DIYALA UNIVERSITY

COLLEGE OF SCIENCE

BIOLOGY DEPARTMENT

Lectures of Mycology

Third class

First semester

2022-2023

Presented By

Mycologist

Dr. Abbas Ammari

Some mycological terms

Anamorph An asexual state of a fungus

Biotrophic Fungi that require living plants to survive; is not a symbiotic relationship

Chlamydoconidium (pl. Chlamydoconidia) A thick-walled, thallic conidium formed within the vegetative hyphae. Chlamydoconidia function as organs of perennation rather than dissemination.

Chytrid A simple, microscopic, aquatic fungus

Coenocytic Infrequently septate, multi-nucleate hyphae as in the Zygomycetes

Columella (pl. columellae) A sterile dome-like structure at the tip of a sporangiophore or within a sporangium.

Conidiogenous cell A cell that forms conidia

Conidiophore A specialized hypha upon which conidia develop.

Conidium (pl. conidia) An asexual reproductive propagule formed in any manner that does not involve cytoplasmic cleavage. Conidia function as organs of dissemination.

Dematiaceous A dark brown, greenish gray or black colour.

Dermatophyte A fungus belonging to the genera Epidermophyton, Microsporum, or Trichophyton with the ability to utilize keratin to infect hair, nail and skin.

Deuteromycetes An artificial subdivision to accommodate those fungi where only the asexual state is known

Dimorphic Having two different morphological forms

Downy Having a short and dense mycelial texture

Ectothrix Natural hair invasion by a dermatophyte characterized by arthroconidia on the outside of the hair shaft.

Endophyte Fungus living inside a plant for part of the plant's life without causing harm that can be seen

Endothrix Natural hair invasion by a dermatophyte characterized by the development of arthroconidia within the hair shaft only

Germ tube The initial hypha that develops from a conidium or spore

Heterothallic A fungus that requires mating between two compatible strains for sexual reproduction to occur

Hyalo- A prefix meaning hyaline to lightly coloured

Hypha (pl. hyphae) A single filament of a fungus

Hyphomycetes A class of mycelial moulds which reproduce asexually by conidia on hyphae or aggregations of hyphae

Lichen An alga or cyanobacterium combined with a fungus

Macroconidium (pl. macroconidia) The larger of two different types of conidia produced by a fungus in the same manner

Microconidium (pl. microconidia) The smaller of two different types of conidia produced by a fungus in the same manner

Mycelium Body of fungus usually hidden inside wood or underneath the ground

Mycorrhizae Fungi that live on plants' roots to help produce necessary nutrients

Peridium The outer wall of an ascocarp.

Phaeo- A prefix meaning darkly pigmented

Rhizoids A short branching root-like hyphae seen in some Zygomycetes

Septum (pl. septa) A cross wall in a hypha

Sporangiolum (pl.) A small sporangium producing a small number of Sporangiospores

Sporangiophore A specialized hypha that bears a sporangium

Sporangiospore An asexual spore produced within a sporangium

Sporangium (pl. sporangia) A sac-like structure producing asexual spores endogenously by cytoplasmic cleavage

Spore a reproductive propagule formed by either meiosis or mitosis. However, if by asexual means, cleavage of cytoplasm is usually involved.

Sterigma (pl. sterigmata) A small pointed structure upon which a basidiospore forms

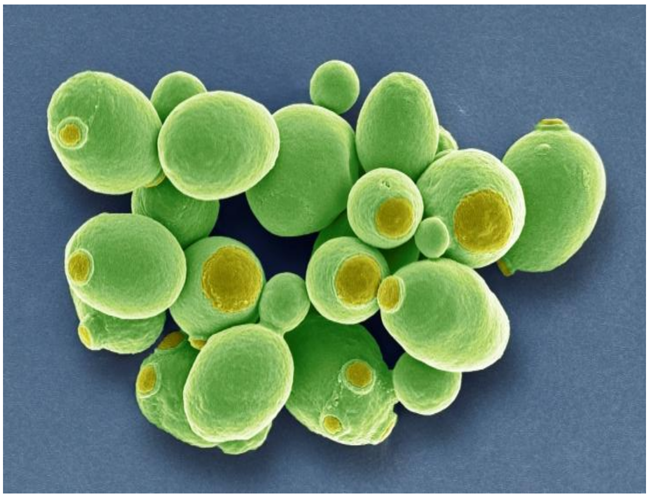
Teleomorph The sexual state of a fungus

Uniserate Phialides arising directly from a vesicle as in Aspergillus

Introduction to mycology:

- ✓ The study of fungi is called mycology, it comes from myco = "fungus" and logy= "science"
- ✓ A person who studies fungi is a mycologist
- ✓ A taxonomic group that includes heterotrophic eukaryotes that are usually filamentous, devoid of chlorophyll, with chitinous cell wall, and produces spores
- ✓ Most species of fungi live as multicellular filaments called hyphae, which form a mycelium while other species live as unicellular.
- ✓ Fungi that reproduce through asexual spores and sexually-produced spores are called perfect fungi whereas fungi that reproduce only by asexual spores are called imperfect fungi (deuteromycetes).

- ✓ Fungi do not have chlorophyll, hence, they are heterotrophic organisms, absorbing foods into their hyphae.
- ✓ Examples of fungi are yeasts, rusts, stinkhorns, puffballs, truffles, molds, mildews and mushrooms .



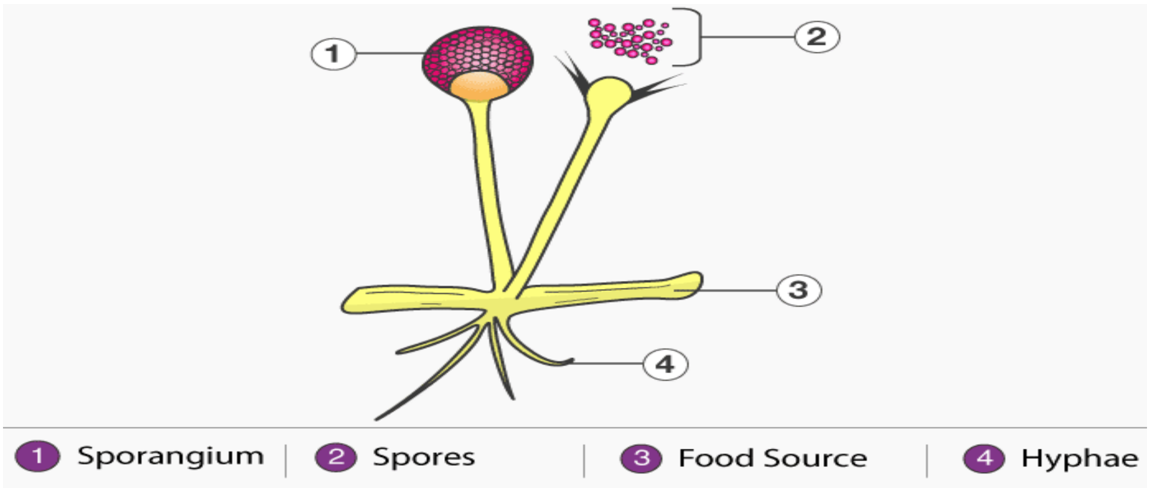
History of Mycology

- Mycology is a relatively new science that became systematic after the development of the microscope in the 16th century.
- Elias Magnus Fries, Christian Hendrik Persoon, Anton de Bary, and Elizabeth Eaton were among the first mycologists. Pier Andrea Saccardo devised a technique for identifying imperfect fungus based on spore colour and morphology, which became the standard approach prior to DNA-based categorization.
- Fungal spores were first observed by Giambattista della Porta in 1588.
- Hendrik Persoon (1761–1836) established the first classification of mushrooms with such skill so as to be considered a founder of modern mycology.
- Pier Antonio Micheli in 1737 marks the beginning of the modern period of mycology.
- Elias Magnus Fries (1794–1878) further elaborated the classification of fungi, using spore color and various microscopic characteristics, methods still used by taxonomists today.

- Anton de Bary(1861) established modern mycology ; he studied slime molds, rusts and late blight of potato diseases.
- Berfield(1875) studied smut disease and he used copper sulphate to control plant diseases caused by fungi.

Characteristics of Fungi

1. They are eukaryotic; cells contain membrane bound cell organelles including nuclei, mitochondria, Golgi apparatus, endoplasmic reticulum, lysosomes etc. They also exhibit mitosis.
2. Have ergosterols in their membranes and possesses 80S ribosomes.
3. Have a rigid cell wall and are therefore non-motile, a feature that separates them from animals. All fungi possess cell wall made of chitin.
4. Are chemoheterotrophs (require organic compounds for both carbon and energy sources) and fungi lack chlorophyll and are therefore not autotrophic.
5. Fungi obtain their nutrients by absorption.
6. They obtain nutrients as saprophytes (live off of decaying matter) or as parasites (live off of living matter).
7. All fungi require water and oxygen and there are no obligate anaerobes.
8. Typically reproduce asexually and/or sexually by producing spores.
9. They grow either reproductively by budding or non-reproductively by hyphal tip elongation.
10. Food storage is generally in the form of lipids and glycogen.



Importance of Fungi

Many fungi can degrade complex organic macromolecules like lignin, which is a more durable component of wood, as well as contaminants like xenobiotics, petroleum, and polycyclic aromatic hydrocarbons. Fungi play an important part in the global carbon cycle by degrading these compounds. Fungi and other creatures that are usually classified as fungi, such as oomycetes and myxomycetes (slime moulds), are frequently economically and socially significant because they cause illnesses in animals (including humans) and plants. Aside from harmful fungus, a variety of fungal species play a significant role in the management of plant diseases caused by various pathogens. For example, members of the filamentous fungus genus Trichoderma are regarded as one of the most significant biological control agents for crop disease management as an alternative to chemical-based treatments.

Beneficial Effects of Fungi:

1. Decomposition - nutrient and carbon recycling.
2. Biosynthetic factories. The fermentation property is used for the industrial production of alcohols, fats, citric, oxalic and gluconic acids.
3. Important sources of antibiotics, such as Penicillin.
4. Model organisms for biochemical and genetic studies. Eg: Neurospora crassa
5. Saccharomyces cerviciae is extensively used in recombinant DNA technology, which includes the Hepatitis B Vaccine.
6. Some fungi are edible (mushrooms).
7. Yeasts provide nutritional supplements such as vitamins and cofactors.
8. Penicillium is used to flavour Roquefort and Camembert cheeses.
9. Ergot produced by Claviceps purpurea contains medically important alkaloids that help in inducing uterine contractions, controlling bleeding and treating migraine.
10. Fungi (Leptolegnia caudate and Aphanomyces laevis) are used to trap mosquito larvae in paddy fields and thus help in malaria control.

Harmful Effects of Fungi:

1. Destruction of food, lumber, paper, and cloth.

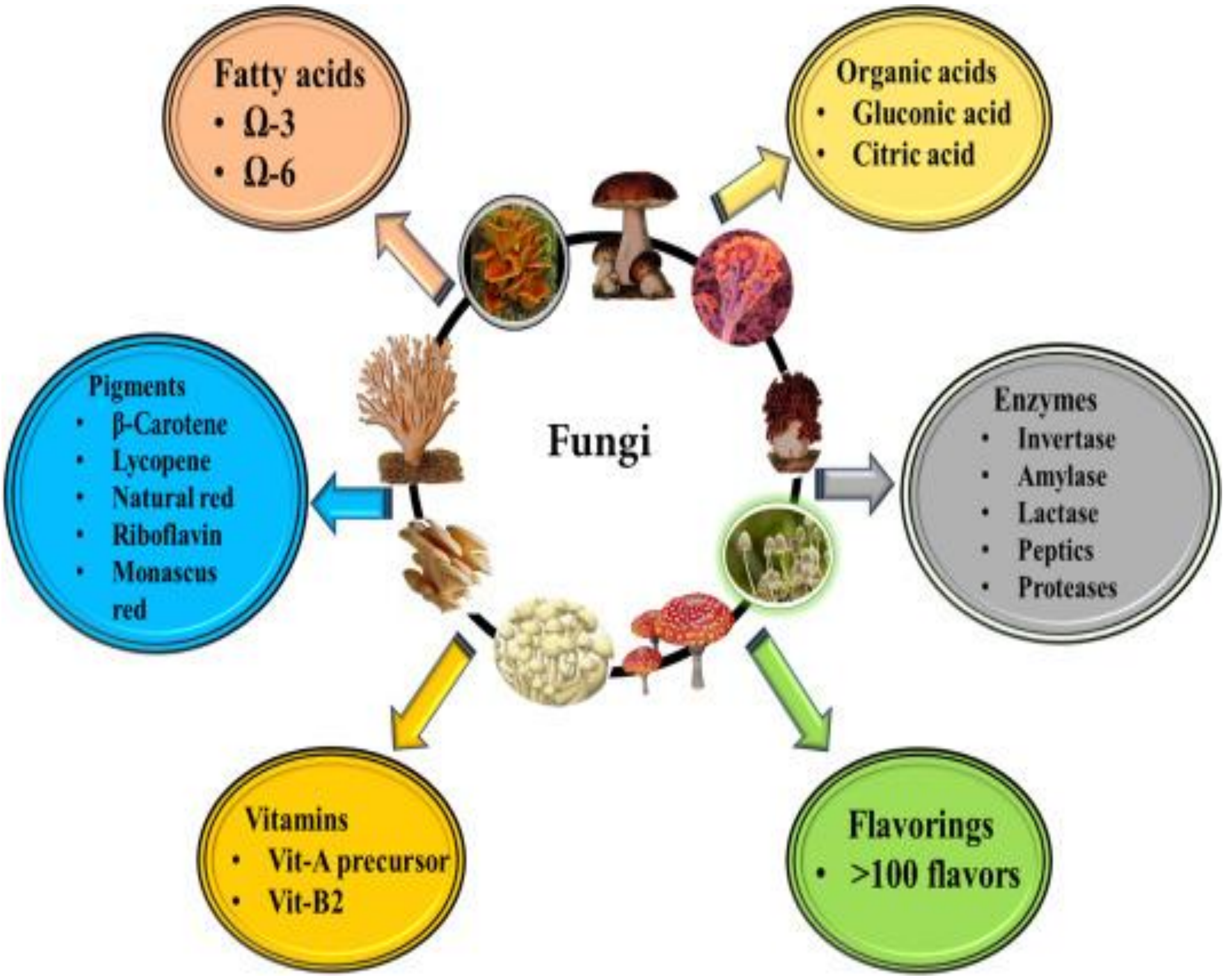
2. Animal and human diseases, including allergies.

3. Toxins produced by poisonous mushrooms and within food (Mycetism and Mycotoxicosis).

4. Plant diseases.

5. Spoilage of agriculture produce such as vegetables and cereals in the godown.

6. Damage the products such as magnetic tapes and disks, glass lenses, marble statues, bones and wax .



Composition of fungal cell

✓ fungal cell has a rigid cell wall in chemical composition are composed mostly chitin (N-acetyl glucose amine) in addition to glucan and cellulose , surrounding the protoplast (nucleus and cytoplasm).

✓ Plasma membrane semi permeable contains protein

and lipid.

✓ nucleus most fungi have very small nuclei, with little repetitive DNA. Surrounding by nulear membrane.

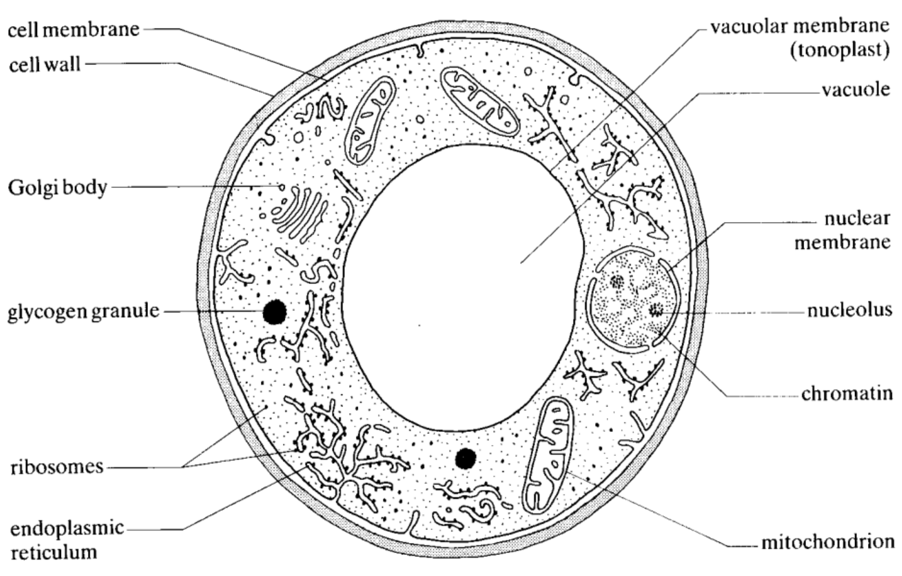
✓ Other organelles Mitochondria—flattened or plate-like mitochondrial cristae in Fungi (similar to animals

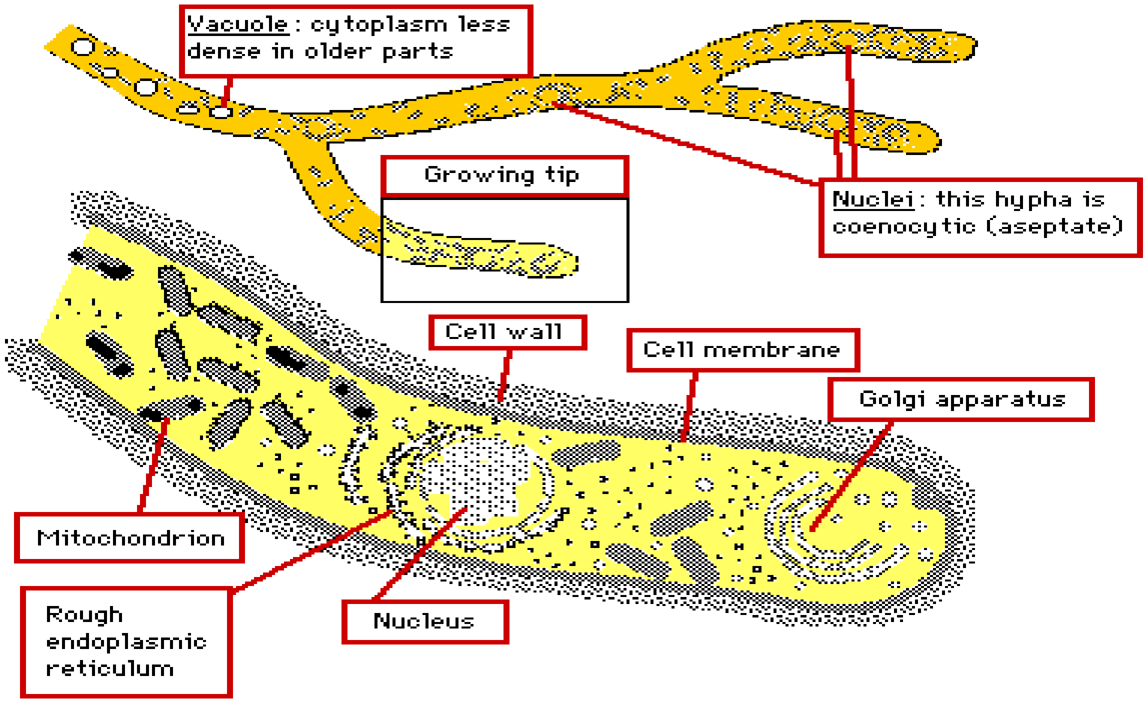
✓ Golgi bodies—consist of a single, tubular cisternal element (stacked, plate-like cisternae in animals and plants)

✓ Other types:

ribosomes, endoplasmic reticulum, vacuoles, lipid bodies, glycogen storage particles, microbodies, microtubules, vesicles

Yeast Cell





Somatic phase of fungi

Mycelium are of three kinds:

1. Vegetative mycelium are those that penetrates the surface of the medium and absorbs nutrients.

2. Aerial mycelium are those that grow above the agar surface

3. Fertile mycelium are aerial hyphae that bear reproductive structures such as conidia or sporangia.

Since hypha is the structural unit of mould, the mycelium imparts colour, texture and topography to the colony. Those fungi that possess melanin pigments in their cell wall are called phaeoid or dematiaceous and their colonies are coloured grey, black or olive. Examples are species of Bipolaris, Cladosporium, Exophiala, Fonsecaea, Phialophora and Wangiella Those hyphae that don't possess any pigment in their cell wall are called hyaline.

Hyphae may have some specialized structure or appearance that aid in identification. Some of these are:

a) Spiral hyphae: These are spirally coiled hyphae commonly seen in *Trichophyton mentagrophytes*.

b) Pectinate body: These are short, unilateral projections from the hyphae that resemble a broken comb. Commonly seen in *Microsporum audouinii*.

c) Favic chandelier: These are the group of hyphal tips that collectively resemble a chandelier or the antlers of the deer (antler hyphae). They occur in *Trichophyton schoenleinii* and T*richophyton violaceum.*

d) Nodular organ: This is an enlargement in the mycelium that consists of closely twisted hyphae. Often seen in *Trichophyton mentagrophytes* and *Microsporum canis*.

e) Racquet hyphae: There is regular enlargement of one end of each segment with the opposing end remaining thin as seen in *Epidermophyton floccosum and Trichophyton mentagrophytes.*

f) Rhizoides: These are the root like structures seen in portions of vegetative hyphae in some members of zygomycetes. G

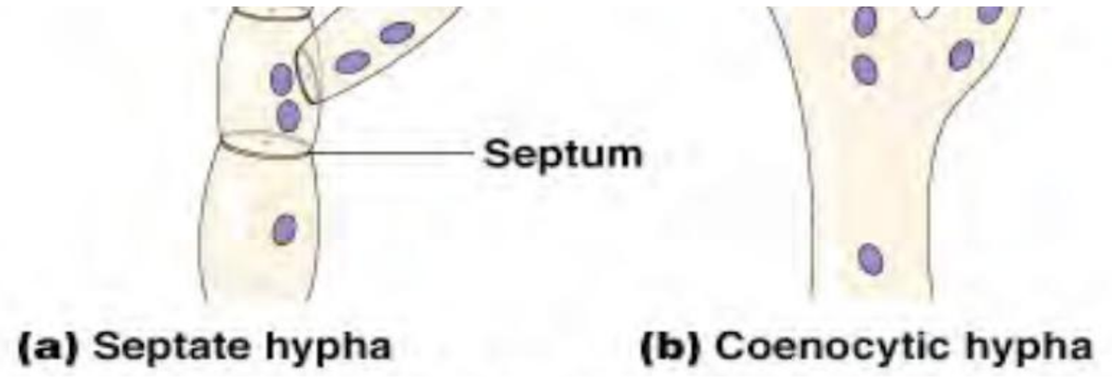
) There are structures in the hyphae, which arise out of modification of a single cell and transform into thick walled resting cells. Chlamydospore (or chlamydoconidia), which are produced by *Trichophyton schoenleinii* and *Trichophyton verrucosum* are thick walled cells that are larger than other cells and arranged singly or in groups. In some fungi such as *Trichosporon beigeilli* and *Coccidioides immitis* some alternating cells become thick walled and subsequently the intervening cells disintegrate leaving behind arthrospores (or arthroconidia).

Yeasts are unicellular spherical to ellipsoid cells. They reproduce by budding, which result in blastospore (blastoconidia) formation. In some cases, as the cells buds the buds fail to detach and elongate thus forming a chain of elongated hyphae like filament called pseudohyphae. This property is seen in *Candida albicans*. The same species also have the ability to produce true hypha, which is seen as germ tube. The difference between the two is that there is a constriction in psueudohyphae at the point of budding, while the germ tube has no Some yeast such as Cryptococcus and the yeast form of *Blastomyces dermatatidis* produce polysaccharide capsule. Capsules can be demonstrated by negative staining methods using India ink or Nigrosin. The capsule itself can be stained by Meyer

Mucicarmine stain. Some yeasts are pigmented. Rhodotorula sps produces pink colonies due to carotenoid pigments while some yeasts such as *Phaeoannellomyces werneckii* and Piedraia hortae are dematiaceous, producing brown to olivaceous colonies. True yeasts such as *Saccharomyces cerviciae* don't produce pseudohyphae. Yeast-like fungi may be basidiomycetes, such as *Cryptococcus neoformans* or ascomycetes such as *Candida albicans*.

✓ Multicellular fungi are composed of filaments called *hyphae* (singular: hypha). contain from Cylindrical, branching filaments composed of a tubular cell wall filled with cytoplasm and organelles . Hyphae are divided into two types according to exist of internal cross walls, called *septa*,

- The septa of many species have pores, allowing cytoplasm to flow freely from one cell to the next. Cytoplasmic movement within the hypha provides a means to transport of materials. The hyphae may be branched. A mass of hyphae that is not a reproductive structure is called a mycelium



Hyphal Morphogenesis

Fungal hyphae originate from a germinating conidium or another hypha during branch formation. An axis of polarity is set and cell surface expansion occurs along the axis to the hyphal tip and linear extension from it. Successive hyphal branching results in a tree-likemycelial network. Nutrients acquired at the colony periphery are distributed in the mycelium. As it grows older parts of the mycelium are recycled to support new growth. In the subkingdom Dikarya (including phyla Ascomycota and Basidiomycota) adjacent branches may fuse (anastomose), forming an interconnected network. The establishment and maintenance of hyphal polarity require microtubule- and microfilament-based motor proteins, chitin deposition, and both cyclic AMP and mitogen-activated protein kinase signaling.

Spitzenkorper are present in growing vegetative hyphal tips and branch points. They are located within the hyphal tip in the direction of hyphal growth. Spitzenk ¨orper are complex, multicomponent structures that function to support directional growth by concentrating and delivering secretory vesicles to the hyphal tip. Some of these vesicles are chitosomes, containing chitin synthase. That enzyme is activated when the chitosome fuses with the plasmalemma, initiating new chitin synthesis and polymerization of chitin microfibrils.

The cytoskeleton is very important in hyphal morphogenesis. Microtubules are responsible for the transport of secretory vesicles to the spitzenk ¨orper, while actin microfilaments control vesicle organization within the spitzenk ¨orper and transport from there to the plasmalemma. The spitzenkorper may be viewed as a supply and distribution center for vesicles involved in apical growth. Pseudohyphae are a feature of Candida species. Pseudohyphal cells bud in a unipolar manner, and the cells remain attached after cytokinesis.

Classification of fungi

Fungi were initially classified with plants and were a subject of interest for botanists; hence the influence of botany can be seen on their classification. In 1969 R.H Whittaker classified all living organisms into five kingdoms namely Monera, Protista, Fungi, Plantae and Animalia. Traditionally the classification proceeds in this fashion: Kingdom - Subkingdom - Phyla/phylum - Subphyla - Class - Order - Family - Genus- Species This classification is too complicated to be dealt here. There are alternate and more practical approaches, one based on sexual reproduction and the other based on morphology of the thallus (vegetative structure).

- **Based on Sexual reproduction:**

1. Zygomycetes: which produce through production of zygospores.

2. Ascomycetes: which produce endogenous spores called ascospores in cells called asci.
3. Basidiomycetes: which produce exogenous spores called basidiospores in cells called basidia.

4. Deuteromycetes (Fungi imperfecti): fungi that are not known to produce any sexual spores (ascospores or basidiospores). This is a heterogeneous group of fungi where no sexual reproduction has yet been demonstrated.

- **Based on Morphology:**

1. Moulds (Molds): Filamentous fungi Eg: Aspergillus sps, Trichophyton rubrum 2. Yeasts: Single celled cells that buds Eg: *Cryptococcus neoformans*, *Saccharomyces cerviciae*

3. Yeast like: Similar to yeasts but produce pseudohyphae Eg: *Candida albicans*

4. Dimorphic: Fungi existing in two different morphological forms at two different environmental conditions. They exist as yeasts in tissue and in vitro at 37^{o} C and as

moulds in their natural habitat and in vitro at room temperature. Eg: *Histoplasma capsulatum, Blastomyces dermatidis, Paracoccidiodes brasiliensis, Coccidioides immitis*

DIMORPHISM

Dimorphism (definition: two forms) is an important characteristic of certain fungal pathogens. Dimorphism is morphogenesis that allows growth to occur in either the mycelial or yeast forms, (mycelium → yeast, or yeast → mycelium conversion); for example, Histoplasma capsulatum is a dimorphic fungus. Fungi causingprimary systemic infections are typically filamentous soil-dwelling molds. The infectious propagules most frequently are conidia that are inhaled, along with hyphal fragments. Morphogenesis to the yeast form occurs during infection of tissues, usually in the lungs. This conversion is temperature sensitive, with the yeast form developing at 37◦ C. In the laboratory, growth at 35–37◦ C on an enriched medium may be used to help identify the fungus by this form change also known as "morphogenesis." There are notable exceptions to the mold-to-yeast dimorphism. The primary systemic pathogens *Coccidioides immitis* and *C. posadasii* grow as a mold formin the environment. The mold form fragments into arthroconidia, which are the infectious propagules. Once inhaled, arthroconidia convert to spherules, enlarge, and segment into endospores. Melanized molds (e.g., *Fonsecaea pedrosoi*, *Cladophialophora carrionii*), the causative agents of chromoblastomycosis, grow as molds in the environment but in the cutaneous and subcutaneous tissues convert to muriform cells—round cells that do not bud but enlarge and divide by internal septation. Growth by enlargement in all directions is called isotropic

Living mode of fungi

In nature fungi obtain their food either by infecting living organisms as parasites or by attacking dead organic matter as saprobes, many also form symbiotic relationships with higher plants as in mycorrhiza (Ectotrophic in **glomeromycetes fungi** and Endotrophic) and with blue green algae as in Lichens, example: **foliose lichen**. Fungi that live on dead matter and incapable of infecting living organisms are called (obligate saprobes example ***Mucor***); those capable of causing disease or of living on dead organic matter (facultative parasites (or) facultative saprobes: leaf curl fungi example ***Taphrina deformans***); and those that can't live except on living protoplasm, (obligate parasites such as **downy and powdery mildews**). A living organisms infected by parasite is known as the host.

Optimal conditions for fungal growth

❖ **Temperature and fungal growth**:

Microorganisms are often grouped into five broad categories in terms of their temperature ranges for growth:

✓ **Psychrophiles** (cold-loving): fungi are defined as having optimum growth at no more than 16°C and maximum growth of about 20°C. In many cases they would be expected to grow down to 4°C or lower, and usually do not survive at temperatures above 20 °C. There are many environments that could suit these organisms, including the deep waters of the oceans. the polar and alpine regions. Because they are active at low temperature, such as *Pseudogymnoascus pannorum Psychrophila Antarctic*

✓ **Mesophiles** (which grow at moderate temperatures),Most fungi are Mesophilic commonly growing within the range 10–40°C, though with different tolerances within this range. For routine purposes these fungi can usually be grown at room temperature (22–25°C). Two important examples shown in Fig. 1 are *Aspergillus flavus*, which produces the potent aflatoxins in stored grain products, and *Penicillium chrysogenum*, used for the commercial production of penicillins.

✓ **Psychrotrophic fungi** would be those that can grow at low temperatures but also above 20°C (5-30°C) .

✓ **Thermophiles** (heat-loving) Organisms that grow at optimum temperatures of 50 °C to a maximum of 80 °C are defined as having a minimum growth temperature of 70°C or above, a maximum growth temperature of 50°C or above, and an optimum in the range of about 40–50°C. *Aspergillus fumigatus*, *Rhizomucor pusillus*

✓ **Hyperthermophiles**: which are characterized by growth ranges from 80 °C to a maximum of 110 °C, with some extreme examples that survive temperatures above 121

°C, the average temperature of an autoclave. The hydrothermal vents at the bottom of the ocean are a prime example of extreme environments, with temperatures reaching an estimated 340 °C ((NO fungi can grow at this temperature)) the cell membrane contains high levels of saturated fatty acids to retain its shape at high temperatures

❖ **Hydrogen ion concentration and fungal growth**

The responses of fungi to culture pH need to be assessed in strongly buffered media, because otherwise fungi can rapidly change the pH by selective uptake or exchange of ions. Mixtures of KH2PO4 and K2HPO4 are commonly used for this purpose. It is then found that many fungi will grow over the pH range 4.0–8.5, or sometimes 3.0–9.0, and they show relatively broad pH optima of about 5.0–7.0. However individual species vary within this "normal" range, as shown by the three representative examples in Fig. 4. Several fungi are **acid-tolerant**, including some yeasts which grow in the stomachs of animals and some mycelial fungi (*Aspergillus*, *Penicillium*, and *Fusarium* spp.) which will grow at pH 2.0. But their pH optimum in culture is usually 5.5–6.0. Truly **acidophilic** fungi, able to grow down to pH 1 or 2, are found in a few environments such as coal refuse tips and acidic mine wastes; many of these species are yeasts.

❖ **Oxygen and fungal growth**

Most fungi are strict aerobes, in the sense that they require oxygen in at least some stages of their life cycle. Even *Saccharomyces cerevisiae*, which can grow continuously by fermenting sugars in anaerobic conditions, needs to be supplied with several preformed vitamins, sterols and fatty acids for growth in the absence of oxygen. *Saccharomyces* also requires oxygen for sexual reproduction. Having established these points, we can group fungi into four categories in terms of their oxygen relationships.

✓ Many fungi are **obligate aerobes**: their growth is reduced if the partial pressure of oxygen is lowered much below that of air. For example, growth of the take-all fungus of cereals is reduced. The thickness of water films around the hyphae can be significant in such cases, because oxygen diffuses very slowly through water, as we saw for the rhizomorphs of *Armillaria mellea* Aerobic fungi typically use oxygen as their terminal electron acceptor in respiration. This gives the highest energy yield from the oxidation of organic compounds.

✓ Many yeasts and several mycelial fungi (e.g. *Fusarium oxysporum, Mucor hiemalis*, *Aspergillus fumigatus*) are **facultative aerobes**. They grow in aerobic conditions but also can grow in the absence of oxygen by fermenting sugars. The energy yield from **fermentation** is much lower than from aerobic respiration, and the biomass production is often less than 10% of that in aerobic culture. However, a few mycelial fungi can use nitrate instead of oxygen as their terminal electron acceptor. This anaerobic respiration can give an energy yield at least 50% of that from aerobic respiration.

✓ A few aquatic fungi are **obligately fermentative**, because they lack mitochondria or cytochromes (e.g. *Aqualinderella fermentans*,Oomycota) or they have rudimentary mitochondria and low cytochrome content (e.g. *Blastocladiella ramosa*, Chytridiomycota). They grow in the presence or absence of oxygen, but their energy always comes from fermentation.

✓ **A few obligately anaerobic** : phylum Neocallimastigomycota they play an important role in the degradation of plant material. such as *Neocallimastix*

❖ **<u>Water availability and fungal growth</u>**

All fungi need the physical presence of water for uptake of nutrients through the wall and cell membrane, and often for the release of extracellular enzymes. Fungi also need intracellular water as a milieu for metabolic reactions. However, water can be present in an environment and still be unavailable because it is bound by external forces.

- **What are the important elements for fungal growth?**

1. Carbon sources: (carbohydrates) such as monosugar (glucose and fructose) or di sugar such as sucrose and maltose and multisugars such as starch

2. Nitrogen sources:

A. Organic source: such as Amino acids and peptone.

B. In organic source: such as nitrate and ammonia. The salts are added according to fungi requirements.

I. Macro elements: which add in large quantities such as Na, Mg, k, Zn.

II. Micro elements: which add in trace quantities such as Sc , M

Reproduction of fungi

Reproduction is the formation of new individuals having all the characteristics typical of the species. Two general types of reproduction are recognized: Sexual and asexual. Asexual reproduction sometimes called somatic or vegetative, does not involve the union of nuclei sex cells or sex organs. Sexual reproduction on the other hand, is characterized by union of two nuclei.

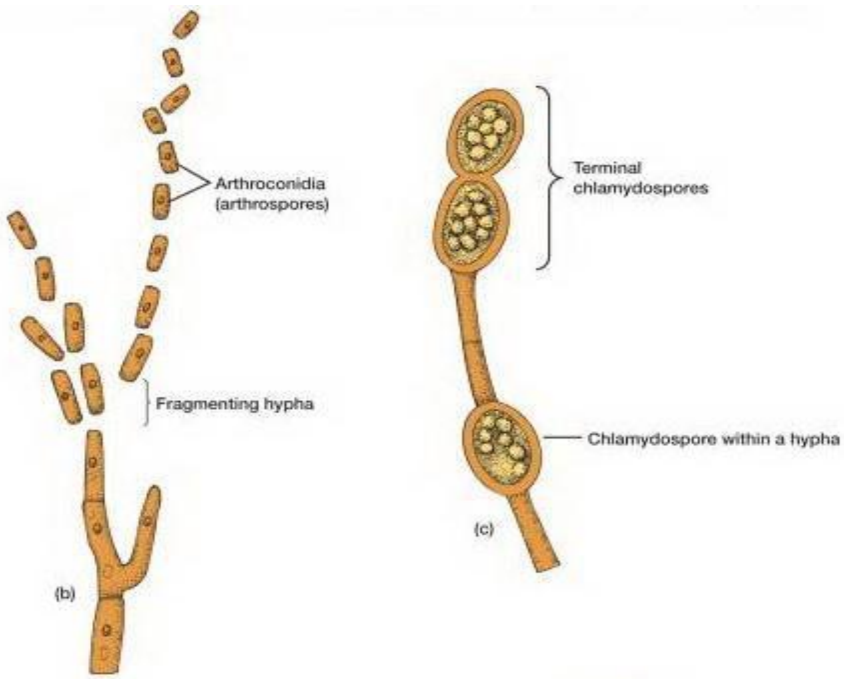
In the formation of reproductive organs, either sexual or asexual, the entire thallus may be converted into one or more reproductive structure, so that somatic and reproductive phases do not occur together in the same individual, fungi that follow this pattern are called **(Holocarpic)** . In the maturity of fungi, however the reproductive organs arise from only a portion of the thallus, while the remainder continuous its normal somatic activities, the fungi in this category are called **(Eucarpic)**.

Asexual reproduction

In general, asexual reproduction is more important for the propagation of the species because it results in the production of numerous individuals, and particularly since the asexual cycle is usually repeated several times during the season, whereas the sexual stage of many fungi is produced only once a year.

❖ Asexual reproduction methods

1) Fragmentation In this process, the mycelium breaks into two or more similar fragments either accidentally or due to some external force. Each fragment grows into a new mycelium. The hyphae may break up into their component cells that behave as spore. These spores are known as **arthrospores**. If the cells become enveloped in a thick wall before the separate from each other or from other hyphal cell, they are often called **chlamydospores**.



2) Simple fission of somatic cells into daughter cells

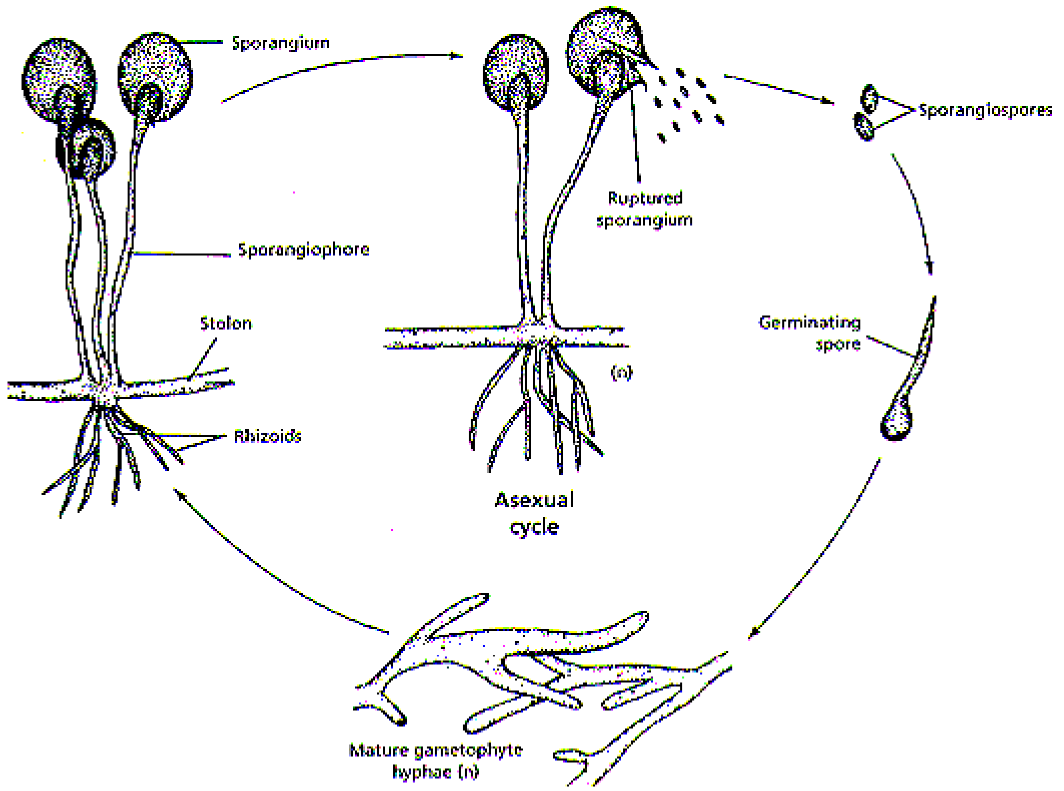
The simple splitting of a cell into two daughter cells by constriction and formation of a cell wall, is characteristic of a number of simple organisms including some yeast.

3) Budding of somatic cell or spores

Each bud producing a new individual. As the bud is formed, the nucleus of parent cell divides and one daughter nucleus migrates into the bud. The bud increases in size while still attached to the parent cell and eventually breaks off and form a new individual, example ***Saccharomyces* .**

4) Spore formation(vegetative reproduction)

The most common method of asexual reproduction in fungi is by means of spores. Spores vary in color from hyaline through green, yellow, orange, red, brown to black; in size from minute to large; in shape from globose through oval, oblong, needle-shape to helical; in number of cell, from one to many; in the arrangement of cells; and in the way in which the spores them-self is borne . Some fungi produce only one type of spore, whereas other produces as many as four types. Fungal spores produced asexually are either borne in **sporangia** (sporangium) and then are called **Sporangiospores** as in ***Rhizopus*** and ***Mucor*** , or are produced at the tips or sides of hyphae in various ways and are then called **conidia** (conidium) as in ***Aspergillus* spp**. Sporangiospores may be motile or non- motile. In simpler fungi the Sporangiospores are usually motile and are called **zoospores**, if non- motile are called **aplanospores**. Fungal zoospores are equipped with one or two flagella (flagellum). There are at least two types of flagella in the fungi: The **whiplash** and **tinsel**. The flagella in fungi are differing in position, types, and number.



Sporangiospores formation in *Rhizopus*

Sexual reproduction

The sexual reproduction in fungi as in other living organisms involves the union of two compatible nuclei. The process of sexual reproduction typically consist of three distinct phases:

1.Plasmogamy: a union of two protoplasts brings the nuclei close together within the same cell.

2.Karyogamy: The fusion of the two nuclei brought together by plasmogamy. **3.Mieosis**: The reduction of chromosomes number to the half. Karyogamy follows plasmogamy almost immediately in many of the simpler fungi. In the more complex fungi, however, those two processes are separated in time and space, with plasmogamy resulting in a binucleate cell containing one nucleus from each parent. Such pair of nuclei we call a **(Dikaryon)**. The sex organs of fungi are called **gametangia** (gametangium), these may form differentiated sex cell called **gametes** or may contain instead one or more gamete nuclei. We use the terms **(isogametangia and isogametes)** to designated gametangia and gametes that are morphologically indistinguishable; we use **(heterogametangia and**

heterogametes) to designate male and female gametangium and gamete that are morphologically different, in the later case , the male gametangium is called the(**antheridium**) and the female is called the (**Oogonium**).

❖ **Sexual reproduction methods**

1.Planogametic copulation:

Planogametic copulation involves the fusion of two naked motile gametes (planogametes) as in ***Allomyces***.

2. Gametangial contact:

Here gametes are not released from gametangia, instead male and female gametangia come in close contact with help of fertilization tube. Then one or more male nuclei migrate in to the female gametangium. Male gametangium is called antheridium and and female gametangium called Oogonium (Ascogonium in Ascomycota) Example: ***Albugo, Aspergillus, Pythium***

3.Gametangial copulation:

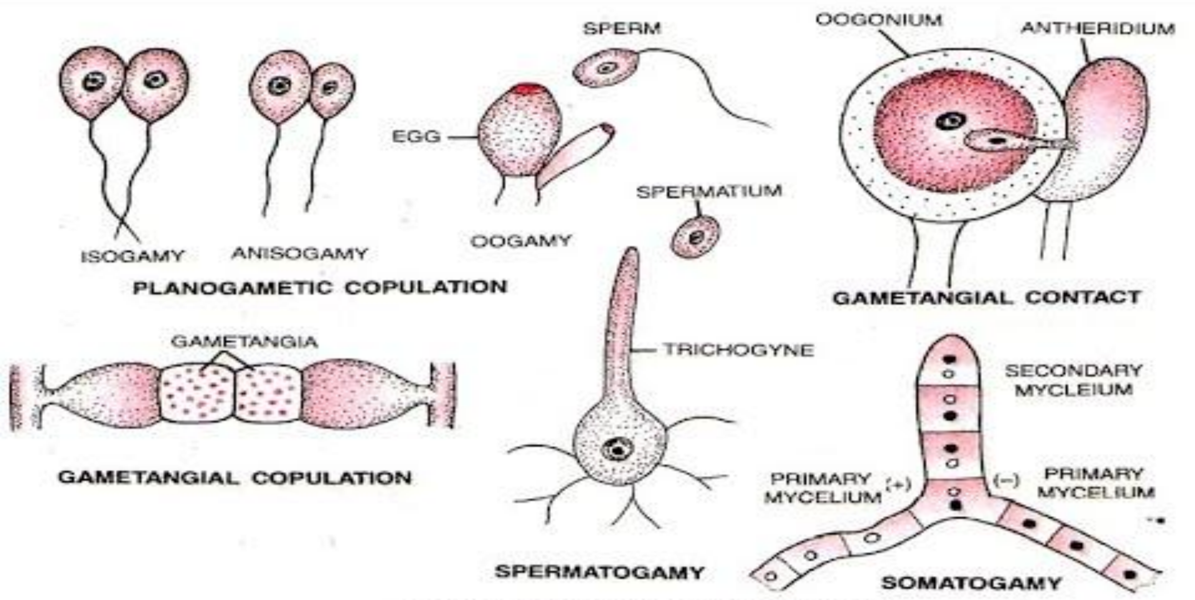
The entire content of two compatible gametangia fuse each other, the gametangia come in close contact, wall at the point of contact dissolves and their contents mix each other and karyogamy is established .Example **Zygomycota**

4.Spermatization:

In some higher fungi sex organs are completely absent, here sexual process is accomplished by minute spore like spermatia and specialized receptive hyphae (trichogyne) acting as male and female structures, respectively. Spermatia are carried by air, water or insect to the receptive hyphae.

5. Somatogamy:

In this method, sex organs are not formed and two vegetative cells or vegetative hyphae take over the sexual function and fuse together advanced fungal groups such as ***Morchella, Peziza, Agaricus***.



Types of sexual reproduction in fungi.

Sexual compatibility

Every thallus reproduces sexually by itself without the aid of anther thallus, these types of fungi are called **(Homothallic fungi).** Whereas the thallus that reproduced sexually and requires the aid of another compatible thallus or a different mating type for sexual reproduction, these types of fungi are called **(Heterothallic fungi).**

HYPHAL AGGREGATION IN FUNGI

In some advanced fungi, hyphae may undergo certain modification in response to functional needs. Hyphal modifications are hyphal aggregations are required to do specific functions during the life cycle of fungi Important hyphal modifications in fungi are:

1. Prosenchyma (Plectenchyma or Proso-plectenchyma)

When the component hyphae is arranged more or less parallel to one another and the whole mass become a felt like structure, the hyphae unite to form a loosely interwoven structure. In prosenchyma, the individuality of fungal hyphae is not lost . ex: ***Claviceps pururea***

2. Pseudoparenchyma

Hyphae are closely intertwined and forms a tissue like structure in cross section and the hyphae lose their individuality and they are not distinguishable from each other. ex: higher fungi.

3. Rhizomorphs

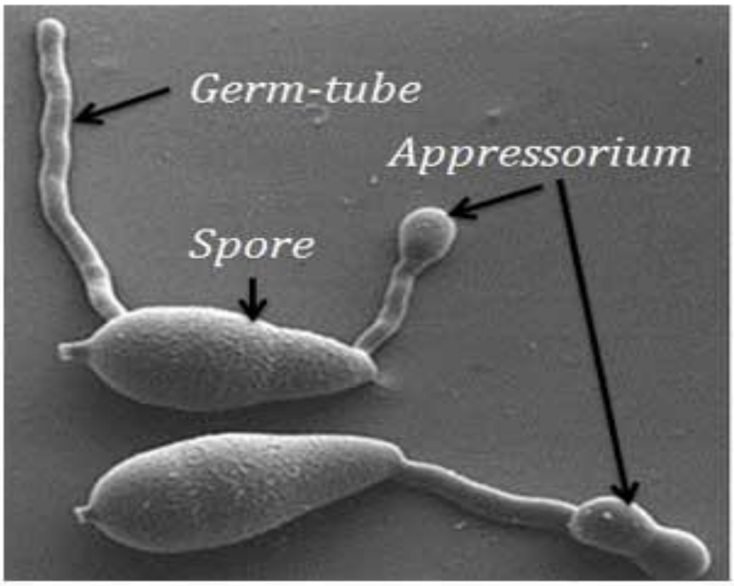
Rhizomorphs (mycelial cords) are thick strands or root like aggregation of somatic hyphae in some fungi, gelatinous, dark brown and rope like coiled structures. the intertwining of hyphae is too tight so that hyphae lose its individuality.

4. Sclerotia

Sclerotium is a compact dark brown with inner cells are colorless globose structure formed by the aggregation of hyphae in some fungi. The interwoven hyphae are very much compact so that the individuality of hyphae is lost and the mass become rounded structures. Sclerotium survives for long periods, sometimes for many years and they represent the resting stage of some fungi. They accumulate food materials and helps in vegetative reproduction. Ex:***Rhizoctonia solani***.

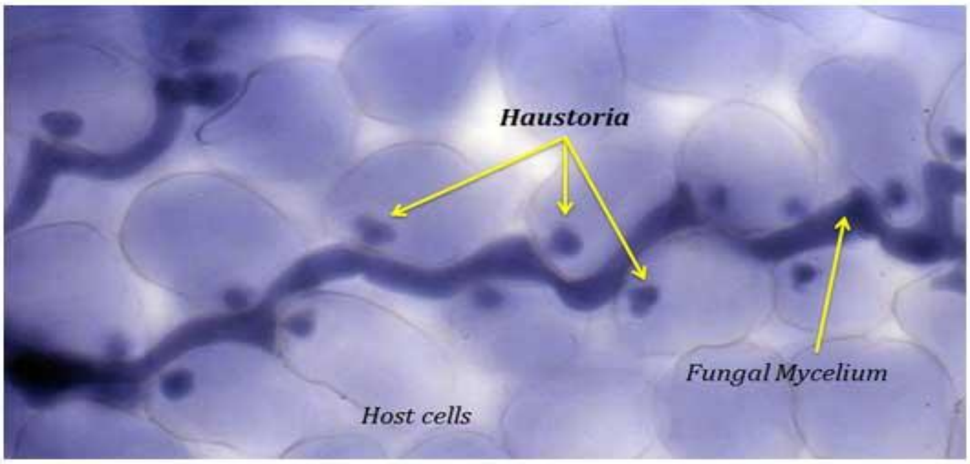
5. Appressorium

Appressorium is a terminal simple or lobed swollen structure of germ tubes on infecting hyphae**,** It adheres to the surface of host and help in the penetrating of hyphae**.** Ex: ***Erysiphae***



6. Haustorium

Is the intracellular absorbing knob like, elongated, finger like or branched structure of obligate parasites , it is the meant for absorbing food materials from the host tissue and They secrete some special enzymes which help in hydrolyzing proteins and carbohydrates of host, ex : ***Albugo***



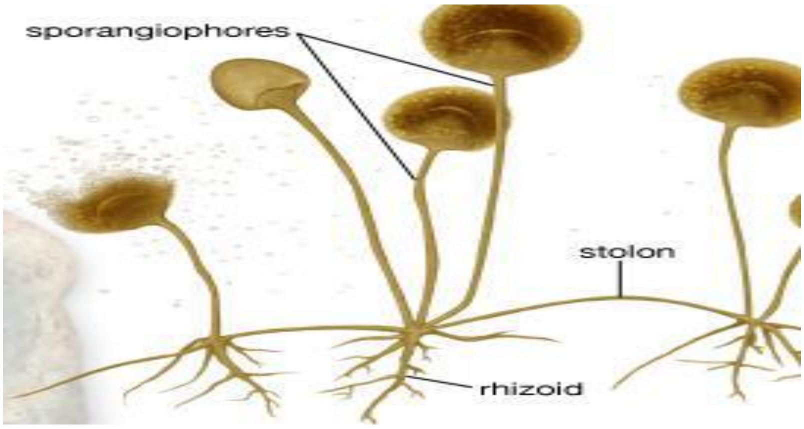
7. Stroma

8. Snares

Snares are trap like structures produced by predaceous fungi to capture small animals such nematodes and protozons.

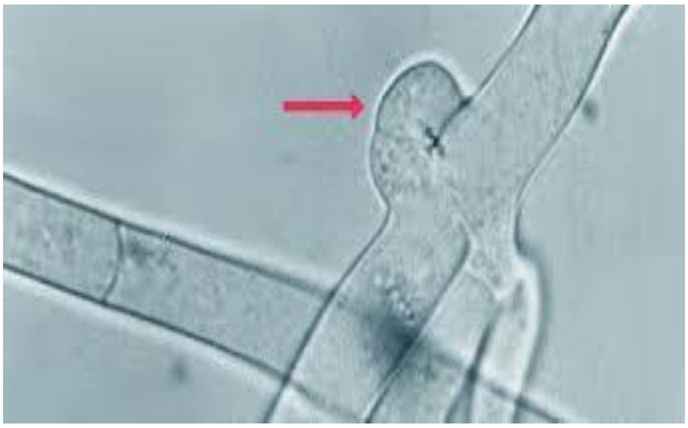
9. Rhizoides

a short, thin filament, resembles a root that anchors the growing (vegetative) body to a substratum and that is capable of absorbing nutrients. It may serve either as a feeding organ (in ***Rhizopus***) or to anchor the thallus to its substratum *(* **in *Chytridium***).



10. Clamp connection:

A bridge like hyphal connection characteristics of the secondary mycelium of many Basidiomycota; involved in maintaining the dikaryotic condition.



Sporocarp (Fruiting body) and spores

In fungi, the sporocarp (also known as fruit body) is a multicellular structure on which spore-producing structures, such as basidia or asci, are borne. The fruitbody is part of the sexual phase of a fungal life cycle. the sporocarp of a basidiomycota is known as a basidiocarp or basidium, while the fruitbody of an ascomycota is known as an ascocarp. Many shapes and morphologies are found in both basidiocarps and ascocarps: these features play an important role in the identification and taxonomy of fungi. The sexual fruiting bodies are :

Basidium: In fungi a basidiocarp, basidium or basidioma (plural: basidiomata) is the sporocarp of a basidiomycota, the multicellular structure on which the spore-producing hymenium is borne. Basidia are found on the surface of the hymenium, and the basidia ultimately produce spores.

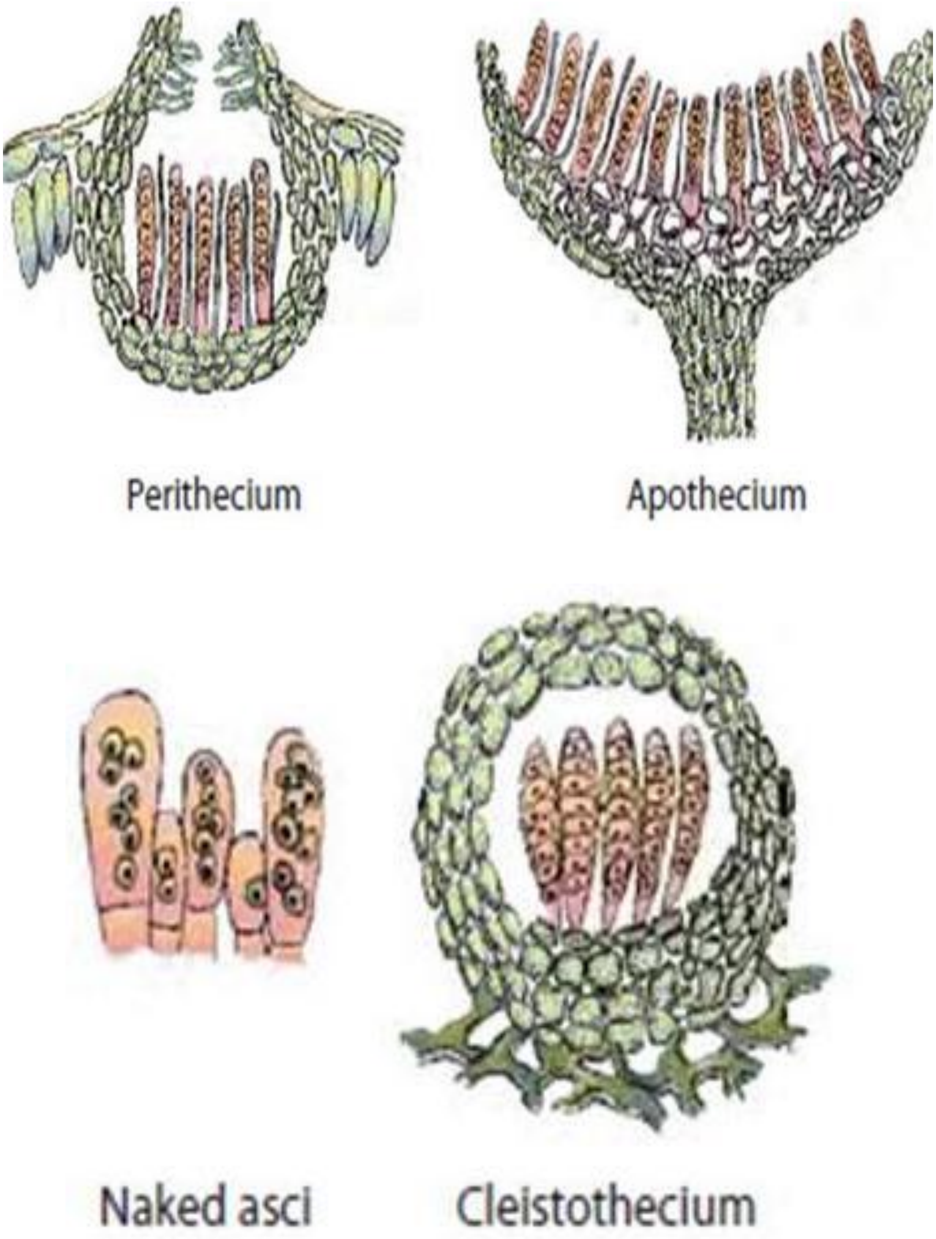
Apothecium: is a wide, open, saucer-shaped or cup-shaped fruit body. It is sessile and fleshy.

Cleistothecium : is a globose, completely closed fruit body with no special opening to the outside. The ascomatal wall is called peridium and typically consists of densely interwoven hyphae or pseudoparenchyma cells.

Gymnothecium : similar to a cleistothecium, a gymnothecium is a completely enclosed structure containing globose or pear-shaped asci. However, unlike the cleistothecium, the peridial wall of a gymnothecium consists of a loosely woven "tuft" of hyphae.

Perithecium: a flask shaped structure opening by a pore or ostiole through which the ascospores escape. The ostiolar canal may be lined by hair-like structures called periphyses.

Pseudothecium (ascostroma): This is similar to a perithecium, but the asci are not regularly organized into a hymenium and they are bitunicate, having a double wall that expands when it takes up water and shoots the enclosed spores out suddenly to disperse them.



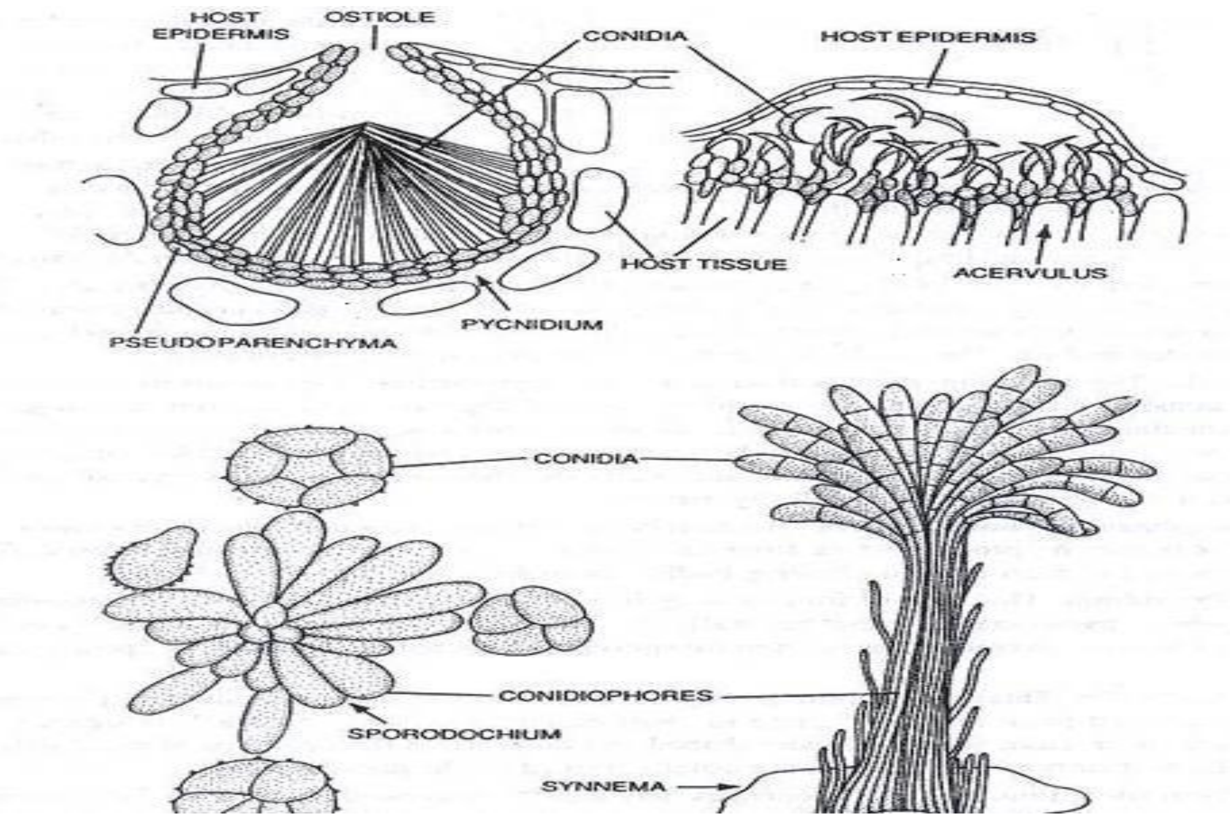
Sporocarps in Imperfect fungi

Pycnidium: a flask-shaped, globose or oval-shaped structure that looks like a cleistothecium or a perithecium but has a cavity filled with conidiophores and conidia instead of asci- and ascospores and often identified incorrectly as an ascocarp.

Acervulus : functionally a structure similar to a pycnidium, but structurally different by being formed by hyphae of plant pathogenic fungi in association with plant tissue

Sporodochium: large mass of short conidiophores and hyphae which arise together from the surface of a structure (multihyphal aggregate) called a stroma (stromata).

A synnema : is a large, erect reproductive structure borne by some fungi, bearing compact conidiophores.



Sporophores and Spores

- **Importance of Spores:**

A. Biological

1) Allows for dissemination

2) Allows for reproduction

3) Allows the fungus to move to new food source.

4) Allows fungus to survive periods of adversity.

5) Means of introducing new genetic combinations into a population

B. Practical

1) Rapid identification (also helps with classification)

2) Source of inocula for human infection

3) Source of inocula for contamination

1- Asexual spores :- Which occurs by the process of mitosis. This is most common process by which spores are reproduced in fungi. There are four types of medically important:

a- Blastospores: The type of spore develop by budding.

b- Chlamydospores: In some fungi the hyphal cell become specialized spore when the cell enlarged and develop thick walls

c- Arthrospores: Other hyphal cells break apart and produce arthrospores. Fragmentation may also happen naturally by the action of wind, soil movement or insects.

d- Conidia: A conidium is produced on a specialized structure called conidiophore. A spore which is produced directly on a hyphae or hyphal tips is called Aleuriospore, when a fungus produce two sizes of aleuriospores : The large one is called Macro-aleuriospore., The smaller one is called Micro- aleuriospore.

2- Sexual spores: Reproduce by meiosis

a- Ascospores: Usually 4-8 spores found in a cell called ascus- asci.

b- Basidiospores: Usually 4- spores found in the surface of cell called basidium.

c- Zygosopores: Large-thick walled spore formed on hyphae.

d- Oospores: This type of spore formed inside cell called Oogonium.

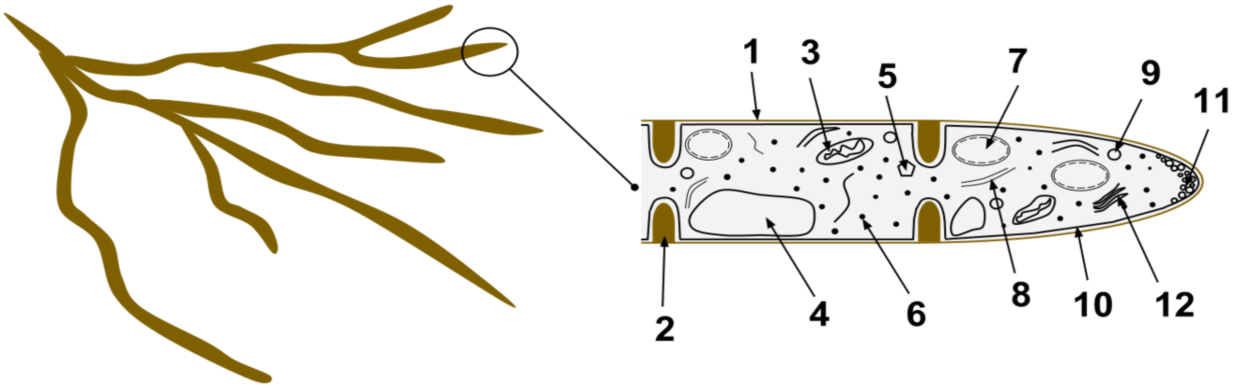
Fungal cell Structure and Function

Overview of the Hyphae

- ✓ The hypha is a rigid tube containing cytoplasm
- ✓ Growth occurs at the tips of hyphae .
- ✓ Many hyphae possess septa
- ✓ Septa contain pores through which cytoplasm flows and Hyphae are actually interconnected compartments, not individual cells.
- ✓ The cell wall of hypha is complex in structure and composition

Fungal Ultra-structure

- ✓ Hyphae show a defined polarity in the arrangement of organelles
- Apical tip
- ✓ Extreme end - no organelles, but numerous membrane-bound vesicles of differing
- ✓ Chitin synthase is present Apical vesicle cluster (AVC)
- ✓ Actin microfilaments
- Apical tip (cont.)
- ✓ Short zone following apex - no organelles, but rich in mitochondria
- ✓ Sub-apical regions contain a diverse array of organelles, septa are present, and the cell walls are less dynamic, more rigid in structure



1- Hyphal wall 2- Septum 3- Mitochondrion 4- Vacuole 5- Ergosterol crystal 6- Ribosome 7- Nucleus 8- Endoplasmic reticulum 9- Lipid body 10- Plasma membrane 11- Spitzenkörper/growth tip and vesicles 12- Golgi apparatus)

The similarities Between Hyphae and Mycelium

- The total collection of hyphae makes the body of a fungus known as mycelium.
- Hyphae and mycelium both appear as thread-like structures that are visible to our naked eyes.
- Moreover, both structures belong to multicellular filamentous fungi.
- They represent the vegetative body of a fungus.
- They have cell walls made up of chitin.
- Moreover, they possess eukaryotic cells.
- Both structures are able to grow by adding new cells.
- Furthermore, they can be septate or aseptate.

Fungal Cell Wall

- ✓ Structural barrier
- ✓ Determines pattern of cell growth and is partly dependent upon: -

-Chemical composition

-Assembly of the wall components

- ✓ Environmental interface of the fungus
- ✓ Protects against osmotic lysis
- ✓ Acts as a molecular sieve
- ✓ Contains pigments for protection
- ✓ Binding site for enzymes

Cell wall components

o Two major types of components

✓ Structural polymers - polysaccharide fibrils that provide rigidity/integrity of the wall

✓ Matrix components - cross-link the fibrils as well as coat/embed them o Main wall components differ between the major taxonomic groups of fungi

- Chitin - straight chain polymers of b-1,4-linked N-acetyl glucosamine residues

- chitosan is de-acetylated chitin

- Glucan - polymers of b-1,3-linked glucose residues with short b-1,6-linked side chains

- Cellulose - b-1,4-linked glucans.

- Matrix polymers: Glucouronic acids , Mannoproteins - mannose attached to protein.

Types of hyphae based on their cell wall

○ **Generative hyphae-** Generative hyphae are characterized by a thin cell wall and greater number of septa

○ **Binding Hyphae-** Compared to generative hyphae, binding hyphae have been shown to have a thicker cell wall and tend to be highly branched

○ **Skeletal hyphae-** Skeletal hyphae are characterized by a long and thick cell wall, but few septa compared to generative hyphae.

Septa

- Septa occur at generally regular intervals along a length of a hypha
- Perforations allow cytoplasm to flow from one cell to another
- When a cell is damaged, a Woronin body or coagulated cytoplasm serves a plug to prevent loss of cytoplasm Coenocytic fungi are more susceptible to cellular damage
- Functions of septa - Structural support of the hypha. - Enables differentiation by dividing hypha into different cells that can undergo separate modes of development.

Types of septa are Simple and Dolipore

Figure 31.3

Cytoplasmic Organelles

Plasma membrane

- Involved in uptake of nutrients

-Anchorage for enzymes/proteins, e.g., chitin synthase, glucan synthase, etc.

-Signal transduction

-Differs in that it contains ergosterol

-Site of action for certain antifungal drugs

-Oomycota contain plant-like sterols

Secretary system :

- Endoplasmic reticulum (ER)

-Golgi apparatus (or equivalent) different in than those found in animals, plants, and the Oomycota in that they lack cisternae

-Involved in fungal tip growth

Chitosomes: microvesicles that are capable of synthesizing chitin

✓ Function primarily within the region of the apical tip

Functions of Vacuoles:

-Storage -Recycling of materials -Contain proteolytic enzymes -Regulation of cellular pH -Possible role in cellular expansion/growth

•Shape of vacuoles are Round and Tubular

Fungal Cytoskeleton

- ✓ Cytoskeleton functions:

-Transport of organelles

-Cytoplasmic streaming

–Chromosome separation

- ✓ Three types of cytoskeletal filaments:

- Microtubules (composed of tubulin)

-Microfilaments (composed of actin) -

Medical mycology

Medical mycology is the study of mycoses of man and their causative agents. Mycoses are disease caused by fungi. There are many factors may be predisposed to fungal infections like: Prolonged antibiotic therapy, Underlying disease (HIV infection, cancer, diabetes, etc.), Age, Surgical procedures and Immunosuppressive drugs. Infection in humans is a chance event, occurring only when conditions are favorable such as inoculum size and resistance of the host , rather than virulence of fungus which includes Ability to adhere to host cells by way of cell wall glycol-proteins, Production capsules allowing them to resist phagocytosis, Ability to acquire iron from red blood cells as in *Candida albicans*, Ability to damage host by secreting enzymes such as (keratinase, elastase and collagenase), Ability to resist killing by phagocytes as in dimorphic fungi and Ability to secrete mycotoxins. On the other hand, there are many factors considered as defense for host include Physical barriers, such as skin (its mucus membranes, the fatty acid content and pH of the skin), presence of normal flora, Chemical barriers and presence of polymorphonuclear leucocytes, monocytes and macrophages.

DETERMINANTS OF PATHOGENICITY

Why are fungi pathogenic for humans? As eukaryotes, fungi use various stratagems to evade host defenses. The list below is a summary of microbial factors that have been shown to influence pathogenicity. Further information specific to each pathogen is discussed in the disease chapters under the section heading “Determinants of Pathogenicity.” • Thermotolerance. Fungi that can grow at 37◦ C are potential pathogens in a suitably susceptible host. • Adaptation to a parasitic lifestyle, sometimes in an intracellular environment. The traditional assumpion is that most primary and opportunistic fungal pathogens are free-living saprobes in nature. ◦ Evidence is accumulating that the ecologic niche of *Cryptococcus neoformans* may include intracellular survival within soil amebae. In that case *C. neoformans* “learned how to become a pathogen.” This theory may also apply to other environmental fungi and may help explain how some fungi have become adapted to intracellular survival within phagocytes ◦ Environmental fungi can infect other mammalian hosts, including small rodents, and have adapted to a parasitic lifestyle. • Adhesins. Pathogenesis of microbial disease proceeds via adherence to host tissues, a process of receptor ligand interaction: for example, BAD-1 adhesin of *Blastomyces dermatitidis*, the Als family of surface adhesins of Candida albicans. • Attack on host tissues using invasion promoting enzymes: ◦ Secreted enzymes that damage host tissues: for example, aspartyl proteinases and phospholipases of *C. albicans*. ◦ Production of catalase that decomposes hydrogen peroxide, thus interrupting the oxidative microbicidal pathway of polymorphonuclear neutrophilic granulocytes: for example, catalase of *Aspergillus fumigatus* and *Histoplasma capsulatum*. • Dimorphism. Morphogenesis to distinct tissue forms confers an advantage to the pathogen. For example, *H. capsulatum* yeast forms are translocated intracellularly within monocytes from the lung to the spleen and liver; spherules and endospores of Coccidioides species spread the infection; yeast forms of *B. dermatitidis* are too large for endophagocytosis. • Evasion of host immune defenses. For example, *Histoplasma capsulatum* survival in the phagosome is linked to preventing phagolysosome fusion and by being a resourceful scavenger of iron from the host through secretion of siderophores, ferric reductase, and directly from host transferrin. • Cell wall molecules are barriers that resist lysis by phagocytes and antifungal agents: for example, cell wall polymers, including α-(1→3)-d-glucan, melanin, and the glucuronoxylomannan polysaccharide capsule of *Cryptococcus neoformans*. To this list we add ß- (1→3)-d-glucan of Candida species, in shielding the yeast from antifungal agents by functioning as a major component of extracellular matrix material of biofilms embedded on intravascular catheters

Mycoses are divided into:

1. Superficial mycoses: Fungi causes infection of outer surface of hair shafts ,skin shafts and nails. They are very rare E.g. Black Piedras

2. Cutaneous mycoses Also called dermatomycoses, ringworms, or tineas: most common fungal infections Infection occurs on the epidermal layer of skin and hair roots , there are include genera Epidermophyton, Microsporum and Trichophyton.

3. Subcutaneous mycoses: Cause infection of the subcutaneous layer of skin, saprophytic species known unable to penetrate the skin, they must be introduced into the subcutaneous tissue by a puncture wound that has been contaminated with soil, ex. chromoblastomycosis and mycetoma

4. Systemic infection: Inhalation of fungal spores (wind dispersed soil borne spores)

◊ lesion formation in lungs

◊ bursting of lesion

◊ diffusion in blood

Blastomycosis and Coccidiodomycosis are saprophytic systemic mycoses

1. Superficial mycoses

I. Tinea versicolor II. Tinea nigra III. Black piedra IV. White piedra V. Otomycosis VI. Occulomycosis

Superficial mycoses refer to the diseases of the skin and its appendages caused by fungi. They possess the affinity for parasitizing stratum corneum of keratin rich tissues like skin, hair & nails and produce dermal inflammatory response and intense itching in addition to a cosmetically poor appearance. The causative fungi colonize only cornified layer of epidermis or suprafollicular portions of hair & do not penetrate into deeper anatomical sites and do not exhibit a pathology (no granulomas, cysts or other lesions) therefore, they do not elicit a host cellular response but the patient usually only becomes particularly concerned for cosmetic reasons and not because of discomfort. Superficial mycoses affect 20% to 25% of the world's population, and the incidence is increasing.

Pityriasis versicolor : pigmented lesions on torso

Tinea nigra : gray to black macular lesions often on palms

Black piedra : dark gritty deposits on hair

White piedra : soft whitish granules along hair shaft

Keratomycosis : Corneal ulcer

Otomycosis: infection of the external auditory canal of the ear.

Tinea versicolor (pityriasis versicolor), a superficial skin infection of cosmetic importance only, is caused by *Malassezia furfur*. The lesions are usually noticed as hypo pigmented areas, especially on tanned skin in the summer. There may be slight scaling or itching, but usually the infection is asymptomatic. It occurs more frequently in hot, humid weather. The lesions contain both budding yeast cells and hyphae. Diagnosis is usually made by observing this mixture in KOH preparations of skin scrapings. Culture is not usually done. The treatment of choice is topical miconazole, but the lesions have a tendency to recur. Oral antifungal drugs, such as fluconazole or itraconazole, can be used to treat recurrences.

Tinea nigra is an infection of the keratinized layers of the skin. It appears as a brownish spot caused by the melanin-like pigment in the hyphae. The causative organism, *Exophiala werneckii*, is found in the soil and transmitted during injury. In the United States, the disease is seen in the southern states. Diagnosis is made by microscopic examination and culture of skin scrapings. The infection is treated with a topical keratolytic agent (e.g., salicylic acid).

White piedra caused by fungi in the *Trichosporon* genus, occurs in semitropical and temperate countries.

Clinical findings: Soft, white to yellowish nodules loosely attached to the hair

Microscopic .: Intertwined septate hyphae, blastoconidia and arthroconidia

Culture: Soft, creamy colonies

Treatment.: **Shaving, azoles**

Black piedra is a condition that is characterized by the presence of firmly adherent black, hard, gritty nodules, which are composed of a mass of fungus cells on the hair shaft, and cause disintegration and breaking of the hair. These stone-hard black nodules are usually localized to the scalp, but may also be seen on hairs of the beard, moustache and pubic hair, with the fungal activity limited to the cuticle.

Fungal infection of the scalp hair

Etiology: *Piedraia hortae*

Frequent in tropical areas

Clinical findings: Discrete, hard, dark brown to black nodules on the hair

Microscopic. Septate pigmented hyphae, and asci; unicellular and fusiform ascospores with polar filament(s)

Culture: Brown to black colonies

Treatment.: **Topical salicylic acid and azol creams**

Otomycosis : Fungus and yeasts infection of the external auditory canal of the ear. Numerous common fungi have been implicated.

Some investigators believe this is not a true disease because tissue invasion and destruction has not been demonstrated

It is a fungal infection of **external ear.**

· It is a very common infection.

· It is usually caused by *Aspergillus niger* and *A.fumigatus*.

· The symptoms are:

1. Itching,

2. Pain,

3. Deafness.

Oculomycosis: The clinical disease is an ocular infection named as mycotic keratitis. It usually follows corneal trauma. Fungal spores colonies the injured tissue and elicit an inflammatory reaction, leading to formation of ulcer and endophthalmitis**.**

KERATOMYCOSIS (Mycotic keratitis).

Posttraumatic / postsurgical corneal infection.

Etiology: Saprophytic fungi (***Aspergillus, Fusarium, Alternaria, Candida), Histoplasma capsulatum***

Clinical findings: Corneal ulcer

Microscopic : Hyphae in corneal scrapings

Therapy:

1-Asolution (5 %) of Natamycin applied topically.

2-Topically applied Amphotericin B, oral Sporanox (1:50 dilution of Sporanox in ophthalmic solution) and ketoconazole in suspension have been used in difficult cases

3-If thermophilic *Aspergillus* spp. are involved the organism may invade the brain. Few drugs are successful in such cases.

How to Avoid Human superficial mycoses??

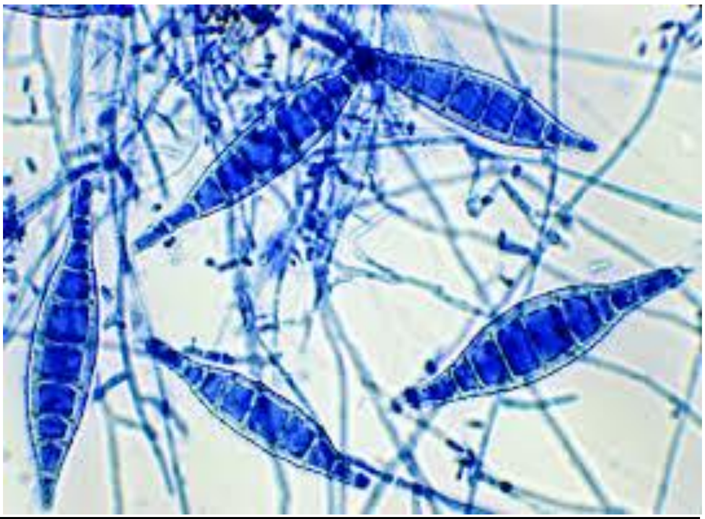
Cleanliness.

Keep your home with well-ventilated.

Use antifungal treatments when infection occurs.

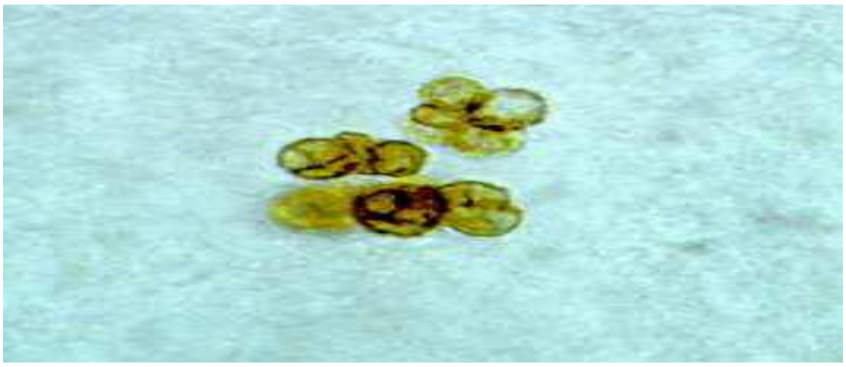
Cutaneous mycoses extend deeper into the epidermis, and also include invasive hair and nail diseases. These diseases are restricted to the keratinized layers of the skin, hair, and nails. Unlike the superficial mycoses, host immune responses may be evoked resulting in pathologic changes expressed in the deeper layers of the skin.

Etiological agents are called dermatophytes - " (i.e., *Microsporum, Trichophyton,* and *Epidermophyton*), are involved in ringworm. —Diagnosis: Microscopic examination of slides of skin scrapings, nail scrapings, and hair. Often tissue suspended in 10 % KOH solution to help clear tissue. Treatment: azoles.

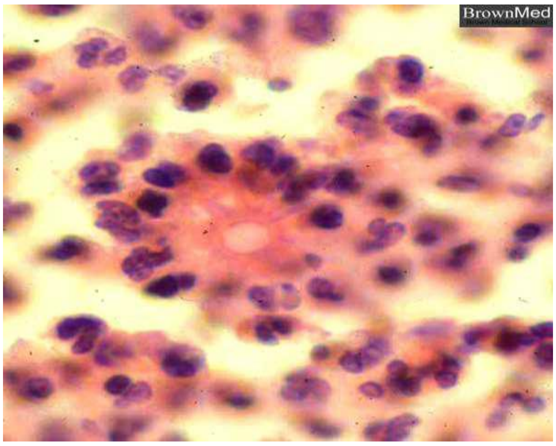


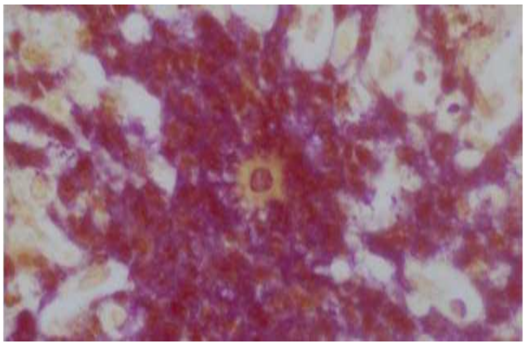
Subcutaneous mycoses: These are caused by fungi that grow in soil and on vegetation and are introduced into subcutaneous tissue through trauma. Clinical manifestations of subcutaneous mycoses are: chromoblastomycosis, sporotrichosis, rhinosporidiasis, mycetoma, subcutaneous phaeohyphomycosis and lobomycosis .

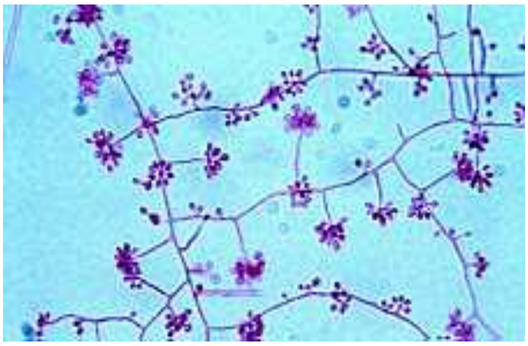
✓ **Chromoblastomycosis:** This is a slowly progressive granulomatous infection that is caused by several soil fungi *Cladophialophora carrionii* (dry and desert areas), *Fonsecaea pedrosoi* (humid areas) and *Phialophora verrucosa*. Conidia or hyphae are dark-colored, either gray or black. Wart like lesions with crusting abscesses extend along the lymphatics. The disease occurs mainly in the tropics and is found on bare feet and legs. **In the clinical laboratory**, dark brown, round fungal cells are seen in leukocytes or giant cells. Sclerotic bodies and Medlar bodies are pathognomonic feature of chromoblastomycosis as these are not seen in any other phaeoid fungal infection. The sclerotic bodies are so characteristic in wet mount as well as in stained smear that other stainings like Gomori methenamine silver stain Treatment with oral **flucytosine** or **thiabendazole**, plus local surgery.



✓ **Sporotrichosis: Etiology*: Sporothrix schenckii*** is a **dimorphic** fungus. Sporotrichosis occurs most often in **gardeners, especially those who prune roses,** because they may be stuck by a rose thorn. **In the clinical laboratory**, round or cigar-shaped budding yeasts are seen in tissue specimens. In culture at room temperature, hyphae occur bearing oval conidia in clusters at the tip of slender conidiophores (resembling a daisy). The asteroid bodies of sporotrichosis consist of central yeast of *S. schenckii*, surrounded by eosinophilic spicules **The drug** of choice for skin lesions is **itraconazole (Sporanox).** It can be prevented by protecting skin when touching plants, moss, and wood.







✓ **Rhinosporidiosis:** Chronic Granulomatus infection of the mucous membrane that causes an inflammatory polyps in the nasal mucous membranes or external eye membranes

Causative agent: *Rhinosporidium seeberi*

Diagnosis: Mainly depends on direct microscopy on histopathological examination and scrape cytology.

Specimen- excision biopsy of lesion or nasal washing

KOH mount and H&E staining-giant cells and lymphocytic reaction around mature sporangium

Systemic mycoses These infections result from inhalation of the spores of dimorphic fungi that have their mold forms in the soil. Within the lungs, the spores differentiate into yeasts or other specialized forms, such as spherules.

Systemic fungi are also called endemic fungi because they are endemic (localized) to certain geographic areas. Systemic mycosis include: Coccidioidomycosis, Blastomycosis, Histoplasmosis and Paracoccidioidomycosis.

✓ **Coccidioidomycosis "valley fever":** Etiology*: Coccidioides immitis* is a **dimorphic** fungus that exists as a **mold** in soil and as a **spherule** in tissue (Stages of *Coccidioides immitis*. **A:** Arthrospores form at the ends of hyphae in the soil. **Endemic** in arid regions of the **southwestern United States** and **Latin America**.In tissue specimens, spherules are seen microscopically. Cultures on Sabouraud's agar incubated at 25°C show hyphae with arthrospores. No treatment is needed in asymptomatic or mild primary infection. Amphotericin B, (Fungizone) or itraconazole is used.

Transient fungemia: The isolation of a commensal or environmental organism

Colonization: Multiplication of an organism at a given site without harm to the host

Infection: Invasion and multiplication of organisms in body tissue resulting in local cellular injury.

Laboratory diagnosis of mycoses:

Specimen collection: specimen collection depends on the site affected. Different specimens include hair, skin scrapings, nail clippings, sputum, blood, CSF, urine, corneal scraping, discharge or pus from lesions and biopsy.

• All specimens must be transported to the laboratory without any delay to prevent bacterial overgrowth. In case of delay specimens except skin specimen, blood and CSF may be refrigerated for a short period.

• Infected hairs may be plucked using forceps. Those hairs that fluoresce under Wood's lamp may be selectively plucked. Hairs may be collected in sterilized paper envelopes.

• Surface of the skin must be disinfected with spirit before specimen collection. The advancing edge of the lesion is scraped with the help of a blunt forceps and collected in sterilized paper envelopes.

• Discolored or hyperkeratotic areas of nail may be scraped or diseased nail clipping may be collected in sterilized paper envelopes.

• Specimens from mucus membranes (oral) must be collected by gentle scraping and transported to laboratory in sterile tube containing saline. Swabs may be collected from vagina.

• Corneal scrapings may be collected using a fine needle and inoculated at bedside.

• Pus may be collected by aspiration; use of cotton swabs may give false positive microscopic results.

• Clean catch urine may be collected in a sterile wide-mouthed container.

• Biopsy specimens must be transported in saline. In certain cases, pus or exudates must be looked for presence of granules.

Microscopy: Microscopy is used to observe clinical specimens for the presence of fungal elements or to identify the fungus following culture. In the latter case, lactophenol cotton blue is stain of choice, which stains the fungal elements blue.

Direct examination of clinical specimens could be stained or unstained.

• Wet mount: Candida may be observed in urine wet mounts

• 10-20% KOH mount: Several specimens are subjected to KOH mount for direct examination. The material is mixed with 20% KOH on a slide and a cover slip is placed. The slide is then gently heated by passing through the flame 2-3 times. The slide is observed on cooling. KOH serves to digest the protein debris and clears keratinised tissue and increases the visibility. Addition of Dimethyl sulphoxide (DMSO) permits rapid clearing in the absence of heat.

• Calcofluor white: This is a fluorescent dye, which binds selectively to chitin of the fungal cell wall. The specimen then can be observed under fluorescent microscope.

• India Ink: Capsules of *Cryptococcus neoformans* can be demonstrated by this negative staining technique.

• Periodic Acid-Schiff (PAS) stain: On staining by this stain, fungal elements appear bright magenta coloured while the background stains green. It is useful in staining tissue specimens.

• Giemsa's stain: It is particularly useful in the detection of Histoplamsa capsulatum in the bone marrow smears.

• Haematoxylin and Eosin (H&E) stain: Useful for staining tissue sections.

• Gomori's methenamine silver nitrate (GMS) stain: Outlines of the fungi are black, internal parts stain pinkblack while the background stains light green. Candida and Aspergillus may be missed in H&E stained sections, therefore GMS stained sections are essential for tissue pathology.

• Gridley's stain: It stains hyphae and yeasts dark blue-pink, tissues deep blue and background yellow.

• Meyer mucicarmine stain: Capsules of C. neoformans and inner walls of Rhinosporidium seeberi's sporangium are stained pink.

• Gram stain: Candida is best demonstrated in clinical specimen by Gram stain.

• Masson-Fontana stain is helpful in staining phaeoid (dematiaceous) fungi in tissue.

• Immunofluorescence: Monoclonal antibody labelled with fluorescent dyes can be used to detect several fungi in the clinical specimens. Culture: One of the most common media used to culture fungi in laboratory is Sabouraud's Dextrose Agar (SDA). It consists of peptone, dextrose and agar. High concentration of sugar and a low pH (4.5-5.5) prevents growth of most bacteria and makes it selective for fungi.

Emmon's modification of SDA contains 2% dextrose and has pH of 6.8. Other basal media to grow fungi include Potato Dextrose Agar, Malt Extract Agar etc. Most fungi are able to grow at room temperature while few pathogenic fungi (e.g, Cryptococcus, dimorphic fungi) can grow at 37o C. Saprophytic fungi grow much quickly than pathogenic fungi (e.g, dermatophytes). In such situations the saprophytic fungi can be inhibited by the addition of cycloheximide (actidione) to the SDA. Addition of antibiotics such as Chloramphenicol, Gentamicin or Streptomycin to SDA serves to inhibit bacterial multiplication. An example of SDA with cycloheximide and Chloramphenicol is Mycosel agar. Other specialized media used for different fungi include:

• Brain Heart Infusion Agar general isolation of fungi and conversion of dimorphic fungi.
• Inhibitory Mould Agar, an isolation medium with Chloramphenicol to suppress most bacteria.

• Caffeic Acid Agar and Birdseed Agar for isolation of *Cryptococcus neoformans*.

• Corn Meal Agar: Enhances production of chlamydospores in Candida albicans and formation of conidia in fungi.

• Trichophyton Agars: Used for selective identification of Trichophyton species. • Dermatophyte Test Medium: Used for recovery of dermatophytes from clinical specimens.

• Sabhi Medium: Isolation of *Histoplasma capsulatum*.

• 'CHROM agar Candida' is useful in identification of Candida species. Conversion of mould to yeast phase must be demonstrated in vitro for identification of dimorphic fungi. Since some fungi grow slowly cultures should not be discarded for 4-6 weeks. Fungi are identified on the basis of colony morphology (including pigmentation) and microscopic observation by tease-mount preparation or slide culture technique.

Serology: Detection of anti-fungal antibody is helpful in diagnosis of sub-cutaneous and systemic mycoses, prognosis and response to anti-fungal drugs. Different serologic techniques that are used include agglutination, immunodiffusion, counter-immunoelectrophoresis, complement fixation test, immunofluorescence, RIA and ELISA. Antigen detection: It is particularly useful in the diagnosis of cryptococcal meningitis from CSF specimens. The test is performed by Latex Agglutination or immunodiffusion tests. It is also helpful in the detection of *Aspergillus* and *Candida* antigens in systemic infections. Skin tests: Delayed hypersensitivity reactions to fungal antigens can be demonstrated by skin tests. A positive skin does not necessarily indicate an active infection; it only indicates sensitization of the individual. Hence, its value is in epidemiological studies than diagnosis. These tests may be performed in Histoplasmosis, Candidiasis, Sporotrichosis, Coccidioidomycosis, Blastomycosis, Paracoccidiodomycosis and dermatophytosis.

Molecular techniques: Newer techniques such as DNA hybridization, PCR are useful in diagnosis of mycoses in a shorter period as well as detect those fungi that are difficult or dangerous to cultivate in vitro.

MYCOTOXNS

 More than 100 known species are capable of elaborating mycotoxins.

 Same mycotoxin can be produced by different fungi & the same fungus can produce different mycotoxins.

 Toxin production occurs only under specific conditions of moisture, temperature, suitability of substrate & appropriate oxygen tension.

 The optimum conditions for toxin production are relatively specific for each fungus. For example, *Fusarium* elaborates its toxin at freezing temperature, while *A. flavus* requires a temperature of 25C

 Insects may also serve as carriers of fungal spores. Therefore, the damage of seed coat by insects, mechanical harvesting may predispose crops to fungal attack.

 The fungi associated with cereal grains have been divided into two types.

♦ **Field fungi** which invade the grains before harvest and require greater water activity for growth (*Fusarium*, *Helminthosporium* and *Cladosporium*)

♦ **Storage fungi** which invade the grains after harvest during drying and in storage (*Aspergillus* and *Penicillium*)

✓ **Mycotoxicosis :** is a toxicosis produced from digesting food containing toxic secondary metabolites (mycotoxins) of fungi .

✓ **Mycotoxins :** are secondary metabolites of fungi that are recognized as toxic to other life forms.

✓ **Allergies:** a disease produced of hypersensitivity from fungal antigens and represent immune response as a result to fungal spores inhalation or direct contact to some fungi such as Aspergillus fumigatus.

✓ **Mycetism:** a toxicosis status produced by digesting smut spores, toxic fruiting bodies or fungal sclerotia.

Characteristics of Mycotoxins

1-Odorless

2-Tasteless

3-Resistant to degradation

4-Production is variable

5-Produced on surfaces of spores

6-Remain active on dead spores

7-Cause short- & long-term effects

8-Multiple exposure routes

9-Produced by numerous species

Factors influencing the production of mycotoxins

1. In Stored (food Conditions of harvesting, Transporting and storage, Moisture content, Temperature, Aeration and Suitable substrate)

2. In Growing crop/pasture including Plant species and Stage of development of plant

3. Modifying factors such as (Species of fungus, Concentration of mycotoxin in food, Susceptibility of animal species, Age, sex, health status and Duration of exposure)

Factors affecting severity of mycotoxicosis in animals

- Species of toxigenic fungus
- Concentration of mycotoxin in the food
- Age, sex and health status of the exposed animal
- Target organs or tissue affected
- Duration of exposure to contaminated feed
- Route of entry-ingestion, inhalation or direct skin contact.
- Mycotoxicosis occurs in two forms.

♦ **Acute**

 Produced when high to moderate amounts of mycotoxins are consumed.

 Causes marked signs of disease or death.

♦ **Chronic**

 Moderate to low levels of mycotoxin intake.

 Cause low productivity, slow growth rate, reduced re-productivity and inferior market quality.

Principles features of mycotoxicosis

1. Outbreaks are often seasonal and sporadic

2. May be associated with particular batches of stored feed or certain types of pasture

3. Susceptibility can vary with the species, age and sex of the animals exposed

4. Clinical presentation may be ill-defined

5. Antimicrobial treatment is ineffective

6. Recovery depends on type and amount of mycotoxin ingested and the duration of exposure to contaminated food

7. Characteristic lesions in target organs of affected animals provide supporting diagnostic evidence

8. Confirmation requires demonstration of significant levels of a specific mycotoxin in suspect feed or in tissues of affected animals

Aflatoxicosis

The name aflatoxin derives from *Aspergillus flavus* toxin.


□Afalatoxins are agroup of approx.20 related toxic compounds produced by some strains of *A. flavus* and *A. parasiticus* during growth on a variety of cereal grains and foods tuffs such as maize, cotton seed & ground nuts.

□ High humidity & high temperature during pre-harvesting, harvesting, transporting and storage, as well as damage to feed crops by insects, drought and mechanical injury during harvesting, favors the growth and toxin production of *Aspergillus flavus*.

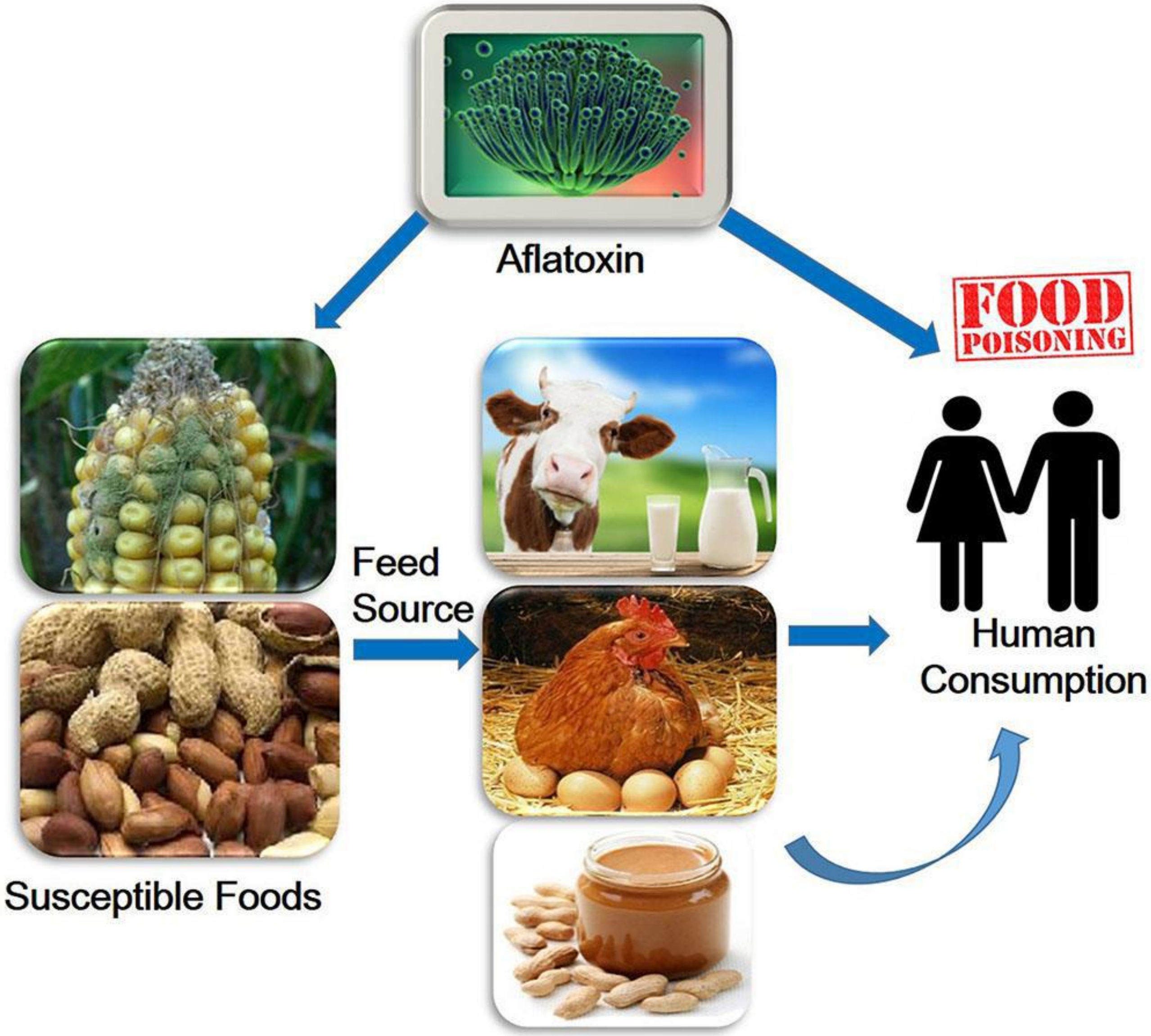
□ Mould growth and toxin formation require a moisture content of the substrate greater than15%, temp.25C and adequate aeration

□Toxic, carcinogenic, teratogenic and mutagenic activity

□ The four major aflatoxins are B1, B2, G1 and G2. These mycotoxins are named according to their position and fluorescent color on thin layer chromatography(TLC).

□B1and B2 produce blue color and G1,G2 gives green fluorescence.

□AflatoxinsM1 and M2 are hydroxylated metabolites of B1andB2, respectively that are excreted in the milk of lactating animals such as dairy cows



Biological effects of aflatoxins

Acute toxicity

Hepatic injury & nervous signs such as convulsions.

Death may occur suddenly.

Chronic toxicity

□Reduction in efficiency of food conversion, depressed daily weight gain, decreased milk production in dairy cattle and enhanced susceptibility to inter-current infections due to immune-suppression

- **Infected lesions**

Principle target organ is liver.

- ✓ Depending on the severity of intoxication)

1. Hepatomegaly
2. Necrosis
3. Marked bile duct hyperplasia

- ✓ Acute hepatic failure
- ✓ In chronic toxicity- degenerative changes in the kidney
- ✓ **Diagnosis**
- ✓ Sample collection-Feed /Tissues
- ✓ Chemical identification of Mycotoxins
- ✓ Concentration of aflatoxin B1 in excess of 100µg /kg of feed are considered toxic for cattle.
- ✓ Thinlayer chromatography
- ✓ HPLC
- ✓ Radio immuno assay

- ✓ ELISA

- Control and prevention

- ✓ Prevention of contamination at all stages of food production, storage and use

- ✓ Decontamination procedures like physical removal and chemical treatment of aflatoxin contaminated feeds such as with acids, alkalies, aldehydes, oxidizing agents of selected gases (ammonia) have been used for degrading aflatoxins.

- ✓ High affitnity inorganic compounds such as benzoic and propionic acid have been widely used as preservatives for stored agricultural products.

Ergotism

 Caused by ingestion of grasses & cereals, particularly rye, infected with fungal species of the genus *Claviceps, notably Claviceps purpurea*

Ergot describes the compacted mass of hyphae that projects as a dark, purplish black, on an infected seed.

 Ergots contain the toxic alkaloids-ergotamine and ergometrine

Two forms of ergotism-gangrenous & convulsive ergotism.

Convulsive ergotism, characterized by neurotoxicity

Towards the end of pregnancy, ergot alkaloids may exert an oxytocin like effect on the pregnant uterus

Abortions have been described in cattle consuming ergotized grass

Diagnosis

Demonstration of fungus on grains

Extraction of ergot alkaloids

Detection by chromatography, or biological testing

Prevention of ergotism

Use of clean seed, crop rotation and deep cultivation

Ochratoxin: is a mycotoxin that comes in three secondary metabolite forms, A, B, and C. All are produced by *Penicillium* and *Aspergillus* species.

Symptoms may be acute, sub acute or chronic depending on the severity of the exposure. OTA has been labeled as a carcinogen and a nephrotoxin, Treatment: activated charcoal and Vitamin D supplements

Trichothecens

Trichothecens are mycotoxins that produced by a variety of fungi particularly *Fusarium* the types of ochratoxins are include deoxynivalenol (DON) and T-2 toxin.

Symptoms Initial symptoms include nausea, vomiting, and burned skin , cough, chest pain, dyspnea, and hemoptysis.

Treatment: activated charcoal.

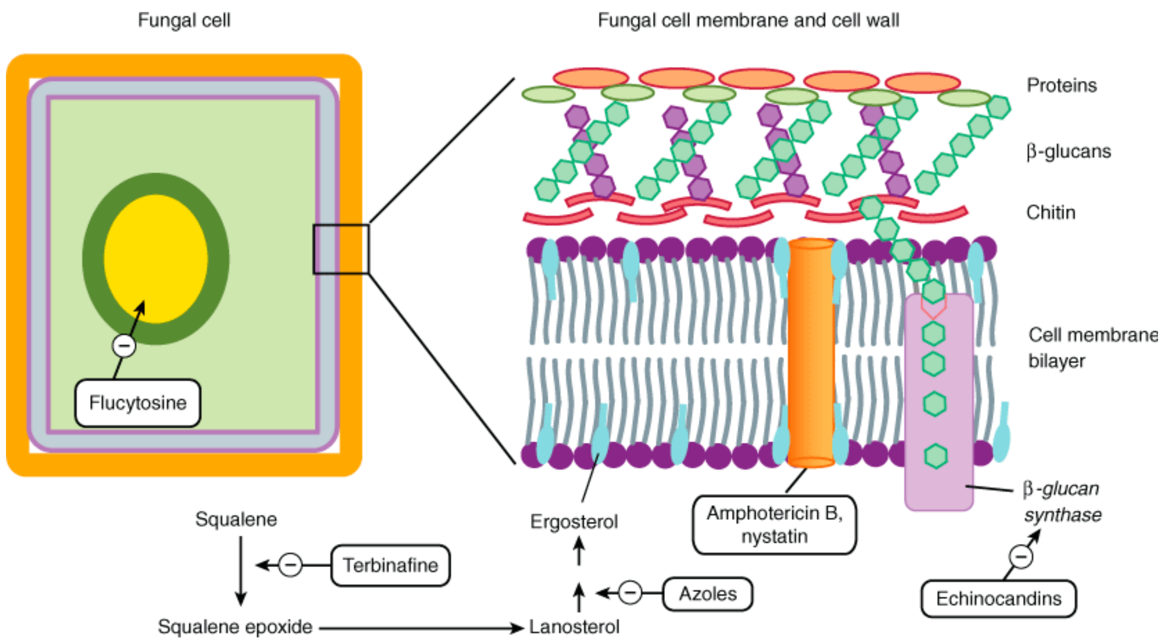
Zearalenone

Zearalenone is produced by several *Fusarium* molds under cool, wet conditions. It grows on grain before harvest, but can worsen in storage. Insect damage increases the susceptibility of crops. Infected corn may be identified by dark purple discoloration and affected wheat by pink tips.

Fungus	Disease	Crop or substrate	Mycotoxin	Animals affected
Aspergillus flavus and *Aspergillus parasiticus*	Aflatoxicosis	Ground nut, maize and nut crops	Afaltoxins (B1, B2, G1 and G2)	Cattle, pig, poultry and dogs
Claviceps purpura	Ergotism	Seed heads of many grasses and grains	Ergotamine and ergometrine	Cattle, Sheep, Pig, Horse and Poultry
Fusarium graminareum	Oestrogenism	Maize, Barley and cereals	Zearalenone	Pigs
Fusarium moniliforme	Leukoencephalomalacia	Maize	Fumonisins (A1, A2, B1 and B2)	Horses and Donkey
Fusarium species	Trichothecent oxicosis	Cereals	T-2 toxin, diacetoxy - seripenol	Many species
A. ochraceus and *P. viridicatum*	Ocharatoxicosis	Barley, wheat and Maize	Ochratoxin-A	Pigs and Poultry

Antifungal drugs

Antifungal drugs are the drugs that use to treat fungal infections. Patients can take antifungal drugs orally, apply them topically, or administer them intravenously. The type of infection has will impact how they receive the drugs.



There are four main types of antifungal drugs.

- azoles
- polyenes
- echinocandins
- allylamines

❖ **Polyenes**

These work by altering the wall of the fungal cells to be more porous, thus making them more likely to burst.

✓ **Nystatin:** It is a polyene macrolide ,similar in structure & mechanism to amphotericin B.

- Too toxic for systemic use.
- Used only topically.
- It is available as creams or ointment.
- Not significantly absorbed from skin, mucous membrane, GIT .

Clinical uses of nystatin are:

- Prevent or treat oral, esophagus, intestinal tract and vaginal candidiasis
- candidiasis
- Can be used in combination with antibacterial agents.

✓ **Amphotericin B:** is a polyene antibiotic binds to ergosterol which present in the membranes of fungal cell and leaks of small molecules (mainly K+) from the cells after formation of “pores” in the membrane.

- -The ultimate effect may be ***fungicidal*** or ***fungistatic*** depending on the organism and on drug concentration.

Amphotericin is the drug of choice for:

- -Disseminated histoplasmosis
- -Disseminated and meningeal coccidioidomycosis
- -Disseminated and meningeal cryptococcosis
- -Invasive aspergillosis
- -Deep candidiasis
- -Mucormycosis

- **Amphotericin is an alternative drug for:**
- -Blastomycosis
- - Sporotrichosis
- - Paracoccidioidomycosis

❖ **Azoles:** there are two sub-categories: imidazoles and triazoles.

Mode of action

1-Inhibit the fungal cytochrome P450 enzyme, (α-demethylase) which is responsible for converting lanosterol to ergosterol.

2- Inhibition of mitochondrial cytochrome oxidase leading to accumulation of peroxides that cause autodigestion of the fungus.

3- Imidazoles may alter RNA& DNA metabolism.

Some examples of imidazoles:

✓ **Clotrimazole:** Skin, oral, and vaginal candida infections.

✓ **Ketoconazole:** Used topically or systematic (oral route only) to treat :

1- Oral & vaginal candidiasis.

2- Dermatophytosis.

3- Systemic mycoses & mucocutaneous candidiasis..

✓ **Miconazole:** Vaginal, skin, and nail infections.

Some examples of triazoles:

✓ **Fluconazole:** Used for the treatment of fungal infections due to Candidiasis

- (is effective in all forms of mucocutaneous candidiasis)
- Cryptococcus meningitis
- Histoplasmosis, blastomycosis, , ring worm.
- Not effective in aspergillosis

✓ **Isavuconazole:** Treatment of invasive aspergillosis and mucormycosis infections.
✓ **Itraconazole:** Blastomycosis, aspergillosis, histoplasmosis, candidiasis, and various superficial mycoses.
✓ **Voriconazole:** Aspergillosis and candida.

❖ **Allylamines**

Allylamines work by inhibiting an enzyme that the membrane of the cell requires to operate correctly. Without this membrane, the cell is likely to be unable to function.

An example of an allylamine is terbinafine, which treats fungal skin infections.

❖ **Echinocandins**

These interfere with an enzyme involved in creating the fungal cell wall.

Echinocandins include:

✓ **Anidulafungin:** Treats esophageal candidiasis and invasive candidiasis.
✓ **Caspofungin:**

Echinocandins	
Caspofungin (Cancidas®)	
- It is a member of a new class of antifungals termed the echinocandins.	
Pharmacokinetics	- Administered IV (An initial dose of 70 mg by IV infusion is given followed by 50 mg IV daily).
Mechanism of action	- Inhibit β(1,3)-D-Glucan synthase enzyme → Decrease synthesis of glucose polymer present in the structure of cell wall. - Fungicidal (Aspergillus and Candida)
Side effects	- Hypersensitivity - GIT → Nausea, Vomiting and abdominal pain. - Anemia and Hypokalemia - Increase plasma creatinine and liver enzymes.
Contraindication	- Pregnancy (Category C) and lactation. - Sensitivity to the drug. - Hepatic dysfunction.

- ✓ **Micafungin:** Esophageal candidiasis and invasive candidiasis.