INTRODUCTION TO PLANT PATHOGENS

PRACTICAL MANUAL
COURSE NO:PATH-171

2012

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ACHARYA N. G. RANGA AGRICULTURAL UNIVERISTY

PRACTICAL MANUAL

B.Sc., (Ag.)

Course No:PATH-171

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COMPLIED BY

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Certificate

Certified that this is a bonafide record of practical work done by Mr. / Ms. _____ID No: _____in B.Sc.,(Ag.) degree program Course No:PATH-171 "INTRODUCTION TO PLANT PATHOGENS" during _____semester,20____-20____.

Date: **Signature of course in-charge**

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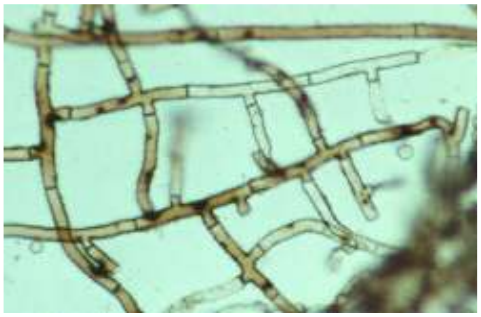
1. STUDY OF VEGETATIVE STRUCTURES OF FUNGI AND THEIR MODIFICATIONS

VEGETATIVE STRUCTURES OF FUNGI:

Fungi produce various types of vegetative structures which are chiefly concerned with nutrition and growth. The vegetative body **(thallus)** of a typical fungus consists of filamentous mycelium, which is made up of branching hyphae. The hyphae may be **aseptate(coenocytic)**, typical of the lower fungi or **septate(multicellular)**with cross partitions, characteristic of higher fungi. The mycelium is **endophytic**(inside plant tissue) in vast majority of cases or **ectophytic**(on the surface of the host) in such fungi that cause powdery mildews. The endophytic mycelium may be either **intercellular** (between the cells) or **intra cellular** (with in the cells) .Special structures known as **haustoria**(drinkers) are produced by inter cellular and ectophytic mycelia and help in obtaining nourishment from the host cells. Some parasitic fungi develop appressoria at the tip of hyphaeby which it attaches to the host surface.



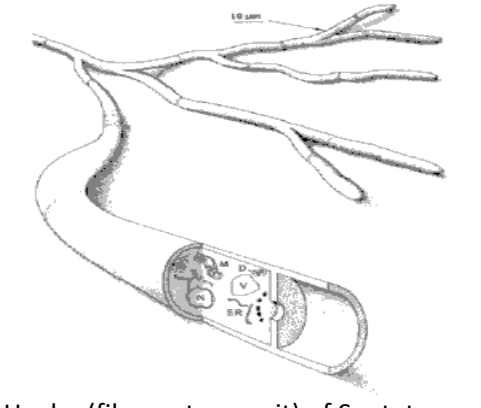
Septate mycelium Ex:*Rhizoctonia*

TERMINOLOGY:

Thallus: (pl. Thalli;Gr.Thanos=shoot) The somatic phase of the fungus. It is the body of the fungus typically consisting of microscopic threads or filaments that branch in all directions, spreading over or with in substratum utilizedfor food.

Mycelium: (pl.mycelia;Gr.Mykes= mushroom,fungus).A mass of hyphae constituting the body (thallus) of a fungus.

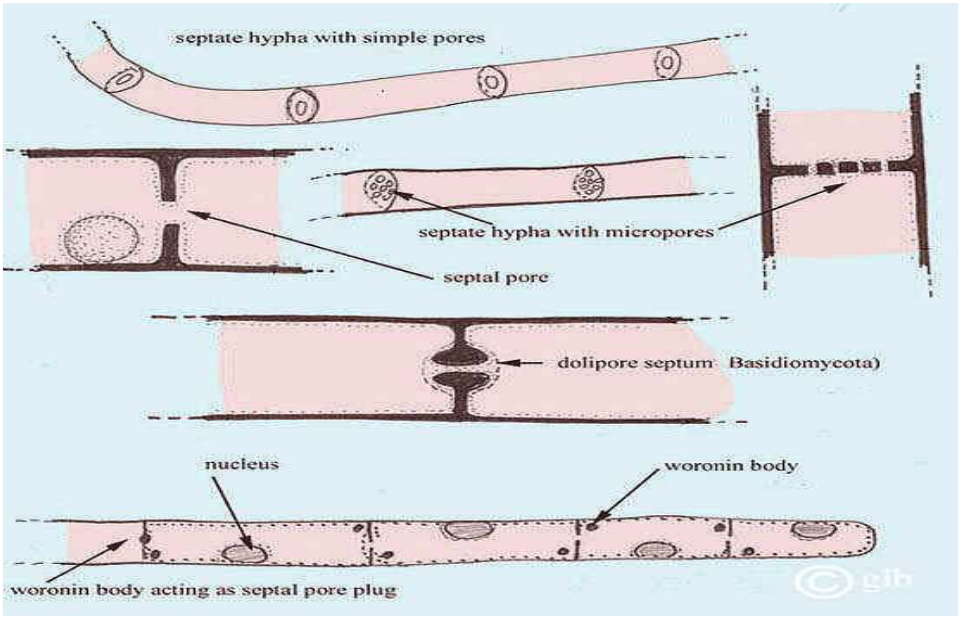
Hyphae:(pl. hyphae; Gr.hypha=web)A thin transparent tubular structure filled with a layer of protoplasm.



Hypha (filamentous unit) of Septate mycelium

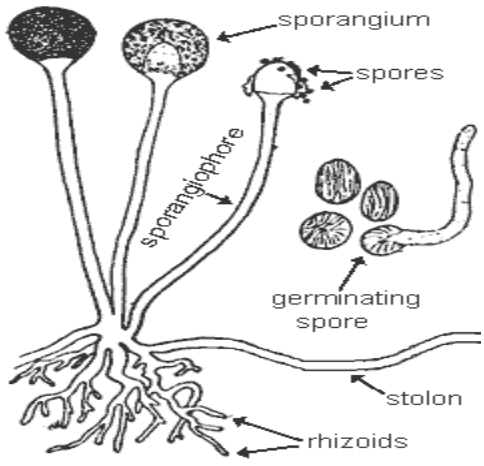
Septum:(pl.septa; L.septum=partition) A cross wall in a hypha.

Septate hypha:A hyphae with more or less regularly occurring cross walls (septa).eg:- *Drechslera.*



Various types of septa in fungi

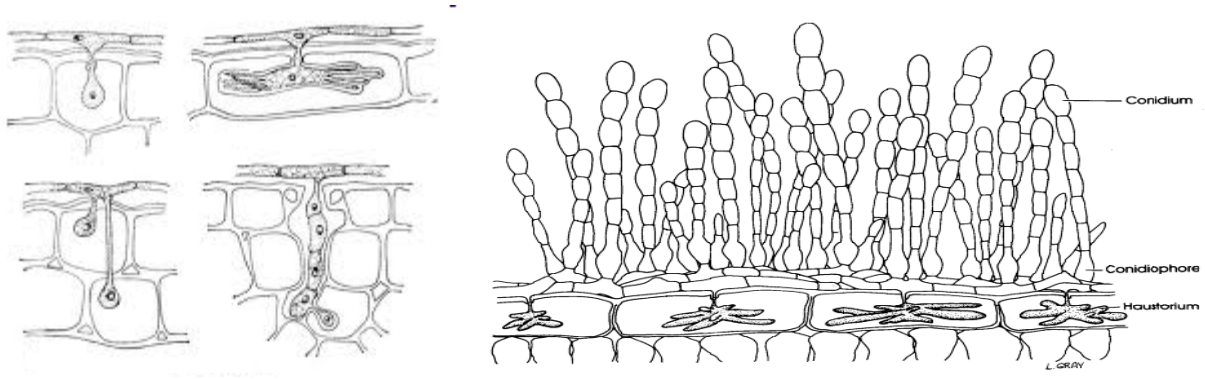
Aseptatehypha:(coenocytic-Gr.koinos=common+kytos = a hollow vessel).A hypha without septa. The nuclei are embedded in the cytoplasm without being separated by cross walls. eg:-*Rhizopus*.



Coenocytic (Aseptate) mycelium in *Rhizopus*sp.

Ectophytic/ Ectobiotic: (Gr.ektos= outside+bios = life). A fungal mycelium living on the surface of the host.*eg:-Oidium.*

Endophytic/Endobiotic:(Gr.endo=inside+bios=life). A fungal mycelium living within the cells of the *host.eg:-Oidiopsis.*



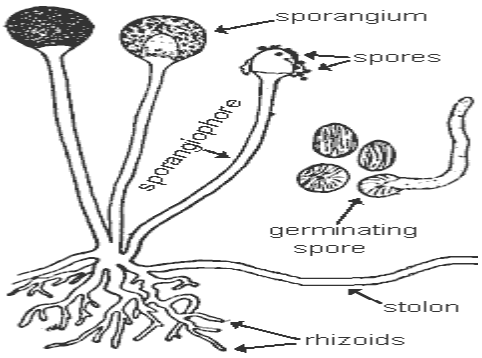
Ectophytic growth in powdery mildew fungi and development of various shapes of haustoria

Ex: *Oidium*sp.

MODIFICATIONS OF VEGETATIVE STRUCTURES

Sometimes special forms of mycelium are formed to perform special functions such as a. obtaining nourishment b. overcoming unfavourable conditions i.e., over overwintering, over summering) and c. reproduction. The following are the various types of modifications of fungal mycelium.

1. Rhizoids:(Gr.rhiza=root +oeides=like). Rhizoids are slender root like branched structures produced by some fungi which are useful for anchoring the thallus to the substratum and for obtaining nourishment *.eg:-Rhizopus stolonifer.*



Rhizoids

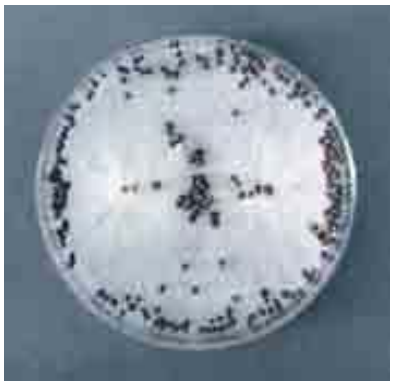
Rhizoids in *Rhizopus*

2. Rhizomorphs:(Gr.rhiza=root + morphe =shape).A thick strand of somatic hyphae in which the hyphae lose their individuality and form complex tissues, which are resistant to adverse conditions and remain dormant until favourableconditions return. The structure of growing tip of rhizomorph somewhat resembles that of a root tip, hence the name *rhizomorph.eg:-Armillariamellea.*

Rhizomorphs Ex:Basidiomycetes fungi

Rhizomorphs Ex: *Armillariamellea*

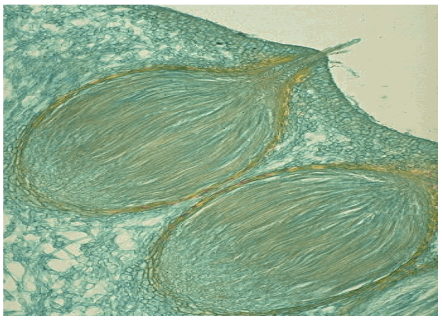
3. Sclerotium:(pl.sclerotia.Gr.skleron=hard) Sclerotium is a hard resting body formed due to aggregation of mycelium, resistant to unfavorable conditions, remain dormant for long periods of time and germinate on the return of favorableconditions.*eg:-Sclerotiumrolfsii and Rhizoctoniasolani.*



Sclerotial bodies in *Rhizoctonia*sp.

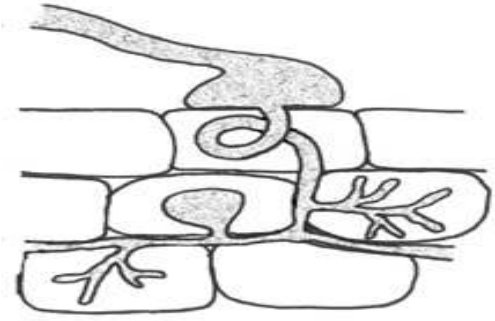
Sclerotial bodies in *Rhizoctonia*sp.

4. Stroma:(pl.stromata Gr .stroma-mattress), Stromais a compact somatic structure much like a mattress or a cushion on which or in which fructifications (spores or fruiting bodies) are usually *formed.eg:-Clavicepspurpurea*and *Colletotrichum. falcatum.*

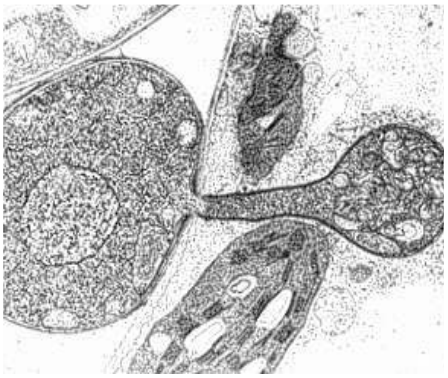


Perithium(Sexual fruiting body) embedded in StromaEx:*Claviceps*

5. Haustorium:(pl. haustoriaL.hauster=drinker). Haustorium is a special absorbing organ produced on certain hyphae by parasitic fungi for obtainin nourishment by piercing into living cells of the host.They may be knob like, elongated or branched like in shape. eg:-knob like-Albugo candida.; elongated-*Erysiphecichoracearum*.

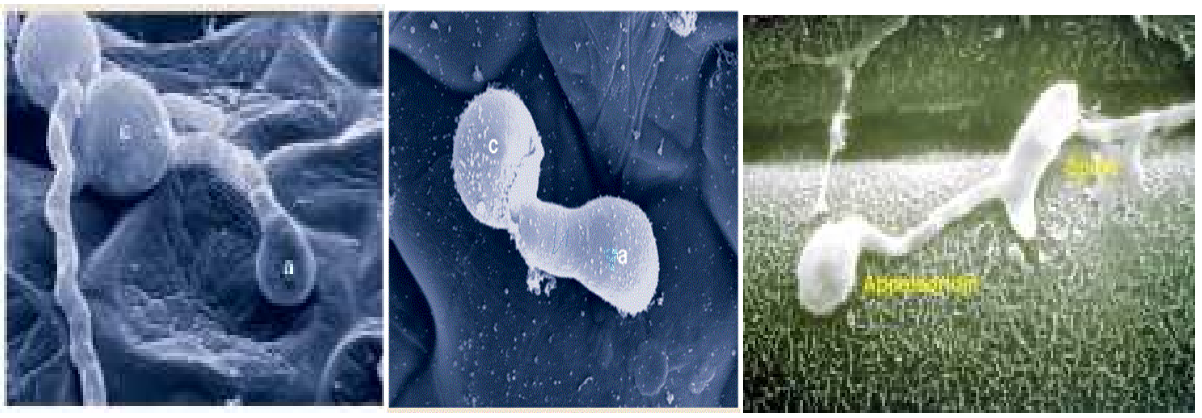


Development of elongated and branched haustoria in *Erysiphe* Sp.



Development of knobbed haustoria in host cell Ex:*Albugo*

6. Appressorium:(pl.appressoriaL.apprimere=to press against). A flattened, hyphal pressing organ from which a minute infection peg usually grows and penetrates the epidermal cells of thehost.*eg:-Pucciniagraminisf.sp. tritici.*



Appressorium

OBSERVATIONS AND RECORD

Observe the different vegetative structures of fungi and make sketches of all the structures and label the parts.

Work done report

Ex No:

Date:

Title:

2. STUDY OF REPRODUCTIVE STRUCTURES OF FUNGI (ASEXUAL AND SEXUAL)

Reproduction is the formation of a new individual. Sexual reproduction involves the fusion of compatible gametes / gametangia. This lacks in asexual and vegetative reproduction. The sexual state is termed as perfect state or teleomorphic state and the asexual state as imperfect state or anamorphic state. Fungi reproduce chiefly by means of spores,which are produced in three ways viz., asexual, sexual and vegetative. Spores may develop on specialized hyphae, exogenously or may be borne in special fruiting bodies or fructifications, endogenously. A fruiting body is any fungal structure that contains either sexual or asexual spores. The fruiting bodies are of great importance to fungi, not only in tiding over conditions of desiccation and freezing, but have vital significance from the point of view of multiplication of inoculum and maintenance. The various fruiting bodies have also been utilized as taxonomic character for distinguishing the various groups of fungi.

ASEXUAL SPORES:

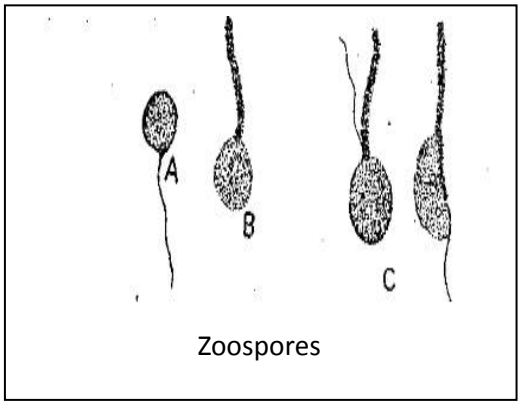
Asexual reproduction occurs usually under conditions that favour growth and several generations may be produced in one season. Asexual spores are capable of immediate germination and bringing about a rapid increase in numbers and spread of the organism under favourable conditions.

The following are the various types of asexual spores produced by fungi.

1.Zoospore(Planospore): Motile asexually produced sporangiospore bearing flagella and capable of moving in water. Sporangium containing zoospore is calledzoosporangium.

Ex: *Pythiumdebaryanum,*

Phytophthorainfestans.



Zoospores

2. Sporangiospore(Aplanospore): Non motile asexual spore found in a sac like structure is called sporangiospore. Sporangia are produced at the tips of special hyphae called sporangiophore

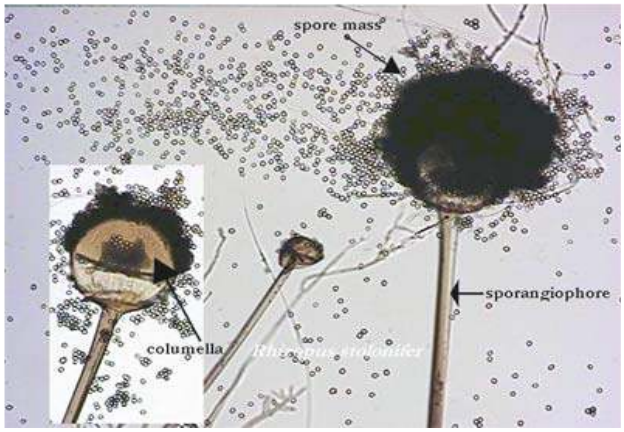
Ex: *Rhizopus stotonifer.*

3.Conidiospore(Conidia): Non motile asexual, single or multicelledspore produced at the tips or sides of a specialhypha called conidiophore.Theconidia may arise directly on the tips of conidiophore*(eg.Drechslera, Helminthosporium, Alternaria)* or inside specialisedfruiting bodies such as pycnidium, acervulus, sporodochium and synnemata.

(a) **Pycnidium:**Itis an asexual, spherical or flask shaped fruiting body lined inside with conidiophores and producing conidia.eg.*Phomalingam, Phomopsisvexans.*

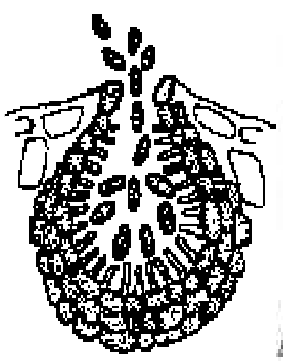
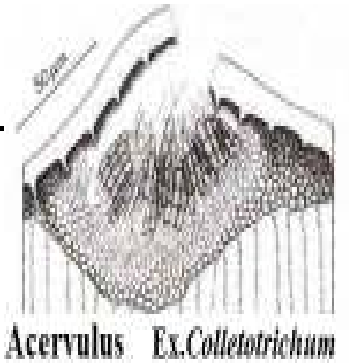
(b) **Acervulus:**A saucer shaped, asexual fruiting body with a mat of hyphae producing conidia on short conidiophores.

Eg:*Colletotrichumfalcatum,Pestalotiapsis palmarum.*



Liberation of Sporangiospores from*Rhizopus* sporangium


Conidia of *Alternaria*

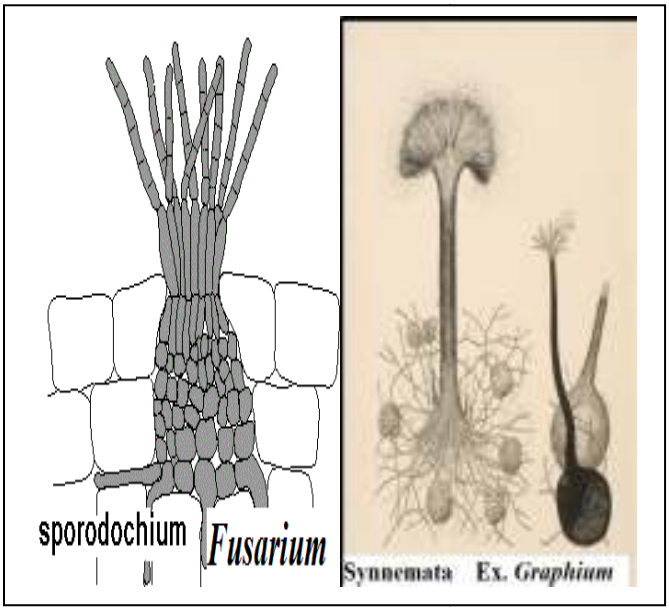
Pycnidium

(c) **Sporodochium:**A cushion shaped asexual fruiting body consisting of cluster of conidiophores woven together on a mass of hyphae

Eg: *Fusariumoxysporumf.sp. ciceri*

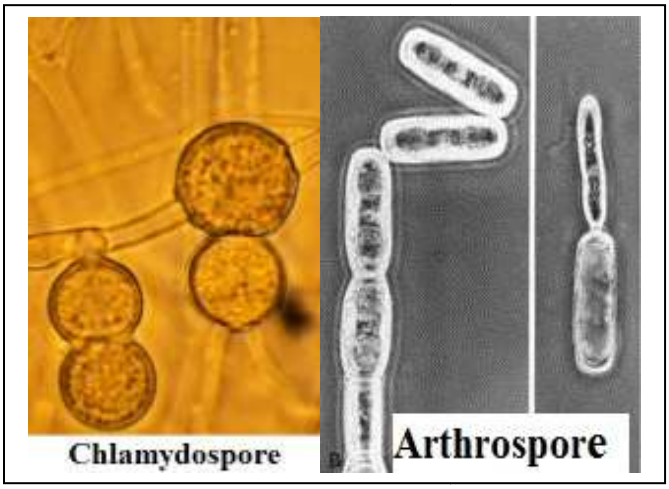
(d) **Synnemata:**A group of conidiophores united at the base producing conidia at its tip or along the length of synnema, resembling a long handled feather duster.

Eg:*Graphiumulmi*



4. **Chlamydospore:**A thick walled vegetatively produced asexual resting spore formed by the thickening of a cell of a hyphal cell.

Eg:*Fusariumoxysporum. f.sp. ciceri.*



5. **Arthrospore:**An asexual spore resulting from the fragmentation of vegetative

hyphae. Eg :*Erysiphaecichoracearum.*

6. **Blastospore:**Spores formed by budding.Budding is the production of small outgrowths (bud) from a parent cell. The bud increases in size and gets pinched off from the parent cell.

Eg:Yeast-*Saccharomyces cerevisiae*.

SEXUAL SPORES:

Sexual spores of fungi are variously known as oospores, zygospores, ascospores and basidiospores depending on the manner of their formation and the class of fungi to which they belong. They are the results of fusion between two gametes of opposite sex and function as resting spores, thus helping the fungus in over-summering or over-wintering. Sexual spores in fungi are less varied and less frequent compared to asexual spores, which are produced in great abundance.

The following are the important types of sexual spores produced by fungi.

1. **Oospore:** A thick walled sexual spore produced by the union of two morphologically different gametangiaEg.*Pythium debaryanum,Phytophthorainfestans.*

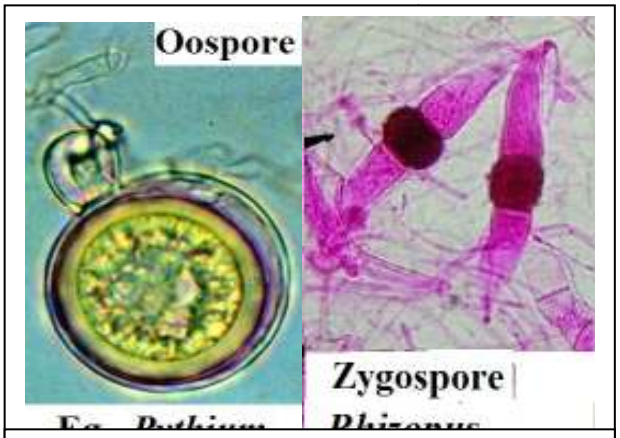
2. **Zygospore**:A thick walled sexual resting spore produced by the fusion of two morphologically similar gametangiaEg.*Rhizopusstolonifer.*

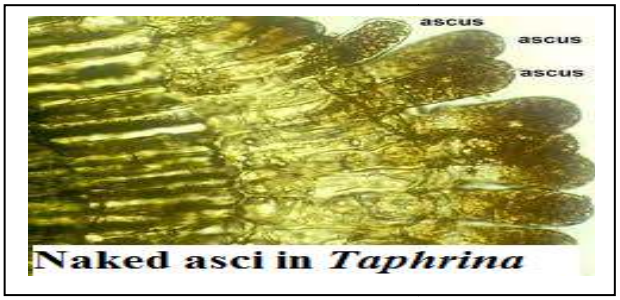
3.**Ascospore:** A sexually produced meiospore borne in an ascus.

Ascus:A sac like structure generally containing eight ascosporesformed after karyogamy and meiosis.

Asci may be produced in a sexual fruiting body called ascocarp or may arise naked without any ascocarp.

Naked ascus: Ascus arising naked without any fruiting body.Eg. *Taphrinadeformans.*





Ascocarp: A sexual fruiting body containing asci. There are four types of ascocarps produced by fungi.

(a) **Cleistothecium**:An entirely closed, more or less spherical shaped ascocarp and is many times provided on its body with appendages of various types, which serve as organs of anchorage and help in dissemination.

Eg.*Erysiphecichoracearum.*

(b) **Perithecium:**A closed, flask shaped ascocarp with a pore at the top ,a true ostiole through which ascospores are released.eg. *Clavicepspurpurea.*

(c).**Apothecium:** An open cup or saucer shaped ascocarp. Eg:*Tuber indicum.*

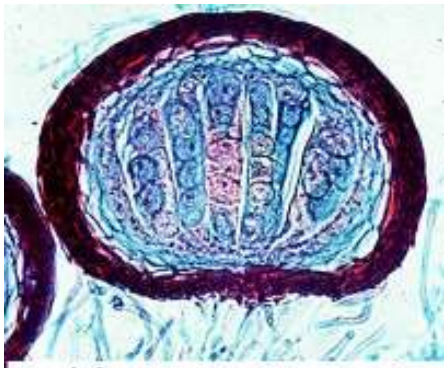
(d) **Ascostromata**:Astromaticascocarp bearing asci directly in loculesor cavities within stroma.eg. *Eisinoefawcetti.*

Pseudothecium:

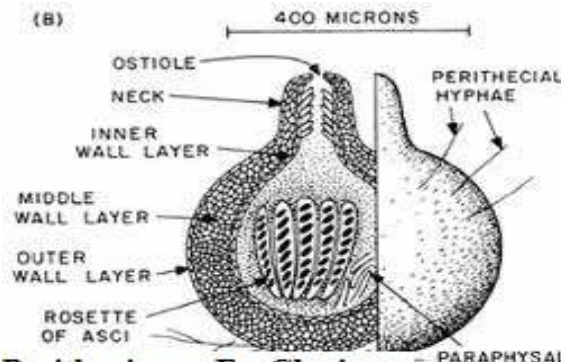
Anuniloculateascostromatais called pseudothecium or pseudoperithecium.

Eg:*Venturiainaequalis*

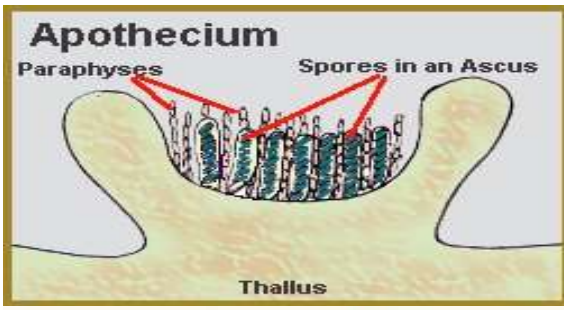
4.**Basidiospore**. Sexual spore borne on a basidiumfollowing karyogamy and meiosis.eg.*Pucciniagraminis.f.sp. tritici, Agaricus.bisporus.*



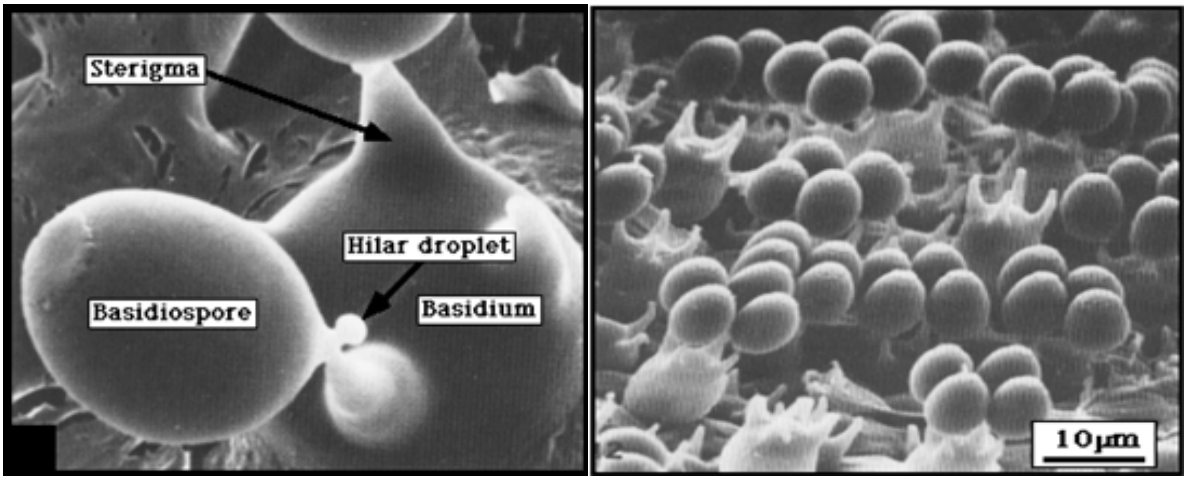
Erysiphe showing cleistothecium



Perithecium Eg.*Claviceps*



Ascostroma Eg. *Elsinoe fawsetti*



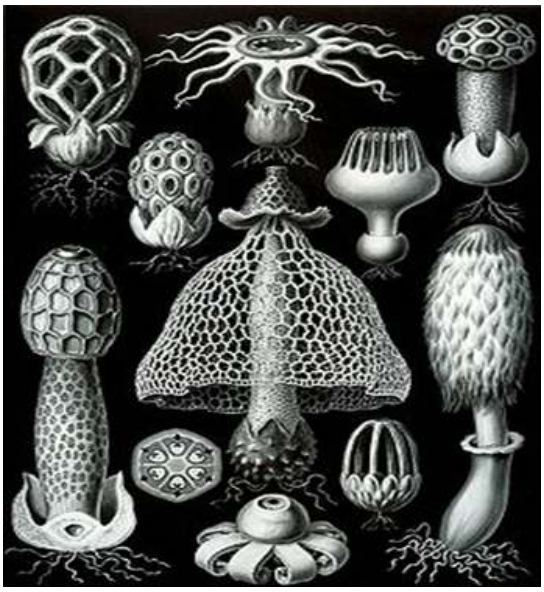
Basidum with four basidiospores on sterigmata

Basidium.A club shaped structure on which basidiospores are borne.

Basidiocarp.Sexual fruiting body that bears basidia and basidiospores.It may be thin, fleshy, spongy, leathery, corky or of any other structure.

Eg.*Agaricus,*

Pleurotus, Volvariella.



Various shapes of basidiocarps

OBSERVATIONS AND RECORD

Observe the different vegetative structures of fungi and make sketches of all the structures and label the parts.

Work done report

Ex No:

Date:

Title:

3. STUDY OF *PYTHIUM* AND *PHYTOPHTHORA*

Division: Eumycota
Sub-division: Mastigomycotina
Class: Oomycetes
Order: Peronosporales
Family: Pythiaceae
eg: *Pythium*

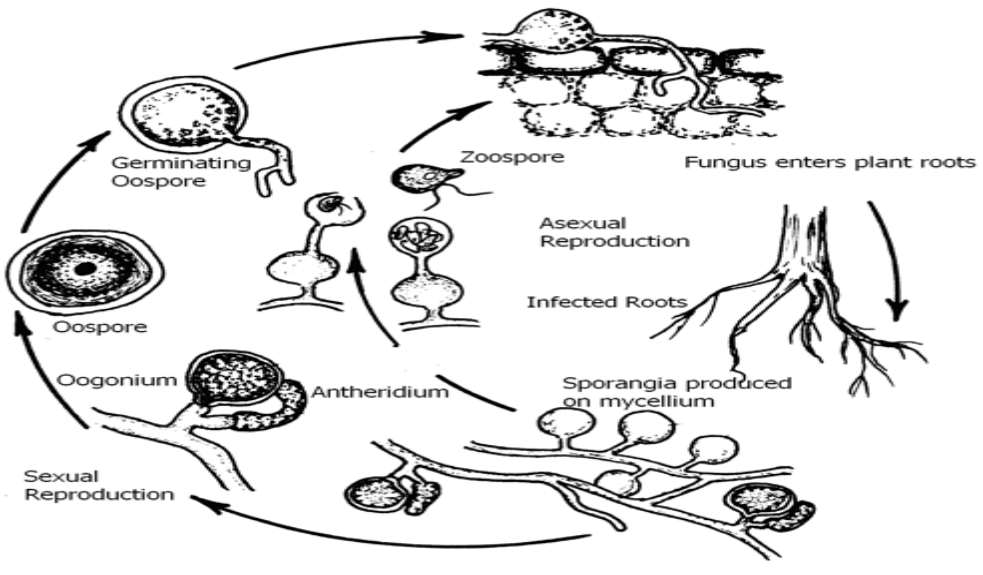
Habitat: Fungus is a facultative parasite and lives in soil on dead organic matter or parasitically on young seedlings of crop plants.

Mycelium: Well developed, branched, coenocytic, hyaline, intracellular mycelium without haustoria.

Asexual reproduction: Sporangia are large, globose(*P.debaryanum*) or irregularly lobed *(P. aphanidermatum*) formed either terminally or intercalary position on somatic. hyphae. Zoospores are produced in a vesicle which emerge out of sporangium.

Sexual reproduction: Gametangial contact. Oospores are smooth, thick walled, round, light brown and aplerotic.

Diseases: Damping-off of vegetable seedlings of solanaceous crops caused by *Pythium debaryanum* or*P. aphanidermatum.*



Pythium debaryanum

OBSERVATIONS AND RECORD

1. Obtain the culture of *P.debaryanum/P.aphanidermatum*.
2. Examine the morphological features of the fungus under a microscope.
3. Look for the mycelium, sporangia and oospores and describe the characteristics.
4. Make sketches of all the structures and label.

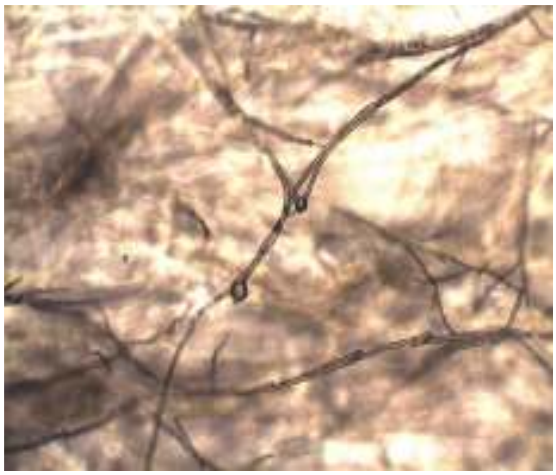
PHYTOPHTHORA

Division: Eumycota
Sub-division:Mastigomycotina
Class: Oomycetes
Order: Peronosporales
Family: Pythiaceae
eg: *Phytophthora*

Habitat: Fungus is a facultative parasite and lives in soil on dead organic matter (parasitically on potato tubers.)

Mycelium: Well developed, branched, coenocytic, hyaline, intercellular mycelium with haustoria.

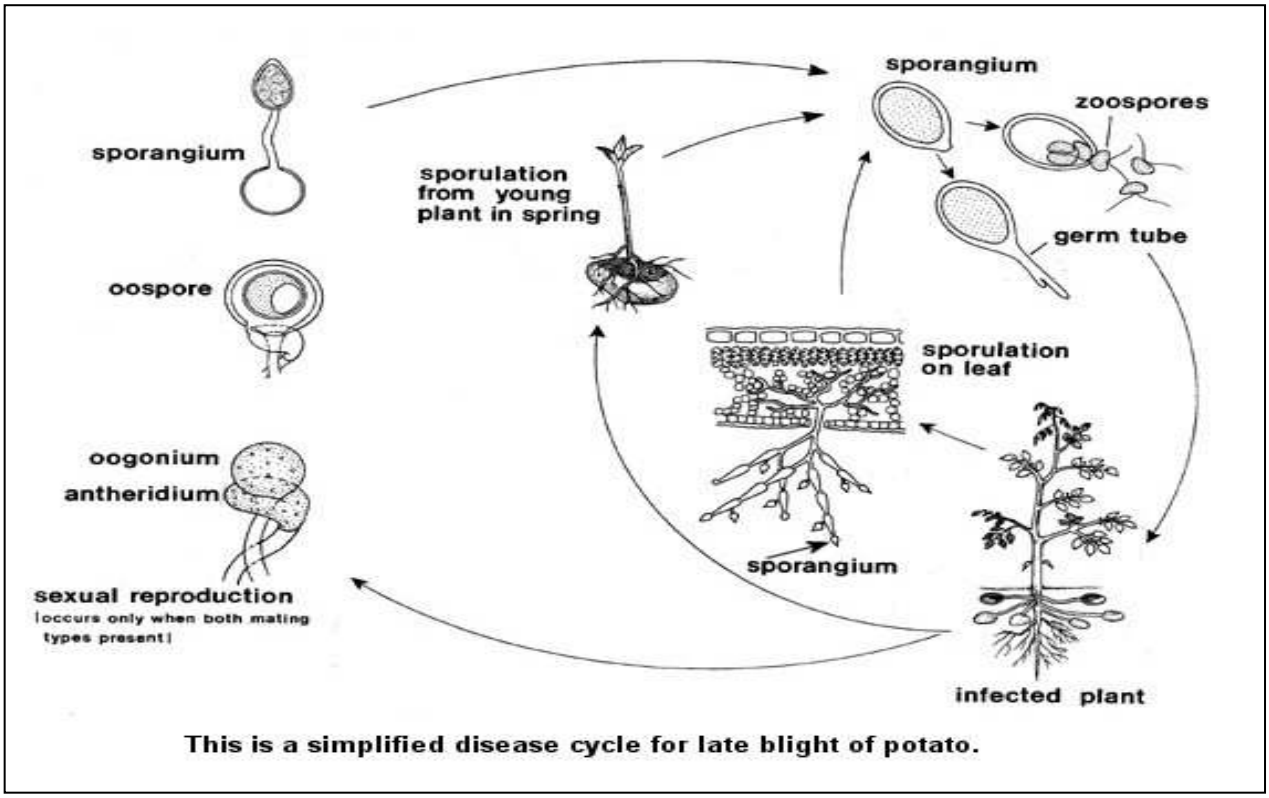
Asexual reproduction: Sporangia are lemon shaped,thin walled, papillate, formed terminally on sympodially branched sporangiophore. Zoospores are produce in sporangium.



Sporangiphore with sporangia

Sexual reproduction: Gametangial contact. Oospores are smooth, thick walled round, dark brown and plerotic.

Diseases: Late blight of potato (*Phytophthorainfestans)*, Leaf blight and black shank of tobacco(*Phytophthoraparasitica var. nicotianae).*



This is a simplified disease cycle for late blight of potato.

OBSERVATIONS AND RECORD

1. Obtain the culture of *P.infestans*or diseased specimens of leaf blight and black shank of tobacco.
2. Examine the morphological features of the fungus under a microscope.
3. Look for the mycelium, sporangia and oospores and describe the characteristics.
4. Make sketches of all the structures and label.

Work done report

Ex No:

Date:

Title:

4. STUDY OF *ALBUGO*

Division: Eumycota
Sub-division: Mastigomycotina
Class: Oomycetes
Order: Peronosporales
Family: Albuginaceae
Eg: *Albugo*

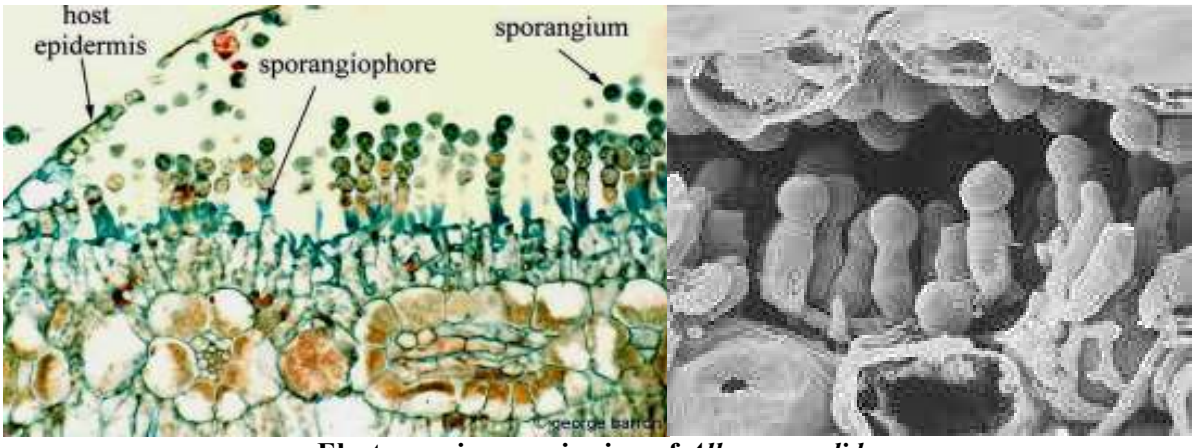
Habitat: Fungus is an obligate parasite on crucifers and lives in soil in the form of oospores or parasitically on plants.

Mycelium: Coenocytic, hyaline, endophytic, intercellular mycelium with knob shapedhaustoria.

Asexual reproduction: Sporangiophores are unbranched, hyaline, clavate, bears sporangia in chains in basipetal succession. Sporangia are spherical, thin walled, sessile, hyaline and germinate by zoospores. Zoospores are hyaline, biflagellate and kidney shaped.

Sexual reproduction: Gametangial contact. Oospores are round, thick walled, dark brown and outer wall warty.

Diseases: White rust on mustard caused by *Albugo candida*



Electron microscopic view of *Albugo candida*

OBSERVATIONS AND RECORD

1.Take out thin sections of diseased leaves.

2.Examine the sections under the microscope.

3.Look for the palisade layer of sporangiophoreswith chains of sporangia and describe the characteristics.

4.Make sketches of all the structures and label.

Work done report

Ex No:

Date:

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5.STUDY OF DIFFERENT GENERA OF DOWNY MILDEW FUNGI- *SCLEROSPORA ,PERONOSCLEROSPORA*

Division: Eumycota
S.D:Mastigomycotina
Class: Oomycetes
Order: Peronosporales
Family: Peronosporaceae
eg:*Sclerospora*
Peronosclerospora

The members of family Peronosporaceae cause downy mildew diseases in several economically important crops. The name downy mildew is given because of soft feathery growth observed on the lower side of affected foliage consisting of sporangiphores and sporangia which comes out through stomata.

Habitat: Fungus lives in the soil in the form of oospores and all the species are obligate parasites of vascular plants.

Mycelium: Mycelium is well developed, branched, hyaline, coenocytic, intercellular and produce haustoria.

Asexual reproduction: Sporangiophores are well developed, characteristically branched and determinate type. Sporangia are deciduous, papillate or non-papillate, germinate by zoospores or in some members germinate by germ tube directly, thus behaving like conidia.

Sexual reproduction: Gametangial contact. Oospores are the sexual resting spores, smooth, round, thick walled, light brown or dark brown in color and germinate by germ tube.

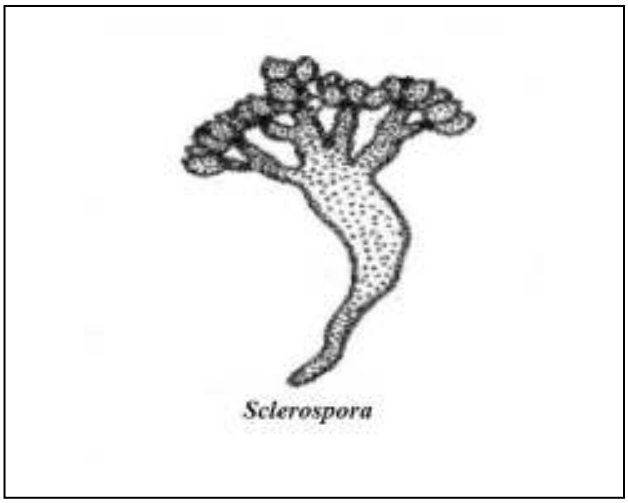
Downy mildew genera are differentiated based on the branching pattern of sporangiophores and method of germination of sporangia.

Distinguishing generic characteristics:

1. Sclerospora:

Sporangiophores are stout, having upright branches, bearing sporangia on sterigmata. Sporangia are hyaline, ovoid, smooth walled, papillate and germinate by zoospores. Oospore is plerotic

Eg:*Sclerosporagraminicola - downy* mildew of bajra



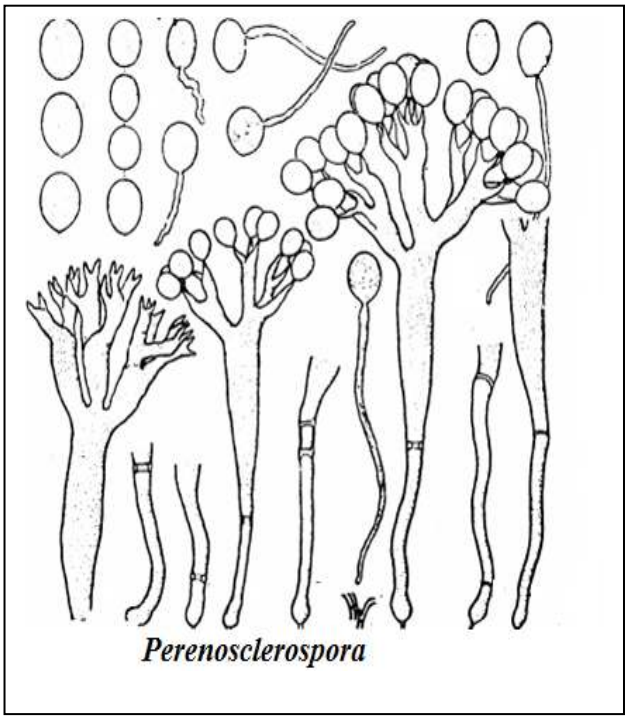
2. Peronosclerospora:

Fungus possess characteristics of both *Peronospora*and *Sclerospora.*

Sporangiophores are erect, short, stout, widening towards upper portion, dichotomously branched 2-5 times at apex bearing sporangia on sterigmata. Sporangia are hyaline, elliptical or ovoid, thin walled, non- papillate and germinate by germ tube like *Peronospora.* Oospore is plerotic type like *Sclerospora.*

Eg:*Peronosclerosporasorghi*- downy mildew of jowar

P. *philippinensis*- downy mildew of maize.



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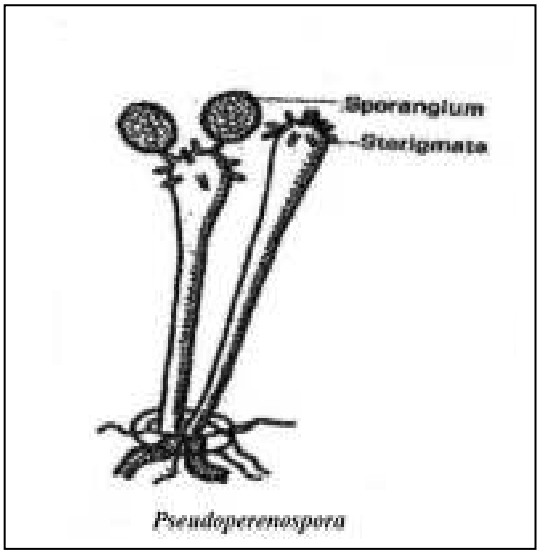
6.STUDY OF DIFFERENT GENERA OF DOWNY MILDEW FUNGI- *PSEUDOPERONOSPORA, PERONOSPORA, PLASMOPARA*, AND *BREMIA* ANDZYGOMYCETES FUNGI - *RHIZOPUS*

Division: Eumycota
S.D:Mastigomycotina
Class: Oomycetes Order: Peronosporales
Family:PeronosporaceaeEg*: PseudoperonosporaPeronospora, Plasmopara, Bremia*

1. Pseudoperonospora:

Sporangiophores are branched at acute angles with curved, blunt tips, bearing sporangia on sterigmata. Sporangia are greyish, ovoid, papillate and germinate by zoospores.

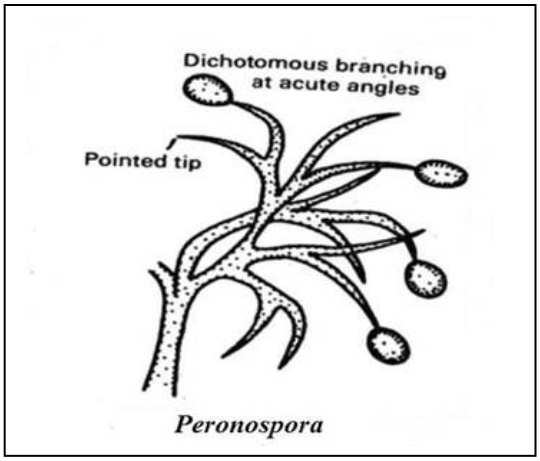
eg.***Pseudoperonosporacubensis-***downy mildew of cucurbits



2. Peronospora:

Sporangiophores are dichotomously branched 2- 7 times at acute angles and tips of branches are curved and pointed bearing sporangia on sterigmata. Sporangia are hyaline, ovoid, non- papillate and germinate by germ tube.

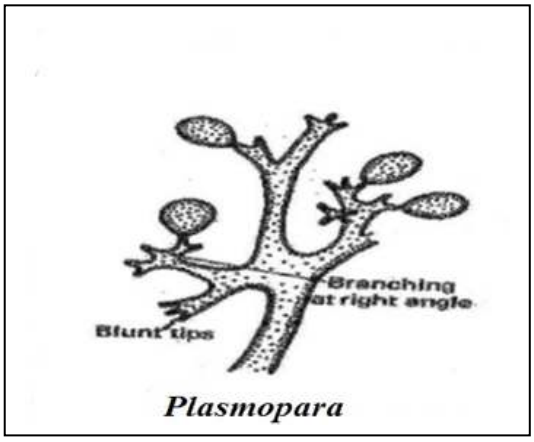
Eg:***Peronospora destructor-*** downy mildew of onion



3. Plasmopara:

Sporangiophores are branched at right angles to the main axis at regular intervals bearing sporangia on sterigmata. Sporangia are ovoid and germinate by zoospores.

Eg:***Plasmoparaviticola-*** downy mildew of grapes

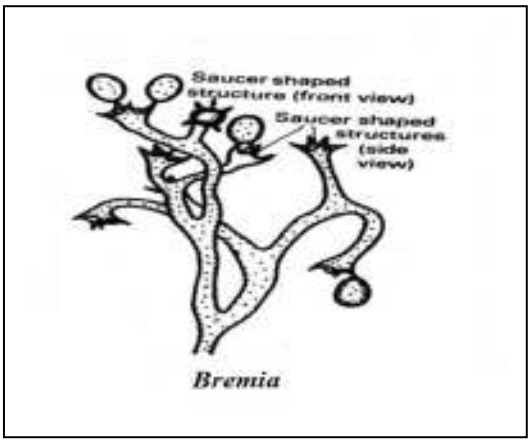


Plasmopara

4.Bremia:

Sporangiophores are dichotomously branched,tips of branches are expanded to cup shaped apophysiswith four sterigmata bearing sporangia. Sporangia are ovoid, papillate and germinate by zoospores.

Eg:***Bremialactucae-***downy mildew on lettuce

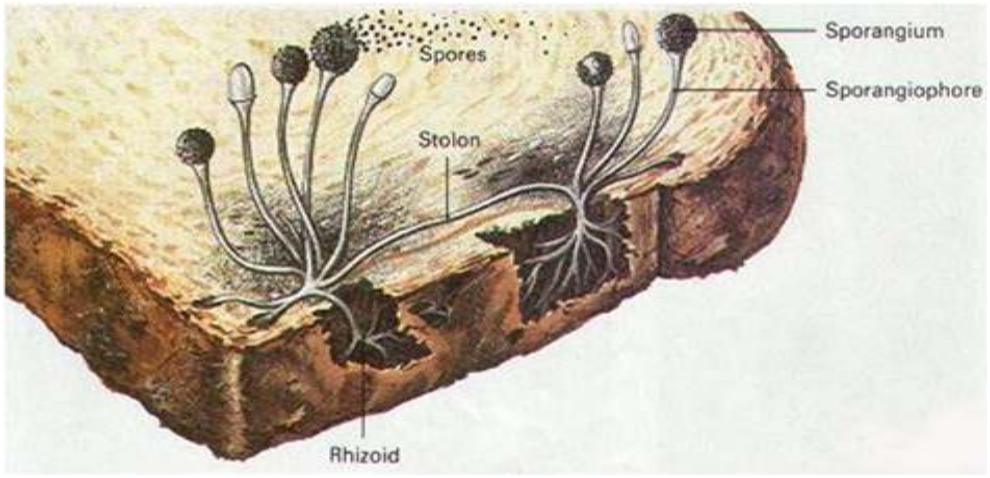


Bremia

ZYGOMYCETES FUNGI-*RHIZOPUS*

Division: Eumycota
Sub- Division: Zygomycotina
Class: Zygomycetes
Order: Mucorales
Family: Mucoraceae
Eg:*Rhizopus.*

Rhizopus stoloniferis known as common bread mold and is a general contaminant of several food materials. The fungus is mostly saprophytic but is a weak parasite. It causes soft rot of potatoes during storage.



Rhizopus stolonifer

Mycelium:

The fungus produces abundant soft cottony mycelium. The mycelium is coenocytic, well developed and is differentiated into rhizoids, stolons and sporangiophores.

Rhizoids/holdfast area cluster of brown, slender, branched root like structures which arise from the lower side of stolons and penetrate into the substratum .These are useful for anchoring the thallus into the substratum and for absorption of nutrients.

Stolons or runners are aerial hyphae which grow on the surface of substratum horizontally and connect the two nodal points (the junction of stolon and rhizoid or the point

from which rhizoids are produced)

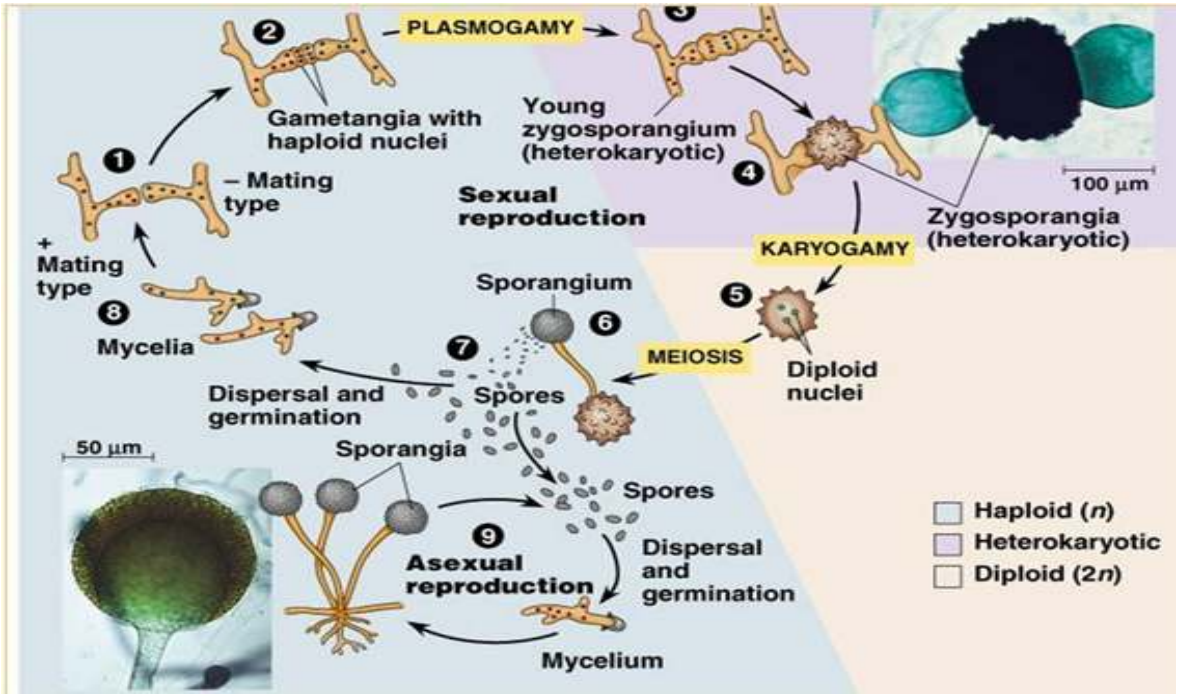
Sporangiophores are erect, unbranched hyphae usually produced in fascicles (groups) only from the nodes during asexual reproduction. Each sporangiophore bears a single sporangium at its tip. Sporangia are large, globose,manyspored with a sterile structure called columella.

Asexual reproduction:

Asexual reproduction is through non motile sporangiospores(aplanospores). Sporangiospores are uninucleate, globose, brown, smooth walled, non motileand are produced inside columellate sporangium. The sporangiospores are liberated by rupture of sporangial wall as a black mass on collapsed columella. The spores germinate under favourable conditions by germ tube that gives rise to mycelium.

Sexual reproduction:

The fungus is heterothallic. Sexual reproduction is by isogametangial copulation. Zygospores are thick walled, dark, warty sexual resting spores that develop in a zygosporangium formed as a result of fusion of gametangiaduring sexual reproduction.



Zygomycetes fungi-*Rhizopus*life cycle

OBSERVATIONS AND RECORD

1. Obtain the culture of *Rhizopus stotonifer.*
2. Examine the morphological features of the fungus under a microscope.
3. Look for the structures viz., coenocytic mycelium, sporangiophores, rhizoids, stolons, sporangia, columella and sporangiospores.
4. Make sketches of all the structures observed and label.

Work done report

Ex No:

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7. STUDY OF POWDERY MILDEW FUNGI –*OIDIUM, OIDIOPSIS* AND *OVULARIOPSIS*

Three types of conidial stages are recognised in powdery mildews.

1.*Oidium*:*(*** syn: *Acrosporium)*

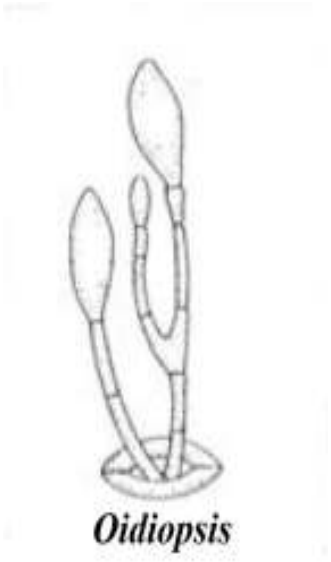
Majority of powdery mildew genera have *Oidium*type conidia. Mycelium is ectophytic, hyaline.Conidia develop from a flask shaped mother cell (spore mother cell) formed on a short conidiophore. Conidia are barrel shaped with flat ends and produced in chains. The conidia are also referred to as meristem arthrospores as these are formed by fragmentation of hyphae. The perfect stages viz., *Erysiphe, Podosphaera, Uncinula, Sphaerotheca*and *Microsphaera*produce *Oidium*as conidial stage.

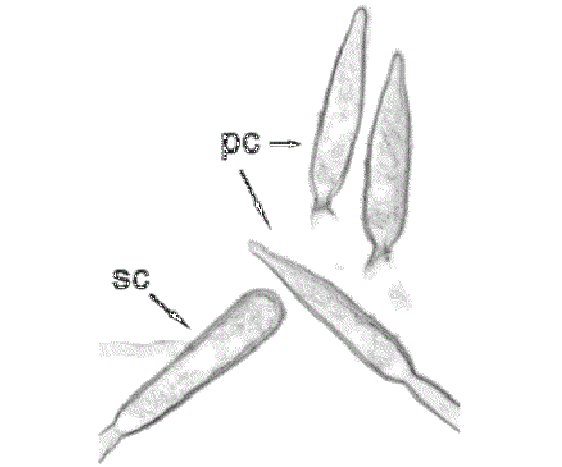
Oidium

2.*Oidiopsis*:

Mycelium is endophytic. Conidiophores may be branched or unbranched, erect, septate, hyaline and emerge through stomata. Conidia are produced singly and cylindrical in shape. Conidia are of two types. a. blunt tip b. pointed tip.

Eg:*Leveillula*sp. produce *Oidiopsis*as conidial stage.



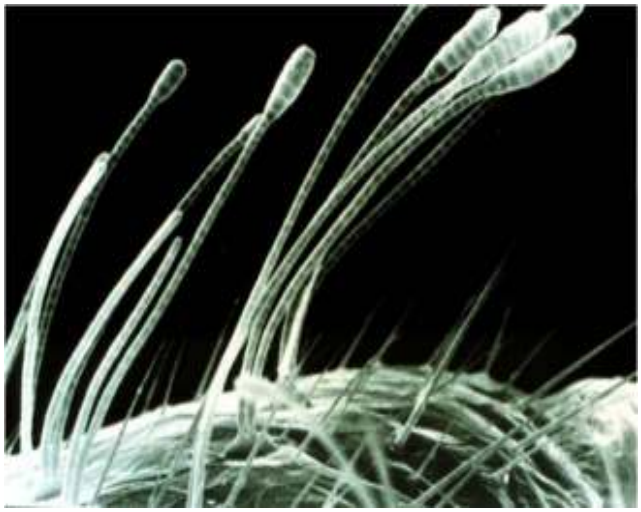


Oidiopsis haplophylli on *Ruta graveolens*: primary (pc) and secondary (sc) conidia (Bar = 20 mm).

***3. Ovulariopsis*:**

Mycelium is partly ectophytic and partly endophytic.The conidiophores are hyaline, septate, unbranched and bear a single conidium. Conidia are rhomboid in shape. In some species, the conidiphores are spiral in shape.

Eg:*Phyllactiniasubspiralis.*



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8. STUDY OF ASCOCARPS OF *ERYSIPHE,PHYLLACTINIA,UNCINULA,PODOSPHAERA*AND *MICROSPHAERA*

Division:Eumycota
Sub- division :Ascomycotina
Class: Plectomycetes
Order: Erysiphales

Sexual state	**Asexual state**
Erysiphe *Uncinula* *Podosphaera* *Sphaerotheca* *Microsphaera*	*Oidium spp.*
Leveillula *Phyllactinia*	*Oidiopsis sp.* *Ovulariopsis sp.*

Powdery mildews are the most common, conspicuous and easily recognisable plant diseases characterized by the appearance of white powdery superficial hyphae on aerial parts of living plants with large, one celled conidia produced terminally on an isolated aerial branched conidiophores with haustoria in the epidermal cells of their hosts. These diseases are most commonly observed on the upper side of the leaf but also affect the underside of the leaves.

Habitat: Obligate parasites of vascular plants.

Mycelium: Mostly ectophytic (superficial), endophytic(internal), or semi- endophytic,

branched, septate, hyaline with globoseor finger shaped haustoria.

Sexual reproduction :

Species are homothallic or heterothallic. Sexual reproduction is by gametangial contact. Antheridia and ascogonia are sex organs.Fruiting body (ascocarp) is a cleistothecium which is produced on superficial mycelium without stroma as a result of gametangial contact. The cleistothecia are first white and finally black in color when mature, globose, completely closed (non-ostiolate), with a wall made up of pseudoparenchymatous tissue of several layers called peridium.

The cleistothecia are provided with characteristic appendages,varyconsiderably in length and character. Asciare released by disintegration of peridialwall of ascocarp. Asci are globose to pyriform, sessile, unitunicate. Ascospores are elliptical or oval, hyaline, unicellular and eight in number in a ascus.

Types of cleistothecial appendages in powdery mildew fungi

1.Myceloid appendage: These are flexible, flaccid and resemble somatic hyphae. Eg:*Erysiphe, Sphaerotheca, Leveillula.*

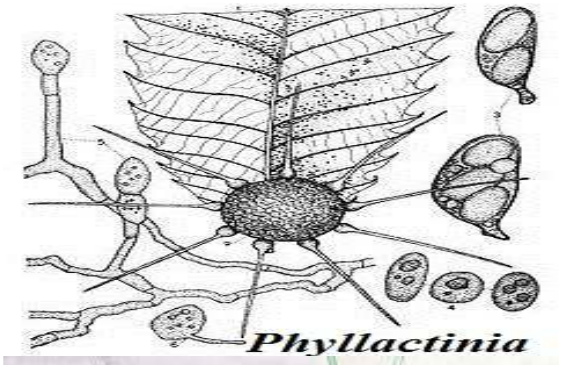
2.Circinoid appendage: (hooked) These are rigid with curled or coiled tips. ego*Uncinula.*

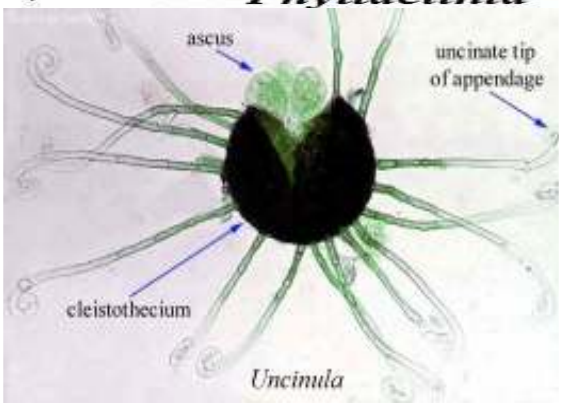
3.Dichotomously branched tips: These are rigid, flattened with dichotomously branched tips.

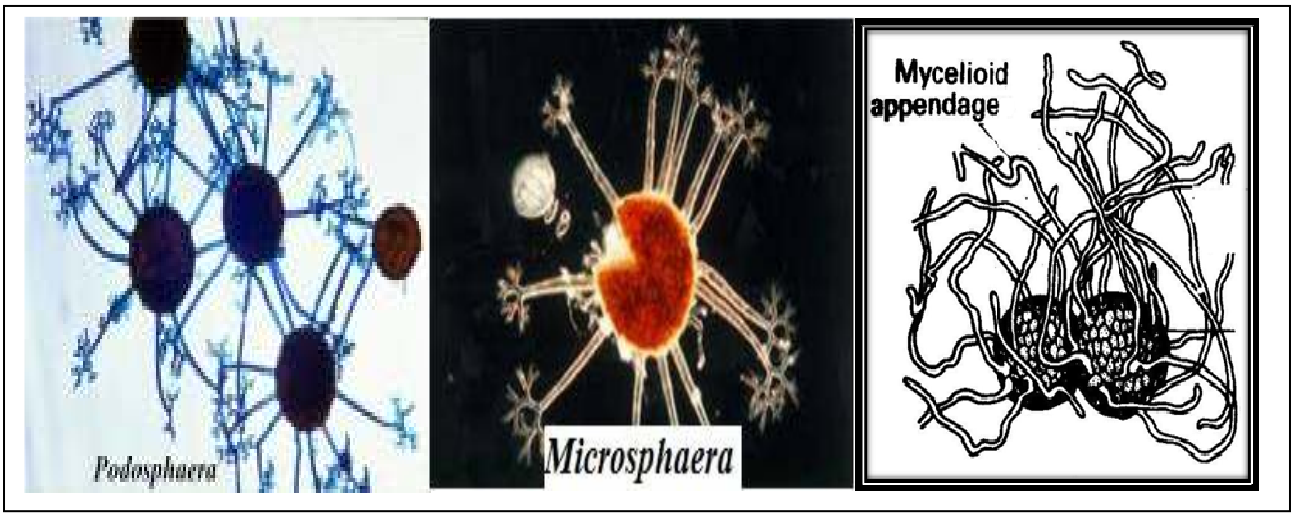
Eg: *Podosphaera, Microsphaera.*

4.Appendages with bulbous base and pointed tip: These are rigid, spear like with bulbous base and pointed tip.

Eg;*Phyllactinia.*







Powdery mildew diseases of various crops are caused by many genera of fungi and these genera are distinguished from one another based on the type of cleistothecial appendage, number of asciper cleistothecium, conidial type and nature of mycelium. Since cleistothecia are encountered rarely in nature, the identification of genera presents a problem. Hence, these fungi are identified by the characteristics of conidia and conidiophores.

KEY FOR THE IDENTIFICATION OF POWDERY MILDEW GENERA:

1. Nature of mycelium

a. Ectophytic b. Endophytic c. Semi- endophytic

2 .Number of asci per cleistothecium

a.One b. Many

3. Type of conidial state

a. *Oidium*b.*Oidiopsis*c. *Ovulariopsis*

4. Type of cleistothecial appendage

a. Myceloid

b. Dichotomously branched

c. Circinoid

d.Bulbous base with pointed tip

1. Ectophytic

a.One ascus per cleistothecium

*Oidium*type conidial state

- Myceloid appendage-
  eg: *Sphaerothecapannosa*- powderymildew on rose
- Dichotomously branched appendage
  eg:*Podosphaeraleucotricha*-Powdery mildew on apple

b. Several asci

*Oidium*type conidial state

Myceloid-eg:*Erysiphecichoracearum*-Powdery mildew on cucurbits / bhendi

Circinoid-eg.:*Uncinulanecator*-Powdey mildew on grapes

Dichotomously branched- eg:*Microsphaeraalni* -Powdery mildew on lilac

2. Endophytic

Many asci

*Oidiopsis*type conidial state

Myceloid–eg:*Leveillulataurica* -Powdery mildew on chillies

3. Semi- endophytic:

Many asci

*Ovulariopsis*type conidial state

Bulbous base with pointed tip- eg:*Phyllactiniacorylea*Powdery mildew on mulberry

Work done report

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9. STUDY OF RUST FUNGI *PUCCINIA* (DIFFERENT STAGES), *UROMYCES AND HEMILEIA*

Division: Eumycota
Sub- Division: Basidiomycotina
Class: Teliomycetes
Order: Uredinales
Family: Pucciniaceae
Eg: 1. *Puccinia*
2. Uromyces
3 Hemileia

The members of the order uredinales are popularly called as rust fungi. The word rust refers to the reddish brown colour of some of the species. It is the brick red colour of the uredia which gives its name i.e. rust and infected field appear rusty. The disease mostly occurs on leaves as pustules and may appear on both the surfaces depending on the host. The characteristic feature of rust fungi is the presence of stalked teliospores. They may be free from each other or variously united but never in the form of layers. The important rust genera are *Puccinia, Uromyces and Hemileia.*

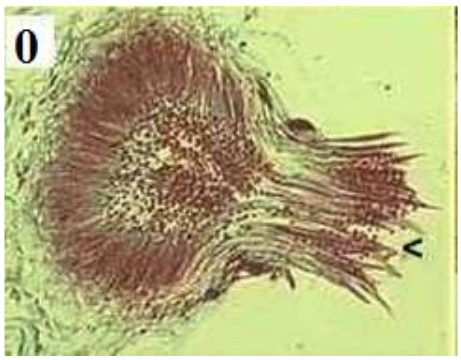
PUCCINIA

Habitat: Biotrophs, heteroeciousmacrocyclic rusts.

Mycelium: Septate, branched, intercellular with haustoria.

The fungus produces 5 distinct stages in its life cycle in a regular sequence.

0 Stage: Spermagonium: Flask shaped, ostiolate, contain numerous spermatia (male sex cells) and receptive hyphae (female sex organs). Spermatia - ovate, hyaline, unicellular, uninucleate and haploid.

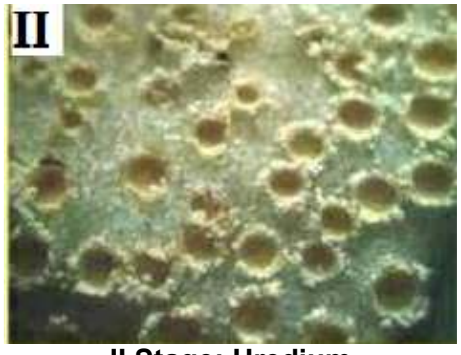


0 Stage: Spermagonium

I Stage: Aecia: Cup shaped, yellow with round to angular aeciospores in chains.

I Stage: Aecia

II Stage: UrediumSub epidermal,uredospores-repeating asexual spores. Oval,binucleate, unicellular, brown, thick walled, spiny, stalked, diploid.



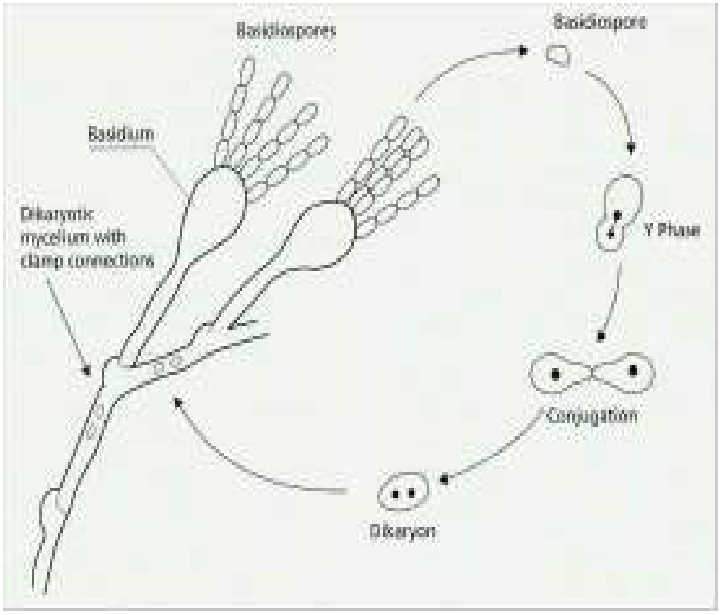
II Stage: Uredium

III Stage: TeliumTeliospores- sexual resting spores, two celled, spindle shaped, round or pointed at apex, constriction at septum, dark brown, thick walled, smooth, stalked, bi nucleate.

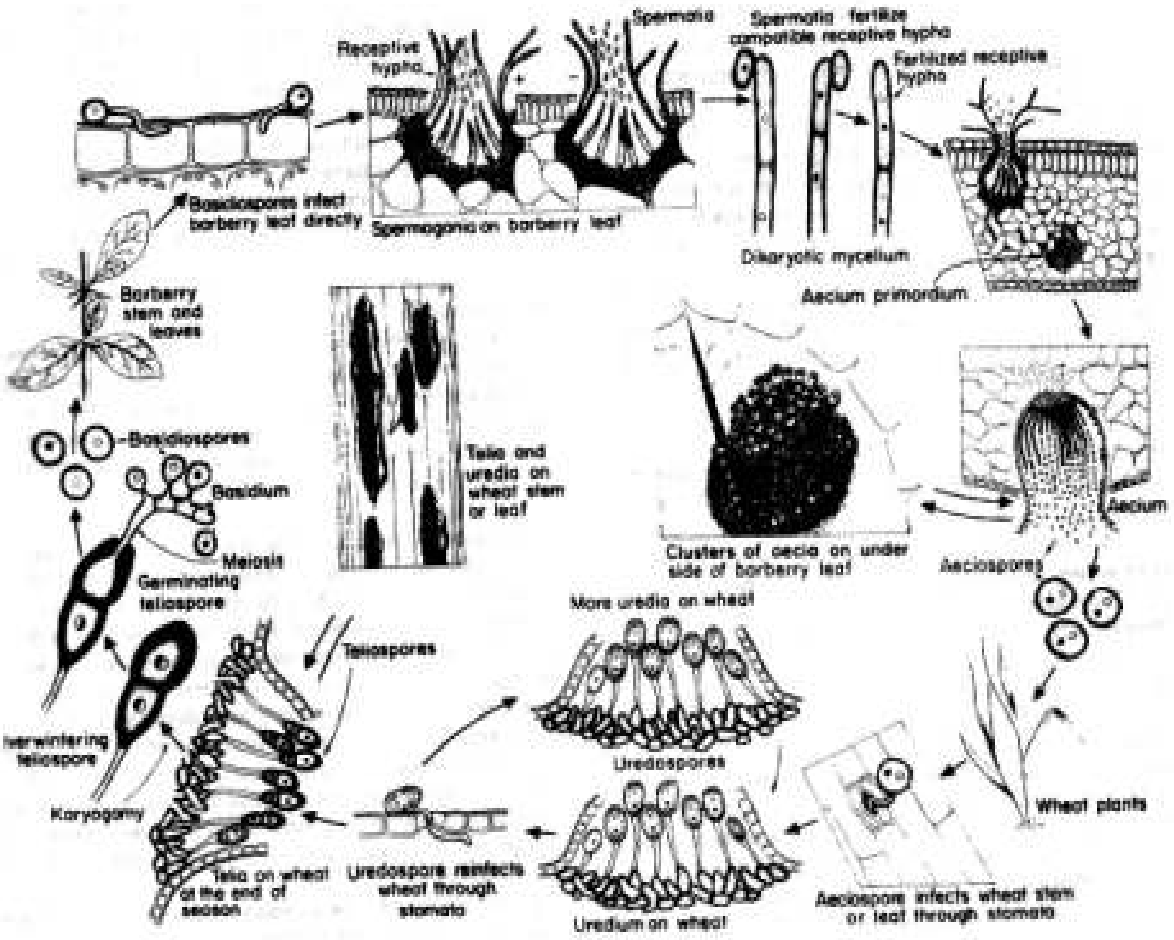
III Stage: Telium

IV Stage: Basidium: Basidiospores- 4 in number produced on sterigmata, uninucleate, haploid.

Eg: 1. *Pucciniagraminis f. sp. tritici*- black stem rust of wheat 2. *Puccinia penniseti*- bajra rust 3. *Pucciniapurpurea*- sorghum rust 4. *Pucciniaarachidis*- groundnut rust.



IV Stage: Basidium



Life cycle of ***Puccinia graminis f. sp. tritici***

UROMYCES

Habitat: Biotroph, autoeciousmacrocyclic rust. **Mycelium**: Septate, branched, intercellular with haustoria.

Spermagonium: Flask shaped, ostiolate with numerous spermatia (male sex organ) and receptive hyphae (female sex organ).

Aecium: Aecia- short, whitish, cup shaped. Aeciospores- round or elliptical, yellow, warty.

Uredium: Uredia- round, yellowish brown, appear

Teliospores of *Uromyces*

on both the surfaces, sub epidermal, Uredospores- ovate, light brown, echinulate, 1 celled, stalked.

Telium: Teliospores- dark brown, ovate, pedicillate, smooth walled, 1 celled, hyaline with papillum at the apex.

Basidium: Basidiospores- 4 in number produced on sterigmata.

Eg:*Uromycesphaseolitypica*-bean rust (syn: *U. appendiculatus*)

HEMILEIA

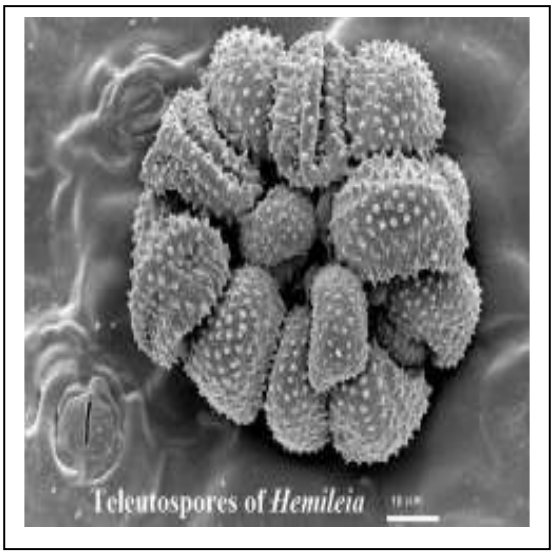
Habitat: Biotroph, autoeciousmicrocyclic rust.

Mycelium: Mostly inter cellular with haustoria,

Spermagonium and Aecium: unknown

Uredium: Uredospores- reniform, looking like an orange segment, convex side echinulate, concave side smooth

Telium: Teliospores- also called as leptospores(a spore which germinate without any resting period), turnip shaped, hyaline, thin walled, stalked, unicellular.



Basidium: Basidiospores 4 in number produced on sterigmata.

Eg:.*Hemileiavastatrix*-coffee rust

OBSERVATIONS AND RECORD

1. Collect the available diseased material
2. Take out thin sections of the diseased leaves
3. Examine the sections under microscope, identify and describe the morphological features of the pathogen.
4. Make sketches of all the structures observed and label.

Work done report

Ex No:

Date:

Title:

10. STUDY OF SMUT FUNGI –*SPHACELOTHECA,USTILAGO*AND *TOLYPOSPORIUM*:STUDY OF *GANODERMA*.

Division: Eumycota
Sub-division: Basidiomycotina
Class: Teliomycetes
Order: Ustilaginales
Family: Ustilaginaceae
Eg:*Sphacelotheca*
Ustilago
Tolyposporium

The smut fungi refer to the characteristic dark brown to almost black soot like powdery mass of teleutospores produced in sori on the affected plant parts. Majority of the fungi attack ovaries, which instead of forming grains, become transformed into sori containing full of teleutospores. Teleutospores or chlamydospores or smut spores commonly called as resting spores are the chief means of survival of the fungus in the absence of the host plant. The important characteristics of smut fungi are i. facultative saprophytes il. Teleutospores are formed from intercalary cells of mycelium iii. Basidiospores / sporidia are not borne on sterigmata. Mycelium is dikaryotic, majority with inter cellular hyphae with haustoria and clamp connections, but without dolipore septum.

Asexual spores :

Budding is the common method of asexual reproduction. The members posses no sex organs.Plasmogamy takes place by fusion of two compatible basidiospores or branches of promycelium. Teleutospores formed from dikaryotic mycelium, germinate and give rise to septate or aseptatepromycelium(basidium) and produce indefinite number of sporidia either laterally and / or terminally. Sporidia are monokaryotic and unable to infect host plant (except *Ustilagomaydis)*. Infection of the host is by means of dikaryotic mycelium.

Sexual spores :

Teleutosporesor chlamydospores are the sexual resting spores. Teleutospores are of taxonomic importance and characteristics of the spores are used as basis for identification of genera.

1. Sphacelotheca:

Majority of the grains are replaced by an oval or cylindrical dirty grey spore sac with small sorus. Sorusis enveloped in a peridium made up of fungal cells with a hard, slender central columella made up of host tissue.

Habitat: Seed borne

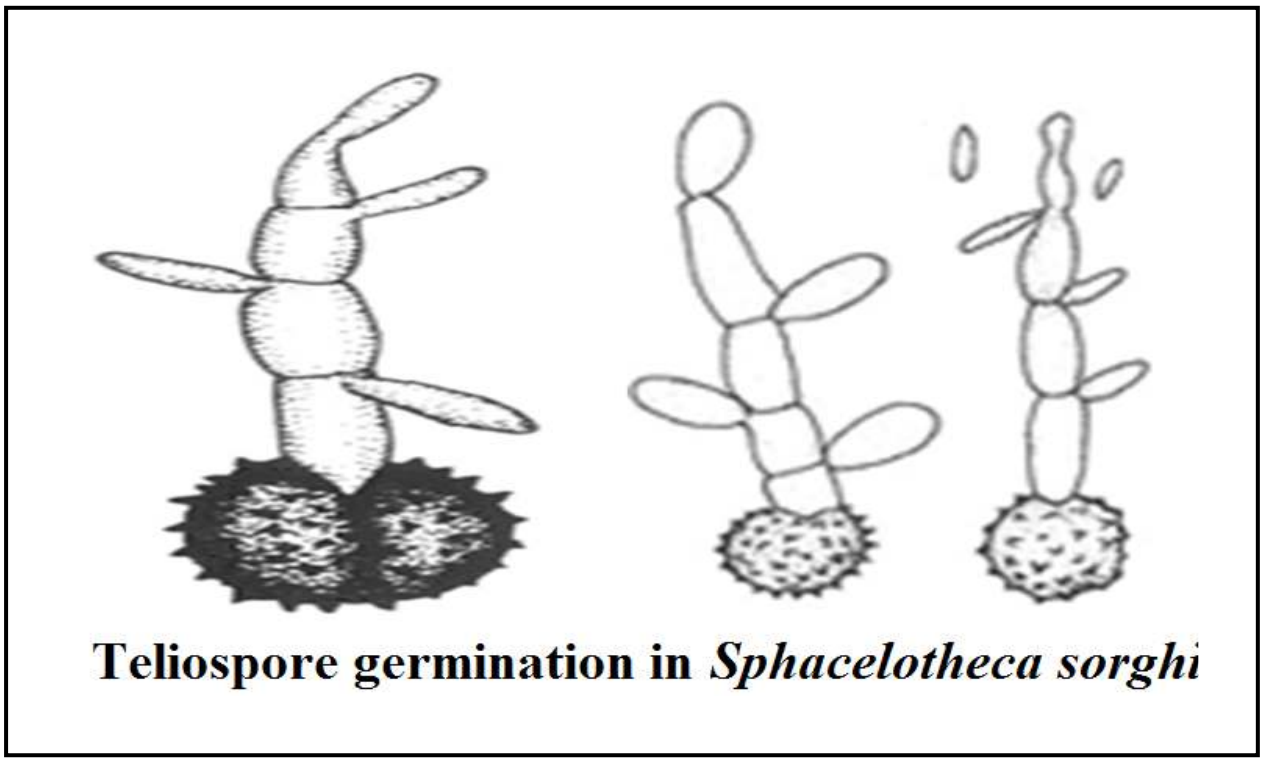
Mycelium: Intercellular

Teleutospores: Dark brown in mass, olive brown singly, smooth walled, oval, often united in loose balls which break up to individual spores when placed in water.

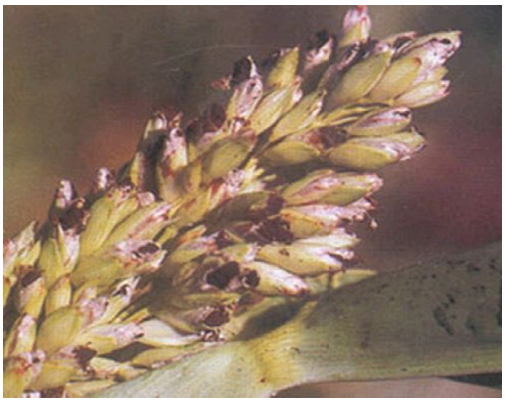
Promycelium / basidium: Septate**,**4celled, produce sporidialaterally and terminally.

Sporidia: Spindle shaped, single celled.

Eg.*Sphacelothecasorghi*- grain / covered / kernal/ short smut of jowar



Teliospore germination in *Sphacelotheca sorghi*



Spacelotheca

2. USTILAGO

Fungus induces gall formation on infected tissue mostly on grains. Sorus is covered by a membrane of host tissue.

Habitat:Seed borne.

Mycelium: Intercellular

Teleutospores: Separate, single celled, spherical, black, tuberculate wall.

Promycelium: Septate, 4 celled, produce sporidia laterally and terminally.

Sporidia: Ovate, single celled, hyaline.

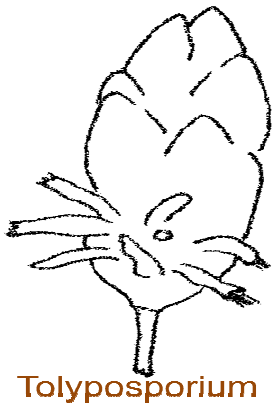
Eg:*Ustilagomaydis*- common smut of maize

3. Tolyposporium:

Only a few grains are transformed into smut sori. Each sorus is surrounded by healthy grains. Sori are long, cylindrical, slightly curved.

Habitat: Soil borne.

Mycelium: Intercellular



Tolyposporium

Teleutospores: Spores united in solid permanent spore balls, globose, brownish genechinulate, 1 celled

Promycelium: Septate, 4 celled, produce sporidia laterally and terminally.

Sporidia: Singles or in chains, spindle shaped.

Eg*Tolyposporiumehrenbergii*- long smut of sorghum

CHARACTERISTICS OF FAMILY GANODERMACEAE:

The fruiting body of the fungus is called bracket fungi which is formed laterally at the base of affected plant as a leathery stalked fan shaped or bracket shaped or without stalk, made up of trimitichyphal system, hymenophoreporoid. The bracket is tough, leathery or woody in texture and size vary from 1 to 20 inches in diameter. The stalk is cylindrical and brown to black in color.

The upper surface of the bracket is reddish brown in color and coated with a hard shiny substance resembling sealing wax while the lower side is white or yellowish in color. When examined with a lens minute holes or pits are seen allover the undersurface.These are the openings of numeral hymenial tubes or pores which are vertically oriented inside the fruiting body. Each basidium gives rise to 4 sterigmata, each of which bears a basidiospore at its tip.

Basidiospores are colored, 2 layered and cystidia are absent in hymenium. Bracket shaped basidiocarp, broadly and horizontally attached to the tree trunks by means of a short stalk or stipe. Ganoderma differs from other bracket fungi in having much longer span of spore release, extending upto 5 months.

Ex: *Ganodermalucidum*- Root rot and wilt of coconutand other palm trees and citrus.

Bracket fungi

OBSERVATIONS AND RECORD:

1.Collect the available diseased material

2.Take out powdery mass containing smut spores from diseased materia! and examine under microscope and describe the morphological features of the pathogen.

3.Make sketches of the structures and label.

Work done report

Ex No:

Date:

Title:

11.STUDY OF DIFFERENT GENERA OF IMPERFECT FUNGI – *SEPTORIA COLLETOTRICHUM* AND *PESTALATIOPSIS*

Division: Eumycota
Sub- Division:
Deuteromycotina
Class 1 :Coelomycetes
Order 1: Sphaeropsidales
Family: Sphaeropsidaceae
eg:*Septoria*
Order 2. Melanconiales
Family: Melanconiaceae
eg. 1. *Colletotrichum*
2. Pestalotiapsis
Class 2. Hyphomycetes
Order: Moniliales
Family 1.Moniliaceae
eg1. *Aspergillus*
2.Penicillium
3.Pyricularia
Family 2.Dematiaceae
eg1.*Alternaria*
2.Drechslera/Bipolaris
3.Helminthosporium
4.Cercospora
5.Phaeoisariopsis
Family 3. Tuberculariaceae
eg 1. *Fusarium*
Class 3.Agonomycetes
Order: Agonomycetales
Family: Agonomycetaceae
eg:1. *Rhizoctonia*
2.Sclerotium

The imperfect fungi technically called "fungi imperfecti" comprise a large group of organisms whose perfect stage (teleomorphic or sexual stage) spores are absent, rare or unknown. These are mostly conidial stages(anamorphic or asexual stage) of Sub- Division Ascomycotina or more rarely Sub- Division Basidiomycotina. Whenever the sexual stage of an imperfect fungus is discovered it is transferred to its systematic position among fungi in Sub- Division Ascomycotina or Basidiomvcotina.

Some of them are saprophytes,many are plant pathogens causing severe losses to the agricultural crops. These fungi are differentiated on the basis of their conidial characteristics and by method of formation of conidiophores bearing conidia.

General characteristics:

Themycelium is septate, freely branched and well developed. The fungi sporulate producing conidiophores on which conidia are produced except in some members. The conidiophores are produced singly in bunches or in compact masses inside characteristic fruiting bodies, suchaspycnidium, acervulus, sporodochiumand synnemata.

The shape, colour size and septation of conidia are highly variable. Some members are characterized by the formation of sclerotial bodies which are a bunch of sterile, thick walled, septatehyphal cells, united together in the form of a ball. These bodies may help the fungus to survive adverse climatic and environmental conditions and to germinate when favourable conditions prevail.

1. Septoria:

Pycnidia: Globose, papillate, ostiolate and brown. Pycnidial wall is consisting of pale brown cells.

Conldliogenous cells: Hyaline, broad and round at base and narrow at tip or barrel shaped or cylindrical.

Conidia: Hyaline, many septate and filiform.

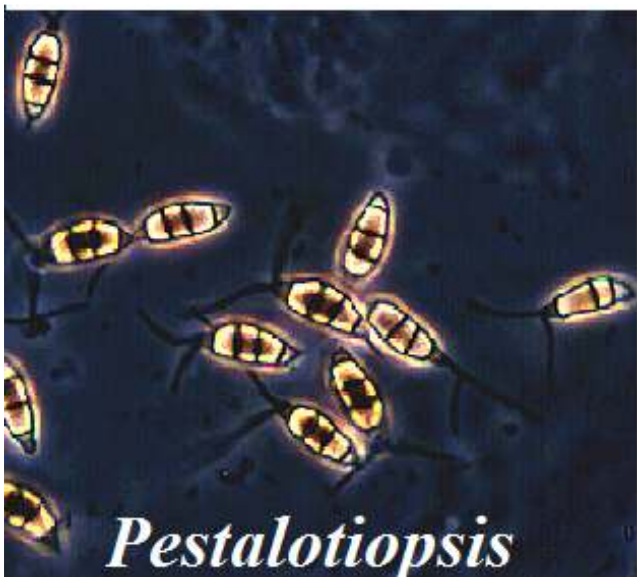
Eg:*Septorialycopersici*- leaf spot of tomato

2.*Pestalotiopsis:*

Acervulus: Epidermal or subepidermal, composed of thin walled polyhedral cells

Conidiophores: Short, branched.

Conidia: Five celled, pointed hyaline end cells with 2 or more apical simple or dichotomously branched appendages, median cells brown, fusiform, ego *Pestalotiopsispalmarum~* grey blight on coconut



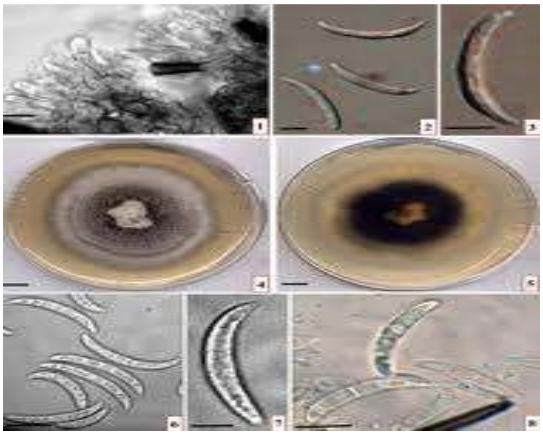
3. Colletotrichum:

Acervulus:Subepidermal, saucer shaped, with dark setae at the edge or among conidiophores, setae- long, darkbrown,septate.

Conidiophores: Simple, elongate, brown, aseptate

Conidia: Hyaline, 1 celled, falcate (sickle shaped)

eg.Colletotrichum falcatum- red rot of sugarcane, C. *lindemuthianum*- bean anthracnose.



Colletotrichum

Work done report

Ex No:

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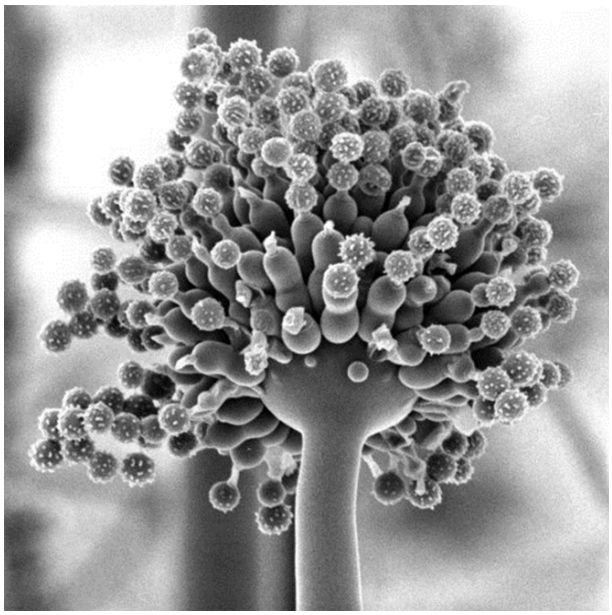
12.STUDY OF DIFFERENT GENERA OF IMPERFECT FUNGI- *ASPERGILLUS*, *PENICILLIUM* AND *PYRICULARIA*

1. Aspergillus :

Well known saprophyte, grown on all types of substrate and also a weak parasite and commonly called as "weed of the laboratory".



Aspergillus

Mycelium: Septate, branched, with multinucleate cells.

Conidiophores: The hyphal cell that gives rise to conidiophore is called foot cell. Conidiophores arise singly on somatic hyphae, long, erect, non septate and bears at its tip a spherical structure called vesicle, which bears two layers of bottle shaped structures called sterigmata or phialides on which conidia are produced in chains. The sterigmata of first layer (lowermost) are called primary sterigmata and the second layer (uppermost) layer are called secondary sterigmata.

Conidia: Globose, one celled, multinucleate, thick, rough walled and black.

Eg:*Aspergillusniger*- collar rot of groundnut

2.Penicillium

The characteristic conidial apparatus technically is called "penicillus" (*L. Penicillium*=small brush)because it resembles a small brush.

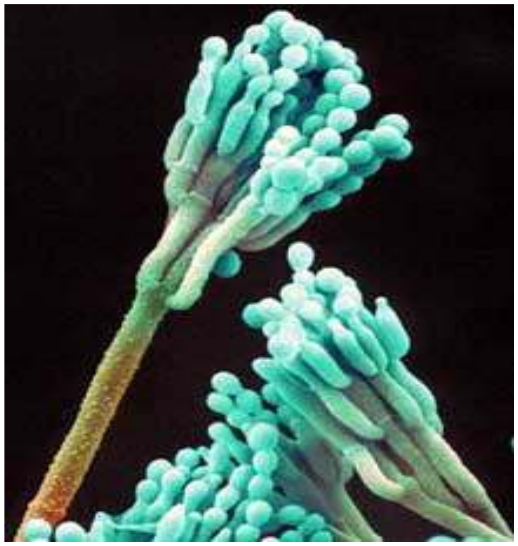
Mycelium: Highly branched, septate.

Conidiophores: Arise from any cell of hyphae (not from foot cell), branch once or twice about 2/3 of the way to the tip in a characteristic symmetric or asymmetric broom like fashion.

The first generation branches are called primary branches or rammi, on which whorls of second generation branches called metulae are produced.Each metula ultimately bears bottled shaped phialides which bear conidia in chains in basipetal succession.

Conidia: Globose, hyaline.

eg: Penicilliumnotatum - citrus blue mold.



Penicillium

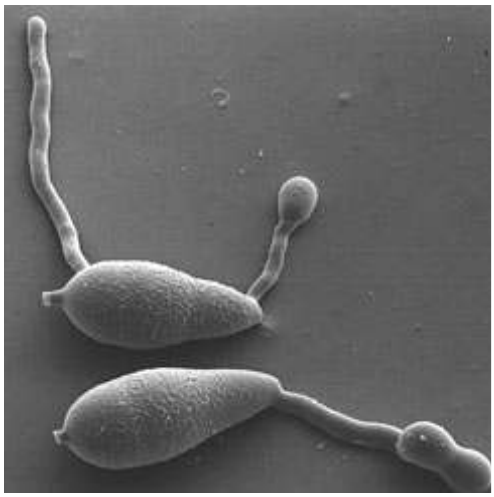
3.Pyricularia

Mycelium: Immersed in host tissue, branched, septate, hyaline or slightly coloured.

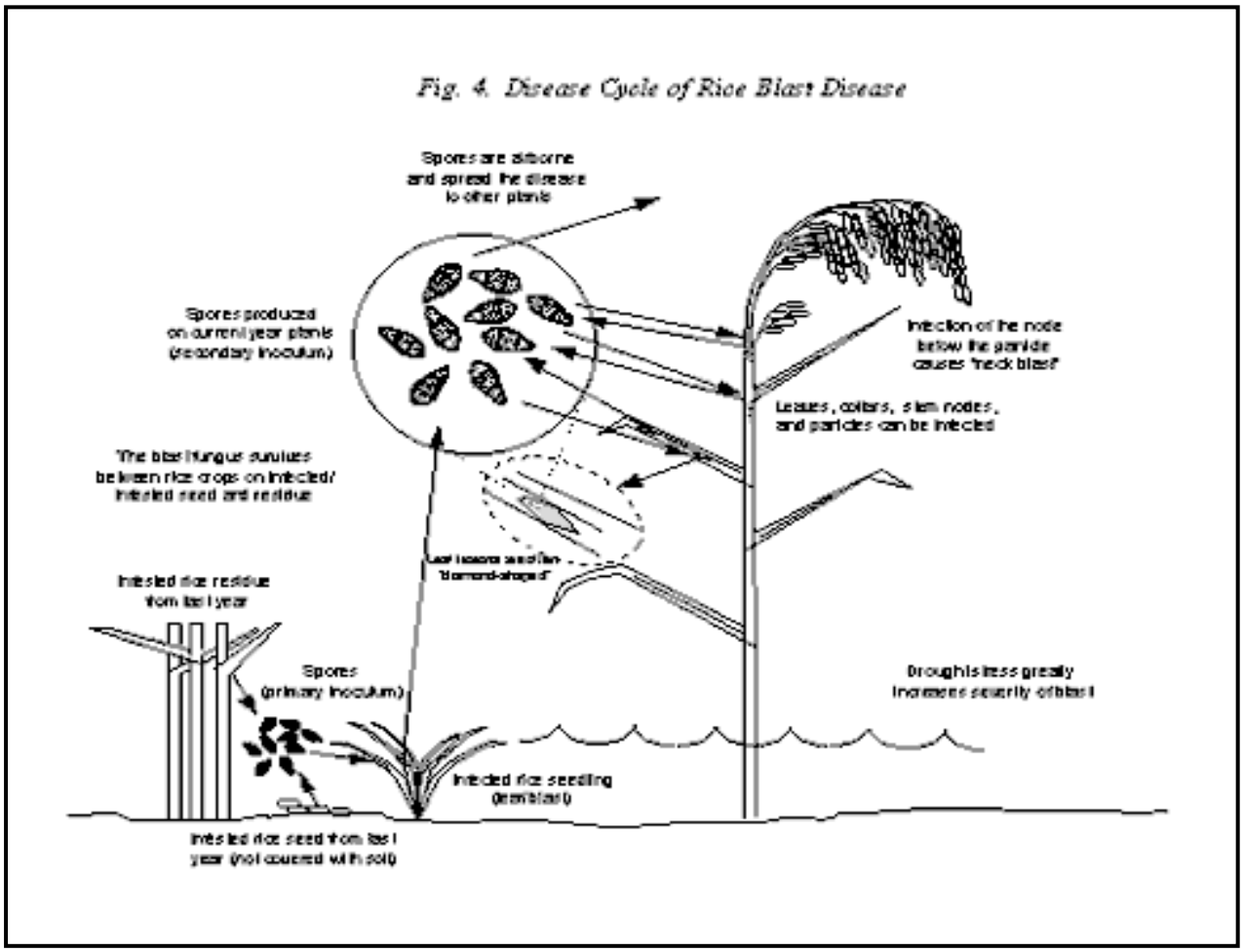
Conidiophores: Emerges through stomata or epidermal cells or cuticle, simple, erect, long, septate, hyaline.

Conidia: Hyaline, pyriform(pear shaped), broader at the base, tapering towards apex, usually 3 celled with a small basal appendage.

eg ..*Pyriculariaoryzae*- rice blast



Conidia of *Pyricularia*



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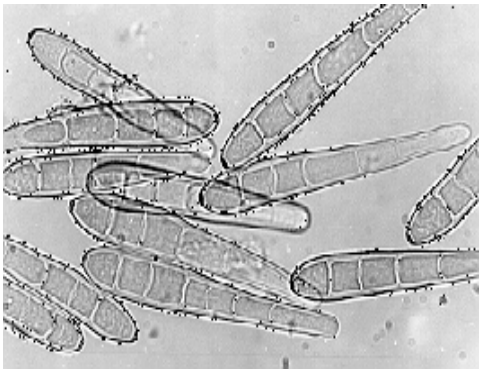
13.STUDY OF IMPERFECT FUNGI- *DRECHSLERA,HELMINTHOSPORIUM, ALTERNARIA, CERCOSPORA* AND *PHAEOISARIOPSIS*

1.Drechslera:

Mycelium: Immersed, branched, septate, brown. **Conidiophores:** Emerge through stomata, brown, erect, septate, simple, producing conidiasinglyat. Apexthrough small pores, sympodialgrowth(continuing growth from a point below apex and then forming a second conidia onnew apex), geniculate (knee joints).

Conidia: Dark, several celled, pseudoseptate, cylindrical, straight, germinating from any or all cells.

Eg: *Drechsleragraminea*- stripe disease of barley



Conidia of *Drechslera*

2.Helminthosporium:

Mycelium: Dark, often in substrate.

Conidiophores: Single or clustered, tall, erect, brown and simple.

Conidia: Develop laterally through pores beneath septa, often appear in whorls, brown, obclavate, pseudoseptate with prominent basal scar.

Eg:*Helminthosporiummaydis*- southern corn leaf blight

Conidia of *Helminthosporium*

3.*Alternaria*:

Mycelium: Branched, septate, dark brown

Conidiophore: Dark, septate, mostly simple bearing a simple or branched chain of conidia

Conidia: Dark, typically with both transverse and longitudinal septa with a simple or branched apical appendage, obclavate, frequently borne acropetallyin chains or borne singly

Eg*Alternariapalandui-onion* blight

Conidia of *Alternaria*

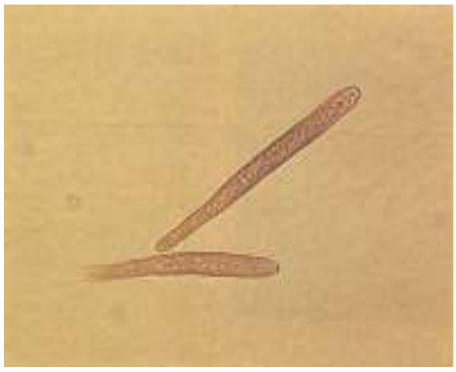
4. Cercospora

Mycelium: Immersed in host tissue. branched, **septate**1 pale brown

Conidiophores: Emerge in clusters through stomata, brown, septate, simple or rarely branched with knee joints, marking the scars of fallen spores

Conidia: Terminal, arise singly from conidiophore, hyaline, filiform, several celled (4 - 12 septate), a scar at the base.

Eg:*Cercosporaarachidicola*- tikka(early leaf spot)on groundnut



Conidia of *Cercospora*

5. Phaeoisariopsis

Mycelium: Septate, intercellular with branched haustoria, pale brown, immersed entirelyinleaf tissue.

Conidiophores : Emerging throughruptured epidermis in clusters, pale to olivaceous brown, smooth, geniculate or not, septate or aseptate, simple with prominent conidial scars.

Conidia: Light colored, cylindrical, usually straight or slightly curved, rounded at ends, base shortly tapered with a conspicuous hilum, mostly 3-4septate.

Eg:*Phaeoisariopsispersonata*(syn: *Cercosporidiumpersonatum*)tikka (late leaf spot) on groundnut

Phaeisariopsis

Work done report

Ex No:

Date:

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14. STUDY OF IMPERFECT FUNGI- *FUSARIUM, RHIZOCTONIA* AND *SCLEROTIUM*

***1. Fusarium*:**

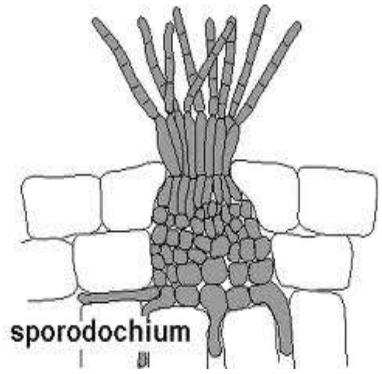
Mycelium: Superficial, cottony in culture, septate, hyaline,grouped into sporodochia.

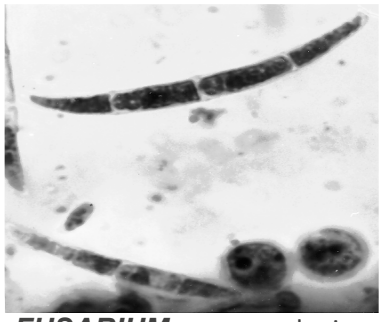
Conidiophores: Slender, simple, stout, branched irregularly bearing awhorl o phialides bearing conidia.

Conidia:Hyaline, 2 types- macroconidia(several celled,slightly curved orbent, pointed at both ends, sickle shaped with a foot cell),microconidia(1or2 celled, ovoid, single or in chains, hyaline) .

Chlamydospores: thick walled, terminal or inter calary, asexual spore formed by modification of hyphal cell

Eg*Fusariumoxysporiumf.sp.ciceri*- wilt of gram .



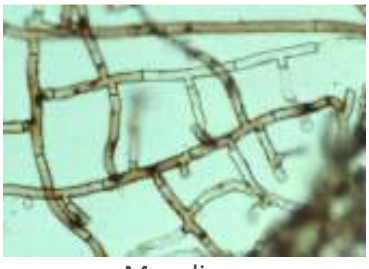


FUSARIUM macro and micro Cconidia

2.*Rhizoctonia*:

No spores

Mycelium: Brown, branches arise at acute angles, hyphal cells barrel shaped and long.



Mycelium

Sclerotia: Brown to black, variable in form (globose, oval or irregular), loosely formed connected by mycelial threads, hard, frequently small (less than 1 mm

diameter), no differentiation of sclerotial tissue.

Eg*Rhizoctoniasolani*- sheath blight of paddy

Sclerotia

3. Sclerotium

Spores lacking

Mycelium: White or light coloured,

Sclerotia: Brown to black, globose or irregular, compact,bigger(more than 1mm diameter in size), hard, tissues are differentiated into rind, central cortex, medullae.

Eg*Sclerotiumrolfsii*- root rot of groundnut


Sclerotia

OBSERVATIONS AND RECORD

1. Collect the available diseased material/fungal cultures
2. Take out thin sections/ scrapings of the diseased material
3. Examine the sections / scrapings/ cultures under the microscope, identify and describe the morphological features of the fungus
4. Make sketches of at! the structures and label.

Work done report

Ex No:

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15. ISOLATION OF PHYTOPATHOGENIC BACTERIA (LOCALLY AVAILABLE DISEASED PLANT MATERIAL) AND STUDY OF COLONY CHARACTERISTICS AND GRAM STAINING.

Isolation and identification of bacteria associated with diseased plant is important to determine whether bacteria are involved in plant disease. The method normally used to isolate phyto-pathogenic bacteria differs from that used for fungi. A suspension of bacteria is prepared from the infected material and loopfuls of this are streaked onto nutrient agar plates. The aim is to produce single colony that can be subcultures pure. Pure cultures are absolutely essential for pathogenicity assays and characterizing the pathogen for identification. The serial dilution method is used for isolating bacteria from diseased tissues contaminated with other bacteria. After surface sterilization of sections of diseased tissues, the sections are ground in small volumes of sterile water and then part of this homogenate is diluted serially. Finally, plates containing nutrient agar are streaked with a loop dipped in each of the different serial dilutions and single colonies of the pathogenic bacterium are obtained from the higher dilutions thatstill contain bacteria.

Choice of Material: Selection of the diseased tissue is important because pathogenic bacteria may occupy different locations in the plant. In isolation of bacteria, it is generally better to use newly collected material. The earliest stages of symptom development should be used. Old lesions and dead areas usually contain few pathogens and many saprophytes. Necrotic diseases usually start with tiny, dark greenish, spots which are excellent for isolations. Cankers and soft roots should either be at an early stage, or if, older lesions only are available, the advancing edge must be used, where the disease is spreading into healthy tissue. When crown gall is suspected in a woody plant a search must be made for young galls on young green stems. With wilts and other vascular infections small pieces of infected stem are usually good for isolation.

Preparation of material: Clan leaves and stems, carefully chosen and handled as aseptically, can often be used without surface sterilization. Roots and parts contaminated with soil should be gently washed with clean water as soon as possible after collection.

Medium: Nutrient agar is suitable for the isolation of most plant pathogens. The medium used for isolationmust have a dry surface. If, water is present the bacteria move around and a carpet of mixed growth results instead of the required single colony.

This exercise deals with the isolation of bacterium, *Xanthomonasaxonopodis*pv. *Citri* - casual agent of citrus canker.

Materials:

Citrus leaves infected by *Xanthomonasaxonopodis*pv. *Citri*(fresh), nutrient agar medium, surface sterliant(I % less toxic sodium hypochlorite), sterile razor blade, glass rod, sterile water, sterile test tubes and Petridishes, sterile pipettes (I ml), inoculation loop.

Procedure:

Put the U.V. lamp of inoculation chamber for 5 mts.Wipe the table topwith rectified spirit. Wash hands with rectified spirit and air dry. Lit the burner/spirit lamp.Arrange sterile petri dishes near the burner.

1.Select a diseased citrus leaf infected by canker and cut out a small portion of the diseased tissue from the advancing lesion using sterile razor blade in a drop of sterile water and after several minutes, examine under microscope. If bacterial ooze is seen, proceed for isolation.

2.Surface disinfect the cut portions by dipping in sodium hypochlorite solution for 60 sec. and then immediately rinse three times with sterile water.

3. Immerse the disinfected cut portions in 1 ml of sterile water taken in a clean sterilized test tube.

4.Crush the cut portions of the leaf with a sterile glass rod. Allow it to stand for 5 minutes to allow the bacteria to diffuse out of the cut tissue and into the water. "

5.Gently lift the lid of a petridishwith left hand and using inoculation loop transfer several loopfulsof the bacterial suspension to sterile pertridishes (three) containing 1 ml of sterile water and mix thoroughly.

6.Hold flask filled with sterile luke warm nutrient agar medium in the right hand and remove cotton plug near the flame and pour about 20 ml of medium into each dish and mix thoroughly by gentle rotation. Allow time for solidification of medium.

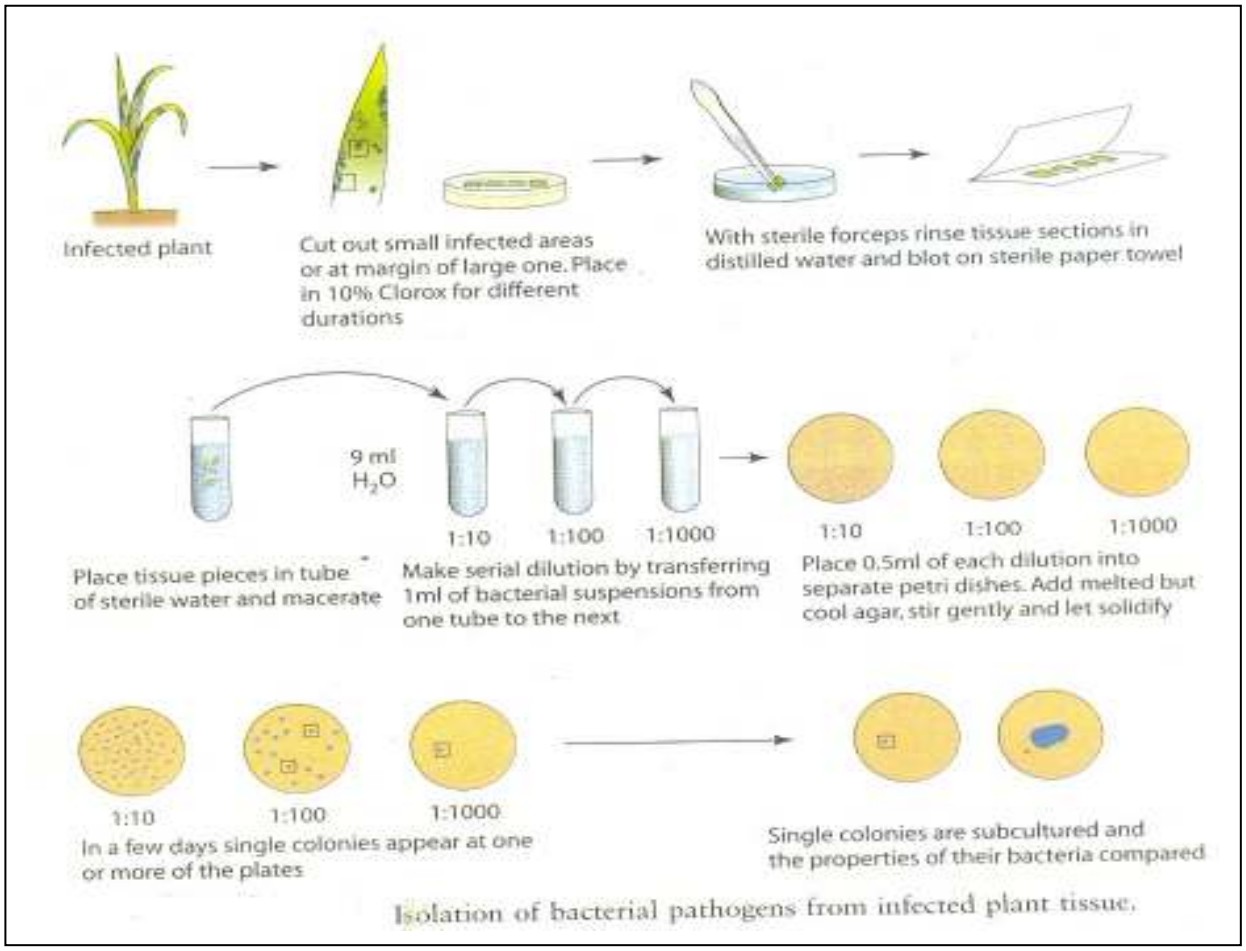
7. Incubate the dishes in an inverted position at 25^0C for 36 to 72 hours.

8.Observation: Observe the dishes for appearance of desired bacterial colonies. If colonies

appear, select consistently found and well isolated colonies of the pathogen, for sub culturing and further studies.

9.Select the isolated colonies and streak on the surface of a solidified medium in a zig-zag manner and incubate the dishes at 25)0C. Bacteria isolated from nature may be contaminated with saprophytic species, hence, re streaking for isolation ensures a pure culture.TransfersomeofthepurifiedcoloniestoNAslants and grow them for further use.

Follow the protocol in the above procedure and isolate the desired bacterial colonies from the given diseasedmaterial and maintain pure culture in petri dishes and slants for further study.



Isolation of bacterial pathogens from infected plant tissue.

STUDY OF COLONY AND MORPHOLOGICAL CHARACTERISTICS AND GRAM'S STAINING REACTION OF A BACTERIUM

The classical approach to bacterial identification as soon as it is isolated from diseased plant material in pure from involves a combination of information from morphological and cultural characteristics, gram staining reaction which would form the basis for the selection of biochemical tests to be performed to identify of the unknown bacterium. The purpose of this exercise is to study the morphological and cultural characteristics and gram staining reaction of the bacterium isolated from the diseased plant material.

A. Study of colony characteristics of bacterium

Note: the following colony characteristics of the pure cultures of the bacterium isolated from the diseased plant material and record the observations in the form of a table.

a) Color : yellow, white

b) Form : Punctiform,circular, rhizoid,irregular, filamentous.

c) Margins : entire, undulate, lobate, filamentous

d) Elevation : flat, raised, convex, pulvinate, umbonate

e) Surface : smooth, glistening, rough, wrinkled, dry, powdery

f) Density : opaque, translucent

B. Study of morphological characteristics of bacterium

Procedure:

1.Take clean glass. slide, wash and dry

2.Prepare the smear of the bacterium culture

Take inoculation loop and sterilize using alcohol lamp/burner. Place a drop of clean water on the slide and mix it with a small portion of culture isolated from diseased plantmaterial. Spread the bacterial suspension thinly to over an area of about 10 paise coin with inoculation loop. This thin layer of bacterial culture spread on a glass slid is called smear or film.

3.Allow the smear on slide to dry in air/heat (by passing the slide smear over the flame for 2-3 times). The main purpose of drying the smear is to fix the bacterial cells on the slide. This is called fixation.

4.Flood the smear with five drops of basic stain (Methylene blue - 60 sec or crystal violet - 30 sec) and allow it to react for designated time.

5. Pour off the stain and wash the smear gently with slowly running tap water.

6. Blot dry the slide using blotting paper (do not wipe the slide).

Observation and results:

Examine the stained slide under microscope and record the shape and arrangement of bacterial cells and make colored drawing of the cells.

Colony and morphological description of bacteria

Colony characteristics	Description	Morphological characteristics	Description
A.Color		a. Shape b. Arrangement:	
B. Form			
C. Margins			
D. Elevation			
E. Surface			
F. Density			

GRAM STAINING OF BACTERIA:

Gram staining developed by Christian Gram in 1884 is one of the most important and widely used differential staining techniques useful in identifying and classifying bacteria into two groups viz., Gram +ve and Gram -Ve. In this process the fixed bacterial smear is subjected to four different solutions.

a. Basic Stain (Primary Stain) : It is an initial stain. The colour is in the positive ion of the stain and reactwith cellular elements which are acidic in nature. Eg. Methylene blue, Crystal Violet.

b. Mordant: It is a substance that increases the affinity between the cell and dye and helps in fixing the dye on cells eg.Iodine.

c. Decolouring agent :It is a substance that removes the dye from a stained celleg.Ethanol (95% alcohol).

d. Counter stain: It is a basic dye of a different colour from the primary stain. The purpose of the counter stain is to give the decolourised cells a colour different from that of the primary stain.
Those organisms that are not readily decolourised retain the colour of the primary stain. Those that are readily decolourised take the colour of the counter stain eg.Safranin.

The first step of Gram staining consists of staining the cells with a basic stain followed by a treatment of these stained cells with a mordant. The cells are then treated with a decolourising agent. The cells that retain the basic stain following decolourisation are called Gram +ve, and those that are decolourised are Gram -ve and. can be restained with a counter stain of contrasting colour. Gram +veisthicker than Gram -ve.

Materials: Fresh solutions of Crystal Violet, Gram's Iodine, 95% ethyl alcohol, inoculation loop, blotting paper, alcohol lamp/burner, clean slides, bacterial culture, immersion oil, microscope.

Procedure:

1) Prepare thin smear of given bacterial culture on a clean glass slide.
2) Stain the smear with crystal violet for 30 sec.

3) Wash the slide with distilled water for a few seconds, using wash bottle.
4) Cover the smear with iodine solution and allow it to act for about 30 sec.
5) Carefully add ethyl alcohol drop by drop on the slide, until no more colorflows from the smear (usually it takes 30 see),

STEPS IN GRAM STAINING:

I. Firstly, take sterile &clean glass slide.

2. Take a drop of water & prepare thin smear using inoculation loop (culture of age <0.4 hr.) & air dry it.

3. Add crystal violet (primary stain) to the smear and keep it for I min &wash it with distilled water.
4. Then add iodine &keep it for 1 min &wash with water.
5. Then wash the smear with alcohol (decolourizer) till color is lost.
6. Add safraninedye to smear. If red colour retained we can conclude bacteria as gram –vebacteria.
   If purple colouris retained it is gram +vebacteria. '

Conclusion:In experiment done, with Xanthomonaspurple color is retained. So, a bacterium is Gram -Ve.

Work done report

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16. DEMONESTRATION OF MECHANICAL TRANSMISSION OF PLANT VIRUSES.

Plant viruses are transmitted from plant to plant in a number of ways. Modes of transmission include vegetative propagation, mechanically through sap, seed, pollen, dodder and vectors like insects, nematodes and fungi.

A. Demonstration of mechanical transmission of plant viruses through sap

Mechanical transmission of plant viruses in nature by direct transfer of sap through contact of one plant with another plant is uncommon. Sap carrying virus from diseased plants is transmitted to healthy plants through leaf injuries caused due to strong winds or by hands or tools during cultural operations. Of the important plant viruses, tobacco mosaic virus and potato virus- x are transmitted through sap in the field.

Materials required

Test plants infected with tobacco mosaic virus, potted healthy test plants, conical flasks, blotting paper, cheese cloth, muslin cloth, abrasive (a substance employed to create micro wounds eg.carborundum (400- 600 mesh) or celite(1 % diatomaceous earth), mortar and pestle, fine washed sand, swabs, distilled water, pieces of card board, vesse! for heating and heater, test tubes with stand, distilled water or phosphate buffer solution (a solution which is added to stabilize the virus) of pH 7.0 (eg. disodium hydrogen orthophosphate - Na_2HPO_4& sodium dihydrogen orthophosphate - NaH_2PO_4(0.1M)or eg.dipotassium hydrogen orthophosphate - K_2HPO_4& potassium dihydroqenorthophosphate - KH_2PO_4(0.1 M), wash bottles.

Procedure

1.Collect two young mosaic infected leaves of test plant, wash with tap water and dry with blotting paper. Weigh the leaves.

2. Prepare the standard extract by grinding the leaves alonqwith finely washed sand using a mortar and pestle. (for preparing standard extract, add distilled water or phosphate buffer equal to the weight of the leaves in to the mortar and qrind).

3.After through grinding, pass the leaf pulp through muslin cloth to get the filtered standard extract of the leaves.

4.Divide the extract into two lots. Heat one lot to boiling and cool. Inoculate the leaves of healthy plants with both the lots separately.

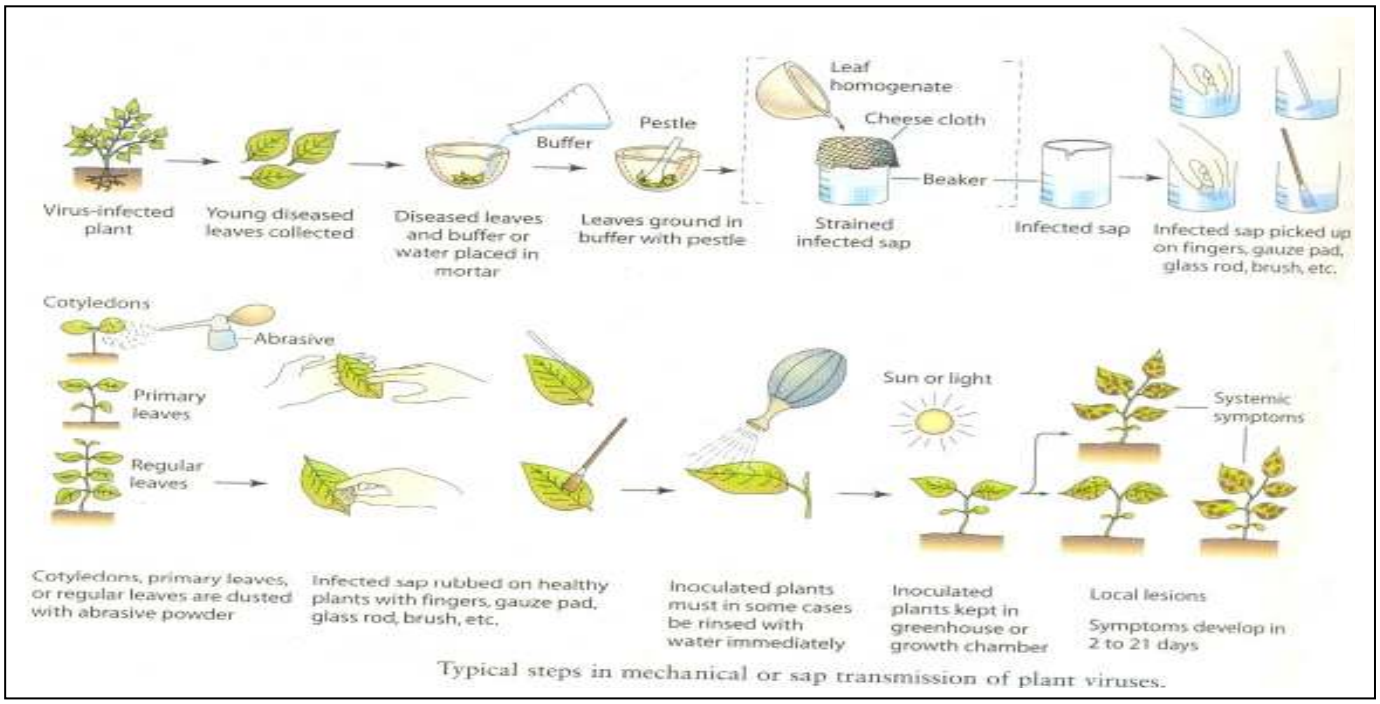
5.Dust the leaves with carborundum lightly or add the celite powder to the sap to create micro wounds.

6.Apply the sap gently by rubbing the leaves (supporting the leaves with a piece of card board or palm) in one direction i.e., only from petiole to the apex of the leaves with a cheese cloth or fingers or painter's brush dipped in sap. The virus enters the leaf cells through the wounds made by the abrasive.

7.After inoculation, wash the leaves with water to remove the excessive inoculum and other extraneous particles if any.

8.Label the plants giving date of inoculation and other details of the host.

9. Place the inoculated plants in a glass house providing optimum conditions for plant growth for observation.



Typical steps in mechanical or sap transmission of plant viruses.

Observations and record

Note the date of inoculation and appearance of symptoms. Symptoms are usually appeared within3- 7 days. Observe the mosaic symptoms in plants inoculated with unboiled extract and absence of symptoms in plants inoculated with boiled extract.

Precautions

1.Inoculum should be obtained from the younger leaves of the infected plants as the younger leaves contain more concentration of the virus particles.
2.Mortar and pestle should be thoroughly cleaned. Washed and sterilized before and after use to avoid contamination.
3.Too much of carborundumpowder should not be used as it may cause lethal injury to the plants.
4.Inoculated plants should be washed thoroughly to remove excess inoculum and other external particles.
5. While inoculation, the leaves should be rubbed gently.

EXERCISE

1. Demonstration of transmission of any given mosaic viral disease through sap and observation of mosaic symptoms on the test plant.

Work done report

Ex No:

Date:

Title: