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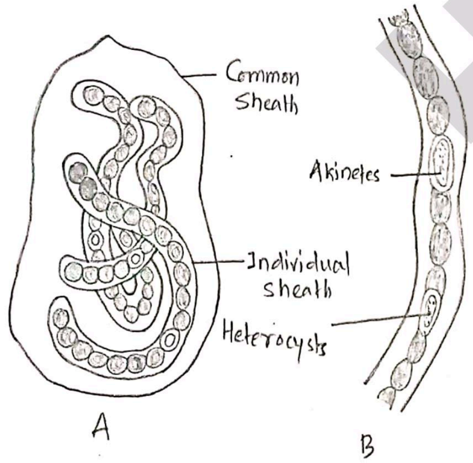

Fig: Nostoc sp. A – Colony
B – Single filament

EXPERIMENT NO – 01

AIM OF THE EXPERIMENT :- Identification and study of vegetative/reproductive structures of the following genera through temporary preparation of slides/ permanent slides –

PROCEDURE :-

(i) A small quantity of sample is taken on the slide and then a drop of cotton blue stain is put on it.

(ii) The slide is passed through a flame 2-3 times to warm up the stain and kept for 3-5 minutes.

(iii) Then on another clean slide a drop of ~~ethanol~~ lactophenol is taken and a portion of stained specimen is transferred on it by a needle.

(iv) Finally, the material is spread over the mountant by two needles and cover by a coverglass.

(v) The excess mountant is blotted off the slide is sealed with paraffin using a turn-table.

(vi) The slide is thereafter used for microscopic study.

1. Cyanobacteria :- Nostoc

Thallus Structure :- Plant body is blue-green in colour, free floating colonial or attached to a substratum. Each colony contains a number of trichomes - embedded within a matrix (common sheath) forming little balls – Nostoc balls. The trichomes consists of a single series of uniform, often torulose, bead-like ellipsoidal cells more or less depressed are often contorted and sometimes form densely interwoven masses. Cells of each trichome are joined end to end to form diffluent. Intercalary heterocysts are present.

Reproductive Structure:-

Akinetes – They are different in appearance from vegetative cells. Akinetes are spherical or oblong and much larger than vegetative cells with dense protoplasm.

IDENTIFICATION :

Thallus blue-green in colour, cells devoid of conspicuous nucleus, absence of any sex organ, presence of gelatinous sheath around the cells, (in most cases) absence of organised cell organelles like plastids.

CLASS : CYANOPHYCEAE

Thallus unbranched, filamentous, presence of hormogonium, heterocyst present in some genera only.

ORDER : NOSTOCALES

Trichomes unbranched, interwoven and surrounded by sheath, presence of heterocysts and akinetes.

FAMILY : NOSTOCACEAE

Trichomes with single series of uniform ellipsoidal (bead-like) cells, presence of intercalary heterocysts and akinetes.

GENUS : NOSTOC

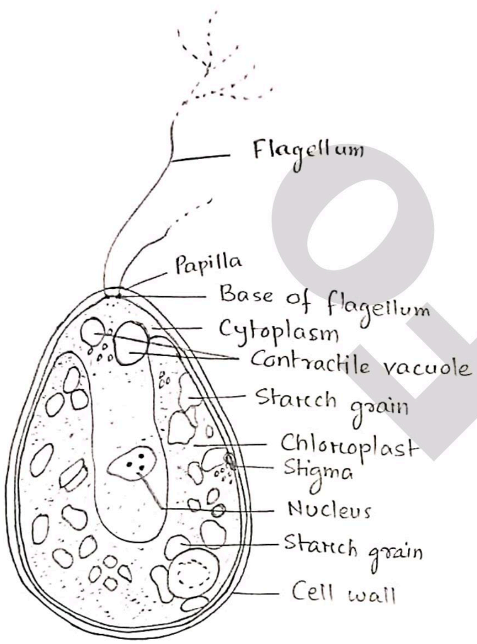

Fig: Chlamydomonas Sp.

2. Chlorophyceae: Chlamydomonas

Thallus Structure:

Plant is unicellular. The vegetative cell is spherical to ellipsoidal or pear-shaped. The cell is surrounded by a cell wall which is differentiated into firmer and mucilaginous portions. The cell contains the following parts (characteristics not visible under ordinary microscope have also been included).

↳ A single large cup-shaded chloroplast, occupying most of the cell, opening at the top and much thicker at the base than elsewhere,

↳ A large pyrenoid embeded in the lower part of the chloroplast.

↳ A single nucleus.

↳ Two contractile vacuoles, situated in the anterior colourless cytoplasm,

↳ An eyespot, situated anteriorly,

↳ A pair of flagella of equal length, attached anteriorly (visible under dark field illumination).

Reproductive Structure:

There may be presence of daughter cells grouped together within the mother cells. This is called 'palmella' stage. This is a kind of asexual mode of reproduction. (sexual reproduction not ordinarily visible).

IDENTIFICATION

Thallus green, chloroplast always possesses pyrenoid (i.e) starch—protein aggregation, presence of cellulosic cell wall.

CLASS : CHOLOPHYCEAE

Thallus unicellular to colonial vegetative cells motile, chloroplast cup shaped.

ORDER : VOLVOCALES

Vegetative body unicellular and motile biflagellate, flagella whiplash type and equal in length.

FAMILY : CHLAMYDOMONADACEAE

Vegetative cells spherical to pearshaped, each cell contains a large cupshape chloroplast with pyrenoid, uninucleate, presence of an eyespot anteriorly, presence of palmella stage.

GENUS : CHLAMYDOMONAS

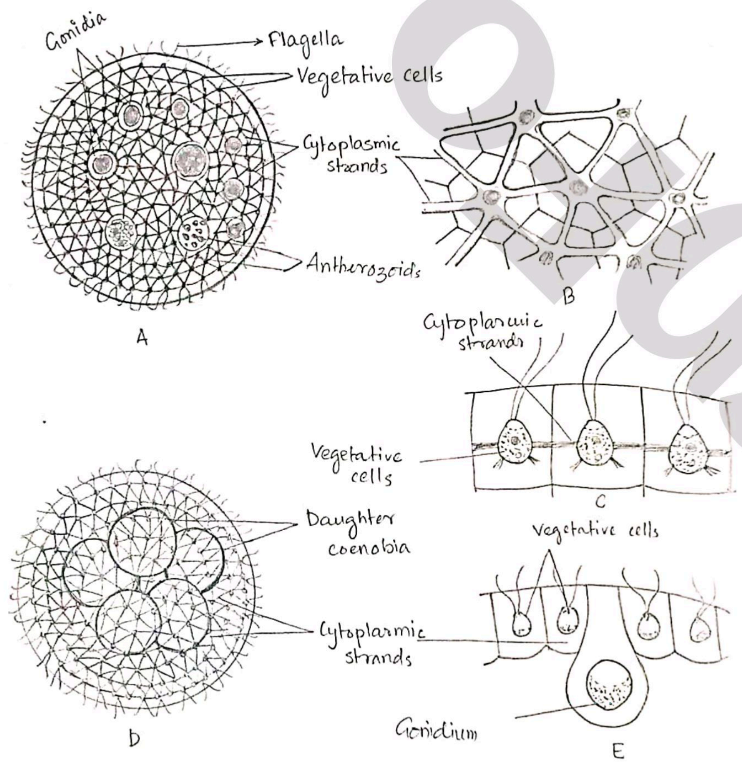

Fig: Volvox species

A – Colony ; B – Cytoplasmic connection
C – Cells with flagella ; D – Daughter colony
E – Gonidium

Volvox :

Thallus Structure : Thallus is colonial and colony is hollow and spherical in shape. The colonial thallus. The colonial thallus (i.e; coenobium) is composed of thousands of small head like spherical cells. Each cell has a cup-shaped chloroplast, single nucleus and contractile vacuoles. In general, cells are arranged in a single layer joined together by fine strands of cytoplasm. The spherical cells of the colonies are biflagellate.

There may be presence of daughter coloniel with-in a mother colony.

Reproductive Structure : Within the coenobium, there are some specialised and larger cells called 'gonidia' which are responsible for the production of daughter colonies asexually or used for sex organ development.

IDENTIFICATION

Thallus green, chloroplast always possesses.

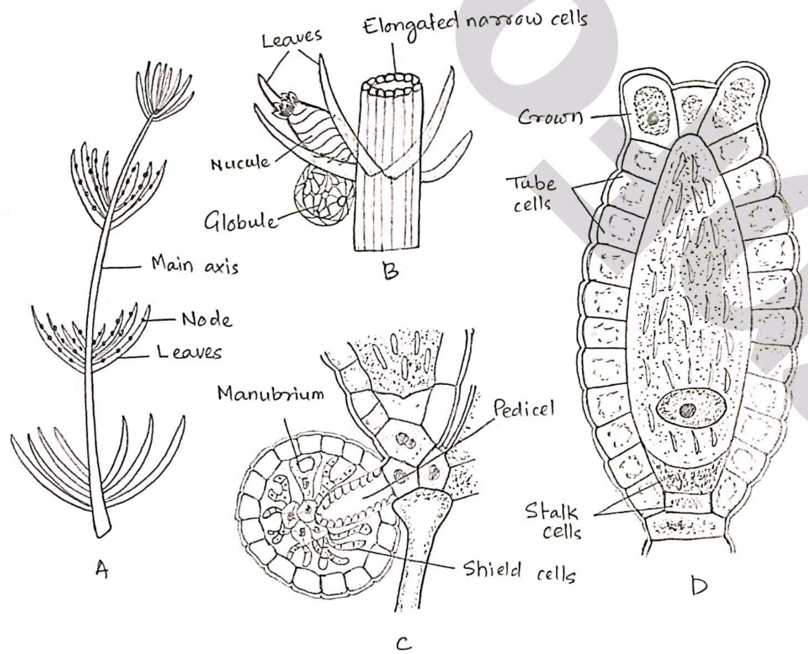

Fig: Chara sp

A – Thallus, B, C, & D – Reproductive structures – Globule (c) and Nucule (D)

Chara :

Thallus Structure : The plant body is slender and flexuous, with individual plants attaining lengths up to 30 cm or more. The thallus has an erect main axis differentiated into a regular succession of nodes and internodes with whorls of lateral branches of limited growth, often termed as 'leaves', arising from the nodes. Branches of limited growth having nodes and internodes just like the main axis may arise axillary to the leaves. The internode is composed of a central cylindrical cell which is many times longer than broad and ensheathed by elongated narrow cells arising from basal nodes constituting the cortex. The lowest node develop dome multicellular rhizoids.

Reproductive Structure : There are two specialized sex organs, viz. globule (i.e; antheridium) and nucule (i.e; oogonium). They are always borne at the nodes with a definite orientation, the nucule lying above the globule.

- Globule – A mature globule is large, stalked, spherical in structure and bright yellow to red in colour. The wall of the globule is composed of eight curved plates, the sheild cells, joined end to end, giving the wall a pseudo-cellular appearance.

- Nucule – It is a stalked oval structure being surrounded by an envelope of five long filaments placed side by side arranged spirally exhibiting a twisted appearance and affording protection to the oogonium. At the top of the nucule there is a conspicuous crown or corona, which is composed of 5 cells.

IDENTIFICATION

Presence of differentiated plant axis containing nodes with lateral branches and internodes, reproductive organ couplex with outer sterile covering.

CLASS : CHAROPHYCEAE

Comparatively complex plant body being undifferentiated into root-like, shoot like or leafy zones, sex organs stalked, multicellular with sterilic ~~covering~~ jackets.

ORDER : CHARALES

Elaborately developed sex organs arising in pairs from nodes.

FAMILY : CHARACEAE

GENUS : CHARA

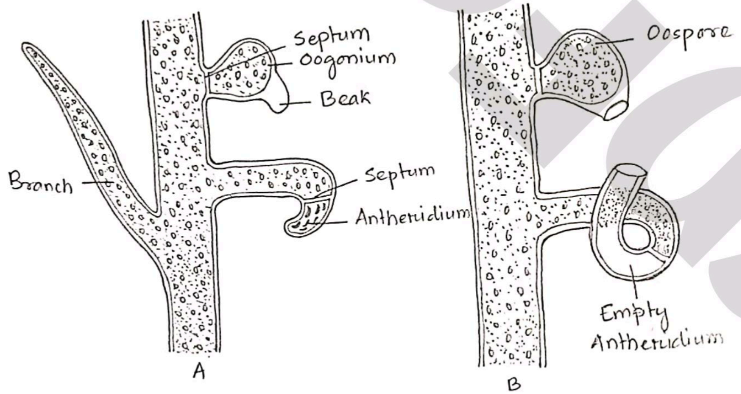

Fig: Vaucheria Sp. A & B – Filament with reproductive structure.

3. Xanthophyceae: Vaucheria

Thallus Structure: The thallus consists of a long, tubular, sparingly branched filament. The filament is coenocytic and multicellular cytoplasm. The central portion of the tube filament is occupied by a large vacuole. The cytoplasmic layer is composed of many small disk-shaped or elliptical chloroplasts with no pyrenoids. The cytoplasm also contains many oil globules. Thallus is yellowish green in colour.

Reproductive Structure: There are two kinds of sex organs, antheridium and oogonium, produced on short branches and then lying close together.

Antheridium:- It is a slender curved hooklike tubular structure which is walled off from the rest of the filament.

Oogonium:- It is a spherical or oval shaped sessile or short stalked body with a subapical beak. The oospore lies within the oogonium and is thick-walled.

IDENTIFICATION

Thallus yellowish green in colour, filamentous, coenocytic, branched; presence of oil as reserve food, sexual reproduction complex oogamous type.

CLASS: XATHOPYCEAE

Plant body coenocytic, filamentous, asexually reproduced by multiflagellated zoospores.

ORDER: HETEROSIPHONALES

Filamentous irregularly branched, antheridia cylindrical, curved, oogonia round to oval.

FAMILY: VAUCHERIACEAE

Filaments long, tubular, sparingly branched, antheridia hook-like curved, oogonia sessile or short-stalked with a beak.

GENUS: VAUCHERIA

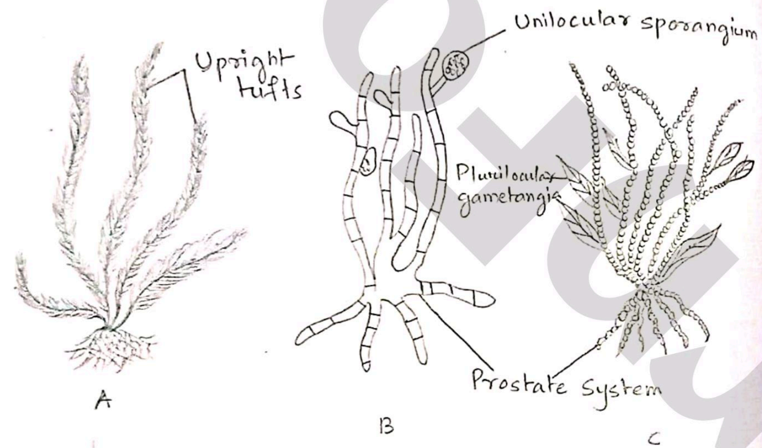

Fig: Ectocarpus Sp. A – Thallus, B & C – Filament with reproductive structure.

4. Phaeophyceae : Ectocarpus

Thallus Structure : The plant body is composed of irregularly prostrate portion and profusely branched filaments which grow upright in tufts. The mature portion of the thallus is multiaxial and pseudoparenchymatous. The ultimate branchlets of the erect portion are uniseriate and attenuated to an acute point. The branchlet cells are uninucleate and contain plate like or band-shaped chloroplasts. The thallus is brownish in colour.

Reproductive Structure :–

(a) Plurilocular Gametangia – Conspicuous plurilocular gametangia are present on tips of the lateral branchets (of haploid plants). The plurilocular gametangium is a narrow, elongated structure with a number of small compartments.

(b) Unilocular Sporangia – There are some filaments which possess more or less oval unilocular sporangia that are developed with an enlargement of the terminal cells of short lateral branchlets (diploid plants). Each sporangium contains a number of zoospore.

(C) Plurilocular Sporangia – Diploid plants also develop pluricular sporangia which are morphologically similar to plurilocular gametangia.

IDENTIFICATION

CLASS : PHAEOPHYCEAE
Mature portion of the thallus multiaxial and pseudoparenchymatous.

ORDER : ECTOCARPALES
Filamentous, branched plant body, gametangia or sporangia borne at the tips of the branchlets.

FAMILY : ECTOCARPACEAE
Gametangia pleurilocular, elogated, borne on the tips of the lateral branches, presence of globose unilocular sporangia with a few spores.

GENUS : ECTOCARPUS.

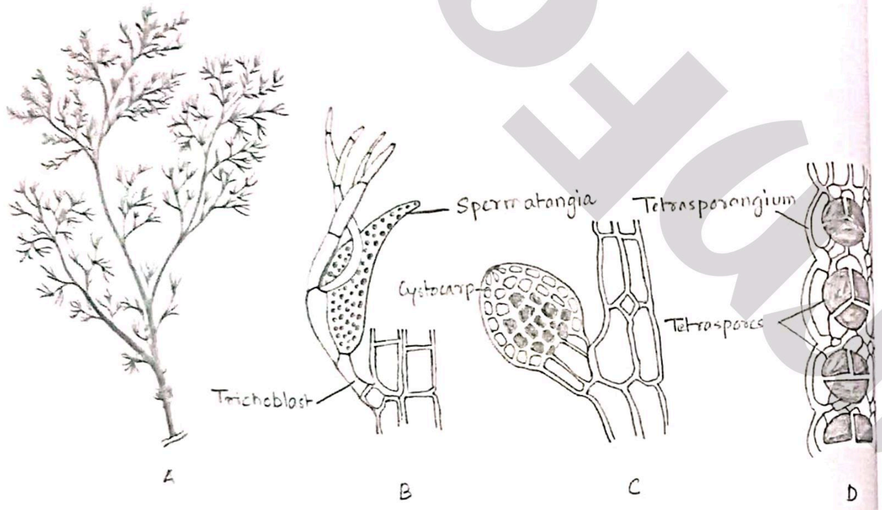

Fig: Polysipheria Sp. A – Thallus
B – Spermatangia
C – Cystocarp
D – Tetrasporophyte

5. Rhodophyceae : Polysiphonia

Thallus structure : The plant body is filamentous, branched latterally or dichotomously & brownish-red to purple red in colour. The main axis and its branches possess a polysiphonous appearance as the central axial cell (siphon) is surrounded by pericentral cells (siphons) of variable number. The cells have prominent cell to cell cytoplasmic connections and each cell has one nucleus and many discoid plastids embedded in dense cytoplasm. Ultimate branches are uniseriate structures and are known as trichoblasts.

Reproductive Structures :-

Spermatangium – The lateral branches of the male plant bear antheridia known as spermatangia in dense clusters. The spermatangia are short-stalked colourless and spherical or oval structures. It contains a single spermatium.

Cystocarp – It is an urn-shaped structures, formed by gonimoblast filaments surrounded by sterile filaments. The terminal cells of the gonimoblast filaments produces a carposporangium with one carpospore.

Tetrasporangia – It is borne on the central axis of the specialised filament called tetrasporophytic filament. Each sporangium contains four tetraspores.

IDENTIFICATION

Presence of gelatinous material in the thallus, cells contain chloroplast with pyrenoids, presence of characteristic post-zygotic structure called cystocarp.

CLASS : RHODOPHYCEAE
well developed branched filamentous thallus-carposporangia developing on filamentous gonimoblasts.

ORDER : CERAMIALES
Polysiphonous, branched thallus, cystocarp, urn-shaped with a pore, tetrasporangia with tetraspore.

FAMILY : RHODOMELACEAE

GENUS : POLYSIPHONIA

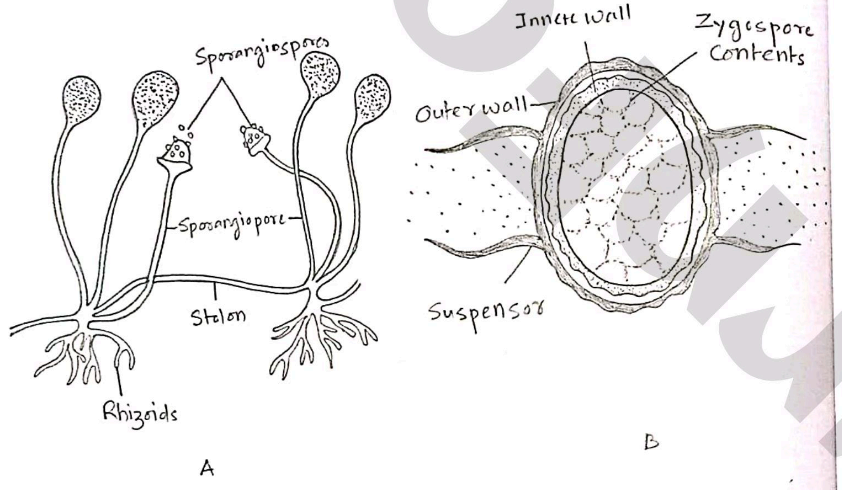

Fig : Rhizopus Sp. : A – Sporangium on Sporangiophore
B – Zygospore

EXPERIMENT NO – 02

AIM :- To study and Identification of vegetative/reproductive structures of the following genera through temporary preparation of slides (by Lactophenol Cotton Blue methods) –

PROCEDURE :-

(i) A thin hand section is made by fine razor or blade and then stained with 1% cotton blue for 5-10 min. After a bit warming over flame.

(ii) Then the specimen mounted on clean slide using lactophenol mountant.

1. RHIZOPUS

Somatic Structure : The thallus is mycelial type. It is composed of numerous, slender, branched and aseptate hyphae. There are two kinds of hyphae – aerial hyphae producing stolons and sporangiophores and prostrate hyphae producing rhizoids.

Reproductive Structure :

Sporangiophore – It is unbranched and arises in tufts from the aerial mycelium. It is terminated by sporangium.

Sporangium – It is small, round and ~~ueck~~ black. Each sporangium has a conspicuous dome-shaped columella, overarched by sporangiospores.

Zygospore – It is dark coloured, rounded and thick-walled. Its surface is warty. It is formed by the fusion of two similar gametangia.

IDENTIFICATION

Thallus mycelial type, mycelium aseptate, spores formed within sporangia, oospore formed sexually.

CLASS : PHYCOMYCETES (PHYCOMYCOTINA)

Gametangia morphologically not distinguishable as male and female, zygospore – formed by gametangial copulation, asexual reproduction by sporangiospores.

SUB CLASS : **ZYGOMYCETES**

Asexual reproduction by sporangiospores formed within sporangium.

ORDER : **MUCORALES**

Sporangiospores liberated by breaking of sporangial wall.

FAMILY : **MUCORACEAE**

Presence of conspicuous stolon forming aerial mycelium sporangiophores formed in tufts from the stoloniform aerial mycelium.

GENUS : **RHIZOPUS**

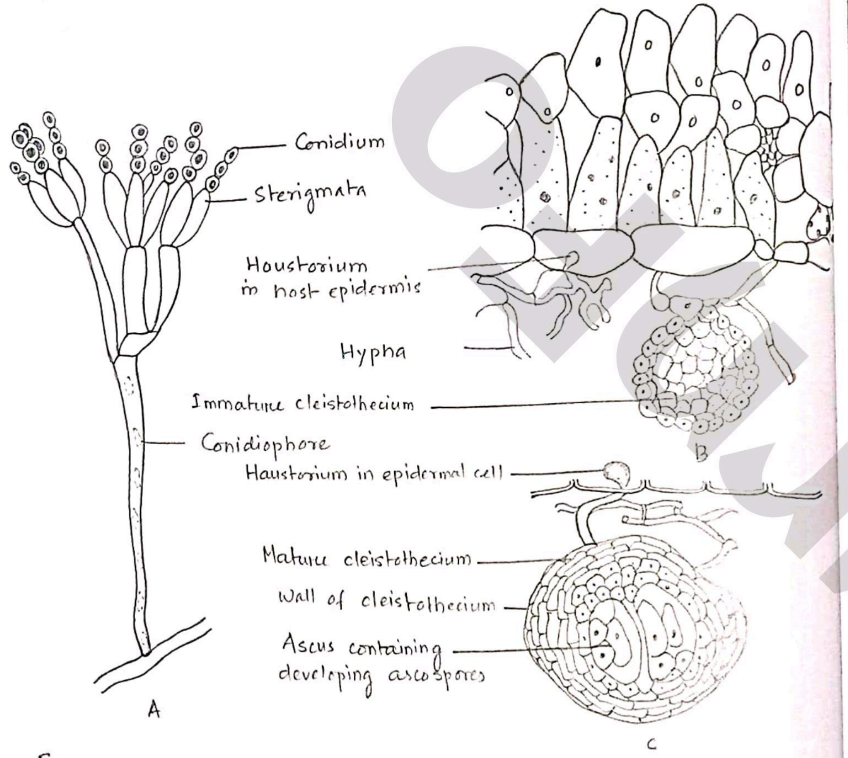

Fig : Penicillium Sp. : A – Conidia on condiophore
B & C – Cleistothecium

2. PENICILLIUM

Somatic Structure :- Thallus is mycelial type. Mycelium is highly branched, hyaline or coloured and septate. Mycelium is anastomosing forming a tuft of cottony mass.

Reproductive Structure :

Conidium – The conidia are developed on condiophores in chain. Conidiophores are branched, slender and the aerial branches are composed of 2-3 cells. Each condiophore is terminated by sterigmata – arranged in a closely packed whorl (penicillus). Conidia are globose to elliptical in shape and have smooth or rough spiny wall.

Cleistotcheium – It is occassionally formed within the mycelial tufts. It is globose to ellipsoidal and devoid of any appendages, cleistotcheium contains many asci.

Ascus – Ascus is slender, tube-like with a row of ellipsoidal ascospores.

Ascospore – Each ascus bears eight ascospores.

IDENTIFICATION

Thallus is mycelial type, hyphae septate, presence of sac-like ascus with endogen-ously formed ascospores, asexual reproduction by conidia.

CLASS : ASCOMYCETES (ASCOMYCOTINA)
Asci produced from ascogenous hyphae and enclosed in well-developed ascospores.

SUB CLASS : EUASCOMYCETES
Asci scattered within the ascocarp, ascocarp cleistothecial type.

SERIES : PLECTOMYCETES

ORDER : EUROTIALES
Presence of sub-aerial ascocarp, mature condia echinulate.

FAMILY : EUROTIACEA
Presence of branched conidiophore which is terminated by sterigmata arranged in a closely packed whorl or like brush; condia borne in chains, globose to elliptical in shape.

GENUS : PENICILLIUM

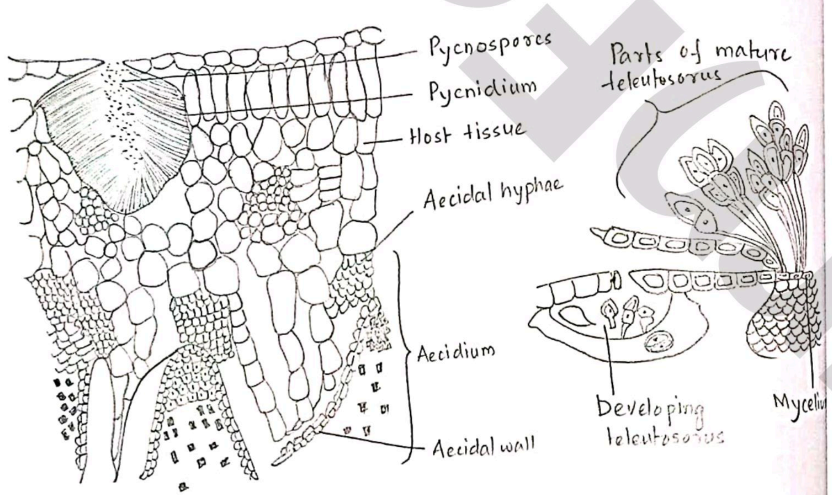

Fig: Puccinia Sp.: A – Aecidium
B – Teleutosorus

3. PUCCINIA

Somatic Structure: The somatic structure of the fungus consists of septate hyphae which penetrate the host tissues and cause destruction but do not become organized into structures of definite form. The hyphae branch intercellularly and produce small haustoria which penetrate the host cells.

Reproductive Structure :- The infected region of the host shows rusty streaks or postules. A T.S. through such region shows various kinds of 'sorus' structure viz. Uredosorus, Teleutosorus, Pycnidial and Aeciosorus stage. All the ~~stage~~ sori show the crust breaking through host epidermis due to pressure of the developing spores from inside the host tissue.

Uredosorus – It is conspicuously present in the upper epidermis of the infected wheat leaf. There is presence of ruptured host epidermal layer with exposed, stalked, one-celled uredospores. Spores are elliptical and minutely echinulate.

Teleutosorus – It is also seen in the upper surface of the infected wheat leaf. The sori are blackish with stalked, bicelled spores exposed from the host tissue after rupture of the host epidermis as seen in T.S through infected region. Cell-wall is smooth.

Pycnidial stage – It is seen in the upper leaf surface of infected barberry plants. In T.S. through the infected region of barberry ~~plant~~ leaf, there is presence of reproductive hyphae exposed outside the leaf tissue through an outside.

Aeciosorus :- It is seen in the lower leaf surface of infected barberry leaf. The sori are just like inverted cups with angular sessile spores formed in chains.

IDENTIFICATION

Mycelium branched and septate presence of basidium and basidiospores.

CLASS : BASIDIOMYCETES (BASIDIOMYCOTINA)

Basidia septate and developed from resting teleutospores.

SUB CLASS : <u>HETEROBASIDIOMYCETES</u>

Basidiocarp lacking

ORDER : UREDINALES

Teleutospores stalked, and germinate by formation of promycelium externally.

FAMILY : PUCCINIACEAE

Polymorphic with various spore forms.

GENUS : PUCCINIA

EXPERIMENT NO – 03

AIM :- To study of plant cell with the help of epidermal peel mount of onion.

INTRODUCTION

The onion peel cell experiments is very popular for observing a plant cell structure. Onion is a eukaryotic plant that contains multicellular cells. We know that the cells is a structural and functional unit of life that builds up living structures.

The bulb of an onion is formed from modified leaves. Like plant cells, onion cells have a rigid cell wall and a cell membrane enclosing the cytoplasm and nucleus. Onion epidermal cells exist as a single layer that serves as a protective skin. It separates thick, juicy leaves of the onion. Thus, the bulb of onion is formed from modified leaves. The epidermal cell of an onion bulb is simple and transperent. Its microscopic observation introduces the general view of plant anatomy to ~~the~~ us.

OBJECTIVES

The main objetives of performing the onion peel cell experiment is to observe the arrangement and structural components of the onion epidermis. The following facts about the onion peel cell experiment play a significant role in educating students:

(i) The epidermis of the onion bulb is a single layer of tissue that is easy to separate. For this reason, onion peel is best for educational and experimental purposes to study the structure of plant cells.

(ii) Due to the large size of onion cells, the cells can be examined under low magnification.

(iii) It is also a simple experiment that we can effecienHy perform, plus they can practise how to use a microscope.

REQUIREMENTS

We need the following glassware and reagents to prepare a temprrary slide of an onion peel.

Materials required to separate onion skin

- Medium-sized onion
- knife
- Forceps

Materials needed to stain and mount the onion peel

- Petri Plate.
- Distilled water.
- Safranin
- Clean glass slide
- Glycerine
- Coverslip.
- Blotting paper.
- Compound microscope.

THEORY

An onion is a multicellular plant. The presence of a rigid cell wall and a large vacuole is a characteristes feature of a plant cell. Thus, onion being a plant, comprises features common to plant cells. Like plant cells, onion cells ~~are~~ consist of a cell wall and cell membrane surrounding the cytoplasm, nucleus and a large vacuole.

- The cell wall is a rigid, protective coat covering the cell membrane, including all the internal components. The rigid cell wall maintains the shape of onion cells and contributes to the compact arrangements of the epidermal cells in onion.

- The cell membrane is interior to the cell wall surrounding the cytoplasm, including all the internal structures.

- The cytoplasm is the cell's inner space that appears jelly-like. It moves the cytosolic material around the cell through cytoplasmic streaming.
- The nucleus is present near the periphery of the cytoplasm. It is the control centre of the cell and the largest organelle in the cell.
- The vacuole is large and prominently seen at the centre of the cell. It stores solid and liquid contents. The basic shape or size of a vacuole differs depending on the needs of the cell.

PROCEDURE

The steps to perform the onion peel cell experiment are as follows:-

Steps to separate an onion peel

A. Take an onion, separate its outermost peel and chop it into two equal halves.

B. Then, take one fleshy scale leaf of a chopped onion bulb and split it into two.

C. Then carefully pull a thin, transparent epidermal peel from the convex surface of the scale leaf using forceps.

D. Then, wash the separated peel in the petri plate containing water. We can cut the onion peel into small rectangular pieces using a blade.

Step to stain and mount an onion peel

E. After that, transfer the onion peels into the Petri Plate containing diluted safranin stain. Leave the peels undistributed for about 3 minutes.

F. Finally, rinse the extra stain of the peel by again developing it the petri plate containing water.

G. With the help of a brush or forcep, transfer the peel to the centre of a clean glass slide.

H. Then to mount the onion peel, add a drop of glycerine over the centre of the slide. Glycerine prevents the peel from drying up.

I. After that, carefully mount a cover slip over the centre of the prepared slide by slowly lowering it with a needle. During this stage, you need to avoid the entry of any air bubbles.

J. Using a piece of blotting paper, remove extra glycerine over ~~the~~ from the margins of a cover slip.

K. Observe temporary slide under the compound microscope.

- First, turn on the microscope's light and ensure the low objective lens is in line with the optical tube. Then, place the prepared slide on the stage of a microscope.

- Looking from the side (not through an eyepiece), lower the tube using the coarse focus knob until the end of the objective lens is just above the cover glass. During this stage, do not crack the, or the objective lens may get damaged.

- Now look through the eyepiece and adjust the smaller, fine focusing knob to move the optical tube upwards until an image comes into focus.

- Then, swap the objective lens to a high objective lens so that ~~you~~ we can notice the cells at greater magnification.

- Prepare an observation table for the cells as seen under a microscope and note the features listed in the observation tube.

OBSERVATION

- Shape of cells: Rectangle
- Arrangement of cells: Compact
- Inter-cellular spaces: Absent
- Nucleus: Present (at the cell's periphery)
- Stained portions: Cell wall and nucleus are darkly stained with less stained cytoplasm
- Unstained portions: Cell membrane and vacuole.

RESULT

Onion epidermal cells are rectangular and compactly arranged (with no inter-cellular space). There is a distinct cell wall, a prominent nucleus and a vacuole.

PRECAUTION

1. Do not overstain the onion skin.
2. Avoid the folding of the peel.
3. The glass slide and cover slip should be dry and clean.
4. Put a coverslip carefully to avoid any air bubbles.
5. Using blotting paper, remove the extra glycerine.

CONCLUSION

Therefore, the onion peel cell experiment is an engrossing activity that can help a student to 'observe and study the plant cell structure. Students can prepare the temporary slide and observe the differences between the slide with stained onion skin and the slide without any stain. The microscopic observation of onion peel cells will allow students to closely examine each component of onion cells.

EXPERIMENT NO – 04 (Part-A)

AIM – To stain nuclear DNA in eukaryotic cells with Feulgen (we will use Onion root tip as the source of eukaryotic cell here).

MATERIALAS REQUIRED

- Onion root
- Formaldehyde.
- 1N HCl
- Schiff's reagents.
- Acetocarmine
- Distilled water

Other equipment's –

- Glass slide & cover slip
- Watch glass
- Dropper
- Forceps
- Blotting paper

PROCEDURE OF DNA-FEULGEN PRACTICAL

1. Fresh onion roots were collected and transferred to a watch glass containing distilled water for 5 minutes.

2. With the help of forceps, the root tip was placed on the glass slide, followed by fixation with Formaldehyde for 5 minutes.

3. The fixed material was treated with 1N HCl for 8-12 minutes in a water bath at 60°C.

4. Afterwards the root was placed in to Schiff's reagents at room temperature for 30 minutes.

5. The root was rinsed in water for 5 minutes, followed by addition of Acetocarmine.

6. Lastly the root was rinsed in water again and blotted dry.

7. Finally, the root was covered with cover slip and slightly pressed.

8. The slide is observed under 40x magnification in compound Microscope.

PRINCIPLE OF DNA BY FEULGEN REACTION PRACTICAL

The fuelgen stain was invented by German Physician Robert Feulgen during 1914 – 1924.

He estimated the nature of Nucleic acid as a polymer of nucleotide with four kind of nitrogenous bases. It is probaby the simplest cytochemical method allowing indication and evaluation of DNA levels into the cell.

Feulgen reaction consists of two steps –

- Initially Acid Hydrolysis is performed for 8–12 minutes, resulting in the cleavage of Nitrogen bases from deoxy ribose and formation of free Aldehyde groups.

- Then the preparation is placed in light yellow Schiff's Reagent, which forms bond with this groups (Free Aldehyde). The red violet product from in this ~~preparation~~ step is evidence of presence of DNA. RNA is not hydrolysed by the HCl treatment and thus this reaction is DNA specific.

The pink colour intensity correlates with DNA content in the cell. However, Feulgen technique is semiconservative in nature. It is becoming quantitative for DNA, if the only aldehyde is remaining in the cell are those produced by Hydrolysis of DNA.

As DNA from any of organism has the same nature, we can use cells from any source (Human, ~~an~~ animal or plant) to demonstrate the presence of Nucleus. We used onion roots to perform this experiment.

Result of DNA Feulgen experiment-

When the slide was visualised under the microscope, some dark coloured along with some Light-coloured regions were seen. Dark colour region indicates the Nucleus as it present in the centre of the cell. While the rest of the light stained regions indicates Cell Cytoplasm as it surrounds the Nucleus.

In proper colour –

- Nucleus – Dark pink colour.
- Cytoplasm – Magenta Colour.

EXPERIMENT NO – 04 (Part-B)

AIM – To study Cell wall in the epidermal peel of onion using periodic Schiff's (PAS) Staining Technique.

Principle of Periodic Acid – Schiff's (PAS) staining –

The periodic acid of the stain reacts with carbohydrates in an oxidative process. In this process, the polysaccharide and the periodic acid reaction form an oxidized compound – aldehyde. Now, the aldehyde reacts with the schiff's reagents, which gives the purple – magenta color. Similarly, the appearance of the pink colour suggests the presence of ~~interna~~ intracellular or extracellular mucin. In contrast, using haemotoxylin or methyl green as counter-stain helps in staining the nuclei. Likewise, a light green coloured counter stain is prefferred to demonstrate the fungal organisms.

Chemical and Reagents

Solutions and Reagents

1. 0.5 % Periodic Acid Solution
2. Periodic acid crystals – 0.5 g
3. Distilled water – 100 ml
4. To prepare 0.5 % periodic acid ~~st~~ solution mix 0.5 g periodic acid crystals in 100 ml distilled water.

Schiff's reagents

Dissolve 5 g basic fuchin in 900 ml boiled water. Once it is cools to 50°c periodic acid crystals in 100 ml 1M HCl to the mixture. Again add 10 g of k2S2O5 once the mixture cools down to 25°c. After completely mixing, shake the solution for 3 minutes and let it incubate for 24 hours in a dark room.

After incubation, add 5g of activated charcoal to the mixture. Then shake the solution for 3 minutes and filter. The refiltration and retreatment of the solution are necessary if the solution is not crystal clear. Store the solution at 40°c in the foil-covered bottle. It is suitable to use the solution for 2-3 weeks if stored properly.

For testing the primary purity of the prepared Schiff's reagent

Pour 10 ml of 10% formalin into a beaker, then add a few drops of the prepared Schiff's reagent. Then change in colour is interpreted as the red-purple color means it is a good Schiff's reagents. Deep blue-purple colour color means poor Schiff's reagent (delayed reaction).

Mayer's Haematoxylin

1. Aluminium potassium sulphate (alum) - 50 gram
2. Distilled water - 1000 ml
3. Haematoxylin - 1 gram
4. Sodium iodate - 0.2 gram
5. Glacial acetic acid - 20 ml

Dissolve alum in distilled water. Add haemotoxylin when alum is completely dissolved. When haemotoxylin is completely dissolved, add sodium iodate and acetic acid. Then boil and cool it.

PROCEDURE FOR PERIODIC ACID-SCHIFF (PAS) STAINING

1. Firstly, remove the paraffin from the tissue sections by washing in distilled water.
2. Then, place the tissue in 0.5% periodic acid solution for 5 minutes. It oxidizes the tissue.
3. Then, cover it with Schiff's reagent for 5-15 minutes which turns into light pink.
4. After that, was the stain for 5 minutes using lukewarm water, which turns it into dark pink.
5. Then counter-stain the tissue using Mayer's Haemotoxylin for 1 minute.
6. After that, wash it with running tap water for 5 minutes and rinse using water.
7. Finally, dehydrate the slide, place the coverslip and mount it using mounting media.

RESULTS INTERPRETATION FOR PERIODIC ACID-SCHIFFS (PAS) STAINING

1. Glycogen, mucin and some basement membranes- Red/ purple.
2. Fungi - Red/ Purple .
3. Background blue .

EXPERIMENT NO – 05

AIM – To measure the size of microscopic objects under microscope.

PURPOSE

The purpose of micrometry is to measure the dimensions (length, breath, diameter and thickness) of microscopic objects (microoganisms, pollen grains, etc.) under microscope.

MATERIALS REQUIRED

Ocular micrometer, stage micrometer, slide, microscope, microbe/pollen grain.

PRINCIPLE

Measurements of the dimensions of microorganisms is done under microscope with the help of two micro-scales called micrometers. Both the micrometers have microscopic graduations etched on their surfaces. One of them, the 'ocular micrometer' is a circular glass disc, which fits into the circular shelf inside the eyepiece. It has arbitrary graduations etched on its surface. However, the distance between the etched graduations is contant for a particular ocular micrometer. The other micrometer, called 'stage-micrometer,' is a special glass slide, which is clipped to the stage of the microscope. It has standard graduations etched on its surface, which are 10 µ apart.

CALIBRATION

Graduations on the ocular micrometer are calibrated against the standard graduations on the stage micrometer. Calibration is required, because the distance between ocular graduations varies depending on the objectives being used, which determines the size of the field.

For calibration, both the micrometer etching are superimposed by rotating the eyepiece. The number of ocular divisions (O.D) coinciding with the number of stage division (S.D.) is found out. From this, the calibration factor for one ocular division (O.D) is calculated as follows:

If 10 O.D. coincide with 2 S.D., then

$10 \text{ O.D.} = 2 \text{ S.D.} = 2 \times 10\,\mu = 20\,\mu$ (since $1 \text{ S.D} = 10\,\mu$)

1 O.D. $= 20/10\,\mu = 2\,\mu$

MEASUREMENT

After caliabration, the stage micrometer is removed and the microbe/pollen grain, whose dimensions are to be measured, is placed on the stage on a slide and focused. Now, the number of ocular divisions occupied by the microorganism is counted. Then, by multiplying this number of divisions with the calibration factor, the size of the microbe is determined as follows. If the microbe/pollen grain occupies 6 O.D. in length, then length of microbe = 6 X calibration factor = i.e. $6 \times 2 = 12\,\mu$.

PROCEDURE

1. The eyepiece is removed from the microscope, and its top lid is unscrewed. The lid is removed. Carefully, the eye lens is removed. The ocular micro-meter (a circular etched glass piece, which slips into the eye piece) is placed carefully into the eyepiece. The eye lens is place back and the top lid is screwed to its original conditions. The eyepiece is placed back in microscope.

2. The stage micrometer is clipped to the stage and the etching centred by moving the mechanical stage.

3. The low power ~~eyepiece~~ objective is taken to position.

4. The eyepiece is ~~routed~~ rotated till the etching on both the micrometers superimpose.

5. The required objective is taken to positions. The required objective is that, using which the microorganism can be viewed and it covers the microscopic field to the maximum possible extent.

6. With the required objective in position (for oil-immersion objective, a drop of oil is put on the stage micrometer) the mechanical stage is moved, so that a line on the stage micrometer coincides with a line on the ocular micrometer. Then, another lines is searched on the ocular micrometer, which coincides with another line on the stage micrometer. The divisions bet-ween the coinciding lines is counted for both the micrometers.

7. The calibration factor for the objective used is calculated.

8. In a similar way, calibration factors are calculated for other objectives.

9. The stage micrometer is removed.

10. The slide containing the microbe to be observed is placed on the stage & focused.

11. The number of ocular divisions covered by the microbe is counted by viewi-ng through eyepiece.

12. The size of the microorganism/pollen grain is determined by multiplying the numbers ocular divisions covered by the microbe with calibration factor

EXPERIMENT NO – 06

AIM – To study the counting of cells per unit volume with the help of haemocytometer.

OBJECTIVE

To determine the concentration of cells in a given sample.

THEORY

For microbiology, cell culture and many of the applications that require use of cell suspensions, it is necessary to determine the concentration of cells. The device used for determining the number of cells per unit volume of a suspension is called a counting chamber. It is the most widely used ~~in~~ type of chamber, since it was mainly designed for performing blood cells counts. It is now used to count other types of cells and other micrscopic particle as well.

The haemocytometer was invented by Louis-Charles Malassez. It is a special type of microscopic slide consisting of two chambers, which is divided into nine (1.0 mm X 1.0 mm) large squares which are separated from one another by triple ~~divisions~~ lines. The area of each is 1 mm^2. Cover glass is supported over the chambers at a height of 0.1 mm. Because of that the entire counting gird lines under the volume of 0.9 mm^2 on one side. The cell suspensions are introduced into the cover glass. The haemocytometer is placed on the microscopic stage and the cell suspension is counted.

The glass microscope slide has a rectangular indentation that creates an 'H' shaped chamber at the centre. This chamber is engraved with laser-etched gird of perpendicular lines. Two counting areas with ruled girds are separated by horizontal groove of the 'H'. There is also a very flat, reusable cover slip. The glass cover slip is held at 0.1 mm above the surface of the counting areas by ground glass ridges on either side of the vertical grooves of the H shape. The device is carefully crafted so that the are bounded by the depth and lines of the chamber is also known. Because the height is constant, the volume of fluid above each other square of the gird is known with precision.

The haemocytometer is used by putting the cover slip on the device, and filling the space with a liquid containing the cells to count. There is a "V" or notch either end which is the place where the cell suspension is loaded into the haemocytometer. The fluid is usually drawn into the space by capillary action. A cover glass, which is placed on the sample, does not simply float on the liquid, but held in place at a specified height. In addition, the gird arrangement of squares of different size allows for an easy counting of cells. It is possible to identify the number of cells in a specified volume by this method.

The ruled area of the haemocytometer consists of several large 1×1 mm ($1 mm^2$) squares, which are subdivided in three ways; 0.25 × 0.25 mm ($0.0625 mm^2$) 0.25 × 0.20 ($0.05 mm^2$) and 0.20 × 0.20 mm ($0.04 mm^2$.) The central, 0.20 × 0.20 mm marked; 1×1 mm^2 is further subdivided into 0.05 × 0.05 mm ($0.0025 mm^2$) squares. Hold the cover slip (0.1) mm at the raised edges of haemocytometer, which gives each square a defined volume.

A number of stain have been employed to distinguish between viable and nonviable cells. This is based on the principle that live cells contain intact cell membranes that eliminate certain dyes, like trypan blue Eosin or propidium. In dead cells, the stain enters the cytoplasm and the cells take on the stain. If more than 25% of the cells are stained, the cell suspension is not likely not a viable one.

To prepare the counting chamber, the mirror-like polished surface is carefully cleaned with 75% ethanol and the cover slip is also cleaned. The cover slips used for counting chambers are specially made, and are thicker than those cover slips used for conventional microscopy, since they must be heavy enough to overcome the surface tension of a drop of liquid. A cover slip is placed on the counting surface prior to putting on the cell suspension. Introduced any of the cell suspension into any of the V-shaped wells with a micropipette. The area under ~~microscope~~ the cover slip fills b[illegible] The charged counting chamber is placed under th[illegible] ing grid is brought into focus at [illegible]

EXPERIMENT NO – 07

AIM :- To demonstrate plasmolysis and de-plasmolysis in peels of Rhoeo leaf in hypertonic and hypotonic solution using sodium chloride.

THEORY

When a plant cell is placed in a concentrated salt solution, water from the cell sap flows out due to exosmosis. The loss of water from the cell sap causes concentration or shrinkage of the protoplasm since the cell wall is firm and less elastic, it cannot keep pace with the concentration of the plasma membrane. Ultimately, the protoplasm separates from the cell wall and assumes a spherical shape. It is called plasmolysis. When a plasmolyzed cell is placed in water or hypertonic solution it absorbs water due to endosmosis and its protoplasm assumes the original shape it is called de plasmolysis. Plasmolysis is, this can be defined as the shrinkage of the protoplasm of a cell from its cell wall due to exosmosis in a hypertonic solution.

MATERIALS REQUIRED

1. Needle
2. Forceps.
3. Droppers.
4. Glass slides.
5. Watch glass.
6. Rhoeo leaf.
7. Coverslip
8. Compound microscope.
9. Sodium chloride 5% solution.
10. Sodium chloride 0.1% solution.

PROCEDURE

1. Take two clean and dried glass slides and place them on a table.
2. Select the fresh and cleaned Rhoeo leaves and place them on the watch glass.

3. Fold the leaves in such a way that it tears from the lower side of the leaf. Or, with the help of clean blade.

4. Extract two small fragments of a fine and transparent layer with the help of forceps from the lower surface of the epidermis of the Rhoeo leaf.

5. Now set up the epidermis of the Rhoeo leaf.

6. With the help of a dropper, add 1 to 2 drop of sodium chloride 0.1% solution to one of the prepared slides.

7. With the help of another dropper, add 1-2 drops of ~~solution~~ sodium chloride 5% solution to the other prepared slide.

8. Now, carefully set a cover slip on the peel of both sides with the help of a needle. Make sure, no bubbles are present.

9. Leave the prepared glass slide undistributed for a few minutes.

10. Now, carefully place the slides under a compound microscope and observe the changes.

OBSERVATION

The cell in dilute solution appears turgid due to endosmosis while the cells in concentrated shows plasmolysis due to exosmosis. When the concentrated solution is replaced with water the protoplast of cells requires its original shape.

CONCLUSION

Plasmolysis is observed when the plant cells are immersed in the concentrated salt solution or sodium chloride 5% solution. During this process, 4 to 5 percent of water passes through the cell membrane into the encircling medium. This occurs as the concentration of water inside the cell is higher than the outside of the cell hence the protoplasm induces shrinkage and takes a spherical shape.

When the plant cells are immersed in a dilute salt solution or sodium chloride 0.1% solution, the water in the plant cells moves from the outside to the inside of the cell as the water concentration is higher outside cell as composed to the inside of the cell which causes the turgidy of the cell.

EXPERIMENT NO – 08

AIM – To study of effect of organic solvent and temperature on membrane permeability.

THEORY

Living beetroot cells are suitable materials to demonstrate the effects of high temperature and chemicals on the permiability of cell membr-anes. Beetroots contains a red pigment called anthocyanin, which is located in the large central vacuoles of the beetroot cells. As long as the cells and their membranes are damaged, anthocyanin will leak out and produce a red colour in the water surrounding the beetroot. The intensity of red colour in the water can be used to assess the degree of damage to living membrames by different factors.

High temperatures and organic solvent eg. alcohols, denature membrane proteins and increase the fluidity of membrane lipids. Organic solvents at high ~~temperature~~ concentration can also dissolve lipids. Acetone, alcohol and chloroform are organic solvents that severely destroy membranes.

A. BY HIGH TEMPERATURE

Procedure

1. Use a cork borere to cut cylinders of tissue from a beetroot.
2. Cut the cylinders of beetroot into thin discs of about 3 mm thick.
3. Rinse the beetroot discs in running water to wash off pigment that leaked out as a result of cutting.
4. Pipette 5 cm^3 of water into six test tubes and labelled as 30, 40, 50, 60, 70 and 80.
5. Use water bath to heat a boiling tube containing water up to 80°c.

6. Gently lower 5 piece of beetroot discs into the hot water and leave them immersed for exactly 1 minute.

7. Carefully remove the discs and place them in the prepared test tube labelled as 80.

8. Leave the tubes for 20 minutes. Shake the tubes occasionally for the pigment to leak out of the cells.

9. Repeat steps (5) to (8) for temperatures at 70°c, 60°c, 50°c, 40°c and 30°c respectively.

10. Remove the discs from each test tube after 20 minutes.

11. Compare the intensity of red colour in each tube. Use a one to ten '+' sign to indicate the relative colour intensity.

B. BY CHEMICALS

Procedure

1. Use a cork borer to cut cylinders of tissue from a beetroot.
2. Cut the cylinders of beetroot into thin disc of about 3 mm thick.
3. Rinse the beet-root discs in running water to wash off pigment that leaked out as a result of cutting.
4. Blot dry the discs with paper towels.
5. Prepare the following test tubes with 5 cm^3 of solutions as below.

Tube	A	B	C	D	E	F
Solution	water	10% alcohol	30% alcohol	50% alcohol	chloroform	Paraffin oil

6. Put equal number of discs into tubes A to F. Stoppered the tubes with cork.

7. Shake the tube occassionally.

8. After an hour, take out all discs from tubes A to D.

9. Take out the discs from tube E (chloroform) carefully without damaging them and blot to remove the solution on the surface of the discs.

10. Put the discs from ~~tube~~ step(9) into another test tube labelled as E1, which contains 5 cm^3 of water.

11. Repeat step (9) and (10) for tube F (paraffin oil).

12. After an hour, take out the discs from tube E1 and F1.

13. Record and compare the colour intensity of the solution in tubes A, B, C, D, E1 and F1

NOTE

1. Chloroform and alcohol can destroy the structure of cell membrane of beetroot, and the red pigment will diffuse out. The pigment is soluble in alcohol and water, but not soluble in chloroform.

2. Paraffin oil and water do not destroy the cell membrane.

PRECAUTION

1. Alcohol and chloroform are volatile and harmful.

2. Alcohol is inflameable. Avoid using naked flame near this solvent.

3. Students could not inhale the vapours from these chemicals when handeling them.

EXPERIMENT NO – 09 (PART-A)

AIM OF THE EXPERIMENT : To study and demonstrate mitosis by preparing the mount of an onion root tip cells.

THEORY OF THE EXPERIMENT :- For entities to mature, grow maintain tissues, repair and synthesize new cells, cell division is required. Cell division is of two types –

→ Mitosis
→ Meiosis

Mitosis :- In mitosis, the nucleus of the eukaryotic cells divides into two daughter cells by splitting of the parent cells. Hence, every cell division involves two chief stages –

- Cytokinesis – cytoplasm division.
- Karyokinesis – Nucleus division.

Stages of mitosis –

① Prophase – The process of mitosis is initiated in this stage where coiling and thickening of the chromosome occurs.

- Shrinking and hence the disappearance of the nucleolus and nuclear membrane takes place.

② **Metaphase** :- Chromosomes turn thick in this phase. The two chromatids from each of the chromosomes appear distinct.

- Each of the chromosomes is fastened to the spindle fibres located on its controller.

③ **Anaphase** :- Each of the chromatid pair detaches from the centromere and approaches the other end of the cell through the spindle fibre.

- At this phase, compressing of the cell membrane at the centre takes place.

④ **Telophase** :- Chromatids have reached the other end of the cell.

- The disappearance of the spindles
- Chromatin fibres are formed as a result of uncoiling of daughter chromosomes.

Post mitosis, the next stage is referred to as Interphase, which is a part of cell division that is non-dividing and between two consecutive cell divisions.

MATERIALS REQUIRED:- Compound microscope, Acetocarmine stain, water, Burner, N/10 Hydrochloric acid, Filter paper, Coverslip, Aceto alcohol, Glass slide, Onion root peel, Forceps Blade, Watch glass, Dropper, Needle, Vial.

PROCEDURE OF THE EXPERIMENT :-

① Placed an onion on a tile.

② With the help of sharp blade, striped the dry roots of the onion.

③ Placed the bulb in a beaker containing water to grow the root tips.

④ Trimmed around 3cm of the newly grown roots and placed them in a watch glass.

⑤ With the help of forceps, shifted the vial holding freshly prepared aceto-carmine.

⑥ With the help of forceps, picked one root and set in on a new glass slide.

⑦ Heated it lightly on the burner in such a way that the stain does not dry up.

8. Excessive stain can be carefully treated using filter paper.

9. The more stained part of the root tip can be trimmed with the help of a blade.

10. Added a droplet of water

11. With the help of needle, a coverslip mounted on it

12. Gently tapped the coverslip with an unsharped end of a needle for the meristematic tissue of the root tip present.

13. The onion root tip cells slide is now prepared, and observed on microscope.

OBSERVATION AND CONCLUSION :-

1. The slide containing stained root tips is placed on the stage of the compound microscope, changes taking place are noted.

2. The different phases of mitosis, such as prophase metaphase, anaphase and telophase can be observed.

EXPERIMENT NO - 09 (PART-B)

AIM OF THE EXPERIMENT :- To study different stages of meiosis.

THEORY:- The word meiosis is derived from the Greek word 'diminution' as the process results in daughter cells with half the number of the chromosomes. It is a specific type of cell division that occurs in the reproductive tissue of diploid organisms and results in the formation of haploid gametes. During meiosis a diploid mother cells give rise to four, haploid daughter cells after two rounds of haploid chromosomes segregation events. These two multistep divisions are called Meiosis I and Meiosis II.

MATERIALS REQUIRED :- Onion buds, Carnoy fixative /Acetic alcohol, 70% alcohol, Dropping bottle of acetic acid, Dropping bottle of acetocarmine, 2N hydrochloric acid. Spirit lamp, slides and cover slips, Pasteur pipettes Teasing needles and fine forceps, Razor blade, watch glass

PROCEDURE :- Ⓐ <u>Pre lab preparation</u>-

① Plucked onion buds and transferred them to carnoy's fixative for 24 hours.

② At the end of the fixation period transfer the buds to a storage solution and kept them vials 4-5°c until required for the experiment.

Ⓑ <u>Preparation of stained squashes</u>:-

① Removed the anthers from the onion buds stored in 70% ethanol.

② Transferred the anthers to a watch glass and washed throughly with water.

③ Drained off the pipe with pasteur pipette. and added a few drops of 2N HCl. Left it for 10-15 min.

④ Removed HCl with a pasteur pipette and washed anthers again with water.

⑤ Transfered the anthers to 2% acetocarmine stain for 10 min.

⑥ Destain the tissue by transfering it to a clean slide and added a few drops of 45% acetic acid from dropping bottle.

7) ~~8~~ Placed carefully a cover slip on the anthers and transferred the slide between the folds of filter paper to remove excess acetic acid.

8) ~~Do not allow~~ Sealed the edges of the cover slip with DPX or nail polish to prevent drying

9) Examined the slide under the compound microscope at 10X and 40X.

IDETIFYNG FEATURES OF MEIOTIC STAGES :-

Meiosis - I :- Cell spends most of their time in Meiosis-I. At the end of this phase the two daughter cells have half the number of the chromosomes. Each cell is genetically identical because of crossing over between homologous chromosome at prophase I and later independent assortment of chromosome at anaphase I due to their random alignment at metaphase I

Prophase - I

ⓐ **Leptotene**:- In leptotene thread-like the chromosome are distincty visible and they have beaded appearance all along their length that is called chromosomes. The terminal portions of the chromosomes attach to the nuclear membrane in many eukaryotes and the chromosomes bear resemblance to 'bouquet of flowers'.

ⓑ **Zygotene**:- The homologous chromosomes begin to pair of synapsed chromosome is called a bivalent or tetrad. Zygonema ends with the completion of pairing.

ⓒ **Pacytene**:- There is an appearance of shorter and thicker form of each paired chromosomes as compared to zygotene. Although genetic exchange between non sister chromatids takes place during phachytene.

ⓓ **Diplotene**:- It is marked by seperation of homologous chromosomes in a bivalent. They are held together at points of physical change. The chromosomes become shorter and thicker

(e) Diakinesis :- Terminazation is completed with the help of coiling of chromosomes, which further become shorter and thicken. The nucleolus disappear. Towards the end of diakinesis spindle formation is initiated and the nuclear envelope breaks down.

Metaphase - I

- The homologous pairs of chromosomes start arranging at the equatorial plane of the spindle.
- The alignment of the homologous chromosomes is random at the metaphase plate that accounts for their independent assortment and enormous genetic variation.
- This is one of the shortest phase.
- The chiasmata between the homologous hold them as bivalents.

Anaphase - I

- Homologous chromosomes separate and move to opposite poles.
- Each homologue has two sister chromatids attached

at their centromeres.

- Two sister chromatids are not identical.

Telophase - I

- A haploid set of chromosomes have reached each pole
- Two nuclei are produced.

Meiosis - II

- The second meiotic division is similar to the mitosis except that haploid cells have chromosomes with two non-identical sister chromatids linked at the centromere.

OBSERVATION AND RESULT:-

EXPERIMENT NO. - 10

AIM :- To perform the gram staining of the given sample.

MATERIALS REQUIRED

Glass slides, Bunsen burner, Cotton, Sample, Microscope.

REAGENTS REQUIRED

Crystel violet dye, Iodine, Alcohol, Safranin dye.

PRINCIPLE

Gram staining is most widely staining technique used by the microscopic observation. It was discovered by Tanish scientist and physcian ~~than~~ Hans Christian Grams in 1884. This technique differentiate bacteria in two groups gram positive and gram negative bacteria. This procedure is based on the ability of microorganism to retain colour of the stain during Gram reaction. Gram positive bacteria are decolourised by alcohol and will remain as purple. After decolourization stop, a counter stain is used to impart pink colour to the gram negative microorganism.

Gram positive bacteria have a thick mesh like cell wall which is made up of peptidoglycan (50-90%) of cell wall, which stain purple. Gram negative bacteria have a thinner layer of peptidoglycan (10% of cell wall) and lose the crystal ~~vio~~ violet iodine complex during decolourisation with alcohol rinse but retain the counter stain safranin thus appearing reddish or purple.

STAIN REACTION :-

(i) Application of crystal violet to heat fixed smear :- Crystal violet dissociates in aqueous solution into CV^+ and Cl^- ions. These two penetrate the cell wall and cell membrane of both gram positive and gram negative. CV^+ interact with negative component of bacterial cell and stain it purple.

(ii) Decolourization of with ethyl alcohol :- Alcohol dissolve the lipid outer membrane of gram negative bacteria, thus leaving the peptidoglycan layer exposed and increase the porosity of cell wall. The CV-I complex is then washed away from the peptidoglycan layer leaving gram negative bacteria colourless. In gram positive bacteria, alcohol has dehydrating effect on cell wall causing cell wall to shrink, then CV-I complex get tightly bound into multi layered leaving the cell with purple colour.

(iii) Addition of gram iodine : Iodine act as a mordant and a trapping agent. A mordant is a substance that increase the affinity of cell wall for a stain by binding to primary stain, thus forming a insoluble complex that get trapped in cell. During the reaction CV-I complex is formed and all the cell turn purple.

(iv) Counter stain with safranin dye : The decolourised gram negative cell can be visible with a suitable counter stain which is usually positively charged safranin, which stained it pink.

PROCEDURE :

(i) Prepare very thin smear of sample on glass slide and heat fixed it.

(ii) Flooded the smeared slide with crystal violet dye. Avoid over flooding and kept it for 1 minute.

(iii) Washed the slide running tap water.

(iv) Applied iodine solution gently all over the slide and kept for 1 minute.

(v) Washed it under tap water.

(vi) Applied 95% ethyl alcohol all over the slide drop wise and kept for 10 second.

(vii) Immediately rinsed with water.

(viii) Finally, flooded the sample with safranin dye to counter stain and kept for 45 secondons.

(ix) Washed the slide with running water.

(x) Observed it under microscope.

OBSERVATION :

Name of the organism	Morphology	Inference
Lactobacillus species	Purple or deep violet colour, spherical, cocci arrange in singles pairs chains and clusters are seen and irregular clusters seen along with pink colour.	The given smear contain both gram positive & gram negative bacteria.

EXPERIMENT NO. - 11

AIM - To study the models of virus - t phage, Lytic cycle (Virulent infection), Lysogenic Cycle (Temperate infection)

Bacteriophage (bacteria-eater)

Bacteriophage (bacteria-eater), as the name suggests, are the viruses that infect and replicate within bacteria. They are commonly called a phage. They are found everywhere. They contain DNA or RNA in their genome, which is encapsulated in a protein coat. They also infect archea.

Bactericidal activity of bacteriophage was first observed in 1896 by Ernest Hanbury Hankin in the water of river Ganges, which could kill cholera bacteria.

William Twort discovered bacteriophage in 1915.

D'Herelle termed them as 'bacteriophage' in 1917, as they showed the ability to kill bacteria.

There are several types of phage virus, which infect only certain bacteria specifically.

They act in the same way as antibiotics by disrupting the cell wall of bacteria and have been used for the same. They have the potential to be used against antibiotics-resistant pathogenic bacteria.

Bacteriophage structure

- A bacteriophage is made up of a protein coat known as capsid, which encapsulates the genome. It consists of a polyhedral head.

- It may be ~~observed~~ enveloped or nonenveloped and have different shapes such as rod-shaped, filamentous, isometric, etc.

- The capsid is made up of many capsomeres. This size and shape very in different species.

- The genome consists of ~~a~~ ss or ds DNA or RNA, which is linear or circular. The genome consists for proteins ranging from 4 to 100. Ms2 bacteriophage genome codes for 4 proteins. The largest genome found in a bacteriophage is 735 kbp.

- The tails may be long or short, contractile or noncontractile. Tail fibers are present, it helps in anchoring the virus to the bacterial cell wall.

Bacteriophage classification and Examples :-

Bacteriophage are classified based on their nucleic acid content and morphological characteristic. There are 19 families of bacteriophage found, of which two families are of RNA bacteriophages. The main families & characteristics of bacteriophages are given below with examples.

Examples	Family of the phage virus	Nucleic acid content	Morphological characteristics
T4 (Escherichia virus T4)	Myoviridae	Linear ds DNA, 169 kbp long	Infects E. coli. Nonenveloped, Icosahedral head, tail is hollow with complex contractile structure and has tail fibers. It undergoes only lytic life cycle, no lysogenic cycle.
T2 (Enterobacteria phage T2)	Myoviridae	Linear ds DNA, 170 kbp	Infect E. Coli. The famous Hershey & Chase experiment to prove that DNA is a genetic material was done with T2 phage infecting E. Coli.
λ (Coliophage, Escherichia virus lambda)	Siphoviridae	Linear ds DNA, 48502 bp	Infected E. Coli. The virus particle is made up of head, tail and tail fibers. Nonenveloped and have a noncontractile long tail. It undergoes both lytic and lysogenic cycles. Commonly used as a vector in recombinant DNA technology.
M13 (Escherichia virus M13)	Inoviridae	Circular ss DNA, 6407 nucleotides long	Filamentous, nonenveloped. Infects E. Coli. M13 plasmids are used in genetic engineering.

Life cycles of Bacteriophage

There are two ways by which bacteriophage infect the host bacterium.

Lytic Cycle (Virulent infection)

They induce complete lysis of bacterial cell, which is known as a lytic life cycle. Examples include T2, T4, T6 (T-even phages), they are also known as virulant phages. The bacterial cell is completely destroyed immediately after replication of the viral genome. This type of infection is called virulent infection and it is mostly used for phage therapy.

The lytic cycle has the following steps :-

1. Adsorption – Anchoring of bacteriophage to the bacterial cell wall with the help of tails fibers.

2. Penetration – The phage DNA gets injected into bacteria.

3. Replication and Synthesis – The bacterial DNA is disrupted and the viral genome takes charge of bacterial machinery. It starts making proteins required for replication and other structural proteins.

4. Assembly – Phage components are assembled into new viral particles.

5. Lysis and release – Bacteria cells are lysed and new viral particles are liberated to infect other cells.

Lysogenic Cycle (Temprate infection)

Bacteriophage that undergo lysogeny known as temperate phages. The viral DNA gets integrated into the host genome and replicates along with the bacterial genome. The integrated viral genome is known as prophage.

It is relatively harmless and continues to remain in the position until the lytic cycle is triggered. It may be spontaneous or due to certain external conditions such as radiations exposure. Then the prophage becomes active and a lytic initiates resulting in the lysis of the cell wall.

After penetration, the phage DNA gets integrated into bacterial DNA and gets replicated along with the bacterial genome.

As the bacterial genome is inserted into the bacterial genome and bacteria continue to perform the normal activities, the viral genome gets transferred to the progenies as well.

Bacterial cells containing a prophage are called lysogenic cells. The lysogenic cells (having a prophage) may exhibit new properties, eg:- Corynebacterium diphtheriae and Clostridum botulinum, when containing certain prophage DNAs, synthesize toxins, which are harmful.

Examples of lysogenic phage include lambda (λ) phage. Due to the ability to insert their genome specifically and replicate, they are used in genetic recombination.

Importance of Bacteriophage

Bacteriophage are used for various purposes. They are widely used in medical and research.

- Phage Therapy – They are used as antibiotics against bacteria due to the same mode of action.
- They are used in the food industry to kill bacteria in meat or cheese products.
- Bacteriophage are used for diagonastic purpose.
- They act as a model in research and studies.
- They are used as a cloning vector in genetic recombination technique.