M.Sc. AGRICULTURE (GENETICS & PLANT BREEDING) LAB MANUAL

3rd Semester

Prepared By
Biological Science Dept.
Agriculture

MIDNAPORE CITY COLLEGE

AST-101

Exercise 1

Diagrammatic and graphic representation – simple, multiple, component and percentage bar diagram – pie chart – histogram. Frequency polygon, frequency curve

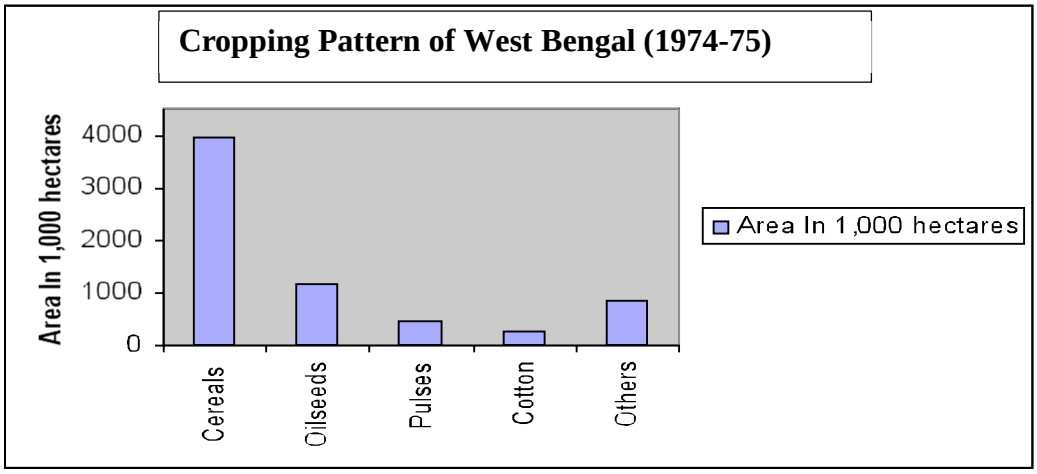
Simple Bar Diagram

Example

The cropping pattern in West Bengal in the year 1974-75 was as follows

Crops	Area In 1,000 hectares
Cereals	3940
Jute	1165
Oilseeds	464
Pulses	249
Others	822

The simple bar diagram for this data is given below:

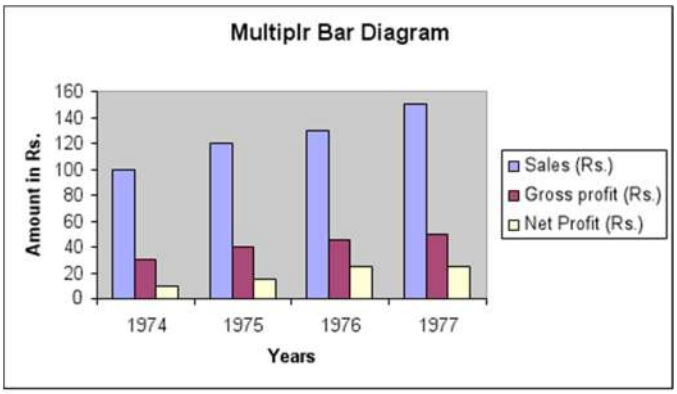


Multiple bar diagram

Example 1

Draw a multiple bar diagram for the following data

Year	Sales (Rs.)	Gross Profit (Rs.)	Net Profit (Rs.)
1974	100	30	10
1975	120	40	15
1976	130	45	25
1977	150	50	25
Total	**500**	**165**	**75**

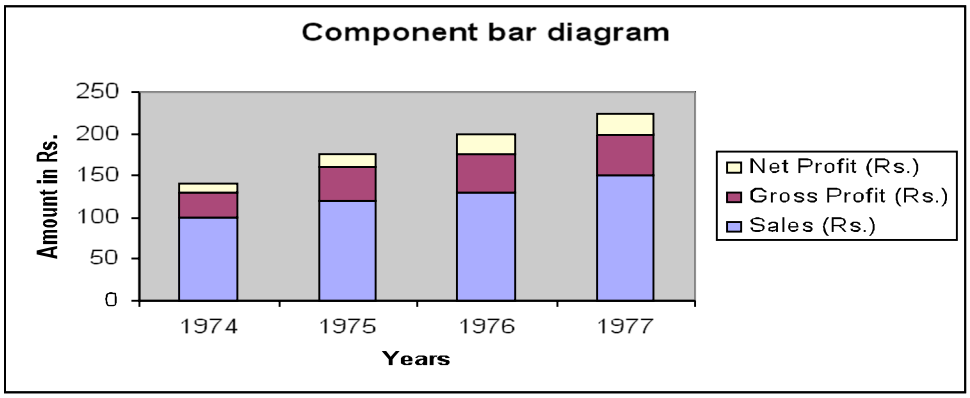


Component bar diagram

Example 2

Draw a component bar diagram for the following data

Year	Sales (Rs.)	Gross Profit (Rs.)	Net Profit (Rs.)
1974	100	30	10
1975	120	40	15
1976	130	45	25
1977	150	50	25



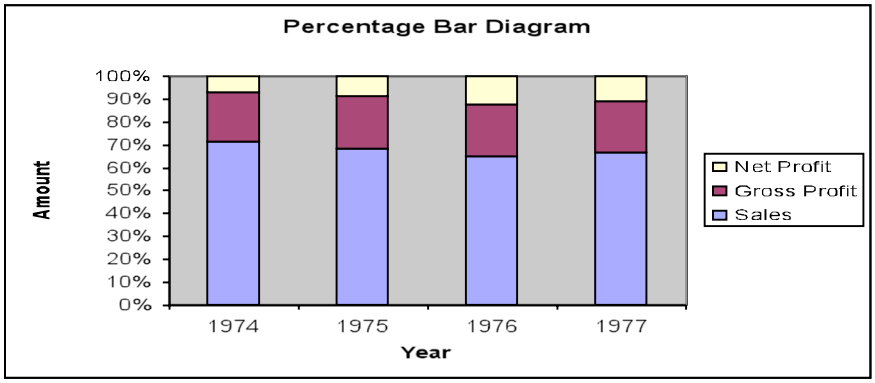
Percentage bar diagram

Example 3

Draw a Percentage bar diagram for the following data

Using the formula Percentage = $\frac{Actual\ value}{Total\ of\ the\ actual\ value} * 100$, the above table is converted.

Year	Sales (Rs.)	Gross Profit (Rs.)	Net Profit (Rs.)
1974	71.43	21.43	7.14
1975	68.57	22.86	8.57
1976	65	22.5	12.5
1977	66.67	22.22	11.11



Pie chart / Pie Diagram

Example 4

Given the population of 1991 of four southern states of India. Construct a pie diagram for the following data

State	Population
Andhra Pradesh	663
Karnataka	448
Kerala	290
Tamil Nadu	556
Total	**1957**

Using the formula

$$\text{Angle} = \frac{\text{Actual value}}{\text{Tota lof the actual value}} \times 360^0$$

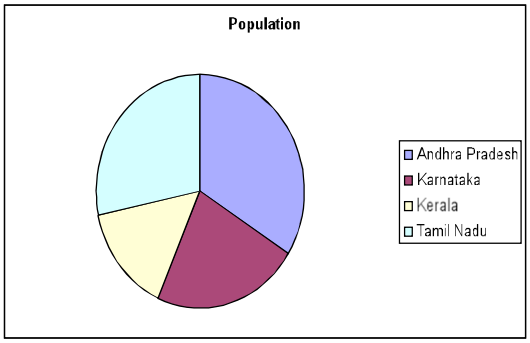
(or)

$$\text{Angle} = \frac{\text{Percentage}}{100} \times 360^0$$

The table value becomes,

State	Population
Andhra Pradesh	121.96
Karnataka	82.41
Kerala	53.35
Tamil Nadu	102.28

Radius = πr2
Here πr^2 =1957
r^2= 1957/π = 623.24
r= 24.96 =25 (approx.)

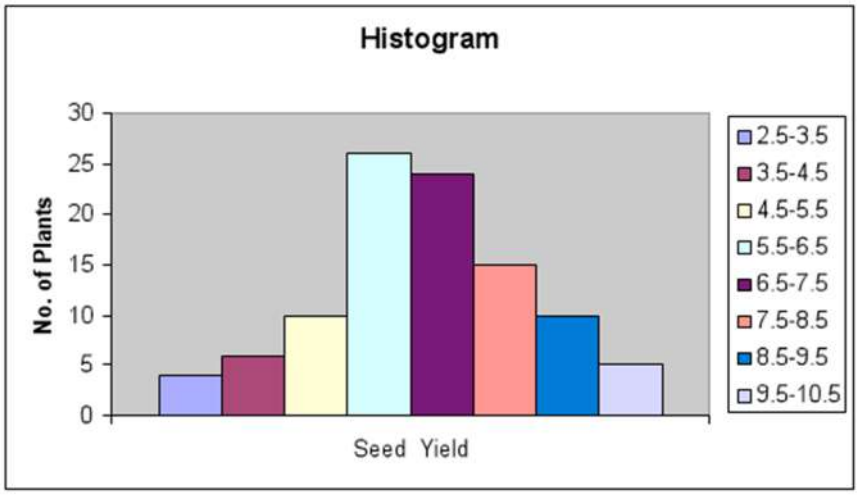


Histogram

Example 5

Draw a histogram for the following data

Seed Yield	No. of Plants
2.5-3.5	4
3.5-4.5	6
4.5-5.5	10
5.5-6.5	26
6.5-7.5	24
7.5-8.5	15
8.5-9.5	10
9.5-10.5	5

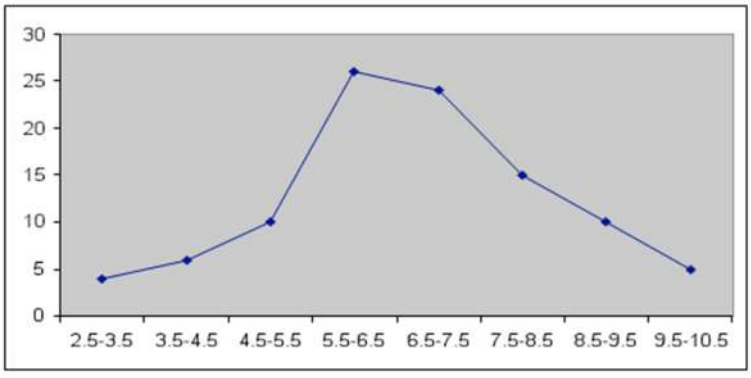


Frequency polygon

Example 6

Draw frequency polygon for the following data

Seed Yield	No. of Plants
2.5-3.5	4
3.5-4.5	6
4.5-5.5	10
5.5-6.5	26
6.5-7.5	24
7.5-8.5	15
8.5-9.5	10
9.5-10.5	5

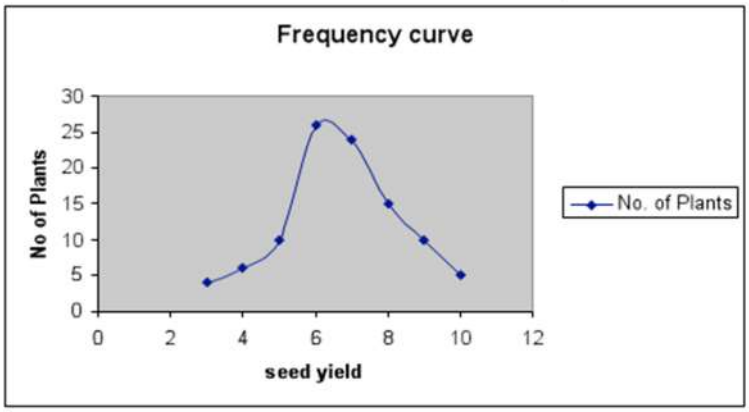


Frequency curve

Example 7

Draw frequency curve for the following data

Seed Yield	No. of Plants
2.5-3.5	4
3.5-4.5	6
4.5-5.5	10
5.5-6.5	26
6.5-7.5	24
7.5-8.5	15
8.5-9.5	10
9.5-10.5	5

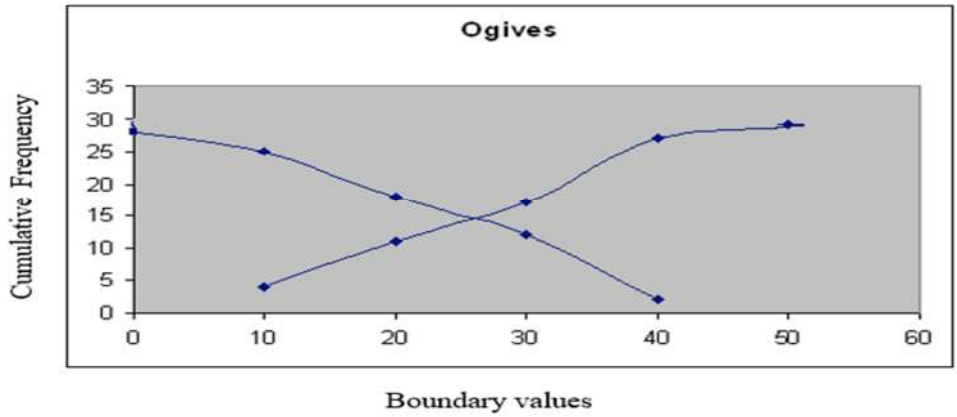


Ogives

Example 8

Draw ogives for the following data

Continuous Interval	Mid Point	Frequency	< cumulative Frequency	> cumulative frequency
0-10	5	4	4	29
10-20	15	7	11	25
20-30	25	6	17	18
30-40	35	10	27	12
40-50	45	2	29	2



Learning Exercise

1. The mean yields of green gram (Kg/hectare) under different weedicide treatment were as follows.

cide	**(Kg/ha)**
azon	
oralin	
furon	
eded Control	

Draw the simple bar diagram.

2. The cropping pattern of Tamil Nadu in 3 different years was as follows

Crops	**Area**		
	2002	**2003**	**2004**
Cereals	3600	3650	3950
Oilseeds	1000	1150	1100
Pulses	400	450	460
Cotton	200	230	240
Others	800	820	820

Draw the multi bar diagram, Component diagram, percentage bar diagram and pie chart.

3. The yields of a crop sorghum from 100 experimental plots are given below. Construct histogram, frequency polygon, frequency curve and ogives.

Grain Yield	**No. of Plants**
65-85	3
85-105	5
105-125	7
125-145	20
145-165	24
165-185	26
185-205	12
205-225	02
225-245	01

Exercise 2
Measures of central tendency- mean, median, mode, geometric mean, harmonic mean for raw data

Arithmetic mean or mean

Example 1

Calculate the mean for 2, 4, 6, 8, 10.

Solution

$$\bar{x} = \frac{2+4+6+8+10}{5} = \frac{30}{5} = 6$$

Short-cut method

Example 2

A student's marks in 5 subjects are 75, 68, 80, 92, 56. Find his average mark.

Solution

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

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

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

$$=68+6.2$$

$$=74.2$$

Median

Ungrouped or Raw data

Example 3

If the weights of sorghum ear heads are 45, 60, 48, 100 and 65 grams. Calculate the median.

Solution

Hera n = 6;

Median = Average of (n/2) and $\{(n/2)+1\}^{th}$ value

$n/2 = 3^{rd}$ value = 60 and $(n/2)+1 = 3+1 = 4^{th}$ value = 65

Median = (60+65)/2 = 62.5 g

Mode

Ungrouped or Raw data

Example 5

Find the mode for the following seed weight 2, 7, 10, 15, 10, 17, 8, 10 and 2 grams.

Mode = 10

In some cases the mode may be absent while in some cases there may be more than one mode.

Example 6

1, 12, 10, 15, 24, 30 (no mode)

2, 7, 10, 15, 12, 7, 14, 24, 10, 7, 20, 10

The modes are 7 and 10.

Geometric mode

Example 7

If the weights of sorghum ear heads are 45, 60, 48, 100, 65 grams. Find the geometric mean for the following data.

N = 5

G.M = $(45 \times 60 \times 48 \times 100 \times 65)^{1/5}$

= 60.968

Harmonic mean

Example 8

Calculate the harmonic mean from the given data: 5, 10, 17, 24, 30

H.M = $n / (\sum_{5} 1/x_i)$

$= \frac{}{\left[\left(\frac{1}{5}\right)+\left(\frac{1}{10}\right)+\left(\frac{1}{17}\right)+\left(\frac{1}{24}\right)+\left(\frac{1}{20}\right)\right]}$

= 11.526

Home work:

The weight of 15 earheads of sorghum are 100, 102, 118, 124, 126, 98, 100, 100, 118, 95, 113, 115, 123, 121, 117.Find

(i) Average of weight

(ii) Median

(iii)Mode

(iv)Harmonic mean

(v)Geometric mean

Practical 3

Measures of central tendency- mean, median, mode, G.M. and H.M. for grouped data

Arithmatic mean or mean

Grouped data

Example 1

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

Direct method

$$\bar{x} = \frac{\sum fx}{N}$$

$$\bar{x} = \frac{3713}{60} = 61.88$$

Short-cut method

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

Here A = 62

$$\bar{x} = 62 - \frac{7}{60} x\ 1 = 61.88$$

Home work

For the frequency distribution of seed yield of sesamum given in table calculate the mean yield per plot.

Yield per plot (g)	64.5-84.5	84.5-104.5	104.5-124.5	124.5-144.5
No of plots	3	5	7	20

Median

Grouped data

Example 3

The following data pertains to the number of members in a family. Find the median size of the family.

Numbers of members x	1	2	3	4	5	6	7	8	9	10	11	12
Frequency f	1	3	5	6	10	13	9	5	3	2	2	1

Solution

X	F	cf
1	1	1
2	3	4
3	5	9
4	6	15
5	10	25
6	13	38
7	9	47
8	5	52
9	3	55
10	2	57
11	2	59
12	1	60
	60	

$$\text{Median} = \text{size of } \left(\frac{N+1}{2}\right)^{th} item$$

$$= \text{size of } \left(\frac{60+1}{2}\right)^{th} item$$

$$= 30.5^{th} \text{ item}$$

The cumulative frequency just greater than 30.5 is 38.and the value of x corresponding to 38 is 6. Hence the median size is 6 members per family.

Example 4

For the frequency distribution of weights of sorghum ear-heads given in the table below. Calculate the median.

Weights of earheads (in g)	No of earheads (f)	Cumulative frequency (m)
60-80	22	22
80-100	38	60
100-120	45	105
120-140	35	140
140-160	20	160
Total	160	

Solution

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

$$\left(\frac{N}{2}\right) = \left(\frac{160}{2}\right) = 80$$

Here l = 100, N=160, f = 45, c = 20, m =60

$$\text{Median} = 100 + \frac{80-60}{45} \times 20 = 108.8 \ gms$$

Geometric mean

Example 5

Find the G.M for the following

Weight of sorghum (x)	No. of ear head(f)
50	4
65	6
75	16
80	8
95	7
100	4

Solution

Weight of sorghum (x)	No. of earhead(f)	Log x	flog x
50	5	1.699	8.495
63	10	10.799	17.99
65	5	1.813	9.065

130	15	2.114	31.71
135	15	2.130	31.95
Total	**50**	**9.555**	**99.21**

Here N= 60

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

$$= \text{Antilog}\left[\frac{99.21}{50}\right]$$

$$= \text{Antilog } 1.9842 = 96.43$$

Example 6

For the frequency distribution of weights of sorghum ear-heads given in table below. Calculte the G.M.

Weights of earheads (in g)	**No of earheads (f)**
60-80	22
80-100	38
100-120	45
120-140	35
140-160	20
Total	**160**

Solution

Weights of ear heads (in g)	No of ear heads (f)	Mid x	Log x	f log x
60-80	22	70	1.845	40.59
80-100	38	90	1.954	74.25
100-120	45	110	2.041	91.85
120-140	35	130	2.114	73.99
140-160	20	150	2.176	43.52
Total	**160**			**324.2**

Here, N= 160

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

$$= \text{Antilog}\left[\frac{324.2}{160}\right]$$

$$= \text{Antilog } [2.02625]$$

$$= 106.23$$

Harmonic mean

Example 7

The marks secured by some students of a class are given below. Calculate the H.M.

Marks	20	21	22	23	24	25
Number of Students	4	2	7	1	3	1

Solution

Marks X	No of Students f	**1/x**	$f\left(\frac{1}{x}\right)$
20	4	0.0500	0.2000
21	2	0.0476	0.0952
22	7	0.0454	0.3178
23	1	0.0435	0.0435
24	3	0.0417	0.1251
25	1	0.0400	0.0400
	18		0.8216

$$\text{H.M} = \frac{N}{\sum f\left(\frac{1}{x_i}\right)} = \frac{18}{0.1968} = 21.91$$

Homework

For the following frequency distribution find the mean, median, mode, G.M and H.M

Weight of earheads in g	**No. of earhead**
40 - 60	6
60 - 80	8
80 – 100	35
100 -120	55
120 -140	30
140 – 160	15
160 – 180	12
180 – 200	9

Practical 4

Measures of dispersion – variance, standard deviation and coefficient of variation for raw data

Variance

The square of the standard deviation is called variance (i.e) variance = $(SD)^2$

Standard deviation

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.

Example 1

Raw data

The weights of 5 ear-heads of sorghum are 100, 102, 118, 124, 126 g. Find the SD.

Solution

x	x^2
100	10000
102	10404
118	13924
124	15376
126	15876
$\Sigma x = 570$	$\Sigma x^2 = 65580$

$$SD = S = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}}$$

$$= \sqrt{\frac{65580 - \frac{(570)^2}{5}}{5-1}} = \sqrt{150} = 12.25\ gms$$

Variance= $(12.25)^{1/2} = 3.5$

Coefficient of variation

C.V= (SD/mean) x 100

Example

Consider the measurement on yield and plant height of a paddy variety. The mean and standard deviation for yield are 50 kg and 10 kg respectively. The mean and standard deviation for plant height are 55 am and 5 cm respectively.

Here the measurements for yield and plant height are in different units. Hence the variabilities can be compared only by using coefficient of variation.

For yield, CV= (10/50) x 100 = 20%

For plant height, CV= (5/55) x 100 = 9.1%

The yield is subject more variation than the plant height.

Homework

1. The weights of 8 earheads of sorghum are 14, 29, 9, 15, 20, 17, 12, and 11. Find Standard Deviation and Variance and coefficient of variation.
2. Find out which of the following batsmen is more consistent in scoring.

Batsman A	5 7	16	27	39	53	56	61	80	101	105
Batsman B	0 4	16	21	41	43	57	78	83	93	95

Practical 5

Measures of dispersion – variance, standard deviation and coefficient of variation for grouped data

Standard deviation and Variance

Example 1

The frequency distributions of seed yield of 50 *Seasamum* plants are given below. Find the standard deviation.

Seed yield in g (x)	3	4	5	6	7
Frequency (f)	4	6	15	165	10

Solution

Seed yieldin gms (x)	**f**	**fx**	**fx^2**
3	4	12	36
4	6	24	96
5	15	75	375
6	15	90	540
7	10	70	490
Total	**50**	**271**	**1537**

Here N = 50, Standard deviation =

$$S = \sqrt{\frac{\sum fx^2}{N} - \left(\frac{\sum fx}{N}\right)^2} = \sqrt{\frac{1537}{50} - \left(\frac{271}{50}\right)^2}$$

$= \sqrt{30.74 - 29.3764}$ =1.1677g

Variance = $(1.1677)^{1/2}$ = 1.081

Example 2

The Frequency distributions of seed yield of 50 *seasamum* plants are given below. Find the standard deviation.

Seed yield in gms (x)	2.5-35	3.5-4.5	4.5-5.5	5.5-6.5	6.5-7.5
No. of plants (f)	4	6	15	165	10

Solution

Seed yield (g) x	**No. of plants f**	**Mid x**	**$d=\frac{x-A}{C}$**	**df**	**d^2f**
2.5-3.5	4	3	-2	-8	16
3.5-4.5	6	4	-1	-6	6
4.5-5.5	15	5	0	0	0
5.5-6.5	15	6	1	15	15
6.5-7.5	10	7	2	20	40
Total	**50**	**25**	**0**	**21**	**77**

A= Assumed mean= 5, N= 50, C= 1

$$S = C \times \sqrt{\frac{\sum fd^2}{N} - \left(\frac{\sum fd}{N}\right)^2}$$

$$= 1 \times \sqrt{\frac{77}{50} - \left(\frac{21}{50}\right)^2}$$

$$= \sqrt{1.54 - 0.1764}$$

$$= \sqrt{1.3636} = 1.1677$$

Variance = $(1.1677)^{1/2}$ = 1.081

Coefficient variation

Example 3

Consider the measurement on yield and plant height of a paddy variety. The mean and standard deviation for yield are 50 kg and 10 kg respectively. The mean and standard deviation for plant height are 55 am and 5 cm respectively.

Here the measurements for yield and plant height are in different units. Hence the variabilities can be compared only by using coefficient of variation.

For yield, CV= (10/50) x 100 = 20%

For plant height, CV= (5/55) x 100 = 9.1%

The yield is subject more variation than the plant height.

Homework

1. From the data given below, find which series is more consistent

ariable	10-20	20-30	30-40	40-50	50-60	60-70
eries A	10	16	30	40	26	18
eries B	22	18	32	34	18	16

2. The yield of a crop sorghum from 31 experimental plots are given below. Find the Range, Standard deviation, Variance, Coefficient of variation.

Grain yield	No. of plots
130	3
135	4
140	6
145	6
146	3
148	5
149	2
150	1
157	1

3. The following table gives the protein intake of 400 families. Find the Range, Standard deviation, Variance, Coefficient of variation.

Protein intake / Consumption unit Per day in grams	No. of Families
15 - 25	30
25 – 35	40
35 – 45	100
45 – 55	110
55 – 65	80
65 –75	30
75 - 85	10

Practical 6

Selection of simple random sampling using lottery method and random numbers

Home work

The following data refers to the Cotton yield of 96 plants.

82	102	88	93	97	38	103	92
102	62	63	72	64	68	59	69
73	65	46	79	87	84	29	52
28	36	37	53	49	51	30	37
56	66	42	37	35	97	32	35
89	99	54	72	26	67	18	27
60	72	33	42	52	82	14	22
57	73	63	61	63	92	40	58
62	61	43	25	42	36	17	30
75	87	47	56	76	36	35	44
56	51	111	73	93	58	49	89
50	80	54	55	91	12	82	76

Select a sample of 25 plants by using simple random sampling method. Also calculate the mean of the 25 samples and verify whether the mean is equal to the mean of the 96 plants.

Practical 7

Students's t test – Paired and Independent t test

Test for single mean (n<30)

Example 1

Based on field experiments, a new variety green gram is expected to give a yield of 12.0 quintals per hectare. The variety was tested on 10 randomly selected farmers' fields. The yield (quintals/hectare) were recorded as 14.3,12.6,13.7,10.9,13.7,12.0,11.4,12.0,12.6,13.1. Do the results conform the expectation?

Solution:

Null hypothesis H_0: $\mu=12.0$

i.e., the average yield of the new variety of green gram is 12.0 q/ha.

Alternate hypothesis: $H_1= \mu \neq 12.0$

i.e., the average yield of the new variety of green gram is not 12.0 q/ha

Level of significance: 5%

Test statistic

$$t_{cal} = \left| \frac{\bar{x} - \mu}{\frac{s}{\sqrt{n}}} \right| \sim t_{(n-1)}\ d.f$$

From the given data

$$\sum x = 126.3 \qquad \sum x^2 = 1605.77$$

$$\bar{x} = \frac{\sum x}{n} = \frac{126.3}{10} = 12.63$$

$$s = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}} = \sqrt{\frac{1605.77 - 1595.169}{9}} = \sqrt{\frac{10.601}{9}}$$

$$= 1.0853$$

$$\frac{s}{\sqrt{n}} = \frac{1.0853}{\sqrt{10}} = 0.3432$$

Now $t_{cal} = \left| \frac{\bar{x} - \mu}{\frac{s}{\sqrt{n}}} \right| \sim t_{(n-1)}\ d.f$

$$= t_{cal} = \frac{12.63 - 12}{0.3432} = 1.836$$

Table value

$t_{(0.05,9)}=2.262$ (two tailed test)

Inference

$t_{cal}<t_{tab}$

We accept the null hypothesis H_0.

We conclude that the new variety of green gram will give an average yield of 12 q/ha.

Note

F-test is used to test the equality of two means

$$F = \frac{S_1^2}{S_2^2} \sim F_{(n_1 - 1, n_2 - 1)}\, d.f \text{ if } S_1^2 > S_2^2$$

where S_1^2 is the variance of the first sample whose size is n_1.

S_2^2 is the variance of the second sample whose size is n_2.

Otherwise

$$F = \frac{S_2^2}{S_1^2} \sim F_{(n_2 - 1, n_1 - 1)}\, d.f \text{ if } S_2^2 > S_1^2$$

Inference

$F_{cal} < F_{tab}$

We accept the null hypothesis H_0; the variances are equal.

Test for equality of two means (Independent samples)

Example 2

A group of 5 patients treated with medicine. A is of weight 42,39,38,60 &41 kgs. Second group of 7 patients from the same hospital treated with medicine B is of weight 38, 42, 56, 64, 68, 69 & 62 kgs. Find whether there is any difference between medicines?

Solution

Ho:., $\mu_1 = \mu_2$ (i.e.) there is no significant difference between the medicines A and B as regards on increase in weight.

H1 $\mu_1 \neq \mu_2$ (i.e.) there is a significant difference between the medicines A and B Level of significance = 5%

Before we go to test the means first we have to test their variability using F-test.

F-test

Ho:., $\sigma_1^2 = \sigma_2^2$

H1:., $\sigma_1^2 \neq \sigma_2^2$

$$S_1^2 = \frac{\sum x_1^2 - \frac{(\sum x_1)^2}{n1}}{n1 - 1} = 82.5$$

$$S_2^2 = \frac{\sum x_2^2 - \frac{(\sum x_2)^2}{n2}}{n2 - 1} = 154.33$$

$$\therefore F = \frac{S_2^2}{S_1^2} \sim F_{(n_2 - 1, n_1 - 1)}\, d.f \text{ if } S_2^2 > S_1^2$$

$$F_{cal} = \frac{154.33}{32.5} = 1.8707$$

$F_{tab}(6,4)$ d.f = 6.16

Means, $F_{cal} < F_{tab}$

We accept the null hypothesis H_0: the variances are equal

Test statistics

$$t = \frac{\left|(\bar{x}_1 - \bar{x}_2)\right|}{S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \sim t_{(n1+n2-2)}d.f$$

Where

$$S^2 = \frac{\left[\sum x_1^2 - \frac{(\sum x_1)^2}{n1}\right] + \left[\sum x_2^2 - \frac{(\sum x_2)^2}{n2}\right]}{n_1 + n_2 - 2} = \frac{330 + 926}{10} = 125.6$$

$$t = \frac{|44 - 57|}{\sqrt{125.6\left(\frac{1}{7} + \frac{1}{75}\right)}} = 1.98$$

Table value

$T_{tab[(5+7-2)=10]}$d.f at 5% is 2.228

Inference:

$t_{cal} < t_{tab}$

We accept the null hypothesis H_0

We conclude that the medicines A and B do not differ significantly.

Example 3

The summary of the results of an yield trial on onion with two methods of propagation is given below. Determine whether the methods differ with regard to onion yield. The onion yield is given in Kg/plot.

Method I	**Method II**
n1=12	n2=12
$\bar{x}_1 = 25.25$	$\bar{x}_2 = 28.83$
SS1=186.25	SS2=737.6667
$S_1^2 = 16.9318$	$S_2^2 = 67.0606$

Solution

Ho:., $\mu_1=\mu_2$ (i.e) the two propagation method do not differ with regard to onion yield.

H_1 $\mu_1 \neq \mu_2$ (i.e) the two propagation methods differ with regard to onion yield.

Level of significance = 5%

Before we go to test the means first we have to test their variability using F-test.

F-test

Ho:., $\sigma_1^2=\sigma_2^2$

H1:., $\sigma_1^2 \neq \sigma_2^2$

$$S_1^2 = \frac{\sum x_1^2 - \frac{(\sum x_1)^2}{n1}}{n1-1} = 16.9318$$

$$S_2^2 = \frac{\sum x_2^2 - \frac{(\sum x_2)^2}{n2}}{n2-1} = 67.0606$$

$$\therefore F = \frac{S_2^2}{S_1^2} \sim F_{(n_2-1, n_1-1)}\, d.f \text{ if } S_2^2 > S_1^2$$

$$F_{cal} = \frac{67.0606}{16.9318} = 3.961$$

$F_{tab}(11,11)$ d.f=2.82

$\Rightarrow F_{cal} > F_{tab}$

We reject the null hypothesis H_0: the variances are unequal.

Here the variances are unequal with equal sample size then the test statistic is

$$t = \frac{\left|(\bar{x}_1 - \bar{x}_2)\right|}{S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \sim t_{\left(\frac{n1+n2}{2}-1\right)}\, d.f$$

Where

$$S^2 = \frac{\left[\sum x_1^2 - \frac{(\sum x_1)^2}{n1}\right] + \left[\sum x_2^2 - \frac{(\sum x_2)^2}{n2}\right]}{n_1 + n_2 - 2}$$

$$S^2 = \frac{SS1 + SS2}{n_1 + n_2 - 2} = \frac{186.25 + 737.6667}{12+12-2} = 41.9962$$

$$t = \frac{25.25 - 28.83}{\sqrt{41.9962\left(\frac{1}{12} + \frac{1}{12}\right)}} = \frac{3.58}{\sqrt{6.9994}} = 1.353$$

t_{cal}=1.353

table value

$$t_{\left(\frac{n1+n2}{2}-1\right)} = t_{\left(\frac{12+12}{2}-1\right)} = t_{11}\, d.f \text{ at 5\% los} = 2.201$$

Inference:

$t_{cal} < t_{tab}$

We accept the null hypothesis H_0

We conclude that the two propagation methods do not differ with the regard to onion yield.

Equality of two means (Dependent samples)

Paired t test

Example 4

In certain food experiment to compare two types of baby foods A and B, the following results of increase in weight (lbs) we observed in 8 children as follows:

Food A(x)	49	53	51	52	47	50	52	53
Food B(y)	52	55	52	53	50	54	54	53

Examine the significance of increase in weight of children due to food B.

Solution

$H_0 : \mu_1 = \mu_2$, there is no significant difference between the two foods.

$H_1 : \mu_1 \neq \mu_2$, there is significant difference between the two foods.

Level of significance = 5%

Test statistic:

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

x	y	d=x-y	d^2
49	52	-3	9
53	55	-2	4
51	52	-1	1
51	52	-1	1
47	50	-3	16
50	54	-4	16
52	54	-2	4
53	53	0	0
Total		**-16**	**44**

$$\bar{d} = \frac{\sum di}{n} = \frac{-16}{8} = -2,$$

$$S = \sqrt{\frac{\sum di^2 - \frac{(\sum di)^2}{n}}{n-1}} = 1.3093$$

$$t_{cal} = \frac{|-2|}{1.3093/\sqrt{8}} = 4.32$$

Table value:

$T_{(8-1)}$ d.f at 5% is = 2.365

Inference:

$t_{cal} > t_{cal}$

We reject the null hypothesis H_0 and accept the alternate hypothesis H_1: there is significant difference between the two foods A and B.

Homework:

1. 10 samples of leaves of the plant are chosen at random from a large population and their weight in grams are found to be as follows

63	63	64	65	66	69	69	70	70	71

From this data mean wt. in universe is 65 g. Can we assume this mean weight?

2. A health status survey in a few villages revealed that the normal serum protein value of children in that locality is 7.0 g/100ml. A group of 16 children, who received high protein food for a period of 6 months had serum protein values shown below. Can we consider that the mean serum protein level of these who were fed on high protein diet is different from that of the general population.

Children	1	2	3	4	5	6	7	8	9	10
Protein level g %	7.1	7.70	8.2	7.56	7.05	7.08	7.21	7.25	7.36	6.59
Children	11	12	13	14	15	16				
Protein level g %	6.85	7.9	7.27	6.56	7.93	8.5				

3. The following data related to the rate of diffusion of CO_2 through two series of differentporosity, find out whether the diffusion rate same for both sides.

Diffusion through finesoil (x_1)	20	31	31	23	28	23	26	27	26	17	17	25
Diffusion through coarse soil (x_2)	19	30	32	28	15	26	35	18	25	27	35	34

4. A new variety of cotton was evolved by a breed. In order to compare its yielding ability with that of a ruling variety, an experiment was conducted in Completely Randomised Design. The yield (kg/plot) was observed. The summary of the results are given below. Test whether the new variety of cotton gives higher yield than the ruling variety.

New Variety	$n_1 = 9$	$=28.2$	$S_1^2=5.4430$
Ruling Variety	$n_2 = 11$	$=25.9$	$S_2^2=1.2822$

5. The iron contents of fruits before and after applying farm yard manure were observed as follows:

Fruit No:	1	2	3	4	5	6	7	8	9	10
Before Applying	7.7	8.5	7.2	6.3	8.1	5.2	6.5	9.4	8.3	7.5
After Applying	8.1	8.9	7.0	6.1	8.2	8.0	5.8	8.9	8.7	8.0

Is there any significant differences between the mean iron contents and in the fruit before and after FYM?

Practical 8

Chi-square test- test for association and goodness of fit

χ^2 – test for goodness of fit

Example 1

The number of yeast cells counted in a haemocytometer is compared to the theoretical value is given below. Does the experimental result support the theory?

No. of Yeast cellsin the square	Obseved Frequency	Expected Frequency
0	103	106
1	143	141
2	98	93
3	42	41
4	8	14
5	6	5

Solution

H_0: the experimental results support the theory

H_1: the experimental results does not support the theory.

Level of significance=5%

Test Statistic:

$$\chi^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i} \sim \chi^2_{(n-1)\,df}$$

O_i	E_i	$O_i - E_i$	$(O_i - E_i)^2$	$(O_i - E_i)^2/E_i$
103	106	-3	9	0.0849
143	141	2	4	0.0284
98	93	5	25	0.2688
42	41	1	1	0.0244
8	14	-6	36	2.5714
6	5	1	1	0.2000
400	400			**3.1779**

$\chi^2 = 3.1779$

Table value:

$\chi^2_{(6-1=5)}$ d.f at 5% significance= 11.070

Inference

$\chi^2_{cal} < \chi^2_{tab}$

We accept the null hypothesis: there is a good correspondence between theory and experiment.

χ^2 test for independence of attributes

Example 2

The severity of a disease and blood group were studied in a research projest. The findings sre given in the following table, know mn as the m x n contingency table. Can this severity of the condition and blood group are associated. Severity of a disease classified by blood group in 1500 patients:

Condition	Blood Groups				Total
	O	A	B	AB	
Severe	51	40	10	9	110
Moderate	105	103	25	17	250
Mild	384	527	125	104	1140
Total	540	670	160	130	1500

Solution:

H0: The two attributes severity of the condition and blood groups are not associated.

H1: The two attributes severity of the condition and blood groups are associated.

Calculation of Expected frequencies

Condition	Blood Groups				Total
	O	A	B	AB	
Severe	39.6	49.1	11.7	9.5	110
Moderate	90.0	111.7	26.7	21.7	250
Mild	410.4	509.2	121.6	98.8	1140
Total	540	670	160	130	1500

Test statistics

$$\chi^2 = \sum_{i=j}^{m} \sum_{j=1}^{n} \frac{\left(o_{ij} - E_{ij}\right)}{E_{ij}} \sim \chi^2(m-1)(n-1) \text{ df}$$

Here, m= 3 and n= 4

Calculations:

O_i	E_i	O_i-E_i	$(O_i-E_i)^2$	$(O_i-E_i)^2/E_i$
51	39.6	11.4	129.96	3.2818
40	49.1	-9.1	82.81	1.6866
10	11.7	-1.7	2.89	0.2470
9	9.5	-0.5	0.25	0.0263
105	90.0	15	225.00	2.5000
103	111.7	-8.7	75.69	0.6776
25	26.7	-1.7	2.89	0.1082
17	21.7	-4.7	22.09	1.0180
384	410.4	-26.4	696.96	1.6982
527	509.2	17.8	316.84	0.6222
125	121.6	3.4	11.56	0.0951
104	98.8	5.2	27.04	0.2737
				12.2347

$\chi^2 = 12.2347$

Table value:

$\chi^2_{(3-1)(4-1)} = \chi^2_{(6)}$ at 5% = 12.59

Inference

$\chi^2_{cal} = \chi^2_{tab}$

We accept the null hypothesis (i.e.) the two attributes severity of the condition and blood group are independent.

2 x 2 contingency table

Example 3

In order to determine the possible effect of a chemical treatment on the rate of germination of cotton seeds a pot culture experiment was conducted. The results are given below

Chemical treatment and germination of cotton seeds

	Germinated	Not germinated	Total
Chemically Treated	118	22	140
Untreated	120	40	160
Total	238	62	300

Does the chemical treatrment improve the germination rate of cotton seeds at 1 % level?

Solution:

H_0:The chemical treatment does not improve the germination rate of cotton seeds.

H_1: The chemical treatment improves the germination rate of cotton seeds.

L.O.S = 1 %

Test statistic

$$\chi^2 = \frac{N(ad-bc)^2}{(a+b)(c+d)(a+c)(b+d)} \sim \chi^2 \text{ (2-1) (2-1) df} = \chi^2 \text{ (1) df}$$

$$\chi^2 = \frac{300(118\times 40 - 22\times 120)^2}{140\times 160\times 62\times 238} = 3.927$$

Table value:

χ^2 (1) df at 1 % L.O.S = 6.635

Inference

$\chi^2_{cal} < \chi^2_{tab}$

We accept the null hypothesis. (i.e)The chemical treatmentwill not improve the germination rate of cotton seeds significantly.

Yates correction for continuity

Example 4

In an experiment on the effect of a growth regulator on fruit setting in muskmelon the following results were obtained. Test whether the fruit setting in muskmelon and the application of growth regulator are independent at 1% level.

	Fruit set	Fruit not set	Total
Treated	16	9	25
Control	4	21	25
Total	20	30	50

Solution:

H0:Fruit setting in muskmelon does not depend on the application of growth regulator.

H1: Fruit setting in muskmelon depend on the application of growth regulator.

L.O.S = 1 %

Tet statistic

$$\chi^2 = \frac{N\left(|ad-bc| - \frac{N}{2}\right)^2}{(a+b)(c+d)(a+c)(b+d)} \sim \chi^2 \text{ (1) df}$$

$$\chi^2 = \frac{50\left[|16\times 21 - 9\times 4| - \frac{50}{2}\right]^2}{25\times 25\times 20\times 30} = 10.08$$

Table value:

χ^2 (1) df at 1 % L.O.S = 6.635

Inference

$\chi^2_{cal} > \chi^2_{tab}$

We reject the null hypothesis. (i.e) Fruit setting in muskmelon is influenced by the growth regulator.

Homework

1. The theory predicts the proportion of beans in the 4 groups A, B, C, D should be 9:3:3:1. In an experiment among 1600 beans, the number in the four groups were 882, 313, 287 and 118. Does the experimental result support the theory.
2. A study was conducted, among 100 professors from 3 different divisions for the preference on beverages of 3 categories test if there is any relationship between the field of teaching and preference of beverage.

	Field of teaching			
Beverage	Business	Social Sciences	Agri	Total
Tea	20	10	10	40
Coffee	10	10	15	35
Cold drinks	10	8	7	25
Total	40	28	32	100

3. A random sample of 600 students from Delhi University are selected and asked their opinion ab out autonomous Status of Colleges. The results were given below. Test the hypothesis at 5% level that opinions are independent of class groupings.

Class grouping	Favour of	Against	
Commerce	120	80	200
Science	130	70	200
Arts	70	30	100
Total	400	200	600

4. In a survey of preference of new coverage 100 persons are collected and taste preference of average was surveyed according to sex of the person. We conclude that the taste preference and sex of the person are associated.

	Male	Female	
Favour	35	25	60
Against	25	15	40
Total	60	40	100

Practical 9

Calculation of Karl Pearson's correlation coefficient

Pearson's Correlation coefficient

Example 1

Compute Pearson's coefficient of correlation between advertisement cost and sales as per the data given below.

Advertisement Cost	39	65	62	90	82	75	25	98	36	78
Sales in lakhs	47	53	58	86	62	68	60	91	51	84

Solution:

H_0: The correlation coefficient r is not significant

H_1: The correlation coefficient r is significant.

Level of significance 5%.

From the data, n = 10;

$\Sigma x = 650$, $\Sigma y = 660$, $\Sigma xy = 45604$, $\Sigma x^2 = 47648$, $\Sigma y^2 = 45784$

$$r = \frac{\sum xy - \frac{\sum x \sum y}{n}}{\sqrt{\sum x^2 - \frac{(\sum x)^2}{n}}\sqrt{\sum y^2 - \frac{(\sum y)^2}{n}}}$$

$$= \frac{45604 - \frac{(650)(660)}{10}}{\sqrt{47648 - \frac{(650)^2}{10}}\sqrt{45784 - \frac{(660)^2}{10}}}$$

$$= \frac{45604 - 42900}{(73.47)(47.1)} = 0.7804$$

Correlation coefficient is positively correlated.

Test statistic

$$t = \frac{|r|}{\sqrt{\frac{1-r^2}{n-2}}} \sim (n\text{-}2)\ d.f$$

$$t = \frac{0.7804}{\sqrt{\frac{1-(0.7804)^2}{10-2}}} = 3.530$$

$t_{tab} = 2.306$

Inference:

$t_{cal} > t_{tab}$, we reject null hypothesis.

The correlation coefficient r is significant. There is a relation between advertisement company and the sales.

Homework

1. Calculate the simple correlation coefficient between wing length & tail length of the following 12 birds of a particular species. Also test its significant.

Wing length (cm)x	1	2	3	4	5	6	7	8	9	10	11	12
	10.4	10.8	11.1	10.2	10.3	10.2	10.7	10.5	10.8	11.2	10.6	11.4
Tail length (cm)y	7.4	7.6	7.9	7.2	7.4	7.1	7.4	7.2	7.8	7.7	7.8	8.3

2. The date refer to the yield of grain in g/plant (y) and the number of productive tillers (x)

Y	37	20	42	36	20	30	26	21	43	44	22	31	26	37	26
X	15	12	17	14	12	13	12	9	24	20	14	18	13	15	7

and 15 paddy plants

Find the correlation.

3. The following data relates to the yield in grams (y) and the matured pods (x) of 10 groundnut plants. Work out the correlation coefficient and test its significance.

X:	14	34	20	16	11	11	20	17	22	17
Y:	16	40	21	18	14	13	20	35	17	27

4. Find the persons coefficient of correlation between price and demand from the following data.

Price	11	13	15	17	18	19	20
Demand	30	29	24	24	21	18	15

Practical 10

Fitting of simple linear regression of y on x

Testing the significance of regression coefficient

Example 1

Form a paddy field, 36 plants were selected at random. The length of panicles(x) and the number of grains per panicle (y) of the selected plants were recorded. The results are given below. Fit a regression line y on x. Also test the significance (or) regression coefficient.

The length of panicles in cm (x) and the number of grains per panicle (y) of paddy plants**.**

S.No.	Y	X	S.No.	Y	X	S.No.	Y	X
1	95	22.4	13	143	24.5	25	112	22.9
2	109	23.3	14	127	23.6	26	131	23.9
3	133	24.1	15	92	21.1	27	147	24.8
4	132	24.3	16	88	21.4	28	90	21.2
5	136	23.5	17	99	23.4	29	110	22.2
6	116	22.3	18	129	23.4	30	106	22.7
7	126	23.9	19	91	21.6	31	127	23.0
8	124	24.0	20	103	21.4	32	145	24.0
9	137	24.9	21	114	23.3	33	85	20.6
10	90	20.0	22	124	24.4	34	94	21.0
11	107	19.8	23	143	24.4	35	142	24.0
12	108	22.0	24	108	22.5	36	111	23.1

Solution:

Null hypothesis H_0: regression coefficient is not significant.

Alternate hypothesis H_1: regression coefficient is significant.

$\Sigma y = 4174$; $\Sigma y^2 = 496258$; $\bar{y} = \Sigma y/n = 115.94$

$\Sigma x = 822.9$; $\Sigma x^2 = 18876.83$; $\ddot{x} = \Sigma x/n = 22.86$

$\Sigma xy = 96183.4$

$$SS(Y) = \sum y^2 - \frac{(\sum y)^2}{n} = 496258 - \frac{(4174)^2}{36} = 12305.8889$$

$$SS(X) = \sum x^2 - \frac{(\sum x)^2}{n} = 18876.83 - \frac{(822.9)^2}{36} = 66.7075$$

The regression line y on x is $\bar{y} = a_1 + b_1 \bar{x}$

$$b_1 = \frac{\sum xy - \frac{\sum x \sum y}{n}}{\sum x^2 - \frac{(\sum x)^2}{n}} = \frac{96183.4 - \frac{(822.9)(4174)}{36}}{66.7075} = 11.5837$$

$\bar{y} = a_1 + b_1 \bar{x}$

115.94 = a + (11.5837)(22.86)

A=115.94-264.8034

A=-148.8633

The fitted regression line is y =-148.8633+11.5837x

$$SS(b) = \frac{\left(\sum xy - \frac{\sum x \sum y}{n}\right)^2}{\sum x^2 - \frac{(\sum x)^2}{n}} = \frac{(722.7167)^2}{66.7075} = 8950.8841$$

ANOVA Table:

Sources of Variation	d.f	SS	MSS	F-value
Replication	1	8950.8841	8950.8841	90.7093
Error	36-2=34	3355.0048	98.6766	
Total	35	12305.8889		

For t-test

$$t = \frac{b}{SE(b)} \sim t_{(n-2)} d.f$$

$$SE(b) = \sqrt{\frac{Se^2}{SS(X)}} = \sqrt{\frac{98.6776}{66.7075}} = 1.2162$$

$$t = \frac{11.5837}{1.2162} = 9.5245$$

Table Value:

$t_{(n-2)}$ d.f=t_{34} d.f at 5% level=2.032

$t_{cal} > t_{tab}$. we reject H_o.

Hence t is significant.

Homework:

1. The following data are using length of 13 sparrows of various ages.

Age (days) (x)	3	4	5	6	8	9	10	11	12	14	15	16	17
Wing length(cm)(y)	1.4	1.5	2.2	2.4	3.1	3.2	3.2	3.9	4.1	4.7	4.5	5.2	5.0

Fit the regression line of y on x. Also test the significance of the regression coefficient.

2. Obtain the regression line of the form y = a + bx between the average number of tilters (x) and the yield in kgs (y) of turmeric crop from the following data

Average number of tillers(x)	3.5	3.2	3.5	3.8	3.6	3.74	2.8	4.2	4.0	4.5
Yield in (Kgs) (y)	2.0	1.8	1.9	2.1	2.0	2.3	1.7	2.5	2.6	3.0

3. Find out the regression equations y on x between the number of root fiber (x) and yields in kg(y) of ginger crop from the following data. Also test the significance of regression coefficient.

Number of root fibre (x)	6	5	3	7	4	9	10	11	15	8
Yield in kgs(y)	1.0	0.8	0.5	1.1	0.6	1.2	1.5	1.6	1.9	0.9

4. Find out the regression equation of y on x and test the significance of regression coefficient.

X	2.1	2.7	3.5	4.6	4.9	5.7	6.0	7.4	8.3	4.8
Y	11.2	12.8	16.4	17.8	19.5	21.1	22.8	26.3	31.0	21.1

Practical 11

Formation of ANOVA table for Completely Randomized Design (CRD) with equal replication and comparison of means using critical difference values

Completely Randomized Design (CRD)

Example 1

The following table gives the yield in kgs per plot of five varieties of wheat after being applied to each of four plots in a completely randomized design.

Varieties	Yield in kgs				Totals	Treatment means
A	8	8	6	10	32 (T1)	8 (T1)
B	10	12	13	9	44 (T2)	11 (T2)
C	18	17	13	16	64 (T3)	16 (T3)
D	12	10	15	11	48 (T4)	12 (T4)
E	8	11	9	8	36 (T5)	9 (T5)
Grand Total					224	

Solution:

Correction factor (C.F) = (Grand total)^2/(r x t); where r is the number of replications per treatment and t is the numbers of treatments.

= $224^2/(4 \times 5) = 2508.8$

Total Sum of Square (TSS) = ΣY_{ij}^2-C.F

= $8^2+8^2+\ldots.+9^2+8^2-2508.8 = 207.2$

Treatment Sum of square = $[(T_1^2+T_2^2+T_3^2+T_4^2+T_5^2)/r]$-CF

= $[(32^2+44^2+64^2+48^2+36^2)/4]-2508.8 = 155.2$

Error Sum of Square = Total sum of square-Treatment sum of square=207.2-155.2= 52.0

ANOVA table:

Source of variation	**D.F.**	**S.S.**	**MS (variance)**	**F (variance ratio)**	**F at 5%**
Between varieties	4	155.2	38.80	11.80*	3.06
Within varieties (error)	15	52.0	3.47		
Total	**19**	**207.2**			

*Significant at 5% level of significances

Here, F test indicates that there are significant difference between the variety means since the observed value of the variance ratio is significant at 5% level of significance. Now we wish to know as to which variety is the best and also which varieties show the significant difference among themselves. This can be done with the help of critical difference (c.d.)

Now, standard error of the difference between two treatment means is

S.E.d = $\sqrt{\frac{2XEMS}{r}}$, Where EMS is the error mean square and r is no. of replications.

=1.32
Critical difference (CD)= SEd x t at 5% for error df
= 1.32 x 2.131 = 2.81

Homework:

1. The following table gives the yields of five varieties of paddy with four replications each by using completely randomized Design.

Varieties	Yield in kg.			
A	8	8	6	10
B	10	12	13	9
C	18	17	13	16
D	12	10	15	11
E	8	11	9	8

Analyze the data to draw your conclusions.

2. Below are given the plan and yield in kg per plot of a completely randomized design for testing the effect of five different fertilizers A, B, C, D & E.

D	E	B	E	D
20	17	21	16	15
A	C	A	D	B
8	17	9	13	17
B	D	E	C	A
12	19	18	18	15
C	A	C	A	E
16	8	18	10	15
E	B	D	B	C
13	16	23	14	19

Analyze the data and state your conclusions.

Practical 12

Formation of ANOVA table for Randomised blocks design (RBD) and comparison of means using critical difference values

Randomised blocks design (RBD)

Example 1

The yields of six nitrogen treatments on a crop in kgs along with the plan of the experiment are given below. The number of blocks is five and the nitrogen treatments have been represented by A, B, C, D, E and F.

Block I	Block II	Block III	Block IV	Block V
D 17 C 12	B 12 C 15	E 23 A 30	A 28 F 64	F 75 C 14
F 70 B 6	E 26 A 26	C 16 D 20	B 9 D 23	D 20 B 7
A 20 E 28	D 10 F 62	F 56 B 10	E 33 C 14	E 30 A 23

It is required to analyse the data.

Solution

The first step in the analysis of data is to tabulate yield figures according to block and treatments in the follow manner.

Varieties	Blocks					Treatment totals	Treatment means
	I	II	III	IV	V		
A	20	26	30	28	23	1277 (T_1)	25.4
B	9	12	10	9	7	47 (T_2)	9.4
C	12	15	16	14	14	71 (T_3)	14.2
D	17	10	20	23	20	90 (T_4)	18.0
E	28	26	23	35	30	142 (T_5)	28.4
F	70	62	56	64	75	327 (T_6)	65.4
Totals	**156 (B1)**	**151 (B2)**	**155 (B3)**	**173 (B4)**	**169 (B5)**	**804 (GT)**	

Sum of Squares for different sources

CF= $GT^2/(b \times t)$; where GT is Grand total, b-blocks and t- no. of treatments

$=804^2/(5 \times 6) = 21547.2$

Total SS= SS of all observations – CF

=10466.8

SS due to blocks=$[(B_1^2+ B_2^2+ B_3^2+ B_4^2+ B_5^2)/t]$-CF = 61.4

SS due to treatments= =$[(T_1^2+ T_2^2+ T_3^2+ T_4^2+ T_5^2)/r]$-CF = 10167.2

SS due to error= Total SS-SS due to block-SS due to treatments = 418.2

ANOVA TABLE:

Source of variation	D.F	S.S	M.S	Variance ratio 'F'	F 15%
Blocks	4	61.4	15.35		
Treatments	5	10167.2	2033.44	97.24*	2.71
Error	20	418.2	20.19		
Total	29	10646.8			

* Significant at 5% level of significance

It is clear from the table that this observed value of 'F' is significant at 5% level of significance which proves that there are significant differences between the treatment means. Now, we have to test the significance of the difference between the individual treatments, and this will be done with the help of CD as usual.

Critical differences:

SEd = 2.89

CD= SEdx $t_{5\%}$= 6.03

Homework

1) The yield of rice (in kg) with five fertilizers tested in four blocks using RBD is given the following layout. Analysis the data & interpret your conclusion.

Block 1	Block 2	Block 3	Block 4
B 10	C 13	A 19	D 20
C 16	A 21	D 24	E 36
A 20	D 21	E 32	B 9
D 23	E 31	B 10	C 13
E 33	B 11	C 14	A 24

2) An experiment was conducted in RBD to study to comparative performance of yield of six varieties of oranges (kg/plot) are given below. Analyse the data and give your conclusion.

Treatments	Blocks				
	B1	B2	B3	B4	B5
V1	5.5	5.9	6.3	6.5	6.7
V2	7.4	7.7	7.9	7.5	8.1
V3	4.6	5.1	5.3	4.9	4.7
V4	5.0	5.8	5.6	6.1	5.3
V5	6.7	6.2	6.9	6.8	6.0
V6	8.2	7.9	7.5	7.2	6.9

Practical 13

Formation of ANOVA table for Latin square design (LSD) and comparison of means using CD values

Latin Square Design

Example 1

Below are given the plan and yield in kg/plot of a 5x5 Latin square experiment on the wheat crop carried out for testing the effects of five, manorial treatments A, B, C, D, and E. 'A' denotes control.

B	15	A	8	E	17	D	20	C	17		R1	=	77
A	9	D	21	C	19	E	16	B	13		R2	=	78
C	18	B	12	D	23	A	8	E	17		R3	=	78
E	18	C	16	A	10	B	15	D	23		R4	=	82
D	22	E	15	B	13	C	18	A	10		R5	=	78

C1 = 82, C2 = 72, C3 = 82, C4 = 77, C5 =80 ; GT = 393

Analyze the data and state your conclusions.

Solution

CF= $GT^2/(r \times c)$ =6177.96

Total SS= $15^2+8^2+....+10^2$-CF = 483.04

SS due to rows= $(77^2+78^2+...+78^2)/t$ -CF = 3.04

SS due to columns= $(82^2+72^2+...+80^2)/t$ -CF = 14.24

SS due to treatments:

Treatment A T_1	Treatment B T_2	Treatment C (T_3)	Treatment D (T_4)	Treatment E (T_5)
8	15	17	20	17
9	13	19	21	16
8	12	18	23	17
10	15	16	23	18
10	13	18	22	15
Total 45	68	88	109	83

SS due to treatment= $(45^2+68^2+...+83^2)/r$ - CF = 454.64

SS due to error= TSS-SSR-SSC-SST = 11.12

ANOVA table:

Source of variation	**Df**	**SS**	**MS**	**Variance ratio F**	**F value at 5% level & 1% level**
Rows	4	3.04	0.76	123.34**	3.26 5.41
Columns	4	14.24	3.56		
Treatments	4	454.24	113.66		
Error	12	11.12	0.92		
Total	24	483.04			

** Highly significant

The observed highly significant value of the variance ratio indicates that there are significant differences between the treatment means.

S.E. of the difference between the treatment means (SED) = $((2*0.92)/5)^{1/2}$= 0.61
CD= SEd*t 5% at df = 0.61*2.179=1.33
Treatment means will be calculated from the original table on treatment totals.

Treatments	**A**	**B**	**C**	**D**	**E**	**CD 5%**
Mean yieldin kg / plot	9.0	13.6	17.6	21.8	16.6	1.33

Conclusion represented symbolically
The treatments have been compared by setting them in the descending order of their yields.

Treatments	**D**	**C**	**E**	**D**	**A**	**CD 5%**
Mean yieldin kg / plot	21.8	17.6	16.6	13.6	9.0	1.33

The treatment 'D' is the best of all. The treatments 'C' and 'E' do not differ significantly each other.
The yield obtained by applying every one of the manurial treatment is significantly higher that obtained without applying any manure.

Homework

1. An oil company tested four different blends of gasoline for fuel efficiency according to a Latin square design in order to control for the variability of four different drivers and four different models of cars. Fuel efficiency was measured inmiles per gallon (mpg) after driving cars over a standard course.

Fuel Efficiencies (mpg) For 4 Blends of Gasoline(Latin Square Design: Blends Indicated by Letters A-D)**Car Model**

Driver	**I**	**II**	**III**	**IV**
1	**D** 15.5	**B** 33.9	**C** 13.2	**A** 29.1
2	**B** 16.3	**C** 26.6	**A** 19.4	**D** 22.8
3	**C** 10.8	**A** 31.1	**D** 17.1	**B** 30.3
4	**A** 14.7	**D** 34.0	**B** 19.7	**C** 21.6

Analyse the data and draw yor conclusion.

2. The numbers of wireworms counted in the plots of Latin square following soilfumigations (L,M,N,O,P) in the previous year were

	Columns				
Rows	P(4)	O(2)	N(5)	L(1)	M(3)
	M(5)	L(1)	O(6)	N(5)	P(3)
	O(4)	M(8)	L(1)	P(5)	N(4)
	N(12)	P(7)	M(7)	O(10)	L(5)
	L(5)	N(4)	P(3)	M(6)	O(9)

Analyse the data and draw your conclusions.

Exercise 14

Non-parametric test

It is clear that whenever the assumption of parametric tests are met, then the non-parametric tests should not be used, as these tests are not as sensitive and powerful as the classical parametric tests, in dealing with the data. When we are unable to apply parametric tests only then we resort to non-parametric tests. In this chapter, various non-parametric tests applicable under different situations are discussed and illustrated with examples.

1. **One Sample Tests:**

These tests lead us to decide whether the population follows a known distribution or the sample has come from a particular population. We can also test whether the median of the population is equal to a known value. A test is also given to test the randomness of a sample drawn from a population as it is the crucial assumption in most of the testing procedures.

1.1. **Runs Test for Randomness:**

One of the most important aspects of all types of statistical testing is that the sample selected must be representative of the population as far as possible since decisions about the population are to be made on the basis of results obtained from samples. Thus the requirement of the randomness of the sample is mandatory. However, in many situations, it is difficult to decide whether the assumption of randomness is fulfilled or not. The assumption of randomness can be tested by runs test, which is based on the theory of runs.

Run and Run Length: A run is defined as a sequence of identical letters (symbols) which are followed and preceded by different letters or no letter at all and number of letters in a run is called run length.

Suppose that after tossing a coin say 20 times, following sequence of heads (H) and tails (T) occur:

HH	TTT	HHH	T	HH	TT	HH	T	HH	TT
1	2	3	4	5	6	7	8	9	10

The first sequence of HH is considered as a run of length 2. Similarly occurrence of TTT is considered as another run of length 3 and so on. So counting the runs in similar way, the total number of runs occurred in above sequence is 10 i.e. R = 10.

The total number of runs (R) in any given sample indicates whether the sample is random are not. Too many or too small number of runs creates doubt about the randomness of sample.

Test Procedure:

Generalizing the problem, let one kind of elements be denoted by plus (+) sign and second kind of elements be denoted by minus (-) sign. The concept of '+' and '-' provides the direction of change from an established pattern. In the above example if 'H' is denoted by '+' and 'T' denoted by '-' then we have

n_1 = number of '+' signs = 11; n_2 = number of '-' signs = 9

and total sample size $n = n_1 + n_2 = 20$, while the number of runs (R) = 10

Now if sample size is small so that n_1 and n_2 are less than or equal to 20 each, then to test the hypothesis:

H_0: Observations in the sample are random against

H_1: Observations in the sample are not random

Compare the observed number of runs (R) in the sample with two critical values of (R) for given values of n_1 and n_2 at a predetermined level of significance (critical values of 'R' for Runs test are available in the Appendix of various statistics books)

Decision Rule:

Reject H0 if $R \leq c_1$ or $R \geq c_2$, otherwise accept H_0 where c_1 and c_2, are two critical values and may be obtained from standard tables.

For the above example the critical value c1 from less than table for $n_1 = 11$ and $n_2 = 9$ is '6' and from more than table the critical value c_2 is 16. So, our acceptance region is $6 < r < 16$. Since the observed value of 'R' is 10, so H_0 is accepted, hence we conclude that our sample observations are drawn at random.

$$E(R) = \mu_r = \frac{2n_1n_2}{n_1 + n_2} + 1$$

$$V(R) = \sigma^2_r = \frac{2n_1n_2(2n_1n_2 - n_1 - n_2)}{(n_1 + n_2)^2(n_1 + n_2 - 1)}$$

$$\text{Thus } Z = \frac{R - \mu_r}{\sigma_r} \sim N(0,1)$$

So, if $|Z_{cal}| \geq 1.96$ then reject H_0 at 5% level of significance and conclude that the sample is not random

< 1.96 then accept H_0 at 5% level of significance

Example-1: A researcher wants to know whether there is any pattern in arrival at the entrance of the shopping mall in terms of males and females or simply such arrivals are random. One day he stationed himself at the main entrance and recorded the arrival of Men (M) and Women (W) of first 40 shoppers and noted the following sequence.

M WW MMM W MM W M W M WWW MMM W MM WWW MMMMMM WWW MMMMMM

Test randomness at 5% level of significance

Solution: H_0 : Arrival of men and women is random

H_1 : Arrival is not random

Here Number of men (n_1) = 25 Number of women (n_2) = 15 Number of runs (R) = 17

Since here $n_1 = 25$ is > 20 so sampling distribution of 'R' is approximated by normal distribution with mean:

$$\mu_r = \frac{2n_1n_2}{n_1 + n_2} + 1 = \frac{2 \times 25 \times 15}{25 + 15} + 1 = \frac{750}{40} + 1 = 19.75$$

$$\text{and } \sigma_r = \sqrt{\frac{2n_1n_2(2n_1n_2 - n_1 - n_2)}{(n_1 + n_2)^2(n_1 + n_2 - 1)}}$$

$$= \sqrt{\frac{2 \times 25 \times 15\,(2 \times 25 \times 15 - 25 - 15}{(25+15)^2\,(25+15-1)}}$$

$$\sigma_r = \sqrt{\frac{750 \times 710}{1600 \times 39}} = 2.39$$

$$\text{Thus, } Z_{cal} = \frac{R - \mu_r}{\sigma_r} = \frac{17 - 19.75}{2.39} = -1.15$$

$\therefore$ Since $|Z_{cal}| < 1.96$ so H_0 is accepted i.e. sample is considered as random.

Note: To test the randomness of a sample of n observations, runs can also be generated by considering positive and negative signs of the deviations of observations from the median of the sample.
Let $x_1, x_2, ….., x_n$ be a sample of size n drawn in that order and we wish to test:
H_0 : Observations in the sample are drawn at random
H_1 : Observations in the sample are not random
Let M be the sample median. For generating runs of positive and negative signs, we compute the deviations $x_1 – M, x_2 – M,……..,x_n – M$ and consider only the signs of these deviations.
Let n_1 and n_2 be the number of +ve and -ve signs so that $n_1 + n_2 < n$ (ignoring the zero deviation) and R be the total number of runs of +ve and –ve signs in the sample.
Decision Rule:
Follow the usual procedure of runs test as given above.
Example-2: Following measurements were recorded in a sample of 20 earheads in a Wheat variety V1: 8.9, 8.4, 10.3, 11.1, 7.8, 9.3, 9.9, 8.2, 10.9, 10.3, 10.8, 8.6, 9.4, 8.9, 9.4, 8.9, 9.5, 9.9, 9.6, 9.7, 9.2 and 10.0. Test the randomness of the sample using runs test.
Solution:
H_0 : Sample is drawn at random
H_1 : Sample is not drawn at random
Let $\alpha = 0.05$
Test Statistic: Median is found to be 9.55, therefore, generating runs of the +ve and –ve signs, we have.

8.9	8.4	10.3	11.1	7.8	9.3	9.9	8.2	10.9	10.3
-	-	+	+	-	-	+	-	+	+
10.8	8.6	9.4	8.9	9.5	9.9	9.6	9.7	9.2	10.0
+	-	-	-	-	+	+	+	-	+

Find the median which is the arithmetic mean of two middle observations 9.5 and 9.6 come out to be equal to 9.55

Consider the signs of the deviation x_i - 9.55, i = 1, 2,·····,20 and count the numberof runs R as the test statistic:

- - + + - - + - + + + - - - - + + + - +

Here number of plus signs (n_1)

= 10Number of minus signs (n_2)

= 10 Number of runs (R) = 10

Conclusion:

Critical value c_1 for $n_1 = 10$ and $n_2 = 10$ (at $\alpha = 0.05$) =

6 Critical value c_2 for $n_1 = 10$ and $n_2 = 10$ (at $\alpha = 0.05$) =

16

The test statistic R (=10) lies in the acceptance region $6 < R < 16$.

Hence H_0 is notrejected and we conclude that the sample is considered as drawn at random.

Non-Parametric Alternative to One Sample t-test:

7.1.1 Sign Test:

The sign test is used for testing the median rather than mean as location parameter of a population i.e. whether the sample has been drawn from a population with the specified median M_o. It is a substitute of one sample t-test when the normality assumption of the parent population is not satisfied. This test is the simplest of all the non-parametric tests and its name comes from the fact that it is based upon the signs of the differences and not on their numerical magnitude.

Wilcoxon Signed Ranks Test:

Wilcoxon signed ranks test (Wilcoxon, 1945, 1949) is similar to sign test as it is used to test the same hypothesis about the median of the population. The sign test is based only on the signs of differences but Wilcoxon Signed Rank test takes into consideration not only the signs of differences such as positive or negative but also the size of the magnitude of these differences. So this test is more sensitive and powerful than the sign test provided the distribution of population is continuous and symmetric.

Let $x_1, x_2, \ldots, x_n$ denote a random sample of size 'n' drawn from a continuous and symmetric population with unknown median M. We are required to test the hypothesis about the median (M) that is:

$H_0 : M = M_0$ against the alternative hypothesis

$H_1 : M \neq M_0$ Choose $\alpha = 0.05$

Take the differences of sample values from M_0 i.e. $d_i = x_i - M_0$, i = 1, 2, ….., n and ignore the zero differences. Then assign the respective ranks to the absolute differences in ascending order of magnitude (after ignoring the signs of these differences) so that the lowest absolute value of the differences get the rank '1' second lowest value will get rank '2' and so on. For equal values of absolute differences, average value of ranks would be given.

After assigning the ranks to these differences assign the sign of original differences to these ranks. These signed ranks are then separated into positive and negative categories and a sum of ranks of each category is obtained. Let T^+ denote the sum of ranks of the positive d_i's and T^- denote the sum of ranks of negative differences.

7.2 Two Sample Tests for Dependent Samples (Non-Parametric Alternative to paired t-test):

7.2.1 Paired Sample Sign Test:

This test is a non-parametric alternative to paired t-test and is used for the comparison of two dependent samples. Here it is desired to test the null hypothesis that the two samples are drawn from the populations having the same median i.e.

$$H_0 : M_1 = M_2 \text{ vs } H_1 : M_1 \neq M_2.$$

The procedure of single sample sign test explained in section 7.1.2 can be appliedto paired sample data.

Here we draw a random sample of n' pairs and observations $(x_1, y_1), (x_2, y_2) \ldots\ldots(x_n, y_n)$ giving 'n' differences

$$d_i = x_i - y_i \quad \text{for } i = 1, 2, \cdots\cdots, n$$

It is assumed that the distribution of differences is continuous in the vicinity of its median 'M' i.e. $P[d > M] = P[d < M] = 1/2$.

All the procedure of one-sample sign test will remain valid for the paired sample sign test, with d_i displaying the role of x_i in one sample sign test.

7.2.2 Paired Sample Wilcoxon Signed Ranks Test:

This test (Wilcoxon, 1945, 1949) deals with the same problem as the paired sample sign test and is an extension of one sample Wilcoxon signed ranks test (Section 7.1.3). But this test is more powerful than paired sample sign test since it takes into account the sign as well as the magnitude of the difference between paired observations.

The observed differences $d_i = x_i - y_i$ are ranked in the increasing order of absolute magnitude and then the ranks are given the signs of the corresponding differences. The null and alternative hypotheses are the same as in paired sample sign test i.e.

$H_0 : M_1 = M_2$ vs $H_1 : M_1 \neq M_2$

If H_0 is true, then we expect the sum of the positive ranks to be approximately equal to the absolute value of the sum of negative ranks. The whole procedure of this test is the same as that of one sample Wilcoxon signed ranks test with the only difference thatin this test d_i's are given by

$$d_i = x_i - y_i, \quad i = 1, 2, \ldots, n$$

Example-6: The weights of ten men before and after change of diet after six months are given below. Test whether there has been any significant reduction in weight as a result of change of diet at 5% level of significance.

7.3 **Two Sample Tests for the Independent Samples:**

Non-Parametric Alternative to two sample t-test:

Mann-Whitney U-test:

This test was developed by Mann and Whitney (1974) and is a non-parametric alternative to the usual two-sample t-test. To apply this test, the two samples that are to be compared should be independent and the variable under consideration should have a continuous distribution. The null hypothesis under this test is that the two population distributions from which the samples have been drawn are identical. Under experimental situation, the null hypothesis may be that two treatments are identical i.e. two treatment effects do not differ significantly.

2. **Kruskal-Wallis H-Test:**

To test whether several independent samples have come from identical populations, analysis of variance is the usual procedure provided the assumptions underlying are fulfilled. But if the assumptions are not fulfilled then Kruskal-Wallis test (Kruskal and Wallis, 1952) is used for one way classification data (i.e. completely randomized design). It is an extension of Mann Whitney U-test in which only two independent samples are considered.

Kruskal Wallis test is an improvement over the median test. In the median test the magnitude of various observations was compared with median

value only. But in this method the magnitude of observations is compared with every other observation by considering their ranks.

Kendall's rank correlation coefficient:

Kendall's rank correlation coefficient (Kendall, 1938) τ is a non-parametric measure of correlation and is based upon the ranks of the observations.

Exercise 15

INTRODUCTION TO MULTIVARIATE ANALYSIS

Multivariate statistical analysis is appropriate whenever several responses are measured on each object or experimental unit. Univariate analysis applied separately to each response leads to incorrect conclusions, since responses measured on the same object are generally correlated. Multivariate analysis can be simply defined as the application of statistical methods that deal with reasonably large number of characteristics or variables recorded on each object in one or more samples simultaneously. It provides statistical tools for the study of joint relationships of variables in data that contains intercorrelations. In other words, multivariate analysis differs from univariate and bivariate analysis in that it directs attention away from the analysis of the mean and variance of a single variable or from the pairwise relationship between two variables, to the analysis of the co-variances or correlations which reflect the extent of relationship among three or more variables. For example, a biometrician concerned with developing a taxonomy for classifying species of fowl on the basis of anatomical measurements may collect information on skull length, skull width, humerus length and tibia length.

Remarks:

1. The term objects in multivariate analysis refer to things, persons, individuals, events or in general entities on which the measurements are recorded. And the measurements relate to characteristics or attributes of the objects that are being recorded and in general are called variables.

Multivariate analysis investigates the dependency not only amongst the variables but also among the individuals on which observations are made.

Important Multivariate Methods:

Multivariate data recorded on a large number of interrelated variables is often difficult to interpret. Therefore, there is a need to condense and sum up the essential features of the data through dimension reduction or some appropriate summary statistics for better interpretation. As a broad classification, the multivariate

techniques may be classified as dependence methods and interdependence methods.

The methods in which one or more variables are dependent and others are independent are called dependant techniques. Multivariate regression, multivariate analysis of variance, discriminant analysis and canonical correlation analysis are the notable dependence techniques. If interest centres on the mutual association across all the variables with no distinction made among the variable types, then such techniques are called interdependence techniques. Principal component analysis, factor analysis, cluster analysis and multi-dimensional scaling are the important interdependence techniques.

Multivariate Regression: It is concerned with the study of the dependence of one or more variables on a set of other variables called independent variables with the objective to estimate or predict the mean values of the dependent variables on the basis of the known values of the independent variables. If there is only one dependent variable and many independent variables, then it is known as multiple regression.

Multivariate Analysis of Variance: It is simply a generalization of univariate analysis of variance, where the primary objective is on testing for significant differences on a set of variables due to changes in one or more of the controlled (experimental) variables.

Discriminant Analysis: It is used to find linear combinations of the variables that separate the groups. Given a vector of p observed scores, known to belong to one of two or more groups, the basic problem is to find some function of the p scores (i.e. a linear combination) which can accurately assign individual with a given score into one of the groups.

Principal Component Analysis: It is a dimension reduction technique where the primary goal is to construct orthogonal linear combinations of the original variables that account for as much of the total variation as possible. The successive linear combinations are extracted in such a way that they are uncorrelated with each other and account for successively smaller amounts of total variation.

Cluster Analysis: The purpose of cluster analysis is to reduce a large data set to meaningful subgroups of individuals or objects. The division is accomplished on the basis of similarity of the objects across a set of specified characteristics. The

individuals of a particular subgroup or cluster are, in some sense, more similar to each other than to elements belonging to other groups.

Canonical Correlation Analysis: The most flexible of the multivariate technique, canonical correlation simultaneously correlates several explanatory variables and several dependent variables. In usual sense, it determines the linear association between a set of dependent variables and a set of explanatory variables. In canonical analysis, we find two linear combinations, one for the predictor set of variables and one for the set of explanatory variables, such that their product moment correlation is maximum.

Physiological and Molecular Responses of Plants to Abiotic Stresses PPH-301

Measurement of Relative water content (RWC) in leaf

Relative water content is described as the amount of water in a leaf at the time of sampling relative to the maximal water a leaf can hold. It is an important parameter in water relation studies, e.g. it allows the calculation of the osmotic potential at full turgor.
The relative water content of a leaf (RWC) is calculated from the following parameters:
RWC = (FW – DW) /(TW – DW)
FW= leaf fresh weight
DW = leaf dry weight
TW = leaf turgit (re-saturated) weight.
Relative water content can be measured in different ways. However, the critical part is the re-saturation of the leaves. Many agricultural scientists use leaf disks to re-hydrate leaves. But especially in stressed leaves that have a relative low relative water content the re-hydration of leaf disks can lead to an over-saturation of the leaf disk because the previously water depleted apoplast is over-saturated. Please note that most leaves will rehydrate to full turgor in a short period of time (btw 1-3 hrs) and that rehydrating leaves for longer periods can lead to serious errors. Esp if rehydrated samples are used for subsequent osmotic relations. A better way of re-saturating leaves is through the petiole of a leaf. The re-saturation follows the normal water pathway, there is no tissue damage (as it occurs with leaf disks), and over-saturation is minimized. Another advantage of re-saturation of intact leaves through the petiole is that the water potential of the leaves after re-saturation can be evaluated using a pressure bomb, thereby allowing an assessment of the success of resaturation (a fully saturated leaf has a water potential close to zero).

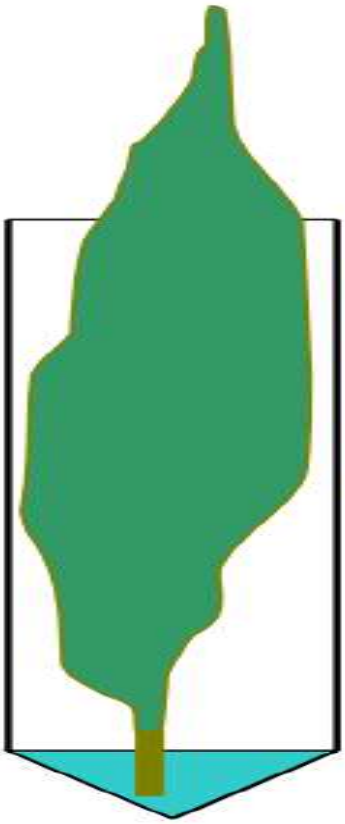
Materials

• Balance (0.000 g)
• Drying oven at 80ºC (can use microwave instead if oven is not available)
• Razorblades • 50 ml Sarstedt tubes (or smaller vials, depending on leaf shape and size)
• Foam rack for 50ml Sarstedt tubes
• Water
• Tissues to dry leaves
• Secateurs or loppers to take branch leaf samples

Method

1. Cut a small twig from a tree
2. Put the twig immediately in a sealable plastic bag (zip-lock) and store in a cool and dry cooler box until further analysis.
3. In the lab fill 50 ml Sarstedt tubes with 5-7 ml of water (tap water is OK or use 50 mM CaCl). Sarstedt tubes are great for eucalypt leaves because the support the long and narrow leaf. It may be necessary to use different tubes or vials for other leaf forms.
4. Cut the leaf at the base of the petiole, i.e. where the petiole enters the twig or the branch using a sharp razor blade.

5. Determine the fresh weight of the leaf.
6. Place the leaf sample in the Sarstedt tube so that the petiole is submerged under the water in the tube. Place the tube in a dark and cool place (like a cupboard).
7. Let the sample re-saturate for some time (4 hours is enough for most samples).
8. Take the leaf out of the tube, dry it with a tissue and determine the turgit (saturation) weight.
9. Put the leaf sample in the drying oven for 24 hrs (oven at 80ºC)
10. Determine the dry weight of the leaf.



Relative water content:

	Measure			Calculate		
Sample ID	FW (g)	TW (g)	DW(g)	RWC	FW/DW	FW/TW

FW = fresh weight
TW = turgit weight
DW = dry weight
RWC = (FW-DW)/(TW-DW)

Artificial saline water preparation

Soils containing an excess concentration of soluble salts or exchangeable sodium in the root zone, it is called as salt-affected soils (Conway 2001; Denise 2003; Jim 2002). Salt-affected soils (Usara/ Kalar) can be broadly categorised into three types based on their salinity and sodicity (Gonzalez et al., 2004) Table-1. When soils contain excessive concentration of water-soluble salts containing positive charge cations such as sodium (Na+), potassium (K+), calcium (Ca2+) and magnesium (Mg2+) along with negative charge anions chloride (Cl-), sulphate (SO42-), nitrate (NO3-), bicarbonate
(HCO3-) and carbonate (CO3 2-), these are called saline (Rhoades and Miyamoto, 1990).
These dissolved salts cause the harmful effect on seed germination, plant growth and yield when the concentration in the root zone exceeds critical level (Conway 2001; Denise 2003).The more soluble salts such as sodium chloride (NaCl), sodium sulfate (NaSO4), sodium bicarbonate (NaHCO3), and magnesium chloride (MgCl2) cause more plant stress than less soluble salts such as calcium sulfate (CaSO4), magnesium sulfate (MgSO4), and calcium carbonate (CaCO3). Irrigation water and saline soils were classified into four and five major groups respectively, depending on salinity levels (Table-2). The electrical conductivity (EC) or EC of the saturated soil paste (ECe) is an important parameter because this value is used to characterise crop salt tolerance. Salt susceptible (glycophytes /sweet plants) and tolerant plants (halophytes/ salt tolerant plants) are classified into four groups viz, sensitive, moderately sensitive, moderately tolerant and tolerant (Fig.1 and Table-3).

Table-1 Classification of salt-affected soils

Class	pH	ECe (dS/m)	SAR	ESP
Normal	6.5 -7.5	<4	<13	<15
Symptom	No visible symptom and normal growth of the plant			
Saline	<8.5	>4	<13	<15
Symptom	White crust on the soil surface. Water-stressed plants. Leaf tip burn/ non-sodic soil with sufficient soluble salts to interfere with the growth of most crops			
Sodic	>8.5	<4	>13	>15
Symptom	Poor drainage. Black powdery residue on soil surface. Soils with sufficient exchangeable sodium to interfere with the growth of most plants, but without appreciable quantities of soluble salts			
Saline-Sodic	<8.5	>4	>13	>15
Symptom	Grey-colored soil. Plants showing water stress. Soils with sufficient exchangeable sodium to interfere with the growth of most plants and containing appreciable quantities of soluble salt			

Table-2. Crop response to salinity, measured as the electrical conductivity of the soil saturation extract (ECe)

Soil depth	Saline Soil Classes/ Interpretation (Classification of irrigation water salinity)				
	Non-Saline/ salt-free	Weakly Saline/ Slightly saline (Low salinity water)	Moderately Saline(Medium salinity water)	Strongly Saline (High salinity water)	Very Strongly Saline (Very high salinity water)
	ECe (dS/m) at 25 oC [(ECw (dS/m)]				
0-60 cm (0-2 ft)	0-2 (up to 0.7)	2-4 (0.7- 2.5)	4-8 (2.5-7.5)	8-16 (7.5-22.5)	>16 (> 22.5)
60-120 cm (2-4 ft)	<4	4-8	8-16	8-16 (7.5-22.5)	>16 (> 22.5)
Crop response	Salinity effects mostly negligible, except in very sensitive plants	Yield of very sensitive crop restricted	Yield of most crop restricted	Only tolerant crop yield satisfactorily	Only a few tolerant crops yield satisfactorily

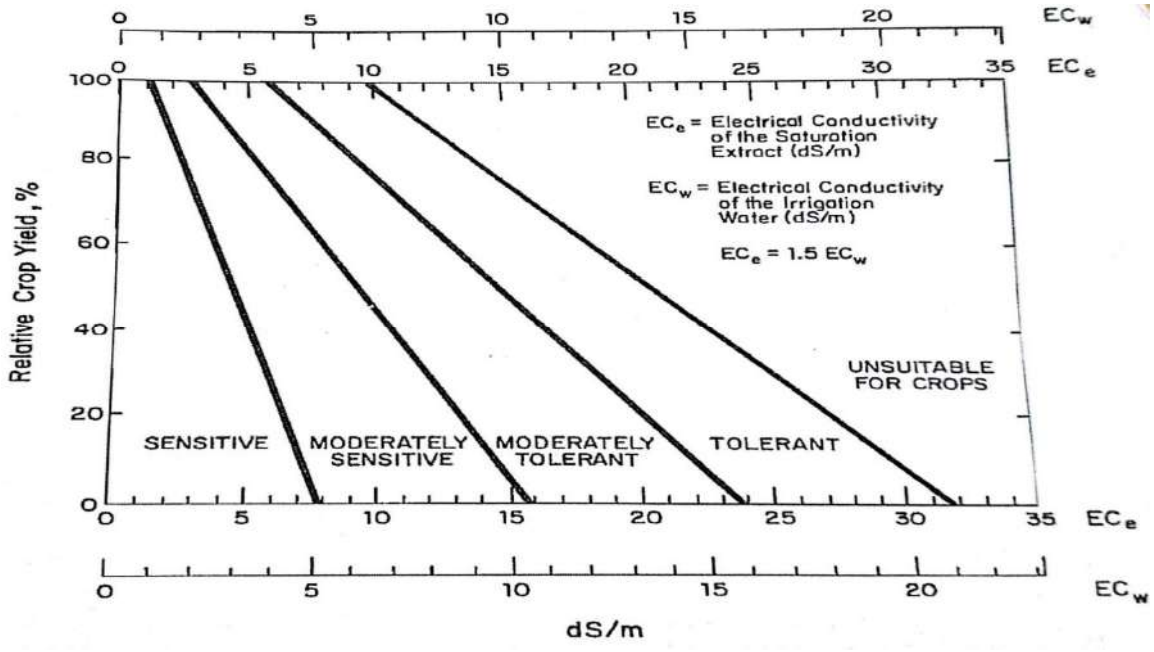


Fig: 1 Relative crop yield (or yield potential) as a function of average root zone salinity (dS/m) grouped according to relative tolerance or sensitive to salinity. Source: Adapted from Maas and Grattan 1999; Grieve et al .2012)

Table- 3 Salt tolerance ratings of various crops

Sensitive	Moderately sensitive	Moderately tolerant	Tolerant
Rice	Chickpea	Sorghum	Barley
Sesame	Corn and Corn (forage)	Soybean	Canola
Gram, Black or urd bean	Peanut	Sunflower	Cotton
Pigeonpea	Sugarcane	Wheat	Guar
Walnut	Alfalfa	Barely (forage)	Oats and forage Oats
Mango	Berseem	Guinea	Rye and forage Rye
		grass	
Banana	Cowpea (forage)	Dhaincha	Triticale
Apple	Buffel grass		Wheat (semidwarf)
			Wheat (durum)
			Kallar grass
			Date palm

Preparation of saline water (Source: USDA Hand book No-60)

Known standard mixtures of salt ratios are used for conducting the experiment under (specify your actual experiment-test tube, hydroponics, pot, and field) for screening the salt tolerant/transgenic cultivars based on Table4, Fig.2 (A and B) and Table 5 Fig-3 using the following formula:

Desired EC = mEq or ME x MW Where,

mEq or ME = milli equivalent for desired EC

MW = molecular weight of the salt

Desired mixture of salts and its ratios: NaCl, Na2SO4, MgCl2, and CaSO4, 13:7:1:4 respectively

Level of desired saline EC (dS/m): 4, 8, 12, 16

Ex: NaCl at 4 EC at 4 EC = 45meq L-1 (Fig.2 (A and B)

$$= \frac{\text{Concentrations of salt (me } L^{-1}) \times \text{salt ratio}}{\text{Total salt ratio}}$$

Total salt ratio

$$ME = \frac{45}{25} \times 13 = 23.43$$

Test the EC of the water before using it to saturate the soil, germination paper (Test the EC of the water before using it to saturate the soil, germination paper (salinity levels raised on germination paper)

Table: 4.Computed salt requirements for desired saline water levels given for various types of experiment (Test tube, hydroponics, pot, and field soils)

EC (dS/m)	ME for all 4 salts	ME for individual salt				MW				Salt required (g) /liter)= ME x MW			
		NaCl	Na_2SO_4	$MgCl_2$	$CaSO_4$	NaCl	Na_2SO_4	$MgCl_2$	$CaSO_4$	NaCl	Na_2SO_4	$MgCl_2$	$CaSO_4$
4	45	23.4	12.6	1.8	7.2	58	142	203	172	1.4	1.8	0.4	1.2
8	95	49.4	26.6	3.8	15.2	58	142	203	172	2.9	3.8	0.8	2.6
12	150	78.0	42.0	6.0	24.0	58	142	203	172	4.6	6.0	1.2	4.1
16	200	104.0	56.0	8.0	32.0	58	142	203	172	6.1	8.0	1.6	5.5

Note: This prepared saline solution/or saline water directly used for germination study in petri dish/germination paper study/ in vitro test tube method or hydroponic study (Hoagland solution) or saline irrigation method- mostly useful/preferable to laboratory conditions, but not good for pot/field conditions. This is why because soil ECe generally comes down into lower than desired or targeted saline soil ECe

Table: 5. Electrical conductivity (EC) of pure solutions at 20°C (dS/m) equivalent with mM solution

Solution	EC (dS/m)
10 mM NaCl	1.0
100 mM NaCl	9.8
500 mM NaCl	42.2
10 mM KCl	1.2
10 mM $CaCl_2$	1.8
10 mM $MgCl_2$	1.6
50 mM $MgCl_2$	8.1

The solutions represent those of salts found in soils or in seawater. Data from the Handbook of Physics and Chemistry (CRC Press, 55th edition, 1975)

Estimation of Cell Membrane Stability Index

A significant impact of plant environmental stress is cellular membrane modification, which results in its total dysfunction of the plant. The cellular membrane dysfunction due to stress is well studied. The dysfunction of membranes is expressed as increased permeability and leakage of ions, the efflux of electrolytes is used to calculate this Index. Hence cellular electrolyte leakage is used to screen for stress resistance. The method was initially developed by the late C.Y. Sullivan (University of Nebraska) in the late 1960's for assessing sorghum and maize heat tolerance. Variations of this methods were developed for cold and desiccation (drought) tolerance. This assay is found in many reports to be associated across diverse genetic materials with yield under stress.

Aim: To estimate the salinity, heat and drought stress tolerance of plant tissue by Sairam

Materials required: leaf sample, beakers, test tubes, water bath, and EC meter

Leaf MSI was determined according to the method of Premchandra et al. (1990), as modified by Sairam (1994). Leaf discs (100 mg) were thoroughly washed in running tap water followed by washing with double distilled water after that the discs were heated in 10 mL of double distilled water at 40 °C for 30 min. Then EC (C1) was recorded by EC meter. Subsequently, the same samples were placed in a boiling water bath (100 °C) for 10 min, and their EC was also recorded (C2) in a conductivity meter

MSI= [1- (C1/C2)] x100

High CMSI corresponded with more stress tolerance

Estimation of chlorophyll stability index and carotenoid stability index in leaf tissue

Carotenoid and chlorophyll pigment content provides valuable information about the physiological status of plants. Chlorophylls a and b are essential pigments to absorb the energy of light and convert it to store chemical energy. Carotenoids have several physiological functions associated with photosynthesis, including a structural role in the organization of photosynthetic membranes, participation in light harvesting and energy transfer, as well as quenching of Ca + b excited state and photo-protection. Carotenoid content is known to be correlated with plant stress and photosynthetic capacity. Green plant pigments are thermosensitive, and degradation occurs when they are subjected toa higher temperature. This method is based on pigment changes induced by heating. The chlorophyll destruction commences rapidly at a critical temperature of 55 ºC to 56 ºC. Thus, chlorophyll stability is a function of temperature. This base has been formerlyused in pine needles immersed in water and heated gradually in a temperature regulated water bath at 58 ºC. Thus, chlorophyll stability is a function of temperature. This property of chlorophyll stability was found to correlate well with drought resistance.

Aim: To estimate carotenoid content and chlorophyll stability index in leaf sample

Materials required:

1. Glass test tube of 2.5 cm in diameter
2. Acetone (80%)
3. Balance
4. Water bath with thermostatic control
5. Spectrophotometer

Procedure:

1. Two clean glass tubes are taken and add 100 mg of representative leaf sample isplaced in them with 10 ml of distilled water.
2. One tube is then subjected to heat on water bath at 56 ºC ± 1 ºC for precisely 30minutes and discard water
3. Add the 10 ml acetone (80%) in both the sample and keep in dark for overnight
4. Take the 1ml sample and add the 2ml acetone (80%) (1:2 ratio)
5. Read the absorbance of the extract at 645, and 663 and 470 nm using acetone (80%) s blank.

Formula:

Total chlorophyll content = 20.2 (A_{645}) + 8.02 (A_{663}) x V/ (1000 x W x a) (mg/g fr. Wt.)

Carotenoid (mg/g): 46.95 (A_{470}- 0.268 x Chl a + b)

Where, A = Absorbance

a = path length of light (3 cm)
V= final volume made (ml)
W= fresh weight of sample (g)
Calculations: CSI = Cs/Cc X 100
Where, CSI = chlorophyll stability index
Cs = Chlorophyll content of stressed plant (mg/g)
Cc = Chlorophyll content of control plant mg/g)
Calculations: CSI = Cs/Cc X 100
Where, CSI* =Carotenoid stability index
Cs = Carotenoid content of stressed plant (mg/g)
Cc = Carotenoid content of control plant mg/g)

* **Carotenoid** **OR**

The chlorophyll stability index (CSI) was determined according to Sairam *et al.* (1997)and calculated as follows:

CSI = (total chlorophyll under stress/total chlorophyll under control) × 100CSI* = (total carotenoid under stress/total carotenoid under control) × 100

*= Carotenoid

Note: Here control and treatment plot is needed

High CSI and CSI* corresponded with more drought tolerance. Thus, CSI, CSI* is directly related with drought tolerance.

Note: Here take the leaf sample 100 to 500 mg or more depend upon the degree of stress

Estimation of Abscisic acid content in leaf and root

Abscisic acid (ABA) is a plant stress hormone that is observed to accumulate under drought stress and mediates many stress responses, like heavy metal stress, drought, thermal or heat stress, high level of salinity, low temperature, and radiation stress. Abscisic acid regulates drought stress responses by mediating stomatal closure, thereby reducing transpiration water loss.

Aim: To determine Abscisic acid content in leaf and root by Titration Method

Materials required: Centrifuge Reagents: 3% dichlorophenol indophenol

Principle:

2,6 dichlorophenol indophenol (2,6-DCPIP) is a blue coloured dye but turns pink when reduced by ascorbic acid. Oxalic acid or metaphosphoric acid may be used titrating medium because it increases the stability of ascorbic acid in the medium

Procedure:

1. Take 0.5 to 5 g of plant sample
2. Add 10-20ml of 3% metaphosphoric acid
3. Centrifuge at 1000xg for 10min
4. Take the supernatant and make the volume upto 100ml
5. Take the 5ml supernatant and add 10 ml of 3% metaphosphoric acid
6. Titrate it against standard 2, 6 dichlorophenol indophenol solution of concentration0.5mg/ml until the pink colour develops completely
7. Note down the difference between final and initial volume of the dye (V2)
8. Take 5ml of the working standard of ascorbic acid (0.1mg/ml concentration) inbeaker add 10ml of 3% metaphosphoric acid and titrate it against the dye.
9. Record the final volume of dye at the endpoint as mentioned above (V1)

The amount of ascorbic acid in mg/100 g of the sample can be calculated as follows:

$$\frac{A}{V1} \times \frac{V2}{B} \times \frac{Total\ volvum\ of\ sample}{Total\ weight\ of\ sample\ taken} \times 100$$

Where,

A = 0.5 mg (the concentration of working standard of ascorbic acid=0.5mg in 5mltaken for titration.

B = 5 ml (volume of sample taken for titration)

V1 = Volume of dye in case of titration with standard solution
V2 = volume of dye in case of titration with the sample solution.

Estimation of proline content in plant tissue

Proline a compatible solute and an amino acid, is involved in osmotic adjustment (OA) and protection of cells during dehydration (Zhang et al., 2009). Cell turgor is maintained due to Osmotic Adjustments which allow cell enlargement and plant growth during water stress. It also enables stomata to remain partially open and CO2 assimilation to continue at water potentials that would be otherwise inhibitory for CO2 assimilation. (Alves and stter, 2004). Proline can scavenge free radicals and reduce damage due to free radicles during drought stress. Growing body of evidence indicated that proline content increases during drought stress and proline accumulation is associated with improvement in drought tolerance in plants (Seki et al., 2007; Zhang et al., 2009).

Aim: To determine the free proline content of plant tissue following Bates et al., (1973) method.

Materials required: test tubes, pestle and mortar, pipettes, funnels, Whatman no. 1 filter paper, water bath, heater, ice bath, separating funnel

Reagents:

3% aqueous sulphosalicylic acid, Glacial acetic acid, Orthophosphoric acid (6M), Toluene, Proline

Acid ninhydrin, warm 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6M phosphoric acid with agitation until dissolved. Store at 4 oC and use within 24 hours.

Principle:

During selective extraction with aqueous sulphosalicylic acid, proline is precipitated as a complex. Other interfering materials are removed by absorption to the protein Sulphosalicylic acid complex. The extracted protein is made with ninhydrin in acidic conditions (pH = 1.0) to form the chromophore (red colour) to read at 520 nm.

Procedure:

1.Extract 0.5 g of plant material fresh by homogenising in 3-5 ml of 3% aqueous solution sulpho-salicylic acid

2.Filter the homogenate through Whatman no. 2 filter paper and make up the volume to 10 ml.

3. Take 2 ml of filtrate in a test tube and add 2 ml of glacial acetic acid and 2 ml acid- ninhydrin

4. Heat the test tube in boiling water bath for one hr.

5. Terminate the reaction by placing the tube in ice bath
6. After attaining room temperature transfer the contents to a separate funnel
7. Add 4 ml toluene to the reaction mixture and stir well for 22-30 sec
8. Take out the lower coloured layer and discard the upper toluene layer
9. Measure the red colour intensity at 520 nm
10. Simultaneously run a blank with 2 ml distilled water instead aliquot.

Calculations:

Express the proline content on fresh-weight basis as follows:

"µmoles per gram tissue = [(µg proline/ml) x ml toluene)/115.5 µg/µmole] / [(g sample)/5]

Or

"µmoles per gram tissue = [(µg proline/ml) x ml toluene x ml salicylic acid]/(115.5 µg µmole x sample (g))

Notes:

1. The colour intensity is stable for at least one hr.
2. The relationship between the amino acid concentration and absorbance is linear inthe range of 0.02 to 0.1 µ M per ml of proline.

Estimation of antioxidant enzymes

Oxidative stress results from conditions are promoting the formation of Reactive Oxygen Species (ROS: Molecular oxygen, singlet oxygen, superoxide anion, hydrogen peroxide, hydroxyl radical, per hydroxyl radical and ozone) that damage or kill cells. Environmental factors that cause oxidative stress includes air pollution (ozone and sulphur dioxide), herbicides (Paraquat) drought, heat, cold, wounding, UV light, intense light, pathogen infection and during senescence. Plant scavenges and disposes of the reactive molecules by use of anti-oxidative defence systems present in several subcellular compartments. The antioxidant defence systems include non-enzymatic and enzymatic antioxidants. Some major antioxidant enzymes Superoxide dismutase (SOD), Peroxidase (PX), Catalase (CAT).

A) Estimation of Peroxidase enzyme

Principle:

The enzyme peroxidase catalyses the oxidation of the substrate by oxygen generatedfrom the decomposition of hydrogen peroxide:

$2H_2O_2 \rightarrow 2H_2O + O_2$

Substrate + O_2 → Oxidized substrate.Reagents:

1. Phosphate buffer (100, mM pH 6.1)

Solution A: Potassium dihydrogen phosphate 6.80g was dissolved in water, and thevolume was made up to 500 ml with doubled distilled water.

Solution B: Dipotassium hydrogen phosphate 8.71g was dissolved in doubled distilledwater, and the volume was made up to 500 ml with doubled distilled water.

Mix 15 ml of sol. A and 85ml of sol. B and final pH 6.1 was adjusted with the help of pH meter:-

1. Hydrogen peroxide (12 mM): Dissolve 124 µl of 30% H_2O_2 in doubled distilledwater and the volume was made up to100 ml
2. Guaicol (96 mM): Dissolve 1075 µl ofanalytical grade guiacol in doubleddistilled water and the volume was made up to100 ml

The reaction mixture contained:

a) Phosphate buffer (100, mM pH 6.1) : 1 ml of 100 mM
b) Guaicol (16 mM) : 0.5 ml of 95 mM
c) Hydrogen peroxide (2 mM) : 0.5 ml of 12 mM
d) Enzyme : 0.1 ml
e) Water : 0. 4 ml to make a final volume of 3 ml.

Absorbance due to the formation of tetra-guaiacol was recorded at 470 nm and enzymeactivity was calculated as per extinction coefficient of its oxidation product, tetra- guaiacol= 26.6 mM^{-1} cm^{-1} Enzyme activity is expressed as µm tetra-guaiacol formed per min per fresh weight orper mg protein

B) Estimation of Catalase enzyme.

Principle

The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water.

Reagents:

1. Hydrogen peroxide: 77754 µl of 30% H2O2 is dissolved in doubled distilled water and make up the volume was made to100 ml to get 75 mM Hydrogen peroxide

2. Phosphate buffer (100 mM, pH 7.0)

Solution A: Potassium dihydrogen phosphate 6.80g was dissolved in water and the volume was made up to 500 ml with doubled distilled water.

Solution B: Di- potassium hydrogen phosphate 8.71 g was dissolved in doubled distilled water and the volume was made up to500 ml with doubled distilled water.

Mix 39 ml of sol. A and 61 ml of sol. B and final pH 6.1 was adjusted with the help of pH meter.

Enzyme Assay:

The reaction mixture contained:

a) Phosphate buffer 50 mM :1.5 ml of 100 mM buffer, pH 7.0
b) Hydrogen peroxide 12.5 mM :0.5 ml of 75 mM Hydrogen peroxide
c) Enzyme : 50µl
d) Water : to make a final volume of 3 ml.

Adding H_2O_2 started the reaction and decrease in absorbance was recorded for 1min. The initial and final content of hydrogen peroxide is calculated by comparing with astandard curve drawn with a known concentration of hydrogen peroxide**.**

Enzyme activity is calculated as the concentration of hydrogen peroxide (initial reading- and final reading = quantity of hydrogen peroxide) per min per mg protein.

PGS-303

1.To study the Safety measures in Laboratory

Chemical Use Guideline

- Never use a product that doesn't have a label to reference.
- Don't mix chemicals without specific authorization from the formulator.
- Always use personal protective equipments.
- When pouring chemicals, pour concentrates into the water and not vice-versa.
- Never pour chemicals into an empty, unlabeled container.
- Don't store flammable chemicals near a source of heat.
- Pesticides, fungicides, etc. always must be stored in a safe and elevated position.

Basically, there are four types of chemicals

- **Toxic chemicals**: These are chemicals that are poisonous to you, and can act upon the body very rapidly. Hydrogen sulfide and cyanide are examples of toxic agents.
- **Corrosives**: This type of chemical is usually an irritant. Corrosives can damage your body by burning, scalding or inflaming body tissues. Examples are chlorine and HCl acid.
- **Flammables**: Flammables are the chemicals that burn readily. They may explode or burn if sparks, flames or other ignition sources are present. Examples are gasoline, benzene and ethyl ether.
- **Reactive**: Reactive chemicals are those that require stability and careful handling. Some of them can explode or react violently if the container is dropped or hit. Nitroglycerine is an example.

Basic Tips of Safe Chemical Handling

- Read the label
- Dress the part
- Follow directions
- Know emergency procedures
- Be careful!
- Report any suspected problems
- Keep your work area neat, clean and organized
- Store everything properly

Basic Rules of Chemical Safety

Be Aware !.....Be Alert !.....Be Alive!.....

- Don't buy or store chemicals you do not need
- Store chemicals in their original container, Original container was designed to hold the chemical without degrading. The original container will have an accurate label, Serious injury can result when people try to identify chemicals with missing or uncertain labels by smelling, tasting or touching.
- Always wear appropriate cloths and work in a safe environment
- Always dispose of chemicals safely

Safety rules of Chemistry Laboratory

- Protect Your Eyes and wear appropriate protective clothing
- Do not apply cosmetics, eat, or drink in the laboratory
- Pour from large containers to smaller ones (Always ADD ACID to water)
- Work with volatile chemicals under a fume hood
- Do not smell any chemicals directly and do not pipet solutions by mouth
- Know the safety about equipments

Basic Laboratory Procedures

- Weighing: Requires careful weighing of all component
- Measuring Liquids: Calibrated glassware, Pipettes should be filled with a hand-operated device called a pipettor etc.
- Cleaning Glassware: Follow proper method of cleaning
- Sterilization: Sterilizing glassware and Instruments, Sterilizing Nutrient Media, Sterilizing, Plant Materials, Sterile Culture Techniques

1. **Acids**: **Strong acids** are very corrosive. They react with metals and can cause severe burns on the skin. Strong acids are Hydrochloric (HCl), Nitric (HNO_3), Sulfuric (H_2SO_4) and Hydrobromic (HBr).
2. **Weak acids** are often organic acids contain a –COOH group. Week acids are Formic acid (HCOOH), Acetic acid (CH_3COOH), Salicylic acid ($C_6H_4(OH)COOH$) and Citric acid ($C_5H_7O_5COOH$).
3. **Bases**: Bases are ionic compounds containing metal ions and hydroxide ions. Bases release hydroxide ions in water solutions. **Common Bases** are Sodium hydroxide (NaOH), potassium hydroxide (KOH), Calcium hydroxide $Ca(OH)_2$, Magnesium hydroxide ($Mg(OH)_2$) and Ammonium hydroxide (NH_4OH)

4. **Salts:** In general, salts are ionic compounds composed of metallic ions and nonmetallic ions. Salts dissociate in water. Salt solutions are generally electrolytes. An electrolyte is a substance that ionizes or dissociates into ions when it dissolves in water.

5. **pH**: The pH scale is a measure of the hydrogen ion concentration. A pH of 7 indicates a neutral solution while, acids are less than 7 and bases are greater than 7.

Know the safety equipments in laboratory

(1) Eye wash fountain (2) Safety shower (3) Fire extinguisher (4) Emergency exits

Personal Safety while handling Pesticides

- Avoid contact with the pesticides
- Wear all designated safety equipments
- Be careful of drips and spills
- Keep hands away from eyes and mouth
- Wash your hands before Smoking, Eating, Bathroom breaks

Use Designated safety equipments (Based on the WPS statement on the label & Regional requirements 1) Long sleeved shirt & long pants of tightly woven material 2) Waterproofed boots 3) Goggles 4) Hard hat 5) Unlined nitrile gloves)

List of Instruments in meteorology

(1) Sunshine Recorder (2) Anemometer (3) Wind Vane Instruments
(4) Pyranometer (5) Stevenson Screen (6) Hygrometer
(7) Ordinary Rain Gauge (8) Self Recording Rain Gauge (9) Thermometer & Barometer

List of Instruments in Microbiology

(1) Microscope (2) Balance/scale (3) Centrifuge
(4) Laminar airflow (5) Spectrophotometer (6) Refrigerator
(7) Freezer (8) Autoclave (9) Hot air oven
(10) Incubator (11) pH Meter (12) Water bath

List of Instruments in Biochemistry/Chemistry

(1) Spectrophotometer (2) Balance/scale (3) Centrifuge
(4) Stirrer (5) pH meter (6) Refrigerator
(7) Freezer (8) Flame photometer (9) Hot air oven
(10) Water bath (11) EC Meter (12) Atomic absorption Spectrophotometer

- **Basic Laboratory Procedures:**

The majority of laboratory operations utilized in the in vitro propagation of plants can be easily learned. One needs to concentrate mainly on accuracy, cleanliness, and strict adherence to details when performing in vitro techniques.

- **Weighing**

The preparation of media requires careful weighing of all components. Even if a commercially prepared medium is used, care must be taken in preparing it and any stock solutions that are required.

Because of the diversity of laboratory balances in use, it is impossible to review the details of their operation. The manufacturer's instructions should be consulted before using any balance. The types of balances most often encountered in the laboratory include top-loading single-pan balance, triple-beam balance, double-pan torsion balance, analytical single-pan balance, and top-loading electronic balance. The last type has become quite popular in recent years due to its accuracy, ease of use, and durability. With certain models of top-loading electronic balances, milligram accuracyis possible. Such accuracy previously required the use of analytical balances.

Several common precautions must be observed to obtain accurate weights. First, the balance should be located on a hard, stable, level surface which is free of vibrationsand excessive air drafts. The balance or weigh area should always be kept clean. Most importantly, the balance should never be overloaded (see manufacturer's specification). It is advisable to use a lightweight weighing container or paper rather than placing the material to be weighted directly on the pan surface.

Measuring Liquids

Calibrated glassware (e.g., beakers, flasks, and pipettes) are required for the preparation of culture media. Graduated cylinders of 10-, 25-, 100-, and 1000-ml capacities are used for many measuring operations, but volumetric flasks and pipettes are required for more precise measurements. Measurement of solutions with pipettesor graduated cylinders is only accurate when the bottom of the curved air-liquid interface is aligned with the measuring mark.

Pipettes should be filled with a hand-operated device, called a pipettor, which eliminated the hazards of pipetting by mouth. Never pipette by mouth!! Three types of pipettors are commonly used. The first is a bulb-type pipettor, which is controlled by a series of valves. The second type of pipettor is operated simply by rotating a small wheel on the side of the handle. Rotating the wheel upward creates a suction bringingthe liquid into the pipette; rotating the wheel in the opposite direction releases the liquid. A third type of pipettor utilizes an electric air pump. Liquid is drawn into the pipette by pressing the top button and released by pressing the lower button.

Cleaning Glassware

The conventional method of washing glassware involves soaking glass in a chromic acid-sulfuric acid bath followed by tap water rinses, distilled water rinses, and finally double-distilled water rinses. Due to the corrosive nature of chromic acid, the use of this procedure has been eliminated except for highly contaminated or soiled glassware. Adequate cleaning of most glassware for tissue culture purposes can be achieved by washing in hot water (70°C+) with

commercial detergents, rinsing withhot tap water (70°C+), and finally rinsing with distilled and double-distilled water. However, highly contaminated glassware should be cleaned in a chromic acid-sulfuric acid bath or by some other proven method such as (1) ultrasonic cleaning, (2) washing with sodium pyrophosphate, or (3) boiling in metaphosphate (alconox), rinsing then boiling in a dilute hydrochloric acid solution, and then finally re-rinsing. Cleaned glassware

should be inspected, dried at 150°C in a drying oven, capped with aluminum foil, and stored in a closed cabinet.

The following general procedure is recommended for cleaning glassware that contains media and cultures after all data has been collected:
Autoclave all glassware with media and cultures still in it. This kills any contaminating microorganisms that may be presents.
After the autoclaved media has cooled, but while it is still in a liquid state, pour it into bio-hazard plastic bags or thick plastic bags, seal, then discard.
Wash all glassware in hot soapy water using a suitable bottle brush to clean the internal parts of the glassware. Any glassware that is stained should be soaked in a concentrated sulfuric acid-potassium dichromate acid bath for 4 hr, then rinsed 10 times before washing it with soapy water.
All glassware should be rinsed three times in tap water, three times in deionized water, three times in double-distilled water, dried, and stored in a clean place.
Wash all instruments and new glassware in a similar manner.

2.To study the identification of Glassware

Beaker

laboratories. Beakers are generally cylindrical in shape, with a flat bottom and a lip for A beaker is a simple container for stirring, mixing and heating liquids commonly used in many pouring. Many also have a small spout to aid pouring asshown in the picture. Beakers are available in a wide range of sizes, from one milliliter up to several liters. Beakers are commonly made of glass(today usually borosilicate glass), but can also be in metal (such as stainless steel or aluminum) or certain plastics (notably polythene, polypropylene, PTFE).Beakers are often graduated, that is, marked on the side with lines indicating the volume contained. For instance, a 250 ml beaker might be marked with lines to indicate 50, 100, 150, 200, and 250 ml of volume. These marks are not intended for obtaining a precise measurement of volume.

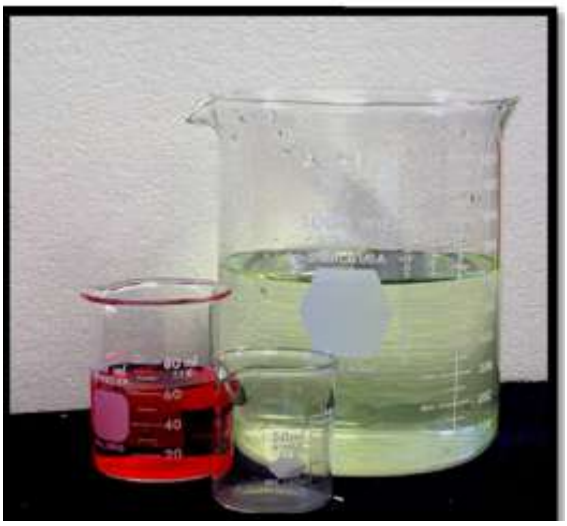


Fig. 1. **Beaker**

2.**Burette (also Buret)**

It is a vertical cylindrical piece of laboratory glassware with a volumetric graduation on its full length and a precision tap, or stopcock, on the bottom. It is used to dispense known amounts of a liquid reagent in experiments for which such precision is necessary, such as a titration experiment. Burettes measure from the top since they are used to measure liquids dispensed out the bottom. The difference between starting and final volume is the amount dispensed. Check the tip of the burette for an air bubble. If an air bubble is present during a titration, volume readings may be in error. Rinse the tip of the burette with water from a wash bottle and dry it carefully. After a minute, check for solution on the tip to see if your burette is leaking. The tip should be clean and dry before you take an initial volume reading. When your burette is conditioned and filled, with no air bubbles or leaks, takean initial volume reading. Read the *bottom* of the meniscus.

Fig. 2. Burette

3.Condenser

In a laboratory a condenser is a piece of laboratory glassware used to cool hot vaporsor liquids. A condenser usually consists of a large glass tube containing a smaller glass tube running its entire length, within which the hot fluids pass. The ends of the inner glass tube are usually fitted with ground glass joints which are easily fitted with other glassware. The upper end is usually left open to the atmosphere. The outer glass tube usually has two hose connections, and a coolant (usually tap water or chilled water/anti-freeze mixture) is passed through it. For maximum efficiency, and to maintain a smooth and correctly directed thermal gradient so as to minimize the riskof thermal shock to adjacent glassware, the coolant usually enters through the lower fitting, and exits through the higher fitting. Maintaining a correct thermal gradient (i.e. entering coolant at the cooler point) is the critical factor. Multiple condensers may be connected in series.

Applications

Condensers are often used in reflux, where the hot solvent vapors of a liquid being heated are cooled and allowed to drip back. This reduces the loss of solvent allowingthe mixture to be heated for extended periods. Condensers are used in distillation to cool the hot vapors, condensing them into liquid for separate collection.

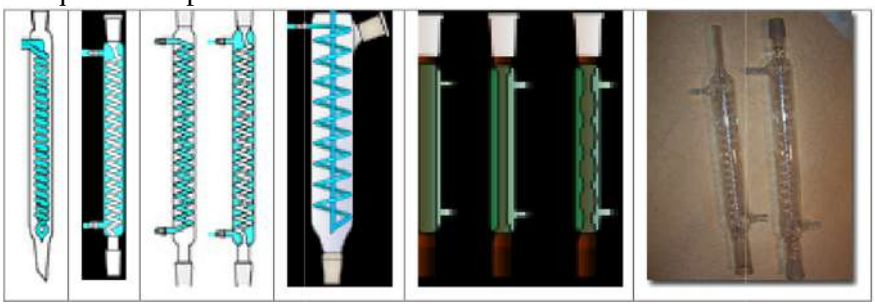


Fig. 3. Condenser

4.Erlenmeyer/ Conical flask

An Erlenmeyer, commonly known as a conical or E-Spot, is a widely used type of laboratory flask which features a flat, conical body, and a cylindrical neck. It's named after the German chemist Emil Erlenmeyer, who created it in 1861. The Erlenmeyer is usually

marked on the side (*graduated*) to indicate the approximate volume of contents, and has a spot of ground glass where it can be labeled with a pencil. It differs from the beaker in its tapered body and narrow neck. The opening usually has slight rounded lips so that the Erlenmeyer can be easily stopper using a piece of cotton wool. The conical shape allows the contents to be swirled or stirred during an experiment, either by hand or by a shaker; the narrow neck keeps the contents from spilling out. The smaller neck also slows evaporative loss better than a bigger neck also Erlenmeyers are used in chemistry labs for titration. Erlenmeyers are used in microbiology for the preparation of microbial cultures. Plastic Erlenmeyer flasks used in cell culture.

Fig. 4. Conical flask

Volumetric flask is a piece of laboratory glassware that is used to measure a very accurate and precise amount of a liquid.. It has a flatter bottom and long neck. The long and narrow neck is marked, at a very accurate measurement. The volume marks are usually made by machine, so it can be more assuredly accurate than hand-made marks.Volumetric flasks come with a stopper or cap. The stoppers are madeof glass and are used for capping the opening at the top of the neck. When a glass stopper is used, the opening at top of the neck has an outer tapered ground glass joint and theglass stopper has a matching tapered inner ground glass joint surface, but often only of stopper quality.

Uses of Volumetric Flask: The volumetric flask is used in two major ways. In one way a solute of known mass is placed in the flask and dissolved. The other technique involves placing an aliquot (sample of precisely known volume) of a solution of known molarity in the flask, then diluting to the mark with solvent. A volumetric flask is used either for making solutions or diluting a liquid to the size of the flask. A volumetric flask is used as a container used to measure the volume of a liquid with extremely high accuracy. Each volumetric flask is designed to measure one particular volume. That's why they come in a variety of sizes, such as 5 ml, 10 ml, 25 ml, 50 ml, 100 ml, 250 ml, 500 ml, 1000 ml, etc.

Measuring/ graduated cylinder

A graduated cylinder, also known as a measuring cylinder, is a pieceof laboratory equipment used to accurately measure the volume of an object. Graduated cylinders are generally more accurate and precise for this purpose than flasks and beakers. Often, the largest graduated cylinders are made of polypropylene for its excellent chemical resistance or polymethylpe tene for its clarity, making them lighter to ship and less fragile than glass. Polypropylene cylinders have excellent chemical high resistance and do not shatter when dropped. Polypropylene is easy to repeatedly autoclave (sterilize); however, autoclaving in excess of 130 °C can wrap or damage polypropylene graduated cylinders depending on the chemical formulation (typical commercial grade polypropylene melts in excess of 160 °C / 320 °F), thus affecting accuracy.

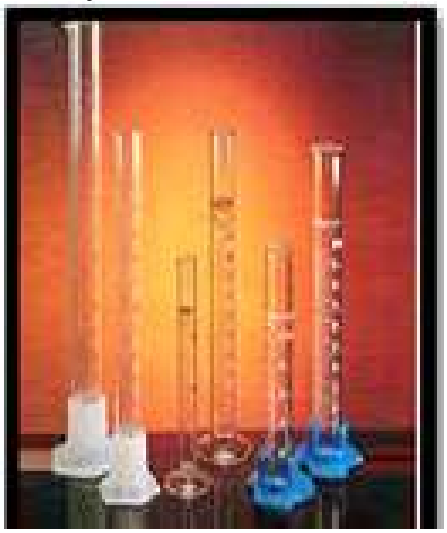


Fig. 5. Measuring/ graduated cylinder

Pipette

A pipette is a laboratory tool commonly used in chemistry, biology and medicine to transport a measured volume of liquid, often as a media dispenser. Pipettes come in several designs for various purposes with differing levels of accuracy and precision, from single piece glass pipettes to more complex adjustable or electronic pipettes.

Volumetric pipettes:

Volumetric pipettes or bulb pipette allow the user to measure a volume of solution extremely precisely. These pipettes have a large bulb with a long narrow portion above with a single graduation mark as it is calibrated for a single volume. Typical volumes are 10, 25,and 50 ml. Volumetric pipettes are commonly used to make laboratory solutions from a base stock as well as prepare solutions for titration

Graduated pipettes:

Graduated pipettes are a type of macro-pipette consisting of a long tube with a series of graduations, as on a graduated cylinder, to indicatedifferent calibrated volumes.

Micropipette:
A Gilson multichannel adjustable micropipette. Pipettes are used to accurately measure and dispense small volumes of liquid. The capacity of a micropipette can range from less than 1µl to 1000µl (1ml), w ile "macropipettes" can measure volumes greater than 1 ml.

Glass micropipette:
These are used to physically interact with microscopic samples, such as in the procedures of microinjection. Most micropipettes are made of borosilicate, alumino silicate or quartz with many types and sizes of glass tubing being available. Each of these compositions has unique properties which will determine suitable applications. Glass micropipettes are fabricated in a micropipette pulle

Separating funnel:
A separating funnel, also known as separation funnel, separatory funnel, or colloquially shape funnel, is a piece of laboratory glassware used in liquid-liquid extractions to separate (*partition*) the components of a mixture into two immiscible solvent phases of different densities.

3.To study the cleaning of glassware

Clean glassware is essential in chemistry. The problem is that the tolerance for contaminants varies with the work you are doing, and sometimes a chemist does not know how important clean glassware is to an experiment until it has failed.
There are two broad degrees of clean in chemistry; quantitative and normal cleaning

- **Quantitatively clean glassware** is required for the most demanding applications where a quantity is being measured at high precision, such in analytical or physical chemistry. At this level of cleanliness there are no residues (e. g., grease) or other impurities on the glassware.
- **Normal clean glassware** is free of most contamination but some contaminates (e. g., grease) is tolerated. Glassware that has been cleaned normally is used where a high degree of precision is not required, such as in a synthesis.
- Clean glassware is essential in chemistry.
- Quantitative and normal washing.
- Quantitatively clean glassware is required for high precision, such in analytical or physical chemistry.
- Glassware at this level of cleanliness has no residues Normal clean glassware is used where high degrees of precision are not required.

General Cleaning Tips

- Disassemble your apparatus as soon as possible after you are finished with it. Triple rinse with an appropriate solvent.
- Graduated cylinders, beakers, Erlenmeyer flasks, burettes and pipettes that were only used to dispense reagents generally only need to be triple-rinsed with a solvent followed by tap water and a final DI (deionized) /distilled water rinse. Air dry on a drying rack.
- Funnels should be rinsed with an appropriate solvent to remove substances that are clinging to them. Follow this by tap water and DI water rinses and air dry.

Health and Safety Considerations

- A task of washing glassware is simple but hazardous.
- You must wear eye protection.
- Gloves are recommended, if glassware contained an irritant, toxic material.
- Before cleaning be sure that any excess reagent has been disposed of properly.

General Cleaning Procedure

Following steps should be followed for glassware for which a simple solvent rinse is not sufficient.

- More aggressive cleaning methods may be required.
- Degrease your glassware's ground glass joints by wiping them with a paper towel soaked in a small amount of ether, acetone or other solvent.
- CAUTION! Wear appropriate gloves and minimize your exposure to the vapors.
- Place the glassware in a warm concentrated aqueous solution of detergent, and letsit for several minutes.
- Scrub with brush.
- Rinse thoroughly with tap water and give a final rinse with DI water.
- If there is clearly a greasy residue on the glass, more aggressive action must be taken.

More Aggressive Cleaning Methods

- If contaminant is a metal-containing compound, soak glassware in a 6M HCl solution.
- DANGER! This solution can cause severe burns! Wear appropriate gloves.
- Once solid has dissolved, copiously rinse the item with tap water, and then repeat general cleaning steps above.
- This method will also remove some organic residues (not grease).
- If contaminant is organic, submerge item in a base bath (a saturated NaOH or KOH solution in ethanol/methanol).

DANGER! The base bath will dissolve skin and alcohols are flammable! Wear gloves and keep ignition sources away from base bath. Be sure that glassware is completely filled with solution and is sitting upright. After several minutes of soaking, carefully remove the item (it will be slippery), and rinse thoroughly. If glassware is not quantitatively clean at this point, general cleaning steps may be repeated/ a longer soaking time in base bath, be needed.

Even More Aggressive Cleaning Methods

- Sometimes 6 M HCl and a base bath are not sufficient, so, more aggressive methods must be employed. CAUTION! All of these methods will do severe damage to eyes, skin, mucous membranes and lungs. Extreme caution should be exercised when using these methods. Wear gloves, eye protection and a lab coat. Work in the hood.
- Aqua Regia is an extremely powerful oxidizing solution (1 HNO_3: 3 HCl) (1 part H_2O be added if this will be stored to minimize generation of Cl_2). It will dissolve gold and will oxidize everything. Extreme caution must be used because it generates Cl_2 and NO_x gases in addition to causing severe tissue damage. Clean glassware before soaking in aqua regia and rinse with water.
- Acidic Peroxide Solution is prepared by dissolving commercially-available "No Chromix" in conc. H_2SO_4.

- An alternative preparation is to prepare a solution by mixing equal proportions of concentrated H_2SO_4 and aqueous H_2O_2 solutions (remember to add the H_2O_2 to the acid).
- A 3% H_2O_2 solution is sufficient, and under no circumstances should H_2O_2 solutions greater than 10% be used. The H_2O_2/H_2SO_4 solution is both a strong oxidant and a strong reductant, so care must be taken when using it.
- Another acidic peroxide solution for cleaning can be prepared by dissolving 36 g $(NH_4)_2S_2O_8$ (ammonium peroxydisulfate) in 2.2 L of 98% H_2SO_4. Take precautions for their use as per aqua regia.
- Chromic Acid a premeasured mix is available under the name "Chromerge", Take precautions as per aqua regia.
- Because high-valent chromium is carcinogenic, teratogenic and causes severe environmental damage, so the use of chromic acid is not recommended.
- Concentrated Hydrofluoric Acid HF will remove everything from glass and will even etch the surface of glass itself.
- It should not be used on calibrated volumetrics.
- HF causes severe, painful burns that do not heal well, and prolonged or intense exposure can lead to a very slow, painful death.
- It is not to be used by any students under any circumstances.

Special Cases

- Cuvettes, you only need to rinse a cuvette in appropriate solvent and wipe outside immediately after use.
- If something has adhered itself to a cuvette, it is best to soak the cuvette in solvent first and gently coax the solid off the side with a cotton swab.
- Never use a brush on a cuvette! If this fails, one of the acidic cleaning solutions mentioned above can be used (but never HF)
- It is not recommended that base bath be used on cuvettes, because it tends to etch glass surfaces.

Protein Contamination: Usually proteins can be removed scrubbing with detergent, but protein defies removal. In that event, you can proceed to the more aggressive acidic solutions, or you can prepare a peptidase solution (an enzyme that degrades proteins). The enzymatic approach is a bit slower than the forcing methods, but it is gentler and so can be used in situations that contaminated item is incompatible with acid.

Common Conversions in Molecular Biology

1 gram (g) = 1 · 10^3 milligrams (mg)	1 liter = 1 · 10^3 milliters (mL)
1 gram (g) = 1 · 10^6 micrograms (μg)	1 liter = 1 · 10^6 microliters (μL)
1 gram (g) = 1 · 10^9 nanograms (ng)	1 liter = 1 · 10^9 nanoliters (nL)
1 gram (g) = 1 · 10^{12} picograms (pg)	1 liter = 1 · 10^{12} picoliters (pL)

1 molar solution = 1 mole/liter
Conversion Exercises
How many grams are there in 0.22 nanograms?
0.5 grams is equivalent to how many micrograms?
0.56 liters equals how many milliters?

4.To study parts of a compound microscope

Principle: Magnified and real images of minute objects are obtained using combination of lenses. A compound microscope is a complex assemblage of such lenses enabling highly magnified images of the microscopic living organisms and intricate details of cells and tissues. A monocular mono-objective compound microscope is normally used in a biology laboratory.
Requirement: A compound microscope, silk cloth, lens cleaning fluid and lens cleaning paper.
Procedure:

- Place the microscope on the working table and remove dust by wiping the body with a silk cloth. Clean the lenses with lens cleaning fluid and lens cleaning paper.
- Identify the various parts of the microscope (Fig. 1.1). Draw a diagram of the microscope and label its various parts.
- Take a permanent slide preparation or a temporary preparation made by you, keep it on the stage, fix with clips after focusing and view. Learn how the microscope can be tilted or inclined by moving the arm of the microscope. Note, how focussing is done by moving the coarse adjustment and fine adjustment knobs.

Observation:

- The microscope has a strong basal foot and a vertical arm joined by an inclination joint. The arm can be tilted to different angles for its convenient usage.
- The stage of the microscope is round/rectangular/square shaped and is fixed to the arm. In the centre of the stage is a small circular hole covered with glass for passage of light.
- The stage is provided with two clips or mechanical device to fix and hold the slide firmly in position. The material to be observed is brought into view by moving the slide and then fixed with the help of clips at desired position.
- A movable (rack and pinion mechanism) or a fixed substage is provided with an iris diaphragm and condensor. The condensor is a system of two or more lenses to recieve parallel light rays and to converge these

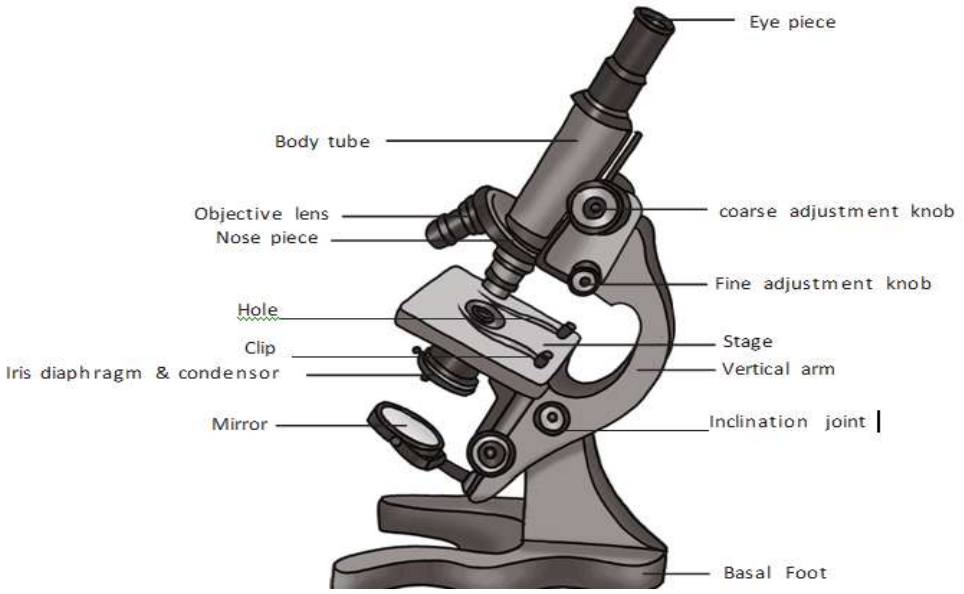


Fig.1. A compound microscope

on to the object through the iris and the hole present in the stage. The diaphragm helps in regulating the aperture size and thereby controls the amount of light that passes through the slide (Fig. 1.2). The needle/ pin is used to increase or decrease the aperture size. Some microscopes do not have the condensor.

Fig.1.2 Iris diaphragm showing different aperture sizes

An adjustable mirror is fitted below the condensor. It has plano- concave surfaces to focus the converging rays of light on the object through iris diaphragm and condensor in order to obtain a brightly illuminated image of the object.

The body of the microscope consists of a movable tubular body tube raised on rack and pinion mechanism. The tube has an ocular or eye piece of specific magnification (which can be changed for lower or higher magnification, i.e., 5X, 10X or 15X. Eye piece with pointer are also available. Two objective lenses 10X, 40X or 45X are mounted on a revolving nose piece at the lower end.

Some microscopes may also have a third objective lens (100X) called oil immersion lens.

The tube with eye and objective lenses can be moved up or down to focus the object sharply with the help of coarse adjustment knob and fineadjustment knob.

The object is first viewed under lower magnification using the coarse adjustment and then under higher magnification by rotating the revolving nose piece on which the objective lenses are mounted. While viewing at higher magnification, only fine adjustment knob is used for fine focus tuning.

Magnification:

Magnification by a microscope is a multiple of the X value of the lenses of the eye piece and objective. For example, a 5X eye piece and a 40X objective will magnify the image 5 · 40 = 200 times the size of the object. Similarly, when a 10X eye piece and a 40X objective are used, magnifying power would be 10 · 40 = 400X. Generally, in a compound microscope, the eye piece lenses are 10X or 15X and the objective lenses are 10X as well as 40X..

Precaution:

- Always clean the lenses before and after using the microscope.
- For cleaning lenses, always use lens cleaning fluid and lens cleaning paper.
- While observing, the objective lens should be carefully adjusted so as to avoid touching the slide lest it breaks the slide.
- Always put back the microscope in its case after use.

5.Use and handling of instruments/ equipments

1. Analytical balance

An analytical balance (often called a "lab balance") is a class of balance designed to measure small mass in the sub-milligram range. The measuring pan of an analytical balance (0.1 mg or better) is inside a transparent enclosure with doors so that dust does not collect and so any air currents in the room do not affect the balance's operation. This enclosure is often called a draft shield.

An electronic balance is a device used to find accurate measurements of weight. It is used very commonly in laborites for weighing chemicals to ensure a precise measurement of those chemicals for use in various experiments.

2.Laminar flow cabinet

A laminar flow cabinet or laminar flow closet or tissue culture hood is a carefully enclosed bench designed to prevent contamination of semiconductor wafers, biological samples, or any particle sensitive materials. Air is drawn through a HEPA (High- efficiency particulate arrestance or high-efficiency particulate arresting or high- efficiency particulate air, is a type of air filter) filter and blown in a very smooth, laminar flow towards the user. The cabinet is usually made of stainless steel with no gaps or joints where spores might collect.

Such hoods exist in both horizontal and vertical configurations, and there are many different typesof cabinets with a variety of airflow patterns and acceptable uses.

Laminar flow cabinets may have a **UV-Germicidal lamp to sterilize** the interior and contents before usage to prevent contamination of experiment. Germicidal lamps are usually kept on for 15 minutes to sterilize the interior and no contact is to be made with a laminar flow hood during this time.

During this time, scientists normally prepare other materials to maximize efficiency. (It is important to switch this light off during use, to limit exposure to skin and eyes as stray ultraviolet light emissions can cause cancer and cataracts.)

3.Incubator

An incubator is a device used to grow and maintain microbiological cultures or cell cultures. The incubator maintains optimal temperature, humidity and other conditions such as the carbon dioxide (CO_2) and oxygen content of the atmosphere inside. Incubators are essential for a lot of experimental work in cell biology, microbiology and molecularbiology and are used to cult re both bacterial as well as eukaryotic cells. Maintenance of uniform temperature within the incubator is essential and is achieved by fan, blower or a water jacket containing heated water.

4.Spectrophotometer

Spectrophotometer is one of the most important instruments in Biochemistry and Chemistry laboratory. It is used for the colorimetric analysis for plant, soil, food etc. It is an instrument that measures the amount of light absorbed by a sample. Spectrophotometer techniques are mostly used to measure the concentration of solutes in solution by measuring the amount of the light that is absorbed by the solution in a cuvette (sample holder) placed in the spectrophotometer**.**

Spectrophotometer has a light source (lamp), monochromator with gratting (which convert polychromatic light into monochromatic light i.e. it convert light of different wavelength into single (desired) wavelength), cuvette and detector.

Spectrophotometer works on Beer- Lambert's Law.

Spectrophotometer is used for the analysis of protein, carbohydrates, phenol, etc.

5.Centrifuge:

For an average laboratory a small table top centrifuge with maximum revolution per minute of 6000 and capable of accommodating l0-12 tubes of l5 ml capacity is sufficient. The tubes should be placed exactly opposite to each other, should be of the same weight and should contain same amount of fluid. The speed is adjusted by a rheostat and should be allowed to rise slowly. A timer for fixed duration of centrifugation is preferred.

A few common uses are:

- Sediment examination
- Separation of serum from clotted blood.
- Concentration of the materials

Magnetic stirrer:

A magnetic stirrer or magnetic mixer is a laboratory device that employs a rotating magnetic field to cause a stir bar immersed in a liquid to spin very quickly, thus stirring it. The rotating field may be created either by a rotating magnet or a set of stationary electromagnets, placed beneath the vessel with the liquid.

Water bath

Water bath is a water container having an electrically operated heating device to provide a fixed and uniform temperature. A thermometer is inserted inside the water bath for recording temperature. A mixer immersed inside water is also desired to maintain uniform temperature throughout the water bath.

It is used to incubate samples in water at a constant temperature over a long period of time. All water baths have a digital or an analogue interface to allow users to set a desired temperature. Utilizations include warming of reagents, melting of substrates or incubation of cell cultures. It is also used to enable certain chemical reactions to occur at high temperature. For all water baths, it can be used up to 99.9 °C. When temperature is above 100 °C, alternative methods such as oil bath, silicone bath or sand bath may be used.

Precautions/handling of water bath

- ¾ It is not recommended to use water bath with moisture sensitive or reactions.
- ¾ Do not heat a bath fluid above its flash/boiling point.
- ¾ Water level should be regularly monitored, and filled with distilled water only.

- This is required to prevent salts from depositing on the heater.
- ¾ Disinfectants can be added to prevent growth of organisms.
- ¾ Raise the temperature to 90 °C or higher to once a week for half an hour for the
- purpose of decontamination.
- ¾ The cover is closed to prevent evaporation and to help reaching high temperatures

Oil bath

An oil bath is a type of heated bath used in a laboratory. These baths are commonly used to heat reaction mixtures. An oil bath is essentially a container of oil that is heated by a hot plate. Generally, silicone oil is used in modern oil baths, although mineral oil, cottonseed oil and even phosphoric acid have been used in the past.

Sand bath

A sand bath is a common piece of laboratory equipment made from a container filled with heated sand. It is used to provide even heating for another container, most often during a chemical reaction. A sand bath is most commonly used in conjunction with a hot plate or heating mantle. A beaker is filled with sand and is placed on the plate.

Fume Hood

This is an air lifting cabinet in which toxic gas releasing reactions during solution preparation and use as well as chemical processes are performed and which provides the safe discharge of environmentally harmful gases.

Earthing

If there is a fault in your electrical installation you could get an electric shock if you touch a live metal part. This is because the electricity may use your body as a path from the live part to the earth part. Earthing is used to protect you from an electric shock. It does this by providing a path (a protective conductor) for a fault current to flow to earth. It also causes the protective device (either a circuit-breaker or fuse) to switch off the electric current to the circuit that has the fault. A key function of equipment earthing is to provide a controlled method to prevent the buildup of static electricity, thus reducing the risk of electrical discharge in potentially hazardous environments.

- ¾ Current - Flowing electricity
- ¾ Earth - A connection to the ground
- ¾ Earthing - A way of preventing electric shocks
- ¾ Electrical installation - a fixed wiring system

6.RULES FOR HANDLING OF CHEMICALS AND IT'S SAFELY

Wide range of chemicals is used in research laboratories of the Institute, each with its own inherent hazards.
An understanding of the potential hazards and precautions required in handling of chemicals is of outmost importance in preventing exposure to chemicals and mishaps.

Routes of entry:

- The main routes of entry of the chemicals into the human body are:
- By mouth (contaminated fingers!)
- By breathing in gases, aerosols or powder
- By skin contact or damage
- By absorption through intact skin
- By splashes into the eyes

Basically, there are four types of chemicals

- Toxic agents
- These are chemicals that are poisonous to you, and can act upon the body very rapidly.
- ・Corrosives
- This type of chemical is usually an irritant. Corrosives can damage your body by
- burning, scalding or inflaming body tissues
- Flammables
- Flammables are the chemicals that burn readily. They may explode or burn if sparks,
- flames or other ignition sources are present
- Reactive
- Reactive chemicals are those that require stability and careful handling. Some of them can explode or react violently if the container is dropped or hit.

Good handling practice

- Obtain the minimum amounts needed for your work
- Ensure that all containers are clearly labelled with their contents
- Toxic materials must be locked away
- Corrosive substances must be stored securely at a low level in trays
- Keep flammable materials in specially designed cupboards and only have out the minimum for immediate use
- Store acids, bases & solvents separately
- Never mouth-pipette
- Always dilute concentrated acids by adding the acid to water, never the reverse
- Never carry Winchesters by the neck – always use a carrier

- Always leave benches, balances etc clean & tidy after use
- Don't mix chemicals without specific authorization from the formulator.
- Always use personal protective equipment. Protect your eyes and hands from exposure to harsh chemicals; gloves, goggles or whatever is appropriate.
- When pouring chemicals, pour concentrates into the water and not vice-versa.
- Never pour chemicals into an unlabeled container.
- Don't store flammable chemicals near a source of heat.
- Pesticides, fungicides, etc. always must be stored in a safe and elevated position.
- Ventilate when engaging in cleaning or other applications using strong chemicals,
- especially dry solvents.

In case of fire:
If your clothing catches fire, immediately drop to the floor and roll to smother the flames and call for help.
If a compound or solvent catches on fire, *if you can*, quickly cover the flames with a piece of glassware

- If it is feasible, use a fire extinguisher to put the fire out.
- Do not put water on an organic chemical fire because it will only spread the fire.
- If the fire is large, do not take chances: evacuate the lab and the building immediately

If you inhale vapors:
Leave the area immediately - at least into the hallway. Tell your Coordinator; they will take you outside into the fresh air, and if necessary provide first aid or take you to get medical attention.
If you spill a chemical on yourself:
Immediately rinse the affected area with lots of water. Use soap if you wish, but never try to "treat" the spill with another solvent or chemical unless directed to do so by your Coordinator. If the affected area remains more than slightly red after the rinsing period, seek medical attention.

7.To study about pH meter

In practice, a pH value is defined by the equation: $pH = - \log_{10} [H^+]$. This equation means that the pH value is a common logarithm expressing the reciprocal of the hydrogen ion concentration. The pH meter consists of a glass electrode and a reference electrode. Reference electrodes are the calomel and silver-silver chloride electrodes. It allows the pH value of the sample to be obtained by measuring the potential difference between the two electrodes with a potential difference meter.

How pH meter works?

- When one metal is brought in contact with another, a voltage difference occurs due totheir differences in electron mobility.
- When a metal is brought in contact with a solution of salts or acids, a similar electricpotential is caused, which has led to the invention of batteries.
- Similarly, an electric potential develops when one liquid is brought in contact with another one, but a membrane is needed to keep such liquids apart.
- A pH meter measures essentially the electro-chemical potential between a known liquid inside the glass electrode (membrane) and an unknown liquid outside.
- The glass electrode measures the electro-chemical potential of hydrogen ions or the potential of hydrogen.
- To complete the electrical circuit, also a reference electrode is needed.
- The pH meter measures the electrical potential. Only the potential between the unknown liquid and the solution inside the glass electrode change from sample to sample.

The potassium chloride inside the glass electrode is a neutral solution with a pH of 7, so it contains a certain amount of hydrogen ions (H+). Suppose the unknown solution you're testing is much more acidic, so it contains a lot more hydrogen ions. What the glass electrode does is to measure the difference in pH between the two solutions by measuring the difference in the voltages their hydrogen ions produce. Since we know the pH of the potassium chloride solution (7), we can figure out the pH of the unknown solution.

To calibrate the pH meter, a standard solution with a known pH value is used. As standard solutions, phthalic acid (pH4.01), neutral phosphate (pH6.86), and borate (pH9.18) are mainly used.

8. To study the Preparation of media and methods of sterilization

Medium/Media: a nutrient blend used to support microbial growth. Culture medium is a liquid or gel designed to support the growth of microorganisms or cells, or small plants.
Culture: Is part of specimen grown in culture media.
Culture Media: is a medium (liquid or solid) that contains nutrients to grow bacteria *in vitro*. There are three physical forms of media: broth, solid, and semisolid.
Liquid (Broth): Mostly used for biochemical tests (blood culture, Broth culture). Growth of bacteria is shown by turbidity in medium. e.g. Nutrient broth, Selenite F broth, alkaline peptone water.
Semisolid agar (soft agar): Contains small amounts of agar (0.5-0.7%).
Solid (agar): Is Broth plus agar (seaweed). Are prepared by adding a solidifying agent (agar 1.5 -3%).
The most common growth media for microorganisms are nutrient broths (liquid nutrient medium). Liquid media are often mixed with agar and poured into Petri dishes to solidify. These agar plates provide a solid medium on which microbes may be cultured.
Properties of Media:

- Support the growth of the bacteria.
- Should be nutritive (contains the required amount of nutrients).
- Suitable pH (neutral to slightly alkaline 7.3-7.4).
- Suitable temperature, and suitable atmosphere. (Bacteria grow at 37^0C)

Types of Culture Media
Simple (basal, ordinary): media that contain the basic nutrients. E.g. Nutrient broth, nutrient agar, peptone water.
Enriched Culture Media: are media that are enriched with: Whole blood e.g. blood agar.
Selective Media: it is a media, which contains substances that prevent or slow the growth of microorganisms other than the bacteria for which the media is prepared for. e.g**TSI** (triple sugar iron agar).
Differential Media (indicators): Contains indicators, dyes, etc, to differentiate microorganisms.e.g. MacConkey agar, which contains neutral red (pH indicator) and is used to differentiate lactose fermenter and non-lactose fermenter. (e.g. *E. coli* and *Salmonella*).

9. To study about tissue culture

Tissue culture: The aseptic culture of plant protoplasts, cells, tissues or organs under conditions which lead to cell multiplication or regeneration of organs or whole plants.
Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture is widely used to produce clones of a plant in a method known as **micro propagation**. Different techniques in plant tissue culture may offer certain advantages of propagation, including:
The production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits.

- To quickly produce mature plants.
- The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds.
- The regeneration of whole plants from plant cells that have been genetically modified.
- The production of plants in sterile containers that allows them to be moved withgreatly reduced chances of transmitting diseases, pests, and pathogens.
- To clear particular plants of viral and other infections and to quickly multiply these plants as 'cleaned stock' for horticulture and agriculture.

Techniques
Preparation of plant tissue for tissue culture is performed under aseptic conditions under HEPA filtered air provided by a laminar flow cabinet. Thereafter, the tissue is grown in sterile containers, such as Petri dishes or flasks in a growth room withcontrolled temperature and light intensity.

Factors Affecting Plant Tissue Culture
Growth Media: Minerals, Growth factors, Carbon source , Hormones
Environmental Factors: Light, Temperature , Photoperiod
Explants Source: Usually, the younger, less differentiated the explants, the better for tissue culture
Genetics: Different species show differences in amenability to tissue culture. In many cases, different genotypes within a species will have variable responses to tissue culture; response to somatic embryogenesis has been transferred between melon cultivars through sexual hybridization

Preparation and standardization of some common reagent solution
The volumetric (titrimetric) analysis, involves essentially in determining the volume of a solution of accurately known concentration which is required to react quantitatively with the solution of the substance being determined. **The solution of accurately known strength is called the standard solution.** It contains a definite number of gram equivalents per litre. The weight of the substance to be determined is then calculated from the volume of the standard solution and the known laws of chemical equivalence.
In chemical analysis, many normal solutions are used. Usually the normality varies. A normal solution of a reagent is one that contains 1 gram equivalent weight per litre of solution.

Standardization is must for accurate analytical results, many process control decisions, legal requirements and analyst confidence.
If a reagent is available in the pure state, a solution of definite normality is prepared simply by weighing out an eq. weight or a definite fraction or multiple thereof, dissolving it in the solvent, usually water, and making up the solution to a known volume. The following is a list of some of the substances which can be obtained in a state of high purity and are therefore suitable for the preparation of standard solutions.

Acid-Base Titration

- Sodium carbonate Na_2CO_3
- Benzoic acid $H(C_7H_5O_2)$

Redox Titration

- Potassium dichromate $K_2Cr_2O_7$
- Sodium oxalate $Na_2C_2O_4$

Complex Formation Titration

- Silver nitrate $AgNO_3$
- Sodium chloride NaCl
- disodium Ehtylenediamine Tetra Acetate dihydrate $Na_2H_2C_{10}H_{12}O_8N_2 \cdot 2H_2O$

Precipitation Titration

- Silver nitrate $AgNO_3$
- Sodium chloride NaCl

When a reagent is not available in the pure form. *e.g.* most alkali hydroxides, some inorganic acids and various deliquescent substances, solutions of the appropriate normality are first prepared. These are then standardized against a solution of a pure substance (as above list) of known normality.

10. Study about sterilization

Sterilization is a term referring to any process that eliminates (removes) or kills all forms of life. Sterilization can be achieved by applying the proper combinations of heat, chemicals, irradiation, high pressure, and filtration.
A widely-used method for heat sterilization is the autoclave. Autoclaves commonlyuse steam heated to 121–134°C. To achieve sterility, a holding time of at least 15 minutes at 121°C or 3 minutes at 134°C is required.
Sterilization methods include; autoclaving, dry-heat, filtration, UV exposure and ethylene oxide.

- The various methods of sterilization are:
- Physical Method
- Thermal (Heat) methods (autoclaving, dry-heat)
- Radiation method (UV exposure)
- Filtration method (Membrane filtration)
- Chemical Method
- Gaseous method (ethylene oxide)

The most tedious parts of in vitro techniques are sterilizing plant materials and media and maintaining aseptic conditions once they have been achieved. Bacteria and fungi are the two most common contaminants observed in cell cultures. Fungal spores are light weight and present throughout our environment. When a fungal spore comes into contact with the culture media used in tissue culture, conditions are optimal for germination of the spore and subsequent contamination of the culture.

Sterilizing Culture Rooms and Transfer Hoods

Large transfer rooms are best sterilized by exposure to ultraviolet (UV) light. Sterilization time varies according to the size of the room and should only be done when there are no experiments in progress. Ultraviolet radiation is harmful to theeyes. Transfer rooms can also be sterilized by washing them 1-2 times a month with a commercial brand of antifungal spirocyte. Smaller transfer rooms and hoods also canbe sterilized with UV lights or by treatment with bactericides and/or fungicides. Laminar flow hoods are easily sterilized by turning on the hood and wiping down all surfaces with 95% ethyl alcohol 15 min before initiating any operation under the hood.
Culture rooms should be initially cleaned with detergent-brand soap and then carefully wiped down with a 2% sodium hypochlorite solution or 95% ethyl alcohol.
All floors and walls should be washed gently on a weekly basis with a similar solution; extreme care must be used to avoid stirring up any contamination that has settled. Commercial disinfectants such as Lysol, Zephiram, and Roccal diluted at manufacturer's recommended rates can be used to disinfect work surfaces and culture rooms.

Sterilizing glassware and Instruments

Metal Instruments are best sterilized using a glass bead sterilizer, Product Number S636 or S637. These sterilizers heat to approximately 275-350° C and will destroy bacterial and fungal spores that may be found on your instruments. The instruments simply need to be inserted into

the heated glass beads for a period of 10 to 60 sec. The instruments should then be placed on a rack under the hood to cool until needed.
Metal instruments, glassware, aluminum foil, etc., can also be sterilized by exposure to hot dry air (130°-170°C) for 2-4 hr in a hot-air oven. All items should be sealed before sterilization but not in paper, as it decomposes at 170°C. Autoclaving is not advisable for metal instruments because they may rust and become blunt under these conditions.
Instruments that have been sterilized in hot dry air should be removed from their wrapping, dipped in 95% ethyl alcohol, and exposed to the heat of a flame. After an instrument has been used, it can again be dipped in ethyl alcohol, reflamed, and then reused. This technique is called flame sterilization. Safety is a major concern when using ethyl alcohol. Alcohol is flammable and if spilled near a flame will cause an instant flash fire. This problem is compounded in laminar flow hoods due to the strong air currents blown towards the worker. Fires commonly start when a flamed instrument is thrown back into the alcohol beaker. In case of fire do not panic. Limiting the supply of oxygen can easily put out fires.
Autoclaving is a method of sterilizing with water vapor under pressure. Cotton plugs, gauze, labware, plastic caps, glassware, filters, pipettes, water, and nutrient media canall be sterilized by autoclaving. Nearly all microbes are killed by exposure to the super-heated steam of an autoclave for 10-15 minutes. All objects should be sterilizedat 121°C and 15 psi for 15-20 min.

Sterilizing Nutrient Media

Two methods (autoclaving and membrane filtration under positive pressure) are commonly used to sterilize culture media. Culture media, distilled water, and other stable mixtures can be autoclaved in glass containers that are sealed with cotton plugs,
aluminum foil, or plastic closures. However, solutions that contain heat-labile components must be filter-sterilized.
Generally, nutrient media are autoclaved at 15 psi and 121°C. For small volumes of liquids (100 ml or less), the time required for autoclaving is 15-20 min, but for larger quantities (2-4 liter), 30-40 min is required. The pressure should not exceed 20 psi, as higher pressures may lead to the decomposition of carbohydrates and other thermo- labile components of a medium.

Sterilizing Plant Material

Obtaining sterile plant material is difficult, and despite any precautions taken, 95% of cultures will end up contaminated if the explant is not disinfected in some manner. Because living materials cannot be exposed to extreme heat and retain their biological capabilities, plant organs and tissues are sterilized by treatment with a disinfecting solution. Solutions used to sterilize explants must preserve the plant tissue but at the same time destroy any fungal or bacterial contaminants.
Once explants have been obtained, they should be washed in a mild soapy detergent before treatment with a sterilizing solution. Some herbaceous plant materials (e.g., African violet leaves) may not require this step, but woody material, tubers, etc., mustbe washed thoroughly. After the tissue is washed, it should be rinsed under runningtap water for 10-30 min and then

be submerged into the disinfectant under sterile conditions. All surfaces of the explant must be in contact with the sterilant. After the allotted time for sterilization, the sterilant should be decanted and the explants washed at least three times in sterile distilled water. For materials that are difficult to disinfect, it may be necessary to repeat the treatment 24-48 hr before making the final explants. This allows previously unkilled microbes time to develop to a stage at which they are vulnerable to the sterilant.

Sterile Culture Techniques

Successful control of contamination depends largely upon the operator's techniques in aseptic culture. You should always be aware of potential sources of contamination such as dust, hair, hands, and clothes. Obviously, your hands should be washed, sleeves rolled up, long hair tied back, etc. Washing your hands with 95% ethyl alcohol is an easy precautionary measure that can be taken. Talking or sneezing while culture material is exposed also can lead to contamination. When transferring plant parts from one container to another, do not touch the inside edges of either vessel. By observing where contamination arises in a culture vessel, you may be able to determine the source of contamination.

In plant tissue culture, small pieces of plant tissue are placed on or in a medium rich in nutrients and sugar. If bacteria or fungi come in contact with the plant tissue or the medium, the culture becomes contaminated. The contaminants (bacteria and fungi) will use nutrients from the medium and the plant, which quickly destroys the plant tissue. Our aim is to surface sterilize the plant tissue and put it on a sterile growth medium without any bacteria or fungi getting on the plant or medium. This is not easy because bacteria and fungal spores are in the air, on us, in us, and under us!

When you see sunlight shining in a window you can, from certain angles, see dust particles in the air. There are hundreds of bacteria attached to each dust particle. A horizontal laminar flow unit is designed to remove the particles from the air. Room air is pulled into the top of the unit and pushed through a HEPA (High Energy Particle Air) filter with a uniform velocity of 90 ft/min across the work surface. The air is filtered by the HEPA filter so nothing larger than 0.3 um (micrometer) can pass through. This renders the air sterile. The flow of air from the unit discourages any fungal spores of bacteria from entering. All items going inside the unit should be sterile or sprayed with ethanol or isopropyl alcohol. They will remain sterile unless you contaminate them.

A transfer cabinet provides an enclosed environment that is not sterile but can be sterilized. A cardboard box lined with aluminum foil or plastic, or a well-cleaned aquarium, provides a shield to reduce contamination. A box that is 20-24 inches wide, 20-24 inches high, and 12-16 inches deep provides a good work area. Working inside any of these does not guarantee success.

The following precautions are necessary for all work areas.

- The room should be swept and if possible, mopped.
- Doors and windows should be closed.
- Air conditioners and fans should be turned off.
- If possible, each student must have a work space that can be properly treated against contamination. For example, the box or aquarium mentioned earlier, or a piece of poster paper lying on the table to indicate the student's sterile workspace.
- Have spray bottles filled with 70% ethanol or isopropanol (never methanol) placed so each student has access to one bottle. Spray everything going into the sterile area.
- Have a central supply area so all necessary items can be picked up and taken to the workspace as needed. Items can be returned to the central supply area after being used.
- Sterile instruments will be needed for each person. One way to accomplish this is to have a ½-pint jar of 70 % ethanol for scalpels and short forceps. When tissue has to be positioned in a vessel, long 10-inch forceps are needed. The long forceps need to be placed deep enough in alcohol so that any part of the forceps that might come into contact with the vessel is sterilized. A 100-ml graduated cylinder can be used to hold the alcohol and long forceps. A ½-pint jar of sterile water is needed for dipping the instruments to remove the residual alcohol that might dry out plant tissues.

- A sterile work surface is needed on which to place the sterile tissue to trim it. The easiest thing to use is a sterile petri dish. If you have glass ones, you can autoclave and reuse them. Presterilized plastic dishes are used and discarded. Spray the bag of dishes with 70 % alcohol before you open it and place the desired number of unopened dishes at each station. Each dish has two sterile surfaces-the inside top and inside bottom.
- Long hair should be tied back or covered.
- Hands should be washed, not scrubbed (scrubbing dries hands and creates flakes of skin that have bacteria) and sprayed with 70 % ethyl or isopropyl alcohol or coated with isopropyl alcohol gel.
- Gloves and masks provide extra protection.
- Do not talk while performing sterile operations.
- Do not lean over your work. Keep your back against the backrest of your chair.
- Try to work with your arms straight: this position may feel awkward, but it will reduce contamination.
- Do not pass non sterile items over sterile areas or items.
- Reach around rather than over. Make your movements smooth and graceful so that you do not disturb the air more than is necessary.

- Work quickly though, the longer it takes to manipulate the tissues the greater the chance of contamination.
- Have only the necessary items in sterile work area. Remove items that are no longer needed as quickly as possible. Act out each step before beginning so that you understand what you are about to do.