Kingdom : Fungi

Fungi: Eukaryotic organisms, structure **Thallus** , spread in different places in wet soil and dry in the fresh and salt water in the air and attacked many of them plant, animal and human also used some of them as a food and is one of the micro-organism-free chlorophyll so fungi are heterotrophy .

Fungi thallus have two phases:

- Somatic phase (vegetative phase): (unicellular , filamentous).
- Reproductive unites (gametes , spores , conidia).

Mycology: the science who study specializes structure and the classification and methods of reproduction of different types of fungi and economic importance to them**.**

Common characteristics between fungi and other living organisms

Organisms	Similarity	differences
Fungi and Plant	1- Contain cell wall . 2- Inability to move . 3- Nutrition absorbency	1- Fungi Don't contain Pigment chlorophyll . 2- Fungi Don't contain complex vascular system 3- Storage carbohydrate in fungi are **Glycogen** while the plants are stored as **starch**
Fungi and Bacteria	1- Both contain a cell membrane and cell wall .	1-bacteria prokaryotic while fungi Eukaryotic . 2- bacteria can be autotrophs as well as heterotrophs while fungi are heterotrophs.
Fungi and Animal	Both use as food sources contain a high percentage of protein	Animals have only plasma membrane while fungi have cell wall in addition to the plasma.

Fungi and Algae	1- Both algae and fungi form thallus . 2- Algae and fungi tend to growth in moist or wet environments.	1- algae are autotrophy containing chlorophylls while fungi are heterotrophy . 2- Algae are incapable of living in the dark while Fungi are capable of living in the dark.

۞morphology of fungi۞

Fungi bodies consists either of unicellular (Yeasts) or filament minutes microscopic size know (**Hypha**) may be divided into cells or undivided , The hypha extends by tip growth, and multiplies by branching , creating a fine network called a **Mycelium** . . Hypha be colorless but in some fungi take several different colors and this is due to the nature of the nutrients stored or to presence some of the different dyes. Each Hypha consists of an outer wall thinning and an internal cavity is full material Protoplasm , and in some fungi to a number of fungal cells separated by cross walls called Septa , And the cross walls between the cells have a small hole central that allows connection Protoplasm between the cell and other. Hypha in some Fungi are undivided or no septa called **Coenocytic** .

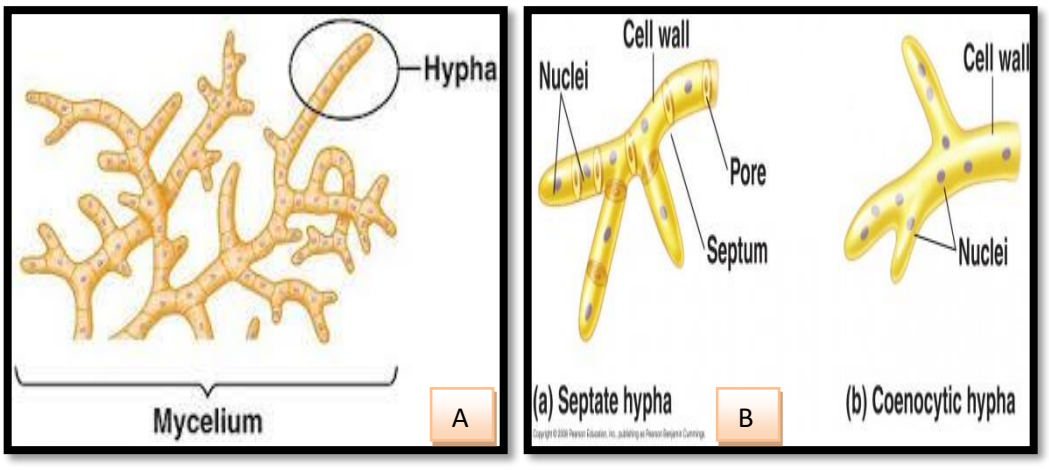


Figure (1) (A) Mycelium (B) Coenocytic

Fungi Nutrition

The nutrition in fungi are **absorptive**, use Enzyme to break down a large Complex molecules in to small organic compounds , then absorbed it.

Type of fungi Nutrition

Nutrition in fungi divided into three divisions :

- **Saprophytism Nutrition:** Living on the remains organisms (plants and animals) live on dead cells **.**
  1-Obligate Saprophytism: Living only on dead cells **ex:** *Penicillium sp.* , bread mold .
  2-Facultative Saprophytism: Usually living parasitic but if not find the host lived Saprophytic ex : Smut fungi .
- **Parasitism Nutrition:** Living on or inside Tissues of organisms causing diseases .
  1-Obligate parasitism: Lived only on living cells ex : Rust fungi , Downy mildew .
  2-Facultative parasitism: Usually living Saprophytic , if not find proper material (dead cells) they lived parasitic ex: *Fusarium sp*.
- **Symbiosis Nutrition:** also called commensal **,** Fungus live beneficial relationship with another organism, such as :
  Lichens: benefit relationship between fungus and algae .
  Mycorrhiza: benefit relationship between fungus and plant root.

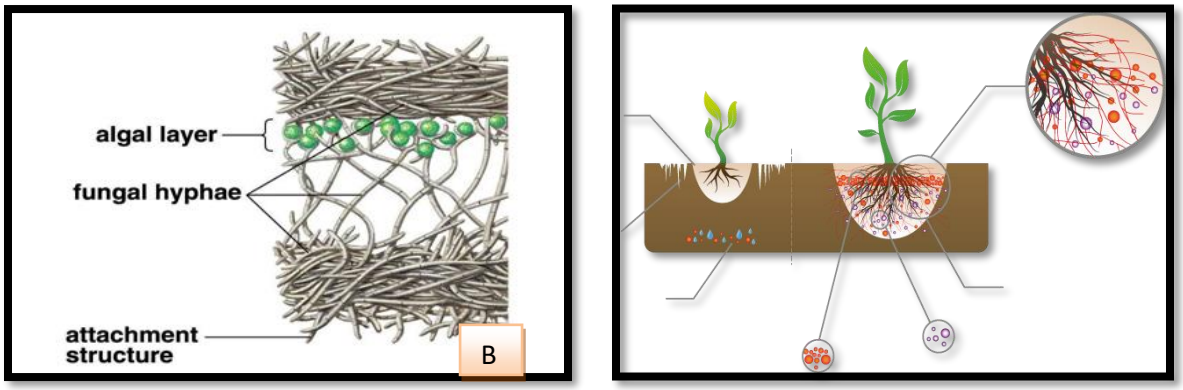


Figure (2) (A) Lichens (B) Mycorrhiza

۞Reproduction in Fungi۞

At the time of reproduction fungi when all somatic phase converted into reproductive structure. Such a condition is known as **Holocarpic** But if only part of the somatic phase converted into reproductive structure , it is called **Eucarpic**.

Types of Reproduction in fungi :

a- Asexual Reproduction(Anamorph) : Also known somatic or vegetative Reproduction , Occurs in all kinds of fungus with the provide proper conditions , This reproduction is achieved in several ways :

1- Fragmentation forming Arthrospore .
2- Simple diffusion like yeast .
3- Budding forming Blastospores .
4- Forming Chlamydospores .
5- Forming Spores .

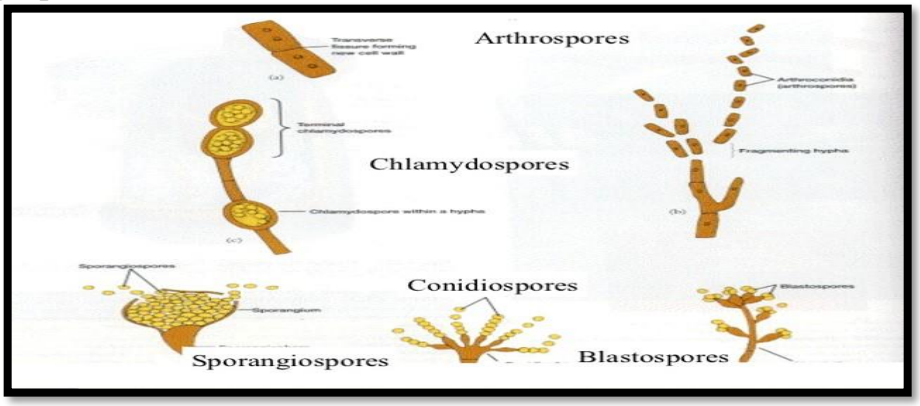


Figure (3) Asexual Reproduction ways

- If the Spores inside Fruit bodies known **Sporangium or Sporangia** called **Sporangiospores,** carried on **Sporangiophore** .
- If the spores produced at the tips or sides the hypha they called **Conidia** carried on **Conidiophore** .

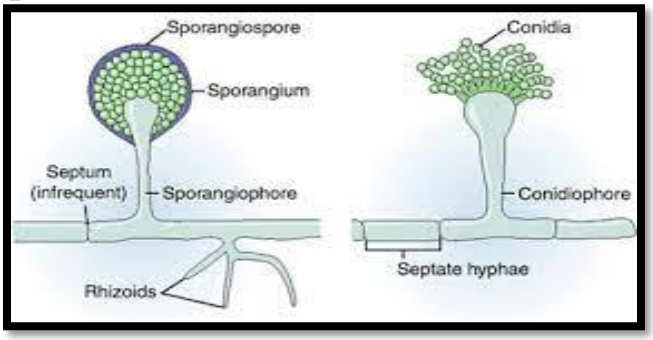


Figure (4) Sporangiospores and Conidia

❖ Type spores are **Zoospores , Aplanospores .**

b- Sexual Reproduction (Telemorph) : Must pass through three stages :

1- Plasmogamy (cell fusion) .
2- Karyogamy (nuclear fusion) .
3- Meiosis (reduction division) .

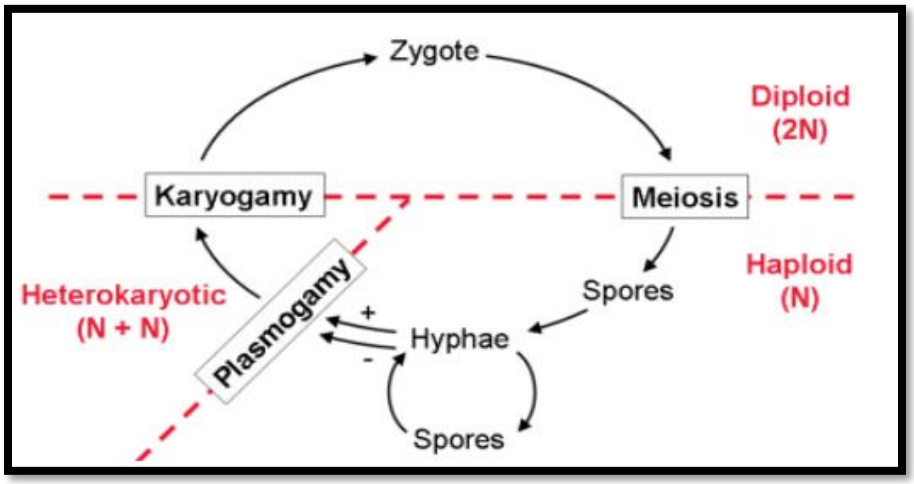


Figure (5) Sexual Reproduction in fungi

❖ **Reproductive organs** called **gametangia** contain a sexual cells called **gametes** , This reproduction is achieved in several ways :

1- Planogamtic Copulation .
2- Gamitangial Copulation .
3- Gamitangial Contact .
4- Spermatization
5- Somatogamy

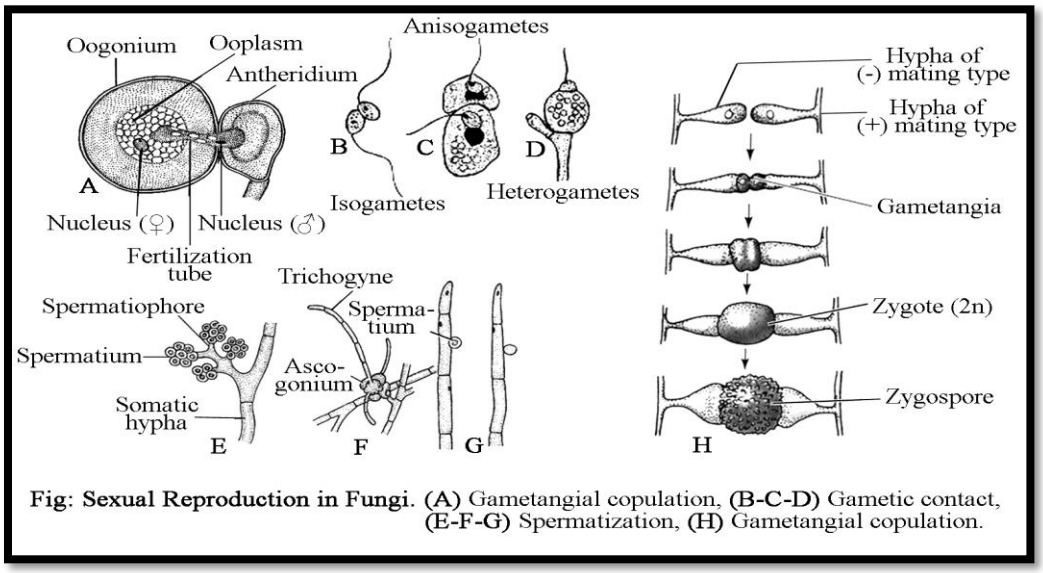


Figure (6) gametangia types in fungi

Economic importance of fungi

Fungi have both positive and negative roles in our daily life. So they are our friends as well as enemy.

❖ Benefit of fungi :

Directly or indirectly fungi are beneficial to human being. Fungi is used in medicine industry, as food, in food preparation, in other industry and also in agriculture. Some of the useful activities are:

1- Preparation of Medicine:

Different types of fungi are used in the production of important numbers of drugs. The most important species are *Penicillium notatum, Claviceps purpurea, Saccharo myces cerevisiae, Aspergillus proliferous* etc.

a- Antibiotics

are the metabolic product of some micro-organisms which are active against other microorganism . wonder drug Penicillin from *Penicillium notatum*. and drug Fusidin(Fusidic acid) from *Fusidium coccineum* .

	Name of antibiotics	Produced fungi	Range of activity
1.	Penicillin	*Penicillium notatum* and *P. crysogenum*	Bacteria (both Gram + and –)
2.	Griseofulvin	*P. nigricans* *P. griseofulvum*	Fungi
3.	Cephalosporin	*Acremonium* sp.	Bacteria (Gram +)
4.	Citrinin	*P. citrinum*	Fungi
5.	Palutin	*P. palutum*	Fungi and Bacteria
6.	Fumagillin	*Aspergillus fumigatus*	Protozoa
7.	Fusidic acid	*Fusidium coccineum*	Bacteria
8.	Viridin	*Trichoderma viridi*	Fungi

b- Vitamins:

Vitamins are the micronutrients required for the growth of living organisms. Vitamin B-complex, Vitamin A and Vitamin B-12 are found respectively from *Saccharomyces cerevisiae*, and *Eremothemium ashbyii*.

(c) Steroid:

Rheumatic arthritis, allergy and some other diseases are con-trolled by steroid. Many fungi have the capacity to synthesize different steroids. Steroid like cortisone is produced by *Aspergillus niger* from plant glycosides by fermentation.

(D) Alkaloid:

Several alkaloids are produced and accumulated in the sclerotium of Claviceps purpurea which causes Ergot disease of rye. Out of several alkaloids, Ergo- metrine and its semisynthetic ana-logues like methyl ergometrine and methyl ergometrine maleate have notable uterine action; those control haemorrhage of mother during child's birth, having side- effect with increase in blood pres-sure and decreased milk secretion

2- Foods

Fungi are used as food by humans from a long time ago. Some fungi have been used directly as food and some are used in food processing:

Direct Use:

Fruit bodies of some fungi, like Mushroom and truffles. are used as food due to their high protein con-tent (21-30% on dry weight) and have good amount of lysine, an amino acid; minerals like Na, Ca, K and P; Vitamins like B, C, D and K and very little amount of fat. These are recommended as ideal foods for heart patients and diabetes. The above-mentioned fungi can grow artificially at the commercial level. Mushroom cultivation has recently gained considerable popularity and has contributed to the national economy in some East Asian countries.

3. Fungi in Industry:

Many fungi are used in the production of alcohol, bread, cheese, enzyme and organic acids.

(a) Alcohol Production:

Alcoholic fermentation by fungi is the basis of brewing industry. The enzyme zymase of microorganisms like yeast is responsible for alcohol production. Wines are produced from grapes or other fruits by *Saccharomyces ellipsoideus* with about 14% alcohol concentration. Beer is brewed from barley malt by *Saccharomyces cerevisiae* with 3-8% alcohol production.

(b) Bread and Cake Production:

During alcoholic fermentation by yeast, CO_2 being released as bubbles are used in baking industry to make the breads and cakes as spongy in appearance.

(c) Cheese Production:

Some species of Penicillium (P. roquiforti and P. camemberti) are used in the production of Roquefort and Camembert cheese by hydrolysis of fats and also to develop specific flavour to cheese.

(d) Enzyme and Organic acid Production:

Many fungi are used in the commercial production of enzymes and different organic acids .

L**ist of some fungi along with produced enzymes and/or acids and their uses are given:**

Enzyme Organic acid	Produced fungi	Uses
Enzymes		
1. Amylase	*Aspergillus oryzae, A. niger*	Alcohol industry and in pharmaceuticals.
2. Cellulase	*Trichoderma reesli*	Production of cheese and hydrolysis of cellulose.
3. Invertase	*Saccharomyces cerevisiae*	Paper industry and confectionary.
4. Zymase	*Saccharomyces cerevisiae*	Ethyle alcohol production.
Organic acids		
1. Citric acid	*Aspergillus niger, A. wentil*	Soft drinks and other foods. Manufacture of ink and leather tanning.
2. Fumaric acid	*Rhizopus stolonifer*	Manufacture of wetting agents.
3. Gallic acid	*Aspergillus gallo-myces. Penicillium glaucum*	Manufacture of ink and dyes.
4. Gluconic acid	*Aspergillus niger, Penicillium purpuro-genum*	In textile, leather, food and photogra-phic industries and also in pharmaceu-ticals.
5. Kojic acid	*Aspergillus oryzae*	As insecticide and antibiotic.
6. Itaconic acid	*Aspergillus terreus, A. itaconicum*	Manufacture of syn-thetic fibre and plasticisers.

4. Soil Fertility:

Decomposition of litter and wood, mainly in the forest, takes place by the combined action of diffe-rent type of fungi. Fungi like *Fusarium, Chaetomium, Chitridium, Penicillium, Aspergillus* etc., can decompose the structural polymers such as cellulose, hemicellulose, lipid, protein, starch etc.

By decomposing the organic matters, fungi help to increase minerals and other sub-stances, thereby the fertility of soil is increased.

5. Plant Nutrition:
Several fungal members like *Rhizoctonia*, *Tricholoma*, *Boletus*, *Phallus*, *Amanita* etc., associated with the roots of higher plants form mycorrhizal relationship. The fungal partner supplies water and minerals and in turn, they take nutrition from the plant.

6. As Insecticide:

Fungi like *Cordyceps sp.* , are used as insecticides to control different types of insects.

7. Biological Research:
Fungi like *Neurospora*, Yeast etc., have been used in genetical and cytological studies. Physarum polysephalum has been used to study DNA-synthesis .

9. Test Organism:
Some strains of *Aspergillus niger* have been used to detect trace elements like Zn, Cu, and Mo, even if the substances are present in very minute quantity in the substrate. These elements when absorbed by the fungus give a particular colour to the conidia. Similarly, *Neurospora crassa* has been used to detect Vitamin B complex .

10. Production plant hormone:

Some fungi are used to produce plant hormone like Gibberellin by soil fungus *Gibberella fujikuroi* .

11. Biological control:

The antagonistic activity of some fungi like *Trichoderma* sp. showed that it is parasitic on many soil-borne and foliage pathogens. *Trichoderma* sp. is being used to control plant diseases in sustainable diseases management systems, *Beauveria bassiana* is a naturally occurring fungus in soils throughout the world and has been researched for control of soil borne insects e.g. the beetle in Europe,

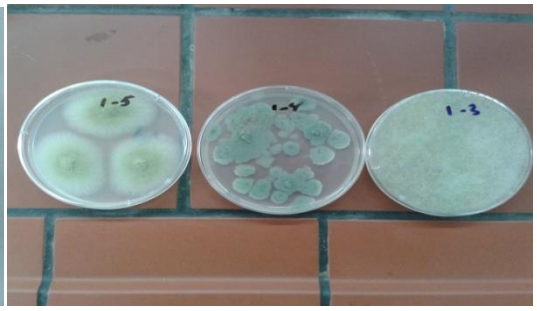
❖ Harmful Activities of Fungi:

Fungi are also harmful to the human beings in various ways, either directly or indirectly. They may cause diseases of plants, human beings, and animals; spoilage of food etc.

1. **Fungi Causing Plant Diseases :**
   Fungi cause several minor and major plant diseases. Some of them also cause famine in different parts of the world. such as late blight of potato diseases cause by *phytophthora infestans* and damping of seeding diseases cause by *pythium debaryanum* white rust cause by family albuginaceae and family peronosporaceae cause downey mildew etc.

2. **storage fungi cause rot in fruit and food :**
   Poor storage of crops and fruits leads to the growth of fungi causing high economic losses like *Penicillium* sp. cause green rot on fruit and *Aspergillus* sp. cause black rot in fruit and *Aspergillus flavus* cause green rot in grains etc.

rot fruit



some storage fungi isolation

3. Fungi Causing Human and animals Diseases:

Some fungi parasitism on humans and animals, causing infections of the skin, hair or nails like Malassezia species ,and dermatophytes which have the ability to use keratin as a nutrient source so have a unique enzymatic capacity [keratinase]by *Trichophyton rubrum* etc.

3. **Production of fungal toxins**
   Some fungi have the ability to produce toxic secondary metabolite call mycotoxins which have a role in the infection of some diseases in both humans and other animals ,The adverse health effects of mycotoxins range from acute poisoning to long-term effects such as immune deficiency , Liver and kidney fibrosis and cancer. such as patulin , aflatoxin , Ergot Alkaloids , etc .

4. **Hallucinogenic Drug:**
   LSD (d-lysergic acid diethylamide), the well-known hallucinogenic drug, is extracted from the sclerotia of *Claviceps purpurea*, the causal agent of ergot disease of rye. Other fungi like *Psilocybe mexicana* produce Psilocin and Psilocybin that have hallucinogenic properties.
5. **damage of clothes :**
   fungi can grow on wet clothes and shoes thus causing damage to them. Clothes made from natural fibers such as cotton, linen, rayon, wool and silk are more susceptible to microbial damage than those made from synthetic fibers. Mold on clothes produce enzymes that breakdown the cellulose or protein to compounds which the mold use as food ex: *Aspergillus niger* .

6. **damage of paper and wood :**
   Filamentous fungi belonging to the Ascomycota phylum are the main microorganisms deteriorating paper-based collections worldwide, being mainly responsible for the appearance of different colour patches with biological origin on paper , including genera *Aspergillus, Penicillium, Chaetomium* etc.

7. **Building materials damage :**
   Stachybotrys chartarum is a black mold that produces its conidia in slime heads. It is sometimes found in soil and grain, but the mold is most often detected in cellulose-rich building materials from damp or water-damaged buildings. It requires very high moisture content in order to grow and is associated with wet gypsum material and wallpaper .

black mold of *Stachybotrys chartarum*

۞Culture media for Fungi ۞

Culture media: Balanced mixture of different nutrients necessary for the growth of microorganisms, it may be simple or complex composition In each case serves to provide the energy and basic units for building cells .

The purpose of using Culture media :

- ❖ Growing and preserving fungi .
- ❖ Study the effect of single nutrients found in media on the growth of fungus.
- ❖ Inducing fungi to produce and forming some material .
- ❖ Classification of fungi and study the cultural characteristics.

۞Division of Culture media۞

a- According to the chemical composition:

- ❖ **Chemically defined media :** Must be known composition**,** consists of metal salts have added some sources of carbon or nitrogen, can be prepared each time the same precision ex: Czapek′s Agar (CZ).
- ❖ **Chemically no defined media:** Not have a specific composition , composition changed depending on the nature of the material prepared, Difficult prepared each time the same precision ex: Potato Dextrose Agar (PDA) ,Corn Meal Agar (CMA) , Malt extract agar.
- ❖ **Natural media :** Use of natural materials without additions, ex: Extracts of the roots of potatoes or carrots , Prepared from wheat or barley or corn.

b- According to the Textures:

- ❖ **Solid media :** It may be natural such as potato chips, Or it may be artificial, such as (PDA) Containing (Agar).
- ❖ **Semi solid media:** Contains a half or a quarter of the amount Agar added to solid media .
- ❖ **Liquid media :** Not contains Agar such as (PD) artificial, (Milk) natural.

c- According to the purpose:

- **General purpose media :** Media are used to growth different types of fungi, such as :
  1. Water Agar (WA).
  2. Potato Dextrose Agar (PDA).
  3. Carrot Agar.
  4. Malt extract agar.
  5. Czapek′s Agar (CZ).
  6. Corn Meal Agar (CMA) .
- **Selective media :** Contains a substance inhibits the growth of some fungi while helping growth another kind , such as add some antibiotics or modify the value of (PH) , or add salt , or use Rose Bengal ex:
  1- Selective *Fusarium* Agar .
  2- *Phytophthora* selective medium .

Notes: Some additives to the culture medium to get a pure Culture, contamination-free :

1. Media with cyclohexamide (cycloheximide is added to inhibit the growth of rapidly growing contaminating molds)
2. Media with or without an antibacterial agent (chloramphenicol, gentamicin and ciprofloxacin are commonly used antibacterial for this purpose).

Preparation of Culture Media General

1- Broth & agar media are prepared by dissolving specified amount of powder in distilled water .
2- Boiling is often required to dissolve the powder by autoclave in 121 C° for 15-20 min .
3- Cool the flask containing the culture media to about 50 C°
4- Pour the culture media on the Petri dishes let it until Solidify .

Sterilization

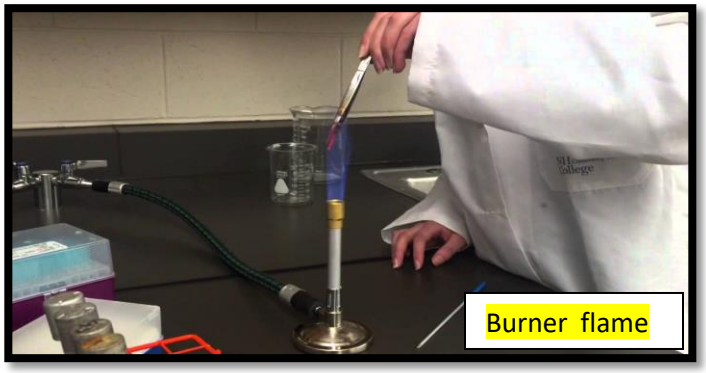
Sterilization: process to remove or kill all microbes in vegetative form or spores found in the media to be sterilized, as well as laboratory tools or solutions or different places , Sterilization be achieved by using the physical or chemical methods.

a- physical methods: High Heat and Radiation the most important physical factors that are used in sterilization.

❖ **Heating :** Be either dry or wet heat

1- Dry Heat :

Red heat and Flaming: by using Bunsen flame ,the red heat sterilize needles , loops ,straight wires, tips of forceps and spatulas ,while the Flaming sterilize mouth of test tubes, flasks, glass slides and cover slip.



Hot air oven : sterilized by exposed to high heat 160°C for 60 min or 180°C for 20 min, this type is used to sterilized Glassware , petri dishes and pipettes, after sterilization leave period until cool and be usable without harm .

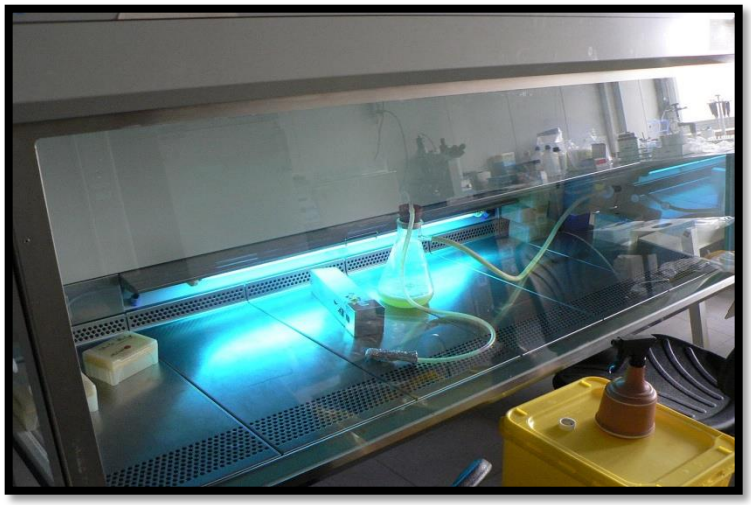
2- Moist Heat :

Means using the water vapor in the sterilization rather of hot air.

Autoclave : Best and fastest ways sterilization of the ability of moist heat to kill the vegetative cells and spores, Being under the temperature of 121 ° C and at a pressure of (1) bar for 15 minutes .

Autoclave

- **Radiation :** The harmful impact of radiation on some microorganisms useful in sterilization some places, such as chambers of surgical operations and in some food industries and sterilizing large surfaces and sterilizing water .



Hood UV radiation lab

1- **Ultraviolet Radiation:** Used more than others in the sterilization due to the impact deadly effect on DNA that any cell , in the laboratory using special lamps with the color violet .

2- **Other Radiation :** Can be used X-ray shortwave and Gamma ray for purposes sterilization.

3- **Filtration :** frequently used to remove cells from the organisms in media , characterized as not change the physical or chemical properties of the materials so used in the preparation of Enzymes , Hormones , Antibiotics solvents ،Serum ، Proteins and some Vitamins.

Millipore filter

b- Chemical methods : Chemicals used solutions for surface sterilization ex:

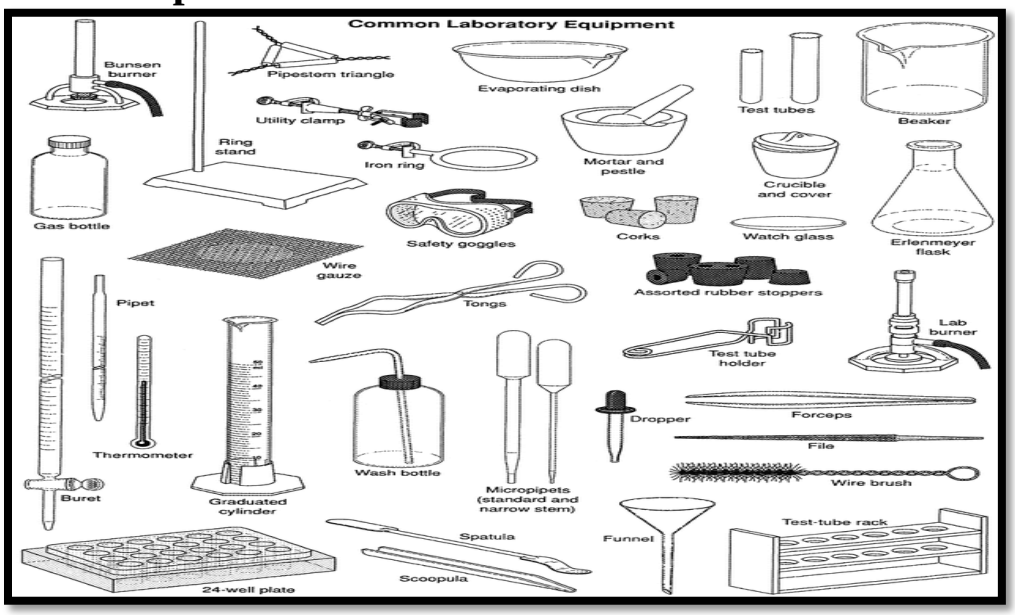
- **Halogens:** It is an effective material against microbial such as Iodine and chlorine .
- **Aldehydes :** One of antimicrobial materials such as Formaldehyde and Formalin by fogging technique , This method is used to sterilize laboratories, incubators and refrigerators .
- **Dettol (chloroxylenol B.F)**

Fungi Isolation:

Fungus spread in many environments heavily where there is no place free of the presence of one or more type of fungus or spores،Which can be isolated from soil, air or water, fungi parasitize humans and plants and less on animal causing diseases and economic losses, so it is necessary to isolation and Diagnosis these fungi to reduce the danger.

Materials and tools:

- **Slides and cover slips**
- **Petri dishes**
- **Burner**
- **Gloves**
- **Lactophenol cotton blue (LPCB)**
- **Pipettes**
- **Inoculating needle**
- **Flask and bakers**
- **Test tube**
- **Media**
- **Microscope**



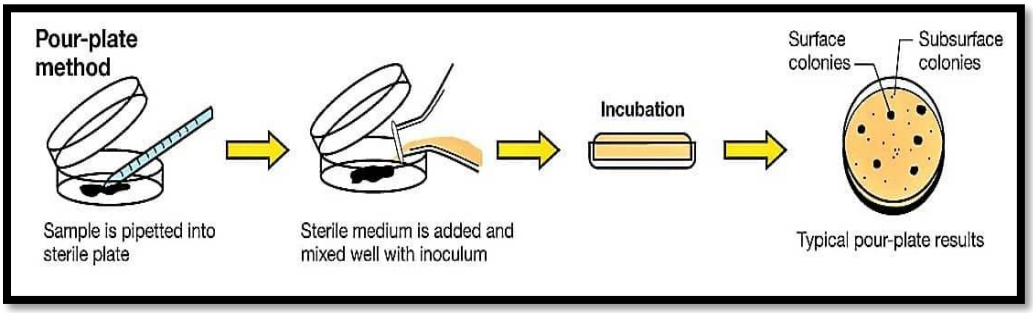
Materials and tools in laboratory

1- Isolation fungi from soil

a- Pour plate methods (Direct Isolation)

- Weighted 0.1 gm of soil and placed it in Petri dish.
- Poured the culture media in Petri dish near flame .
- Moved the Petri dish with a circular movement right and left for mixing the soil with media .
- Leave the plate until it solid , then incubated for 5 days at 25-28 °C.

Not: Can distribution the soil sample on the surface of solid culture media .



Pour plate method

b- Indirect isolation :

It's a modified method of poured plate methods, use to Purification fungi from bacteria .

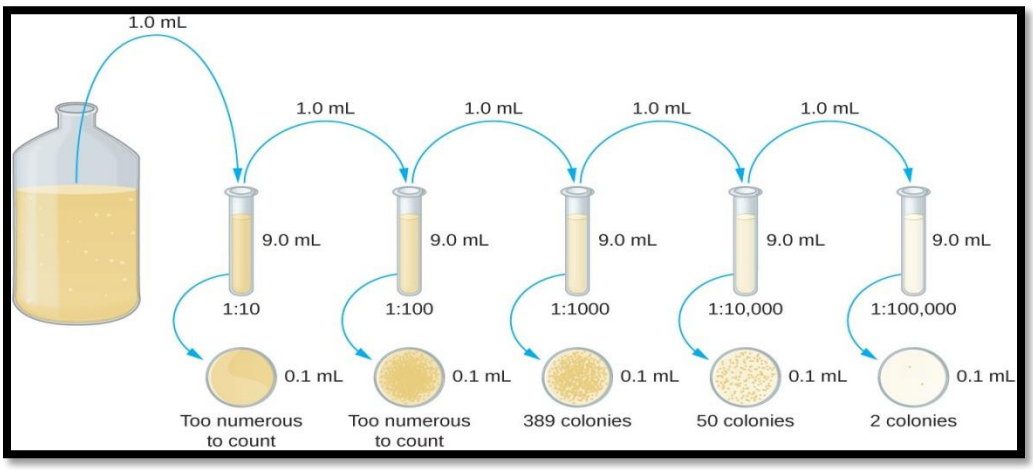
- Add 10 ml of culture media in Petri dish and left to solid.
- Placed 0.1 gm of soil sample above the culture media .
- Poured 5 ml of culture media on the plate and left to solid.

In this case hyphal fungi grow to high because it **Aerobic** organisms while the bacteria (**anaerobic**) stay within the culture media .

c- Dilution method:

- Weighted 1 gm of soil and placed it in test tube contain 10 ml Distilled water(DW) (stoke), Mixed it well.
- Prepare 5 test tube each contain 9 ml of (DW).
- Take 1ml of stoke to the first tube that will be first dilution 1/ 10 (10^{-1}).
- Take 1ml of the first dilution to the second tube that will be the second dilution 1/ 100 (10^{-2}).
- Take 1ml of second dilution to the third tube that will be the third dilution 1/ 1000 (10^{-3}).
- Take 1ml of third dilution to the fourth tube that will be the fourth dilution 1/ 10000 (10^{-4}).
- Take 1ml of fourth dilution to the fifth tube that will be the fifth dilution 1/ 100000 (10^{-5}).
- Placed 1 ml of each filtrate dilution in a Petri dish and then poured the culture media, moved the dish for mixing the sample with the culture media.
- Leaf it until solid , then incubated for 5 days at 25-28°C.

  Not: The dilution (1ml) can be distributed on surface of solid culture media .



Dilution method

2- Isolation fungi from air:

- Poured the media on Petri dishes and leave it until solid .
- Open the dished in several different places for 30 min.
- Incubated for 5 days at 25-28 °C.

3- Isolation fungi from water:

a- Dilution method:

- Take 1ml of water sample placed it in test tube contain 9ml of (D.W) , that will be first dilution 1/ 10 (10^{-1}).
- Take 1ml of the first dilution to the second tube that will be the second dilution 1/ 100 (10^{-2}).
- Take 1ml of second dilution to the third tube that will be the third dilution 1/ 1000 (10^{-3}).
- Take 1ml of third dilution to the fourth tube that will be the fourth dilution 1/ 10000 (10^{-4}).
- Take 1ml of fourth dilution to the fifth tube that will be the fifth dilution 1/ 100000 (10^{-5}).
- Placed 1 ml of each filtrate dilution in a Petri dish and then poured the culture media, moved the dish for mixing the sample with the culture media.
- Leaf it until solid , then incubated for 5 days at 25-28°C.

  Not: The dilution (1ml) can be distributed on surface of solid culture media .

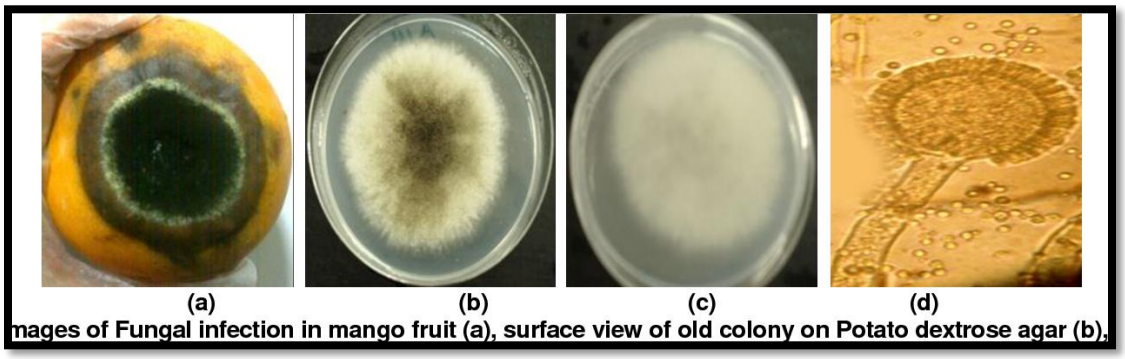
b- Filtration methods:

- Filtrate the water sample using cellulose filter Placed in a sterile suppression.
- After the passage the sample, Filter paper taken by sterile forceps and placed on surface of culture media then incubated the dishes for 5 days at 25-28 °C.

Filtration methods

4- Isolation fungi from fruits and vegetables:

- Placed the media into the plate and left it until solid .
- Surface sterilization to rotten part by **Sodium Hypochlorite or** Potassium Permanganate (2%) .
- Washing the sample by (D.W) 2-3 times .
- Transfer part rotten to the Petri dish by a sterile needle.
- Incubated the dishes for 5 days at 25-28 °C.



Isolation fungi from fruits

Not: Sometimes **baits** used to attract certain types of fungi, where a growth appear clear on these baits then transferred to the culture media , the baits may be some type of fruits , seeds, skin, hair .

purification of fungal culture :

A simplified method of purification of fungal isolates in a single petri dish and obtaining pure isolation for one species of fungi, As well as the study of the history of life of these fungi and methods of parasitism and nutrition. Look figure (1)

A- Show Petri dish before purification .
B- Show Petri dish after purification .

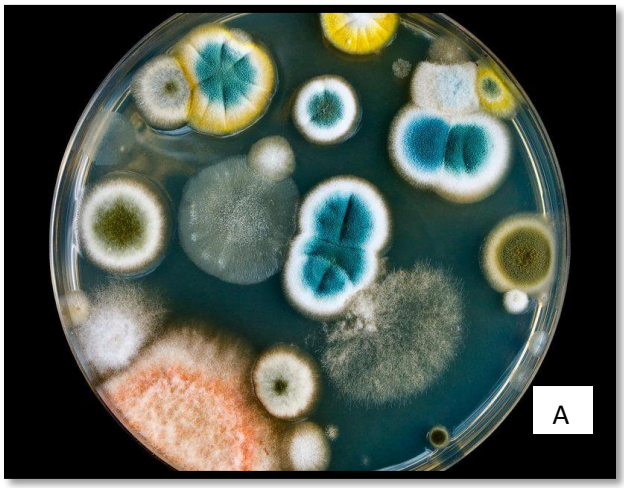


figure (1)

Types of fungal purification

a- Single spore culture:

Obtain pure culture growing from single spore , as follows:

- ❖ Preparation fungal suspension.
- ❖ Dipping a needle vaccination(Swab) in fungal suspense and then being a simple streak on the surface of solid plate.
- ❖ Streak 3-4 solid plates in the same Swab.
- ❖ Incubating dishes 25-28 ° C For 5 days.
- ❖ Re-cultivation spores in new solid plate .
- ❖ Saved on the slant culture media.

b- Hyphal tip culture :

This method used in the case of fungi that do not produce spores easily .

- ❖ Using Capillary glass tube to separate a small part of the hyphal tip.
- ❖ Transfer hyphal tip to the solid plate.
- ❖ Incubating dishes 25-28 ° C For 5 days.

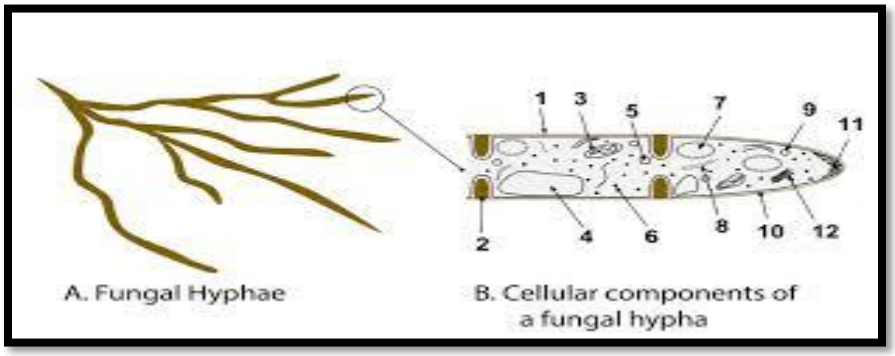


figure (2)

Diagnosis of fungal :

Definition of fungi isolated through morphological characteristics and microscopic characteristics of the farm fungal .

- ❖ **Morphological characteristics :** Examination fungal growth on the culture media morphologically and registration morphological characteristics such as the quality of the growth on

culture ، color culture ، growth density، tip shape and watching the color change of fungal growth.

- **Microscopic characteristics:** Prepare slides of fungal growth ، staining the slides and examine under Microscopic to see the hyphal shape ، spores shape ، conidial shape .

Special Dyes in slides fungi :

- **LactoPhenol Cotton Blue (LPCB):** This type of Dyes used to staining most types of fungi ، consist of
  1- **Phenol :**which it kill any microorganism.
  2- **Lactic acid :** protect the fungal structure **.**
  3- **Cotton blue :** staining the chitin in the fungal cell wall.
  4- **Distell water**
  5- **Glycerol**
- **Potassium hydroxide preparation :**used to staining the fungi that infected the hairs and nails (Dermatophytosis) ، consist of :
  1- **Potassium hydroxide:** melting the keratin found in samples (hairs ،nails) .
  2- **Distell water**
  3- **Glycerol**

Pathogenicity : the ability of a microorganism to produce disease in a host.

Pathogenicity test :

1- **Use a seeds :**

- **Preparation spore suspension to The fungus specific to pathogenicity test .**
- **Bring certified seeds.**
- **Providing clean soil in pots ، With three pots as follows:**

  1 – The First Pot is a control (just put the seed).

  2 – The second pot contains seed soaked spore suspension

  3 - The third pot contains a soil sprinkled with spore suspension .

- **Note : the seedling growth in a week and record results.**

Figure(1) Pathogenicity test use seeds

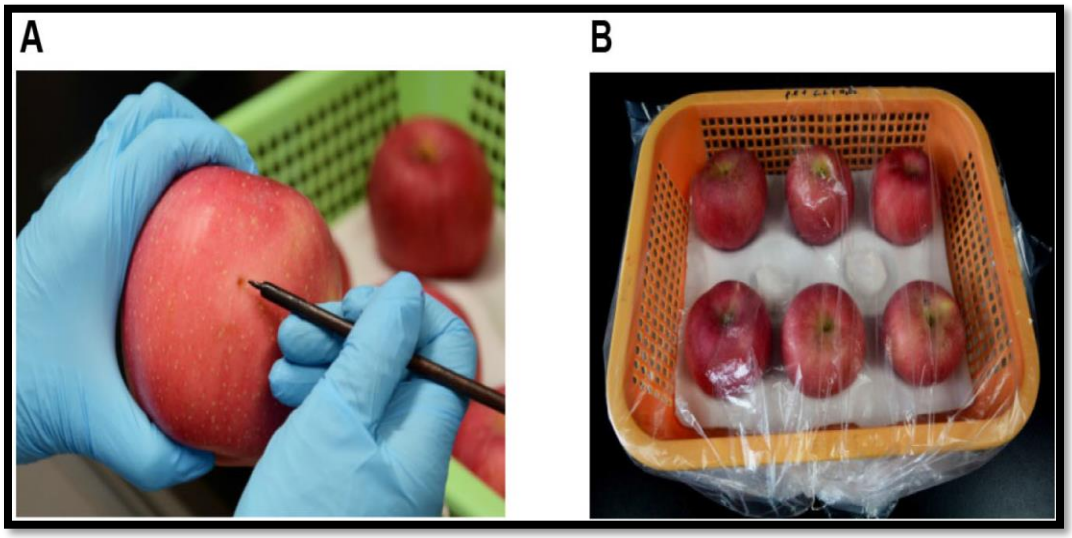
2- Use a fruits:

- **Preparation spore suspension to The fungus specific to pathogenicity test .**
- **Bring fruits sound and free from any scratch (Any type).**
- **Hole the fruit of three equal places by needle.**
- **Each type of fungus two fruit as follows:**

1 - The First fruit is a control (just hole three equal places).

2 - The second a fruit exposed to spray spore suspension on the holes areas .

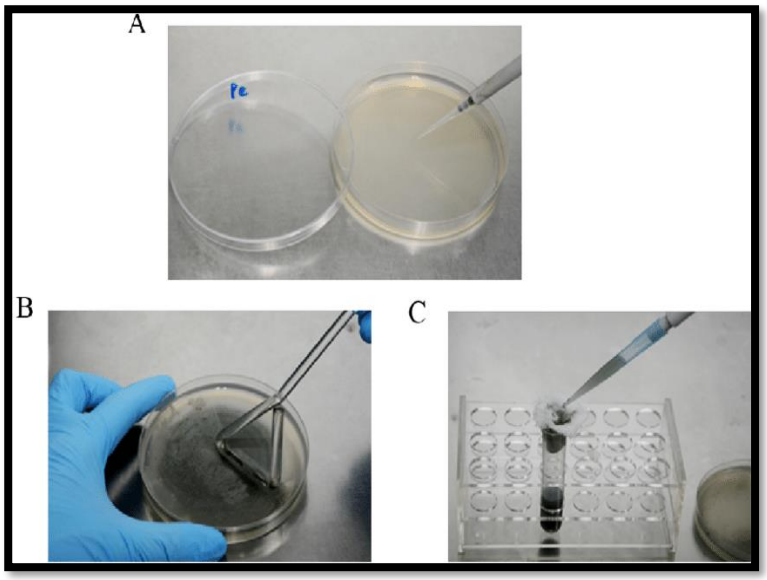
- **Put fruits inside a sterile bag ، and then inside the incubator for 5 to7 days at 25° with note appearance spots on the fruit, measured and compared with control.**



Figure(2) Pathogenicity test use fruit

Spore suspension preparation :

1- Pour 10 ml of distilled water on the petri dish container fungal isolates under study .
2- By a glass slide is harvested fungal inside dish .
3- Filter by filter paper .
4- Mixing tube by Vortex .



Figure(3) Spore suspension

Or

1- Cutting disc the size of 1 cm from the petri dish containing the fungal .
2- Placed inside the tube container 10 ml of distilled water.
3- Mixing tube by Vortex .

Classification of fungi :

Classification : is the systematic arrangement of organisms into groups based on specific standards .

Standard Endings :

Division ……....mycota

Sub division …….mycotina

Class …….mycetes

Subclass…….mycetidae

Order …….ales

Family ……..aceae

Writing scientific name: The first letter of the genus written with a capital letter , while the species with small letter, The scientific name written in *italics* or underlined .ex: aspergillus niger

Aspergillus niger ***OR*** **Aspergillus niger**

The fungal characteristics that use in Classification :

- **Cell wall**
- **The cell wall chemical component**
- **Somatic phase**
- **Reproduction**
- **The structural that formed by fungi**
- **Fruiting bodies**
- **Spores**

Fungal Webster , Weber and Hibbett Classification(2007):

1- Kingdom : protozoa (protista)

Phylum : myxomycota
Phylum : plasmodiophoromycota

2- Kingdom : Chromista (Stramenopila)

Phylum : Hyphochytriomycota
Phylum : Labrinthulomycota
Phylum : Oomycota

3- Kingdom : Fungi

Phylum : Chytridiomycota
Phylum : Zygomycota
Phylum : Ascomycota
Phylum : Basidomycota
Phylum : Deutromycota

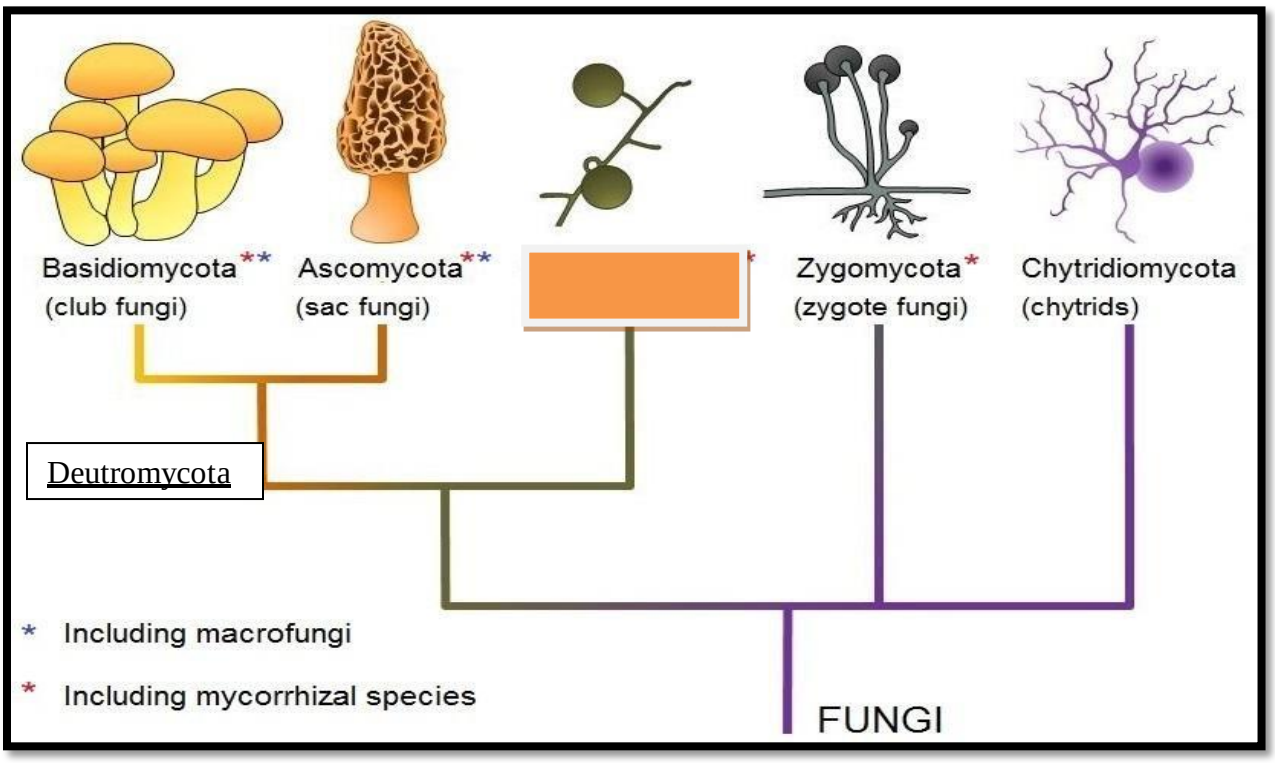


Figure (1) kingdom fungi

Kingdom : protozoa (protista)

1- No cell wall so also called Gymnomycota
2- Phagotrophic nutrition .
3- Somatic phase are **plasmodium** .
   - ❖ **Plasmodium:** protoplasmic mass Similarity Amoeba , multinucleated also called true slime mold.
   - ❖ **Pseudo Plasmodium :** Accumulation of Amoeba cells surrounded by membrane , also call cellular slime mold.

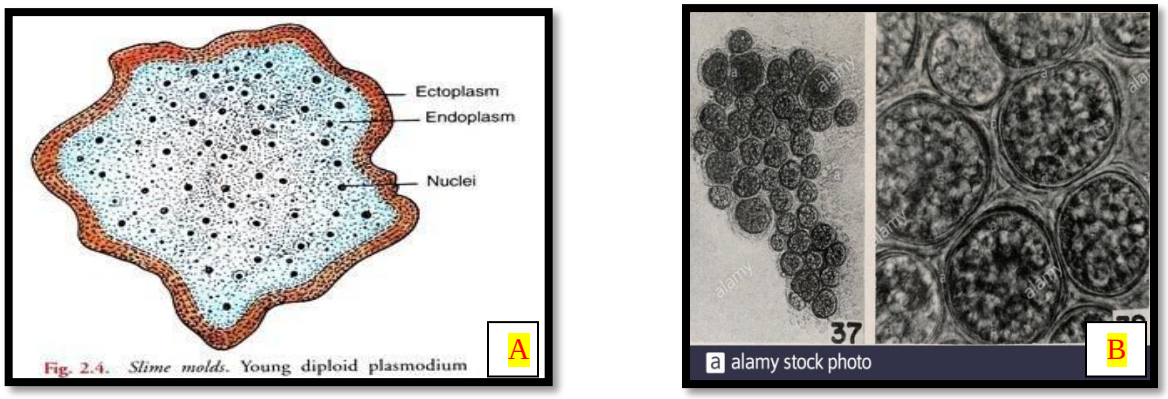


Figure (2) (A)plasmodium (B) Pseudo Plasmodium

Type of plasmodium

1- **Protoplasmodium** : microscopic,slow movement,produce one sporangium .
2- **Aphanoplasmodium** : grow as Protoplasmodium at the beginning , and then it will change into net work .
3- **Phaneroplasmodium** : grow as Protoplasmodium at the beginning then it will change into granular and more density plasmodium . it is visible .

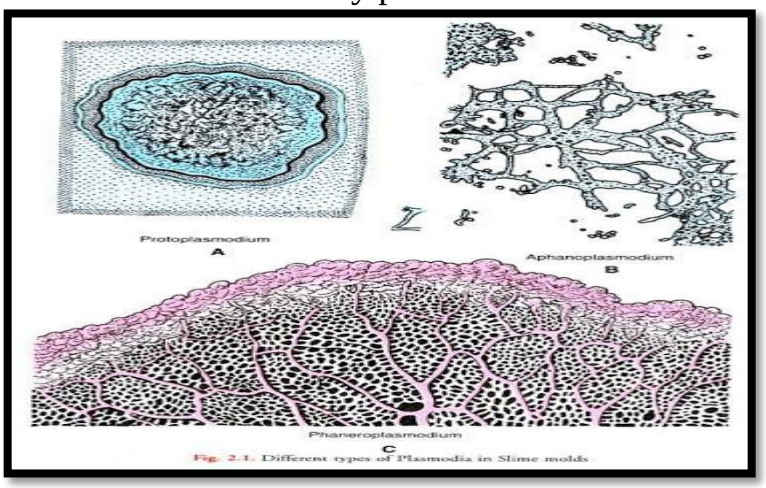


Figure (3) type of plasmodium

Phylum : myxomycota
Class: myxomycetes

1- True slime mold
2- No cell wall.

Life cycle are four stages or four types of cells:

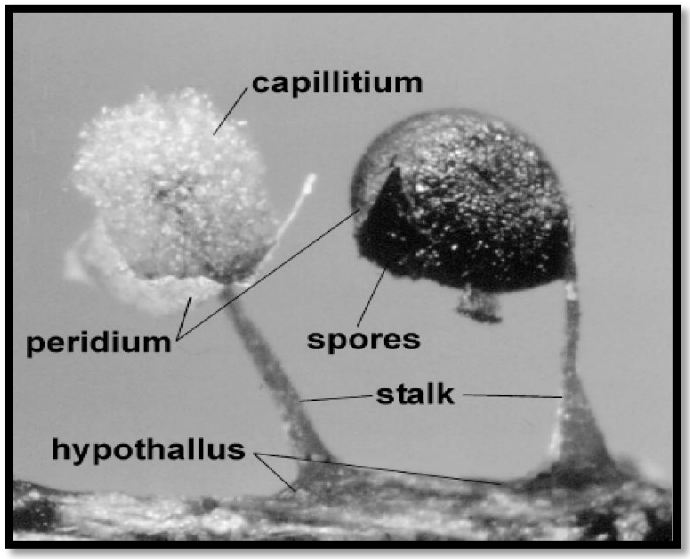
1- Three cells uninucleated ,one of them flagellated
2- Somatic phase as plasmodium multinucleated
3- Somatic phase resisted to environment condition called **sclerotium .**
4- Reproductive phase as sporophores which contain inside of it spores that have cell wall .

❖ **Peridium** : non-cellular envelope covers spores inside sporophore.

Type of sporophores (fruiting body)

1- Sporangium : consist of

a- Peridium
b- Columella
c- Capillitium
d- Stalk
e- Hypothallus
f- Spores



Figure(1) Sporangium consist of slime molds

2- **Aethalium :** big like cushion shape **.**

3- **Pseudoaethalium :** accumulation of several sporangium .

4- **Plasmodiocarp :** like plasmodium .

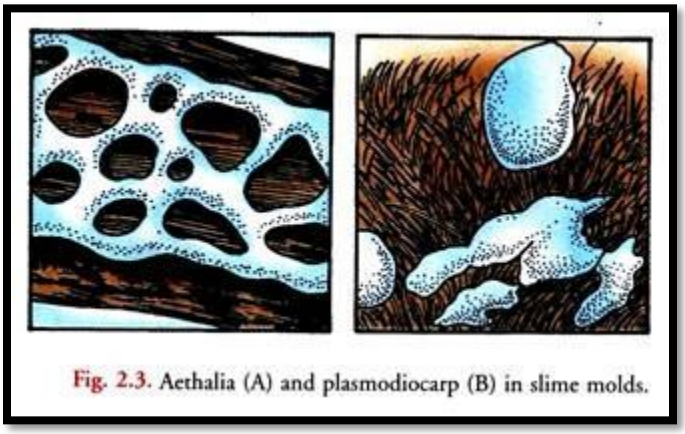


Figure (2) Aethalia and plasmodiocarp of slime molds

1- Sub class : ceratiomyxomycetidae

Order : Ceratiomyxales

Family : Ceratiomyxaceae

Genus : *Ceratiomyxa sp.*

❖ Forming exospores , no sporophores . cell wall

2- Sub class : Stemonitomycetidae

Order : Stemonitales

Family : Stemonitaceae

Genus : *Stemonitia sp.*

❖ Plasmodium kind Aphanoplasmodium , sporangium , violet spores

3- Sub class : Myxogasteromycetidae

1- Order : Liceales

Licea sp.

Lycogala sp.

❖ Plasmodium kind Protoplasmodium and Aphanoplasmodium, spores light colors, Aethalium.

2- Order : Trichiales

Metatrichia sp. (wasp nest slime mold)

Trichia sp.

Arcyria sp.

❖ Plasmodium kind Protoplasmodium and Aphanoplasmodium , sporangium ,spores light or red colors.

Phylum 2 : plasmodiophoromycota
Class : plasmodiophoromycetes
Order : plasmodiophorales
Family : plasmodiophoraceae

General characteristics of this Division:

1- Somatic phase is plasmodium , no cell wall .
2- Multi nuclei .
3- Endobiotic or Endoparasitic on vascular plants or on kingdom Stramenopila.
4- Necrotrophic meaning :kill the host cell before feeding . not phagotrophic .
5- Forming zoospore, have two flagella type whiplash and unequal length .
6- Presence nuclear division Cruciform .

Economic importance :

1- ***Plasmodiophora brassicae*** parasitic on Cabbage causing club root.

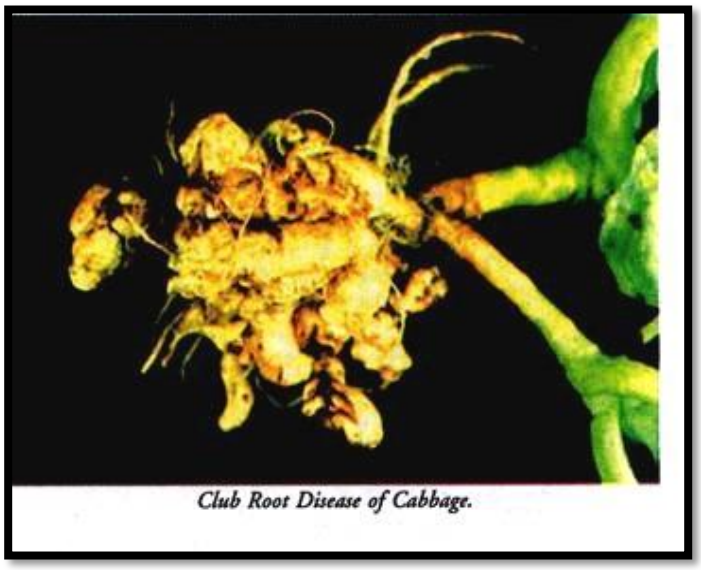


Figure (3) *Plasmodiophora brassicae* causing club root on Cabbage

2- ***Spongospora sp***. parasitic on potato causing powdery scab .

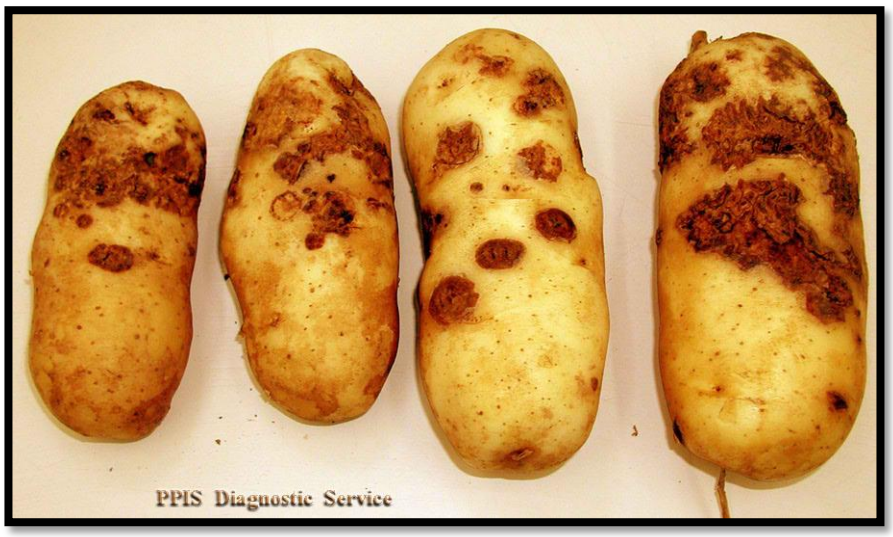


Figure (4) *Spongospora sp*. causing powdery scab on potato

3- Parasitic on water mold (Oomycota) ***Saprolegnia sp.*** which parasitic on fish and their eggs, so it used as biological control .
4- Viruses transporter that cause plant disease .
5- Some of them are parasitic on fresh water algae .

Kingdom : Chromista

Phylum: Oomycota

Class : Oomycetes

- lack chitin in their cell walls
- often referred to as **water molds**
- Have large round Oogonia , structures containing the female gametes .
- rarely have septa

Order: peronosporales

1- Cause plant diseases such as **white rust** and **downey mildew** .
2- All its members obligated parasitism .
3- Mycelium non septet .
4- Asexual reproduction by forming zoospore Binary flagella, Kidney shape , Flagella get out through the concavity region .
5- The shape of sporangiophore is club , There are arranged vertically under the epidermis of host plant .
6- Sporangio in the appropriate environmental conditions are divided into many **spores** Possible to germinate and occur a new infections.
7- Sporangio in the inappropriate environmental conditions behave **conidia** occur a new infections.
8- This order have many families divided according to
   a- presence sporangio within the host.
   b- shape and regularity asexual units on sporangiophore.

Family 1- : pythiaceae :

grow continuously for sporangio on single thread (hypha).

G1 : *pythium debaryanum* (causes damping of seedling disease)

Asexual reproduction : sporangio are circular carry on the thin stalk like hypha, not release zoospore directly, but forming tube at the end of the tube forming vesicle have all the contents of sporangio than forming zoospore and when

release loses flagella and becomes kidney shape than spores germinate forming a new hypha .

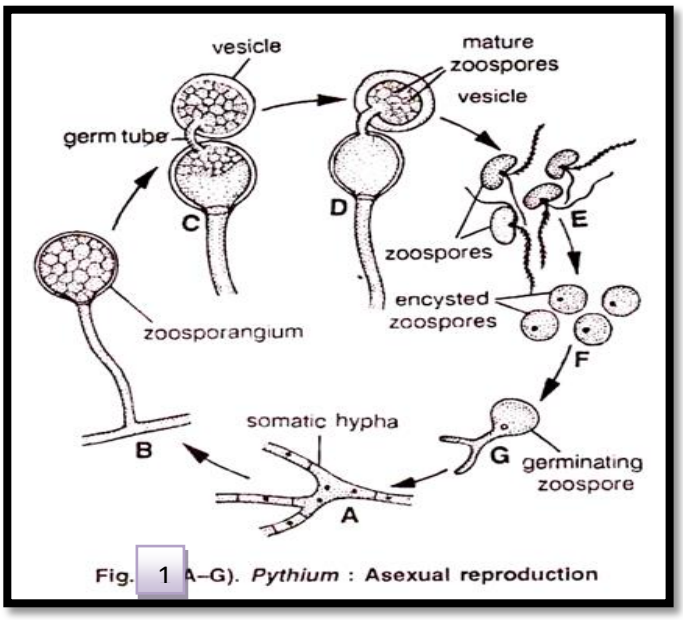


Fig. 1 (A–G). *Pythium* : Asexual reproduction

Sexual reproduction: fertilization between male gametes (Anthridium) and female gametes (oogonia) forming **Oospore** which germination to a new hypha when temperatures are high , But if temperatures have decreased forming vesicle and release zoospore and germination to a new hypha.

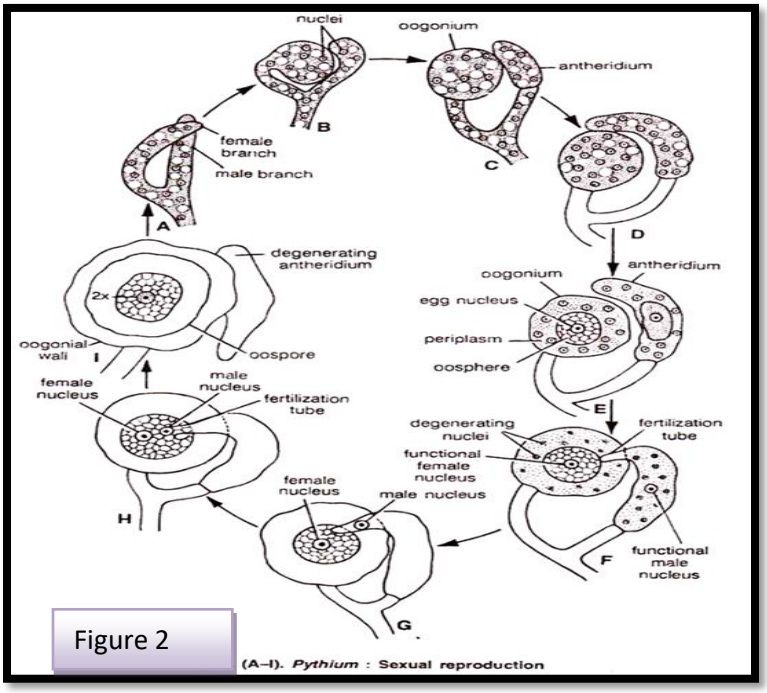


Figure 2 (A–I). *Pythium* : Sexual reproduction

G 2: ***phytophthora infestans*** (causes late blight of potato disease)

	Phytophthora	***Pythium***
1-	Shape the sporangio are lemon	Shape the sporangio are circular
2-	Zoospore release from papilla	Zoospore release from vesicle
3-	Sporangiophore are clearly	Sporangiophore are Thin
4-	Anthridium arise from base oogonium	Anthridium be side-Site

Family 2- : albuginaceae

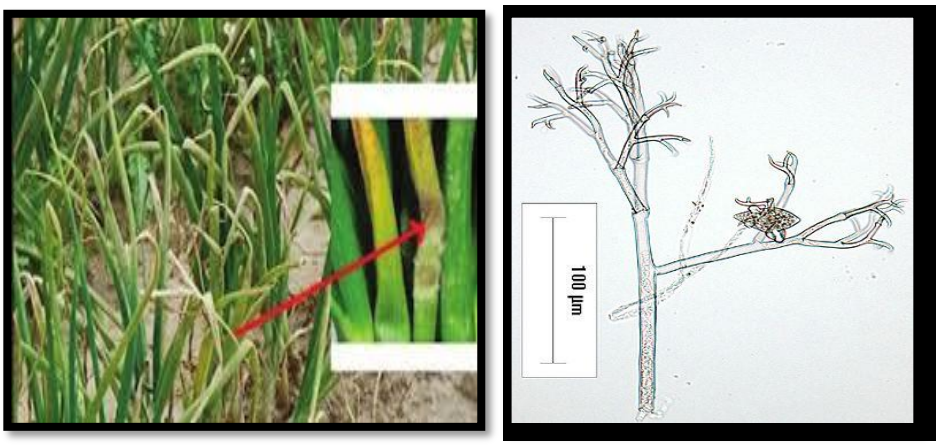
1- All its members obligated parasitism .
2- Cause plant diseases such as **white rust** (White pustules glossy on Plants leaves).
3- The shape of sporangiophore is club .
4- Infects members plants of Family Cruciferae .
5- G : ***Albugo candida***


Figure 3 white rust

Family 3-: peronosporaceae

1- Members obligated parasitism on many economic plants .
2- Cause plant diseases such as **downey mildew(**Disease symptoms appear shaped yellowing spots on the upper surface of the leaves.
3- sporangiophore are clearly and branches.

G 1- *Peronospora destractor* ---- infects Onions and tobacco, Binary Branches with acute angle in endes carry sporangio.



G 2- *Plasmopora viticola* -------- infects grapes , The ends of the branches there is a small bump called **sterigma** carry sporangio .

G 3- *Bremia lectucae* ---------- infects lettuce**,** The ends of the branches have 3-4 sterigma carry sporangio .