M.Sc. BOTANY LAB MANUAL

4th Semester

Prepared By

Biological Science Dept.

Botany

MIDNAPORE CITY COLLEGE

SEMESTER-IV
PRACTICAL

PAPER: BOT 494 Full Marks: 25

FOREST MENSURATION & SURVEYING

1. Measurement of diameter and Girth.

The size of the tree is best described with the diameter of the tree. The diameter of the tree provides a measure of tree performance and is a useful starting point for estimating tree volume. It is the linear measurement, the main objective of which is to estimate the volume of the trees. The volume of a tree is dependent on diameter or girth at breast-height, total height and form factor.

It is not only necessary for calculation of volume of logs, but also necessary for making inventory of growing stock as well as to correlate height, volume, age, increment of trees. In forestry, diameter should always be used for the description of the tree. In the past, girth has been used but this is no longer recommended.

A carefully defined point should be used for measuring the diameter of the tree. In many countries, especially India, this will be at 1.37 m above the base of the tree where the tree meets the ground. By convention, the diameter of forest trees is measured in centimetres at 1.37 m above the ground and is termed the "Diameter at Breast Height" or DBH. Because trees are measured with the bark on, this is also called the Diameter at Breast Height over Bark (DBHOB).

When measuring live trees most information is presented as over bark dimensions. Diameter at Breast Height (DBH) is the most common parameter used in the measurement of standing timber. Generally DBH is not measured on dead trees or on those of less than 7 cm DBH. Universally adopted standard height for measuring girth, diameters and basal area of standing trees is 1.37 m and being practiced in India, Burma, America, Union of South Africa and other British Colonies. In Europe, Australia and UK, DBH is taken as 1.3 m which is recommended by FAO as standard. In New Zealand, DBH is measured at 1.4 m.

The Significance of DBH:

i. Convenient height for taking measurement.

ii. Avoids the fatigue caused unnecessarily.

iii. Saves extra expenditure from not clearing the base.

iv. Abnormalities, e.g., root swell, disappear below breast-height.

v. Standardizes diameter measurement giving a uniform point of measurement. Diameter measurement at stump height is preferred, but standardization is lost because height of stump depends upon skill of the labour and the commercial value of the tree.

2. Girth class distribution.

Standard Rules Governing Breast Height Measurement DBH should be measured using callipers or tapes and the following conventions:

i. Breast height point should be marked by intersecting vertical and horizontal lines 12 cm long, painted with white paint.

ii. On flat ground, breast height (BH) should be marked by means of a measuring stick on standing trees at 1.37 m or 4 ft 6 inch above the ground level.

iii. On sloppy ground, BH should be measured from uphill side after removing any dead leaves or needles lodged there.

iv. In case of leaning trees on flat ground, BH is measured along the tree stem and not vertically on the side of the lean of the trees. On sloppy ground, BH on leaning trees should be measured from the uphill side along the leaning side of tree stem but not vertically.

v. In abnormal trees, BH mark should be shifted little up or down as little as possible to a more normal position of stem and diameter is measured.

vi. If tree is forked above BH, it should be considered as a single tree. If tree is forked below the BH, each fork should be treated as separate tree. If forking renders the BH point abnormal, foregoing rule should be applied and the tree is counted as one or two depending on the place of measurement.

vii. BH of the tree with aerial root/ buttress formation is measured by shifting BH mark a little above the buttress formed.

viii. Callipers should be held in such a way that the arm of the calipers is at right angles to the stem.

ix. Tapes should maintain a loop, at right angles to the stem, around the tree. Obstacles such as branches, climbers, loose bark etc. should be removed before measurement so that they do not distort the result.

DBH vs. Girth

DBH = Diameter at Breast Height = 2r

Where, r is the radius of stem

GBH = Perimeter of Stem at Breast Height = $2\pi r$

Thus, GBH = DBH x π

Under Bark vs. Over Bark:

DUB = DOB – 2t

GUB = GOB – $2\pi t$ where, t is the bark thickness

Instruments such as diameter callipers, tapes and wooden scales are most commonly used and the details of their descriptions are presented below:

1. Diameter Callipers:

It is generally made of wood and consists of a graduated rule and two arms. One is fixed at right angles to one end of the rule and the other movable parallel to the fixed rule. Size more than 120cm in length is rarely used. The use depends on the desired accuracy.

Callipers marked are painted to differentiate the reading. In some callipers, the arms are made parallel by screw adjustment. Metal callipers made of aluminium alloy are now in use. They are not heavier than wooden callipers and easy to keep clean and adjustment.

The different types of callipers are:

i. Flurry Callipers:

Arms from aluminium and graduated rule from wooden and bound with brass of sizes available in lengths of 80, 60, 35cms.

ii. Fommes Callipers:

All aluminum and same size as Flurry.

iii. Indian Aluminum Callipers:

They are variable in the length of 50 cm, 75 cm & 100 cm and they are graduated to show cm and mm.

Callipers are used to measure diameter of standing trees and logs. Diameters can be read directly in cm and mm thus making the instrument applicable for precise scientific work. If just one measurement is taken, an assumption is made that the cross-section at that point on the stem is perfectly circular. More than one measurement may be taken at that point on the stem. The average reading of these measurements is used as an estimate of the mean diameter. The measurement of more than one diameter and the subsequent estimation of mean diameter are made easier with the use of electronic calipers. A single calliper measurement has the potential to both over or under estimate the true mean diameter. With increasing numbers of measurements using calipers, evenly spread around the stem, the deviation of the estimate of mean diameter from the true mean will decrease.

2. Tapes:

It is a band of cloth, reinforced cloth, plastic or steel about 1.5 cm wide and of varying length and is used to measure girth of trees and logs. It is usually graduated one side in cm and mm but sometimes it is graduated on both sides to give measurements in metric system on one side and those in British system on the other.

The ends of the tape are always plated some metal to prevent their tearing off but in case of longer tapes which are kept encased in some cover by winding it in or in some other cases too, the beginning of tape has a metal ring to hold it. In western countries, tapes are often provided with hook enables one person to measure large trees with tapes lying flat in correct position on the tree.

The different types of tapes are:

i. **Cloth Tape:**

The tapes are made of cloth, though they may be painted with some paint on both sides to give better look and correct them from the influence of water. They are also affected by fluctuations in length due to expansion in use.

ii. Metallic Tape:

The better quality of tapes are usually reinforced inside by metal wires and are, therefore called metallic tapes. They are painted with some durable paint. So, they are more durable and reliable.

iii. Steel Tape:

Steel tapes are used for precise work and are mostly used in forest for measurement in sample or research work.

iv. Nylon and Fiberglass Tapes:

In western countries, nylon coated steel tapes and fiberglass tapes are available.

The tree measuring tapes are generally 3 m long or at the most 5 m. But land measurement tapes may be 5 m, 10 m, 30 m or even 50 m long. The tree measuring tapes, which are usually small, do not generally have cases to cover them, though some steel tapes are 2 or 3 m long are kept in cases with some spring device to wind them back when not in use. Diameter is best measured with a diameter tape, which is a tape marked with units that convert the girth to the diameter. It is important to make sure that a correctly calibrated tape is used.

3. Biltmore Stick:

The Biltmore stick is a rule graduated to indicate the diameter of a tree. The rule is placed against the trunk of the tree at a tangent. With the eye about 25 inches away (average arm length), the diameter may be read by lining up the end of the stick with the line of vision to one side of the trunk and sighting across the rule to the other side. At the point where the line of vision crosses the stick, the graduation will indicate the diameter of the tree (CCC 2009).

4. Wooden Scales:

It is a flat wooden piece marked in cm and mm. wooden scales are used to measure diameter of stumps or end sections of logs exposed as a result of cross- cutting. It is also used in stump and stem analysis for measuring radius at successive decade marks. The diameter should be measured along the passing through pith. In case of eccentric stumps or logs, two diameters, one along major axis and other at right angles to it should be measured.

As the end of the scale gets worn off by continuous use, the measurement should be taken from first centimeter and not from zero and one cm is deducted from the reading. The scale should be placed on edge so that the ends of the line to be measured coincide with marks of the scale. While reading measurement, the eye should be just above the mark. If this is not done, some error will creep in the measurement which is called error of parallax.

Two types of wooden scales are available provided with folding arrangements at every 15 cm length:

i. 30 cm long and 3 cm width

ii. 60 cm long and 1.5 cm width

5. Wheeler's Pentaprism Caliper:

The pentaprism is a moderately expensive instrument used for measuring the diameter of the stem at heights normally above reach from the ground. The Wheeler's pentaprism is an optical caliper that has two pentaprisms: a fixed one and a movable one. The construction allows generating parallel beams that correspond to the arms of a mechanical caliper. The sighting is situated at the position of the fixed prism and is separated into two parts.

Through the upper part, the observer aims directly at the left hand side of the trunk. The second beam goes through the two prisms where the movable prism is moved back and forth in such a manner that the second beam aims at the right hand side of the stem. This is achieved by simultaneously looking through the lower part of the sighting where the picture of the trunk in the prism is mirrored to the left along the left side of the tree.

6. Spiegel Relaskop:

The Spiegel Relaskop, also known as a Relaskop, is a sophisticated instrument that can be used to measure stand basal area and tree height and diameter at any point up a tree bole. In conjunction with other equipment, the Relaskop can be used in the estimation of distance (range) to an object and the number of trees per ha. The Relaskop has a peep-hole at the rear and a clear window at the front.

Three additional windows in the lower half of the instrument allow light to enter and illuminate the scale. A brake button, bottom half at the front of the instrument, allows a weighted wheel within the Relaskop to rotate. When looking through the peephole, a circular field of view is seen. The scales are seen in the bottom half of this field of view and scale readings are taken where the scale touches the line halfway up the field of view.

3. Measurement of height of a tree.

Determination of the Stem Diameter for a Known Height on the Tree:

First depress the brake button and move back until the tree stem appears to cover all of band 1 and the 4 quarter bands, i.e. the left side of the tree is aligned with the left of band 1 and the right side of the tree is aligned with the right side of the right most quarter band. Measure the horizontal distance (D in m) to the tree (directly beneath the point of interest). This method provides a maximum precision for determining diameter with the Relaskop.

Calculate the stem diameter (d in cm) as $d = 4 \times D$

Measurement of both height and diameter at same time at one or more points up the stem:

1. Measure out a horizontal distance (D in m) appropriate for the height of the tree.

2. Sight to the base of the tree and the point of interest and determine height using the appropriate scales and calculations.

3. With the brake button depressed, align the right side of the tree with the right side of band 1 and ensure that the left side of the tree falls within the quarter bands. If the left side of the tree goes beyond the quarter bands, you will need to move further away from the tree – you will need to be 5 m away for every 20 cm of diameter. If the left side does not make it to the

quarter bands, i.e., it falls within band 1, align the right side of the tree with the left side of band 1.

4. Estimate the number of quarter bands that are covered by the tree bole (between 0 and 4).

Experienced operators can read down to fifths of a quarter band:

1/5 just into the quarter band

2/5 almost half a quarter band

3/5 at least half a quarter band

4/5 almost the whole quarter band

5. Calculate diameter (d in cm) as:

$d = 2 \times D \times [b + (q/4)]$

Where

b equals 1 if band 1 is covered and 0 otherwise,

q denotes the number of quarter bands (read down to one fifth of a quarter band).

6. The precision (p) in per cent of the estimate depends on the distance from the tree (D in m) and the estimated diameter (d in cm):

$p = 10 \times D/d$

How to Measure the Height of a Tree ?

Total height of the tree is the straight line distance from the tip of the leading shoot (or from the highest point of the crown where there is no leader) to the ground level, usually measured on slopes from the uphill side of the tree. It is the straight-line distance from the highest point of the crown to the ground level. It is used to find out tree volume. It is also essential to read volume tables, form factor table, yield tables, etc. Tree height is normally used to find out productive capacity of the site and site quality of a locality.

There are different measurements of height on trees:

i. Bole Height:

It is the distance from the ground level to the position of the first crown forming living or dead branch.

ii. Commercial Bole Height:

The height of bole that is usually fit for utilization as timber is called commercial bole height.

iii. Height of Standard Timber Bole:

It is the height of the bole from the ground level up to the point where average diameter over bark is 20cm.

iv. Timber Height:

It is the vertical distance from the base of the tree to the point on the main stem where the diameter is 7 cm for conifers. For hardwoods, timber height is the vertical distance from the base of the tree to the point on the main stem where the diameter is 7cm or where the main stem becomes the crown, whichever is the lower.

v. Stump Height:

It is the height of the top of the stump above ground and it is normally 20-30 cm.

vi. Crown Length:

It is the vertical measurement of the crown of a tree from the tip to the point half way between the lowest green branches forming green crown all round and the lowest green branch on the bole.

vii. Crown Height:

It is height from the ground level to the point half way between the lowest green branches forming green crown all round and the lowest green branch on the bole.

viii. Breast Height:

If refers to the usual point of measurement of standing tree or stem diameter (1.37 m or 1.3 m) above ground on the uphill side of the tree.

ix. Mean Crop Height:

The mean height of all the trees growing in forest.

c. Top Height:

Mean height of the trees with the largest dbh in a stand.

Height Measurement Principles:

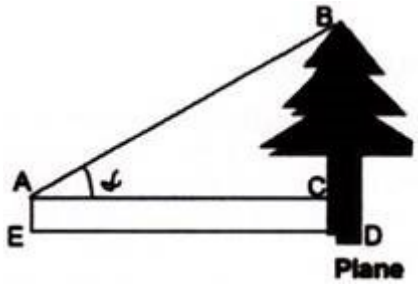
Trigonometric Principle:

Trigonometry is a branch of mathematics dealing with measurements of the angles and sides of triangles and functions based on these measurements.

Tangent Law:

The height of the tree is calculated with the help of the tangents of the angles to the top and base of the tree and the distance of the observer from the tree. Simple example is of standing tree in plane ground.

Tree Height (BD) = AE + ED Tan α



Sine Law:

In trigonometry, in any triangle, sines of angles are proportional to the opposite sides. The three basic trigonometric functions that we are concerned with here (sine, cosine and tangent) are ratios of the lengths of two sides of a triangle. These ratios are the trigonometric functions of an angle, theta, such that here, opposite side refers to tree height.

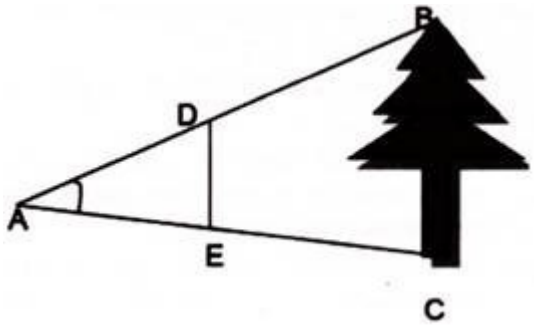
Principle of Similar Triangles:

Two triangles are said to be similar when the corresponding angles are equal and the corresponding sides are proportional. ADE and ABC are similar triangles.

Thus, BC: DE = AC: AE

Tree Height (BC) = DE x (AC/AE)

Where, DE and AE are known and AC can be measured in ground



Height sticks are used to measure the height of small trees. Hypsometers, altimeters and clinometers are used to measure height of tall trees (CCC 2009).

i. Hypsometers:

Used for determining the height of standing tree from observations taken at some distance from the tree.

ii. Altimeters:

Generally altitude measuring instruments, which can be devised to determine heights of tree.

iii. Clinometers:

It measures angle of slope. Any instrument which measures angles of slope can be used for height of tree by trigonometrical methods.

iv. Laser Dendrometers:

They basically rely on the same geometric principles as the hypsometers.

Some clinometers designed for this purpose called hypsometers are based on geometr-ical principles of similar triangles or based on relations between the sides of right angled triangles. Christen Hypsometer, Improved Callipers, Smythies' Hypsometer, Improved Smythies' Hypsometer are the instruments based on similar triangles. They are very slow, fatigue, heavy

and rough information. These instruments are very easy to use manually in the field even by unskilled labour.

Abney's Level, Brandis Hypsometer, Relaskop, Topographical Abney's Level, Haga Altimeter, Blume-leiss Hypsometer are the height measuring instruments based on trigono-metrical principles. They are manufactured scientifically and repair is difficult on spot. They are used only by skilled labour and limited use and expensive. It is fast, easy to carry and accuracy is maintained. They are easily available in markets and adopted by many countries.

1. Christen Hypsometer:

The Christen hypsometer is a simple instrument consisting of a rule or scale about 10 inches long which may be folded and carried in the pocket. It is made of metal, thin wood or even card board of about 2.5 cm thickness having two flanges to be used with a staff of known length. Upper flange will suspend by thread and next one to be suspended with weight to prevent swinging. It is based on similar triangles and usual length of instrument is 33cm (1.3 feet) used with a staff of 3.6 m (12′).

The estimator, facing the tree at sufficient distance to permit him to see its top and base, holds the instrument vertically before him. An assistant holds a 12-feet pole upright at the base of the tree. The estimator moves the scale nearer or away from the eye until the whole length of the scale just covers the entire view (in height) of the tree. The marking on the scale which is on the line of sight with the top of the upright pole indicates the height of the tree.

2. Abney's Level:

It is commonly used tree height measuring instrument based on trigonometrically principles. It gives accurate angle of elevation and depression. Readings can be taken after sighting the tree without disturbing the index arm. The instrument is small and light and can be used even in hills without difficulty. It has eye piece (hollow telescopic piece), magnifier glass, protector, wire and screw. It is an instrument with hollow tube with an eye piece at one end and a short sighting tube fitted at the other.

Eye piece consists of 2 or 3 telescopic hollow tubes and a sighting tube is a small detachable tube fitted with a horizontal wire at the centre. A mirror behind the horizontal wire covering only half of the tube so fitted that it makes 45°. A spirit level is fitted to the main tube, which

can be rotated by one screw. Wheel is for quicker movement and screw is for final adjustments. An index arm is also attached to the spirit level.

As the spirit level rotated, index arm moves on a graduated semi-circular arc. The angles of elevation and depression are noted in graduated arc in degrees up to 90°. On either side of the zero mark at middle, each division gives the reading of 10′.

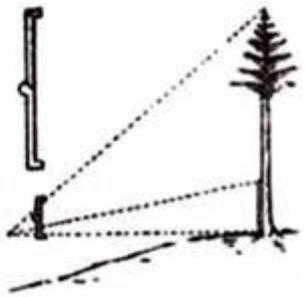


Fig. 12.4: Height Measurement using Christen Hypsometer

The horizontal distance from the base of the tree is measured to a location where the required point on the tree (e.g., tree tip) can be seen. The tree tip is sighted through the eye piece and this makes the instrument inclined and the bubble is not seen in the mirror.

Therefore, while sighting the top, the screw is rotated to bring the spirit level in a horizontal position. The spirit level is continued to be moved slowly to the position when the bubble image is bisected by the line of horizontal wire on the mirror and in the other half the tree top is seen touching the horizontal wire.

The spirit should be balanced by tuning with screw as well as moving to and back ward. The degrees and minutes for angle of elevation- (a) are read. Again the base of the tree is sighted through eye piece and angle of depression (P) is measured.

If the base of the tree is level with the observer or below observer (i.e., observer on the upward slope), total tree height is calculated using following formula:

Tree Height = Horizontal Distance x [Tan (α) + Tan (β)]

If the base of the tree is above the observer (i.e. observer on the downward slope), the following formula is used to get a total tree height:

Tree Height = Horizontal Distance x [Tan (α) – Tan (β)]

3. Suunto Clinometer:

The Suunto Clinometer (clino) is a tool commonly used by foresters to measure tree heights and also slope angles. Tree height is measured using the principle of triangulation with a clinometer. Of all the forestry tools, the clinometer requires the most practice and skill. It is assumed that the tree grows at a right angle to the ground (even on a slope). At the rear of the clino is a peephole, which shows a percentage scale and a horizontal line. First, the horizontal distance between the base of the tree and the operator is measured.

Looking through the peephole, the horizontal line is lined up with the top of the tree and the corresponding number from the percentage scale, which is on the right hand side is read off (top reading). The scale on the left is in degrees and should not be used. Similarly, the corresponding percentage scale for base of the tree (bottom reading) is measured. If the base of the tree is level with the observer or below observer (i.e., observer on the upward slope).

Total tree height is calculated using following formula:

Tree Height = Horizontal Distance x [Top Reading + Bottom Reading]

If the base of the tree is above the observer (i.e., observer on the downward slope),

The following formula is used to get a total tree height:

Tree Height = Horizontal Distance x [Top Reading – Bottom Reading]

4. Haga Altimeter:

Haga altimeter is based on the trigonometrically ratio. It is very easy to use and read height directly. This instrument is based on the principle of ‘sight the object and shoot’. It has eye piece, pronges, scale, trigger button, release trigger button and tuning knob. It has several percent of usual scales: 15, 20, 25, and 30 for the corresponding horizontal distance from the tree: 15 m, 20 m, 25 m, and 30 m.

For example, if the observer is standing at a distance of 20 m from the base of the tree, then he must use the percentage scale of 20 for measuring the reading. First, the observer stands at a distance of 20m from the tree and looks the top of the tree through eye piece. He sees the tip of the tree through eye piece and coincides it with the pronge of the instrument.

At this position, trigger is pressed to arrest the pointer on the scale and now the reading on percent scale 20 is noted (top reading). Now the pointer is released by pressing trigger release button. Again the base of the tree is viewed through eye piece and corresponding reading on 20 percent scale is noted (bottom reading).

The total tree height is calculated using the formula:

Tree Height = Top Reading + Bottom Reading

Ravi altimeter/multimeter and Blume Leiss altimeter also work with the above same principles and readings are taken in the same manner of Haga Altimeter.

5. Vertex IV and Transponder T3:

The vertex IV is primarily designed to measure the height of standing objects and most often trees. The instrument can also be used to measure distance, horizontal distance, angle and inclination. The vertex instrument with its ultrasonic measuring technique proved to be specially useful in dense terrain with thick undergrowth, where conventional methods such measuring tapes, laser instrument and mechanical height measures are difficult to use.

To define a reference point in a secure and reliable way, the vertex IV works with the transponder T3. The vertex IV communicates with the transponder. This communication eliminates any mix up of signal from other instruments or places (echos) in an efficient way.

The measuring operation will not be disturbed by objects in between the vertex IV and the transponder T3 in any significant way. The reference point i.e., the T3 is used as a sight mark for height measuring and can be placed at optional height. The reference point height is set in a special menu in the vertex instrument and automatically added to the measured height.

The vertex IV uses ultrasound to measure distance. Unlike measuring tapes and laser instrument, ultrasound can be used also when there is no free aim to the reference point. The ultrasound will not pass through an obstacle, but looks for the shortest way. Heights are calculated trigonometrically using the variables contained when measuring angle and distance.

The vertex IV automatically assumes that the measuring object is perpendicularly positioned to the ground. With the vertex IV, an unlimited number of heights or objects can be measured. The instrument display can show the four lastly measured heights per object at a time. When

using a telescopic method to measure, an in built BAF function can be used for the vertex IV instrument to control the minimum diameter for trees.

The function is useful when some trees in an area are covered by other, making the decision whether to include the tree or to exclude it from the area, difficult. By simply measuring the distance between the tree and plot centre, the vertex IV can calculate the minimum diameter the tree should have in order to be included into the counting. Data can be sent through IR or Bluetooth and results can be stored and processed in the digitech professional calliper, other PC or handheld computer.

6. Spiegel Relaskop:

The standard metric Relaskop has three scales for measuring (vertical) height. The appropriate scale will depend on the horizontal distance from the tree.

i. Left-most scale – 20 m from the tree.

ii. Middle-left – 25 m from the tree.

iii. Middle-right – 30 m from the tree.

After depressing brake button, one should look straight up or down and the appropriate distance values can be seen alongside their scales. A point must be selected that is 20, 25 or 30 m (horizontal) from the tree of interest. Other horizontal distance can be selected for which the final scale reading must be doubled or halved or multiplied with respective conversion factors. The base and tip (or any other points of interest) must be clearly visible from the selected point.

If the tree is leaning, the point where you observe the tree should be at 90 degrees to the plane of the lean. If the lean is severe (i.e., more than 10 degrees from vertical), the point directly beneath the tip of the tree should be located (e.g. using a plumb-bob) and the horizontal distance taken from this point. The actual distance chosen is related to an initial estimate of the tree height.

Once the observation point is found and the appropriate scale selected, sight through the peephole to the base of the tree. Tap the brake button several times until the scale settles then read the height directly from the appropriate scale. If you are looking down towards the base of the tree, this reading is the vertical height that the base of the tree is beneath your eye.

Sight to the top of the tree (or other point) and again tap the brake button until the scale settles.

The scale is a direct reading of the height above your eye to this new point. Add the two heights together if the base of the tree was below your eye to determine total (vertical) height. If the base of the tree was initially above your eye (i.e., the base if above you on sloping ground) subtract the initial height from the upper height to determine (vertical) height.

4. Volume calculation

Volume is the most widely used measure of wood quantity and is usually estimated for the assessment of economic value or commercial utilization potential. The wood volume of a tree includes stem, branches, stump and roots. For standing trees, aboveground volume production is generally based on stem wood volume for conifers, but may include branch volume for broad-leaved tree species.

Depending on measurement objective and local traditions, measurements or predictions of wood cubic volume may refer to, for example, total stem volume, total tree volume (stem and branches), or volume above a certain merchantable limit. Volume estimates may include or exclude bark and, for aboveground estimates, include or exclude the stump. Volume is always a cubic measure and usually expressed in cubic meters. Merchantable volume, however, is sometimes expressed in other units related to commercial use.

Volume is usually estimated for standing trees from such measurements as diameter, or diameter plus merchantable height, using a volume equation or a log rule. Volume may be measured directly on felled trees or logs, but is often estimated from dimensions such as minimum diameter or piece length. Direct measurement of volume is usually done by sectioning the tree into smaller pieces assumed to be cylinders.

The general formulas for calculating the volume of standing trees are:

i. Volume = BA x H x Form Factor

ii. Volume = $\pi r2H$ x Form Factor

iii. Volume = D2H x Form Factor

Where,

BA = Basal area of the tree

r = radius of the tree

H = Height of the tree

D = Dbh of the tree

Stem/Log Volume Measurements:

It is possible to utilize geometric relationships to approximate volume. The volume of a cylinder is simply the area of the base times the height, and the volume of a cone is one-third of the volume of a cylinder with the same area of the base and height. Trees are neither cones nor cylinders, but empirical analyses often indicate that the volume of a single-stemmed tree is between that of a cone and a cylinder, with tree volume often lying between 0.40 and 0.45 times that of an equivalent cylinder.

Volume of Cylindrical Stem = S x L

Volume of Conical Stem = 1/3 x S x L

Volume of Paraboloid Stem (Huber's Formula) = Sm x L

Volume of Paraboloid Stem (Smalian's Formula) = (S1 + S2) x L/2

Volume of Neiloid Stem (Newton's Formula) = (S1 + 4Sm + S2) x L/6
Where,

V = Volume of log

L = Length of log

S = Cross-Sectional area of cylinder or cone

Sm = mid cross-sectional area of log

S1 = Cross-sectional area at the smaller end

S2 = Cross-sectional area at the larger end

G = Mid Girth of log in inches

Volume Calculation of Sawn Timber

V = L x B x H (cubic meter or cubic feet)

Volume Calculation of Firewood/Stacked Wood:

Volume may be estimated for stacks of logs or processed products by measuring dimensions. In these cases, local knowledge is often needed for appropriate estimation of volume.

Firewood is stacked in the form of rectangular parallel piles and the volume of the stacked firewood is calculated by:

Volume = Length (L) x Height (H) x Breadth (B) of the stack expressed in cubic meter or cubic feet.

Stem Form:

Form is defined as the rate of taper of a log or stem. Taper is the decrease in diameter of a stem of a tree or of a log from base upwards. The taper varies not only with species, age, site and crop density but also in the different parts of the same tree.

Form Factor:

Form factor is defined as the ratio of the volume of a tree or its part to the volume of a cylinder having the same length and cross-section as the tree.

The form factors are of the following types:

i. Artificial Form Factor:

This is also known as the breast height form factor. Here, the basal area is measured at breast height and the volume refers to whole tree both above and below the point of measurement.

ii. Absolute Form Factor:

For this form factor, basal area is measured at any convenient height and the volume refers only to that part of the tree above the point of measurement.

iii. Normal Form Factor:

In this form factor, basal area is measured at a constant proportion of the total height of the tree.

Form Quotient:

It is the ratio of diameter to diameter at breast height.

Form quotients are of two types:

i. Normal Form Quotient:

It is the ratio of mid-diameter of a tree to its diameter at breast height.

ii. Absolute Form Quotient:

It is the ratio of a stem diameter at one half its heights above the breast height to the diameter at breast height.

Form Height:

It is the product of form factor and the total height of the tree.

Form Height = Volume of tree/Basal area

Form Class:

It is one of the intervals in which the range of form quotient of trees is divided for classification and use.

Form Point Ratio:

Form point is the point in the crown where wind pressure is estimated to be centered. Form point ratio is the relationship of height of the form point above ground level to the total height of the tree, usually expressed as percentage.

Tree Basal Area Measurements:

Tree Basal Area (TBA) is the cross-sectional area (over the bark) at breast height measured in square meter (m2). TBA can be used to estimate tree volumes and stand competition. To determine tree basal area simply measure the diameter at breast height in centimeters (DBHOB) and calculate the basal area (m2) using an equation based on the formula for the area of the circle (area = πr2 where r = radius). The formula below also converts the diameter in centimeters to the basal area in m2. The same technique can be used to calculate the cross sectional area of the tree at any point along the stem.

Tree Basal Area (TBA) in m2 = (DBH/200)2 x 3.142

Where, DBH is the Diameter at Breast Height in centimeters and 3.142 is π

5. Chain surveying.

Chain survey is a linear measurement. In this method, the actual ground is set on a paper by a system of straight lines. These lines are called as chain lines which are prerequisite in chain surveying. This is a convenient method for surveying small areas and open ground with simple details. But it is not suitable where the area cannot be divided into triangles. Instruments used in chain surveying 1. Chain :-The chain is composed of 100 or 150 pieces of galvanized mild steel wire 4mm in diameter called links. The end of each link are bent into a loop and connected together by means of three oval rings. The ends of the chain are provided with brass handles for dragging the chain on the ground. The length of link is the distance between the centers of the two consecutive middle rings. The end links includes the handles. Metallic tags or indicators are fixed at various distinctive of the chain to facilitate quick reading. The following chains is used in surveying a. Metric surveying chains: The chains are made in lengths of 20 and 30 meters. To enable the reading of factious of a chain, tallies (tags) are fixed at every five meter length and small brass rings are provided at every meter length. To facilitate holding of the arrows in position with the handle, a groove is cut on the out side surface of the handle. The handle joints are flexible. the tallies used for marking the distances in a metric chain are marked with letters ‘Me’ and ‘m’. b. Steel Band Chain:-It

consists of a ribbon of steel with bras handle at each end. It is 20 or 30 long and 16 mm wide. It is wound on an open steel cross or on the metal reel in a closed case. The graduations are etched as meters decimeters, centimeters on one side and 0.2 m links on the other. Brass tallies are fixed at every 5 m length of the band. c. Günter's Chain:-It is 66 fit long and is divided into 100 links. Each link is 0.66 ft long. It is very convenient for measuring distance in miles and furlongs. Also for measuring area and when the units of area is an acre d. Revenue Chain:-It is commonly used for measuring fields in cadastral survey. It is 33 ft long and divided into 16 links. Each link is 2.0625 ft long. e. Engineer's chain:-It is 100 ft long and it is divided into 100 links. Each link is 1 ft in a length. Used in all Engineering surveys. 2. Arrows:-They are also called as marking or chaining pins and are used to mark the end of chain during the process of chaining. They are made up of good quality hardened and tempered steel wire of 4mm in diameter. The arrows are made 400 mm in length. They are pointed at one end of inserting in to the ground. The other end is in to a ring. 3. Tapes:-Used for taking subsidiary measurements, such as offset. It is very light and handy. Tape is available in 1, 2, 10, 30, and 50 meters. The tape is marked with a line at every five millimeters, centimeters, decimeters, and meter. 4. Wooden Pegs:-These are used to mark the positions. They are made of hard timber and tapered at one end. They are usually, 2.5 cm square and 15 cm long. But in soft ground 40 to 60 cm long and 4 to 5 cm square is suitable. They should be driven in the ground with about 4 cm lengths, projecting above the ground. 5. Ranging rods and pole:-Used for making the positions of stations and for ranging. They are made of seasoned timber or steel. They are circular or octagonal in cross section of 3 cm diameter. Lower shoe is 15 cm long. They are made in two sizes as 2 meters and 3 meters and are divided in equal parts each 0.2 m long. They are painted alternatively black and white or red and white. Ranging poles are 4m or more heighted hollow rods used in the case of very long chain lines.

6. Plane table survey.

Instruments Required:

- Tripod
- Plane Table
- Plumbing Fork
- Level
- Magnetic needle compass
- Alidade
- Measuring Tape
- Ranging Rods (For demonstration purpose)
- Other accessories

- 28in x 22in drawing sheet
- Scotch Tape
- Chisel pointed Pencil
- Eraser

Procedure:

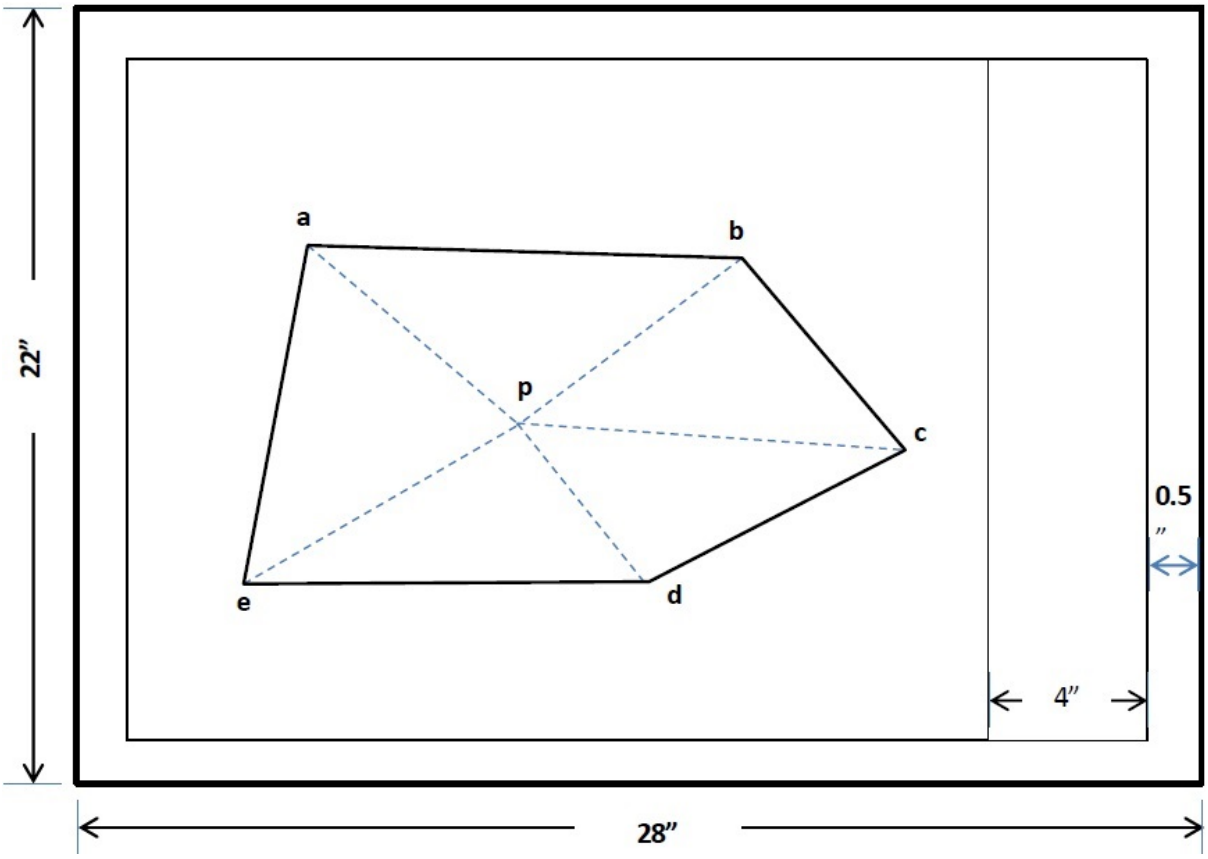
1. Select a suitable point **P** on the ground such that all the details are visible from it
2. Centre and level the plane table over P
3. Mark the direction of the North on the sheet by using compass
4. Locate instrument station p on the sheet by using plumbing fork, such that p on sheet is exactly over P on ground
5. Cantering the alidade on point p sight various details step by step and draw a ray from each detail along the fiducially edge of the alidade
6. Let the details be named as A, B, C, D, E etc.
7. Now measure the distances of each point from P i.e. PA, PB, PC, PD, PE and plot them to scale on the sheet as pa, pb, pc, pd, pe respectively
8. Joint a, b, c, d, and e to give the outline of the details

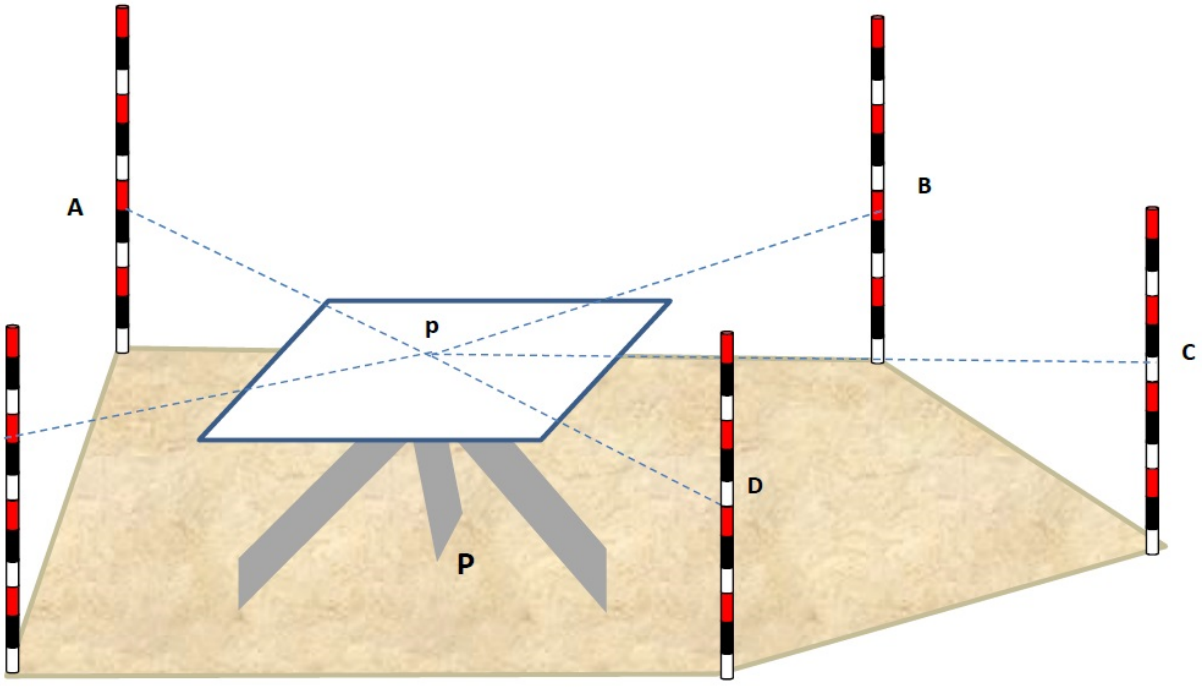
NOTE: *These details may be building corners, electric towers, tree, manhole etc. But for demonstration purpose we will put ranging rods.*

Significance and Applications

This is the easiest method in plane tabling. It is used when:

- All the details are visible and accessible from one instrument station
- The ground is level and smooth
- Distances are so small that can be measured with single tape


a
b
p
c
e
d
22”
0.5
”
4”
28”


A
B
p
C
D
P

PAPER: BOT – 495D

SPECIAL PAPER PRACTICAL MICROBIOLOGY

Full Marks: 50

1. Study of fermentation of sugar by different bacteria.

Carbohydrate Fermentation Test: Uses, Principle, Procedure and Results

Principle

When microorganisms ferment carbohydrate an acid or acid with gas are produced. Depending up on the organisms involved and the substrate being fermented, the end products may varies. Common end-products of bacterial fermentation include lactic acid, formic acid, acetic acid, butyric acid, butyl alcohol, acetone, ethyl alcohol, carbondioxide and hydrogen. The production of the acid lower the pH of the test medium, which is detected by the color change of the pH indicator. Color change only occurs when sufficient amount of acid is produced, as bacteria may utilize the peptone producing alkaline by products.

Table 1. pH Indicators for Carbohydrate Fermentation Media

pH Indicator	Uninoculated Media		Acid (fermentation)		Alkaline (negative)	
	pH	Color	pH	Color	pH	Color
Andrade's	7.1-7.2	Light pink	5.0	Pink-red	12.0-14.0	Yellow, colorless
Bromcresol purple (BCP)	7.4	Deep purple	5.2	Yellow	6.8	Purple
Bromthymol blue (BTB)	7.0	Green	6.0	Yellow	7.6	Deep Prussian blue
Phenol red	7.4	Reddish-orange	6.8	Yellow	8.4	Pink-red

Phenol red is commonly used as a pH indicator in carbohydrate fermentation tests. Other pH indicators such as bromocresol/bromocresol purple (BCP), bromothymol/bromothymol blue (BTB), and Andrade's can be used.

Durham tubes are inserted upside down in the test tubes to detect gas production. If the test organism produce gas, the gas displaces the media present inside the tube and get trapped producing a visible air bubble.

Based on the characteristics reactions observed, bacteria can be classified as:

- Fermenter with acid production only
- Fermenter with acid and gas production
- Non-fermenter

Test procedure

Phenol Red Carbohydrate Broth is commonly used in carbohydrate fermentation test. The carbohydrate source can varies based on your test requirements.

1. Phenol Red Glucose Broth
2. Phenol Red Lactose Broth
3. Phenol Red Maltose Broth
4. Phenol Red Mannitol Broth
5. Phenol Red Sucrose Broth

Preparation and Composition of the media

Get specific Phenol Red Carbohydrate Test media from the commercial suppliers or Phenol Red Broth Base and add specific carbohydrate source based on your test requirements, or you can prepare media mixing the following ingredients.

Composition of Phenol Red Carbohydrate Broth

- Trypticase or proteose peptone No. 3: 10 g
- Sodium Chloride (NaCl): 5 g
- Beef extract (optional): 1 g
- Phenol red (7.2 ml of 0.25% phenol red solution): 0.018 g
- Carbohydrate source: 10 g

A. Preparation of the media

- Prepare broth media by mixing all ingredients in 1000 mL of distilled/deionized water and heating gently to dissolve it *(Note: Use single carbohydrate source based on your requirements).*
- Fill 13 x 100 mm test tubes with 4-5 ml of phenol red carbohydrate broth.
- Insert a Durham tube to detect gas production.
- Autoclave the prepared test media (at 121°C for 15 minutes) to sterilize. The sterilization process will also drive the broth into the inverted Durham tube. *(Note: When using arabinose, lactose,maltose, salicin, sucrose, trehalose, or xylose, autoclave at 121°C for only 3 minutes as these carbohydrates are subject to breakdown by autoclaving)*

The prepared broth media will be a light red color and the final pH should be 7.4 ± 0.2.

Alternatively, prepare Phenol Red Broth Base, heat sterilize and cool to 45°C. Prepare specific carbohydrate solution separately, filter the solution using membrane filter (pore size: 0.45 µm). Add carbohydrate solution to the broth base and mix it. The preferred carbohydrate concentration is 1%.

B.Inoculation and Incubation

- Aseptically inoculate each test tube with the test microorganism using an inoculating needle or loop. Alternatively, inoculate each test tube with 1-2 drops of an 18- to 24-hour brain-heart infusion broth culture of the desired organism .
- Incubate tubes at 35-37°C for 18-24 hours. Longer incubation periods may be required to confirm a negative result.

C. Interpretation of the results:-

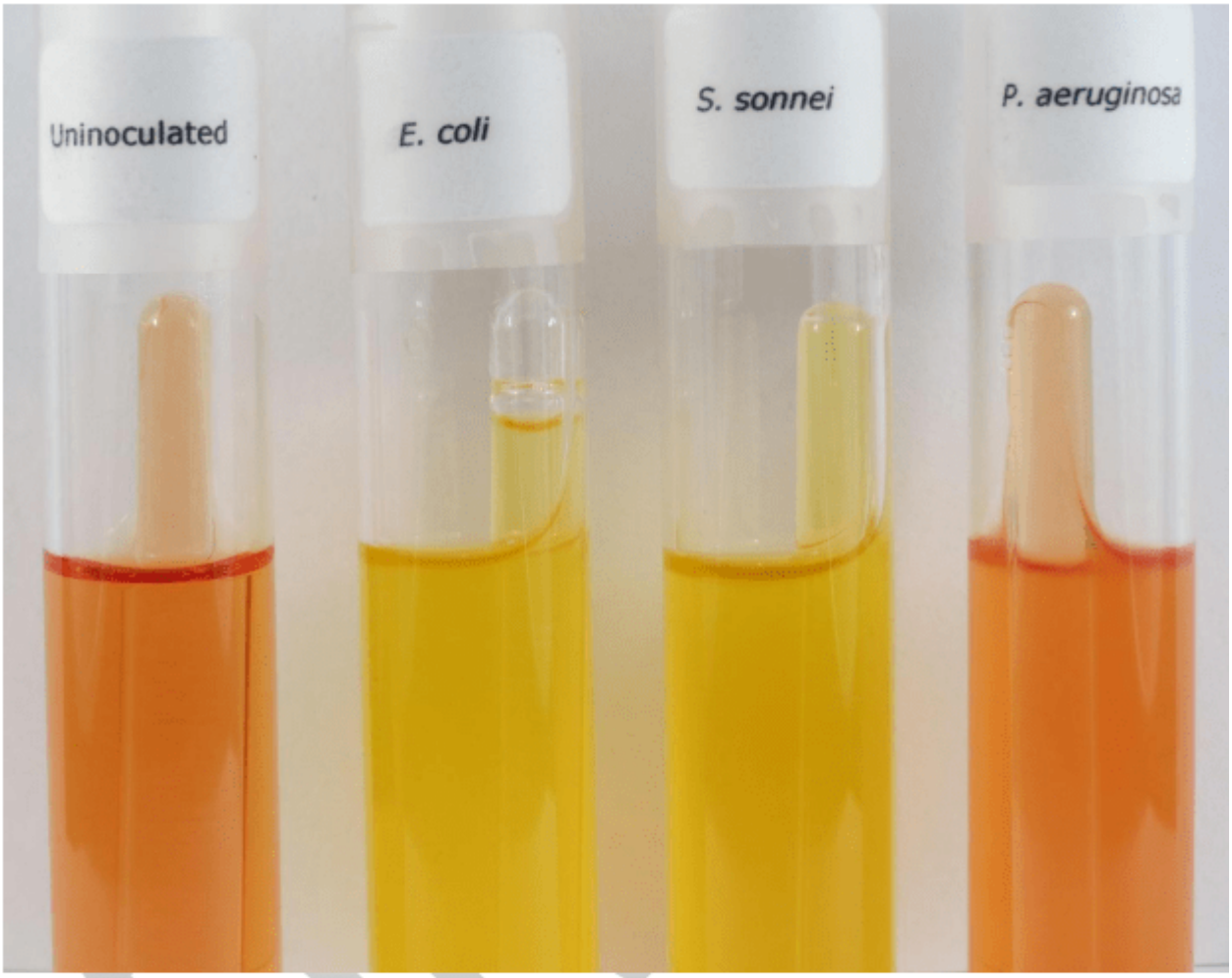


Figure 1. Peptone media with phenol red indicator. From left to right: Uninoculated tube; glucose fermenter with gas production (visible air bubble in the inverted Durham tube); glucose fermenter without gas production (no visible air bubble in the inverted Durham tube); non-fermenter.

1. Acid production:

1. Positive: After incubation the liquid in the tube turns yellow *(indicated by the change in the color of the phenol red indicator).* It indicates that there is drop in the pH because of the production of the acid by the fermentation of the carbohydrate (sugar) present in the media. NOTE:**If you are using other pH indicators please refer to Table 1 for their corresponding colors in particular pH.*

2. Negative: The tube containing medium will remain red, indicating the bacteria cannot ferment that particular carbohydrate source present in the media.

2. Gas Production

1. Positive: A bubble (small or big depending up the amount of gas produced) will be seen in the inverted Durham tube.
2. Negative: There won't be any bubble in the inverted Durham tube i.e. bacteria does not produce gas from the fermentation of that particular carbohydrate present in the media i.e. anaerogenic organism.

2. Starch and protein hydrolysis.

The purpose of experiment is to observe amylase enzyme in different environment and detect of each environment by helping colour changes. Enzymes are biological molecules that catalyze many different chemical reactions. With few exceptions, all enzymes are proteins and each enzyme is specific to a certain chemical reaction. Enzymes must maintain a specific three dimensional structure in order to function properly. If an enzyme's structure is altered (by heat or harsh chemicals) it may not function at all. This breakdown (denaturation) of an enzyme's structure may be fatal

Amylase Enzyme

Amylase, which is commonly found in saliva and germinating seeds. It catalyzes the breakdown of starch. When amylase reacts with starch, it cuts off the disaccharide maltose (two glucose molecules linked together). As the reaction progresses, less starch will be present and more sugar (maltose) will be present.The activity of amylase can be observed by using iodine.Because iodine reacts with starch to form a dark brown/purple color. As amylase breaks down starch, less and less starch will be present and the color of the solution (if iodine is added) will become lighter and lighter. The color change was observed using spot-plates as illustrated on the diagram below.

Amylase activity was observed under four different treatments:

effect of temperature

effect of pH

effect of substrate concentration

effect of enzyme concentration

The Effects Of Temperature

Amylase is an important metabolic enzyme. Its function is to catalyze the hydrolysis of starch into glucose. At high temperatures, Amylase becomes denatured, denatured amylase no longer catalyzes the hydrolysis of starch into glucose.

EFFECT OF pH:

Based on these results, what is the optimal pH for amylase? Is this optimal pH considered acidic, basic/alkaline, or neutral? Why does the activity decrease when the pH is too low or too high?

APPARATUS:-

-Starch

-Amylase Enzyme

-KH2P04

-Na2HP04

-HCI

-Heater

-Beaker

-Falcon tube

-Spectrophotometer

-Iodine

PROCEDURE:-

1.0.27 g KH2P04 buffer solution PH 5 was prepared with 20ml

2.0.27g KH2P04 PH6 was prepared with 20ml

3.0.27g KH2P04 PH7 was prepared with 100ml

4.0.282g Na2HPO4 PH8 was prepared with 20ml

5.0.282g Na2HP04 PH9 was prepared with 20ml

6.20g Starch was also prepared with 50ml cold water

7. To test amylase activity with PH difference, 5ml starch, 5ml buffer (PH5, 6,7,8,9 is used each) and 1ml amylase were mixed each other.

8.10min later, 0.5ml prepared sample was put into 5ml HCI.

9. At 620nm, the results were measured at spectrophotometer.

10. Second part temperature effect, 5ml starch, 5ml PH7 buffer and 1ml amylase were mixed.

11. Prepared sample was put into different temperature 30, 50, 70 and 90C.

12.10 min later, 5ml HCI was put into 0.5 ml prepared sample.

13.2-3 min later, 5ml iodine was added into 0.5ml new sample

14. Absorbance of each was measured at spectrophotometer.

OBSERVATIONS:-

In this experiment, we tried to create different environment to examine amylase enzyme activity. The environment differences could be provided by PH differences. Therefore we prepared different medium also different pHs.K2.The graph was gained foam our results. One of them is a graph that related to amylase activity at different PH. The other one is real ted to amylase activity at different temperatures at constant PH. With K2HPO4 PH 5.6and 7 were

prepared and with Na2PO4 8and 9.Each preparation procedure was applied.5ml starch, 5ml buffer,1ml amylase were added each other and then waited 10 min.after 10min,5ml HCI was added into 0.5 ml sample mixture. In a same way, the mixture for temperature observation was prepared pH 7.And added iodine to end of procedure. Absorbance results were taken from spectrophotometry. This measurement was at 620nm.

pH buffer sample with amylase
According to the results,
The smallest one can be think as a best one. How much enzyme is used is more essential point. If it is less one ,it means starch cannot be used adequately. High starch amount means that complex amount is also high. The opposite one shows best activity amylase at smallest concentration. The colour is more light, smaller absorbance could be think as best amylase activity.
Temperature sample with amylase
At 30C the colour is slightly orange.
At 50C the colour is extra light like iodine colour.
At 70C the colour is slightly purple.

At 90C the colour is more purple than at 30C one like orange-purple.At constant PH ,the small concentration ,at 50C.Because small absorbance formed by small complex.It means that amount of starch was decreased also.Best activity is 50C at constant PH.

RESULTS

Our aim is to be related to activity of amylase.To detect it, we prepared different PH from KHP04 and Na2HP04 by adding acid or base. Usage both of them is related to interval of buffer.After preparation buffer,we measure absorbance at spectrophotometry.At different PH absorbance give also different concentration.If amylase enzyme concentration with sample is small, it means enzyme is used complex is more small so activity of ezyme is best one in there.At different PHs ,smallest concentration is at PH 7.And then we did second part of experiment by using PH7.The chosen of PH7 is related to observation best amylase activity at first part.At PH7 we took sample with amylase enzyme concentration at different PHs.The smallest concentration is at 50C in second part.The concentration is 0.006.The colour is more light like iodine colour.Starch is used with amylase and therefore complex colour is more light also.The amylase enzyme activity is best one at 50C.This measurement is done at 620nm.

DISCUSSION AND CONCLUSION

Why is measured at 620nm ? Why HCI is used for preparation ? What does Light color mean?How does more heat affect rxn? During experiment ,we want to distinct purpose of experiment by answering these question.In this experiment,we related to effect of different buffer and temperature.We prepared buffers at different PH.KH2P04 was prepared for PH 5 ,6 ,7and Na2HP04 for 8and 9.In first part , at constant temperature (room temperature) sample with amylase concentration was measured.At PH 7,we measured the smallest

one.Small concentration means less complex less starch and enzyme is used enzyme activity is high.Our result from measurement at PH 7 is 0.026.As a second part ,constant PH,temperature was changed and then observed the effect of it.At 50 C ,smallest absorbance (0.0060)was found and the colour was extra light.It means more less complex there.In this experiment ,iodine is used to detect starch molecules by observing color change.Iodine and starch were combined and then formed complex.The another point is why HCI is used.The acid stops the enzymatic reaction and iodine reacts with starch to produce blue color.Activity of enzyme is also essential.It can be used for denaturation detection.Starch reacts with iodine which is yellow to form blue compound Amax=620nm.The intensity of the blue color can be quantified spectrophotometrically by measuring its absorbance at 620nm.

3.Microbial assay of streptomycin (agar cup, disk and turbidity method).

Antibiotic Assay Medium No. 5 (Streptomycin Assay Agar w/

Yeast Extract)

Intended use

M006

Antibiotic Assay Medium No.5 (Streptomycin Assay Agar w/Yeast extract) is used for microbiological assay of Dihydrostreptomycin, Framycetin and Kanamycin B using Bacillus subtilis

Composition**

Ingredients Gms / Litre

Peptone 6.000

HM Peptone B # 1.500

Yeast extract 3.000

Agar 15.000

Final pH (at 25°C) 7.9±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 25.50 grams in 1000 ml purified /distilled water. Heat to boiling to dissolve the medium completely. Sterilize by

autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates. Advice:

Recommended for the Microbiological assay of Dihydrostreptomycin, Framycetin, Kanamycin

Principle And Interpretation

This medium is commonly used for assaying Streptomycin by cylinder plate method using Bacillus subtilis as test organism.

This method is used in the assay of commercial preparations of antibiotics, as well as for antibiotics in body fluids, feeds

etc. Medium composition is in accordance to the specifications detailed in the FDA (5) and numerically identical to the

name assigned by Grove and Randall (1).

Peptone, yeast and HM Peptone B provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins

and other necessary growth nutrients for the test organism like Bacillus subtilis.The medium provides solidified

substratum for growth of organims. The pH-7.9 maintained in this medium- provides optimum growth conditions for

Bacillus subtilis (4). This medium is used to prepare the base as well as seed layer in the microbiological assay of

antibiotics such as Dihydrostreptomycin, Framycetin and Kanamycin B.

To perform the antibiotic assay the Base Agar should be prepared on the same day as the test. For the cylinder method, a base

layer of 21 ml is required. Once the base medium has solidified, seed layer inoculated with the standardized test culture can

be overlaid. Even distribution of the layer is important.

Type of specimen

Pharmaceutical preparations

For pharmaceutical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Specimen Collection and Handling:

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection.

Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per

established guidelines should be followed while handling specimens. Safety guidelines may be referred in

individual safety data sheets.

Limitations :

1. Freshly prepared plates must be used or it may result in erroneous results.

HiMedia Laboratories Technical Data

Organism Inoculum

(CFU)

Growth Recovery Antibiotics

assayed

Bacillus subtilis subsp.

spizizenii ATCC 6633

(00003*)

50-100 good-luxuriant >=70% Dihydrostreptomycin, Framycetin, Kanamycin B

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Medium amber coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 2.55% w/v aqueous solution at 25°C. pH : 7.9±0.2

pH

7.70-8.10

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Storage and Shelf Life Store between 10-30°C in a tightly closed container and use freshly prepared medium. Use before expiry date on the

label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.

Product performance is best if used within stated expiry period.

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must

be decontaminated and disposed of in accordance with current laboratory techniques (2,3). Disposal

6. Study of microbial growth curve.

Objectives:

1. To study the different phases of bacterial growth.
2. To plot standard growth curve of *Staphylococcus aureus.*
3. To determine the generation time of given bacteria.

Principle:

The increase in the cell size and cell mass during the development of an organism is termed as growth. It is the unique characteristics of all organisms. The organism must require certain basic parameters for their energy generation and cellular biosynthesis. The growth of the organism is affected by both physical and Nutritional factors. The physical factors include the pH, temperature, Osmotic pressure, Hydrostatic pressure, and Moisture content of the medium in which the organism is growing. The nutritional factors include the amount of Carbon, nitrogen, Sulphur, phosphorous, and other trace elements provided in the growth medium. Bacteria are unicellular (single cell) organisms. When the bacteria reach a certain size, they divide by binary fission, in which the one cell divides into two, two into four and continue the process in a geometric fashion. The bacterium is then known to be in an actively growing phase. To study the bacterial growth population, the viable cells of the bacterium should be inoculated on to the sterile broth and incubated under optimal growth conditions. The bacterium starts utilising the components of the media and it will increase in its size and cellular mass. The dynamics of the bacterial growth can be studied by plotting the cell growth (absorbance) versus the incubation time or log of cell number versus time. The curve thus obtained is a sigmoid curve and is known as a standard growth curve. The increase in the cell mass of the organism is measured by using the Spectrophotometer. The Spectrophotometer measures the turbidity or Optical density which is the measure of the amount of light absorbed by a bacterial suspension. The degree of turbidity in the broth culture is directly related to the number of microorganism present, either viable or dead cells, and is a convenient and rapid method of measuring cell growth rate of an organism. Thus the increasing the turbidity of the broth medium indicates increase of the microbial cell mass (Fig 1) .The amount of transmitted light through turbid broth decreases with subsequent increase in the absorbance value.

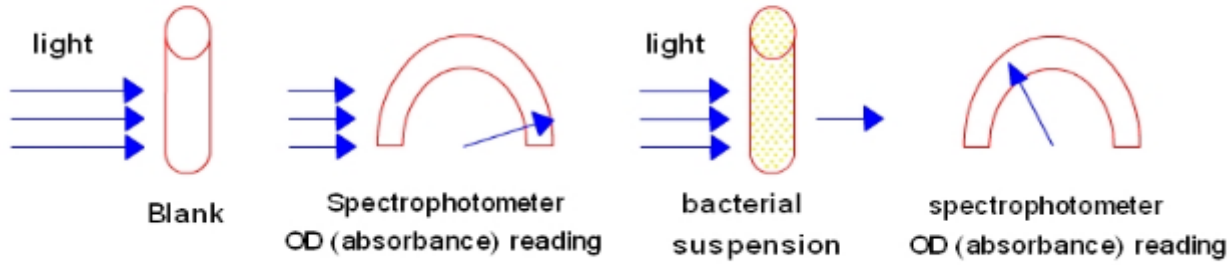


Fig 1: Absorbance reading of bacterial suspension

The growth curve has four distinct phases (Fig 2)

1. Lag phase

When a microorganism is introduced into the fresh medium, it takes some time to adjust with the new environment. This phase is termed as Lag phase, in which cellular metabolism is accelerated, cells are increasing in size, but the bacteria are not able to replicate and therefore no increase in cell mass. The length of the lag phase depends directly on the previous growth condition of the organism. When the microorganism growing in a rich medium is inoculated into nutritionally poor medium, the organism will take more time to adapt with the new environment. The organism will start synthesising the necessary proteins, co-enzymes and vitamins needed for their growth and hence there will be a subsequent increase in the lag phase. Similarly when an organism from a nutritionally poor medium is added to a nutritionally rich medium, the organism can easily adapt to the environment, it can start the cell division without any delay, and therefore will have less lag phase it may be absent.

2. Exponential or Logarithmic (log) phase

During this phase, the microorganisms are in a rapidly growing and dividing state. Their metabolic activity increases and the organism begin the DNA replication by binary fission at a constant rate. The growth medium is exploited at the maximal rate, the culture reaches the maximum growth rate and the number of bacteria increases logarithmically (exponentially) and finally the single cell divide into two, which replicate into four, eight, sixteen, thirty two and so on (That is 2^0, 2^1, 2^2, 2^3.....2^n, n is the number of generations) This will result in a balanced growth. The time taken by the bacteria to double in number during a specified time period is known as the generation time. The generation time tends to vary with different organisms. *E.coli* divides in every 20 minutes, hence its generation time is 20 minutes, and for *Staphylococcus aureus* it is 30 minutes.

3. Stationary phase

As the bacterial population continues to grow, all the nutrients in the growth medium are used up by the microorganism for their rapid multiplication. This result in the accumulation of waste materials, toxic metabolites and inhibitory compounds such as antibiotics in the medium. This shifts the conditions of the medium such as pH and temperature, thereby creating an unfavourable environment for the bacterial growth. The reproduction rate will slow down, the cells undergoing division is equal to the number of cell death, and finally bacterium stops its division completely. The cell number is not increased and thus the growth rate is stabilised. If a cell taken from the stationary phase is introduced into a fresh medium, the cell can easily move on the exponential phase and is able to perform its metabolic activities as usual.

4. Decline or Death phase

The depletion of nutrients and the subsequent accumulation of metabolic waste products and other toxic materials in the media will facilitates the bacterium to move on to the Death phase. During this, the bacterium completely loses its ability to reproduce. Individual bacteria begin to die due to the unfavourable conditions and the death is rapid and at uniform rate. The number of dead cells exceeds the number of live cells. Some organisms which can resist this condition can survive in the environment by producing endospores.

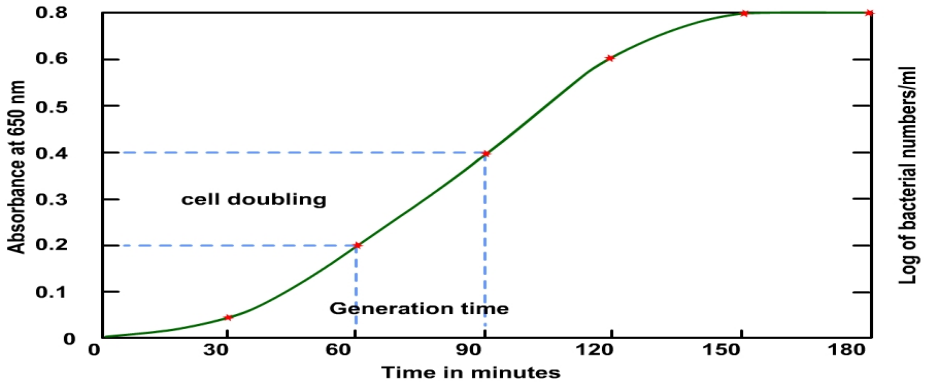


Fig2: Different phases of growth of a bacteria

CALCULATION:

The generation time can be calculated from the growth curve(Fig 3).

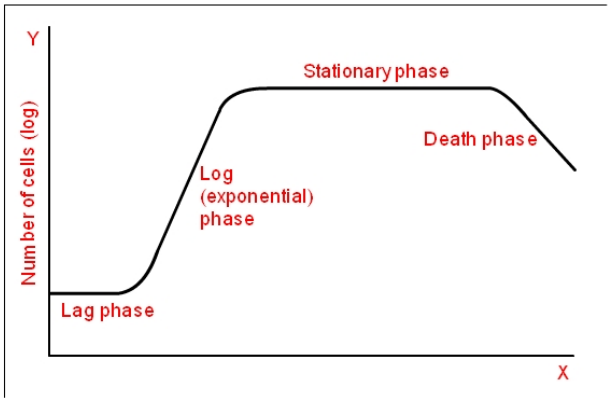


Fig 3: Calculation of generation time

The exactly doubled points from the absorbance readings were taken and, the points were extrapolated to meet the respective time axis.

Generation Time = (Time in minutes to obtain the absorbance 0.4) – (Time in minutes to obtain the absorbance 0.2)

= 90-60

= 30 minutes

Let No = the initial population number

Nt = population at time t

N = the number of generations in time t

Therefore,

$$Nt = No \times 2^n \ldots\ldots\ldots (1)$$

$$\log Nt = \log No + n \log 2$$

Therefore,

$$n = (\log Nt - \log No) / \log 2$$

$$n = (\log Nt - \log No) / 0.301 \ldots\ldots\ldots (2)$$

The growth rate can be expressed in terms of mean growth rate constant (k), the number of generations per unit time.

$$k = n / t$$

$$k = (\log Nt - \log No) / (0.301 \times t) \ldots\ldots\ldots\ldots (3)$$

Mean generation time or mean doubling time (g), is the time taken to double its size.

Therefore,
$Nt = 2No$.....(4)

Substituting equation 4 in equation 3

$$k = (\log Nt - \log No) / (0.301 \times t)$$
$$= (\log 2N0 - \log No) / (0.301 \times t)$$
$$= \log 2 + (\log No - \log No) / 0.301\ g$$

(Since the population doubles t= g)

Therefore,
$k = 1/g$

Mean growth rate constant, $k = 1/g$ Mean generation time, $g = 1/k$

6.Estimation of protein, sugar, DNA and RNA.

Determination of RNA:DNA Ratio in Bovine Liver Tissue

Introduction:

Concentration determination is a valuable measurement in the biological sciences. In particular,
nucleic acid concentration, although it may seem relatively small compared to the macroscopic
world, is worth measuring. Nucleic acids are polymers composed of nucleotides, which in living
organisms are based on one of two sugars, ribose or deoxyribose, yielding the terms ribonucleic
acid (RNA) or deoxyribonucleic acid (DNA) (Karp, 2013). However, isolating the nucleic acids
from the rest of the cells is the first obstacle. In 1957 W.C. Schneider developed a method for the
determination of nucleic acid inside cells (Bregman, 2002). First, the chilled tissue must be well
mixed so there is disruption at the cellular level also known as homogenization. The specific type
of homogenization used for this experiment was by physically shearing the chilled tissue sample
with a chilled Waring Blender to reduce the sample size (Burden, 2008). Next, the desired
nucleic acids must be extracted from the homogenized sample using trichloroacetic acid (TCA)
and ethanol. Cold TCA precipitates the nucleic acids, proteins and lipids from the rest of the
homogenized sample. This precipitate containing the desired nucleic acids can be separated from

the acid soluble supernatant by centrifugation in which the pellet contains the unpurified desired product (Bregman , 2002). For purification, ethanol which is a polar solvent is used to dissolve the polar fats. Finally, Schneider's method requires the use of hot TCA to dissolve out and partially degrade the DNA and RNA into solution.

Once the nucleic acid containing solution is obtained, specific nucleic acids, DNA or RNA can be colored so the compounds absorb light. Two colorimetric reactions were necessary for this experiment. The Diphenylamine reaction uses the diphenylamine reagent that contains acetic and sulfuric acids to cleave the DNA at some of the phosphodiester bonds and hydrolyze the glycosidic linkages between the sugars and purines when the DNA is heated. The sugars of the DNA, 2-Deoxyribose, are then converted to ω-hydroxylevulinyl aldehyde by the acid to react with the diphenylamine to produce the blue colored compounds that absorbs visible light. The intensity of the blue solution depends on the concentration of the DNA in solution; larger DNA concentrations correspond to more intense blue colored solutions. The other reaction uses orcinol reagent that contains hydrochloric acid to cleave some of the phophodiester bonds and hydrolyze the glycosidic linkages between the sugars and purines of RNA. The acid also converts sugars of the RNA, ribose, to furfural that can react with the orcinol in the presence of Iron (III) ions to produce green colored compounds in solution. The major difference between the two reactions is that the orcinol reaction colors all five membered sugar rings including the RNA rings and some of the DNA rings. 10% of the intensity of the green colored solution is caused by the DNA concentration (Bregman , 2002). The color reactions were also performed on known concentrations of pure DNA and pure RNA to set up a colorimetric assay in which there are solutions prepared with increasing concentrations.

Once isolated, the extracted, purified, and colored nucleic acid samples of DNA and RNA are prepared for a light absorption reading. The study of electromagnetic radiation emitted or absorbed by a given chemical species that can be used for quantitative analysis is known as spectroscopy (Zumdahl, 2010). Of the variety of spectroscopic techniques, this experiment used a spectrophotometer to measure the amount of visible light absorbed by the colored DNA and RNA samples of nucleic acid extract from a bovine liver sample. The spectrophotometer used in this experiment is an instrument that spreads light from a tungsten lamp into its component wavelengths between 340 and 1000nm due to a diffraction grating. The sample absorbs wavelengths of light and the light that is not absorbed strikes the phototube, which converts the transmitted light energy to an electric current that is read by a meter (Bregman, 2002).

To determine the concentration of the extract samples absorbance values were measured for known concentrations of pure DNA and pure RNA prepared solutions to be able to create a graph. This graph is known as a standard curve. The extracted sample absorption values could

then be superimposed on this standard curve to determine the unknown DNA and RNA concentrations from the extract. The ability to use this direct relationship graph between absorbance and concentration is due to the Beer-Lambert law that states that absorbance is directly proportional to solute concentration and to the length of the light path (Bregman, 2002).

Procedure:

Extraction of the Nucleic Acids

This experiment used 30g of frozen calf liver as the source of nucleic acids. This sample was homogenized by mixing the cubed sample in a chilled Waring Blender with 120mL of distilled water. Each lab group received 2.0mL of the liver homogenate.

5.0mL of cold 10% trichloroacetic acid (TCA) was added and mixed to the chilled homogenate.

The solution was centrifuged at high speed for 2 minutes and the supernatant was drawn off using a Pasteur pipet. The pellet remaining in the centrifuge tube was resuspended in 5.0mL of chilled 10% TCA and spun in the centrifuge for another 2 minutes. Again the supernatant was drawn off and discarded. Then 10mL of 95% ethanol was added to resuspend the pellet. The sample was centrifuged for 2 minutes, and the supernatant was decanted to obtain the desired pellet. This ethanol wash was performed twice for further purification. 5.0mL of 5% TCA was added to the tube to disperse the purified pellet and the tube was placed in a 90°C water bath for 15 minutes during which the tube was agitated three times. After the warm water bath, the tube was centrifuged for 2 minutes and the desired supernatant was decanted into a test tube. A final wash of the pellet with 5.0mL of 5% TCA was done to ensure all of the nucleic acids were decanted into the test tube.

Diphenylamine Reaction

To perform the colorimetric assay a diphenylamine reaction was done with the nucleic acid extract. The preparation of the five-sample assay required six test tubes to be set up as show in the table below.

Tube	[DNA] (µg/mL) in each standard	Volume (mL) of each DNA stock solution	Nucleic acid extract (mL)	5% TCA (mL)	Diphenylamine reagent (mL)
Blank	0	-	-	2.0	4.0
1	100	2.0	-	-	4.0
2	200	2.0	-	-	4.0
3	300	2.0	-	-	4.0
4	400	2.0	-	-	4.0
5	-	-	2.0	-	4.0

After all six tubes received the diphenylamine reagent dispensed from a buret, the tubes were inverted to mix the solutions. The tubes were placed in a boiling water bath for 10 minutes, cooled quickly, and then transferred to six cuvettes. The spectrophotometer was set to a wavelength of 600nm, the blank cuvette was put in the sample holder and the absorbance was adjusted to zero/100% Transmittance. The absorbance values of tubes 1-5 were measured and recorded as shown below.

Tube	[DNA] (µg/mL)	A_{600}
1	100	0.193
2	200	0.355
3	300	0.524
4	400	0.648
5 (nucleic acid extract)	unknown	0.123

Orcinol Reaction

To determine the unknown RNA concentration a separate colorimetric assay was done using the orcinol reaction. Again, six tubes were set up according to the table below.

Tube	[RNA] (µg/mL) in each standard	Volume (mL) of each RNA stock solution	Nucleic acid extract (mL)	5% TCA (mL)	Orcinol reagent (mL)
Blank	0	-	-	3.0	3.0
1	100	0.4	-	2.6	3.0
2	200	0.4	-	2.6	3.0
3	300	0.4	-	2.6	3.0
4	400	0.4	-	2.6	3.0
5	-	-	0.4	2.6	3.0

Once all tubes received 3.0mL of orcinol reagent dispensed from a buret, the tubes were inverted to mix. The tubes were placed in a boiling water bath for 20 minutes, cooled quickly, and transferred to six cuvettes. The wavelength was set to 660nm on the spectrophotometer, and the blank cuvette was placed in the sample holder so the absorbance was set to read A = 0 and 100% Transmittance. The absorbance values of tubes 1-5 were measured and recorded and shown below.

Tube	[RNA] (µg/mL)	A_{660}
1	100	0.126
2	200	0.263
3	300	0.382
4	400	0.514
5 (nucleic acid extract)	unknown	0.182

Results:

Measuring and recording the absorbance values for both colorimetric assays allowed the data to be organized and analyzed. Since all the stock solution concentrations that were added to tubes 1-4 were known and the corresponding absorbance values were measured a standard curve was created to visualize the data. The standard curve plots absorbance versus known concentrations

of the pure substance because the Beer-Lambert law states that absorbance is directly proportional to solute concentration and to the length of the light path, which in this case was held constant (Bregman, 2002).

The standard curve for the diphenylamine reaction using the known DNA stock solutions is shown below. This curve abides by the Beer-Lambert law and displays a direct relationship between the concentration and absorbance. As the concentration of the DNA increased the absorbance value increased, resulting in a positively sloped graph where the slope equaled 1.7×10-3 cm2/µg, which represents the molar absorption coefficient (α) in the Beer-Lambert law.

An important note is that on this plot the point of zero DNA concentration and zero absorbance,

the blank cuvette's DNA concentration and absorbance, was graphed. The line on the graph is a

line of best fit starting at the origin. From this graph it was found that the concentration of the previously unknown DNA extract was 72.35µg/mL. To determine this value the slope equation

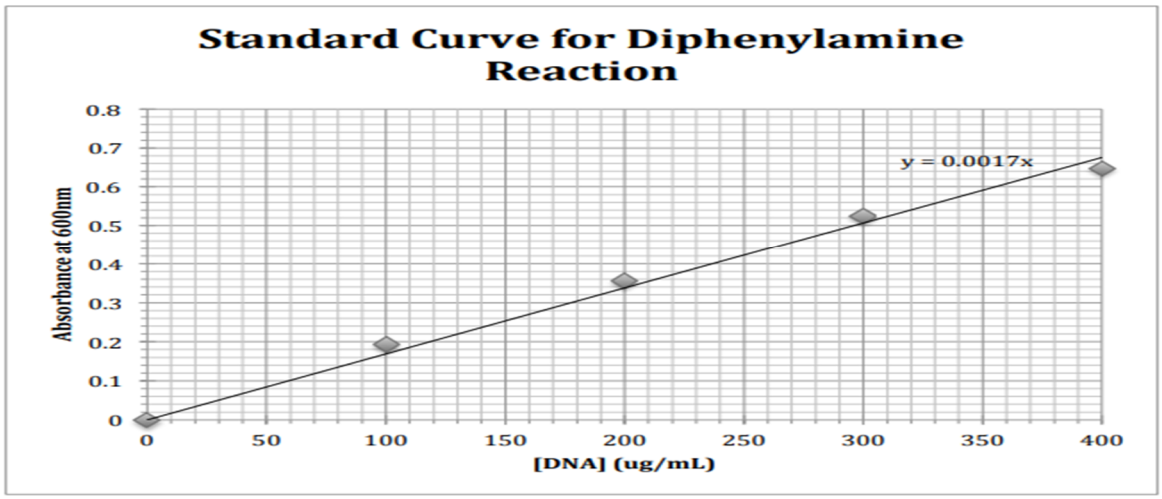
on the graph was used to calculate the concentration at an absorbance of 0.123. Graphically to

find the concentration the absorbance value of 0.123 was located on the graph and then moving

horizontally from that point until the line of best fit was hit. From the line of best fit, a vertical

line downward was followed to the DNA concentration axis to read the extracted DNA concentration. Using the mass and volume from the homogenization of 30g bovine liver and 120mL distilled water the concentration of DNA in the original bovine liver was calculated to be

0.29µg of DNA per milligram of bovine liver.



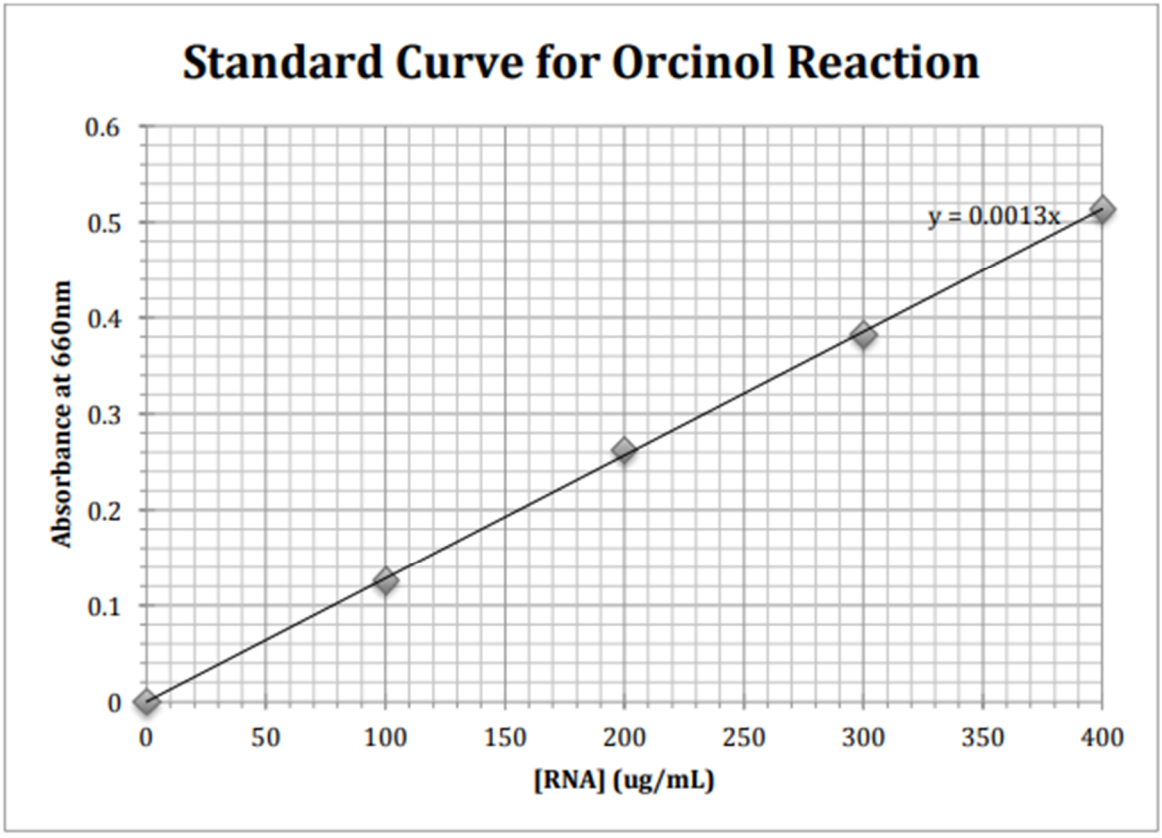
The standard curve for the orcinol reaction that colored the RNA is also shown below. This positively sloped data plot of absorbance versus the known RNA concentrations also supports the direct relationship of these two variables of the Beer-Lambert law. The molar absorbance coefficient for the orcinol reaction was 1.3×10-3 cm2/µg. On this graph, too, the point of zero absorbance and zero RNA concentration was plotted and a line of best fit was produced to go through the origin. The process of determining the unknown RNA concentration of the nucleic

acid extract for the orcinol reaction graph was different from the Diphenylamine reaction

standard curve. The reason being is that there is a slight color contribution from the DNA concentration of the extract that was measured in the unknown "RNA" absorbance value (Bregman, 2002). Ten percent of the recorded absorbance of the unknown extracted RNA concentration is actually an absorbance value from the extracted DNA concentration.

Reaction

determine the absorbance of just the unknown RNA from the extract, 10% of 0.097, the absorbance at the concentration of DNA in the extract, was subtracted from the nucleic acid extract absorbance value 0.182 (absorbance value from the orcinol reaction). This computed the
unknown RNA absorbance to be 0.172. Using the slope equation on the graph the unknown RNA concentration was found to be 132.54µg/mL. The ratio of RNA concentration to DNA concentration from the extract was 1.83. Initially the RNA concentration was 0.53µg of RNA per
milligram of bovine liver.



Discussion

One of the simplest and most widely used methods to determine the amount of protein or nucleic
acid present in a given solution is to measure the amount of light of a specific wavelength that is
absorbed by that solution (Karp, 2013). A spectrophotometer was the instrument used during this
experiment to measure the absorbance values of concentrated, colored solutions of nucleic acids

Standard Curve for Orcinol Reaction from a bovine liver sample. However, in order to measure the amount of light absorbed by DNA and RNA extracts the nucleic acids were isolated and purified from the rest of the liver tissue. Using known concentrated stock solutions of DNA and RNA the unknown concentrations of the extract were determined by colorimetry, a procedure in which the concentration of a compound is measured by the intensity of the color produced when the appropriate color reagents were added (Bregman, 2002). The blank cuvette used as a part of the colorimetric assay served to adjust the spectrophotometer prior to absorbance readings. This blank cuvette contained all of the same substances in the sample except the nucleic acids in order to set the instrument to compensate for the absorbance of the solvent. Therefore, the absorbance values recorded are indeed the amount of light the nucleic acids in solution absorbed.

The results from the Diphenylamine and Orcinol reactions that were organized into the standard curves above support the Beer-Lambert law. The direct relationship between the absorbance values and the concentration of nucleic acids resulted in a positive sloped plot because with more moles of nucleic acids in the 6mL of sample solution the more photons of light the sample could absorb. This result corresponds to a previous experiment done in the Cellular Biology laboratory at SUNY Cortland in which an absorption spectrum was created of different concentrations of erythrosine (Parsons and Schapiro, 1975). The higher concentrated sample had an overall larger curve than the less concentrated sample especially at the absorption maximum, the wavelength at which the absorption spectrum exhibits a peak (Bregman, 2002).

The unknown DNA extract concentration value that was determined, 72.35µg/mL and the absorbance value of 0.123 qualitatively correlated well with the other known DNA stock concentrations and those absorbance values. When preparing the colorimetric assay it was noted that the blue color intensity observably increased as the concentration of the DNA increased and the unknown DNA concentration sample color was the least intense blue color.

The unknown RNA extract concentration value, which was greater than the DNA concentration of the extract, was 132.54µg/mL. The ratio between this RNA and DNA is 1.82, indicating that RNA has a higher concentration in bovine liver tissue cells than DNA concentration. These results are consistent with other experimental results regarding the concentration of nucleic acids in liver tissue. Musafa and Mittal performed a similar experiment in 1981 that measured the weights of proteins and the concentrations of RNA and DNA in the livers and brains of catfish to observe how nutrition influenced these measurements. In fact this lab used the same RNA extraction technique of Schneider. It was found that with normal feeding the concentration of RNA in the liver was 437.4µg/100mg, the concentration of DNA was 93.8µg/100mg, and the

protein concentration was 17.6mg/100mg. This produced a 4.660 RNA/DNA ratio in the liver. In
the brain the concentration of RNA was 113.8µg/100mg, the concentration of DNA was 44.5µg/100mg, and the protein concentration was 8.8mg/100mg. This ratio in the brain of RNA/DNA was 2.552. One key point made was that high RNA/DNA ratios correspond with actively synthesizing and accumulating protein individuals (Musafa and Mittal, 1982). Thus, the
liver is more involved in protein synthesis than other tissues of a body such as the brain. This explains the high ratio because it is the RNA strands of nucleic acids (mRNA, rRNA, and tRNA)
that are more directly involved in protein synthesis (Karp, 2013).
A corresponding experiment that would be a logical follow up to the bovine liver experiment would be one in which other tissues of a calf were tested. It would be interesting to extract, purify, color and create standard curves of absorbance versus concentration for other tissues of
the body. One could test where RNA concentration was larger or smaller and therefore where protein synthesis was more increased or decreased. Also, in these future experiments it would be
advantageous to measure the protein concentration as well just at Musafa and Mittal had done.
With a protein concentration one could directly make the connection that where there is a higher
RNA concentration there is a higher protein concentration because RNA is involved in synthesizing proteins.
Nucleic acids are one of the four macromolecules of biology that hold the genetic information that allow cells to function (Sternfeld, 2015). The concentrations of DNA and RNA that make up
nucleic acids do vary from tissue to tissue to allow cells to function properly and efficiently.

7. E. coli Plasmid DNA MiniPrep Protocol

Introduction

Isolation of plasmid from bacteria.

The isolation of plasmid DNA from E. coli using an alkaline lysis is a well-established method. E. coli with plasmid is cultured in media with antibiotics to a high cell density, harvested, and then lysed with a SDS/NaOH solution. Rapid acidification using concentrated potassium acetate causes the precipitation of protein and chromosomal DNA. Plasmid DNA, which is supercoiled, remains in solution and can be captured on a silica spin column. The plasmid DNA is washed with an ethanol solution and then eluted in water or TE buffer.

Material:-

Microfuge tubes
Resuspension buffer (50 mM Tris HCl, pH 8, 10 mM EDTA, 100 µg/ml RNase A)
Lysis buffer (0.2 N NaOH, 1% SDS)
Neutralization buffer (3/5 M Potassium acetate, pH 6)
Spin columns (Product Number SSC 100-01)
Isopropanol

Wash buffer (70% Ethanol)
Elution buffer (water or TE buffer- 10 mM Tris, pH 8, 1 mM EDTA)
Equipment
Vortexer
Centrifuge
Protocol

Culture E. coli with plasmid in LB media with antibiotic selective pressure, overnight on a shaker at 37°C.

Pellet 1.5 ml of bacterial culture in a microfuge tube by centrifuging for 2 minutes at 10,000 rpm.

Decant the supernatant and add 200 µl of the resuspension buffer. In order to resuspend the pellet you may have to vortex.

Add 250 µl of the lysis buffer, invert the tube 10 times to mix thoroughly. The solution should become clear and viscous.

Add 350 µl of the neutralization buffer, invert the tube 10 times or until a precipitate forms. The precipitate is a mixture of protein and chromosomal DNA.

Centrifuge the tube for 10 minutes at 10,000 rpm. Transfer the supernatant to a microfuge tube and add 0.7 isopropanol. Incubate at -20°C for 15 minutes.

Transfer the solution to a spin column.

Centrifuge the spin column for 1 minute at 7,000 rpm. Discard the flow through.

Add 400 µl of the wash buffer and centrifuge for 1 minute at 7,000 rpm. Discard the flow through. Repeat this step.

Centrifuge for an additional 2 minutes at 10,000 rpm to remove residual wash buffer.

Transfer the column to a clean microfuge tube. Add 50 µl of elution buffer and centrifuge for 1 minute at 10,000 rpm.

Results:-

Sample Name	Concentration (ng/µl)	260/280	260/230
E. coli DH5α with pSVβ	1143.41	2.06	2.38

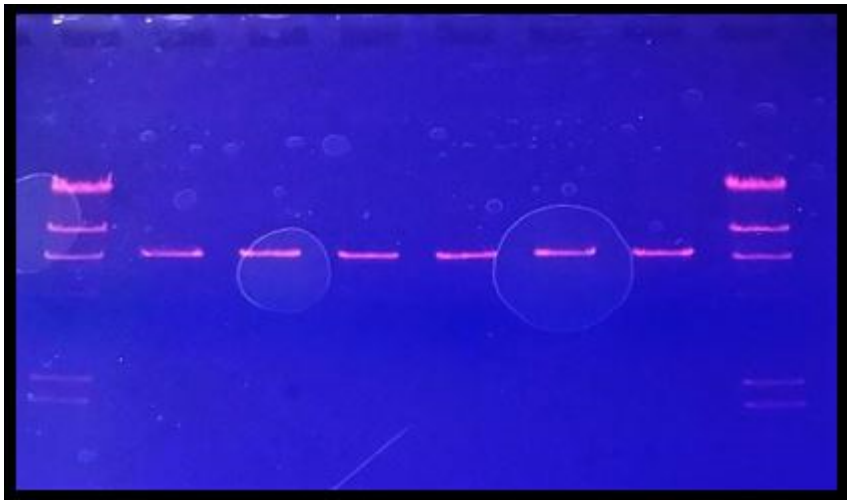


Figure 1: The isolated plasmid under went an enzymatic digest with HindIII (sourced from NEB) and was run on an Agarose Gel with Ethidium Bromide, along side two lanes of digested Lambda ladders

10. Plasmid DNA Extraction and Agarose Gel Electrophoresis

A. Plasmid DNA Extraction

Plasmids have been found to be wide distribution in bacteria. They are autonomously replicating extrachromosomal elements which are not essential for the growth of their host cells. However, they may encode a wide range of genetic products which may permit their host to adapt better to adverse conditions, for example, in the presence of antibiotics.

In cloning work, very often the recombinant plasmids have to be isolated from their transformed hosts in order to characterize by restriction analysis and sequencing. The information from these analyses provides a basis for the mode of their presentation to the transformants and the planning of future experiment for the recombinant molecules. Among the various methods available for the preparation of plasmid DNA for

rapid screening, a protocol involving the use of an alkaline solution to lyse the cells, salt precipitation to remove cell debris and chromosomal DNA and application to hibind DNA column to eliminate proteins and other contaminants has been widely employed. Gel electrophoresis, which is easily performed, rapid, inexpensive and reproducible, has become the most popular resolution technique in nucleic acid research. Gel electrophoresis using agarose, a highly purified linear polysaccharide derived from agar, has been widely used in the detection and characterization of plasmids, also the linear DNA fragments. Plasmids of sizes ranging from less than one kilo-base (kb) to over a few hundred kb can resolved by conventional agarose gel electrophoresis. Since all the DNA molecules have the same charge to mass ration, electrostatic

charge is not a factor in electrophoretic mobility. Different DNA molecules will nevertheless move through a gel at different rates on the basis of size and conformation. The electrophoretic mobility of a DNA species through a gel is described in the following equation:

Electrophoretic mobility= d/Et . where d is the distance travelled in cm. E is the electric field strength in V/cm and t is the time in seconds. The mobility of a DNA band is subject to alterations resulting

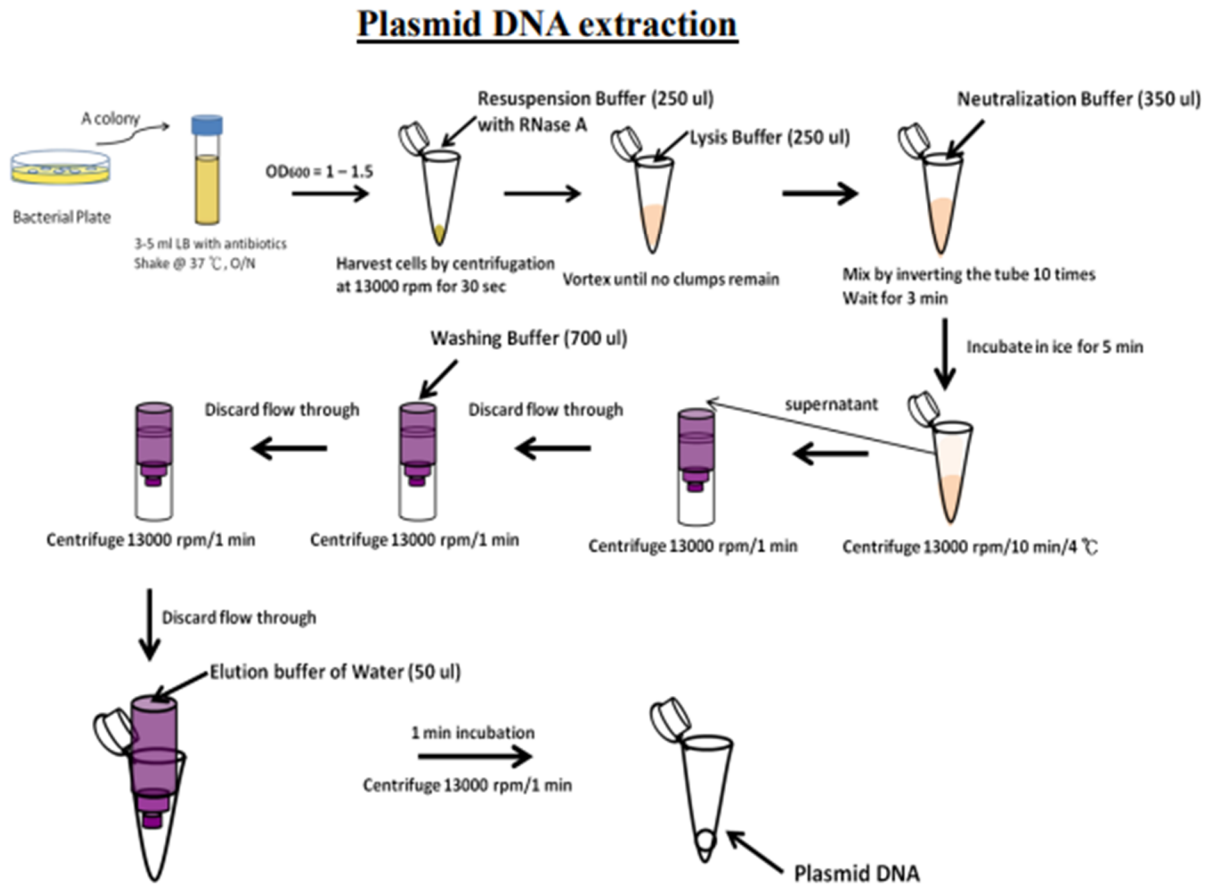
from variation in voltage supply, gel concentration, ionic strength, pH of the

electrophoresis buffer and temperature. Under the influence of an electric field, the motilities of different DNA species through a gel are inversely related to their respective molecular sizes. Therefore, those
with larger sized will move more slowly. If it so happens that two DNA species are of the same size, but different conformation, for example a covalently closed circular (CCC) species versus its open circular (OC) counterpart, agarose gel electrophoresis can still be used to separate them as CCC molecules are more compact, so less retarded than those of OC form.

Materials:-

Mini-prep plasmid DNA extraction kit
ddH2O (deionized, sterile, DNase-free)
DNA ladder (25bp/ 100bp/ 1kb)
6□/10□ DNA loading dye
DNA staining dye (SYBR Green/ Red Safe/ Gel Red…DO NOT use Ethidium Bromide)
Agarose gel (use DNA grade agarose)
TAE buffer (Tris-Acetate-EDTA buffer)
Gel imager: UV trans-illuminator
(Beware of UV damaging your skin, eye and your DNA samples)

Procedures



Plasmid DNA extraction

B. Agarose gel electrophoresis

1. Setting up an agarose gel:
1. For a small gel (the one used in our lab), add 20 ml 1□TAE buffer to a conical flask. (If there is none, dilute the 50□TAE buffer by 50 times.)
2. Then, add 0.2 g agarose (1%) to the conical flask and heat it by microwave oven by 30-45 s to dissolve it until it becomes a clear and transparent liquid.
3. Cool it down a little bit by running water for around 15 s.
4. Add about 1 µl (for 20 ml TAE) of DNA staining dye, red safe (20000□).
5. Pour the solution to the white tightened tank with gates to allow it to solidify. Add the gel comb so as to create wells for the gel. Wait >15-30 min until it is gel-like and ready to use.
2. Running agarose gel:
1. Orient the gel with wells (comb removed) facing the BLACK negative electrode. Check if the gel is covered by TAE buffer in the tank.
2. Add 6□/10□ loading dye to the DNA to a total volume of <25 µl (depended on the well) before adding to the wells. Mix loading dye to DNA to make the solution colored.
3. Load the sample to the wells (<25 µl/ well)
4. Add 3-5 µl DNA ladder to a separate well.
5. Connect the electrodes to the power supply with correct color, black to black, red to red. Apply power supply with 120 V. Check if there are bubbles on the negative electrodes.
6. Allow it to run for about ~30 min (the time is variable based on the gel concentration and the size of interested DNA. Be aware the samples run into the gel by checking if the blue band stays on the gel.
7. After electrophoresis for 30 min, disconnect power, take the gel to imager, and turn UV on to observe bands.
*REMINDER: Never run a gel with >200V, as the heat so generated can melt the gel and also easier to cause electric leakage. Range from 80 – 160 V is acceptable. Usually it needs at least 100 ng DNA for a band to be seen and visualized on the UV trans-illuminator.

11. Plate count of bacteria

Food Microbiology experiment 5 standard plate count method

OBJECTIVES:-

After attending to this experiment, we shall be able to:
• undertake enumeration of viable microorganisms by SPC;
• perform the technique of serial dilution;
• do pour plating; and
• count colonies using colony counter.

INTRODUCTION:-

Often it is necessary to know the number of bacteria in a specimen, for example, to ensure that water, milk or other foods are safe to consume. Enumerating microbial populations is also important for evaluating products such as antibiotics, vitamins, and preservatives. Several methods can be used to determine bacterial concentrations. These include direct counts, plate counts, filtration, and turbidimetric measurements.
The plate count is one of the most accurate means of enumeration of viable microbes because you get a visual indicator for every cell in the specimen.
The technique stems from Robert Koch's insight gained from viewing colonies

growing on the surface of a spoiling slice of potato. In practice, a small aliquot of a liquid suspension of microbes is spread on the surface of solidified nutrient medium, which when incubated, leads to each cell 'developing' into a visible colony through repeated fission.

The pour plate technique can be used to determine the number of microbes/ml or microbes/gram in a specimen. It has the advantage of not requiring previously prepared plates, and is often used to assay bacterial contamination of foodstuffs. Each colony represents a "colony forming unit" (CFU). For optimum accuracy of a count, the preferred range for total CFU/plate is between 30 to 300 colonies/plate.

PRINCIPLE:-

The number of bacteria in a given sample is usually too great to be counted directly. However, if the sample is serially diluted and then plated out on an agar surface in such a manner that single isolated bacteria form visible isolated colonies (as shown in Fig.5.1), the number of colonies can be used as a measure of the number of viable (living) cells in that known dilution.

However, keep in mind that if the organism normally forms multiple cell arrangements, such as chains, the colony-forming unit may consist of a chain of bacteria rather than a single bacterium. In addition, some of the bacteria may be clumped together. Therefore, when doing the plate count technique, we generally say we are determining the number of Colony-Forming Units (CFUs) in that known dilution. By extrapolation, this number can in turn be used to calculate the number of CFUs in the original sample.

Normally, the bacterial sample is diluted by factors of 10 and plated on agar. After incubation, the number of colonies on a dilution plate showing between 30 and 300 colonies are determined.

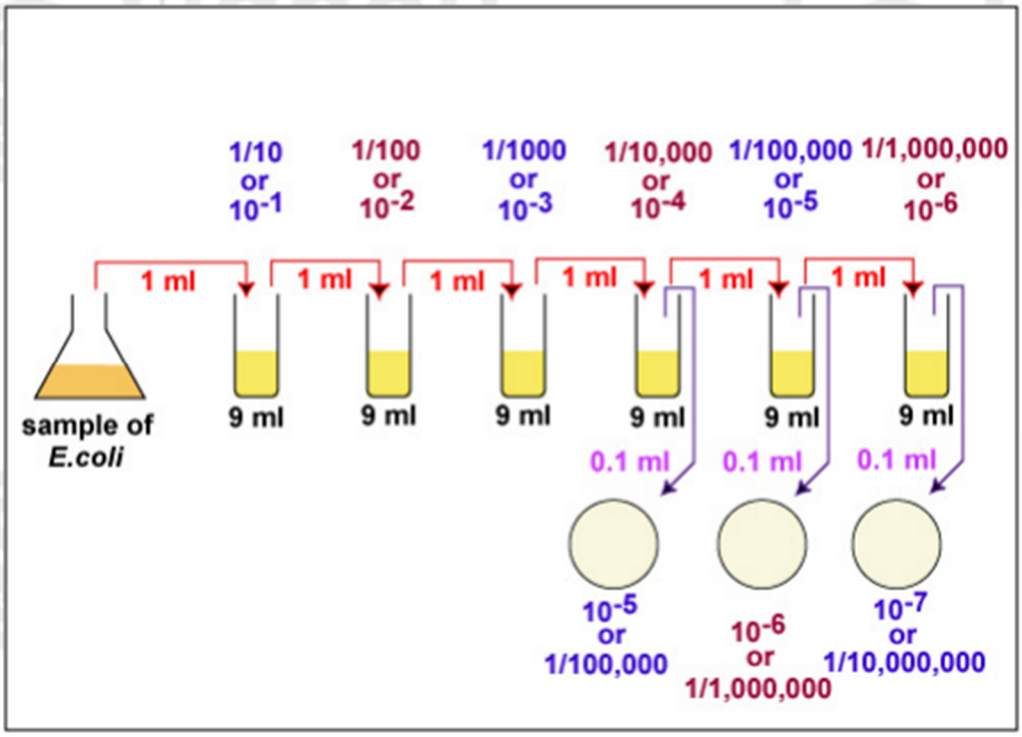


Fig. 5.1: Series of dilution used in SPC.

A plate having 30-300 colonies is chosen because this range is considered statistically significant. If there are less than 30 colonies on the plate, small

errors in dilution technique or the presence of a few contaminants will have a drastic effect on the final count. Likewise, if there are more than 300 colonies on the plate, there will be poor isolation and colonies will have grown together. Generally, one wants to determine the number of CFUs per milliliter (ml) of sample. To find this, the number of colonies (on a plate having 30-300 colonies) is multiplied by the number of times the original ml of bacteria was diluted (the dilution factor of the plate counted). For example, if a plate containing a 1/1,000,000 dilution of the original ml of sample shows 150 colonies, then 150 represents 1/1,000,000 the number of CFUs present in the original ml. Therefore the number of CFUs per ml in the original sample is found by multiplying 150 x 1,000,000 as shown in the formula below:

number of CFUs per ml of sample =
number of colonies × dilution factor of the plate counted
ml of sample plated

In the case of the example above,150 x 1,000,000 = 150,000,000 CFUs per ml. For a more accurate count it is advisable to plate each dilution in duplicate or triplicate and then find an average count.

One disadvantage of pour plates is that embedded colonies will be much smaller than those which happen to be on the surface, and must be carefully enumerated so that none are overlooked. Also, obligate aerobes may grow poorly if deeply embedded in the agar.

5.3 MATERIALS REQUIRED

Cultures: 18-24 hour old nutrient agar slant or nutrient broth cultures of E.coli

Reagents: Sterile Dilution blanks (containing 9ml of 0.9% NaCl2), Plate count agar (standard methods)

Equipment and glassware: Petri dishes, glass or plastic (at least 15 x 90 mm), Pipettes with pipette aids, Pipette and petri dish containers, Incubator, Colony counter, blender or stomacher (for food sample), autoclave

PROCEDURE

E. Coli Culture

1. Label the bottom of six petri plates 1-6. Label four tubes of saline 10-2, 10-4, 10 -6 and 10-8.
2. Using aseptic technique, the initial dilution is made by transferring 1 ml of E. coli sample to a 9ml sterile saline blank (Figure 5.1). This is a 1/100 or 10-2 dilution.
3. The 10-2 blank is then shaken by grasping the tube between the palms of both hands and rotating quickly to create a vortex. This serves to distribute the bacteria and break up any clumps.
4. Immediately after the 10-2 blank has been shaken, uncap it and aseptically transfer 1ml to a second 9ml saline blank. Since this is a 10-2 dilution, this second blank represents a 10-4 dilution of the original sample.
5. Shake the 10-4 blank vigorously and transfer 1ml to the third 9ml blank. This third blank represents a 10-6 dilution of the original sample. Repeat the process once more to produce a 10-8 dilution.
6. Shake the 10-4 blank again and aseptically transfer 1.0 ml to one petri plate and 0.1 ml to another petriplate. Do the same for the 10-6 and the 10-8 blanks.
7. Remove one agar pour tube from the 48 to 50o C water bath. Carefully remove the cover from the 10-4 petri plate and aseptically pour the agar

into it. The agar and sample are immediately mixed gently moving the plate in a figure-eight motion or a circular motion while it rests on the tabletop. Repeat this process for the remaining five plates.
8. After the pour plates have cooled and the agar has hardened, they are inverted and incubated at 37o
C for 24 hours.
9. At the end of the incubation period, select all of the petri plates containing between 30 and 300 colonies. Plates with more than 300 colonies cannot be counted and are designated too many to count (TMTC). Plates with fewer than 30 colonies are designated too few to count (TFTC) . Count the colonies on each plate. A Quebec colony counter should be used. Standard Plate Count Method
10. Calculate the number of bacteria (CFU) per milliliter or gram of sample by dividing the number of colonies by the dilution factor multiplied by the amount of specimen added to liquified agar.
number of bacteria per ml = number of colonies
dilution × amount plated
11. Record your results.

5.4.2 Food Samples

1. Using separate sterile pipettes, prepare decimal dilutions of 10-2, 10-3, 10-4, and others as appropriate, of food homogenate. (For food homogenate. Add 450 ml phosphate-buffered dilution water to blender jar or stomacher sterile bag containing 50 g analytical food sample and blend for 2 min. This results in a dilution of 10-1.)
2. Make dilutions of original homogenate promptly, using pipettes that deliver required volume accurately.
3. Prepare all decimal dilutions with 9 ml of sterile diluent plus 1 ml of previous dilution, unless otherwise specified, by transferring 1 ml of previous dilution to 9 ml of diluent.
4. Pipette 1 ml of each dilution into separate, duplicate, appropriately marked petri dishes.
5. Add 12-15 ml plate count agar (cooled to 45 ± 1°C) to each plate within 15 min of original dilution.. Pour agar and dilution water control plates for each series of samples.
6. Immediately mix sample dilutions and agar medium thoroughly and uniformly by alternate rotation and back-and-forth motion of plates on flat level surface.
7. Let agar solidify. Invert solidified petri dishes, and incubate promptly for 24 ± 2 h at 37°C.
8. Repeat step 9, 10 and 11 from culture procedure above.

5.5 OBSERVATIONS

1. Choose a plate that appears to have between 30 and 300 colonies.
2. Count the exact number of colonies on that plate using the colony counter (as demonstrated by your instructor).
3. Calculate the number of CFUs per ml of original sample as follows:
The number of CFUs per ml of sample = The number of colonies (30-300 plate) × ml of dilute sample plated
_____ = Number of colonies
_____ = Dilution factor of plate counted
_____ = Number of CFUs per ml

RESULTS:-

Record your results and find the average number of cfus/ ml by adding the results from all of your plates and dividing by the number of plates.

PRECAUTIONS:-

1. Avoid sampling foam.
2. Do not deliver less than 10% of total volume of pipette. For example, do not use pipette with capacity greater than 10 ml to deliver 1 ml volumes; for delivering 0.1 ml volumes, do not use pipette with capacity higher than 1.0 ml.

Isolation of Soil Microorganisms

A Project for Elementary Grades

Title : Isolation of soil microorganisms

Objective : To isolate and count the microorganisms found in a sample of soil by the dilution method using aseptic techniques.

Materials:

Erlenmeyer flask containing 50ml of sterile agar (0.1%)
cup containing 0.5g of soil
small vials containing 4.5ml of sterile agar (0.1%)
sterile 1ml pipettes
sterile glass stirring rod
Petri plates containing about 10ml of PDA (potato dextrose agar)
strips of Parafilm
paper towel disinfectant marking pens

Method:

Mark the dilutions on the vials (10-3

2.Add the 0.5g soil to the Erlenmeyer flask containing 50ml of agar (this is the 10-2 dilution).

3.Shake well for at least one minute.

4.Using a sterile pipette, take 0.5ml and place it in a vial containing 4.5ml of 0.1% agar. (this is the 10-3
dilution).

5.Shake well for at least one minute.

6.Repeat steps 4 and 5 for the three other dilutions (10-4

7.Starting with the weakest dilution (10-6), pipette 1ml onto each of 2 Petri plates containing PDA. Spread over the entire surface using the sterile glass stirring rod. (The 10-6
dilution first.) Follow the same steps for the 10-5 and 10-4 dilutions.

8.Seal the Petri plates with the Parafilm.

9.Incubate the Petri plates at room temperature.

10. Observe after 24, 48 et 72 hours.

Results:

Record the number of colonies/Petri plate/dilution.

Calculate the number of microorganisms per gram of soil using the following formula :# of colonies/plate ×
the dilution factor = # organisms / 1 gram of soil

Record the characteristics of each colony.

Take your time – you should have about an hour; the dilutions take around half an hour.

Ask students to wash their hands and take a seat before beginning the lesson.

Ask them to READ the instructions, and ask questions if anything is not clear.

If the students would like to practice pipetting, there are NON-STERILE pipettes available with water.

The vials and Petri plates are already labeled. Ask that the students write their initials on their Petri plates.

The first thing to do is to clean up their workplace with disinfectant, and work on a damp towel.

Remind them that once they have finished to collect their vials, etc., and place them in the sink. To clean

their work place if messy, and to return to the classroom. There, they may watch the demonstration

BOT 495A

ANGIOSPERM TAXONOMY & MOLECULAR SYSTEMATICS

(Practical)

Drawing and Description of a specimen from locally available representative families, identification up to species :

Study of taxonomic specimen –A

Specimen – Supplied from the college laboratory .

Habitat –Terrestrial .

Habit –Herbaceous .

VEGETATIVE PARTS

Root – Not supplied .

Stem –Erect , nodes and internodes are conspicuous ,quadrangular ,deep green in colour with swollen nodes , minute hair present ,inter-nodal length -1.5 cm.

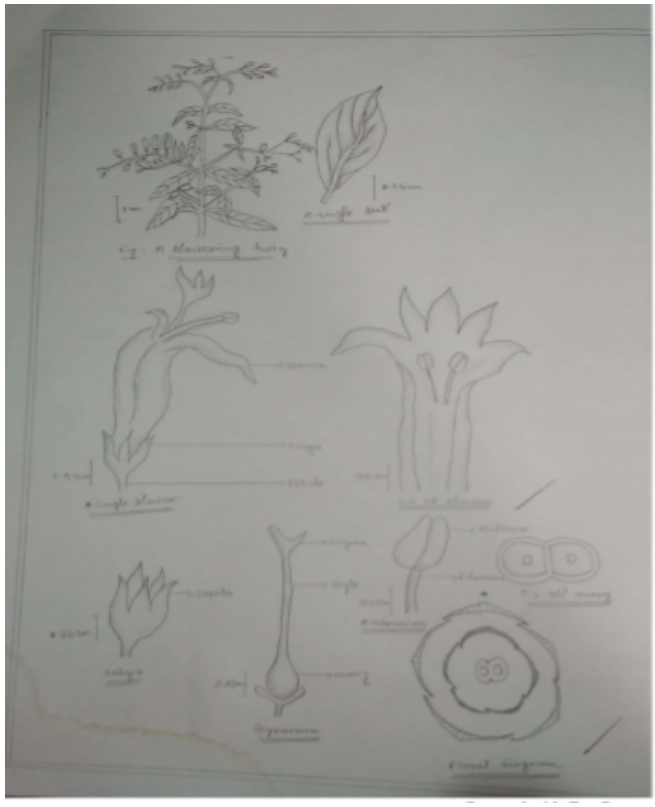
Leaf –Opposite decussate ,leaf margin entire ,leaf apex acute with equal leaf base ,lanceolate ,length of lamina 2.5 cm ,deep green in colour ,reticulate venation .

REPRODUCTIVE PARTS –·

Inflorescence – Recemose ,panicle .

- Flowers are pedicelled, biliped, white-purple or spotted purple and solitary. Pedicel is 2.5–10 mm in size, slender and glandular pubescent. Bracts are acicular and 2.5 mm long.
- Calyx - sepals 5,,gemosepalous ,valvate, lobes are subacute, 2.5-3.7 mm long and glandular. Corolla - petals 5, bilabiate , gamopetalous ,7.5-12.5 mm in size, tube about half as long as the corolla. Androecium - Stamens 4 ,epipetalous,didynamous ,filaments are attached nearly to the corolla tube . Filaments are hairy and anthers are two celled and exerted , purple beared at base.

- Gynoecium – carpels 2 ,syncarpous ,style slender ,stigma shortly two lobed ,ovary contains very minute hair ,two locules in ovary ,axile placentation ,ovules 2-6 in each locule.
- Fruit is a capsule, oblong, 18-20X4.5–5.0 mm, young ones sparsely glandular and hairy; when mature it is glabrous. Seeds are subquadrate, yellow to brownish in colour and rugose.



Identificatio-(1) stem with conspicuous nodes and internodes ,nodal region swollen

(2)leaves opposite decussate. lanceolate.

(3)Inflorescence – recemose panicle type .flowers bracts and bracteoles ,zygomorphic,bisexual and small .

(4) corolla bilabiate , stamen epipetalous ,didynamous ,anther bi-celled and exerted ,carpels -2, syncarpous, stigma shortly two lobed , style slender ,ovary with minute hair ,axile placentation ,seeds with retinacula .

……..hence .the plant belongs to the family Acanthaceae.

Erect or prostrate ,not climbing ,calyx distinct ,bracts are not sub-connate ,ovules more than two in each cell .

Seed supported on hard upward curving retinacula, ovules 2 ,not collateral or if more than two superposed in one row or else arranged alternately in each cell .

Corolla lobes, imbricate in bud .

Capsule compressed at right angles to the septum, seeds ovoid hardly compressed .

…….Genus – Andrographis .

Capsule nearly glabrous ,linears.
Leaves lanceolate ,glabrous.
Pedicels distinct.
……species – A. paniculata

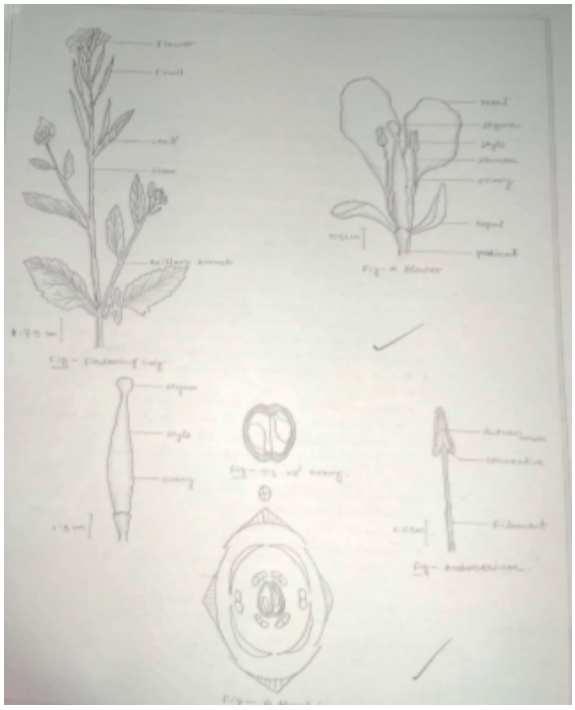
Hence the supplied plant specimen –**A** is *Andrographis paniculata* L.

Study of Taxonomic specimen –B

Specimen – Supplied from the college laboratory .
Habitat –Terrestria
Habit –Erect herb
VEGETATIVE PARTS
Root – Not supplied .
Stem –Solid, soft , terete, nodes and internodes are distinct ,alternate branching green in colour juice pungent.
Leaf –Exstipulate, sessile ,simple , alternate serrate ,leaf apex acute with equal leaf base ,glabrous ,reticulate venation, unicostate .
REPRODUCTIVE PARTS –·
Inflorescence – Recemose ,receme,axillary or terminal , ebracteate.

- Flowers – Actinomorphic .bisexual ,complete ,hypogynous ,tetramerous .
- Calyx-sepals 4, polysepalous, imbricate, deciduous, greenish.
  Corolla - petals 4, polypetalous ,cruciform ,distinctly clawed at the base, vavate ,yellow . Androecium - Stamens 6 , free, tetradynamous (2 –outer and 4-inner)filament long ,anther bi-celled ,basifixed dehiscing longitudinally .
- Gynoecium – carpels 2 ,syncarpous ,style short ,stigma simple ,capitate, ovary superior ,2 celled by a false partition - replum ,ovules many in parietal placentation .
- Fruit -Dry dehiscent ,elongated capsule (siliqua).
- Identificatio- (1) Plants herbaceous .
- (2)leaves simple, alternate ,reticulate ,unicostate ..
- (3) Inflorescence – recemose ebracteate, flower –bisexual ,complete ,regular ,sepals -4, free ,imbricate ,petals -4 ,cruciform ,clawed ,stamens -6 tetradynamous , carpels -4 ,syncarpous , superior , ovules in parietal placentation , fruit long dehising capsule (siliqua),seeds globose .
-
- ……..hence .the plant belongs to the family Cruciferae.
- Plant dehiscing ,narrow long ,seed-1 ,seriate,flower-yellow .
- …….Genus – *Brassica* .

- Leaves of the stem auriculate ,leaves without hairs ,less lobed.
- ……species – *B. napus* .
- Hence the supplied plant specimen – **B** is *Brassica napus* .
-
- ***N. B :- Drawing ,measuring and labeling are mandatory part of plant taxonomic description*** .
-
-



Study of taxonomic specimen –C

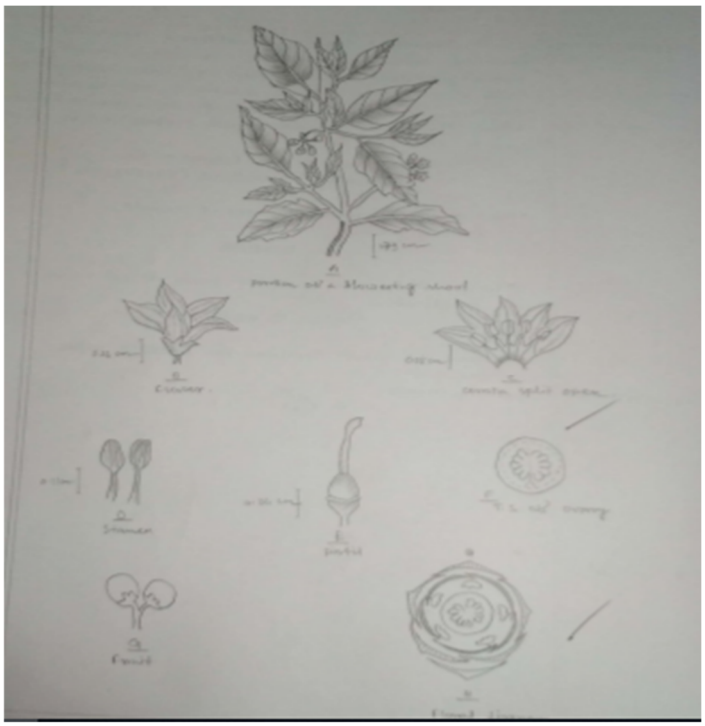
Specimen – Supplied from the college laboratory .
Habitat –Terrestrial .
Habit –Herbaceous

VEGETATIVE PARTS

Root – Not supplied .
Stem –Woody,erect ,cylindrical ,branched distinct nodes and internodes ,solid ,glabrous , length of internodes varies from 4-5 cm ,green in colour , minutely hairy .
Leaf – ,simple , alternate petiolate, exstipulate ,ovate , serrated ,leaf apex acute ,membranous ,green in colour ,length of each leaf 3-4 with equal leaf base ,glabrous ,reticulate venation unicostate .
REPRODUCTIVE PARTS –·
Inflorescence – Flowers in dichotomous ,extra axilary ,cyme , ebracteate.

- Flowers – Actinomorphic .bisexual ,complete ,hypogynous ,pentamerous ,small, white in colour ,ebracteate ,pedicillate.

- Calyx - sepals 5,,gamosepalous ,imbricate, regular ,persistant ,small ,oblong ,acute ,hairy ,greenish. Corolla - petals 5 gamopetalous ,rotate , imbricate,small ,white in colour . Androecium - Stamens 5, epipetalous,alternate to petalous ,filament short ,anther 2- celled ,oblong ,basifixed ,dehiscent by apical pores .
- Gynoecium – carpels 2 ,syncarpous, ovary superior .style terminal,stigma simple and small , axile placentation ,slightly bifid..
- Fruit -Berry ,simple ,indehiscence with star shaped ,persistant calyx ,round ,purplish black when ripe and shiny .



Identification- .

1. Inflorescence – flowers in extra axillary cymes .
2. leaves simple, alternate ,exstipulate ..
3. Sepals united ,persistant.
4. Corolla campanulate ,rotate.
5. Stamen -5 epipetalous ,alternate with porous dehiscence.
6. Gynoecium bicarpellary ,ovary superior ,2- chambered with many ovule in each
7. Fruits are berry.

……..hence .the plant belongs to the family solanaceae

Fruit indehiscent berry ,corolla rotate ,leaves entire ,anther connivent in a cone ,dehiscent by apical poress,embryo curved or sub -spiral.

…….Genus – *Solanum*

Leaves ovate or oblong ,sinuately lobed .

……species – *Solanum nigram* .

Hence the supplied plant specimen – C is *Solanum nigram* .

Study of taxonomic specimen –D

Specimen – Supplied from the college laboratory .

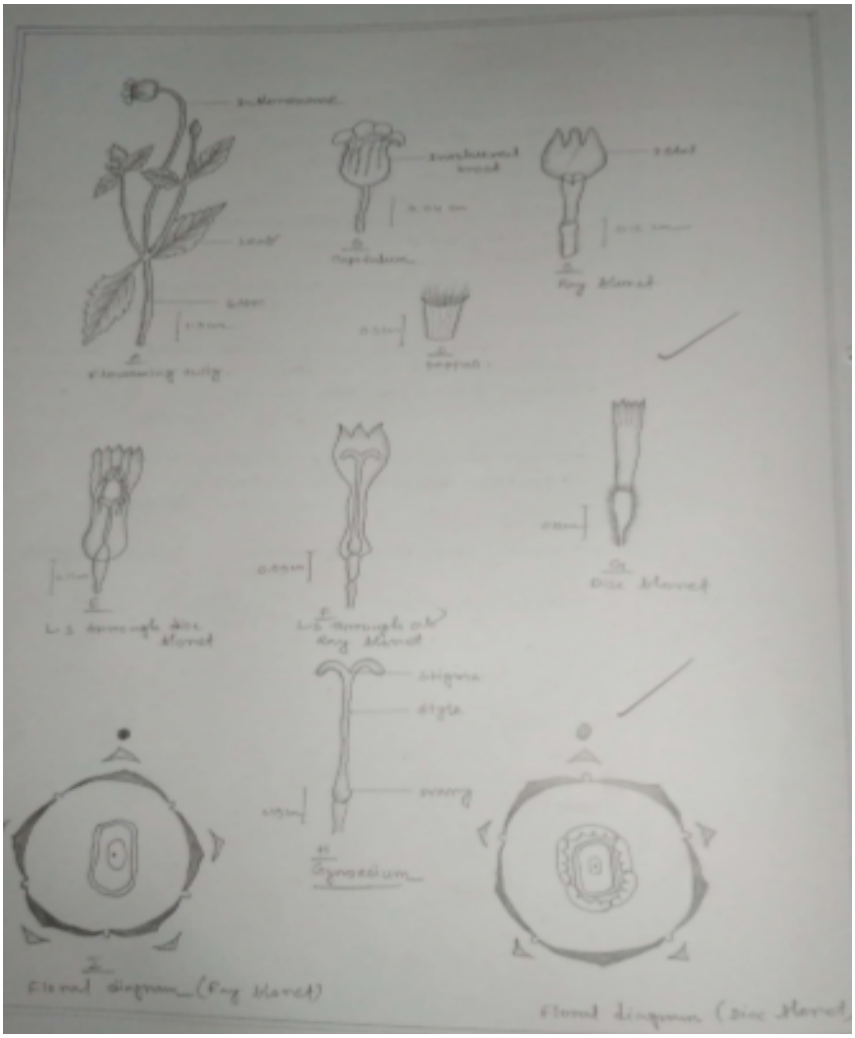
Habit –Herb ,prostate .
VEGETATIVE PARTS

Stem –Aerial prostrate ,presence of hair ,green in colour . .
Root – Not supplied
Leaf –Simple ,exstipulate ,hairy ,leaf margin serrate, 1-2 cm long both the surface consist of hair ,deep green in colour ,reticulate venation .
REPRODUCTIVE PARTS –·
Inflorescence – Capitulum
Involucre –Many bracts and bracteoles basally fused together on the outer surface forming an involucres . It is green in colour and hairy .

- Flowers –Bracteate ,sessile ,two types of florets (1) Ray florets (2) Disc florets .
- Ray florets –Bracteate ,sessile ,unisexual ,dichlamydeous ,zygomorphic ,epigynous ,yellow in colour .
- Calyx - sepals ∞ ,free , highly reduced ,hairy and scale like structure called pappus ,superior . Corolla - petals 3, gamopetalous ligulate ,yellow ,superior ,

.

- Gynoecium – carpels 2 ,syncarpous , ovary inffferiorb,locule -1 ,one ovule in the basal placentation ,style single, terminal ,stigma bifid ,hairy ,coiled
- Disc florets – Bracteate ,sessile complte ,actinomorphic epigynous ,dichlamydeous ,pentamerous ,yellow.
- Calyx –Same as ray florets .
- Corolla – Petals -5 ,gamopetalous ,tubular ,superior ,yellow .
- Androecium –Stamen -5 ,epipetalous ,syngenesious ,bicelled superior .
- Gynoe cium –Carpels -2 ,syncarpous ,ovary inferior ,locule -1, basal placentation ,style single ,terminal ,stigma labid ,hairy ,coiled .



Identification –Ovary inferior ,stamen equal in number to corolla lobes ,alternate with lobes stamens attached to the corolla ,anther coronate ,syngenesious ,ovary -1 locular ,ovule -1 ,calyx reduced to pappus ,basal placentation ,flowers in heads surrounded by an involucres .

……..hence .the plant belongs to the family Asteraceae.

Corolla of all the flowers fused to near the mouth or if any expanded from a tubular base (ligulate)then only the marginal florets of the fiower –head (ray-florets) so, expanded .style long distinct or style sub entire then so only in the sterile flowers of heads with dissimilar .

Flowers dissimilar (heterogamous) disc.

Pappus of numerous scales ,head radiate .

Scales of pappus –feathery ,head medium .

…….Genus -*Tridex*

Only one species recorded in the key .

……species T- procumbens .

Hence the supplied plant specimen – **D** is *Tridex procumbens* .

Study of taxonomic specimen –E

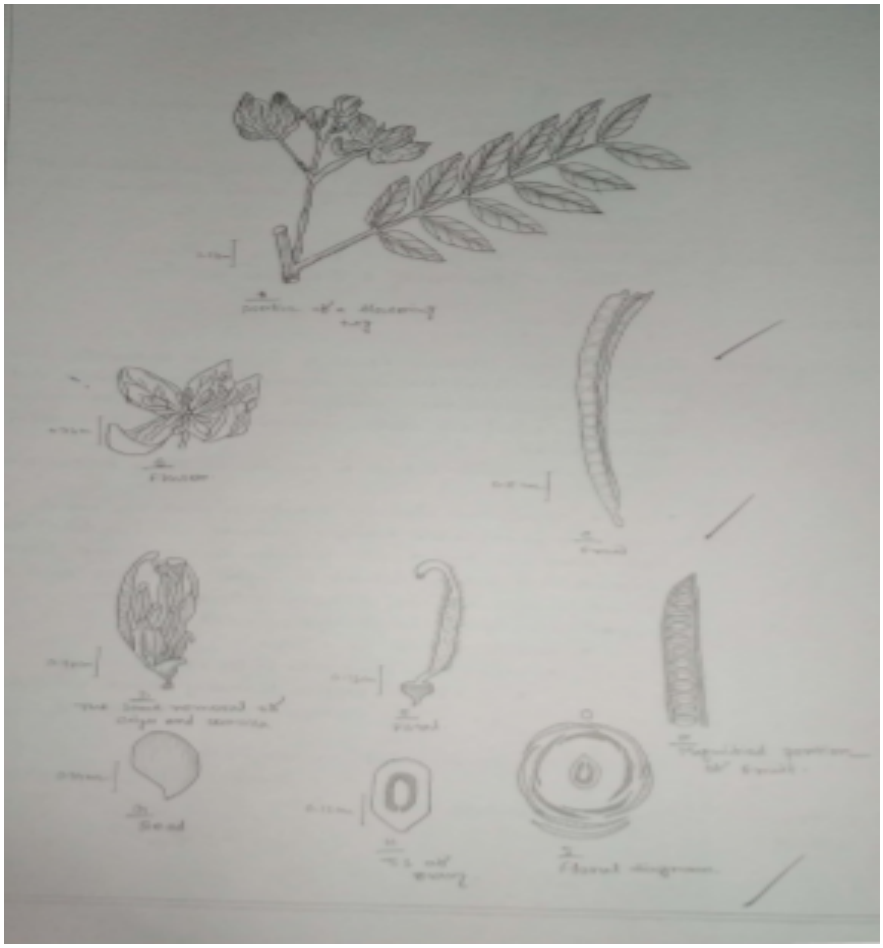
Specimen – Supplied from the college laboratory .
Habitat –Terrestrial .
Habit – Herbaceous .

VEGETATIVE PARTS

Root – Not supplied .
Stem –Solid, woody, erect, arial, branchedcyylindrical ,glabrous , nodes and internodes are distinct ,inter-nodal length 2.4-3.4 cm ,green in colour .
Leaf –Pinnately compound ,stipulate ,number of leaflet 6-12, reticulate venation unicostate entire ,ovate ,acute gland dotted ,lanceolate ,length of the leaf varies 10-12cm .
Leaflet – Ovate ,unicostate ,entire ,acute ,reticulate ,length of each leaflets varies from 3-5 cm .
REPRODUCTIVE PARTS –·
Inflorescence - Axillary racemes ,bracts and bracteole present ,corymb .

- Flowers – Bisexual regular ,pedicillate ,slightly zygomorphic ,pentamerous ,perigynous yellowish . .bisexual ,complete ,hypogynous ,tetramerous .

- Calyx - sepals 5,polysepalous ,imbricate, broad ,greenish .
  Corolla - petals 5 , polypetalous ,slightly irregular, imbricate,yellow .
  Androecium - Stamens 10 unequal ,filamentous, 6-fertile and 4- sterile,out of six , four small and two large ,anther 2-celled ,basifixed ,dehiscent longitudinally .
- Gynoecium – carpel- 1 ,apocarpous ,style short ,stigma terminal , ovary superior ,unilocular ,large curved slightly pubescent ,greenish , marginal placentation .
- Fruit -Woody ,dry dehiscent ,pod, lomentum .

- 

Identificatio- .

(1)leaves pinnatly compound .

(2) Flower bisexual ,irregular ,zygomorphic .

(3)Calyx of 5 sepals , free irregular

(4)Androecium of 10 stamens .

(5) Gynoecium monocarpellary, placentation marginal .

……..hence .the plant belongs to the family Caesalpiniaceae.

Leaves simple ,foliate or pinnate ,anther basifixed ,petals -5 ,leaves even pinnate .

…….Genus – *Cassia* .

Leaflets 3-5,ovate –oblong

Pods flattened ,impressed between the seeds .

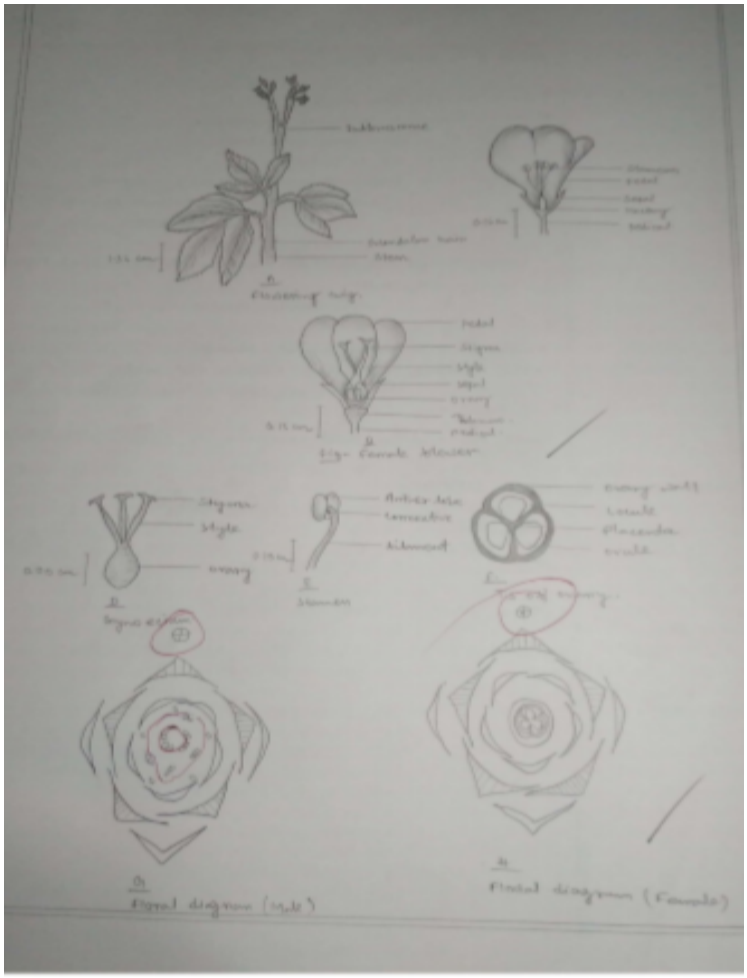
……species C- *occidentalis* .

Hence the supplied plant specimen – E is *Cassia occidentalis*

Study of taxonomic specimen –F
Specimen – Supplied from the college laboratory .
Habit –Under shrub, milky latex through the vegetative part .
VEGETATIVE PARTS

Stem –Aerial , erect ,solid ,reddish brown ,having gland differentiated into nodes and internodes , nodes swollen length of the internode -3 cm .
Root – Not supplied
Leaf –Simple ,palmate ,petiolate,reddish –green ,tri-penta foliate upper surface –brownish green ,shiny stipulate , stipule modified into glands ,gland are present on the upper surface .margin- entire, apex –acute ,base-cordate,venation –multicostate reticulate .texture of lamina –membranous ,glutinous dorsiventral alternate ,lower –glabrous ,upper-shiny .length of the the petiole 5-6.5 cm .
REPRODUCTIVE PARTS –·
Inflorescence –peniculate cyme .

- Flowers –Bracteate , unisexual ,actinomorphic ,hypogynous ,pentamerous ,dichlamydous ,red in colour ,length of flower -0.8 cm .
- Male flower –
- Calyx - sepals 5 ,gamosepalous, lanceolate ,imbricate ,glandular ,inferior ,green ,persistant .length of the calyx -0.3 cm .
  Corolla - petals 5,obovate ,imbricate-quencuncial,inferior ,reddish brown,length of the corolla -0.4 cm .
- Androecium – Stamens -8 in two bundle,3 in inner whorl ,3- in outer whorl,anther –reniform ,flattened ,dehiscence ,transversely .
- Female flower –
- Calyx – Sepals -5 gamosepalous ,lanceolate ,imbricate ,glandular ,inferior ,persistant .
- Corolla –Petals -5 obovate ,imbricate ,quincuncial ,inferior ,reddish brown .
- Gynoecium – Carpels -3 ,syncarpous ,triangular ,superior ovary ,ovule in each locule ,pendular ,axile placentation .
- Fruit –Regma ,seeds carrunculate .



Identification – Plants under shrubs ,leaves alternate ,stem with latex ,flower unisexual ,actinomorphic ,poly petalous ,,steams free mostly , carpel-3 ,united ,superior, ovule in axial placentation , fruit dehiscent –capsular type ,leaves simple,stipulate ,seeds with copious albumen

……..hence the plant belongs to the family Euphorbiaceae.

Flower monoecious ,stamen biserate ,stamens alternate with sepals .

Flowers in terminal cymes .

Leaves digitateiy lobed .

Central flower female .

Calyx regular 5- lobed .

Stamens many outer 5 in a series opposite the petals ,the rest in column in centre of flower .

…….Genus – *Jatropha* .

Leaves palmately 3 -5 lobed ,lobes widened in the middle ,glandular toothed ,bracts ,sepals and stipules glandular ,petiole glandular .

……species J -*gossipifolia*

Hence the supplied plant specimen – F is *Jatropha gossipifolia*