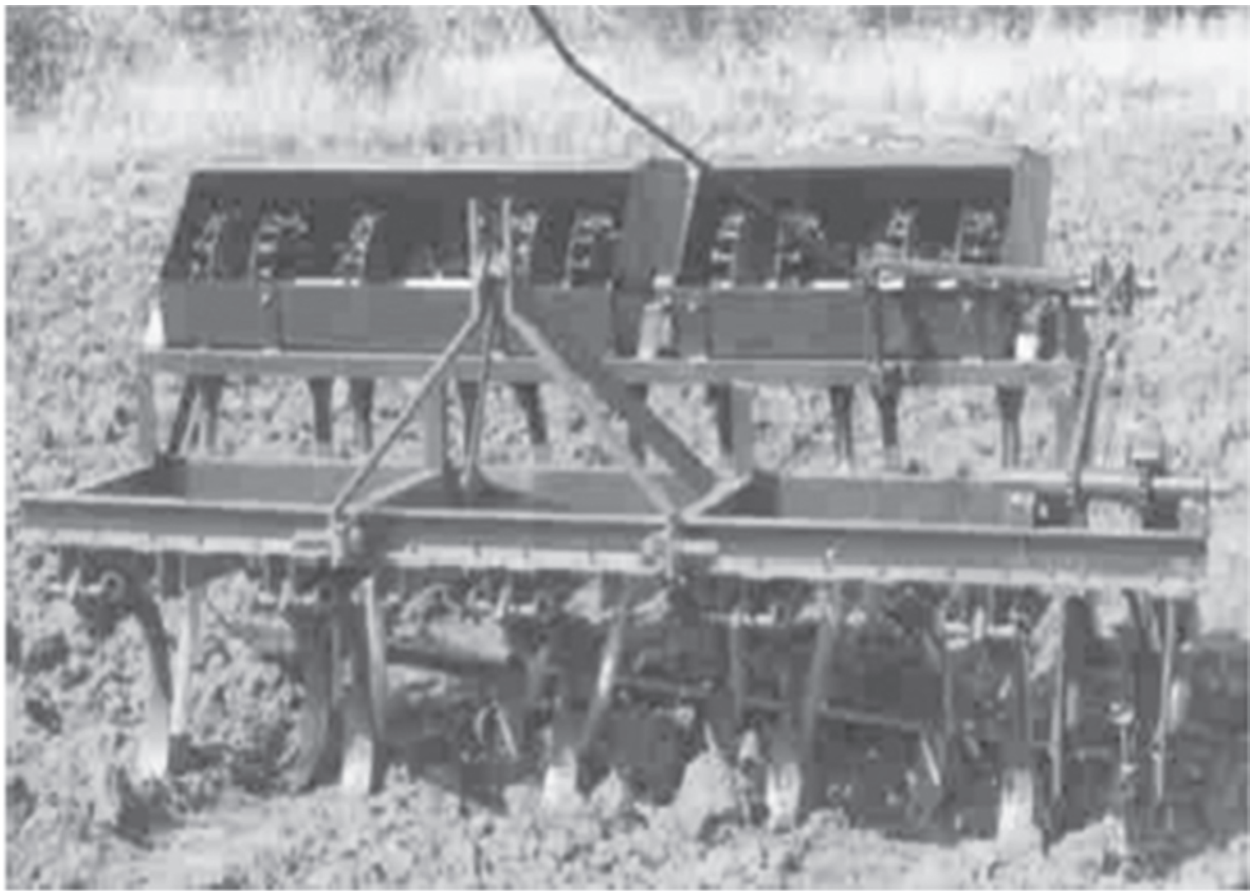


Figure : Tractor drawn seed drill

EXERCISE - 9
MEASUREMENT OF FIELD CAPACITY, BULK DENSITY AND INFILTRATIONRATE

Field capacity

Field capacity is the moisture content in percentage of a well drained soil on oven dry basis, of few days after completer saturation when down ward movement of execs water has practically ceased. Such a stage is reached generally in 48 to 72 hours after saturation. The field capacity is the upper most limit of available moisture range in soil water and plant relationship. The force with which moisture is held at this point varies from $1/10^{th}$ to $1/3^{rd}$ of atmospheric pressure (atm).

Materials Required :

1. Straw mulch
2. Black polythene sheet
3. Spade
4. Water
5. Screw auger
6. Moisture
7. Balance
8. Oven

Procedure :

1. Select the representative spot in the field.
2. Ensure that water table is not within 2 meter from the layer of which field capacity is to be determined.
3. Bund an area of about 2.5 m^2 at all the four sides and removes all weeds to avoid transpiration.
4. Pond the water till all the desired layers gets sufficiently wet.
5. Spread straw much of at least 40 cm thickness on the surface of bonded plot to prevent evaporation and cover it with gunny bags or polyethylene sheet.
6. Put sufficient weight over material to protect it against blowing away due to winds.
7. Take soil sample from different layers up to the root zone depth with auger and determine the soil moisture content at every 24 hour Intervals till the values of two successive samples are nearly equal.
8. The lowest reading in each layer can be taken to represent the value of field capacity of the soil.

Observations for determination of Field capacity (FC):

Depth	Sr. No.	Wt. of empty moisture box (g)(W_1)	Wt. of empty moisture box + Moist soil (g) (W_2)	Wt. of empty moisture box + Dry soil (g) (W_3)	Wt. of moist soil (g) (W_3-W_1) WS_1	Wt. of dry soil (g)(W_3W_1)WS_2	Moisture Percentage	F.C.
1	2	3	4	5	6	7	8	9
0-15	1							
	2							
	3							
15-30	1							
	2							
	3							
30-45	1							
	2							
	3							
45-60	1							
	2							
	3							

Bulk density

Bulk density is the ratio of bulk weight of the soil to its bulk volume. The bulk volume includes volume of air in pore space and soil particles. The value of bulk density is always lower than the real density because air has relatively more volume.

Objectives :

1. To determine bulk density of the given soil

Materials Required :

1. Core sampler
2. Moisture boxes.
3. Vernier calipers
4. Physical balance
5. Oven

Procedure :

1. At field capacity, take the soil of the cores from the desired depths with the help of a core sampler. Whose volume is pre – determined by measuring height and diameter of the core.
2. Transfer the soil of the core in a moisture box and oven dry it.
3. Record the oven dry weight.
4. Calculate the bulk density by using following formula :-

$$\text{B.D (gm/cc)} = \frac{\text{Weight of oven dry soil}}{\text{Volume of wet soil or weight of water}}$$

Observations for determination of Bulk Density (B.D.) :

Depth	Sr. No.	Date of sampling	Wt. of empty moisture box (g) (W_1)	Wt. of empty moisture box + Moist soil (g) (W_2)	Wt. of empty moisture box + Dry soil (g) (W_3)	Wt. of moist soil (g) (W_2-W_1) WS_1	Wt. of dry soil (g) (W_3-W_1) WS_2	Moisture Percentage	B. D.
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
0-15	1								
	2								
	3								
15-30	1								
	2								
	3								
30-45	1								
	2								
	3								
45-60	1								
	2								
	3								

Infiltration rate

Determination of infiltration rate of soil by double ring infiltrometer

The downward entry of water into the soil through the soil surface is called as infiltration.

It is an important soil property because it partitions rain in to the soil water and runoff. It depends on many factors such as soil texture, moisture content, soil cover and soil management.

- ✓ **Infiltration characteristics of soil are practical significance in irrigation soil and water conservation and watershed management.**

Aim :To measure the water intake rate of the soil using double ring infiltrometer.

Principle :The main principle is to measure the amount of H_2O entering the soil profile as a function of time.

- ✓ During infiltration appreciable lateral movement of**H_2O may also occure to avoid errors due to the lateral movement to concentrate iron rings (Infiltrometers) are used. Water level in both rings should be kept nearly equal. The rate of fall off H_2O level in the inner rings is measured.**

Apparatus : Double ring infiltrometer / two rings made from 14-16 gauge iron sheet rolled into a cylinder, outer ring of 16 cm diameter, both 30 cm height, spade, bucket, polythene sheet, watch, hook guage or scale, driwing plate and hammer.

- ✓ The lower edges of the rings are sharpened to fecilitate easy drive of the rings. The top of the rings are provided with steuardy rims.

Procedure : Describe the texture, surface condition, structure, compaction soil content and layering sequence in the profile.

1. Determine the initial soil moisture content.
2. Install infiltrometer rings in a uniform and nearly levelled plot to a depth of 15 cm.
3. Pond 10-15 cm of water in outer as well as inner ring.
4. Record the fall off H_2O level in the inner ring with hook guage at 1, 3, 5, 10, 20, 30, 40, 60, 80, 100, 120 min and there after on hourly basis till the water intake is constant.
5. However, the time intervals of observations can be vary according to objectives of study and soil permeability.

6. More H_2O should be added into the rings when water level falls by 4-5 cm in order to check drastic H_2O level fluctuations which may affect constant intake rate.
7. Using scale or hook guage record the H_2O level and time just before and after preponding.
8. Keep the intervals between these two observations as short as possible to avoid errors caused by intake during the retailling period.
9. plot the infiltration rate and cummulative infiltration as function of time.

Precautions :

1. Drive the rings straight down with minimum soil surface disturbance.
2. Cover the soil surface in the ring with polythene sheath while pouring H_2O in the rings for the first time to avoid dispersion of surface soil and cloging of pores.
3. Try to keep the level identical in inner as well as outer ring.

Observations and calculations :-

- ✓ Location, texture of the surface, soil surface conditions (kind and extent of vegetation, crust, salts)
- ✓ Layering sequence in the profile
- ✓ Antecedent, soil H_2O content
- ✓ Specific problem of any (erosion, salinity, ponding)
- ✓ Area of inner ring

Sr. No.	Descriptive term	Infiltration rate	
		Inches per hr	Millimeter/hr
1.	Very rapid		
2.	Rapid		
3.	Moderately rapid		
4.	Moderate		
5.	Moderately slow		
6.	Slow		
7.	Very slow		

Sr. No.	Cummulative time (t) min	Water level reading (r) cm	Cummulative intake (cm)	Infiltration rate cm/min
1.				
2.				
3.				
4.				
5.				

If we convert thesedr/at values that is cm/min to mm/hr the values are as

Sr. No.	Cum time (t) (min)	Infiltration rate mm/hr

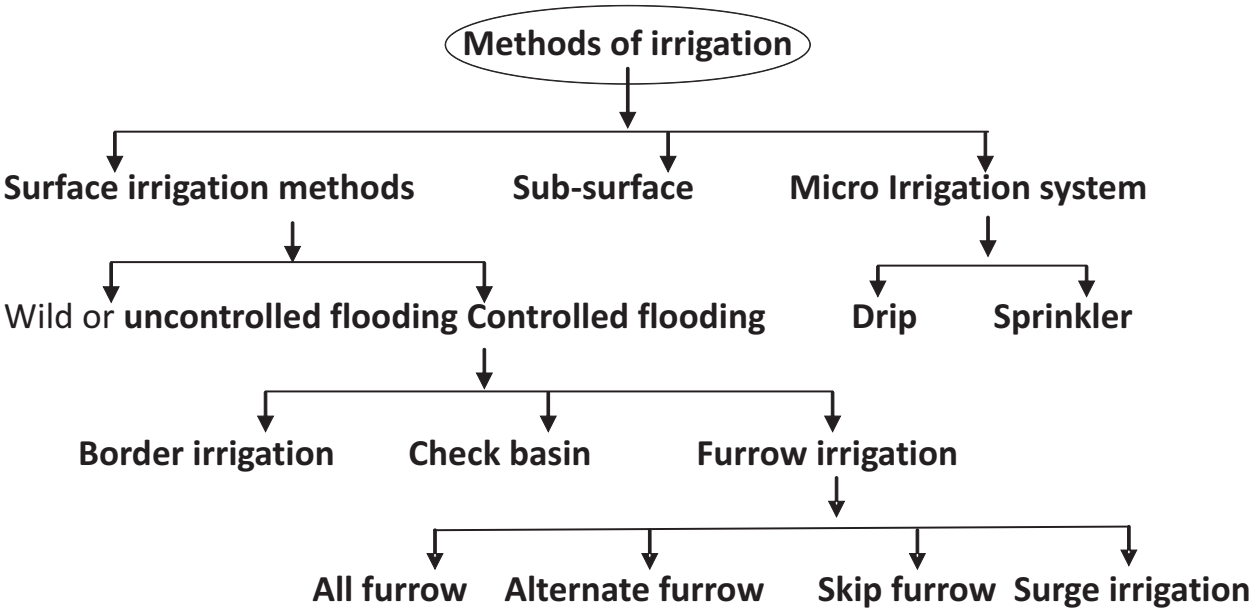
EXERCISE - 10

FIELD LAYOUT OF VARIOUS IRRIGATION METHODS

There is a need to bring more and more cultivable land under irrigation for assuring crop production to meet the food requirement of our ever-increasing population. It is therefore, necessary to attain maximum efficiency in the application of the existing irrigation potential in the country. Although some improved methods of application of irrigation water like sprinkler system and drip system are being popularized yet, comparatively a larger irrigated area in this country is still irrigated by conventional surface methods.

Factors affecting the adoption of irrigation methods

1) Topography
2) Soil type and conditions
3) Crops to be grown, nature and its habit of growth
4) Value of the crop
5) Cultural practices are to be required
6) Season of the crops
7) Sources of water
8) Skill and expenditure involved in method and its operation
9) Cost of installation
10) Efficiency that can be achieved



A. Surface irrigation methods

Surface irrigation methods are being classified based on slope, shape and size of the field, the end conditions and how water flows into and over the field.

1. Wild or uncontrolled flooding

In this method water is applied over the smooth or flat field without much control over the flow or prior preparation. The water is applied into the field from the ditch excavated either on the contour or up and down the slope. The water distribution is quite uneven.

Advantages

- Minimum labour requirements.
- System does not interfere for the use of farm implements in standing crop.
- Rough levelled field may also be irrigated under wild flooding.

Disadvantages

(i) Wastage of water,

(ii) Non-uniform distribution of water,

(iii) Excessive soil erosion and

(iv) Require drainage arrangement to reduce flooding.

2. Controlled flooding

In this method, irrigation is applied by flooding but with some control as per demand of soil, crop and water supply. The following are most common methods used under field conditions :

1. Border irrigation

The land is divided into number of long parallel strips called borders. These borders are separated by low ridges. The border strip has a uniform gentle slope in the direction of irrigation. Each strip is irrigated independently by turning the water in the upper end. This method is suitable to all close growing crops like wheat, barley, fodder and legumes. A uniform moisture distribution can be achieved by this method with good water use efficiency.

Advantage

1. Border ridges can be constructed with simple farm implements like bund former.

2. Labour requirement in irrigation is reduced as compared to conventional check basin method.
3. Uniform distribution of water and high water application efficiencies are possible.
4. Due to longer strip size inter-cultivation is possible.

2. Check basin irrigation

Check basin irrigation is most common (popular) method of irrigation in India and in many other countries. The field is divided into smaller plots to bring unit area forming basins within which irrigation water can be controlled. The basins are filled to the desired depth and water is retained until it infiltrates in to the soil. The size and shape of the check basin depends upon soil type, infiltration rate, size of stream and crop to be grown. The shape of check basin may be square or rectangular.

Basins for orchards (wide spaced crops) prepared generally in ring (circular around tree) and the rings are connected with water channel. The rings prepared in orchards are small during young stage.

Advantages

1. Simplest method
2. Helps in conservation of rainfall in the field and reduce erosion of soil due to run-off.
3. Helps in removal of salts from soil profile through leaching requirement.
4. Uniform distribution of water is possible.

Disadvantages

1. Wastage of land in preparation of bunds and channels is more over other surface methods.
2. Interfere in carrying out agricultural operations due to bunds and channel.
3. Labour requirement is higher.
4. Not suitable for crops which are sensitive to wet soil conditions around the stem.

3. Furrow irrigation

In furrow irrigation method, water is applied through small stream running between the crop rows. This method of irrigation is suitable for row crops like sugarcane, tobacco, potato, cotton and some vegetable crops.

In general, small plants require small furrows, larger plants permits large furrows. Furrows of 7.5 to 12.5 cm depth are appropriate for vegetables, while some row crops and orchards require much deeper furrows.

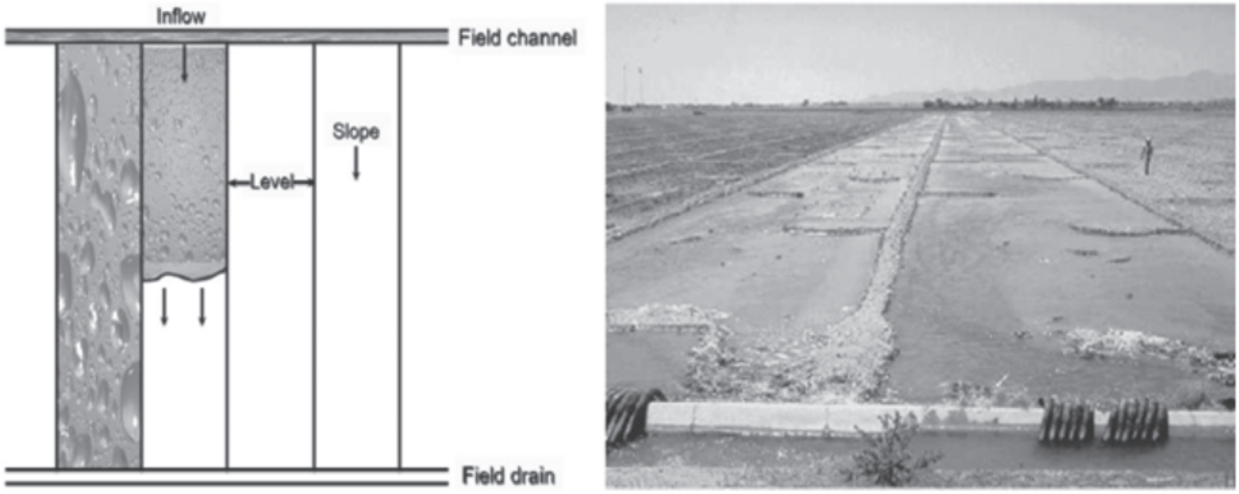


Fig.: Border strip method of irrigation

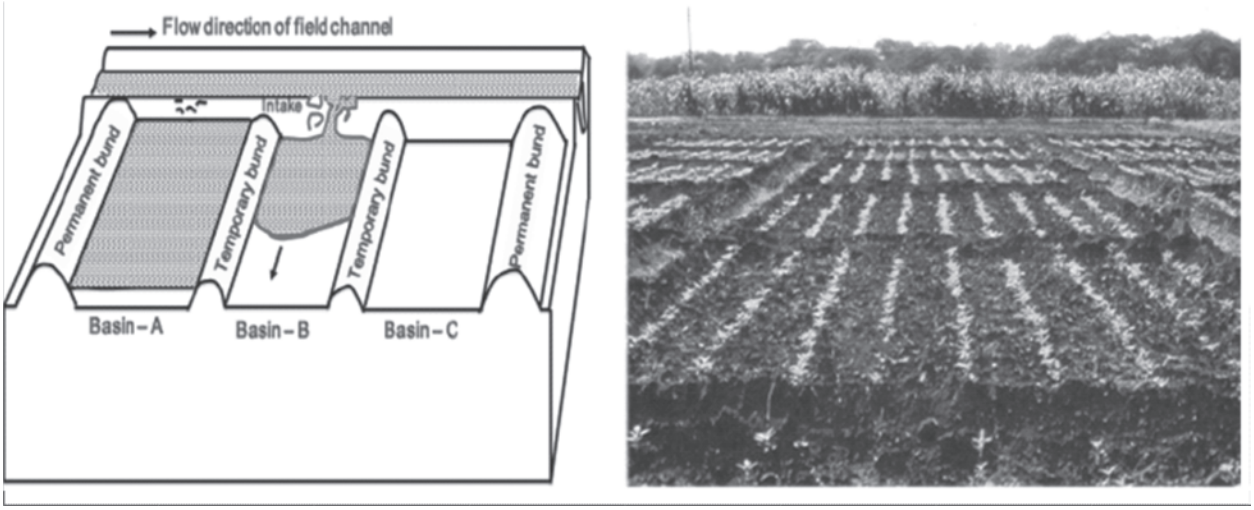
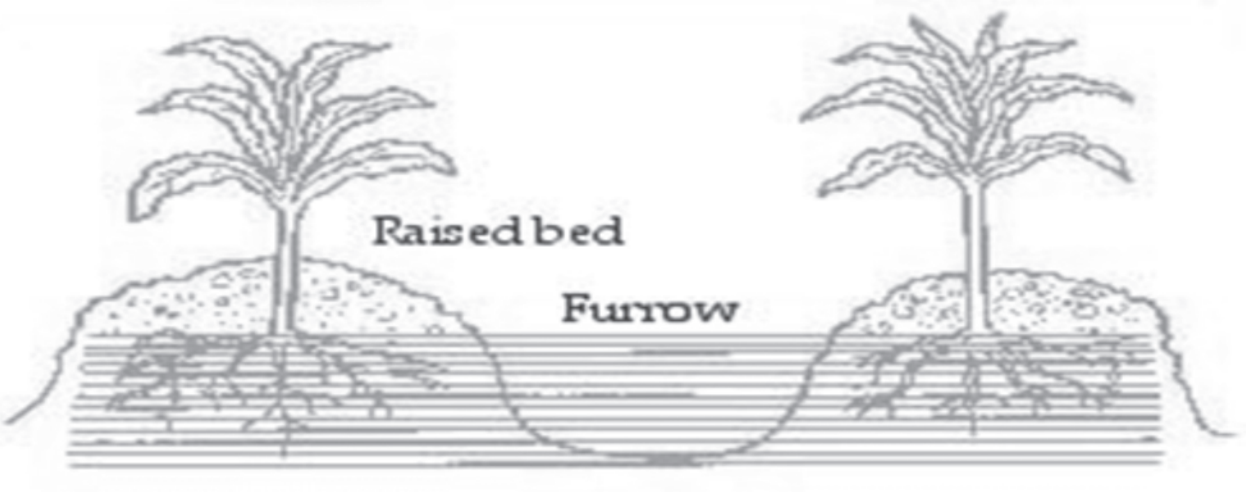


Fig. : Check basin method of irrigation

Advantages

- Both large and small streams can be used.
- The furrow can be used to disposed- off excess water of irrigation or run-off from rainfall rapidly.
- Wastage of land is very low.
- Labour requirement is very low for layout and application of water.
- Wastage of water is very low, only a limited surface of land is wetted and hence WUE is much high as compared to all other surface methods.

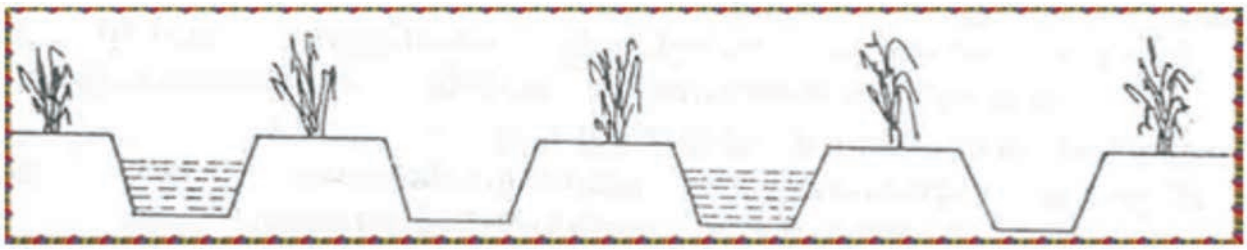
- Weeds can be controlled mechanically, between the beds, early in crop cycle.
- Less lodging occurs.



Type of furrow

A. All furrow irrigation: Water is applied evenly in all the furrows and are called furrow system or uniform furrow system.

B. Alternate furrow irrigation: It is not an irrigation layout but a technique for water saving. Water is applied in alternate furrows for eg. During first irrigation if the even numbers of furrows are irrigated during next irrigation the odd number of furrows will be irrigated.



C. Skip furrow irrigation: They are normally adopted during the period of water scarcity and to accommodate intercrops. In the skip furrow irrigation a set of furrows are completely skipped out from irrigation permanently. The skipped furrow will be utilized for raising intercrop. The system ensures water saving of 30-35 per cent. By this method the available water is economically used without much field reduction.

D. Surge irrigation: Surge irrigation is the application of water in to the furrows intermittently in a series of relatively short On and OFF times of irrigation cycle. It has been found that intermittent application of water reduces the infiltration rate over surges thereby the water front advances quickly, hence, reduced net irrigation water

requirement. This also results in more uniform soil moisture distribution and storage in the crop root zone compared to continuous flow.

B. Sub-surface irrigation

- In subsurface irrigation water is applied beneath the ground by creating and maintaining an artificial water table at some depth. Usually 30-75 cm below the ground surface.
- Moisture covers upwards towards the land surface through capillary action.
- Water is applied through underground field trenches laid 15-30 m apart.
- Open ditches are preferred because they are relatively cheaper and suitable to all types of soil.
- The irrigation water should be good quality to prevent soil salinity.

Advantage

1. Minimum water requirement for raising crops.
2. Minimum evaporation and deep percolation losses.
3. No wastage of land.
4. No interference to movement of farm machinery.

Disadvantages

1. Requires a special combination of natural conditions.

2. There is danger of water logging.

3. Possibility of choking of the pipes lay underground.

4. High cost.

C. Micro irrigation system

The term micro irrigation is commonly used to describe several low - pressure irrigation systems including drip/trickle.

1. Sprinkler irrigation

In the sprinkler method of irrigation, water is sprayed into the air and allowed to fall on ground surface somewhat resembling rainfall. The spray is developed by the flow of water under pressure through small orifices or nozzles. The pressure is usually obtained by pumping. With careful selection of nozzle sizes, operating pressure and sprinkler spacing the amount of irrigation water required to refill the crop root zone can be applied nearly uniform at the rate to suit the infiltration rate of soil.

Components of sprinkler irrigation system

A. sprinkler system usually consists of the following components :

i) A pump unit

ii) Tubing's- main/sub mains and laterals

iii) Couplers

iv) Sprinkler head

v) Other accessories such as valves, bends, plugs and risers

Adaptability

- When water is a scarce (limited)
- Uneven topography of land
- Nursery of crops, which require rainfall type irrigation
- Sandy soils that have a high infiltration rate
- Most field crops except paddy and Jute

Advantage of sprinkler irrigation

1. Water saving to an extent of 35-40% compared to surface irrigation methods.

2. Sprinkler method is suitable for soils having high infiltration rate.
3. Land grading and land levelling is not essential.
4. Suitable for high value crops, especially the fruits and vegetable.
5. Nutrients can be applied with the irrigation water, as per requirement of the crop, and hence increases the fertilizer use efficiency by more than 40%.
6. Controlled water application leading to higher application efficiency.
7. Irrigation is possible in area at higher elevation than the source.
8. Drainage problems eliminated.

Disadvantages

1. High initial cost.
2. Efficiency is affected by wind.
3. Higher evaporation losses in spraying water.
4. Not suitable for tall crops like sugarcane.
5. Poor quality water cannot be used (Sensitivity of crop to saline water and clogging of nozzles).
6. Operation and maintenance require technical manpower.
7. Pollination of crop is adversely affected (Paddy and Jute).

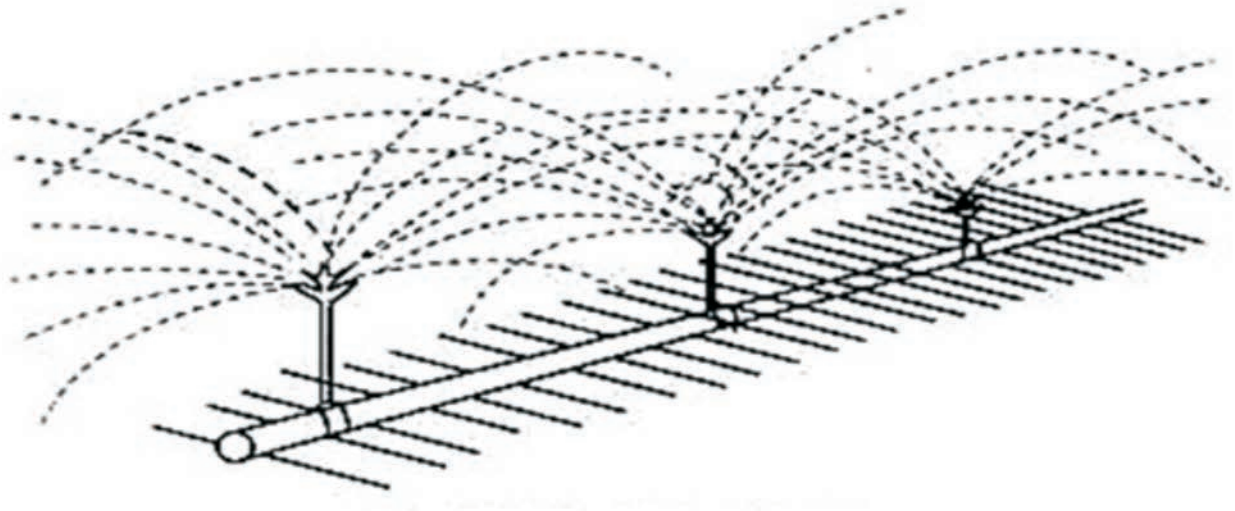


Fig.: Sprinkler system

2. Drip Irrigation

Drip irrigation also known as trickle irrigation system is a technique by which water and fertilizer including chemicals can be placed at the direct disposal of the root zone with the help of a specially designed dripper. Water is applied as continuous drops or fine spray through emitters placed along a low pressure delivery system. Such system provides water precisely to plant root zones and maintains ideal moisture conditions for plant growths.

Components of drip irrigation

A. Head control unit:

i) Pump/Overhead Tank

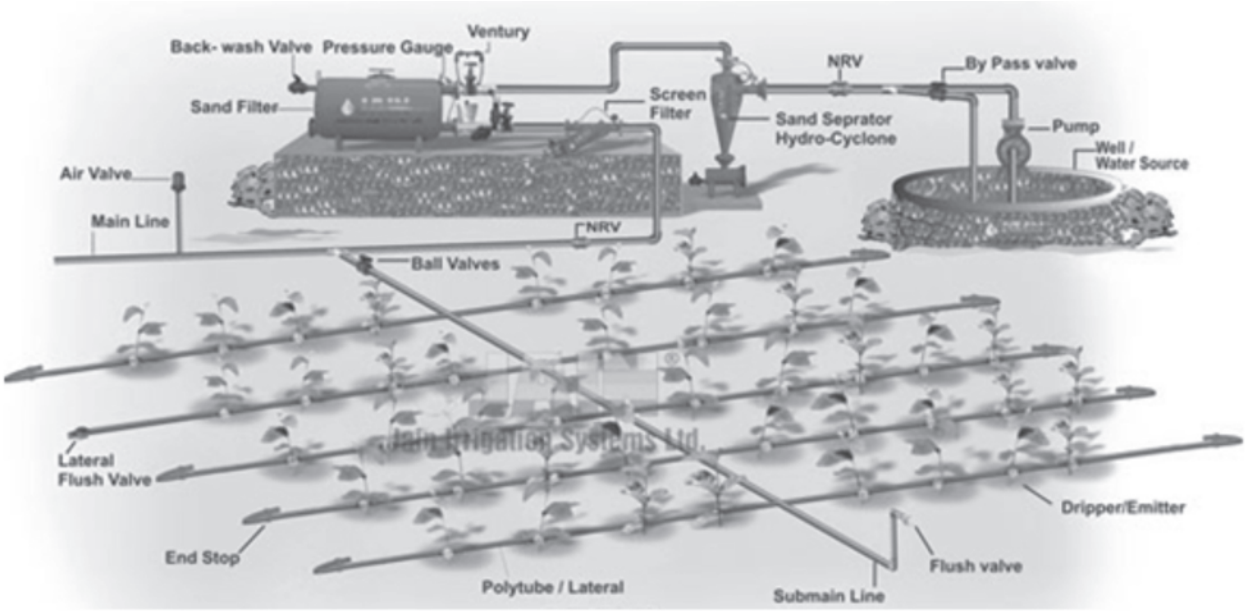
ii) Fertilizer Applicator iii). Filters (Gravel or Media Filter, Screen Filters, Disk Filters)

B. Distribution network :

i). Mainline ii).Sub-mains iii). Laterals iv). Emitters / Drippers (Online Pressure Compensating drippers, Online Non-Pressure Compensating drippers, In-Line Drippers or Inline tubes)

C. Accessories

i) Gate valve ii) tee iii) elbow iv) end cap v) grommet take off



Layout plan of Drip irrigation system

Advantages

i. Water saving

ii. Enhanced plant growth and yield of better quality

iii. Reduced salinity hazards to plants

iv. Fertilizer and other chemicals application

v. Higher efficiency of all chemicals

vi. Reduced weed growth

vii. Labour saving

viii. Highly suited to poor soils

ix. Efficiency in cultural operations

Disadvantages

i. High initial investment

ii. Clogging of drippers by suspended materials, salts oxides of iron, organic material

iii. Interferes with farm operations and movement of implements and machineries

iv. Operational difficulties: Installation, maintenance

v. Problem of theft, rodents etc.

vi. Not highly suited to closed spaced crops like wheat, rice etc.

Q.1. Write difference between drip and sprinkler irrigation method.

Ans. _____

_____

_____

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_____

_____

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Q.2. Write difference between surface and sub-surface irrigation method.

Ans. _____

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Q.3. Under what situation drip method of irrigation is used ?

Ans. _____

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Q.4. Under what situation sprinkler method of irrigation is used ?

Ans. _____

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_____

Q.5. Draw a neat sketch of drip irrigation method.

Q.6. Draw a neat sketch of Check basin method of irrigation.

EXERCISE - 11
TO WORK OUT THE LABOUR UNIT AND UNIT OF WORK FOR VARIOUS OPERATIONS

Unit of labour :

Amount of work done by one labourer employed for any farm operation in a day of 8 (eight) hours.

Labour unit :

Number of labourers required working for 8 (eight) hours a day to complete any field operation for an area of 1(one) hectare or one acre.

$$\textbf{Unit of labour} \text{ (In sq.m)} = \frac{8 \times \mathbf{A}}{\mathbf{T}} = \mathbf{C}$$

Where **A**= Length x Width (m)

T= Time required for area of work (hour)

$$\textbf{Labour unit} \text{ (No. of labourer/ha/day) } \mathbf{C} = \frac{\text{(One hectare = 10,000 sq.m.)}}{\mathbf{(}\text{sq.m.)}}$$

10,000 (sq.m.)

= 2.47 acres

= 100 are

(0.4 hectare = 1 acre)

Calculate the Unit of labour (work) and Labour unit :

Example - 1

Weeding operation is carried out by 1 labourer in 15.0 m x 10.0 m plot of pearlmillet crop. Weeding operation completed in 3.0 hrs. Calculate Unit of labour and Labour Unit.

$$\text{Unit of labour} = \frac{8 \times A}{T} = C$$

Where A = 15 m x 10 m = 150 sq. m.

T = 3.0 hrs.

$$= \frac{8 \times 150}{3} = \mathbf{400\ sq.m.}$$

C = 400sq.m.

$$\text{Labourunit} = \frac{10{,}000}{C} = \frac{10{,}000}{400} = \mathbf{25\ labourers/ha/day}$$

Q.1 Interculturing operation is carried out by 1 labourer in 20.0 m x 10.0 m Plot of maize crop. Interculturing operation completed in 4.0 hrs. Calculate Unit of labour and Labour Unit.

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NAVSARI AGRICULTURAL UNIVERSITY

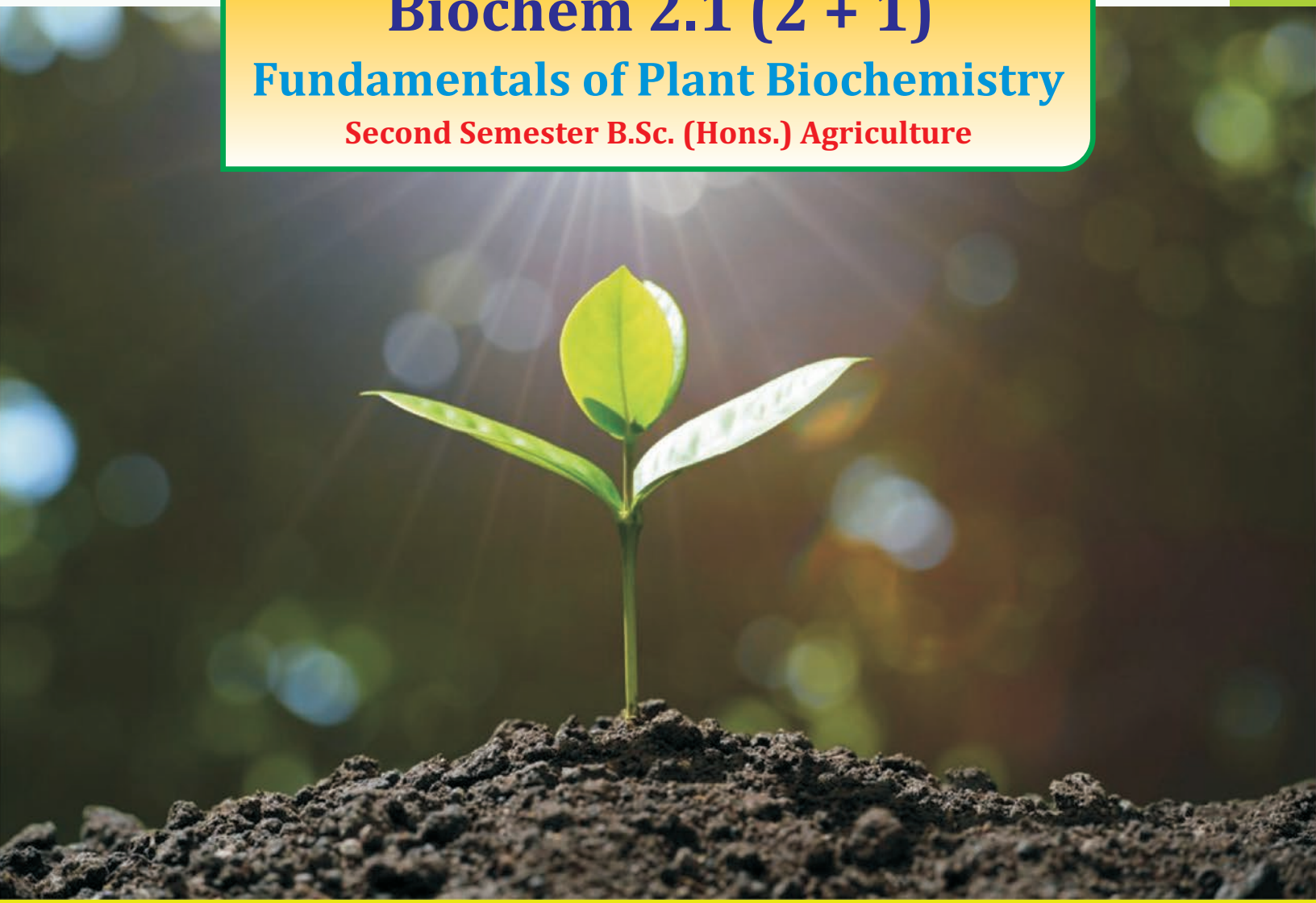
Practical Manual

ICAR

Biochem 2.1 (2 + 1)

Fundamentals of Plant Biochemistry

Second Semester B.Sc. (Hons.) Agriculture



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FOREWORD

Agriculture is a vast subject which includes many disciplines and the discipline of biochemical approaches, 'Biochemistry' is being adopted in almost all fields related to agriculture. Biochemistry is a quantitative science of biochemical investigations, especially based on laboratory experimentation. Fresh students and research scholars usually lack the required confidence to perform practicals. They have to refer many books and periodicals to select procedures for their research works. In order to perform the laboratory operations smoothly, it is imperative to provide the beginners with necessary guidelines before hand. Provision of written practical manuals prepared with due consideration to the level and syllabus would obviously be of great help both to students and instructors in ample measure. Manuals, such as this, would especially assist students to tide over the common problems and carry out their practicals with great ease and accuracy. In this regard, a well documented procedure manual will help the scholar to save lots of time and energy.

This manual entitled "***Fundamentals of Plant Biochemistry***" A Practical Manual provides biochemical methods related to important constituents in various crops like wheat, maize, oilseed crops, cotton, vegetables, fruits, legumes including many crops. Therefore, it will be useful for the students, teachers and scientists engaged in teaching and research in agriculture and allied subjects.

I appreciate and congratulate *Prof..R. P. Bambharolia and Prof. H. P. Patel for their commendable efforts in bringing out this practical manual* for their efforts.

January, 2019

(Z. P. Patel)

NAVSARI AGRICULTURAL UNIVERSITY

ICAR

Practical Manual
Biochem 2.1 (2 + 1)

Fundamentals of Plant Biochemistry

Second Semester B.Sc. (Hons.) Agriculture

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Preface

Biochemistry is one of the fundamental subjects of life science and knowledge of its practical aspects is absolutely essential for students and the researcher. Practical manual on "***Fundamentals of Plant Biochemistry***" is a fundamental book which highlights and makes the readers aware of the important techniques of biochemistry. This book has been especially designed keeping in view the latest syllabus prescribed by the ICAR as per the 5th dean committee recommendation for second semester undergraduate agriculture students.

However, it is equally useful for the post graduate students, research scholars, teachers and scientists working on the basic and applied aspects of biochemistry. The genesis of this manual lies in the problem faced by the students and their teachers to carry out the practical classes, as no standard study material was available for it. Keeping in view, the authors along with the Principal (CAW, NAU, Waghai) decided to provide printed practical manual of the said course (Biochem.2.1) to the students from the academic year 2019. The format of the exercises is appropriate for use as a workbook. It is hoped that this practical manual will be highly useful for the students in learning the fundamental techniques of biochemistry. The editors welcome suggestions from users, students as well as instructors/teachers for its future improvement.

R. P. Bambharolia
H. P. Patel

Certificate

Reg. No. : _____ **Batch No.: _____**

Roll No. : _____ **Uni Seat No.: _____**

This is to certify that the practical exercises duly signed were performed in the subject of ***Biochemistry, Course No. Biochem. 2.1 (2+1) [Fundamentals of Plant Biochemistry] as a part and partial requirement of the Course by Mr./Ms.*** _____

Roll No. _____ of ***Second Semester*** *class during academic year 20___.*

The numbers of practical performed were _____ out of _____.

Course Teacher **Professor & Head**

Examiner
(Internal Exam.)

Examiner
(External Exam.)

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Introduction and Scope of Biochemistry

Biochemistry is the branch of life science which deals with the study of chemical reactions occurring in living cells and organisms. Life is a chemical process involving thousands of different reactions occurring in an organized manner. These are called metabolic reactions. The term 'Biochemistry' was first introduced by the German Chemist Carl Neuberg in 1903. It takes into account the studies related to the nature of the chemical constituents of living matter, their transformations in biological systems and the energy changes associated with these transformations. Biochemistry may thus be treated as a discipline in which biological phenomena are analyzed in terms of chemistry. The branch of Biochemistry for the same reason has been variously named as 'Biological Chemistry' or 'Chemical Biology'. Modern biochemistry has two branches, descriptive biochemistry and dynamic biochemistry. Descriptive biochemistry deals with the qualitative and quantitative characterization of the various cell components and the dynamic biochemistry deals with the elucidation of the nature and the mechanism of the reactions involving these cell components. Many newer disciplines have been emerged from biochemistry such as enzymology (study of enzymes), endocrinology (study of hormones), clinical biochemistry (study of diseases), molecular biochemistry (study of biomolecules and their functions), etc. Along with these branches certain other specialities have also come up such as agricultural biochemistry, pharmacological biochemistry etc.

Biochemistry is related to almost all the life sciences and without biochemistry background and knowledge, a thorough understanding of health and well being is not possible. Those who acquire a sound knowledge of biochemistry can tackle the two central concerns of the biomedical sciences (1) the understanding and maintenance of health, and (2) the understanding and treatment of diseases.

Biochemistry is the study and application of substances, reactions and processes in animals, plants, bacteria and viruses. Biochemistry has vast scope. Biochemists work in hospitals, industry, drug design and development, environmental sciences, forestry, agriculture, dietetics, hormone production, vaccine research, virology, immunology, microbiology, toxicology, food science, plant science and associated areas and in areas from marine biology to entomology not just to carry out R&D work and develop new products but also to monitor the production, quality and safety of the product. Biochemists provide diagnostic service, carrying out tests on blood, urine and other body fluids, while researching the underlying causes of disease and methods of treatment. They find jobs in pharmaceutical and agrochemical companies, food brewing and biotechnology industry. The postgraduate degree in biochemistry also provides opportunity for advanced teaching in universities, colleges, medical, dental and veterinary schools and consulting or allied work. Observational, organizational, computational skills, planning and team work are important for biochemists.

Scope and importance of Biochemistry in agriculture

1. To evaluate nutritive value of cereals, pulses, poultry and cattle feed.
2. Development and exploitation of better genotypes.
3. Removal and inactivation of toxic or anti nutritional factors present in food grains in general and grain legumes in particular by breeding and chemical treatments. e.g. BOAA in Lakh dal, trypsin inhibitors of soybean, aflatoxins of groundnut.
4. Food preservation and processing technology and post-harvest physiology of fruit crops and vegetables and their nutritional quality.
5. Biochemistry of disease and pest resistance.
6. Biochemistry of drought resistance: Proline and hydroxyproline imparts drought resistance to Jowar.
7. Formulation of balanced diet.
8. Use of nonconventional sources of protein foods viz., single cell proteins, fish protein concentrates, mushrooms and leaf proteins.
9. Developments in the field of intermediary metabolism i.e. synthesis and degradation of constituents of living tissues.

General Information: Keeping a Laboratory Notebook

Students will be **required** to maintain a laboratory notebook. The notebook will be used for the recording of laboratory data and calculations, and will be critically important for writing your lab reports.

The purpose of a laboratory notebook is to allow anyone with some biochemical knowledge to understand **exactly** what you did. You need to record the information in sufficient detail so as to be able to repeat it, and you must be able to understand exactly what your results were. You will need good notes to be able to write your lab reports; in addition, as your understanding of biochemistry improves, your notebook may allow you to figure out why some parts of your experiments did not work as expected.

In your notebook, each experiment should begin with a **title**, a **date**, and a statement of the **objective** of the planned work. You should also **record exactly what you did at each step** (being sure to mention anything that you did that differed from the information in the Manual). In addition, you should record any numerical information, such as the weights of reagents used, absorbance readings, protein concentrations, and buffer concentrations. Most experiments will extend over several days, and over several pages in your notebook. To allow you to keep track of what you have done, you should include the day's date at the top of each page. Including sub-titles for each page may make it easier to keep track of what you did at each step.

Everything you do should be recorded **directly** into your lab notebook in **pen**. If you make a mistake, draw a line through it, and write the correction next to the mistake. (It may turn out that the original information was correct after all, so do not obliterate the original information by erasing it, or by removing the page from your notebook.) Any calculations performed should be written directly into your book.

Writing important information on scrap paper, and then recording it in your notebook later is **not** acceptable. **If you are writing something while in the laboratory, you should be writing it directly into your notebook.**

At each step in your experiment (after each assay or measurement), in addition to the results, record your thoughts regarding the experiment and how you think it is going. Record your mistakes, and your attempts to rectify them. Record the calculations involved in any type of data analysis, as well as explanations for both what you did and what you think it means.

It is also a good idea to look over your notebook periodically during the semester and make notes of things that you do not understand, so that you can ask questions before the lab reports are due.

Do not say "well, I will remember what this means"; instead, ***write it down***! Do not say "I will remember what I was thinking while I did this experiment"; instead, ***write it down***! If you use your lab notebook properly, you will find that writing your lab reports is much easier, and you will be developing good habits for the future.

SAFETY IN THE LABORATORY

Virtually all experiments conducted in a biochemistry laboratory present a potential risk to the well-being of the investigator. In planning any experiment it is essential that careful thought be given to all aspects of safety before the experimental design is finalised. Health hazards come from a variety of sources:

- ✓ ***Chemical hazards:*** All chemicals are, to varying extents, capable of causing damage to the body. They may be irritants and cause a short-term effect on exposure. Alternatively they may be corrosive and cause severe and often irreversible damage to the skin. Examples include strong acids and alkalis. Thirdly they may be toxic once they have gained access to the body by ingestion, inhalation or absorption across the skin. Once in the body their effect may range from slight to the extremes of being a poison (e.g. cyanide) and carcinogen (e.g. benzene and vinyl chloride). Finally there is the special case of the use of radioactive compounds.
- ✓ ***Biological hazards:*** Examples include human body fluids that may carry infections such as HIV, laboratory animals that may cause allergic reactions or transmit certain diseases, pathogenic animal and cell tissue cultures, and all microorganisms including genetically engineered forms.
- ✓ ***Electrical and mechanical hazards:*** All electrical apparatus should be used and maintained in accordance with the manufacturer's instructions. Electrophoresis equipment presents a particular potential for safety problems. Centrifuges, especially high-speed varieties, also need careful use especially in the correct use and balance of the rotors.
- ✓ ***General laboratory hazards:*** Common examples include syringe needles, broken glassware and liquid nitrogen flasks.

Routine precautions that should be taken to minimise personal exposure to these hazards include the wearing of laboratory coats, which should be of the high-necked buttoned variety for work with microorganisms, safety spectacles and lightweight disposable gloves. It is also good practice not to work alone in a laboratory so that help is to hand if needed. In the UK, laboratory work is subject to legislation including the Health and Safety at Work Act 1974, the Control of Substances Hazardous to Health (COSHH) Regulations 1994 and the Management of Health and Safety at Work Regulations 1999. This legislation requires a risk assessment to be carried out prior to undertaking laboratory work. As the name implies, a risk assessment requires potential hazards to be identified and an assessment made of their potential severity and probability of occurrence. Action must be taken in cases where the potential severity and probability are medium to high. Such assessments require knowledge of the toxicity of all the chemicals used in the study. Toxicity data are widely available via computer packages and published

handbooks and should be on reference in all laboratories. Once the toxicity data are known, consideration may be given to the use of alternative and less toxic compounds or, if it is decided to proceed with the use of toxic compounds, precautions taken to minimise their risk and plans laid for dealing with an accident should one occur. These include arranging access to first-aiders and other emergency services. It is normal for all laboratories to have a nominated Safety Officer whose responsibility it is to give advice on safety issues. To facilitate good practice, procedures for the disposal of organic solvents, radioactive residues, body fluids, tissue and cell cultures and microbiological cultures are posted in all laboratories.

EXPERIMENT NO. 1

Quantitative determination of total carbohydrates from plant material by Anthrone Method

Principle

The Anthron reaction is the basis of a rapid and convenient method for the determination of hexoses, aldopentoses and hexuronic acids either free or present in polysaccharides. Carbohydrates are dehydrated by conc. H2SO4 to furfural. Furfural condenses with anthron to form a blue green colored complex which is measured colorometrically at 630 nm.

Reagent

- 2.5 N HCl
- Ethanol or Absolute Alcohol – 80% Solution.
- Anthrone Reagent (0.2%) – Dissolve 200 mg Anthrone (crystalline powder) in 100 ml of ice cold, 95% Sulphuric Acid (H_2SO_4, handle acid with care and prepare fresh before use).
- Glucose or Dextrose Standard Stock Solution (1.0 mg/ml): Dissolve 50 mg in 50 ml of distilled water an hour before doing the experiment, using standard volumetric flask.
- Working Standard solution (0.1 mg/ml): Dilute 10 ml of Standard Stock Solution to 100 ml with distilled water.

Procedure

- Weigh 100 mg of sample into a boiling tube.
- Hydrolyze the sample using 5.0 ml of 2.5 N HCl and place tube in a boiling water bath for 3 hours. Cool contents of the tube to room temperature.
- Neutralize it with solid sodium carbonate until the effervescence ceases (check pH using paper strip of narrow range i.e. pH 5.0 – pH 7.0).
- Make up the volume to 100 ml and centrifuge (at 5000 r.p.m. for 10 minutes).
- Collect the supernatant (liquid part) by decanting it into a dry, clean vessel and use it for analysis – take 0.5 & 1.0 ml aliquots for assay.

Note: In case of young, soft tissues like plant leaf materials, weigh 100 mg tissue, homogenize it in mortar-pestle using 10 ml of 80% absolute alcohol (Ethanol). Centrifuge homogenate at 5,000 rpm for 10 min. Collect the clear supernatants for assay (see Observation table).

Observation Table (Assay)

Sr. No.	Aliquot of Standard/ Sample (ml)	Distilled Water (ml)	Ice cold Anthrone Reagent (ml)		Dark Green color Absorbance at 630 nm
1	0.0	1.0	4 ml	Incubate tubes (at 100° C) for 8 min. And cool rapidly	
2	0.2	0.8			
3	0.4	0.6			
4	0.6	0.4			
5	0.8	0.2			
6	1.0	0.0			
Samples					
1	0.5	0.5			
2	1.0	0.0			

Calculation:

Draw a standard graph by plotting concentration of the standard (in mg or µg) on X-axis v/s Absorbance (or optical density) on Y-axis. From the graph, derive quantity of sugars (carbohydrate) present in give sample. Amount of sugars present

Amount of carbohydrate present in sample (Gm %) =

$$\frac{\text{Graph Factor x O.D. x Total Volume (ml)}}{\text{Aliquot of test sample x Wt of sample (gm) x 10,000}}$$

Results:

(Signature of Teacher)

EXPERIMENT NO. 2

Estimation of soluble proteins from plant leaf or grain samples by Lowry's Method

Principle

Proteins react with the Folin-Ciocalteau (FC) Reagent to give a blue coloured complex. The colour so formed is due to the reaction of alkaline copper with the protein (as in Biuret test) and the reduction of phosphomolybdate-phospotungustic compounds (of FC reagent) by tyrosine and tryptophan (Tyr and Try) residues present in the protein. A blue-purple colour is produced which can be quantified by its absorbance at 660 nm.

Materials

- 2% Na_2CO_3 in 0.1 N NaOH (Reagent "A").
- 0.5% $CuSO_4.5H_2O$ in 1% potassium sodium tartrate, aqueous (Reagent "B").
- Alkaline $CuSO_4$ solution – Mix 50 ml of 'A' and 1.0 ml of 'B' (as above 1 & 2) prior to use (working reagent 'C').
- Folin-Ciocalteau Reagent (Rgt. 'D') – Available commercially (dilute one part of the reagent with one part of dist. water i.e. 1:1 ratio prior to adding).
- Standard Protein solution - (Stock) Weigh 50 mg of Bovine Serum Albumin (BSA, Fr. V) and dissolve in distilled water, make up volume to 50 ml in a standard flask.
- Working standard – Dilute 10 ml of the stock solution (as prepared above) to 50 ml with distilled water in a standard volumetric flask. One ml of this solution contains 200 µg protein.
- All necessary glassware be kept dry, clean (and free from finger impressions) e.g. test-tubes and stands, filter paper, glass rod, pipettes (1.0 ml, 2.0 ml, 5.0 ml and 10.0 ml), measuring cylinders (50 ml/100 ml), volumetric and standard flasks etc.
- Vortex mixer, Spectrophotometer.

Extraction of protein from plant sample:

Usually, buffers are employed to extract proteins from plant material, esp. for enzymes. Weigh 500 m g sample and grind well at RT using mortar-pestle, wetting with 5-10 ml of the buffer. Centrifuge at 8,000 rpm for 10 min. Collect the supernatant for protein estimation. Perform the assay as given below.

Observation Table (Assay)

Sr. No.	Std/sample Aliquot (ml)	Dist. Water (ml)	Rgt. 'C' (ml)		Rgt. 'D' (ml)		Readings 660 nm
1	0.0	1.0					
2	0.2	0.8					
3	0.4	0.6		**Incubation 10 min**		**Incubation (in dark) 30 min.**	
4	0.6	0.4			0.5 ml		
5	0.8	0.2	5 ml				
6	1.0	0.0					
Samples							
1	0.2	0.8					
2	0.4	0.6					

Calculations:

Draw a standard curve on a graph paper and calculate the amount of protein in the sample (Take into account the dilutions made while conducting assay). Express the amount of protein in mg/g (or 100 g of sample, as the case may be).

Amount of Protein present in sample (Gm %) =

$$\frac{\text{Graph Factor x O.D. x Total Volume (ml)}}{\text{Aliquot of test sample x Wt of sample (gm) x 10,000}}$$

Results:

(Signature of Teacher)

EXPERIMENT NO. 3

To observe protein denaturation by various factors

Principle

Proteins tend to lose their biological activity due to an irreversible damage to their native structural conformations i.e., loss of organized tertiary (3D) structure and exposure to numerous hydrophobic groups in the aqueous environment provided. This is called denaturation and can be caused by heat, mineral acids (HCl, HNO_3), organic acids (perchloric and trichloroacetic acids), alkalis (NaOH) and heavy metals (sub-molar solutions of copper sulphate and lead acetate), as provided in this experiment. As a matter of principle, students will also learn how to prepare molar, normal, ppm and percent solutions that are routine in biochemical work.

Materials

- BSA (Fr. V) or Egg albumin (white portion) or Casein (hydrolysate) or any other protein source.
- Test tubes and stands, beakers (50 ml/100 ml/200 ml), pipettes (5.0 & 10.0 ml), measuring cylinders (50 ml, 100 ml), glass-rod, conical flasks, standard volumetric flasks etc.
- Solutions of hydrochloric acid (HCl, 1 to 5N), nitric acid (HNO_3 conc.), sodium hydroxide (NaOH, 1 to 5N), perchloric acid (5 to 10% PCA), trichloroacetic acid 910 to 20% TCA).
- Water bath (maintained at 60°C.
- Filter papers square (ordinary), reagent bottles (125 – 250 ml), other routine glassware.

Methodology

- **Test – I (Heat Denaturation)**

  Solution of Egg-white – (Sample protein) : The white from hen's egg is separated from the yolk (yellow part) and dissolved in 20 fold volume of distilled water, filtered through several layers of steel gauze and stored in refrigerator.

  OR

  Prepare a 5% aqueous saline solution of BSA or any other/purified protein preparation (e.g. casein powder, egg albumin flakes).

Take 5.0 or 10.0 ml of the prepared test (protein) solution in a clean, dry test tube. Take replications. Place the tube in the hot water bath (T=60°C) for 10 minutes. Remove the tubes and allow to cool at R.T. (26°-32°C) and observe coagulation of the proteins in solution (Note observations in Table).

- **Test – II (Denaturation by mineral acids)**

  1N – 5N solutions of HCl (about 5 drops) are added to a 2.0 to 5.0 ml protein test solution. Mix contents. Stand at R.T. for 10 to 15 minutes. Note the observation (Table).

  Add to a 2.0 ml protein test solution taken in a clean, dry tube, 2.0 ml of concentrated HNO_3 slowly by the sides of the tube. A muddy or off-white ring appears at the interface of both liquids indicating presence of protein. Shake the contents and allow to settle at R.T. (10-15 min.). Note the observation (Table).

- **Test – III (Treatment with NaOH)**

  To a 2.0-5.0 ml test protein solution, add about 5 drops of a 1-5N NaOH solution. Mix contents. Stand at R.T. for 10 min. Note the observation. Is your observation on the contrary to that seen for test-II ? State why, if so. (Record in Table).

- **Test – IV (Denaturation by organic acids)**

  Organic acids (such as 5% solution of PCA and 20% solution of TCA given) are added 9about 5 drops) individually to a 2.0 to 5.0 ml test protein solution taken in separate tubes. Mix contents. Stand at R.T. for 15 min. and note observations (Table).

- **Test – V (Denaturation by heavy metal salt solutions)**

  Add about 5 drops of the given heavy metal solutions (0.1 M each of copper sulphate and Lead acetate0 to a 2.0 to 5.0 ml of the test protein solution. Mix the contents. Stand at R.T. for 15 min and take observations (Table).

Observation Table

Tube No.	Samples taken (ml)	Treatment done	Observations	Remarks
1	5.0	Heat (60°-80°C) for 10 min.	Protein in solution coagulated.	
2	2.0 to 5.0	5 drop of 1 to 5N HCl, mix.	Protein in solution precipitated.	
3	2.0 to 5.0	5 drops of 1 to 5N NaOH mix.	Protein in solution dissolved.	
4	2.0	2.0 ml conc. HNO_3 added, by side of test tube. Mix contents.	Muddy ring ppt. of protein at interface; Off white ppt. turn yellow with time.	
5	2.0 to 5.0	5 drops of 5% PCA. Mix contents	White ppt.	
6	2.0 to 5.0	5 drops of 20% TCA. Mix contents	White ppt.	
7	2.0 to 5.0	5 drops of 0.1M copper sulphate solution Mix contents	White ppt.	
8	2.0 to 5.0	5 drops of 0.1 M Lead acetate solution. Mix contents.	White ppt.	

Results: According to observations noted in Table above.

(Signature of Teacher)

EXPERIMENT NO. 4

Estimation of Free Fatty Acids from Oil/Fat samples

A small quantity of free fatty acids is usually present in oils along with the triglycerides. The free fatty acid content is known as acid number/ acid value. It increases during storage. The keeping quality of oil therefore relies upon the free fatty acid content.

Principle

The free fatty acid in oil is estimated by titrating it against KOH in the presence of phenolphthalein indicator. The acid number is defined as the mg KOH required to neutralize the free fatty acids present in 1g of sample. However, the free fatty acid content is expressed as oleic acid equivalents.

Materials

- 1% phenolphthalein in 95% ethanol
- 0.1 N Potassium hydroxide (KOH)
- Neutral solvent: Mix 25mL ether, 25mL 95% alcohol and 1mL of 1% phenolphthalein solution and neutralize with N/10 alkali.

Procedure

- 1 Dissolve 1-10 of oil or melted fat in 50mL of the neutral solvent in a 250 mL conical flask.
- Add a few drops of phenolphthalein.
- Titrate the content against 0.1N potassium hydroxide.
- Shake constantly until a pink colour which persists for fifteen second is obtained.

Calculation

- Acid value (mg KOH/g) = $\dfrac{\text{Titre value x Normality of KOH} \times 56.1}{\text{Weight of the sample (g)}}$

The free fatty acid is calculated as Oleic acid equivalents using the equation 1mL N/10 KOH=0.028g oleic acid

❖ Free fatty acid content (gm) (Oleic Acid Equivalents) = $\frac{\text{Titre value (mL) x 0.028}}{\text{Wt. of the sample (g)}}$

Results:

(Signature of Teacher)

EXPERIMENT NO. 5

Estimation of Total Phenol content from Plant samples

Phenols, the aromatic compounds with hydroxyl groups are widespread in plant kingdom. They occur in all parts of the plants. Phenols are said to offer resistance to diseases and pests in plants. Grains containing high amount of polyphenols are resistant to bird attack. Phenols include an array of compounds like tannins, flavonols etc. Total phenol estimation can be carried out with the Folin-Ciocalteau reagent.

Principle

Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce blue colored complex (Molybdenum blue).

Materials

- 80% Ethanol Solution
- Folin-Ciocalteau Reagent
- 20% Na_2CO_3 solution
- Standard (100 mg Catechol in 100ml water)
- Dilute 10 times Standard Stock for preparing working standard.

Procedure

- Weigh exactly 0.5 to 1.0g of the sample and grind it with a pestle and mortar in 10-time volume of 80%ethanol.
- Centrifuge the homogenate at 10,000rpm for 20 min. save the supernatant. Re-extract the residue with five times the volume of 80% ethanol, centrifuge and pool the supernatants.
- Evaporate the supernatant to dryness.
- Dissolve the residue in a known volume of distilled water (5mL).
- Pipette out different aliquots (0.2 to 2 mL).
- Add 0.5mL of Folin-Ciocalteau reagent.
- After 3 min, add 2mL of 20% Na_2CO_3 solution to each tube.
- Make up the volume in each tube to 3mL with water.
- Mix thoroughly. Place the tube in a boiling water for exactly one min, cool and measure the absorbance at 650nm against a reagent blank.
- Prepare a standard curve using different concentration of Catechol.

Calculation

From the standard curve find out the concentration of phenols in the test sample and express as mg phenols /100g material.

Result:

(Signature of Teacher)

EXPERIMENT NO. 6

Estimation of titrable acidity from different samples

Principle

Acidity of foods is usually determined by acid, base titration using standard sodium hydroxide. The reaction being between a weak acid and a strong alkali, phenolphthalein is used as the end point colour indicator, which produces a faint pink colour around pH 8. It is determined by titration with an alkali, where the amount of alkali required to change the pH from acidic to slightly alkaline is found. This change in pH is noted with the help of an indicator which gives different colours reaction with various pH.

Organic acids react with sodium hydroxide to form their corresponding sodium salts. The common organic acids are mono- carboxylic (acetic acid, lactic acid), dicarboxylic (malic and tartaric acids) and tri-carboxylic (citric acid) acids. The general reaction between an organic acid and sodium hydroxide is as follows.

$$R\text{-}(COOH)n + n\ NaOH = R\text{-}(COONa)n + n\ H_2O$$

By knowing the equivalent weight of the acid, the acid content can be calculated based on the alkali required for neutralization.

For judging the sourness of any fruit, acidity determination is necessary. In this method, simple neutralization is carried out using standard (0.1 N) alkali in presence of phenolphthalein as an indicator. The degree of acidity is determined as the number of ml of standard alkali (0.1 N NaOH) required to neutralize known volume of juice.

Equipment and Apparatus

- Chemical balance
- Conical flask – 250 ml - 3 each
- Beaker – 100 ml - 1 each
- Volumetric flask – 100 ml - 1 each
- Burette – 10 ml - 1 each
- Pipette – 5 and 10 ml - 1 each

Reagents

- 0.1 N NaOH: Dissolve 4 gm NaOH in 1000 ml distilled water.
- 1% Phenolphthalein indicator: Dissolve 1 g phenolphthalein in 100 ml alcohol.

Sample preparation

- **Thin Juices, RTS beverages etc.:** Mix thoroughly and filter through previously washed and dried muslin cloth. Use 5-10 ml for titration.
- **Fresh fruits & vegetables, dried fruits, preserves, jams, marmalades pickles etc.:** Pulp the material in a blender or mortar and mix thoroughly. Accurately weigh 10 to 20 g of the pulped material in a beaker, add about 50 ml distilled water and boil gently for 15 to 30 min replacing the water lost by evaporation. Cool, transfer to a volumetric flask (say 100 ml) and make up the volume. Filter through Whatman No.1 filter circle, if necessary.
- **Fruit pulps, squashes, syrups, cordial etc.:** Weigh 10-20 g of the material, mix with distilled water, heat on steam bath to dissolve, cool and make up the volume in a volumetric flask (say 100 ml).

Procedure

- Pipette out suitable aliquot (5-10ml) of the prepared sample (quantity depending on the acidity of the sample) into a 250 ml. conical flask.
- Add about 50 ml of distilled water and few drops of phenolphthalein indicator.
- Titrate to light pink end point with 0.1 N NaOH solution.
- Products like juices and beverages may be directly weighed (5-10g) and transferred into 250 ml conical flask with about 50 ml distilled water and titrated.
- At the end point, the colour changes to the light pink. Calculate the percent titrable acidity present in the lime juice.

Observations

Where samples are boiled with water and made up to volume:

- Weight of sample = W = ---------- g
- Volume made up to = V_1 = ----------ml
- Volume of aliquot taken for titration = V_2 = ----------ml
- Volume of NaOH required = V_3 = ---------- ml
- Normality of the NaOH solution = 0.1

Where sample is weighed and directly taken for titration:

- Weight of sample = W_1 = ---------- g
- Volume of NaOH required = V_4 = ---------- ml
- Normality of the NaOH solution = 0.1

Calculations

1000 ml 1N NaOH = One gram equivalent of Organic acid.

Calculate the acidity in terms of the predominant acid present in the product. The equivalent weights of some common organic acids and the foods in which they are the major acids are given below. However, unless specifically required, it is customary to calculate the acidity of food materials as anhydrous citric acid.

Organic Acid Equivalent Wt. (E.W.) & Respective Foods

Anhydrous citric acid	64	Citrus fruits
Malic acid	67	Raw mango, apple
Tartaric acid	75	Grapes, tamarind
Lactic acid	90	Milk foods
Acetic acid	60	Vinegar containing foods

For samples boiled with water and made up to volume:

$$\% \text{ Acid} = \frac{\text{E.W. of acid} \times \text{Titer} \times \text{Normality of NaOH} \times \text{Volume made up} \times 100}{1000 \times \text{Aliquot taken} \times \text{Weight of sample}}$$

$$= \frac{\text{E.W. of acid} \times V_3 \times 0.1 \times V_1}{10 \times V_2 \times W}$$

For sample taken directly for acidity estimation:

$$\% \text{ Acid} = \frac{\text{E.W. of acid} \times V_4 \times 0.1}{10 \times W_1}$$

For carbonate beverages, expel carbon dioxide by warming or just heating to boil, cool and then titrate.

Result

Sample Detail:

Percent Acidity of the given Sample (w/w) = _____

(Signature of Teacher)

EXPERIMENT NO. 7

Estimation of Vitamin-C by titrametric method

Ascorbic acid or Vitamin C is sugar acid and an antiascorbutic. It is required for normal formation of connective tissue collagen., specifically for hydroxylation of certain proline and lysine residues. It is required for normal iron metabolism. It is strong reducing agentlosing hydrogen atom readily to become dehydroascorbic acid which has also Vitamin C activity. Ascorbic acid acts as an antioxidant and pro tects the cell membrane from the toxic action ofpowerful oxidizing agents. Ascorbic acid foumd abundantely in barriers, fresh fruits like citrus, guavas, chilles and green leafy vegetable.

Principle

The titrimetric method is a redox titration method which depends on the reduction of the blue dye 2,6 dichlorophenolindophenol to a colourless leuco compound by ascorbic acid. Ascorbic acid, is a strong reducing agent because of which it reduces the dye 2,6 dichlorophenolindophenol and itself gets converted to dehydroascorbic acid. After equivalence point is reached the next drop of dye gives a pink colour to the solution indicating the end point. The dye in this titration is coloured in the oxidized form and colourless in the reduced form. Also the dye is pink in acidic solution and blue in alkaline solution.

In acidic solution the pink dye is reduced to a colourless substance in presence of ascorbic acid. The solution remains colourless as more dye is added until all the ascorbic acid has reacted. As soon as the next drop of dye solution is added the solution becomes light pink due to excess dye indicating that the end point of the titration has reached.

Reagents

- **4 % oxalic acid:** Dissolve 4 gm of oxalic acid in 100 ml of water (10 ml = 1 mg)
- **2, 6 – Dichlorophenol indophenol (Dye solution):** Dissolve 0.05 gm of 2,6 – dichlorophenol indophenol in 50 ml of distilled water to which 42 mg of sodium bicarbonate is added. Make up the total volume to 200 ml.
- **Standard stock ascorbic acid solution:** Dissolve 100 gm of commercial L – ascorbic acid solution in 100 ml of 4 % oxalic acid.
- **Working standard:** Dilute 10 ml of stock standard solution to 100 ml of 4% oxalic acid. The concentration of working standard is 100 μg/ml.

Procedure

- Pipette out 5ml of the working standard solution into a 100 ml conical flask.
- Add 10 ml of 4% oxalic acid and titrate against the dye (V1ml). End point is the appearance of pink colour which persists for few minutes. The amount of the dye consumed is equivalent to the amount of ascorbic acid.
- Extract the sample (0.5 g – 5.0 g depending on sample) in 4% oxalic acid and make up to a known volume (100 ml) and centrifuge.
- Pipette out 5 ml of supernatant, add 10 ml of 4% oxalic acid and titrate against the dye (V2 ml).

Calculation

Amount of Ascorbic acid

(mg/100g sample) = [0.5mg/V_1 ml] X [V_2/1.5 ml] X [100 ml/Wt. of the sample] X 100

Result

Sample Detail:_____

Vitamin C (Ascorbic Acid) content of the given Sample = _____ mg per 100 ml

(Signature of Teacher)

EXPERIMENT NO. 8

Estimation of Chlorophyll 'a' and 'b' content in green leaves

Principle

The estimation of chlorophyll A and B is based on their absorption at 642.5 and 666 nm. Both the pigments absorb at both the wavelengths but the absorption of chlorophyll 'a' is more at 663 nm and that of 'b' is at 645 nm. Other pigments of leaves do not absorb at these wavelengths.

Apparatus, Equipments & Reagents

- Pestle – Morter
- Spectrophotometer
- Vol. Flaks
- Acetone (85 %)

Procedure

- Weigh 100 mg of green leaves and grind in a pestle mortar. Add 5 ml of 85 % acetone and again grind. Grind the residue again with 15 ml of 85 % acetone and transfer the supernatant to a 50 ml vol. flask. Suspend the residue in 10 ml of 85 % acetone, transfer the clear supernatant with other supernatant and make up the total volume to 50 ml with 85 % acetone.
- Measure the absorbance of the supernatant at 663 and 645 nm on spectrophotometer and calculate chlorophyll a and b as well as total chlorophyll.

Observations

- Fresh weight of tissue extracted (in g): W: _____
- Final Volume of extract in 80% Acetone (in ml): V:_____
- Absorbance at 663: _____
- Absorbance at 645: _____

CALCULATIONS

- mg Chlorophyll 'a'/g tissue = $12.7\ (A_{663}) - 2.69\ (A_{645}) \times \frac{V}{1000 \times W} = \_\_\_\_\_$

- mg Chlorophyll 'b'/g tissue = $22.9\ (A_{663}) - 4.68\ (A_{645}) \times \frac{V}{1000 \times W} = \_\_\_\_\_$

- mg Total Chlorophyll /g tissue = $20.2\ (A_{663}) + 8.02\ (A_{645}) \times \frac{V}{1000 \times W} = \_\_\_\_\_$

Result:

(Signature of Teacher)

EXPERIMENT NO. 9

Extraction of Plant Genomic DNA by CTAB Method

Principle

Isolation of DNA is a pre-requisite for its use in various experiments of molecular biology (e.g. cloning, preparation of genomic library and DNA hybridization). Isolation of DNA is accomplished by the rupturing of cell walls and nuclear membranes (from given plant sample) at the cellular level, followed by deporteinization and precipitation of the nucleic acid component using ethanol. Whichever method is followed, the recovery of DNA from plant material taken is very important. For this, the gentlest possible technique of cell rupture must be followed to prevent DNA from fragmenting by mechanical shearing when power-driven shear forces are employed along with use of chemicals like EDTA (which chelate the Mg^{2+} needed for DNases). Tissue disruption followed by other subsequent steps should be performed at 4°C, using glasswares and solutions that have been autoclaved.

Materials:

- Buffer Solution (To prepare 100 ml, take following components)

Chemical components	Stock conc.	Amount taken	Final concentration
Tris. pH 8.0	2.0 M	5.0 ml	100 mM
EDTA (in above buffer)	0.5 M	4.0 ml	20 mM
NaCl	5.0 M	28 ml	1.4 M
Double Dist. Water	-	63 ml	
Total		100 ml	

- 2% CTAB (N,N,N,N-ethyl trimethyl ammonium bromide): Take 2.0 g (or less depending upon requirement) of CTAB powder, first dissolved in 1.0 ml of Distilled water and make up to 100 ml by adding buffer solution as prepared above.
- ß-mercaptoethanol: Prepare a 14.4 M stock and take 70 µl to make a final concentration of 10 mM (add freshly prepared solution).
- Chloroform : Isoamyl Alcohol solvent mixture (in the ratio of 24:1).

- 5M NaCl (aqueous).
- 3M Sodium Acetate (aqueous).
- Chilled 70% Ethanol or absolute alcohol or ethyl alcohol (EtOH, aqueous).
- 1 x TE buffer :

Chemical components	Stock conc.	Amount taken from stock (for 100 ml)	Final Concentration
10 mM Tris, pH 8.0	2.0 M	0.5 ml	10 mM
1 mM EDTA (in buffer)	0.5 M	200 µl	1 mM

- RNase (10 mg/ml per stock).
- 95% EtOH (aqueous)
- 0.1 x TE buffer :

Chemical components	Stock conc.	Amount taken from stock (for 100 ml)	Final Concentration
1 mM Tris, pH 8.0	2.0 M	0.5 ml	1 mM
0.1 mM EDTA (in buffer)	0.5 M	20.0 ml	0.5 M

Methodology

- Grind 1.0 g tissue in Liq. N_2 (commercially available in regulated-flow cylinders / cans).
- Transfer the powder in 50 ml centrifuge tube containing 10 ml of 2% CTAB buffer and add 7 µl of ß-mercaptoethanol. Keep at 60°C for about half an hour.
- Perform Chloroform : Isoamyl Alcohol (24:1) extraction once/twice and centrifuge at 6000 rpm for 15 min. at room temperature (RT).
- Pipette out supernatant to a fresh tube and add ½ volume of 5M NaCl and 1/10 volume of 3M Sodium Acetate.
- Precipitate DNA with 5.0 ml of chilled 70% EtOH.
- Mark centrifuge tubes with the probable location of pellet formation.
- Centrifuge for 3 min. at 3000 rpm and continue for next 3 min. at 5000 rpm.
- Wash DNA pellet in chilled 70% EtOH.

- After drying, suspend the DNA pellet in 1 x TE and transfer the suspension to Micro-centrifuge tube using wide-bore pipette tip.
- Centrifuge again at 3000 rpm at 4°C for 5 min to remove any left debris.
- Add 1 µl of RNase (from 10.0 mg/ml stock) to 100 µl DNA suspension and incubate at 37°C for one hour.
- Add MQ (autoclacesd double dist. water) water to make up the volume to 500 µl.
- Perform Chloroform : Isoamyl Alcohol (24:1) using it's equal volume (=500 µl) extraction; extract twice and remove upper phase using wide bore tips.
- Precipitate DNA in 1/10 Sodium Acetate and 95% EtOH (add 5.0 M NaCl, if necessary).
- Centrifuge at 10,000 rpm for 10 min at 4°C and wash pellet thrice with 95% EtOH.
- Dry pellet and elute in 0.1 x TE and quantify on gel or spectrophotomatrically.

Precautions

- Grind plant tissue in liquid nitrogen rapidly and transfer the powder to extraction buffer without thawing.
- Carefully observe precipitation of DNA while adding isopropanol.
- Mark the centrifuge tube at the bottom towards outside of centrifuge rotor where pellet will be lodged during centrifugation.
- Do not agitate solution of DNA to avoid shearing. DNA pellet should be dried after ethanol wash before dissolving in 1 x TE.

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EXPERIMENT NO. 10

Quantification of Plant Genomic DNA (Spectrophotometric Method)

Principle

DNA quantification is an important step in many procedures where it is necessary to know the amount of DNA that is present when carrying out restriction digestion or performing other exercises such as polymerase chain reaction (PCR). For quantitating the amount of DNA or RNA, readings are taken at wavelengths of 260 nm and 280 nm. The reading at 260 nm allows calculation of the concentration of nucleic acid in the sample. An optical density (OD) value of 1 corresponds to ca.50 µg ml^{-1} for double-stranded DNA, 40 µg ml^{-1} for single-stranded DNA and RNA, and ca.33 µg ml^{-1} for single stranded oligonucleotides. The ratio between the readings at 260 nm and 280 nm (OD_{260}:OD_{280}) provides an estimate of the purity of the nucleic acids. Pure preparations of DNA and RNA have OD_{260}:OD_{280} values of 1.8 and 2.0, respectively.

Materials

- Genomic DNA
- Tris – EDTA (TE) buffer
- Spectrophotometer with UV range

Methodology

- Take 2 µl of the DNA sample in a quartz cuvette. Make up the volume to 1 ml with T E buffer.
- Measure absorbance of the solution at wavelengths at 260 and 280 nm.
- Calculate DNA concentration using the relationship for double stranded DNA, 1 OD at 260 nm = 50 µg ml^{-1}.
- Calculate the ratio OD_{260}: OD_{280}.

Observations:

Sample No.	Optical Density (OD)	Concentration of DNA ($\mu g\ ml^{-1}$)	Concentration of DNA ($\mu g\ \mu l^{-1}$)

[

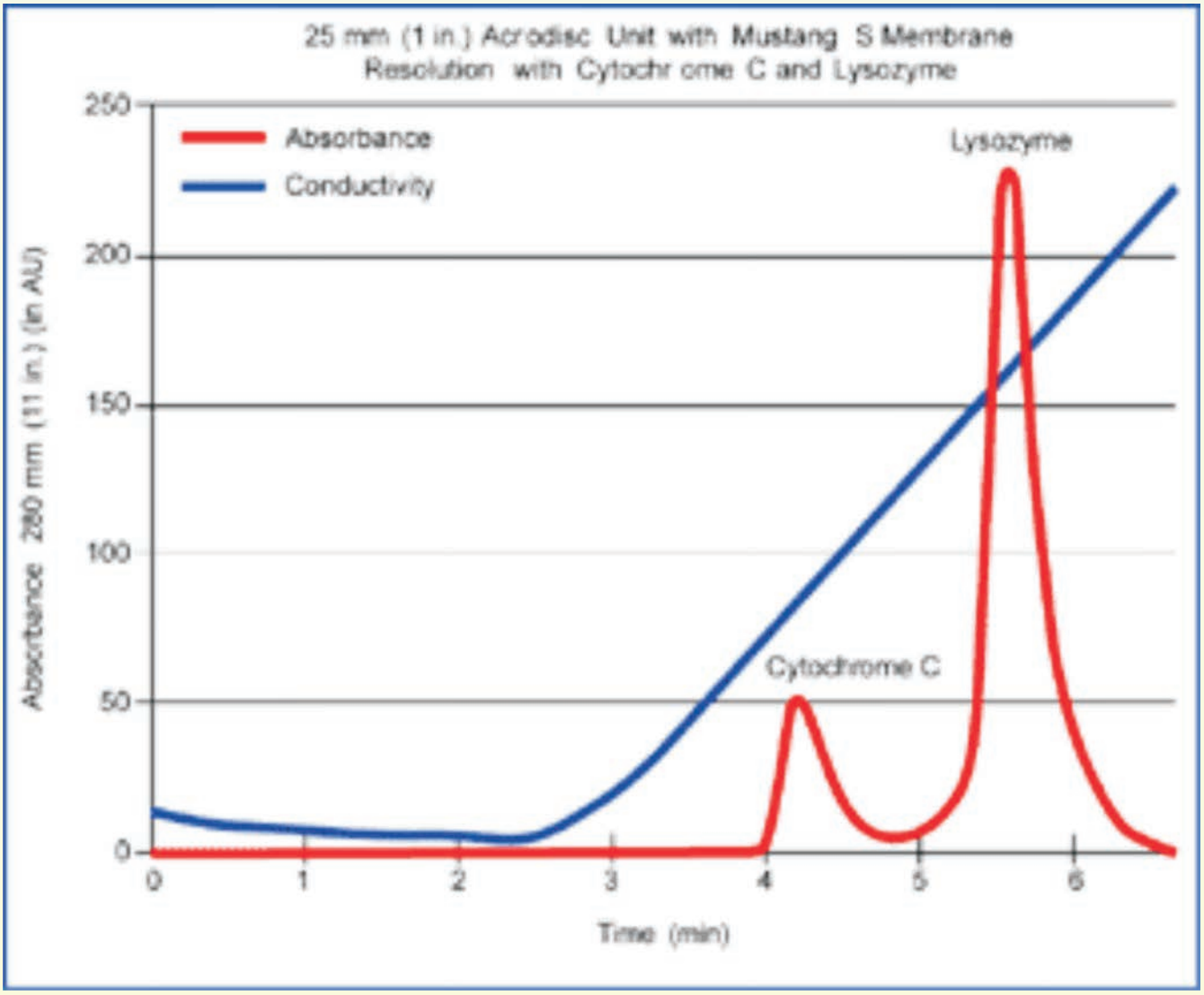
Calculation

- If 1 OD at 260 nm = 50 µg ml^{-1} then _____ OD = _____ X 50 µg/ml
- Dilution factor = 1 ml/2 µl = 500.
- DNA Concentration (µg/µml) = 50 x OD x Dilution factor / 1000

Result:

(Signature of Teacher)

BIOCHEMISTRY


25 mm (1 in.) Acrodisc Unit with Mustang S Membrane
Resolution with Cytochr ome C and Lysozyme
Absorbance
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PRACTICAL MANUAL

ICAR

Production Technology for Fruit and Plantation Crops (1+1)

HORT. 2.2

Department of Horticulture
College of Agriculture
Navsari Agricultural University
Waghai-394 730 (Dangs), Gujarat

Prepared and Compiled By:
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NAVSARI AGRICULTURAL UNIVERSITY

Practical Manual

ICAR

Production Technology for Fruit and Plantation Crops

Course No.: Hort. 2.2

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FOREWORD

It is a matter of great pleasure to write and forward the **Practical Manual** of the course entitled **"Production Technology for Fruits and Plantation Crops"** prepared by **Dr. Binayak Chakraborty, Assistant Professor (Horticulture), College of Agriculture, NAU, Waghai**. Indian agriculture is witnessing a gradual change particularly in the cropping system, land use system, input utilization, marketing and above all the monetary returns. The horticulture has gained importance in recent years as a significant component of agriculture in India as it has emerged as one of the potential enterprises in accelerating economic growth and alleviating the poverty of country. Besides providing wide range for crop diversification to the farmers, it also plays very important role for sustaining large number of Agro-industries creating a huge employment opportunity for the people.

This practical manual would certainly provide complete information regarding botanical aspects, planting material and propagation method, field management as well as post harvest technology for fruits and plantation crops. This manual has been prepared following the latest syllabus and knowledge; so probably fulfils the requirements of B.Sc. (Hons.) Agriculture students for the above mentioned course. However, it is equally useful for the post graduate students, scholars, teachers and scientists working on the applied aspects of these dollar earning crops.

Place : Waghai
January, 2019

Dr. Z. P. Patel
Principal & Dean
College of Agriculture, Waghai
Navsari Agricultural University

CERTIFICATE

Uni. Seat No._____

Registration Number_____

This is to certify that Mr./Miss. _____ of third semester B.Sc. (Hons.) in Agriculture has satisfactorily carried out _____ exercises as shown in the practical manual of **Hort. 2.2 (Production Technology for Fruits and Plantation Crops)** at Horticulture Department during the year 20_____.

(Course Teacher)

Head of Department

Signature of External Examiner

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Experiment No. 01	Date:

Description and Identification of Fruit Crops

Objective: To know the major and minor fruit crops cultivated in India and acquire knowledge regarding origin and botanical description of those fruit crops.

Sr. No.	Common Name	Botanical Name	Family	Origin	Type of Fruit	Edible part
1	Mango	*Mangifera indica*	Anacardiaceae	Indo-Burma	Drupe	Mesocarp
2.	Banana	*Musa paradisiaca*	Musaceae	Indo-Burma	Berry	Mesocarp + Endocarp
3.	Acid lime	*Citrus aurantifolia*	Rutaceae	Probably India	Hesperidium	Juicy placental hair
4.	Mandarin Orange	*Citrus reticulata*	Rutaceae	Indo-China	Hesperidium	-do-
5.	Sweet Orange	*Citrus sinensis*	Rutaceae	Indo-China	Hesperidium	-do-
6.	Pumello	*Citrus maxima* syn. *C. grandis*	Rutaceae	Indo-China	Hesperidium	-do-
7.	Grape	*Vitis vinefera*	Vitaceae	Caspean sea reigon	Berry	Pericarp
8.	Papaya	*Carica papaya*	Caricaceae	Mexico	Berry	Mesocarp
9.	Guava	*Psidium guajava*	Myrtaceae	Mexico	Berry	Pericarp
10.	Pomegranate	*Punica granatum*	Punicaceae	Iran	Balausta	Aril
11.	Sapota	*Achrus sapota*	Sapotaceae	Mexico	Berry	Mesocarp
12.	Custard apple	*Annona squamosa*	Annonaceae	Mexico	Aggregate fruit	Mesocarp
13.	Aonla	*Phylanthus emblica*	Euphorbiaceae	India	Drupe	Epicarp and mesocarp
14.	Ber (Indian)	*Zizyphus mauritiana*	Rhamnaceae	India	Berry	-do-
15.	Jamun	*Syzygium cumini*	Myrtaceae	India	Berry	-do-
16.	Date palm	*Phoenix dectylifera*	Palme	Iraq	Berry	-do-
17.	Pineapple	*Ananus comosus*	Bromeliaceae	Brazil	Multiple fruit (Sorosis)	Perianth
18.	Jack fruit	*Artocarpus heterophyllus*	Moraceae	India	Multiple fruit (Sorosis)	Perianth
19.	Litchi	*Litchi chinensis*	Sapindaceae	South China	Nut	Aril
20.	Apple	*Malus* × *domestica*	Rosaceae	Asia Minor	Pome	Thalamus
21.	Strawberry	*Fragaria* × *annanasa*	Rosaceae	(Cultivated strawberry developed in France)	Etario of achene (Aggregate Fruit)	Thalamus/ Receptacle Tissue

Question:

1. **List out the fruit crops belongs to the family Rutaceae and Rosaceae.**

2. **List out the fruit crops having aggregate and multiple type of fruit.**

3. **List out the fruit crops of Indian and Mexican origin.**

4. **Write down the edible part of pomegranate, mango, sapota, acid lime and strawberry.**

5. **List of the drupaceous and berry fruits cultivated in India.**

Experiment No. 02	Date:

Description and Identification of Plantation Crops

Objective: To know the major and minor plantation crops cultivated in India and acquire knowledge regarding origin and botanical description of those plantation crops.

Sr. No.	Common Name	Botanical Name	Family	Origin	Chromosome No.	Economic part
1	Coconut	*Cocos nucifera*	Palmae	South East Asia	2n = 32	Copra and liquid Endosperm,
2.	Areca nut	*Areca catechu*	Palmae	Philippines	2n = 32	Kernel
3.	Cashewnut	*Anacardium occidentale*	Anacardiaceae	Brazil	2n = 42	Kernel
4.	Coffee	*Coffea Arabica* and *Coffea canephora*	Rubiaceae	Ethiopea	2n = 44 2n = 22	Kernel
5.	Tea	*Camellia sinensis*	Theaceae	Assam (India)	2n = 45	Two leaf and a bud
6.	Rubber	*Hevea brasilensis*	Euphorbiaceae	Brazil	2n = 36	Latex
7.	Oil palm	*Elaeis guineensis*	Palmae	West Africa	2n = 32	Kernel and Mesocarp
8.	Cocoa	*Theobroma cacao*	Sterculiaceae	Central America	2n = 20	Karnel
9.	Palmyrah Palm (Toddy palm)	*Borassus flabellifer*	Palmae	India	2n=36	Mesocarp and Kernel
10.	Betel vine	*Piper betle*	Piperaceae	India	2n = 26, 32, 42, 52, 58, 64, 68, 78	Leaf

Question:

1. **Differentiate between Arabica and Robusta coffee**

2. **Enlist the masticatory plantation crops.**

3. **Enlist plantation crops which are used as beverages.**

4. **Write the uses of coconut palm.**

5. **Write the full form of CNSL and list out the uses of CNSL.**

Experiment No. 03	Date:

Description and Identification of Varieties of Some Important Fruit Crops

Objective: To study the characteristics features of different varieties of fruit crops cultivated in India.

• MANGO

1. **Alphonso**: (Mid-season variety)
   Growing belt: Maharashtra (Ratnagiri Hill areas) and South Gujarat
   Other names: Badami, Gundu, Khader, Appas, Happus and Kagdi Happus

   Other characters: This is only the export quality variety of mango grown in India. The fruit of this variety is medium in size, ovate oblique in shape, orange yellow in colour and excellent in taste. It has been found good for canning purpose. It is susceptible to spongy tissue disorder.

2. **Bangalora**: (Mid-season variety)
   Growing belt: South India
   Other names: Totapuri, Kallamai, Burmodilla, Killi Mukku and Gilli Mukku

   Other characters: The fruit size is medium to large with prominent sinus. Fruit quality is poor but keeping quality is very good. It is widely used for processing.

3. **Banganapalli**: (Early-season variety)
   Growing Belt: South India, Main commercial variety of Andhra Pradesh and Tamil Nadu
   Other names: Chapta, Safeda, Baneshan and Chapai

   Other characters: Fruit is large in size and obliquely oval in shape. The colour of the fruit is golden yellow. Fruit quality and keeping quality are good. It is good for canning.

4. **Bombai:** (Early-season variety)
   Growing belt: Bihar and West Bengal
   Other names: Malda

   Other characters: Fruit size is medium, shape ovate oblique and colour is yellow. Fruit quality and keeping quality are medium.

5. **Bombay Green**: (Earliest variety in North India)
   Growing belt: Uttar Pradesh, Bihar and West Bengal
   Other names: It is also known as Malda

   Other characters: Fruit size is medium, shape ovate oblong and fruit colour is spinach green. Fruit quality is good and keeping quality is medium. It is highly susceptible to mango malformation.

6. **Chausa:** (Late-season variety)
   Growing belt: North India

   Other characters: It is the sweetest mango variety grown in India. Fruits are bright yellow with soft pulp. It is shy bearer.

7. **Dashehari**: (Mid-season variety)
   Growing belt: Uttar Pradesh and Bihar

   Other characters: This variety derives its name from the village Dashehari near Malihabad district of Lucknow. It is a leading commercial variety of north India and one of the best varieties of our country. The fruit size is small to medium, shape is oblong oblique and fruit colour is yellow. Fruit quality is excellent and keeping quality is good.

8. **Himsagar**: (Early-season variety)
   Growing belt: West Bengal

   Other characters: This variety is indigenous to Bengal. This is one of the choicest varieties of Bengal and has gained extensive popularity. Fruit is of medium size, ovate to ovate oblique in shape. Fruit colour is yellow. Fruit quality and keeping quality are good.

9. **Kesar**: (Early-season variety)
   Growing belt: Gujarat

   Other characters: It has red blush on the shoulders. Fruit size is medium, shape oblong and keeping quality is good. This variety also has export quality.

10. **Langra**: (Mid-season variety)
    Growing belt: North India

    Other characters: This variety is indigenous to Varanasi area of Uttar Pradesh. It is extensively grown in northern India. Fruit is of medium size, ovate shape and lettuce green colour. Fruit quality is good but keeping quality is medium. Highly susceptible to fruit drop.

11. **Mulgoa**: (Late season variety)
    Growing belt: South India

    Other characters: It is quite popular among the lovers of mango owing to high quality of fruits. Fruits are large in size, roundish oblique in shape and yellow in colour.

12. **Neelum**: (Late season variety)
    Growing belt: South India

    Other characters: This commercial variety indigenous to Tamil Nadu. It has excellent keeping quality and good combing ability with other varieties. Hence, it is used in breeding purposes.

HYBRIDS:

Name	Place of origin	Parentage	Important characters
Amrapali	IARI, New Delhi	Dashehari x Neelum	It is dwarf, regular bearing and late maturing variety. The variety is suitable for high density planting as about 1600 plants may be planted in a hectare. It yields on an average of 16 tonnes/hectare
Mallika:	IARI, New Delhi	Neelum x Dashehari	It is from a cross of. Its fruit is large in size, oblong elliptical in shape and cadmium yellow in colour. Fruit and keeping quality are good. It is a mid season variety
Arka Aruna	IIHR, Bangaluru	Banganapalli x Alphonso	It is dwarf, regular bearing and precocious. Fruits are large having attractive skin colour with red blush and free from spongy tissue. Suitable for homesteads as well as high density planting.
Ratna	Fruit Research Station, Vengurla	Neelum x Alphonso	This hybrid is from a cross of. Tree moderately vigorous, precocious, fruits are medium sized, attractive in colour and free from spongy tissue
Sindhu	FRS, Vengurla	Ratna x Alphonso	It is from a cross of. It is regular bearer, fruits medium sized, free from spongy tissue with high pulp to stone ratio and very thin and small stone (Seedless)

• BANANA

1. Dwarf Cavendish: (AAA)

Growing belt: Maharashtra, Gujarat, Bihar and West Bengal. Also popular in Tamil Nadu, Karnataka and Andhra Pradesh

Other Names: Basrai, Bhusawal, Kabuli, Pacha Vazhai, Singapuri

Important Character: It is a popular commercial cultivar grown extensively for table and processing purposes. The bunch size, the fruit length and size is quite good though the keeping quality is rather poor. The thick rind of the fruits retains to some extent the greenish colour even when the fruits are ripe. In combination with high-density planting and drip irrigation,

Dwarf Cavendish is becoming a highly successful cultivar. It is highly susceptible to Sigatoka leaf spot disease and resistant to wind damage due to dwarf stature.

2. Robusta: (AAA)

Growing belt: Tamil Nadu, Andhra Pradesh, Maharashtra and some parts of Karnataka
Other Names: Bombay Green, Pedda Pacha Arati

Important Character: It is a semi-tall variety. It is a high yielding and produces bunch of large size with well developed fruits. Dark green fruits turn bright yellow upon ripening depending on ripening conditions. It requires propping. Fruit has a poor keeping quality leading to a quick breakdown of pulp after ripening, hence not suited for long distance transportation. Robusta is highly susceptible to Sigatoka leaf spot disease in humid tropics.

3. Rasthali: (Silk AAB)

Growing belt: Tamil Nadu, Andhra Pradesh, Kerala, Karnataka, Bihar and West Bengal
Other Names: Mortaman, Malbhog, Amrithpani, Rasabale

Important Character: Its unique fruit quality and aroma has made Rasthali popular and a highly prized cultivar for table purpose. Longer crop duration, severe susceptibility to *Fusarium* wilt, requirement of bunch cover to protect fruits from sun cracking and formation of hard lumps in fruits make crop production more expensive.

4. Poovan: (Mysore AAB)

Growing belt: Tamil Nadu, Andhra Pradesh, Kerala, Karnataka, Bihar and West Bengal
Other Names: Chini Champa, Champa, Dora Vazhai, Karpura Chakkarakeli

Important Character: It is also commercially cultivated for leaf industry throughout Tamil Nadu and in certain parts of Kerala. Fruit is slightly acidic, firm and has typical sour-sweet aroma. Fruits turn to attractive golden yellow on ripening. Medium sized bunch, closely packed fruits, good keeping quality and resistant to fruit cracking is its plus points. But it is highly susceptible to Banana Bract Mosaic Virus and Banana Streak Virus.

5. Nendran: (AAB)

Growing belt: Kerala and Tamil Nadu
Other Names: French Plantain, Rajeli and Bhorot

Important Character: Bunch has 5-6 hands weighing about 12-15 kg. Fruits have a distinct neck with thick green skin turning buff yellow on ripening. Fruits remain as starchy even on ripening. It is leading culinary variety in the world and very much suitable for chips making. Nendran is highly susceptible to Banana Bract Mosaic Virus (BBMV), nematodes and borers.

6. Red Banana: (AAA)

Growing belt: Kerala, Tamil Nadu, Andhra Pradesh and Karnataka
Other Names: Lal Velchi, Chandra Bale, Lal Kela

Important Character: The colour of the pseudostem, petiole, midrib and fruit rind is purplish red. It is a robust plant with bunches weighing 20-30 kg under good management practices. Fruits are sweet, orange yellow coloured and with a pleasant aroma. It is highly susceptible to bunchy top, fusarium wilt and nematodes.

7. Monthan: (ABB)

Growing belt: Tamil Nadu, Kerala, Andhra Pradesh, Orissa and West Bengal
Other Names: Bontha, Karibale and Kacha Kela

Important characters: It is used in processing. Used as vegetable throughout India. Monthan is a fairly tall and robust plant bearing bunches of 18-20 kg after 12 months. Fruits are bold, stocky, knobbed and pale green in colour. The skin is usually green. Apart from its culinary use of fruits, pseudostem core is a highly relished vegetable with many medicinal properties. Monthan is also cultivated for production of leaves in Trichy and Tanjore districts of Tamil Nadu. It has many desirable qualities like immunity to Banana Bunchy Top Virus (BBTV) diseases, salt tolerance and normal bunch mass even under marginal condition, but it is highly susceptible to *Fusarium* wilt disease.

8. Karpuravalli: (ABB)

Growing belt: Tamil Nadu, Kerala, Andhra Pradesh, Orissa and West Bengal
Other Names:Kanthali, Pisang Awak, Kosta Bontha

Important characters: It is a popular variety grown for table purpose in medium rich soils. It is also the sweetest among Indian bananas. Karpuravalli is occasionally seeded depending on the seasonal variability. Its ash coated golden yellow and sweet fruits have good keeping quality. This variety is also used to make banana wine. Karpuravalli is highly susceptible to wilt disease, tolerant to leaf spot disease and well suited for drought, salt affected areas and for low input conditions.

• CITRUS:

a) Mandarin Orange (*Citrus reticulata*)

1. Coorg Mandarin

Growing belt: Karnataka

Important Characters: Fruits oblate, colour bright yellow and uniform, rind medium thick with little adherence, segments usually between 9 to 11, pulp yellow with fine texture and abundant juice

2. Darjeeling Mandarin

Growing belt: Darjeeling (West Bengal) and Sikkim

Important Characters: Fruits are comparatively smaller in size somewhat flat in shape, colour yellowish to orange when fully ripe, rind thin, adherence little, juice abundant and sweet flavour.

3. Khasi Mandarin

Growing belt: Assam and Meghalaya

Important Characters: Fruits globose to oblate, surface smooth, colour orange-yellow to bright orange, rind thin with very little adherence, segments usually 10, pulp vesicles uniformly orange, texture coarse, juice abundant with well-blended flavour.

4. Kinnow

Growing belt: Punjab and western Rajasthan

Important Characters: Developed through hybridization between [*Citrus nobilis* (King) x *C. deliciosa* (Willow leaf)]. The fruit is medium-size somewhat oblate in shape, rind moderately thick, adherence with the pulp quite strong, thick mesocarp, easily peelable surface, smooth and glossy, fruit colour yellowish orange at full maturity, segments 9 to 10, firm, pulp yellowish orange, very juicy somewhat acidic. The variety is cold resistant.

5. Nagpur Mandarin

Growing belt: Maharashtra and Tamil Nadu

Important Characters: This is the finest mandarin orange variety grown in the world. Fruits are yellowish green to orange, oblate, rind thin, fine texture and good flavour and taste. Size is medium and the skin is easily peelable.

b) Sweet Orange (*Citrus sinensis*)

1. Jaffa: (Mid-season variety)

Growing belt: Punjab, Haryana and Rajasthan

Important Characters: It is a clone of the Palestine *beledi* seedling group identified by H.S. Sanford in 1883 in Florida. Being a cold tolerant, high-quality, mid-season cultivar, it quickly increased in popularity. However, 'Jaffa' has several serious faults including a strong tendency toward alternate bearing and susceptibility to *Alternaria*

2. Hamlin: (Early season variety)

Growing belt: Punjab, Haryana and Rajasthan

Important characters: It is a chance seedling near Glenwood by A.G. Hamlin in 1879 in Florida. Fruits are smaller in size, globule to slightly oblate, seedless (0-4) and sweet in flavour. The peel is smooth and thin. Well adopted and cold tolerant.

3. Valencia: (Latest maturing variety)

Growing belt: Punjab, Haryana and Rajasthan

Important characters: It is a United State origin variety identified by English nursery man Thomas Rivers in 1865. Tree vigrous upright and large, having alternate bearing tendency and very wide range of adaptation. Fruit possess abundant juice, good flavour but somewhat acidic. Fruit holds well on the tree with little deterioration in quality well past maturity. Well adapted in wide range of agro ecological conditions sub-tropical and high heat index areas.

4. Sathgudi:

Growing belt: Andhra Pradesh and Tamil Nadu

Important Characters: It is originated from Sathgur in Tamil Nadu. Fruit is medium, subglobose, areole absent. Rind is medium thick, smooth and finely pitted. The pulp is straw yellow in colour, juicy with good flavor.

5. **Blood Red Malta:**
Growing belt: Haryana and Punjab

Important Characters: it is introduced from Mediterranean region. Tree is comparatively dwarf. The colour of the pulp is streaked blood red when fully ripe, juicy and sweet. The fruits are sometimes dried on tree due to physiological disorder.

6. **Mosambi: (Early variety)**
Growing belt: Maharashtra

Important characters: Originated in Mozambique, highly popular in central India and Pakistan. Trees are vigorous, productive, upright, large and round in shape. Fruit surface is moderately to roughly pebbled, striped with narrow, longitudinal grooves and ridges. Flesh is straw-yellow in colour; somewhat firm and juicy. The flavour is insipid because of very low acidity.

7. **Pineapple (Mid-season variety)**
Growing belt: Punjab, Haryana and Rajasthan

Other characters: This variety originated as a chance seedling on the place of J.B. Owens at sparr in 1860 in Florida. It is thornless and more sensitive to frost than other orange cultivars. Flesh colour light orange; tender, juicy, richly flavoured. Fruit matures during Dec-Jan. Excellent for processing. Well adapted to subtropical climate.

c) Acid Lime (*C. aurantifolia*)

1. **Pramalini:** It bears fruits in cluster of 3-7 and yields 30% more than the normal kagzi lime. The fruits have 57% juice, which is higher than Vikram (53%) and normal lime (52%).

2. **Vikram:** This also bears fruits in clusters of 5-10 and some off season fruits during September, May and June. It gives 30-32 % more yield over normal lime.

3. **Chakradhar:** It is a seedless strain of acid lime. The plants are erect, compact and dense in habit. Fruits are round, with thin papery rind, containing 60-66 % juice and almost seedless compared with 52-62% juice and 6-8 seeds/fruit in others. Bearing starts by 4^{th} year of planting. It bears fruit during January-February, June-July and September-October.

• GRAPE

1. **Anab-e-Shahi: (White seeded)**
Growing belt: Andhra Pradesh

Important Characters: The vines are very vigorous and heavy yielder. The fruit bunches are very attractive, medium large to large (400-600 g) medium long, cylindrical to long conical, neither shouldered nor winged, well filled to moderately compact. Berries are greenish yellow when raw and amber when fully ripe in colour, ovoid elongated in shape and medium large in size. Juice is clear and sweet. It is highly susceptible to rust, powdery mildew and downy mildew, moderately susceptible to anthracnose and resistant to *Cercospora* leaf spot. In Hyderabad, it has been found susceptible to stem borer even in very well maintained vineyards.

2. **Bangalore Blue: (Coloured seeded)**
Growing belt: Bangalore and Mysore area

Important Characters: It is a *vinifera* and *labrusca* hybrid. It is being extensively used for making juice and wine in Mysore State. In Mysore State, two commercial crops are being taken by the grape growers each in February-March and August-September. It is known for its hardiness and resistance to diseases. It has been found to be almost resistant to anthracnose and *Cercospora* leaf-spot, but susceptible to both powdery and downy mildews.

3. **Beauty Seedless: (Coloured seedless)**
Growing belt: Punjab, Haryana, Delhi and Uttar Pradesh

Important characters: It is an introduction from California. Ripening is fairly uniform, very early, end of May to first week of June in North India. It is likely to prove useful in the processing industry for juice and raisin making. It is not susceptible to cracking. Keeping quality is good. It is resistant to rust, moderately susceptible to anthracnose and *Cercospora* leaf-spot.

4. **Perlette: (White seeded)**
Growing belt: Punjab, Himachal Pradesh, Haryana, Delhi and Uttar Pradesh

Important characters: This variety was evolved at the University of California, Davis by Dr. H. P. Olmo. It is a hybrid of colokertek hiralynoje 26 x Sultanina marble and resulted from a cross made in 1936. Because of the transluscence of the mature fruit being its most striking feature, a name describing this character was sought. The French name 'Perlette' signifying 'little pearl' was selected. One of its major defects is the compactness of clusters. The defect increases thinning costs. It is susceptible to rust and downy mildew. It has been found to be moderately susceptible to *Cercospora* leaf-spot moderately to highly susceptible to anthracnose, but moderately resistant to powdery mildew.

5. **Pusa Seedless: (White seedless)**
Growing belt: Delhi, Punjab, Haryana, Rajasthan and Uttar Pradesh

Important characters: A selection made at the Indian Agricultural Research Institute, from Thompson Seedless. The berries are greenish white, ellipsoidal or ellipsoidal elongated, small, size moderately variable; bloom thin, easily removable; apex pointed, adherence medium strong. Pulp melting, watery, mild in flavour, veins prominent, very sweet. Juice clear, greenish, sweet. It is susceptible to rust and downy mildew, highly susceptible to anthracnose and *Cercospora* leaf-spot.

6. **Thompson Seedless: (White seedless)**
Growing belt: Maharasthra

Important characters: It is a *vinifera* grape, which originated in Asia Minor and was first grown in California by William Thompson near Yuba city. It is also called Oval Kishmish in the eastern mediterranean regions, and Sultana in Australia and South Africa. It is believed to be grown in every viticultural country of the world. It is a multipurpose grape. More than half of the world's raisins and about 95% of Californian raisins are made from this variety. Besides being a major raisin variety of the world, it is a leading table grape. Large quantities of white

dessert wines are also made from this variety. It is susceptible to rust and downy mildew and highly susceptible to anthracnose.

GUAVA

1. **Allahabad Safeda: (White fleshed)** This is considered as the finest guava variety grown in the world. It is popular among growers of Uttar Pradesh. Fruits are medium to large (average weight 150-200 g), round, smooth with yellowish skin at maturity. Fruits are soft when ripe with pleasant flavour and good keeping quality. The TSS, acidity and ascorbic acid contents are 12.6 and 0.41% and 172 mg/100 g pulp.

2. **Apple Colour: (Red fleshed)** This cultivar has been originated from Allahabad district of Uttar Pradesh. It is not heavy bearer, but grown because of its attractive colour and good quality fruits.

3. **Sardar (L-49): (White fleshed)** It has been evolved through selection made at poona. Trees are semi dwarf, vigorous, heavy branching type with flat crown. Plup is white with many seeds.

4. **Chittidar: (White fleshed)** Trees are tall with rounded crown, spreading branches and moderate bearing habit. Fruits are small to medium, subglobose in shape, straw yellow in colour with red spots of pin head size on fruit skin.

5. **Red Fleshed**: Vigorous and tall tree with spreading branches. Fruits are elepitic to oblong, medium sized, dawn pink flesh and smooth skin with few red dots on surface. Keeping quality is poor to medium.

LITCHI:

1. **Bombai:** This is an important commercial variety cultivated for table purpose in West Bengal. It ripens during first to second week of May. Average yield is 80 -90 kg/tree. Fruits are large in size, mostly obliquely heart-shaped colour at maturity with carmine red tubercles on uranium-green background. In this variety each developed fruit has another tiny under-developed fruit attached to the fruit stalk.

2. **Calcutta (Syn. Kalkattia):** It is the best of all the varieties grown in northern parts of India for table purpose. It can be successfully cultivated even in hotter areas provided there is protection from strong hot winds and provision for plenty of water for irrigation. The average yield is 80-100 kg/tree. It is a late season variety and the fruits ripen in the last week of June.

3. **Dehra Dun:** This variety is mainly grown for table purpose in Uttar Pradesh and Punjab. In Bihar, it is cultivated under the name of 'Dehra-Rose'. It is a late-season variety and fruits start ripening in third week of June. Fruit yield is 80-90 kg/tree. This variety is highly susceptible to sunburn and cracking.

4. **Early Seedless (Syn. Early Bedana):** It is the earliest variety and is mainly grown for table purpose and processing in Uttar Pradesh and Punjab. It is a medium to poor-yielding variety but bears regularly. The yield of fruit varies from 50-60 kg/tree.

5. **Kasba:** It is mostly grown in eastern part of Bihar. Fruits medium-large, heart-shaped with red tubercles on reddish background at maturity. It ripens during the first week of June. Average yield is 80/100 kg/tree.

6. **Late Seedless (Syn. Late Bedana):** This variety is not completely devoid of seed but it is rather shrivelled and small in size. It can be successfully cultivated even in hotter areas provided there is protection from strong hot winds and provision for plenty of water for irrigation. The average yield is 80-100 kg/ tree. It is cultivated for table and processing purpose.

7. **Rose Scented:** It is commercially cultivated for table purpose in Muzaffarpur area of Bihar. Besides high fruit quality, it is famous for distinct rose aroma and hence called Rose Scented. It is a mid-season variety that starts ripening in the first week of June. Average yield is around 80-90 kg/tree.

8. **Shahi:** This is one of the commercial cultivar cultivated for table purpose in Muzaffarpur area of Bihar, which matures, in the third week of May. Fruits are oval and oblong conical in shape and crimson-red tubercles appear on uranium-green background at maturity. It is heavy bearing variety with large fruits and average yield of 90-100kg/tree.

9. **Swarna Roopa:** It is a selection in litchi identified and released by CHES, Ranchi with high T.S.S and resistant to fruit cracking. This is the first variety developed in India.

PAPAYA

1. **CO. 5:** It is a selection from Washington and isolated for its high papain production. It produces consistently 14-15 g dry papain/fruit. It gives 75-80 fruits/tree in two years with an average yield of 1,500-1,600 kg dried papain/ha.

2. **Coorg Honey Dew:** Popularly known as 'Madhubindu' and is cultivated for table as well as processing purpose. The variety bears greenish-yellow oblong-shaped fruits with orange thick flesh and good flavour. The variety can be maintained pure by growing in isolation. Due to its excellent fruit quality it fetches good market value.

3. **Pusa Delicious (Gynodioecious):** This is a medium-tall plants, starts yielding 8 months after planting and has good quality fruits (10°-13° Brix). The fruit is medium-sized (1-2 kg) with deep orange flesh having excellent flavour. It is grown as a table purpose variety.

4. **Pusa Dwarf (Dioecious):** It is a dwarf plants and medium-sized (1-2 kg) oval fruits. The plant starts bearing from 25 to 30 cm above-ground level and is comparatively drought hardy. This variety is very suitable for high-density planting.

5. **Pusa Dwarf (Dioecious):** Medium size fruits, oval in shape and suitable for high-density planting.

6. **Pusa Giant (Dioecious):** Plants are vigorous, sturdy and tolerant to strong wind. It is a dioecious cultivar with big-sized (2.5-3 kg) fruits, suitable for canning industry.

7. **Pusa Majesty (Gynodioecious):** This variety is tolerant to viral diseases and root knot nematodes. The variety is suitable for papain production and is comparable to C0.2 variety for papain yield. The fruits are medium-sized, 1- 1.5 kg in weight, round in shape and have better keeping quality. It starts fruiting 146 days from the time of transplanting. The variety is tolerant to root knot nematode.

8. **Ranchi:** It is a variety from Bihar and popular in south India. The fruits are oblong with dark yellow pulp and sweet taste.

9. **Solo:** It is a table purpose variety. The fruits are small with deep pink pulp and a sweet taste. Excellent for kitchen garden.

10. **Washington (Dioecious):** It is a table purpose variety. Fruits are round to ovate, medium-large in size with few seeds. When ripe, skin attains a bright yellow colour. The average weight of fruit ranges from 1.5-2 kg.

SAPOTA

1. **Calcutta Round:** It is commercially grown in West Bengal, Karnataka and other states. The foliage is light green in colour. Fruits are large but the flesh is gritty and of moderate quality.

2. **Chaatri:** It is similar to Ka1ipatti but the branches have a droop- ping nature similar to that of an umbrella. The branches appear in all directions horizontally in whorls. The leaves are light green in colour. Fruits are similar to Kalipatti but the fruit quality is not as good as in Kalipatti.

3. **Cricket Ball:** It is also known as 'Calcutta Large'. It is grown in Tamil Nadu, Karnataka, Maharashtra, West Bengal and Andhra Pradesh. The leaves are light green. This bears the largest sized fruits which are round in shape. The pulp is gritty, granular and not very sweet.

4. **Kalipatti:** It is a leading variety of Maharashtra, Gujarat, and north Karnataka. It has dark green, broad and thick leaves. Fruits are oval, less seeded with a sweet, mellow flesh of excellent quality. Fragrance is mild. Each fruit has 1-4 seeds. Fruits appear singly and the main harvest is in winter.

5. **Oval:** The fruits are small to medium sized and oval to egg shaped. The pulp is coarse-grained and less sweet.

Questionnaires:

1. **List down hybrids of mango with their parentage.**

2. **Cite some examples of dioecious and gynodioecious varieties of papaya.**

3. **On the basis of your own observation write the best varieties of important fruit crops (Mango, Banana, Guava, Mandarin Orange, Sapota, Grape and Litchi) suitable for growing in India.**

4. **Write cracking resistant varieties of litchi and papain yielding varieties of papaya.**

5. **List down early, mid and late season varieties of mango and sweet orange.**

6. **Write some varieties of mango, papaya and banana suitable for high density planting.**

7. **Briefly describe distinguishing features of mandarin orange, acid lime and sapota varieties (at least three each).**

8. **Suggest some grape varieties which could be suitable for growing in nothern and southern parts of India separately.**

9. **Separate mango varieties suitable for growing in northern, southern, eastern and western parts of India.**

10. What are the white and red fleshed varieties of guava, give a complete list of them.

Experiment No. 04	Date:

Description and Identification of Varieties of Some Important Plantation Crops

Objective: To study the characteristics features of different varieties of fruit crops cultivated in India.

A. COCONUT:

1. East Coast Tall (ECT):

Soil: Well drained deep sandy loam, alluvial and red loamy soils are ideal

Time take for bearing: 6 to 8 years

Average Yield: 70 nuts / palm / year

Copra content: 125 gram / nut, the range between 100 and 140 gram

Oil Content: 64 per cent

Growing Belt: TN, AP, Bihar, Pondicherry, Orissa, MP, Andamans and WB.

Special Features: The nuts are smaller than West Coast Tall

2. West Coast Tall (WCT) :

Other Name: Ordinary or Common Tall Variety

Soil: WCT palm grows in all type of soil, especially grow well in littoral sand as well as in the interior and is somewhat tolerant to moisture stress in the soil

Time take for bearing: 6 to 7 years

Average Yield: 80 nuts / palm / year

Copra content: 176 gram/ nut, the range between 135 and 200 gram

Oil content: 68 per cent

Growing Belt: TN, Kerala, KA, Gujarat, BR, MP, Lakshadweep, Orissa and Tripura.

Special Features: It can prefer for both edible purpose and soap manufacture. Toddy is also prepared.

3. Chowghat Orange Dwarf (COD):

Time take for bearing: 3 to 4 years

Average Yield: 65 nuts / palm / year

Copra content: 150 gram / nut

Oil content: 66 per cent

Growing Belt: Kerala, Karnataka and Tamil

Special Features: Susceptible to high wind and drought.

4. Chowghat Green Dwarf (CGD):

Time take for bearing: 3 to 4 years

Average Yield: 66 nuts / palm / year

Copra content: 60 gram / nut

Oil content: 66 per cent

Special Features: The leaf petioles, leaves and nuts are dark green in colour. The nuts have the characteristic 'beak' when fully mature. Root wilt disease tolerant variety.

B. ARECANUT

1. Mangala (VTL - 3): It is a selection, from China and released for cultivation by Arecanut Research Station, Vittal (Kerala). It is semi-tall type and bearing starts after 3-5 years of planting. It has a number of desirable characters such as early bearing, early stabilization, high fruit set and yield. Its mean yield is 2 kg *Chali* (10 Kg ripe nuts) per palm per year. The nut possesses good quality attributes.

2. **Sumanagala (VTL-11):** It is introduced from Indonesia. It has all the desirable characters of VTL. Its mean yield is 33 Kg., Challi (17:5 Kg rupee nuts) /palm /year. This selection is also reported to be tolerant to burrowing nematode, *Radopkolus similes.*

3. Sreemangala (VTL - 17): It is a selection from Singapore. Its annual mean yield *is* 3.1 Kg Chali (15.6 Kg raw nuts)/palm/year. It also tolerates *Radopholns similes.*

4. Mohitnagar: This is grown in the Northern part of West Bengal. The nut is very big and uniform and much similar to Kahikuchi. It yields better than other selections. At Kidu farm in Karnataka it yielded 3.7 kg. chali (19.5 Kg. ripe nuts) per palm per year.

C. COCOA: Types of Cocoa

1. Criollo: This is considered to be the "Prince of Cocoas" and used as an ingredient in the very finest of chocolates. It is native to Central and South America as well as the Caribbean islands and contributed only 5.0 % of total world production. Since, Criollo is extremely susceptible to environmental threats. The pod colour is red and beans are white to pale pink colour.

2. Forastero: This is the most widely grown cocoa in the world and contributed about 80.0 % of total world cocoa production. It is thought to be native to the Amazon basin. Forastero is mainly grown in Africa, Ecuador and Brazil. The popularity of this cocoa is due to it is harder than Criollo and less susceptible to diseases. The yield is also higher than Criollo. Forastero cocoa has purple-coloured beans and is mainly used to give chocolate its full-bodied flavor. The pod colour of Forastero cocoa is yellow. There are many Forastero subspecies: Amelonado, Cundeamor and Calabacillo. Amelonado cocoa is the most extensively planted Forastero cocoa.

3. Trinitario: Trinitario is a natural hybrid of Criollo and Forastero. It was first came into existence on the Island of Trinidad. Trinitario combines the best of the two other main varieties: the hardiness and high yield of Forastero and the refined taste of Criollo. The quality of the cocoa varies between average and superior. It is the predominant fine flavor cocoa. Trinitario populations are usually variable in pod and bean characteristics because the parents have highly contrasting characters. They can now be found in all the countries like Mexico, the Caribbean islands, Colombia, Venezuela, and in parts of Southeast Asia.

D. CASHEW NUT

Name	Apple Colour	Kernel weight (g)	Shelling %	Selection /hybrid	Nut yield (kg/tree)	Region
Jhargram-1	Yellow	1.50	30.00	Selection	8.50	WB
Bhubaneswar-1	Pinkish Yellow	1.67	27.99	Selection	12.00	Orissa
Goa-1	Yellow	2.20	30.00	Selection	7.60	Goa
Vengurla-1	Yellow	1.39	31.00	Selection	19.00	MH
Vengurla-4	Red	1.91	31.00	Hybrid	17.20	MH, GJ
Vengurla-7	Yellow	2.90	30.50	Hybrid	18.50	MH, GJ
Chintamani-1	Yellowish Red	2.10	31.00	Selection	7.20	KA
NRCC-1	Red	2.10	28.80	Selection	10.00	KA
NRCC-2	Pink	2.15	28.60	Selection	9.00	KA
Ullal-1	Yellow	2.05	30.70	Selection	16.00	KA
Ullal-2	Red	1.83	30.50	Selection	9.00	KA
Ullal-3	Dark Red	2.10	30.00	Selection	14.70	KA
Ullal-4	Yellow	2.10	31.00	Selection	9.50	KA
BPP-1	Yellow	1.37	27.50	Hybrid	10.00	AP
BPP-2	Yellow	1.04	25.70	Hybrid	11.00	AP
BPP-3	Yellow	1.34	28.10	Selection	11.00	AP
BPP-4	Yellow	1.15	23.00	Selection	10.40	AP
BPP-5	Yellow	1.25	24.00	Selection	11.00	AP
BPP-6	Yellow	1.44	24.00	Selection	10.50	AP
BPP-8	Yellow	1.89	29.00	Hybrid	14.50	AP
Akshaya	Yellow	3.12	28.36	Hybrid	11.78	KE
Amrutha	Yellow	2.24	31.58	Hybrid	18.35	KE
Anagha	Orange Red	2.90	29.00	Hybrid	13.73	KE
Anakkyam-1	Pinkish Yellow	1.67	27.99	Selection	12.00	KE
Dhana	Yellow	2.44	29.80	Hybrid	10.66	KE
Dharasree	Yellowish Pink	2.40	30.50	Hybrid	10.66	KE
Kanaka	Yellow	2.08	30.58	Hybrid	12.80	KE
Madakkathara -1	Yellow	1.64	26.80	Selection	13.80	KE
Madakkathara -2	Red	1.88	26.00	Selection	17.00	KE
Priyanka	Yellowish Red	2.87	26.57	Hybrid	17.03	KE
Sulabha	Light Orange	2.88	29.40	Selection	21.90	KE
Vridhachalam-1	Yellow	1.40	28.00	Selection	7.20	TN
Vridhachalam-2	Pinkish Yellow	1.45	28.30	Selection	7.40	TN
Vridhachalam-3	Red	2.16	29.10	Selection	11.68	TN

WB=West Bengal, MH= Maharashtra, GJ= Gujarat, KA= Karnataka, AP= Andhra Pradesh KE= Kerala, TN = Tamil Nadu,

E. OIL PALM: Types of Oil palm

1. Dura: Thick Shelled

2. Pisifera: Shell-less

3. Tenera: Thin Shelled

1. List out the Tall and Dwarf varieties of coconut

2. Write down the introduced and Indian varieties of areca nut

3. Enlist the cashew nut varieties suitable for growing in Gujarat.

4. List out the hybrid varieties of cashew nut

5. Enlist the types of oil palm and cocoa

6. Differentiate between Criollo and Forastero cocoa

Experiment No. 05	Date:

Seed Propagation: Scarification and Stratification of Seeds

Objective: To study the seed propagation of fruit crops

Seed: A seed is a small embryonic plant enclosed in a covering called the seed coat, usually with some stored food. Seed in fruits forms after fusion of male and female germ cells or gametes. Characters of both parents are inherited by the seed. Therefore the new individuals formed in this way are not true-to-type i.e., they are not like parents and moreover they are unlike each other.

Parts of a Seed:

1. **Seed Coat/Testa**: The seed coat consists of one or more protective layers that cover the seed.
2. **Embryo:** An embryo is the young, minute/rudimentary multicellular plant structure that present in seed. Embryo has two parts:
   i. **Epicotyl:** Epicotyls is the portion of the embryonic stem above the point at which the stem is attached to the cotyledon(s)
   ii. **Hypocotyl:** Hypocotyl is the portion below the point of attachment. The hypocotyl is connected to the radicle.
3. **Endosperm:** The endosperm is a source of stored food, consisting primarily of starches.
4. **Cotyledon:** The cotyledon is described as a seed leaf that stores food in the form of starch and protein for use by the embryo. In some seeds cotyledon act as endosperm.

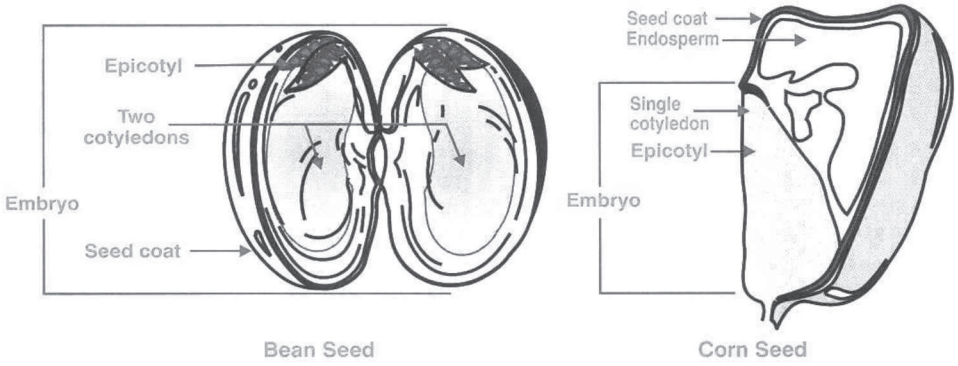


Fig. 1: Parts of Seed

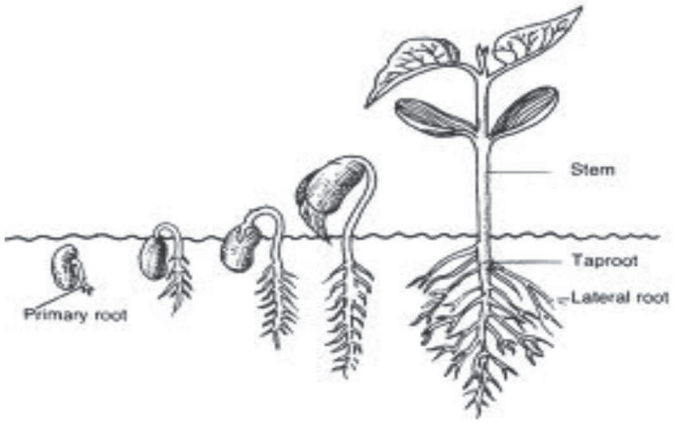
Terms Related to Seed Propagation:

1. **Germination:** Germination incorporates those events that commence with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis.
2. **Viability:** Capacity to germinate is known as viability
3. **Vitality:** The health and vigour of embryo is called as vitality
4. **Emergence:** Appearance of seedling above the soil surface is known as emergence

5. **Dormancy:** Seed dormancy is defined as the failure of an intact viable seed to complete germination under favorable conditions
6. **Hypogeal germination:** Seeds leave the cotyledons in the soil upon germination. Examples: *Pisum*, *Zea*.
7. **Epigeal germination:** Cotyledons are elevated above the soil upon germination. Examples: Phaseolus *Linum* and many other dicots.

Events during Seed Germination:

1. Absorption of water
2. Activation of enzymes
3. Degradation of complex starchy material
4. Translocation of food
5. Increase respiration and elongation of cell
6. Growth of radicle followed by plumule



Seed dormancy: The viable seed do not germinate even under favourable condition like moisture, air, temperature and light. Some seed have very hard seed coat which does not easily become soft.

Methods to overcome seed dormancy:

1. **Scarification: (Breaking of seed coat):** Scarification is the process of physically or chemically altering the seed coverings to improve germination in dormant seeds. It is a horticultural necessity for species with physical dormancy (hard, impermeable seed coats) to permit water uptake. Three types of treatments are commonly used as scarification treatments. These include mechanical, chemical, and heat treatments.
   a) Mechanical Treatment: Breaking of seed coat
   b) Chemical Treatment: Thiourea, KNO_3, Gibberellic acid are used
   c) Heat Treatment: In nature, physical dormancy appears to be relieved most often by high temperature exposure. This process can be mimicked by placing seeds on moist or dry sand at temperatures above 35°C (95°F).
2. **Soaking in water**: Seeds of some species are soaked in water for a particular period to enhance germination
3. **Stratification**: **(Cold temperature treatment):** Stratification is a method in which seeds are subjected to a period of chilling or warm temperatures to alleviate dormancy conditions in the embryo. eg. Seeds like apple, peach, pear etc.

1. **Justify acid lime is propagated through seeds.**

Experiment No. 06	Date:

Commercial Method of Propagation of Fruit Crops

Objectives: To study the commercial methods of major fruit crops grown in India

A. Propagation methods of Mango:

1. **Inarching :**
   For the actual inarching operation, the stock is brought close to the scion. A thin slice of bark about 6-8 cm long about 8mm in thickness at a height of about 20 cm above ground level is removed with a sharp knife from the stock. A similar cut is made in scion. Thus the cambium layer of both stock and scion are exposed. These cuts area brought together and tied firmly with the help of poly ethane strip.
2. **Softwood grafting:**
   In this technique, grafting is done with mature, procured scion on the emerging soft coppery red shoot of rootstock. The leaves on the stock must be retained while grafting to high success. The technique is effective on dry, hot weather or in area of low ppt. Arid and semi arid regions in arching grafting are not well established due to disturbing of tap root system so that in situ soft grafting is recommended.

B. Propagation of Banana:

(1) ***Suckers:***

Sword suckers Leaves are pointed, narrow & upright. Rhizome is conical with sound heart**.** Growth is vigorous and fast. Bearing is early (11 months.).Sword suckers are the best.

Water suckers: Leaves are Broader, spreading with roundish tip. Rhizome is flat with non-sound heart. Growth is slow. Late bearing (15 months).

Planting of suckers: Sword suckers of 3-4 months old separated from mother rhizome and planted.

(2) ***Rhizome:*** Except coastal region of Saurashtra, usually rhizome planting is followed.

Planting of Rhizome:- Pseudostem of sucker is completely removed from rhizome. Such rhizomes are stored under shade in cool & dry place for 2 months. The conical rhizomes with sound heart & few side buds are used for Planting.

C. Propagation of Acid lime:

1. *Seed.* Sour lime is mainly propagated by seed. Propagation by seeds is followed due to polyembryonic nature of it. In sour lime about 78 per cent seeds possess polyembryos. Each polyembryonic seed produces three to four seedlings. Among them, one is sexual origin and remaining are nucellar. Nucellar or apogamic seedlings breed true to type. Polyembryonic.

Raising of seedling:- Freshly extracted seeds are sown on well prepared nursery bed at the distance of 20 x l0 cm and 1.5 to2cm deep. Germination completes within 3 weeks. The sexual

seedlings, which are usually stunted and poor in growth compared to apogamic or nucellar seedlings, are uprooted carefully. Removal of sexual seedlings is essential because they do not breed true to type. The apogamic seedlings are identical to the parent in growth and production. Seedlings are ready for transplanting 6 to 9 months after sowing. However, usually one year old seedlings are preferred for transplanting. Seeds are sown in seedbed during July- August.

D. Propagation of Guava:

1. **Air layering:** During rainy season (June to September) because of high rooting and survival of layers (80-85%). For air layering 1 year old branches of 1 to 1.5 m length are selected.

   A ring of bark of 2.5 to 3.0 cm long is removed from 45 cm away from the tip. For better rooting IBA (root promoting plant growth hormone) @ 4000-5000 ppm is applied at the upper cut end of girdled area. The girdled area is covered with moist sphagnum moss and wrapped with polythene tape. Rooting starts within 3-5 weeks.

   After 6 to 8 weeks when sufficient roots are visible through the transparent tape, the layer is removed from the branch by means of cutting the stem below the girdled area in stages.

   The polythene film is removed from the finally severed rooted stem and some of the top is headed back which is then potted and kept in the shade until new leaves appear. When the new flushes are produced, the plants can be transplanted in the field.

E. Propagation of Sapota

Time of grafting: Approach grafting should be preferably done in the beginning of rainy season or during February-March.

Preparation of rootstock: In this method 2-3 year old 'Khirni' or 'Rayan' plants raised in polythene bags are used as rootstocks due to their strong root system. These seedlings should be at least 45-60cm in height and 1 cm in diameter at the time of grafting.

Preparation of Scion: The mother plants selected for approach grafting should be vigorous true-to-type and between 10-15 years old. The lower most branches are usually bent and tied to a peg near the ground. 1-1 / year old 2 branch (scion) of pencil thickness is selected for grafting.

The formation of union: The seedling of 'Khirni' (rootstock) is brought closer to the branch near the ground. The cambium layers of one side of this plant at 10-15cm from the base of the plant is exposed by taking a 5-6cm long and 0.5cm wide cut with a sharp knife. Similar cut is taken on the selected branch of the mother plant. The exposed portion of both the plants are brought together and secured firmly with polythene strip.

Care and management of grafts:

1. Avoid gap between the joints of rootstock and scion.
2. The union of the scion and the stock takes place in about 2-3months. However, the scion should not 2 be separated for at least 3-4 months.
3. The root stcok should be watered every day for the first 15 days and thereafter, at an interval of 3 days till the graft is separated from the mother plant. T

4. The rooted graft is slowly detached by giving 2-3 successive cuts 2cm below the union over a period of month before finally detaching from the parent plant.
5. The grafts are maintained in a nursery under shade for few days.

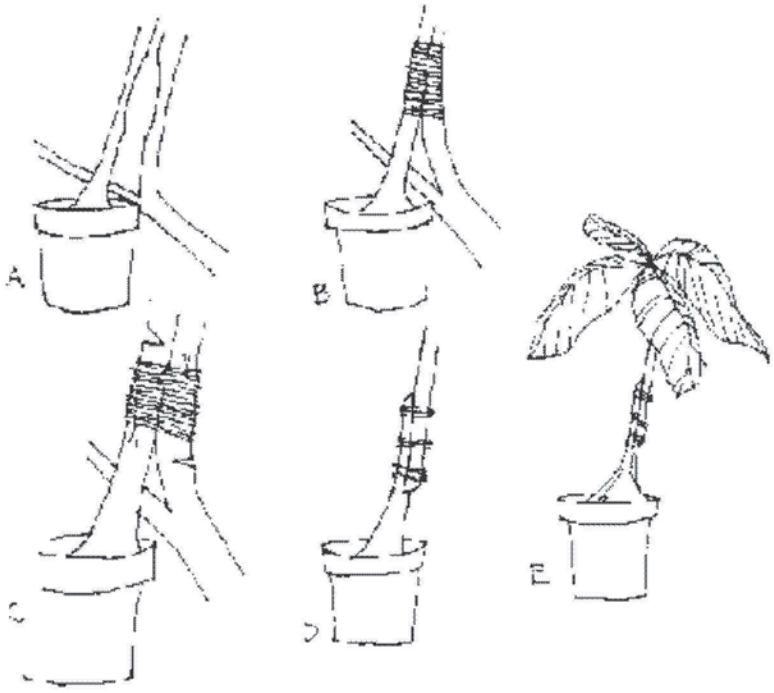


Fig. 1: Inarching in mango

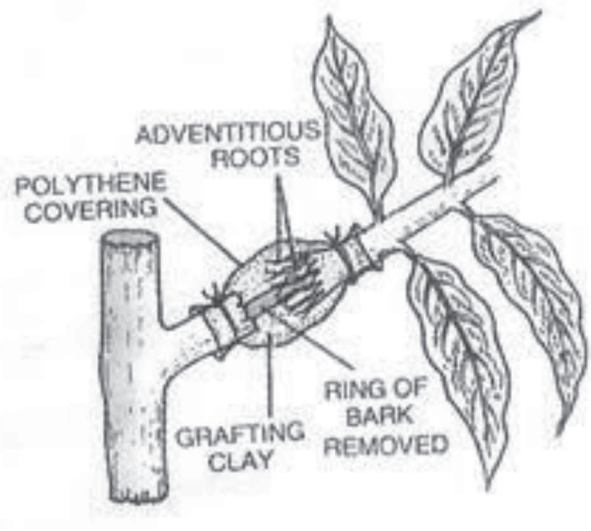


Fig. 2: Air layering in guava

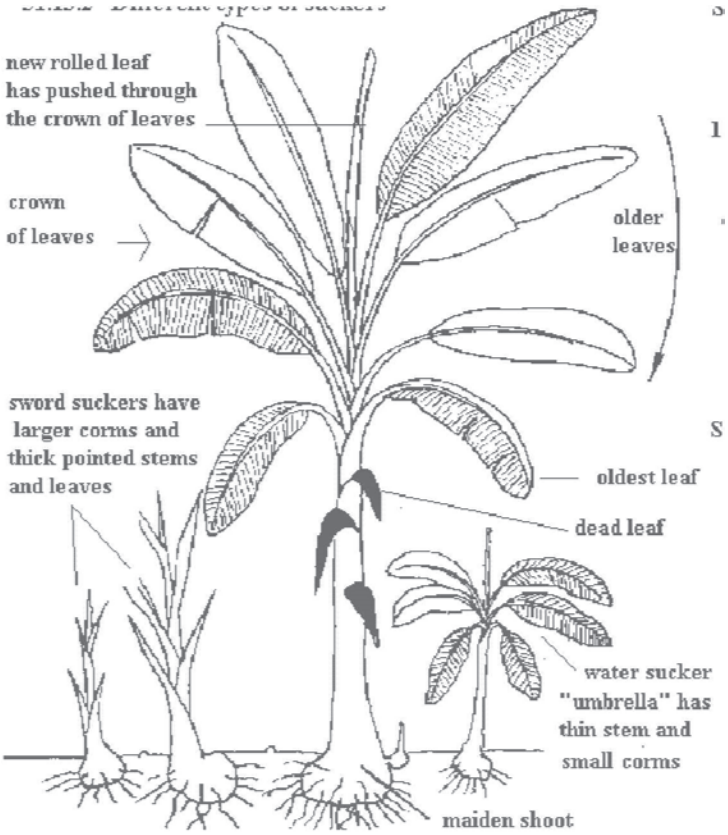


Fig. 3: Different types of suckers in banana

1. **Justify papaya is propagated through seeds**

2. **Justify *Khirni* is the best rootstock for sapota.**

3. **Differentiate between Scion and Rootstock.**

Experiment No. 07	Date:
Commercial Method of Propagation of Plantation Crops	

A. Propagation method of Coconut:

Propagation - 1. Sexual – Seeds 2. Asexual - Tissue culture

It is commercially propagated through sexually (seed nut). Seeds are selected from elite mother palm because the crop is cross pollinated; variation is there in sexual propagation. To avoid variation up to some extent or to get genetically superior material it is required to select the mother palm and selection of seed nut and seedlings.

Selection of Mother palm:

1. Crown should be spherical, drooping. Erect crown should be avoided.
2. Length of petiole and Bunch stalk should be short and stout.
3. Nut should be round and spherical medium in size.
4. Trees producing barren nuts should be avoided
5. Palm should have 30 to 40 fully opened leaves and 12 to 15 bunch with a high setting of female flowers.
6. Palm should be in age group of 25 to 40 years, yield is more than 80 nuts/ year
7. Palm growing close to house, cattle sheds compost pit should be avoided.
8. Mother palms selected should be free from pest and diseases.

Collection of nuts:

1. The nut should be 11 to 13 months old with full maturity.
2. Nut should be oblong in shape medium in size. Rest for 1 to 2 months.

Nursery management of coconut:

Coconut being cross pollinated plant, mother plant is selected with utmost care. Nursery should be near a water source. Field should be worked to fine tilth. Field is laid out into raised, long and narrow beds to accommodate 4-5 rows at 30 x 30 cm. Seeds are planted in the nursery at the beginning of monsoon in shallow trenches of 20-25 cm depth at 30 x 30 cm. Planted either horizontally or vertically with husk visible. Horizontal planting is preferable because seedlings will be robust. Suffer less damage, exhibit higher germination and vigorous growth. Irrigated adequately after planting. Nursery needs regular watering, weeding and plant protection for getting early and maximum germination. Seed nuts treated with 0.2%. BHC is applied against termites.

Nuts germinate within 10-12 weeks. In 5 months maximum seed nuts would germinate. Seedlings are distributed for planting at 9-12 months.

Selection of seedling for planting:

1. Early germination
2. Rapid growth,
3. Early splitting leaves
4. Vigour of growth
5. Free from pests and disease.

6 Thick collars

7. Seedling is of 9 to 12 months old.

B. Propagation method of Cashew nut:

Different methods of propagation have been tried in cashew nut *viz*. epicotyl grafting, soft wood grafting, veneer grafting, side grafting, layering, patch budding etc. Among them, soft wood grafting has been found to be the best for commercial multiplication of cashew since success rate is the maximum over other propagation methods.

Various steps involved in soft wood grafting are given below

Selection of Seed Nuts

1. Seed nuts may be collected during the peak period of harvest (February-March) and sundried for two to three days.
2. Viable seed nuts may be selected by immersing the nuts in water or 10% saline solution. Seeds which sink in water may be selected.
3. Medium sized nuts (7-9 g) may be selected to get vigorously growing seedlings.
4. Fresh seed nuts are to be used for raising root stocks. More than one- year old seed nuts may be avoided.

Raising Root Stocks

1. The seed nuts should be soaked in water overnight before sowing
2. Use polythene bags of size 25 cm x 15 cm and 300 gauge thickness.
3. Punch about 16-20 holes on the polythene bags to ensure good drainage
4. Prepare potting mixture with soil, sand and compost @1:1:1 ratio and mix with rock phosphate @ 5 g per 2 kg potting mixture.
5. Fill the polythene bags up to the brim of the bag.
6. Place the soaked nuts in the centre of the bag with stalk end up, at a depth of 2.0-2.5 cm.
7. Water the bags immediately after sowing and daily thereafter. Avoid excess irrigation.

8. Nuts usually germinate within 15-20 days after the sowing during the monsoon months and within 8-10 days during the dry months.
9. Prevent damage to germinating nuts from squirrels, birds etc.
10. Nuts should be sown at weekly intervals to get continuous supply of root stocks. During the summer, provide partial shade to the seedlings till they change their bronze colour to green and then keep them in the open.
11. The seedlings will be ready for grafting 40-50 days after germination.
12. During the rainy season, damping off of young seedlings is common. To control this disease, spraying/ drenching Bordeaux Mixture (1 %) is effective.

Selection of Root Stock

1. Select 40-50 day old healthy seedlings having unbranched main stem and growing in the centre of the polythene bag, as root stock.

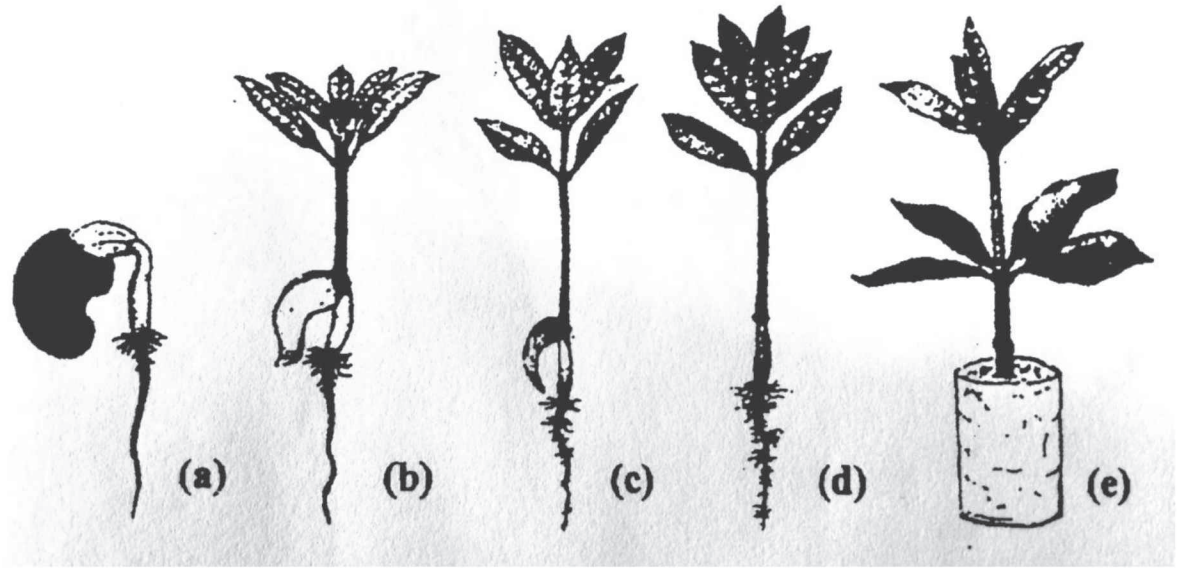


Fig. 1: Different Stages of development of Rootstock

Selection of Scions

1. Select a high yielding variety of cashew as a mother plant to collect adequate number of scions.
2. Select three to five month old non-flowering lateral shoots of current season's growth the selected scions should be 10-12 cm long, straight, uniformly round, pencil thick and brown having dormant plumpy terminal bud. The top four to five leaves will be dark green indicating proper maturity of the scion

Pre-curing

Pre-cure the selected scions by clipping off 3/4 portion of the leaf blades.

The scions will be ready for grafting in seven to ten days thereafter.

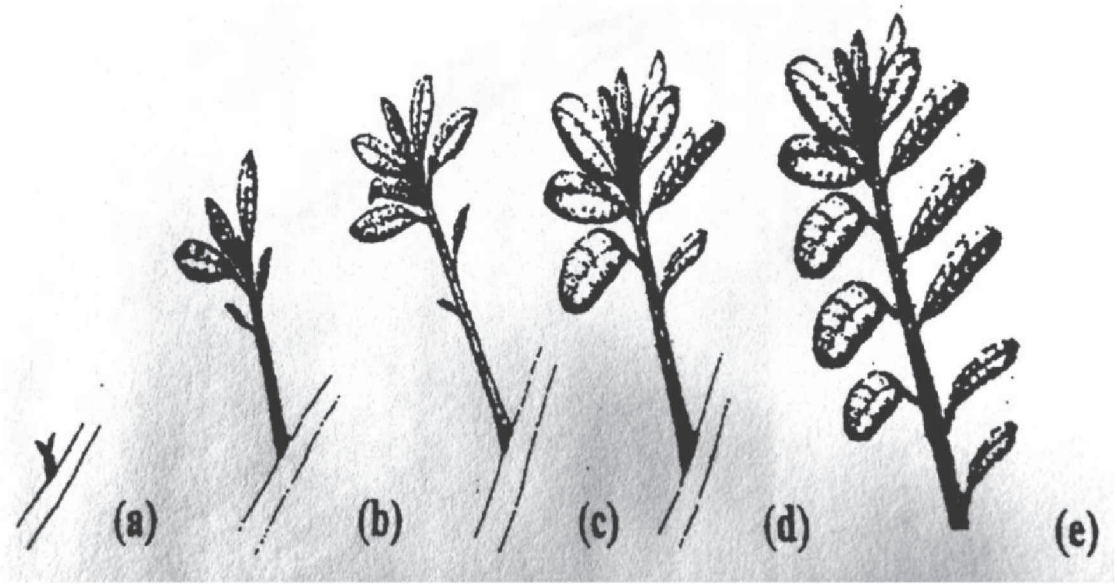


Fig. 2: Precuring Technique of Cashew nut Scion

Collection of Scions

The pre-cured scions are to be cut early in the morning to avoid desiccation.

The scions should be collected before the terminal buds sprout.

Wrap the scions in moist cloth and put in polythene covers as soon as they are cut from the mother tree and bring them to the nursery for grafting. If necessary, they can be stored for three to four days and used for grafting

Preparation of Root Stock

Retain two pairs of bottom leaves and remove the others from the selected seedling, using a sharp knife.

Give a transverse cut on the main stem, 15 cm above ground level

Make a cleft of 4-5 cm depth in the middle of the de-capitated stem of the seedling by giving a downward cut

Preparation of Scion

Select a matching scion stick (same thickness as that of the root stock) * Shape the cut end of the scion to a wedge of four to five cm long by chopping the bark and wood from opposite sides.

Grafting

Insert the wedge of the scion into the cleft of the root stock, taking care to ensure that the cambium layers of the stock and the scion are in perfect contact with each other.

Secure the graft joint firmly by a polythene tape (1.5 cm wide and 30 cm long).

Cover the scion with a wet polythene cap (15 cm x 12.5 cm, 100 gauge thickness) and tie at the bottom to maintain humidity inside and to protect the apical bud from drying.

The polythene cap should not touch the terminal bud.

Keep the grafted plants under shade for 10-15 days to enable sprouting of the terminal buds.

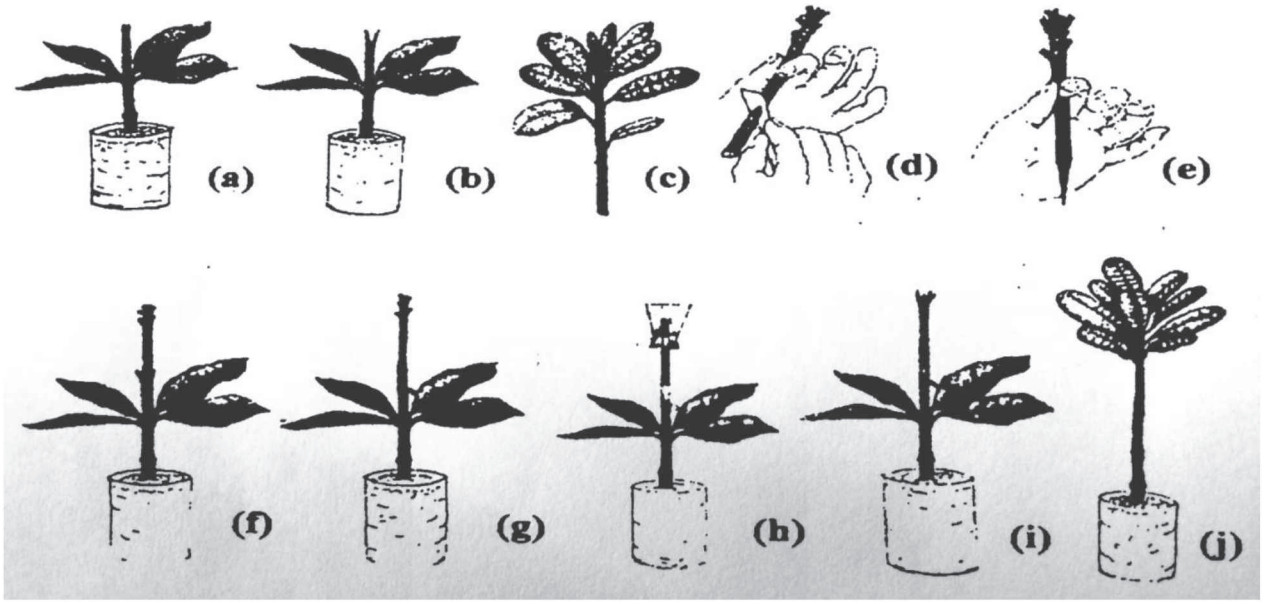


Fig. 3: Grafting Technique in Cashewnut

Remove the polythene caps and the grafts are shifted to open. The successful grafts show signs of growth within 3-4 weeks after grafting

The grafts will be ready for planting 5-6 months after grafting.

The success percentage in softwood grafting is more during March to September, than the other seasons, under Kerala conditions.

Care in the Nursery

Water the grafts regularly using a rose can or micro-sprinklers.

Remove the polythene tape from the graft joint about three months after grafting to avoid girdling.

Remove the new sprouts emerging from root stock at frequent intervals.

Remove panicles, if produced by the grafts, as and when observed.

Shift the grafts frequently from one place to another to prevent them from striking roots into the ground.

Spray insecticide (depending upon requirement) for controlling the infestation of sucking insects.

C. OIL PALM:

It is propagated by seed. Seedlings area raised in nursery. It is highly cross pollinated crops so it leads high variation. To avoid variation following steps should be followed.

- Selection of mother palm: The palm which has high yield, early bearing and gives more than 50% fruit set should be selected.
- Selection of nuts: Fully ripe nuts are to be collected. Heavier seed nuts (above 35 g) selected as they give higher germination and better vigour than lighter one. All under sized and malformed are rejected.

Seed treatment: Newly ripe seed nuts are collected and kept in container in which the temperature is maintain 35 to 40 ^{o}C for about 80 days & then kept in tap water for about 48 hours. By this treat. the seeds are begins to germinate after three days from sowing & continue up to 30 to 40 days. Nursery management of oil palm:

Primary Nursery: Selected & treated seeds are sown at 5 cm a part in sand bed under partial shade with their stalk ends keeps upward. Sand is spread over nuts just to cover the nuts. The bed may be irrigated daily**.** Seed nuts are also sown in polythene bags (size 22x10 are & 200 guage) filled with media (Soil + FYM 50:50).

Secondary Nursery: When seedling are of three months old & with two to three leaf stage can be transplanted in secondary Nursery at 30x30 cm in beds of 150 cm width & of convenient length.

Time of transplanting: At onset of monsoon. Seedlings are ready for transplanting in main field in about 12 to 18 months. The seedlings which have 5 or more number of leaves should be selected for planting.

Planting: Planting can be done during rainy season. Polybag is cut and seedling is separated from the poly bag intact with all of earth and root system. Seedling planted in the centre of pH. Collar region of plant should not be buried into the soil. Care is to be taken that collar region is level to land surface. Deep planting is avoided. After planting, it is watered and seedling is protected and mulching can be done in basins. After establishment of seedling, manure and fertilization can be done.

Pit size 60 cm x 60 cm x 60 cm or 90 cm x 90 cm x 90 cm should be dug out well in advance.

Planting distance: 9 m x 9 m.

D. ARECANUT

It is propagated through sexually (seed). Seeds are selected from elite mother palm because the crop is cross pollinated; variation is there in sexual propagation. To avoid variation up to some extent or to get genetically superior material it is required to select the mother palm and selection of seed nut and seedlings.

Selection of mother palm:

- It is necessary to select the mother palm which has high productivity.
- Higher per cent of fruit set
- The mother palm which is early and give high percentage of fruit set more than 50 % should be selected.
- Stability in yield

- Early bearing
- More than 10 open healthy leaves.
- Four or more than four inflorescences per year.
- Free from pest and diseases.

Selection of seed nut: Nuts should be fully ripe, should be heavier (above 35 g) to get high percentage of germination and high vigour. Medium to heavy nuts are selected and are soaked in water. Lighter nuts which float on water are rejected and heavier nuts are used for multiplication.

Raising of seedling in nursery:

Seed nuts are sown immediately in the nursery beds raised 5 to 6 cm apart in sandy soils. Their stalk ends pointing upward. Germination commences in about 40 days after sowing and the sprout are ready for transplanting, when they are three months old. 2-3 months old sprouts with 2 to 3 leaves are transplanted to the secondary nursery.

Secondary nursery beds of 150 cm width and convenient length are prepared. Sprouts are planted at 30 x 30 cm spacing with the onset of monsoon. Provide shade by erecting pandal or by growing banana. Beds should be supplied with basal dress of well decomposed cattle manure @ 5 t per acre. Irrigate the beds; provide facility for draining excess water. Weeding, mulching should be done as per the requirement. Seedlings are transplanted at 12-18 months old. Seedlings with good number of leaves (5-7) and having minimum height are selected as they are high yielder. Seedlings are lifted along with a ball of earth.

Characters of seedling to be selected for planting:

- Age 12 to 18 months old.
- Having more than 5 leaves.
- Dwarf seedling with more number of leaves.
- Free from pest and diseases

1. Write down the mother palm selection criteria of coconut and areca nut.

2. **What is precuring of scion? Write down the advantages of precuring of scion in grafting.**

Experiment No. 08	Date:
Micropropagation of banana	

Objectives: To study the micropropagation techniques of banana

Presently banana is multiplied through tissue culture techniques. The benefits of tissue culture technique in banana are as follows:

Merits

1. Virus free planting material could be generated.
2. Multiplication of maximum numbers of plant could be achieved by following this method.
3. Plants could be generated throughout the year.

Demerits

1. High establishment cost of tissue culture laboratory
2. Skilled labour is required.
3. The mortality of tissue culture plant lets under filed condition.

The steps of micropropagation in Banana

Tissue Culture Technique in Banana

1. **Selection of explants**: Parental pseudostem, small suckers, inflorescence apex and axillary flower buds are suitable explants for banana tissue culture. Generally healthy suckers (60-80 days old) are used as a source of explants. From the selected sucker a cube of growing meristem of about 1-2 cm^3 containing the apical meristem is excised. Subsequently a shoot tip of about 3 × 5 mm, consisting of the apical dome covered with several leaf primordia and a thin layer of corm tissue, is aseptically dissected.

2. **Disinfection of the selected explants:** 0.5-1.0% NaCl or 0.1% $HgCl_2$ are the common disinfectant. The block of tissue is dipped in 70% ethanol, surface sterilized in a 2% sodium hypochlorite solution, and after 20 min rinsed three times for 10 min in sterile water.

3. **Preparation of tissue culture media:** In most banana micropropagation systems, semi-solid media are used. As a gelling agent agar (5-8 g/l) is frequently added to the culture medium. Sucrose is the most preferred carbon source used at a concentration of 2-4% (W/V).Vitamins like Thiamine (Vitamin B_1), Nicotinic acid (Vitamin B_3) and Pyridoxine (Vitamin B_6) are frequently used. Amino acid glycine is being used as an immediate source of nitrogen cultured tissues.

4. **Antioxidants in tissue culture media:** Banana tissue cultures often suffer from excessive blackening caused by oxidation of polyphenolic compounds released from wounded tissues. These undesirable exudates form a barrier round the tissue, preventing nutrient uptake and

hindering growth. Therefore, during the first 4-6 weeks, fresh shoot-tips are transferred to new medium every 1-2 weeks. Antioxidants, such as ascorbic acid or citric acid in concentrations ranging from 10-150 mg/l, are added to the growth medium to reduce blackening, or the explants are dipped in antioxidant solution (cysteine 50 mg/l) prior to their transfer to culture medium.

5. **Growth regulators in tissue culture media:** Auxins and cytokinins are commonly used for rooting and shooting respectively. The widely used auxins are IAA, NAA and IBA. BAP is the cyotkinin of choice for in vitro shoot bud proliferation. The media pH should be maintained at 5.8.

6. **Maintenance of Temperature and Light Intensity in Tissue Culture laboratory**: The optimum incubation temperature should be between 24-26°C.Generally the light intensity maintained ranges from 1,500-3,000 lux. Higher levels of 3,000-10,000 lux during the later stages improve the survival rate of plant lets up on transfer to soil.

Culture Ploriferation

First subculture is done after 4 weeks of inoculation. By this time, explants enlarge to about double the original size. The blackened surface is scraped off and corm base is reduced to 0.5cm thickness.

Reducing the thickness of basal corm tissue decreases blackening, eventually chances of proliferation of non-meristamatic tissue outgrowing the meristamatic tissue. Any growing apical shoots, is cut to activate the axillary buds. By the end of first subculture shoots may be seen with 1-3 side buds. By the end of second subculture cycle is repeated at 4 weeks interval to increase the proliferation rate. After 5-6 subculture cycles, the proliferated buds are put in a regeneration medium containing 1/10th BAP. The shoots develops are then rooted in 1/2MS medium with IBA and activated charcoal. After a month, the rooted plants are ready for hardening.

Hardening:

Micropropagated plants are delicate in nature as they are grown under artificial conditions of High humidity and optimum light intensity. Rooted shoots of 6-10 cm tall with well ramified roots are washed free off agar medium and taken to the micropots containing soilrite and then shifted to growth tunnels for further establishment. After an appropriate period of hardening, the plants are taken out of the micopots roots are dipped in a fungicidal solution to reduce the risk of damage by fungal diseses.Then repotted in a mixture containing 1:1:1:1 of soil:sand:soilrite and FYM.

Once after repotting they are shifted under 75% shade nets where they are exposed to 60-70% RH slight intensity of 40-45 mol/m2/sec for about 10-12 days. Next stage , they are maintained at 50-60% RH and light intensity of 200 mol/m2/sec for about 10-15 days and at an intensity of 600 mol/m2/sec for another month. These plantlets are shifted to polybags containing

1:1:1 of sand:soil:FYM and maintained in green house (light intensity of 600-700 mol/m^2/sec) until field planting. During primary and secondary hardening plants should be roughed for somaclonal variations. This could be for vegetative deformities, foliar deformities like variegation, rosette foliage others like dwarfism etc.Important points to be taken care during secondary hardening of tissue culture plants are as follows:

1. The rooting media should be 100 per cent free from pathogen.
2. Water used for irrigating the plants should be free from pathogens and nematodes.
3. Strict rouging by trained personnel should be done to remove the types once in 7 days
4. Sample plants from each batch should be randomly virus indexed (atleast 10 plants from each batch/explants).
5. While shifting of primary hardened plants, two longitudinal cuts of net pots should be given to facilitate further corm growth.

Manuring and plant protection in nursery

- Plantlets should be 2-3 weeks old before any fertilizer application is taken up.100ml water containing 05.kg Urea, 2g Super phosphate and 1g Muriate of potash can be applied per plant.The manuring is repeated by doubling the dosage after three weeks.
- Spraying of commercially available micronutrient mixtures during sixth week would help in better establishment both in nursery and field.Sanitary measures are strictly adopted in the nursery to avoid the risk of damage by pests and diseases.
- Pest and diseases encountered during the process of hardening are to be given due attention at appropriate times especially foe soil borne nematodes and aphids which transmit BBTV. The maximum number of subculture cycles should be adhered to 7 or less to check off types to the minimum.

Ideal tissue culture plant

1. A well hardened plant should be minimum of 30 cm in height and should have a pseudostem circumference of 5.0-6.0 cm after 45-60 days of hardening.
2. .The plant should have 5 photosynthetically active leaves and inter foliar space must be not less than 5.0 cm.
3. The plant should have approximately 25-30 active roots at the end of secondary hardening stage.
4. The length of active roots should be more than 15 cm with a good number of secondary roots.

5. The poly bag should be size (20.0cm in length and 16 cm in diameter)with potting media filled to ¾ full of the bag.

6. The media/potting mixture approximately should weight about 750-800g.On dry weight basis.

7. Plantlets should be free from any visual symptoms of leaf spot, pseudostem rot and physical deformations.

8. Plantlets should be free from the presence of root pathogens like *Erwinia* rot symptoms, nematode lesions and root knots. Random checking of roots is very essential at the time of procurement.

9. Those exhibiting abnormal growth must be discarded.

1. Draw a schematic diagram of micropropagation techniques followed in banana.

Experiment No. 09	Date:

Physiological disorders of fruit and plantation crops

Objectives: To study the symptoms, causes and remedies of various physiological disorders of fruit and plantation crops.

A. <u>Physiological Disorders of Mango</u>:

1. **Black Tip:** The distle end of fruit becomes black & hard. Premature ripening of fruit which is not marketable.More in Pujab, UP, Bihar and West Bengal.

   Causes*:*

   1. It is due to fumes of brick klines near 630 m perifery of mango orchard.
   2. CO_2, SO_2 & acetylene are constituents of this fume.

   Control Measures:

   Spray Borax 0.6 or Caustic soda 0.8 %.

2. **Leaf Scorch:** Young leaves show characteristic symptoms like burning. In severe case the older leaves also burned off. It is common in saline soils.

   Causes:

   1. Due to excess chloride in the soil

   Control Measures:

   1. Use potassium sulphate (K_2SO_4) in place of murate of potash
   2. Reduce the level of salinity in soil

3. **Spongy Tissue:** Ripe fruit look normal, but on cutting, spongy development found in flesh, which is bed in odor, ceramic white in color & unpalatable. Commonly found in Alphonso (30 %) and Zamadar.

   Causes:

   It is due to accumulation of radiant heat in fruit tissues during April-May.

   Control Measures:

   1. Mulching dry leaves, straw etc. under the tree.
   2. Avoid harvesting during hot period of day.
   3. Harvest fruit at ¾th maturity.

B. <u>Physiological Disorders of Banana</u>

1. **Choke throat:** This order is called choke throat because the distal part of the inflorescence comes out but the basal part tapered at the throat. The low temperature at the time of flowering is responsible for this type of bunch formation. Due to low temperature, the leaves become yellowish and under severe condition necrotic spot can be seen on the leaves. The maturity time of bunch is extended up to 5-6 months than 3.5-4 months.

Causes: This disorder results from low temperature.

Management

1. Use such varieties which are capable to resist the cold temperature.
2. Use wind break trees like *Eucalyptus* to check the bad effect of cold waves

2. **Kotta vazhai:** *Kotta* means seed and *Vazhai* means banana. The formation of seeds in the banana pulp makes the fruits inedible. However, this disorder cannot be detected at earlier stage but later it could be characterized by the presence of sharp, tapered and ill filled fruits. This is a serious disorder in Poorna variety of banana.

Management

It can be managed by spraying 20 ppm 2, 4-D.

3. **Finger drop:** It is a disorder in which the pedicle becomes soft and weak. The individual fruits can be dissociated from the bunch very easily during ripening.

Management

The ripening chamber of banana should be maintained as follows

- Temperature: 14-18 ^{o}C
- Relative Humidity 90-95 %
- Ethylene 1ml/litre for 24-48 hrs.

4. **Peel splitting:** In this disorder the peel of the fruit is splitted into bisects and consequently the pulp is exposed as the cracks widens.

Management

The ripening chamber of banana should be maintained as follows

- Temperature: 14-18 ^{o}C
- Relative Humidity 90-95 %
- Ethylene 1ml/litre for 24-48 hrs.

C. Physiological Disorder of Citrus

1. **Granulation** Granulation is a serious problem of citrus, especially under North Indian conditions. This abnormality is initiated at the stem end of the fruit which gradually extends towards the stylar end. The affected juice sacs become hard and dry, fruits become grey in colour, enlarged in size, have flat and insipid taste and assume a granular texture. Granulated fruits contain less extractable juice as most of it turns into gelatinous mass. This results in more quantity of rag and thus low pulp/rag ratio. The terms granulation, crystallization and dry end are used to describe this trouble. It is much more prevalent in larger sized fruits than in small fruit, in young than in old trees and in humid than in dry areas.

Probable causes:

- Luxuriant growth habit of the plant
- Age of plant (More in younger plants)
- Rootstock (Vigourous like Rough lemon increases the incidence)

- Variety
- Frequent irrigation
- Mineral constituents in plant tissue (high Ca and Mn, and low P and B)
- Time of harvest (More in younger fruits)
- Exposure to sunlight
- Cold temperature during the maturity period increases the incidence.

Management

- Spraying of 300 ppm NAA in the moth of August, September and October could be reduced the incidence by 50%.
- Giberellic acid 15 ppm followed by NAA 300 ppm in October and November also reduce granulation.

D. Physiological Disorders of Pomegranate

1. **Fruit Cracking:** Serious physiological disorder.

   Causes:

   Young fruits crack due to boron deficiency.

   Larger fruits crack due to soil moisture fluctuation and day-night atmospheric moisture.

   Control Measure:

   1. Maintain soil moisture.
   2. Cultivate tolerant variety-Bedana Bosec and Khog.
   3. Harvest early.
   4. Spray $Ca(OH)_2$ and borax after fruit set and fruit initiation.

E. Physiological Disorder of Guava

1. ***Fatio*:** Old branches & trunk splits. Common in Bhavnagar

   Causes: Unknown

F. Physiological Disorder of Strawberry

1. **Albinism:** (Lack of fruit colour during ripening) It is a physiological disorder in strawberry. It is probably caused by certain climatic conditions and extremes in nutrition. Fruits remain irregularly pink or even totally white and sometimes swollen. They have acid taste and become less firm. Albino fruits are often damaged during harvesting and are susceptible to Botrytis infection and decay during storage.

G. Physiological Disorder of Coconut

1. **Barren nut:** It refers that without or with imperfectly developed kennel is as ancient as the coconut cultivation. Nut generally oblong in shape and the quality of the flask produced is very much less compared to normal fruit. The embryo in the Barren nut is mostly absent or when present it is in running stage of decay, often these nuts are seen with the shell and kernel improperly developed. Fungal infection in the embryo is also noticed.

Cause:

1. Defective fertilization
2. Malformation of Embryo, female flowers unfertilized.
3. Nutritional deficiency
4. Potash / Boron deficiency
5. Excessive bearing

2. Shedding of buttons and immature nuts

Causes:

1. Diseases and pests infection
2. Nutritional deficiency, immaturity of trees. 'N' has good effect on setting of fruits yield, nos. of female flowers, mechanical strength etc.
3. Unfavorable soil and climatic factors, moisture stress
4. Hormonal imbalance more severe in heavy soil / than light
5. Defective pollination - Heredity, lack of pollination.
6. Formation of abscission layer at attachment place due to severe drought.

Control: 20 ppm 2-4-D sprayed on female flowers at weekly interval for a month starting just after completion of fertilization proved very useful and resulted more than double the setting of buttons. This is recommended by CPCRI (Central Plantation Crops Research Institute), Kasaragad.

H. Physiological Disorder of Cocoa:

1. Cherelle Wilt: Cacao beans are produced in pods (fruits) of the trees of *Theobroma cacao* L. Cacao pods are produced after pollination of the small cauliflorous flowers covering the branches and trunk of the tree. Despite abundant flowering, only 0.5–5 % of cacao flowers become pollinated. Pollinated flowers then develop into immature pods, commonly known as cherelles. Despite abundant flowers and pod set of trees, few cherelles develop into mature pods. Up to 75 % of cherelles are lost to a thinning condition known as cherelle wilt. Additionally, cherelles can be lost at an early stage to insect, stramenopile, and fungal pests.

List down the physiological disorder of mango, banana, strawberry and coconut

Experiment No. 10	Date:

Preparation of Plant Growth Regulators

Objectives: To study uses of plant growth regulators and method of preparation and calculation to prepare a desired concentration of plant growth regulators.

Plant Growth Regulator: Plant growth regulators may be defined as any organic compounds other than nutrients synthesized in one part of the plant and translocated to another part, where in very low concentrations either promote or inhibit or modify the growth and development processes of plant.

The different types of plant growth regulators (PGR_s) are as follows:

1. Auxins
2. Gibberellins
3. Cytokinins
4. Ethylene
5. Growth Inhibitors [Abscisic Acid (ABA)]
6. Growth Retardants [CCC, AMO, 1618, Phosphon - D, Morphacting, MH]
7. New plant growth regulators [Jasmonates/Jasmonic acid derivatives (JA_s), Salicylic acid, Brassinosteroids (BR_s), Polyamines (PA_s) etc.]

These compounds have been extensively used in fruit production at various growth and development stages of plants. Here some of the examples are given below:

1. Propagation:

In propagation, plant growth regulators are applied in paste, solution or powder form. The concentration of the chemical varies with plant species and types of cutting and method of application. The most common plant growth regulators used in rooting are Indole Acetic Acid (IAA), Indole Buteric Acid (IBA) and Napthalene Acetic Acid (NAA).

2. Seed Germination/Breaking of Dormancy:

Gibberellic acid (GA_3) significantly accelerates seed germination in many plant species. Presoaking the seed with GA_3 enhances germination of deciduous temperate fruit (Apple, Peach, Plum etc.) trees.

3. Induction of Flowering:

Pant growth regulators like NAA at 10 to 50 ppm causes early flowering in pineapple. 2, 4 D at 6 to 10 ppm has used to induce flowering in pineapple. Flowering can be delayed by 1 to 2 weeks NAA at 200 to 800 ppm application in apple, cherries, pears, peaches, and plums.

4. Sex Expression:

Plant growth regulators can change the sex of the flowers. Male sterility can be induced in corn by MH Plant growth regulators may be defined as any organic compounds other than nutrients synthesized in one part of the plant and translocated to another part, where in very low concentrations either promote or inhibit or modify the growth and development processes of plant. The different types of plant growth regulators (PGR_s) are as follows:

5. Flower and Fruit Thinning:

Many fruit trees produces heavy flowering and fruit in one year and few or one in next year. By using G.R the normal bearing can be maintained NAA at 5 to 10 ppm and NAA at 5 to 7 ppm for thinning of apple, peaches and grapes.

6. Pre Harvest Drop of Fruits:

Flower and fruit drop is a problem in many fruit crops. Application of NAA 10 - 50 ppm in mango, citrus and chilies reduce fruit drop by preventing formation of abscission layer.

7. Fruit Development:

Application of 50-60 ppm GA in grapes increases the size of berry.

8. Early Maturity:

Early maturity fetches higher prices in the market. In pine apple application of 20 ppm NAA induces early flowering and early maturing at least by two months. Spraying of 50 ppm NAA reduces maturity in grapes, use of 250–400 ppm of Ethrel induces early maturity in Ber.

9. Early Ripening and Colour Development:

Fruits like mango, banana and papaya ripe after harvest. Dipping of fruits in 20-50 ppm Ethrel solution induces golden yellow colour to fruit induces early maturity.

10. Delayed Maturity:

Delay in ripening is required when fruit are to be sent to long distance market. Dipping of fruit in 2,4-D, 2,4- 5- T or MH- 40 extends storage life of fruits.

11. Sprouting of Bud:

Ethrel, GA_3, IBA and Cytokinin spray induces sprouting of buds. MH (Maleic Hydrazide) is used to retard the sprouting of potato and onion. It is used in plant breeding for induction of male sterility. Application of NAA, IAA and GA at 50 to 100 ppm increases female flowers in pumpkin, cucumber to get more yield.

Preparation of Growth Regulators: Synthetic growth regulators are available in market and those are used to prepare the desired concentration for a particular purpose in horticultural applications. Usually, the concentration of prepared plant growth regulators is expressed in parts per million (ppm).

Forms of Preparation of Plant Growth Regulators

1. **Powder form**
2. **Lanolin Paste form**
3. **Solution form**

How to calculate:

General formula:

100 ppm IBA in powder form= 100 mg IBA in 1 kg of chalk powder (powder form) = 100 mg IBA in 1000000 mg (10^6 = one million) of chalk powder

200 ppm NAA in lanolin paste form = 200 mg NAA in 1 Kg of lanolin paste (paste form) = 200 mg NAA in 1000000 mg (10^6 = one million) of lanolin paste

500 ppm BA in solution form = 500 mg BA in 1 litre of distilled water (solution form)

We know, weight of 1 ml pure water is 1 g

i.e., 1 ml pure water = 1 g water = 1000 mg water

1 litre pure water = 1000 ml = 1000 × 1000 = 10^6 mg (One million)

Therefore, if we dissolve 500 mg BA in 1 litre water it becomes the concentration of 500 ppm BA

Formula for preparation of working solution:

$$\mathbf{N_1V_1 = N_2V_2}$$

Where, N_1= Concentration of stock (given) solution,

V_1 = ?,

N_2= Concentration of working solution,

V_2= Volume of working solution

Examples:

1. **Prepare a 50 ppm 500 ml IBA solution from a 1000 ppm stock solution**

   Here, N_1= 1000

   V_1 = ?

   N_2 = 50

   V_2 = 500 ml

   So,

$$V_1 = \frac{N_2 \times V_2}{N_1}$$

$$V_1 = \frac{50 \times 500}{1000}$$

$$V_1 = 25 \text{ ml}$$

   Therefore, take 25 ml of 1000 ml IBA and make the volume upto 500 ml with distilled water (Ans.)

2. **Prepare a 150 ppm 2 litre NAA solution from a 1500 ppm stock solution**

   Here, N_1= 1500

   V_1 = ?

   N_2 = 150

   V_2 = 2 litre = 2000 ml

   So,

$$V_1 = \frac{N_2 \times V_2}{N_1}$$

$$V_1 = \frac{150 \times 2000}{1500}$$

$$V_1 = 200 \text{ ml}$$

   Therefore, take 200 ml of 1500 ml IBA and make the volume upto 2000 ml with distilled water (Ans.)

3. **Prepare a 40 ppm 250 ml GA_3 solution from a 1000 ppm stock solution**

4. **Prepare a 250 ppm 1.5 litre IBA solution from a 1500 ppm stock solution**

5. **Prepare a staock solution of 1500 ppm IBA**

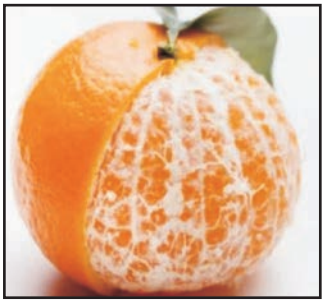
6. **Prepare a stock solution of 2000 ppm BAP.**

APPENDIX-I


Mango


Banana


Acid lime


Mandarin orange


Sweet Orange


Pummelo


Grapes


Papaya


Guava


Pomegranate


Sapota

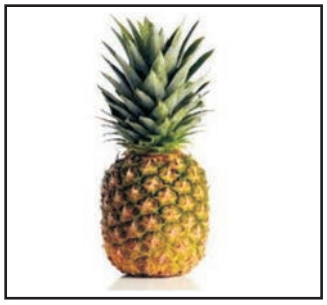

Custard apple


Aonla


Ber


Jamun


Date


Pineapple


Jackfruit


Litchi


Strawberry

APPENDIX-ll

Coconut

Areca nut

Oil Palm

Cashew Nut

Coffee

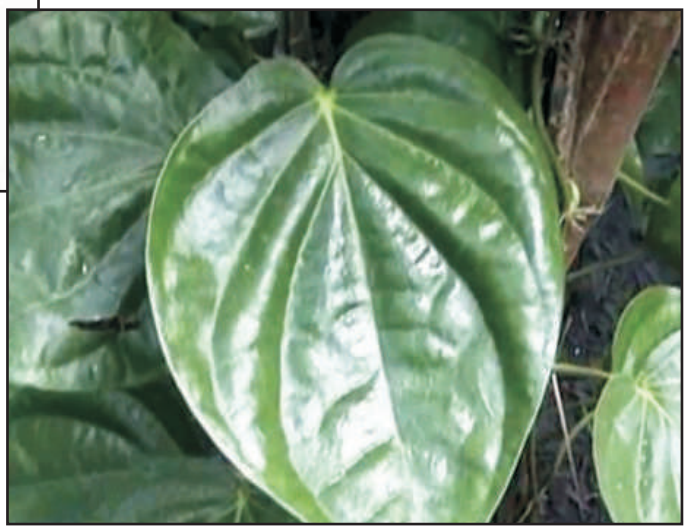
Tea

APPENDIX-ll

Rubber Tree

Cocoa

Palmyrah palm



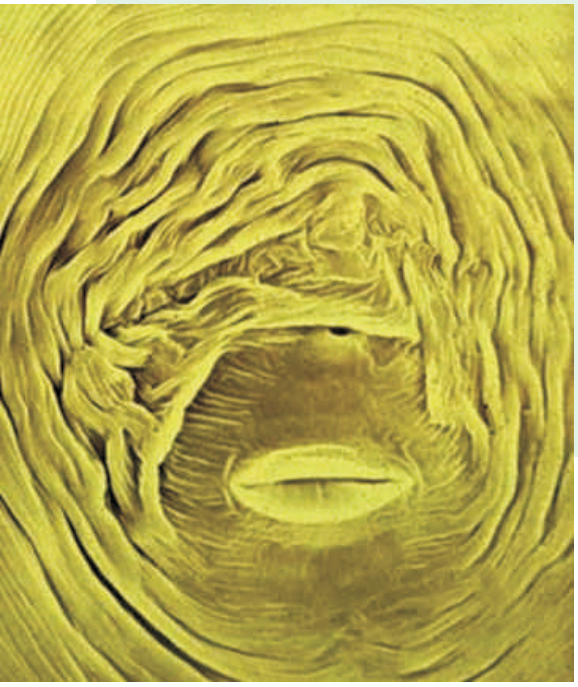
Betel Vine

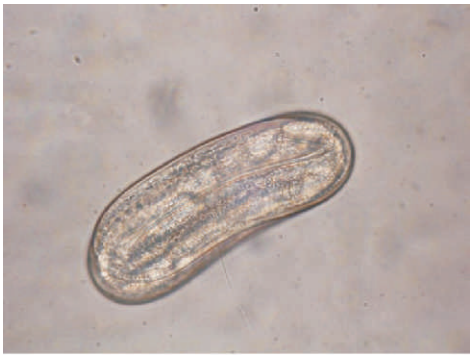
PRACTICAL MANUAL

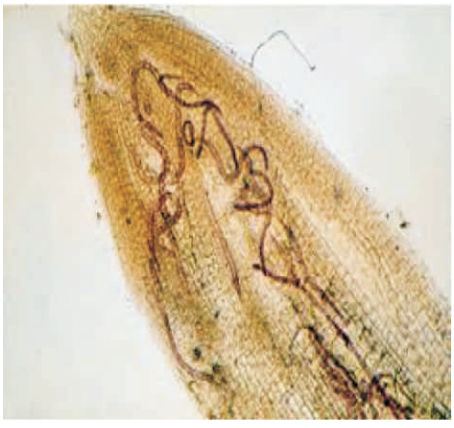
Pl. Path. 2.2 (1 + 1)

Introductory Plant Nematology

Second Semester B.Sc. (Hons.) Agriculture



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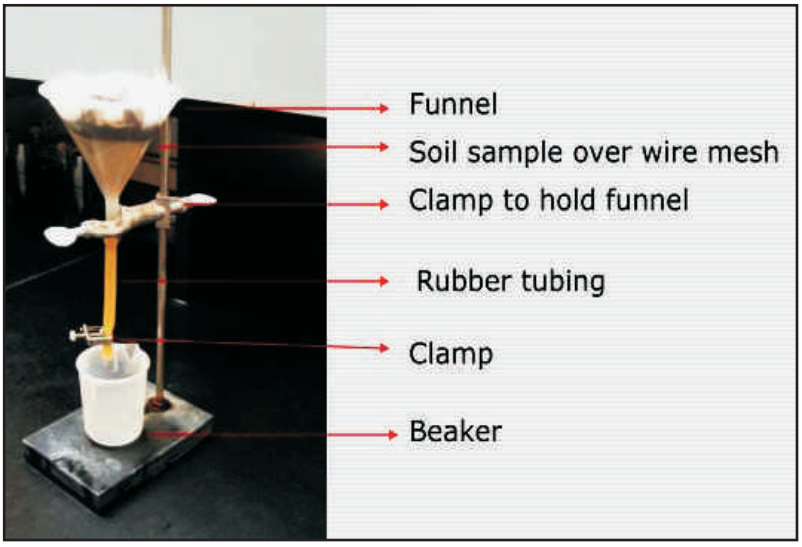
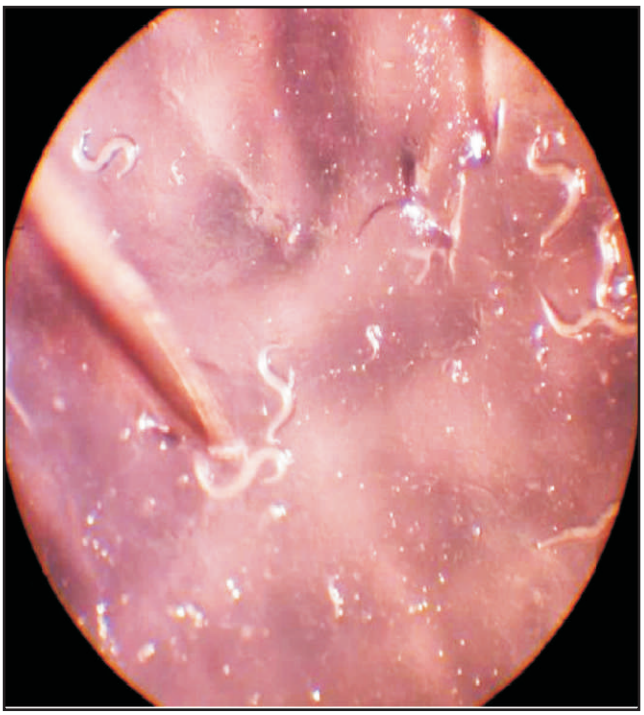
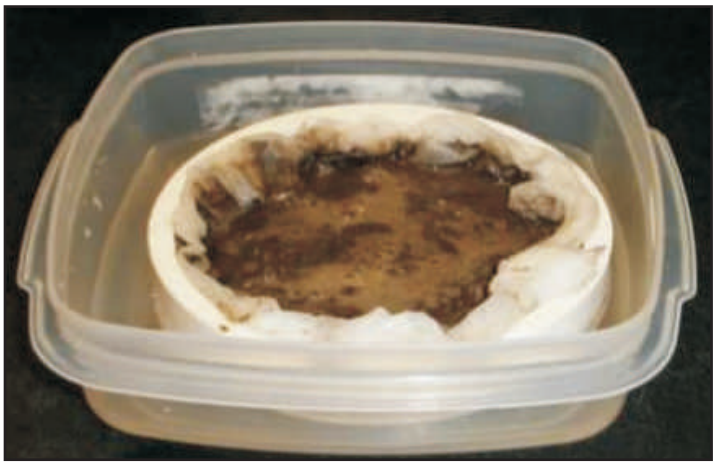
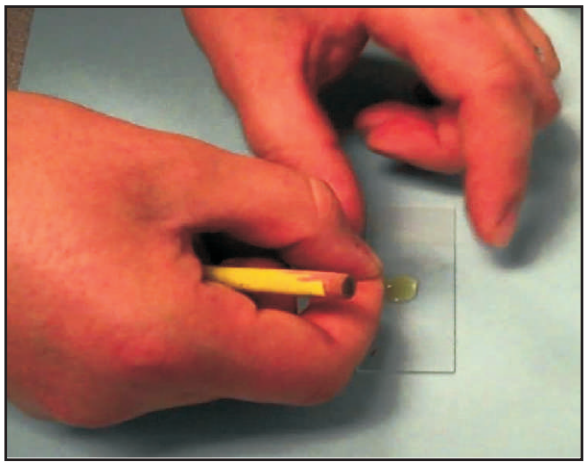

Funnel
Soil sample over wire mesh
Clamp to hold funnel
Rubber tubing
Clamp
Beaker







PRACTICAL MANUAL

Pl. Path. 2.2 (1 + 1)

Introductory Plant Nematology

Second Semester B.Sc. (Hons.) Agriculture

NAVSARI AGRICULTURAL UNIVERSITY

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:: FOREWORD ::

It gives me great pleasure to write the foreword of the Laboratory Manual of "Introductory Plant Nematology" prepared by Dr. Amol J. Deshmukh, Dr. Pushpendra Singh and Prof. R. P. Bambharolia of the Department of Plant Pathology. As a matter of fact, I have always been of view and opinion that the students must get a laboratory guide where what to do, how to do and finally what to get are precisely described as it would save time that is otherwise unnecessarily wasted. This manual is being brought out of the efforts of the teachers of the Department of Plant Pathology, College of Agriculture, NAU, Wagahi (Dangs). This is one of the most unique and fundamentals practical books available for fulfilling the requirements of the undergraduate students and has been prepared as per the latest syllabus prescribed by the ICAR. However, it is equally useful for the Post-graduate students, scholars, teachers and scientists working in the basic and applied aspects of Fundamentals of Plant Pathology.

This manual entitled "Introductory Plant Nematology" A Practical Manual consists exercises which are comprehensive and exhaustive in enriching the knowledge of fundamental techniques of Plant Nematology.

I appreciate and congratulate *Dr. Amol J. Deshmukh, Dr. Pushpendra Singh* and *Prof. R. P. Bambharolia for their commendable efforts in bringing out this practical manual* for their efforts.

(J. J. Pastagia)

:: PREFACE ::

Plant Pathology is one of the important subjects of the life science and knowledge of its practical aspects is absolutely essential for students and the researcher. Practical manual on " **Introductory Plant Nematology** " is a fundamental book which highlights and makes the readers aware of the important techniques of Plant Nematology. This book has been especially designed keeping in view the latest syllabus prescribed by the ICAR as per the 5th dean committee recommendation for first semester undergraduate agriculture students.

However, it is equally useful for the post graduate students, research scholars, teachers and scientists working on the basic and applied aspects of Plant nematology. In this manual the exercises are followed by questions which will be helpful to the readers in enriching their knowledge about the subject. The genesis of this manual lies in the problem faced by the students and their teachers to carry out the practical classes, as no standard study material was available for it. Keeping in view, the authors along with the Principal (CAW, NAU, Waghai) decided to provide printed practical manual of the said course (Pl. Path. 2.2) to the students from the academic year 2020. The format of the exercises is appropriate for use as a workbook. It is hoped that this practical manual will be highly useful for the students in learning the fundamental techniques of Plant Pathology. The editors welcome suggestions from users, students as well as instructors/teachers for its future improvement.

A. J. Deshmukh
Pushpendra Singh
R. P. Bambharolia

Reg. No. : .....

Batch No.: .....

Roll No. : .....

Uni Seat No.: .....

:: CERTIFICATE ::

This is to certify that the practical exercises duly signed were performed in the subject of ***Plant Pathology, Course No. Pl.Path. 2.2 (1+1) [Introductory Plant Nematology]*** *as a part and partial requirement of the Course by Mr./Ms.*_____

Roll No. _____ *of* ***Second Semester*** *class during academic year 20*_____.

The numbers of practical performed were _____ *out of* _____.

Course Teacher

Professor & Head

Examiner
(Internal Exam.)

Examiner
(External Exam.)

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Exercise -1: Survey of nematode infestation

Survey = latin word; Sur= over, vey=Video i.e. to see over

Definition

Survey:

To move over a specific place for some observation.

Surveillance:

Repeated or sequential survey of some place/ locality for some observation to ascertain the changes /fluctuations in the objects of study e.g. in this case to ascertain the changes in plant status due to suspected nematode infestation.

Principle of Survey:

Principle of survey relates that no management practice should be followed against a pest unless its presence is confirmed at the population level that exceeds the economic threshold to make the management tactics economically desirable.

Objectives of Survey:

1) To obtain a general idea about the occurrence of nematode fauna in a particular crop/ locality.
2) To obtain information regarding distribution and frequency of a particular disease or a nematode species in a given crop/ locality.
3) To have an idea about association of one or more nematode species and their population densities during different periods of time.

Kinds of Survey:

1. Qualitative Survey:

includes identification of different species of nematodes.

2. Quantitative Survey:

involves the estimation of population of one or more nematode species present.

Types of Survey:

1) Random:

Samples are collected from any crop at any place suspected to be infested.

2) Planned:

It is further of two types

a. Extensive Survey: A vast area is taken in to consideration to obtain a preliminary assessment of nematode population.

b. Intensive Planned: A particular area is surveyed to obtain accurate and detailed knowledge about the pest status in that region and sometimes in a particular crop.

Methods of Survey:

Five steps are involved.

1) Collection of plant and soil samples and recording of relevant information like locality, crop, crop status, soil type, previous crop (optional), time of collection, collector's name etc.
2) Storage of samples at around 5°C in temperate regions, 10–16°C in warmer regions of the middle latitudes and 16–18°C in the tropics and subtropics.
3) Processing of samples for nematode extraction
4) Estimation of nematode population of different genera
5) Identification of important and prevalent species for further studies.

Presentation of Survey Data:

After identification, the data accumulated during survey can be represented in number of ways:

1) Mapping
2) Quantitative indexing (1 to 5); 1= rare or 1% infestation, 2= infrequent or 2% infestation, 3=Frequent i.e. 3-10% infestation, 4= Abundant i.e. 11-50% infestation, 5= Very abundant i.e. >50% infestation.
3) Gall indexing (in case of root-knot nematode infestation) 1 to 5.

The frequency and Density of nematode population can be calculated by using following formulas:

$$\textbf{Absolute Frequency} = \frac{\text{No. of samples infested with a particular species}}{\text{Total number of samples collected}} \times 100$$

$$\textbf{Relative Frequency} = \frac{\text{Frequency of a particular species}}{\text{Sum frequency of all species}} \times 100$$

$$\textbf{Relative Density} = \frac{\text{Total of all individuals in a sample}}{\text{No. of individuals of a particular species in the sample}} \times 100$$

Questions:

1. Define survey? Enlist out objectives of survey?

2. What is the difference between survey and surveillance? And give principle of survey?

3. Calculate absolute frequency of following nematode species which were extracted from the samples collected of the Dang Region

Sr. No.	Name of species	Numbers of soil sample collected	Numbers of samples found infested
1	*Meloidogyne* sp.	100	75
2	*Nacobbus* sp.	100	100
3	*Pratylenchus* sp.	200	36

Exercise -2: Sampling of Plant Parasitic Nematodes

Plant parasitic nematodes are minute unsegmented worms that live in soil or plant parts that they infect. They are harmful to host plants and reduce the yield and/or damage the quality of crops. These pests have to be detected in the soil or in the plant parts before proper control actions are taken.

Objective:

To be aware of the procedure of collecting samples from soil and plant parts for qualitative and quantitative analysis of suspected nematode populations in soil and plant roots with minimum possible error.

What is a sample?

Sample is a representative of the particular area (which may be a field, district, state or country) for which information is required.

When to Sample?

The most appropriate time to sample depends on the crop and the purpose of the sampling.

Predictive assays:

In general, nematode populations are highest at the end of growing season and drop as the soil temperature declines. For annual crops, sample at or immediately after harvest. For orchards and nurseries, collect samples during early and mid-season. For turf and ornamental plants, sample before planting.

Diagnostic assays:

Sample the soil of living plants showing symptoms at the onset of symptoms during growing season. Separate samplings from healthy and diseased plants, help to compare the population densities. Also include roots, if possible, because some nematodes spend part of their life cycle inside the roots and more accurate diagnosis of nematode damage can be made from samples including roots.

Materials required:

Sampling tools like soil auger/ hand hoe/ cheese sampler, polythene bags, aluminum foil, labels, rubber bands.

Sampling tools to be used in collecting the samples

1. **Soil augers:** Advantages of an auger include the ability to sample in drier and heavier soil types.
2. **Oakfield tubes:** These devices are excellent for obtaining soil samples to depths of approximately 3 feet (if soil texture and moisture are conducive)
3. **Viehmeyer tube:** can be used to collect sample from greater depths than that of oakfield tubes
4. **Shovel:** Take sample from shallow rooted crops

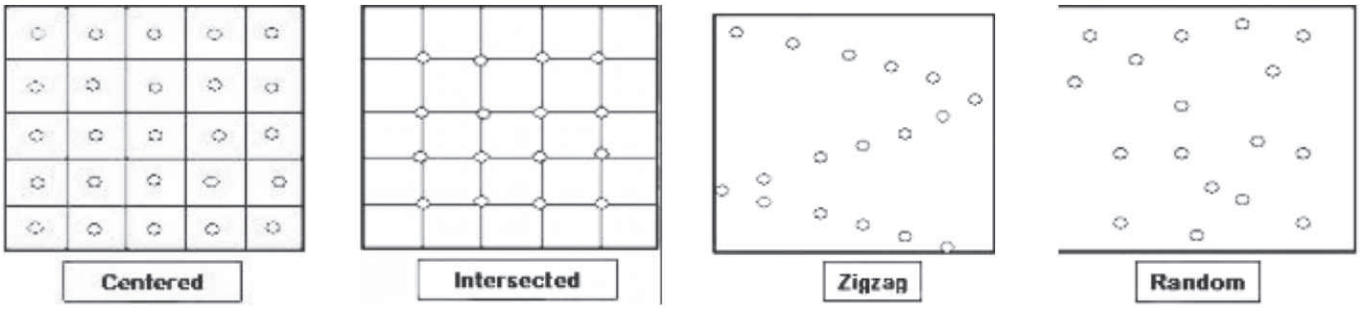
How to sample soil?

Sampling techniques should have backing from diagnostic and identification laboratory to obtain quick and reliable nematode identification. Auger samplers are used to obtain soil cores for nematode population studies at different depths. The soil cores should be obtained from top first foot (15-20 cm) where most of soil inhabiting nematodes live.

How many soil samples should be collected?

Obtaining twenty soil cores per five acres will cover four cores per acre is a scheme of sampling that is highly recommended but it is vary from crop to crop and area. Various patterns to obtain soil samples are depicted below.

Sampling patterns: (O = one subsample).



Labeling

Sample labeling with crop detail, age of crop, location, variety, date of sample collection should be necessary

How to preserve the samples?

The soil samples should be protected from extreme temperature, direct sun light, drying and freezing. Sample bags are made out of paper lined with plastic however plastic zip lock bag can be used as sample bag instead. Store in a cool dark place and send the samples to the laboratory as soon as possible (within 24 hours if possible for a good result)

Where to sample?

The sampling pattern for soil and root samples depends upon the type of crop and pattern of planting. Use the following guidelines to determine the sampling pattern.

Sampling Methodology:

Plant parasitic root nematodes are present either in or around the roots of their host plants and some genera prevail the above ground parts like stem, leaves, spike or buds. Samples are procured differently from annuals and perennials.

Sampling from vegetable field

- Select six rows of a field (two from the beginning, two from the middle and two from the far end of the field).
- Collect 8-10 sub samples up to a depth of 20-30 cm (active feeder root zone) from each pair of rows in a criss-cross (zigzag) manner.
- Place all the sub samples in the same polythene bag and label it for the crop, locality, crop status, visible symptoms if any, crop history (if known as previous crop), Date of collection and farmers name .
- Label information should be written separately in a note book also.

Sampling from field crops

- Leave about one meter periphery of the field.
- Remove two-three cm upper layer of the soil with the help of an auger/ hand hoe.
- Collect 50-100 cc soil along with feeder roots up to depth of 15-20 cm. this will make one sub sample.
- Draw 10-20 such subsamples from an area of one hectare area in a zig-zag manner (fig. 3)
- Place all the subsamples in one polythene bag so as to make one composite sample.
- Put an aluminium foil label bearing the sample number in the polythene bag and tie it with a rubber band.
- Write the details of labeling in a separate note-book also.

Sampling from an orchard

- Take two subsamples from one tree up to a depth of 30 to 60 cm (feeder root zone) depending upon the type and age of the tree.
- At least ten trees should be sampled at random to cover an area of one hectare.
- Pool all the subsamples in polythene bag and label it properly for the fruit, orchard site, orchard age, symptomatic information, orchardist name, date of collection etc

Sampling from a single plant e.g. Tree/shrub

- Collect 10 sub samples, five near the trunk and five from drip line i.e. from inner and outer rhizosphere of the tree by the method described above.
- The depth of the sampling will vary with the kind and age of the tree and depth of its feeder root system. Spatial distribution; horizontal as well as vertical should be standardized.
- Put all the subsamples in the same polythene bag and label it.

Sampling from a fallow area

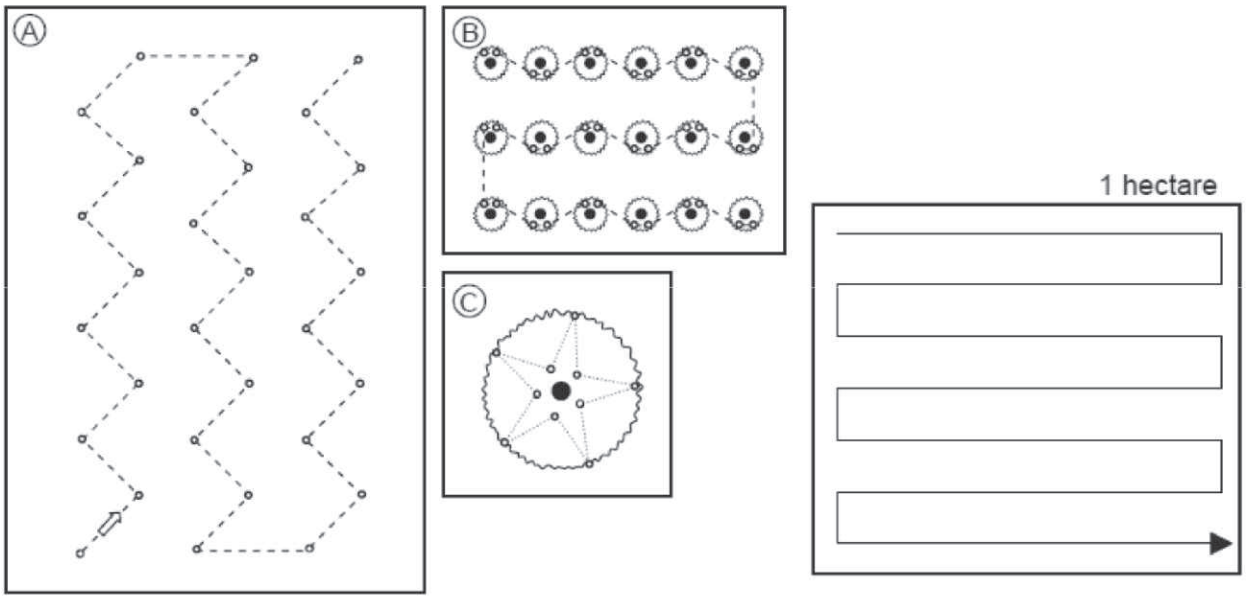
This type of sampling is done in the areas showing a poor crop yield history. Sampling should be carried out at the depth between 8-12 inches below the soil surface.

Precautions should be taken at sampling:

- Samples should always be collected from diseased and apparently healthy plants separately. Dead plants should be avoided.
- Sampling should always be done from optimum depth where maximum nematode fauna is expected.
- Extremely dry and wet soils should be avoided for sampling. Soil collected for sampling should have optimum moisture and sample should be sealed properly to maintain moisture.
- Sample should be washed as early as possible. Refrigeration is a must if samples are to be stored for some period

Storage of the samples:

- Samples should be stored in a refrigerator (about 10^0C) for a few days only if immediate processing is not possible.



Patterns of sampling: A-on fallow land, B- in an orchard, C-for a single tree

Questions:

1) With what objective will you collect soil and plant samples?

2) Write down the steps to be followed while collecting samples from vegetable field.

3) Discuss the steps to be followed while collecting samples from field crops.

4) Discuss the method of sampling for suspected nematode population in a tree.

5) How will you do sampling for nematode population in an orchard?

6) Draw out figures of sampling pattern?

Exercise -3: Extracting nematode from Soil with Cobb's Flotation and decanting method

This technique is mainly used for the extraction of motile nematodes from soil and was introduced by Cobb (1918) and is mainly used for Tylenchid species.

Principles

- Differential sedimentation rate i.e. the soil particles and nematodes settle at different rates due to differences in their specific gravity.(specific gravity of nematodes is 1.05)
- Different sized nematodes are retained on sieves of different pore sizes.

Materials

- Take out the composite sample in a pan, mix it thoroughly and take 200 g for processing. Store the remaining sample in a refrigerator.
- Transfer 200 g soil to Pan A and add about one litre water, mix well with hands, breaking clods and clumps.
- Wait for a 15 seconds and pass this soil suspension through a 20-mesh sieve (pore size 840µm), collecting the filtrate in pan B. Wash the Pan A.
- Collect roots present on the 20-mesh sieve in a beaker and discard the remaining material.
- Stir the suspension of pan B gently, wait for a few seconds and pour it through a 60-mesh sieve (pore size 250µm) in pan A. Collect the residue left over 60-mesh sieve in a beaker and label it as 60. This residue will contain cyst nematodes in case of their infestation and can be viewed directly under a stereo microscope.
- Pass the contents of pan A through a 300-mesh sieve (pore size 53 µm). Discard the suspension passed through the sieve.
- Collect the residue left over 300-mesh sieve in a beaker and label as 300.
- Further process 300-mesh residue by either of the following techniques.

Advantages

- Simple and rapid
- High extraction efficiency
- All nematode genera are recovered at different sieves
- No elaborate apparatus needed

Disadvantages

- Maximum of 200 gm soil
- Not suitable for clay soil because light particles remain in suspension leading to a dirty final suspension.

Remarks:

Aperture size of sieves can be adapted to size of target nematodes, e.g. 350 µm for *Xiphinema* and *Longidorus* and likewise

Questions:

1. Enlist out principles of Cobb's floatation and sieving method?

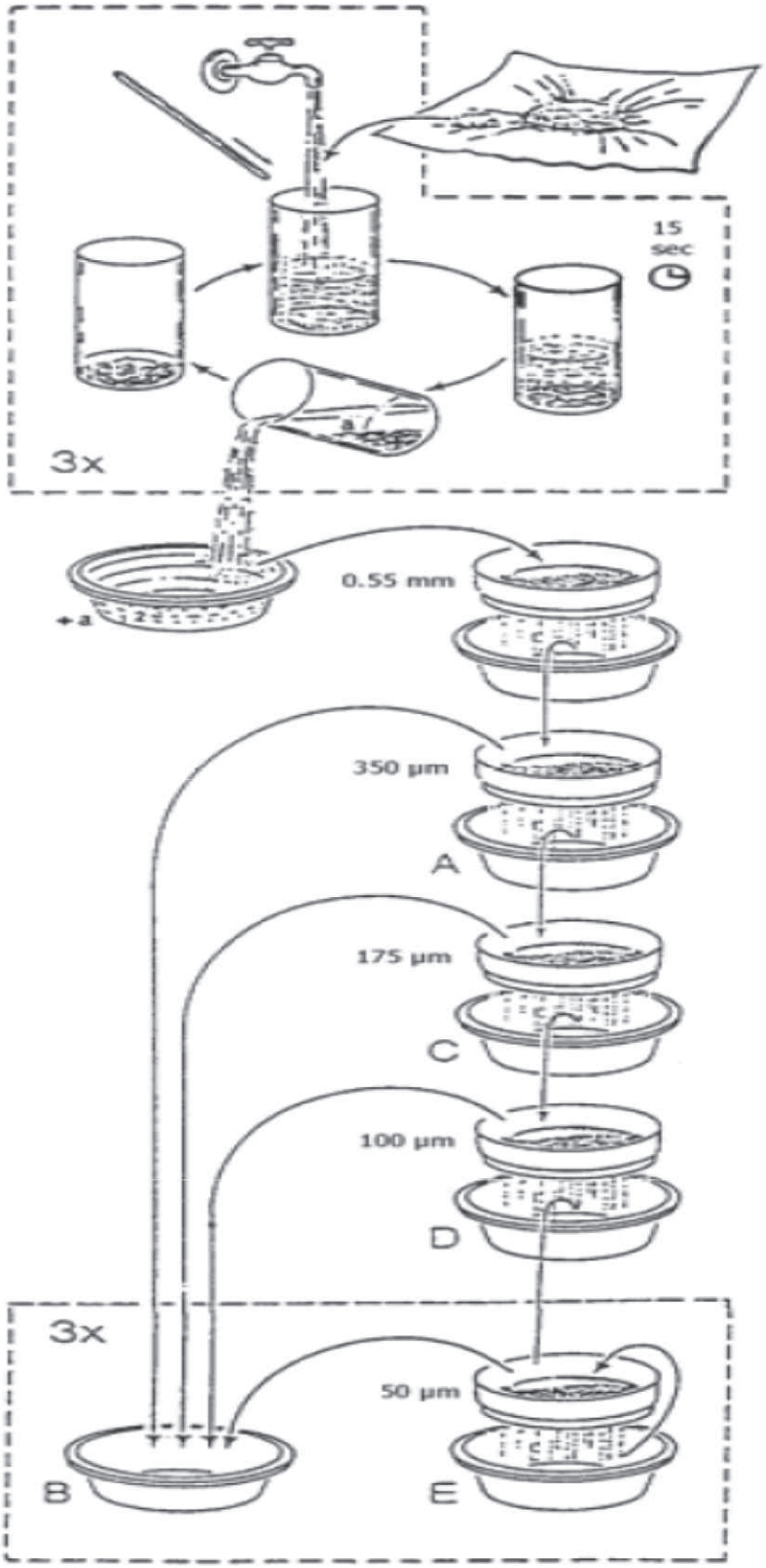


Illustration of Cobb's floatation and decantation technique

2. Enlist out merits and demerits of Cobb's floatation and decanting method?

3. Draw out a well labeled figure of Cobb's floatation and decanting method?

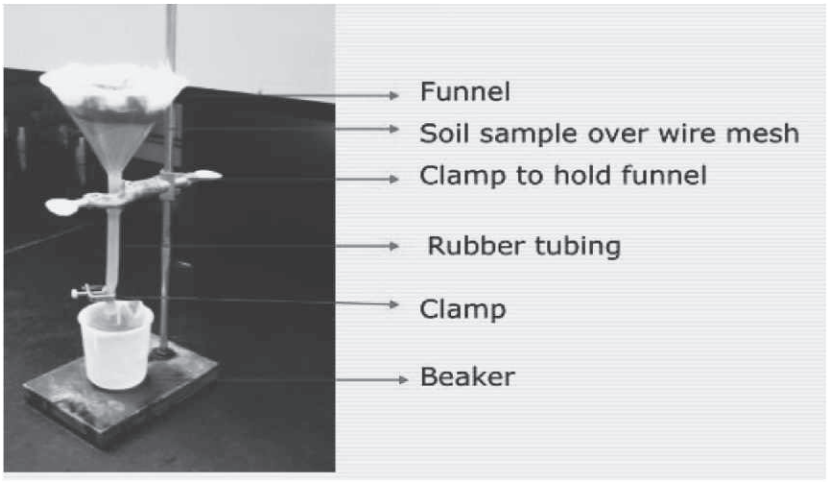
Exercise -4: Extracting nematode from Soil with Baerman's funnel technique

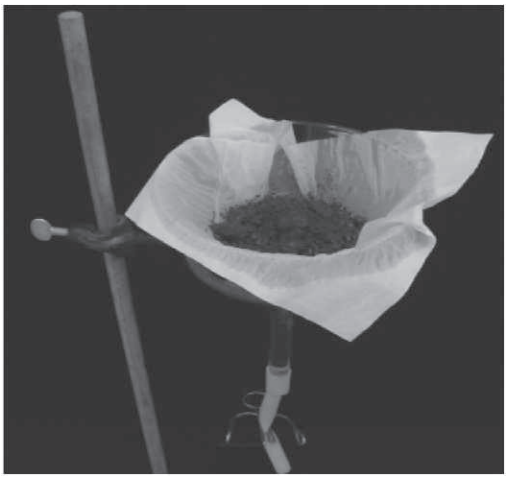
Principle:

- The active and motile nematode pass through the tissue paper and get collected at the base of rubber tube/specimen vial due to movement and gravitational force whereas inert soil particles/debris remain on the tissue paper.

Procedure:

1. Assemble the Baerman's funnel apparatus.
2. A clamped rubber tube is placed below the funnel
3. A piece of window screen (or similar material) is placed in the mouth of the funnel
4. Place a tissue-paper wrapped soil sample (200cc / 200 g composite representative sample) onto the screen material.
5. The funnel is placed into a rack or holder
6. Add water to the funnel setup until the screen and soil sample are immersed.
7. Wait overnight (or longer 24-48 hrs if desired).
8. Gather the first couple of drops of water from the bottom of the tube by slowly releasing the clamp on the tubing.
9. Examine under the microscope. Note that this technique will work only with actively mobile, living nematodes.





Baerman's Funnel set up

Advantages

- Clear nematode suspension, free of dirt and debris is obtained.
- The nematodes are collected in a small amount of water, thus making further working with them easy.

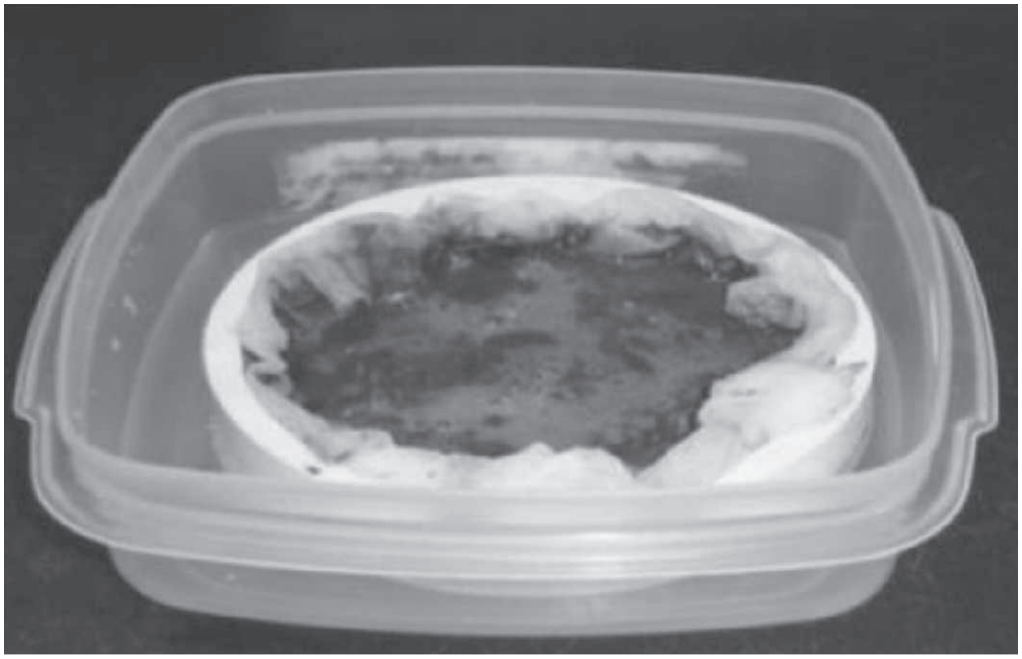
Disadvantages

- It is a time consuming.

- Nematodes may lose their activity/viability due to lack of oxygen.
- Sedentary and slow moving nematodes cannot be extracted

Modified Baermann's funnel Technique (Schindler's modification)

In this technique Petri-plate is used in place of funnel. Other steps are same as described above for Baermann's funnel method and the nematodes get collected in Petri-plate



Advantage:

- Maximum recovery of active nematode is achieved by thi1s method because the nematodes do not lose their activity/viability since the oxygen is always available.

Precautions:

- Thorough mixing of the soil and breaking of clods is important.

11 • While pouring nematode suspension on the tissue paper, a thumb should be placed in between to avoid tearing of the tissue paper.

Questions:

1. What is Baerman's funnel technique give its principle ?

2. Give advantage of modified Baerman's funnel technique over simple Baerman's funnel technique ?

3. Draw out well labeled fig of Baerman's funnel technique and write down its procedure in brief ?

Exercise 5: Extraction of Nematode from Plant Material

Objectives:

To study the nematode fauna present in the plants and their quantitative estimation.

Material required:

Infested plant material, scissors, Petri-plates, wire gauge, facial tissue paper, warring blender, acid fuchsin, lactophenol, etc.

i. Direct examination technique

Procedure:

- Wash the infested plant material thoroughly and chop it into small pieces and place it in a Petri-plate filled with water.
- The migratory forms semiendo/endoparasitic nematodes come out of the chopped material into water by 24 hours and can be observed directly under stereomicroscope.
- Alternatively the chopped material can be processed by modified Baermann's technique.

ii. Waring blender technique

Procedure:

- Wash the roots under tap water so that adhering soil particles are removed.
- Chop the roots into small pieces, and put them in a blender jar. Add about 100 ml water. Cover the jar with lid.
- Operate the blender just for 10-15 seconds.
- Take out this material from the blender and place it on the modified Baermann's funnel assembly as described earlier.

iii. Acid fuchsin staining technique (for semi-endoparasites and endoparasites)

Procedure:

- Wash the roots gently under the tap water to remove the soil particles.
- Place them on a bloting paper so that excess water is removed.
- Chop the roots in to small pieces of about 1-2 cm.
- Take a weighed quantity of chopped roots (0.5 to 1 g) and wrap them in a piece of muslin.
- Boil them in 0.1% acid fuchsinlactophenol solution for 1-3 min depending upon the hardness of the root.
- Remove the excess stain under running tap water.
- Leave the stained root bits in plain lactophenol (lactic acid 1 part+ glycerine 2 parts + phenol 1 part + water 1 part) overnight to distain the roots.
- Examine the roots under a stereomicroscope. The nematodes appear red in more or less transparent root tissues.
- The stained but dead nematodes can be dissected out of the roots under a stereomicroscope.

Precautions:

- Plant material should be chopped into small pieces for easy and efficient extraction.
- Excess stain should be washed off and destaining should be for appropriate period.
- Roots should not be boiled excessively.

Questions:

1. Enlist out techniques useful for examining the nematodes from plant sample and give procedure and precautions for Acid fuchsin staining technique?

Exercise -6: Counting and picking of plant parasitic nematodes

Objectives:

1. To estimate the number of the nematodes in a given suspension.
2. To transfer nematodes from nematode suspension to other solution to be used for further investigations.

Materials Required:

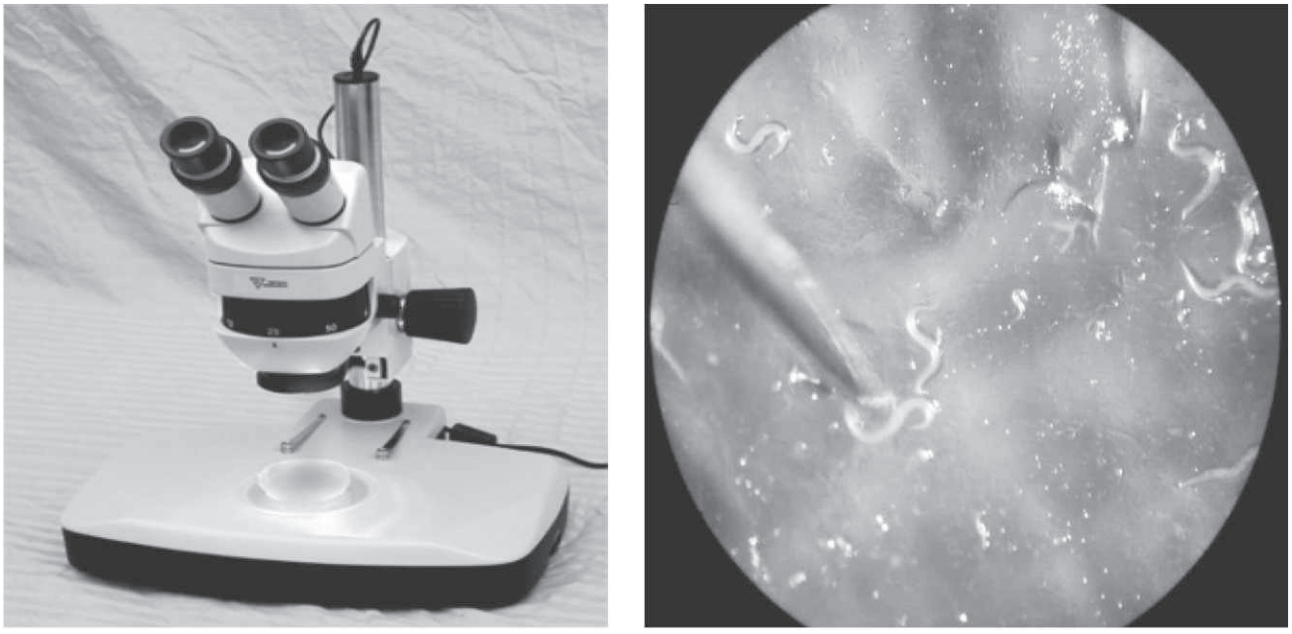
Counting dish, tally counter, nematode suspension, pipette, stereo zoom microscope, nematode pick etc.

a) Direct counting method:

This method is used when the nematode population in the suspension is very low.

Procedure:

- Measure the volume of total nematode suspension with a measuring cylinder.
- Bubble the suspension with pipette and transfer five ml of suspension to the counting dish without disturbing the dish.
- Place the counting dish on the object stage of the stereo microscope and allow the nematodes to settle at the base of the dish without disturbing. This will take a few seconds.
- Adjust one corner most small square of the counting dish from one side under stereo microscope and record the nematode number using tally counter.
- Proceed to the next square and likewise count from all the squares having nematode suspension.
- Repeat the process with another five ml nematode suspension until nematodes are counted in whole suspension.
- Aggregate of all the counts will give nematode population in the quantity of soil washed.



Stereoscopic microscope with nematode counting dish and nematode pick

b) Dilution method:

This method is used when the nematode population in the suspension is very high.

Procedure:

- Take the nematode suspension in a beaker and dilute it by adding water so as to make the final volume of 100ml.
- Bubble the diluted suspension vigorously with a pipette.
- Transfer 5ml of nematode suspension to a counting dish and count the nematodes as suggested in the direct counting method.
- Repeat the process three times by taking another 5 ml of suspension after rigorous bubbling.
- Calculate the average number of nematodes per ml.
- Multiply with 100 to compute the total nematodes count in the sample. This is the nematode population in the given quantity of soil.

Precautions:

- Suspension should be bubbled properly before pipetting out the suspension for nematode counting.
- Nematodes should be allowed to settle at the bottom of counting dish for a few seconds before initiating counting.
- While counting, all the squares of the counting dish having any amount of suspension should be viewed.
- There should be no duplicity in viewing of the squares while counting.
- Counting from a sample should be repeated at least three times and average be taken to calculate number of nematodes per sample size.

ii. Nematode picking

Procedure:

- Place the nematode suspension in counting dish / cavity block / Petri-plate and focus the nematode under a low magnification of stereo zoom microscope.
- Gently give a slight jerk to the nematode intended to be picked with a pointed nematode pick so that the nematode starts floating in the water column.
- Refocus the floating nematode under microscope.
- Immediately bring the pick underneath the nematode and lift it with a jerk.
- Transfer it to a drop of water placed over a glass slide or to a few ml of clean water in the cavity block as per the requirement.

Precautions:

- Picking of the nematodes should be done at lower magnifications.
- The focus of microscope should not be lost until nematode is picked out of suspension.

Questions:

1. Enlist out advantages of dilution method over direct count method

2. Write down the procedure of dilution method and enlist out the precautionary measures

Exercise 7: Preparation of Fixatives with their compositions

Objectives:

To prepare different types of fixatives and solutions required for preparing temporary and permanaent whole mounts of the neamatodes.

Fixatives:

Fixatives are the chemicals used to preserve the nematode specimens without any postmartum changes and also help in optical differentiation of the tissue.

Types of fixatives: Following are some of the common fixatives.

1. **Formalin 4%**

   Formalin (= 37% formaldehyde) 10.8 ml
   Distilled water 89.2 ml

   The body structure of some species becomes grainy when using formalin as a fixative. This is probably due to the formation of 'free formic acid', and can be prevented by adding a bit of calcium carbonate to the stock solution (Baker 1945).

2. **F.A. 4:1 or F.A. 4:10**

   Formalin (= 37% formaldehyde) 10.8 ml
   Glacial acetic acid 1 or 10 ml
   Distilled water Add to obtain a total volume of 100 ml

   Acetic acid neutralizes the shrinking effect caused by formalin. The disadvantage of this mixture (especially 4:10) is that nematodes become brown and the posterior part of the spear fades.

3. **F.P. 4:1**

   Formalin (= 37% formaldehyde) 10.8 ml
   Propionic acid 1 ml
   Distilled water 88.2 ml

   Propionic acid neutralizes the shrinking effect caused by formalin, and slightly enhances the contrast. F.P. is useful for year-long fixation, although Netscher and Seinhorst (1969) prefer fixing in formalin 4%, after killing with hot F.P. 4:1 (or F.A. 4:1).

4. **F.G. 4:1**

   Formalin (= 37% formaldehyde) 8.5 ml
   Glycerine 2 ml
   Distilled water 89.5 ml

   If other components of the liquid evaporate (e.g. if the jar was not properly closed), nematodes kept in this fixative stay in the glycerine, preventing them from drying out.

5. **T.A.F.**

   Formalin (= 37% formaldehyde) 7.6 ml
   Tri-ethylamine 2 ml
   Distilled water 90.4 ml
   Fixing in T.A.F. (Courtney et al. 1955) gives good results as specimens remain more or less life like, but Hooper et al. (1983) detected degeneration of the cuticle of some nematodes after 20 years of storage. Therefore, Southey (1986) does not recommend T.A.F. as a long-term fixative.

6. F.A.A. (Ditlevsen's)

Formalin (= 37% formaldehyde) 6.5 ml
Ethanol 96% 20 ml
Glacial acetic acid 1 ml
Distilled water 39.5 ml

Because of the alcohol, F.A.A. has a dehydrating, thus shrinking, effect. This may be useful, among others, for making annulation or natural indentations more visible (Southey 1986).

7. Bouin's Fixative

Formalin (= 37% formaldehyde) 26.5 ml
Saturated aqueous Picric acid, 75 ml
Glacial acetic acid 5 ml]

Nematodes are picked and placed in any of the above mentioned fixatives heated to 50°C.

Double strength (DS) of all above mentioned fixatives can be prepared by reducing the quantity of water to just half of the above mentioned quantities. DSFs are used for fixing nematode suspension. The suspension is poured in equal volume of double strength boiling fixative for this purpose.

Infested plant material is fixed in 5-10% formaldehyde after thorough washing with clean water. However, plants loose their natural colour , when fixed in formaldehyde. If natural green colur of plants is to be retained, they should be fixed in aqueous Copper sulphate solution ($CuSO_4$ 14.0g mixed in 2.5 l water) bubbled with Sulpher Dioxide gas (SO_2) until solution turns clear.

Questions:

1. Define fixative? Enlist out different types of fixatives used in nematode mounting?

Exercise-8: Preparation of Temporary and Permanent Mounts

Objectives:

- Temporary mounts of nematodes are prepared to study those structures which become obscure after fixation but may form important identification characters.
- Semi-permanent mounts are prepared to study those structures which do not remain clear for a very long period after fixation.
- Permanent mounts are prepared to preserve the slides for detailed morphological studies and to maintain the specimens for a long time for future investigations.

Materials required:

Nematode suspension, desiccators, Calcium chloride (anhydrous), cavity blocks, ethyl alcohol, formalin, lactophenol, glycerol, glass wool, glass slides, cover slips, Whatmann filter paper/ blotting paper, glyceel/nail enamel.

Procedure:

Procedure involves steps like killing, fixing and mounting of nematodes.

a. Killing.

- A temperature of 50°C is optimum for killing the nematodes
- For killing a few individual specimens, place the nematodes in a drop of water on a glass slide and warm the slide slightly while moving it regularly on the burner.
- Be cautious that the drop does not dry. Add one more drop of water.
- This method of killing is useful for making temporary mounts.

b. Killing and Fixing

- Take measured quantity of live nematode suspension in a test tube.
- Add 8% boiling formaldehyde solution/ FA4:1/TAF as fixative in equal volume to it so that the temperature of suspension is not more than 50°C.
- Leave this fixed suspension in specimen vial and cork it. Let it stand for minimum 24 hours.
- If not required for use immediately, fixed nematodes can be preserved for any period of time without further deterioration.
- This method is generally used for making semi-permanent and permanent mounts.

Temporary mounts:

Procedure:

- Place a drop of water on the centre of slide.
- Pick the freshly killed nematode specimens and place it in this water drop.

- Place a coverslip in such a way that no air bubble remains.
- Such specimens can be observed under microscope only for a few minutes until water dries up.

2. Clearing

Internal structural of nematodes specially, the reproductive organs are obscured due to the presence of food material in the intestine. To make the contents of intestine transparent, clearing is done by either of the following methods.

a) Rapid Lactophenol Method (*For semi-permanent mounts*)

Procedure

- Take a drop of lactophenol on a slide.Lactophenol can be prepared by mixing lactic acid, phenol, glycerin and water in a ratio of 1:1:2:1.
- Warm it to 55-60oC for 1-2minute.
- Transfer the desired nematode to a drop of warm lactophenol.
- The nematode will become transparent and is ready for mounting in lactophenol (semi-permanent mount).

b) Glycerol- ethanol (*Seinhorst's*) method (*for permanent mounts*)

Procedure:

1• Take Seinhorst's solution – I (96% ethanol-20ml, glycerine-1ml, distilled water-79 ml) in a cavity block and transfer the desired nematodes into it.
- Cover the cavity block partly and put it in a glass vessel containing 96% alcohol.
- Place the glass vessels in an oven 40°C for 12hours.
- Refill the cavity block with Seinhorst's solution- II (Glycerin-5 parts, 96% alcohol-95 parts).
- Cover the cavity block partly and leave it in oven at 40°C for 4 hours
- The nematodes are left in the pure glycerin and are ready for mounting in glycerin (permanent mount)

3. Mounting

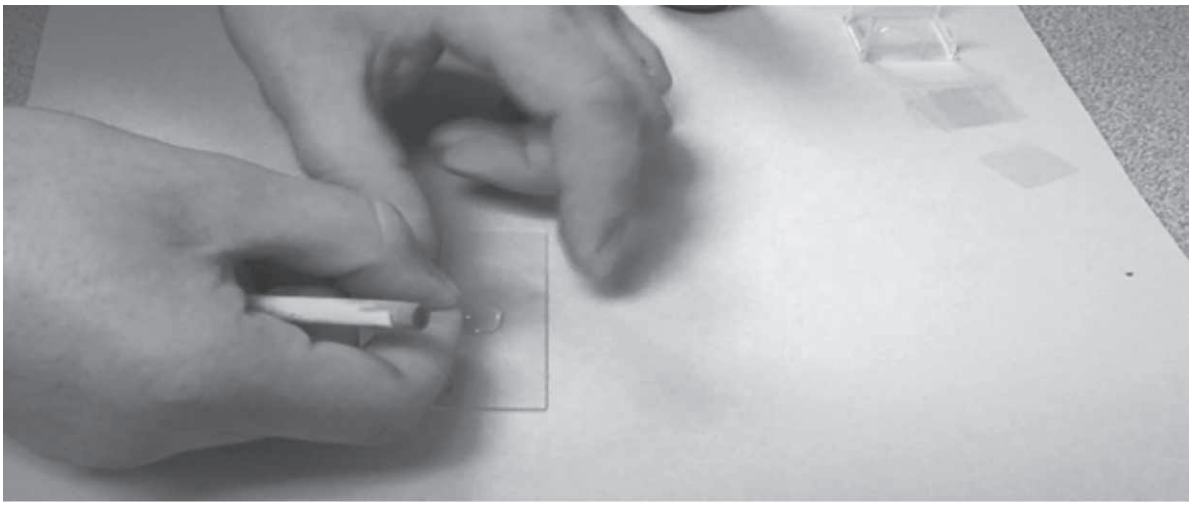
Procedure

- Take a drop of anhydrous glycerin in the centre of a glass slide. Glycerin can be made anhydrous by keeping it in an open vial in a desiccators having anhydrous calcium chloride.
- Pick 2-3 cleared nematodes and transfer them on to the slide.
- Arrange them parallel (under stereomicroscope) ensuring that nematodes are resting on the surface of the slide.
- Pick three glass wool pieces (diameter equal to nematode) and arrange in the drop on the slide radially.
- Take a cover slip and put it over the drop of lacto phenol/glycerin and tap it gently.

- Remove the excess of lacto phenol/glycerin with the help of a blotting paper.
- Seal it with glyceel/nail polish first at three places and after a few minutes ring it completely with the nail polish. After 5 minutes each, give two coating of nail polish again.
- Label the slide for nematode species, host, locality and date.

Precautions:

- While killing temperature should not exceed 50°C as high temperature distorts various body structures permanently.
- Glycerine drop should be placed in the centre and needs to be just enough to cover the cover slip completely. A big drop may slide the specimens out of cover slip area.
- The thickness of the glass wool should be almost same or slightly more than the thickness of the nematode.
- The cover glass should be placed slowly in a sliding phase so that no air bubble remains.



Preparation of Temporary and Permanent Mounts

Questions:

1. Give advantage of permanent mount over temporary mount?

2. Enlist out precautions to be taken at the time of nematode mounting

Exercise -9: Pathogenicity of Root Knot Nematode

Pathogenecity:

Capacity of a nematode species to cause infestation in a host plant.

The pathogenicity of nematode species was also carried out by koch's postulates.

1. The nematode species must be **associated** with the examined symptoms in diseased plant part.
2. The nematode species must be **extracted** and grown in pure form
3. The nematode species must produce the similar diseased symptoms as observed earlier after **inoculation** on healthy host plant.
4. The nematode species must be **re extracted** in pure form and similar as observed earlier.

Procedure followed to prove Root Knot Nematode Pathogenecity

1. **Extraction of nematodes:** The *M. incognita* eggs that served as inoculum were extracted from rhizospheric soil and roots by Cobb's sieving and decantation method from any one of the susceptible infected crop.
2. **Planting of crop:** seeds of the crops will be sown in the pots containing well manured soil, recommended irrigation and needful resource facility
3. **Inoculation:** The extracted egg mass of *M. incognita* will be inoculated at rhizosphere of 30 days old plant. The plants will be maintained as it is by providing proper irrigation, fertilizer maintaining an untreated/ un-inoculated control
4. **Observation:** the observation will be recorded after 15 days of inoculation by observing root gall index and foliar symptom development
5. **Reisolation:** infeted galls and rhizospheric soil was then subjected to extract the nematode again by Cobb's sieving and decantation method and confirmation will be made for the presence of similar types of nematode

Question:

1. Define pathogenicity? Write down procedure to prove nematode Pathogenicity?

Exercise -10: Nematicides, formulations and their application

Definition:

Nematicides are the chemicals used to kill the nematodes or suppress their population.

Nematicides may be

1. Plant/ animal originated nematicides
2. Synthetic nematicides

1. Plant originated nematicides (*botanicals*):

The toxic principal in botanicals is mostly alkaloids. Plants like marigold, neem, darek, karanj, castor etc. have been known to have nematicidal principles. Poor shelf life and fast degradation or exposure to moisture, heat and light are their negative points. High safety and low residual hazards are their positive features. Recent strategy is to include some of the plants and their products for suppression of nematode population in IPM. Neem being toxic to nematodes due to azadirachtin and nemandiol is one of the most commercialized nematicides available in Indian market. Some of the commonly available formulations are: Commercially available Neem formulations.

Neem product	Dose/ha
Neemark	2.5 l
Nimbecidine	0.5 l
Neemplus	1.0 l
Neem rich	0.5-1.0 l
Achook	0.5-1.0 l
Neem Gold	1.0 L

Synthetic nematicides fall under two categories:

1. Fumigant nematicides
2. Non fumigant nematicides

1. Fumigant nematicides: belong to two chemical groups

a) Halogenated Hydrocarbons: e.g. D-D mixture, EDB, DBCP etc

b) Methyl isocynate and its releasers: e.g. MIT, Metam, Dazomet etc.

Some of the commercially available halogenated hydrocarbons used as nematicides are listed below.

Common name	Trade Name	Dose
D-D mixture (1,3 dichloro propene, 1, 2 dichloro propane)	Telone, Vidden D	250-500 l/hectare
DBCP (1,2 dibromo-3 chloropropane)	Nemagon, Fumazone	25-50 l/ha
Chloropicrin (Tear gas), Trichloronitromethane)	Picfume, Larvicide	12fl.oz/100sq.ft
EDB (Ethylene Dibromide) 1, 2 Dibromoethane	Nemex, Dowfume, Bromofume, Soilfume	69 l/ha
MBr (Methyl Bromide)	Bromomethane, Bedfume Pestmaster	1-2 lb/sft

Non fumigant Nematicides: Contact and systemic nematicides with little fumigant action as well. Mainly belong to two groups:

1. Carbamates

Common name	Trade Name	Formulation	Dose
Carbofuran	Furadan	3G, 50 WP	Soil application@2-3kg a.i./ha Bare root dip@500-1000ppm for 30 min
Oxamyl	Vydate	40 EC	Foliar spray @4-8 l a.i./ha
Methomyl	Lannate	90 WP	-do-
Metham Sodium	Vapam	32-35% aq. sol. Soil	drench @40 fl. Oz./sq ft.

2. Organophosphates

Common name	Trade Name	Formulation	Dose
Fensulfothion	Dasanit	5&10G,EC, WP	Soil application @ 3-6 kga.i./ha Bare root dip @ 500-1000 ppmfor 30 min.
Fenamiphos	Nemacur	10 G, EC	-do-
Thionazin	Nemaphos	-do-	-do-
Ethoprophos	Mocap	-do-	-do-
Dichlofenthion	Hexanema	5G, 75 EC	-do-
Phorate	Thimet	10G, EC	-do-
Sebuphos	Rugby	-do-	-do-
Dimethoate	Rogor	30 EC	Bare root dip @500-1000 ppm for 30 min.
Carbosulfan	Marshal	25 EC	-do-
Phosphamidon	Dimecron	85 SL	-do-

Nematicide Formulations

Since 85% of the phyto parasitic nematodes are root parasites dwelling in soil, most of the nematicides find their application in soil/ plant rhizosphere in the form of granules, drenching (EC formulation) or dust. When used for seed dressing ST formulations (seed treatment formulations) and when used for bare root dips, liquid-able formulations are preferably used. However, foliar sprays and liquid-able formulations are used for the nematodes feeding on above ground parts of the plants.

Nematicide Application Methods

Fumigation

Soil fumigation requires prior preparation to be effective. Prior to fumigant or nonfumigant application, soil is often turned or tilled to increase porosity and uniformity and promote decomposition of residual plant roots, which can serve as hiding places for nematodes or interfere with fumigant movement. Adequate but not

excessive soil moisture is critically important to the success of some fumigants. Fumigants are typically injected with chisels or shanks into the upper 15–40 cm of soil, with the actual depth a function of compound, soil structure, and crop. Although deep injection is often required to minimize the escape of fumigant into the surrounding air, inadequate levels of nematicide in the upper soil layers may result in some situations. Following fumigation, the soil surface is often compacted in order to retard fumigant loss from the soil surface. The design of injection equipment modified for minimization of fumigant escape into the surrounding air is an active research area. Because the shallow chisel traces left in treated soils provide a means for fumigant to escape into the atmosphere, some nematicide labels mandate that the traces be covered with soil. Experimental chisels angled to the side 45? in order to eliminate chisel trace formation have provided control of root-knot nematodes on tomato equivalent to conventional chisels. Another example of minimizing atmospheric loss is through use of single chisel injections for crops traditionally fumigated with dual chisels. Fumigation usually involves the use of plastic tarpaulins to minimize atmospheric losses and deliver nematicide to the target organism.

Irrigation

Liquid and emulsifiable formulations of nematicides can often be applied through surface or drip irrigation systems. The goal of delivering sufficient nematotoxic materials without excessive leaching is researchable but sometimes difficult to achieve. Drip irrigation in particular offers a means of precisely controlling the amount of active ingredient delivered to a field, as well as regulating the amount of water, so that leaching of active ingredient beyond the root zone and into groundwater can be eliminated. Drip irrigation also is useful for postplant applications, and it avoids the use of granular materials that may pose risks to birds. Use of drip irrigation also reduces the amount of personal protective equipment required for field workers.

Granules and Broadcast Sprays

The most widely practiced method of applying nonfumigant nematicides is with granular formulations. Methods for application of nonfumigants to soil have been thoroughly reviewed. In some cases, adequate control can be achieved by band application of nematicides at or before sowing. In band application, plant roots may eventually grow beyond the treated area at a time when the root system will be sufficiently vigorous to not suffer serious damage. In-furrow application sometimes is practiced but may result in lack of delivery to the root zone; in other cases, in-furrow application may be preferable. In some cases, sidedress applications of nematicides are useful replacements or additions to at-plant applications. In other cases, broadcast application of granules or sprays followed by a thorough mixing of the soil may be effective. Tillage is necessary to distribute nematicide to a broad enough area to provide control, and a thorough mixing is particularly important for nematicides with poor soil mobility characteristics. Use of broadcast sprays instead of granules often promotes greater uniformity in distribution. For many annual crops, incorporating nematicides into the upper 10–15 cm of soil provides the best balance of efficacy, expense, ease, and safety to wildlife.

Seed Dressing and Bare Root Dip

Seed-transmitted nematodes can be successfully treated with nematicidal treatment of seeds. Much experimental research with biocontrol organisms or nematicidal natural products is performed with seed formulations. The principle behind bare root dips is similar to that for seed dressings; i.e., sufficient nematicide is applied to transplants to protect them at a highly vulnerable time. Root dips have provided nematode control in several situations.

Soil fumigation

Seed treatment

Seedling dip treatment

Questions:

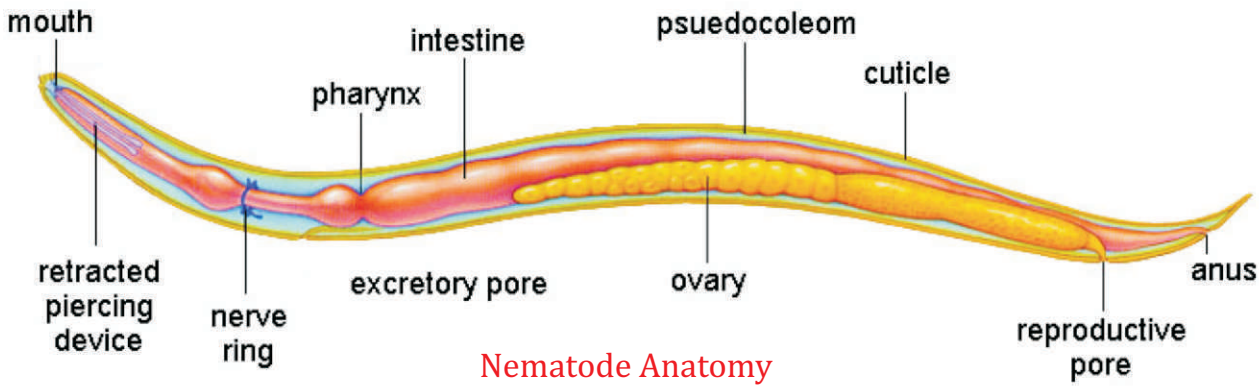
1. Define Nematicide? Enlist out their types with two examples of each?

2. Enlist out different formulations of the nematicides with their examples?

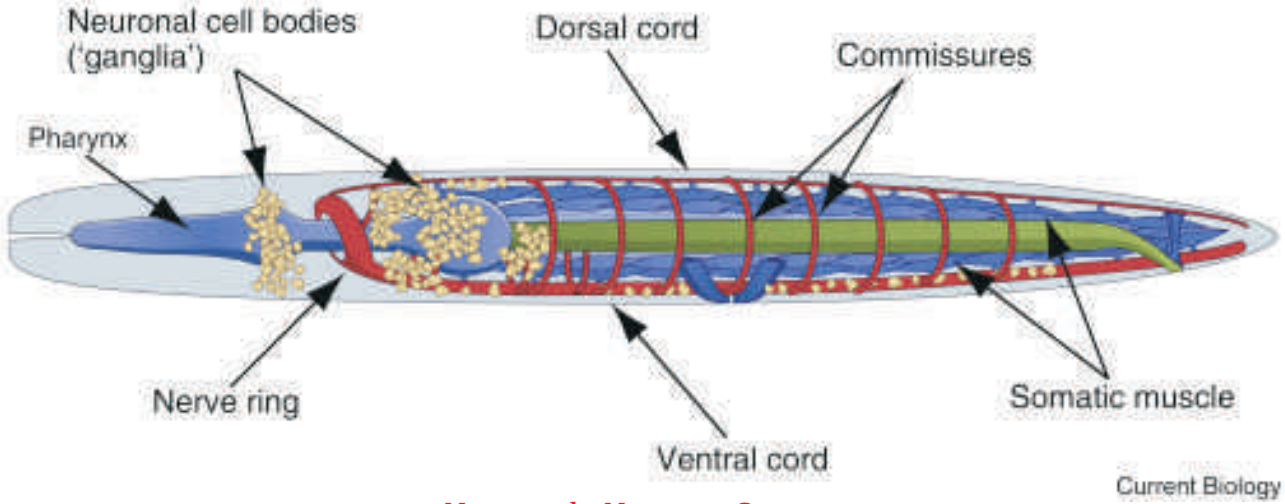
3. Enlist and explain nematicide application methods in brief?

4. Why fumigant nematicides are generally used?

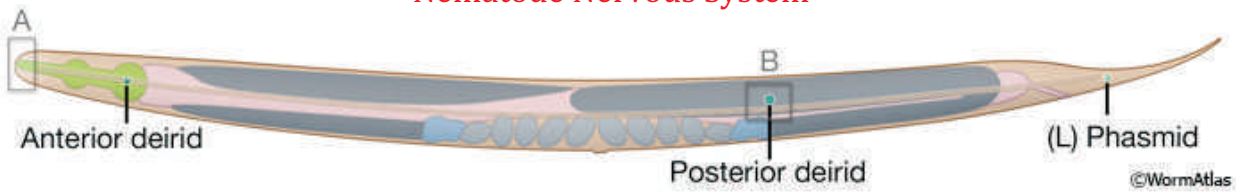
NOTES



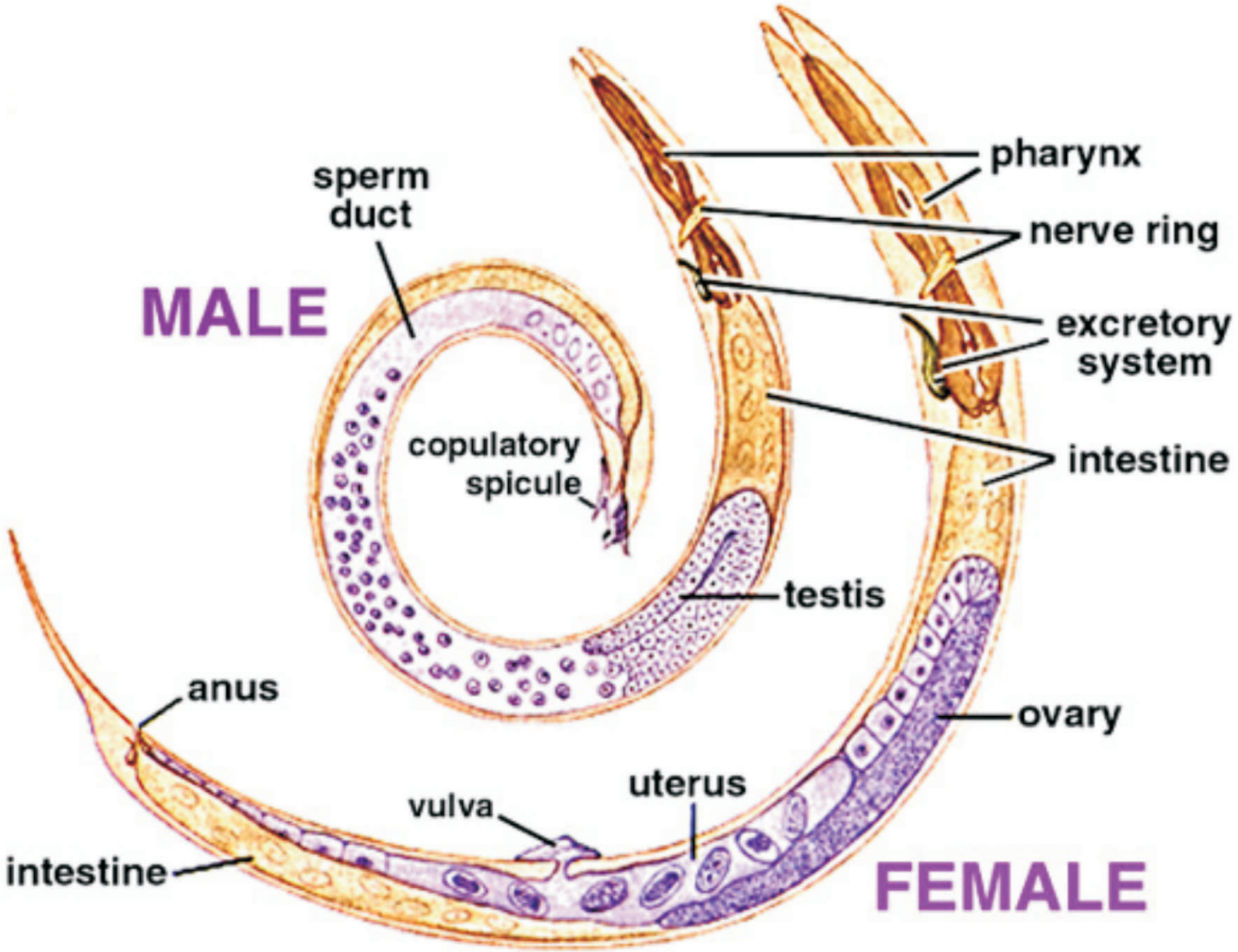
Nematode Anatomy



Nematode Nervous System



Nematode Sensory Structures


pharynx
sperm duct
nerve ring
MALE
excretory system
copulatory spicule
intestine
testis
anus
ovary
uterus
vulva
intestine
FEMALE