EMMY

T-56

LECTURE NOTE ON MYCOLOGY

BY

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MYCOLOGY

OBJECTIVES

1. WHAT DO YOU UNDERSTAND BY MYCOLOGY?
2. TYPICAL STRUCTURE OF THE FUNGAL CELL
3. GENERAL PROPERTIES OF FUNGI
4. FUNGAL REPRODUCTION
5. FUNGAL TAXONOMY: CLASSIFICATION
6. FUNGAL DISEASES AND SELECTED CAUSATIVE AGENTS (MYCOSES)
7. ANTI-FUNGAL THERAPY
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MYCOLOGY

Mycology refers to the scientific discipline devoted to the study of fungi. Fungi are important decomposers that break down organic matter, live as parasites on animals, humans and plants. Fungi also play an important role in many industrial processes and are used as research tool in the study of fundamental biological processes. Fungi secrete enzymes outside their body structure and adsorb the digested food. Scientists who study fungi are called **mycologists.** The study of fungal toxins is called **mycotoxicology** and the diseases caused by fungi in animals are called or known as **mycoses.** Fungal toxins are harmful chemicals produced by fungi under certain conditions that are capable of causing disease and death in both humans and plants eg patulin. Fungi unlike bacteria are eukaryotes in the sense that they contain membrane bound organelle such as nucleus, lysozymes, microtubules, golgi apparatus, mitochondria e.t.c. Consequently there are many similarities between the biochemistry of fungal cells and vertebrate (human) cells. They are spore bearing organism that has absorptive nutrition and lacks chlorophyll, they reproduce sexually, asexually and in rare cases parasexually.

Fungi are widely distributed in nature. They have a global distribution from Polar to tropical regions. They occur as part of the normal flora on the body of warm blooded animals, as animal and plant pathogens. Fungi are important decomposers of organic matters, they degrade complex organic materials to

simple organic compounds and inorganic molecules. They are saprophytes, securing nutrients from dead organic material by releasing degradative enzymes into the environment. Medically, fungi are an extremely important group of microbes, being responsible for a number of potentially fatal diseases in humans, but a significant number of fungi are of great benefit to humanity in terms of production of alcoholic beverages, bread, enzymes, antibiotics and recombinant proteins. Fungi have also been utilized for a range of molecular biological applications.

TYPICAL STRUCTURE OF THE FUNGAL CELL

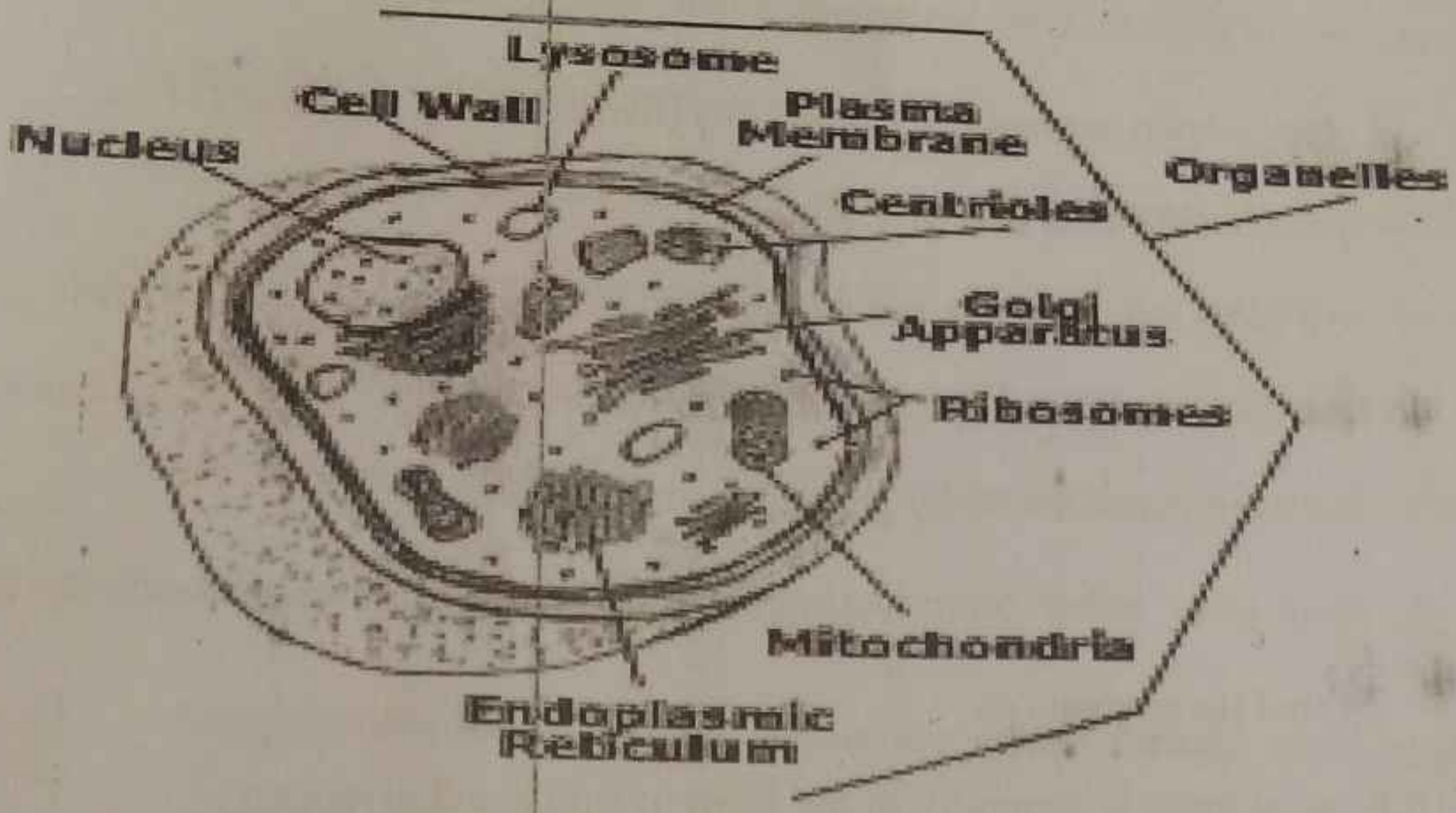
The body or vegetative structure of a fungus is called a **thallus**. It varies in complexity and size. Single-cell microscopic fungi are referred to as yeasts, while multicellular masses are called moulds. Fungi also include macroscopic puffballs and mushrooms. Like most bacteria, fungi possess cell walls; however, fungal cell walls are usually made of **chitin**. Chitin is a strong but flexible nitrogen containing polysaccharide consisting of N-acetylglucosamine residues. Instead of Chitin, some fungi cell walls are composed of other polysaccharides such as mannans, galactosans or cellulose. The dominant sterol in fungal cell membranes is the ergosterol. Sterols are important components of the plasmalemma and represent regions in the phospholipid bilayer. Actively respiring fungal cells possess a distinct mitochrondria which has been described as the power house of the cell. Electron transport and oxidative phosphorylation occur in the mitochondrion inner membrane. The outer membrane contains enzymes involved in lipid biosynthesis. The fungal cell also consists of ribosomes which are usually present in the form of polysomes. Ribosomes are the site of protein biosynthesis. The golgi apparatus, endoplasmic reticulum and the plasmalemma mediates the export of proteins and lipids into vesicles for delivery to targeted destinations. The vacuole is employed as a storage space where nutrients, hydrolytic enzymes or metabolic intermediates are retained until required. Yeast is an oval shaped unicellular fungus with a single nucleus

that reproduces either asexually by budding and transverse divisions or sexually through spore formation. Each bud that separates can grow into a new cell, and some group together to form colonies. Generally, yeast cells are larger than bacteria and are commonly spherical to egg-shaped. They lack flagella and cilia but have most other eukaryotic organelles.

A typical yeast cell is surrounded by a rigid cell wall which contains a number of structural polysaccharides that accounts for 25% of the dry weight of the cell. These polysaccharides include **Chitin**, **mannans** and **glucan**. Glucan is the major structural component of the yeast cell wall. It is a branched polymer of glucose which exists in three forms in the cell; β-1,3-glucan, β-1,6-glucan and β-1,3,- β-1,6- complexed with chitin. Mannan is a polymer of the sugar mannose and it is found in the outer layers of the cell wall. Proteins and lipids are also present in the cell wall and under certain conditions may represent up to 30% of the cell wall contents.

The **thallus** of a mould consists of long, branched, thread-like filaments of cells called **hypae** that form a tangled mass called a **mycelium**(mycelia). In some fungi, protoplasm streams through hyphae, uninterrupted by cross walls. These hyphae are called "**Coenocytic or Aseptate hyphae**". The hyphae of other fungi have cross walls called Septa (septum) with either a single pore or multiple pores that enable cytoplasmic streaming. These hyphae are termed "**Septate hyphae**".

Hyphae are composed of an outer cell wall and an **inner lumen**, which contains the **cytosol** and **organelles**. A plasma membrane surrounds the cytoplasm and lies next to the cell wall. The filamentous nature of hyphae results in a large surface area relative to the volume of cytoplasm. This makes adequate nutrient absorption possible.



A DIAGRAMMATIC REPRESENTATION OF A TYPICAL FUNGAL CELL

GENERAL PROPERTIES 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. Fungi have ergosterols in their membranes and possesses 80S ribosomes
3. Fungi 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. Fungi are chemoheterotrophs (require organic compounds for both carbon and energy sources) and fungi lack chlorophyll and are therefore not autotrophic
5. Fungi are osmiotrophic; they obtain their nutrients by absorption

6. They obtain nutrients as saprophytes (live off 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 fungi reproduce asexually and/or sexually by producing spores and even parasexually by genetic recombination.
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

FUNGAL REPRODUCTION

Fungi undergo three major modes of reproduction;

1) Asexual means

2) Sexual Means

3) Parasexual means

1) ASEXUAL REPRODUCTION

This is the commonest mode in most fungi. Asexual reproduction is achieved in several ways namely

a) A parent cell undergoes mitosis and divided into two daughter cell by a central constriction and formation of a new cell wall

OR

b) Mitosis in vegetative cells maybe concurrent with budding to produce a daughter cell. This is very common in yeasts.

The formation of asexual spores often accompanies asexual reproduction and is usually used as a means of dispersal. A spore is a unit of sexual or asexual reproduction that may be adapted for dispersal and for survival in microbes. Spores are known to survive extreme unfavourable conditions.

There are many types of asexual spores namely

i) Arthroconidia (arthrospores): These are spores formed when hyphae fragment, through the splitting of the cell wall or septum

ii) Sporangiospores: These are spores that developed or enclosed within a sac at a hyphal tip.

iii) Conidiospores: These are spores that are not enclosed in a sac unlike the sporangiospores but produced at the tips or sides of the hypha

iv) Blastospores: These are spores that are produced from a vegetative mother cell budding .

2. SEXUAL REPRODUCTION

Sexual reproduction in fungi is achieved by the fusion of two compatible nuclei that generally undergo meiosis. In some fungi, the nuclei in the fused hypae immediately combine to form a zygote. In others, the two genetically distinct nuclei remain separate forming pairs that divide synchronously.

There are three main Steps associated with sexual methods of reproduction and they include:

a) Plasmogamy: This is the cytoplasmic fusion of two cells, a union of two protoplasts brings the nuclei close together within the same cell.

b) Karyogamy: This stage follows the first stage almost immediately in many of the lower fungi. This is the fusion of two compatible nuclei, which results in the production of diploid or zygote nucleus

c) The third final step is genetic recombination and meiosis; nuclear fusion. Which eventually takes place in all sexually reproducing fungi as described above is sooner or later followed by **genetic recombination and meiosis,** which again reduces the number of chromosomes to the haploid and which constitutes the third stage of sexual reproduction.

In summary **plasmogamy** brings two haploid nuclei together in one cell; **karyogamy** unites them into one diploid zygote nucleus; and **genetic recombination** and **meiosis** restores the haploid condition in the four nuclei which results from it. In other words meiosis reduces again the number of chromosomes to the haploid. In a true sexual cycle these three processes occur in a regular sequence and usually at specified points.

The resulting four haploid spores are said to be sexual spores e.g. **zygospores, ascospores** and **basidiospores**.

Note: If a sexual spore is produced only by fusion of a nucleus of one mating type with a nucleus of another mating type (+ and – strains), the fungus is said to be **heterothallic**.

In contrast, **homothallic** moulds produce sexual spores following the fusion of two nuclei from the same strain.

Sexual reproduction requires two compatible isolates to occur

2) PARASEXUAL REPRODUCTION

This process involves genetic recombination without the requirement of specific sexual structures. This mode of reproduction was first seen in Aspergellus species and it is also known to occur in basidomycetes, ascomycetes and denteromycetes.

IMPORTANCE OF SPORES

i) Spores aids in reproduction

ii) Spores aids the fungus to move to new food source

iii) Spores aids the fungus to survive periods of adversity

iv) It serves as a means of introducing new genetic combination into a population

v) Spores aid in rapid identification which helps with classification

vi) Spores can be a source of inocula for human infection

vii) Spores can be a source of inocula for contamination

FUNGAL TAXONOMY: CLASSIFICATION

Fungal taxonomy is the classification system of fungi; fungal taxonomy is extremely difficult because of the number and inter-relatedness of fungal classes. Mycologists classify these fungi in diverse ways but there are six basic classifications of fungi with distinct characteristics attributed to each class and they include the following;

1. ASCOMYCOTA

Characteristics

i. Sexual reproduction involves meiosis of a diploid nucleus in an ascus, giving rise to haploid ascospores;

ii. Most fungi in this class also undergo asexual reproduction with the formation of conidospores with specialized aerial hyphae called conidiophores.

iii. Most fungi in this class produce asci within complex fruiting bodies called **Ascocarps**.

iv. Fungi here Includes saprophytic, parasitic forms; Most fungi in this class form mutualisms with phototrophic microbes to form lichens.

v. This class of fungi comprises of four monophyletic subdivisions: *Saccharomycetes, Pezizomycotina, Taphrinomycotina* and *Neolecta*.

Examples of fungi that belong to this class are Ascobolus, Aspergillis, Candida, Crinula, Neurospora, Penicillium, Pneumocystis and Saccharomyces.

2. *BASIDIOMYCOTA*

Characteristics

i. They Include many common mushrooms and shelf fungi.

ii. Sexual reproduction involves formation of a **basidum** (a basidium is a small, club-shaped structure that typically forms spores at the ends of tiny projections) within which haploid basidiosphores are formed. This is why members of this group are called club fungi, usually, four spores per basidium but can range from one to eight.

iii. Sexual reproduction involves fusion with opposite mating type resulting in a dikaryotic mycelium with parental nuclei paired but not initially fused.

iv. In this class, no subdivisions is recognised.

v. They include plant pathogens (rusts and smuts) belonging to *Urediniomycota and Ustilaginomycota*. Examples of fungi in this class are Agaricus, Dacrymyces, Lycoperdon, Uromyces, Boletes and polyporus .

3. *CHYTRIDIOMYCOTA*

Characteristics

i. The chytridiomycetes consists of Flagellated cells in at least one stage of life cycle; they may have one or more flagella.

ii. They consists of cell walls composed of chitin and β-1,3/1,6-glucan;

iii. Sexual reproduction often results in a zygote that becomes a resting spore or sporangium;

iv. In this class fungi are either saprophytic or parasitic.

v. The recognised subdivisions in this class include *Blastocladiales, Monoblepharidales, Necallimasigaceae, Spizellomycetales* and *Chytridiales.* Examples of chytridomycetes are Allomyces, Blastocladiella, Coelomomyces, Physoderma, Synchytrium

4. *ZYGOMYCOTA*

Characteristics

i. *In this class* the thalli are usually filamentous and nonseptate, without *cilia.*

ii. *Sexual reproduction* gives rise to thick-walled zygospores that are often ornamented.

iii. They Include seven subdivisions: *Basidiobolus, Dimargaritales; Endogonales, Entomophthorales, Harpellales, Kickxellales, Mucorales,* and *Zoopagales.*

iv. Human pathogens in this class are found in *Mucorales* and *Entomophthorales.*

Examples are Amoebophilus, Mucor, Phycomyces, Rhizopus, and Thamnidium.

5. GLOMEROMYCOTA

Characteristics

i. They are Filamentous, most are endomycorrhizal and arbuscular.

ii. They lack cilium.

iii. They form asexual spores outside of host plant.

iv. They lack centrioles, conidia, and aerial spores.

v. In this class no subdivisions is recognised just like in the class of basidiomycota

Examples of fungi in this class are Acaulospora, Entrophospora and Glomus.

6. MICROSPORIDIA

Characteristics

i. They are obligate intracellular parasites usually of animals.

ii. They Lack mitochondria, peroxisomes, kinetosomes, cilia, and centrioles;

iii. Their spores have an inner chitin wall and outer wall of protein;

iv. They produce a tube for host penetration.

v. In this class, their subdivisions are currently uncertain.

Examples of fungi in this class are Amblyospora, Encephalitozoon, Enterocytozoon and Nosema.

Mycoses are diseases caused by fungi. There are so many types of fungal diseases associated with their causative agents that are named based on the location of their occurence and they are;

FUNGAL DISEASES AND SELECTED CAUSATIVE AGENTS (MYCOSES)

Type of Mycosis	Disease	Species name
Superficial	Pityriasisversicolor(neck and shoulder trunk rashes) White piedra(hair shaft)	*Malassezia furfur* *Trichosporonbeigelii*
Cutaneous	Tineapedis (ahtlete's foot) Onychomycosis (nail infection) Tineacapitis (scalp ringworm)	*Trichophytonrubrum* *Trichophytonrubrum* *Trichophytontonsurans*
Subcutaneous	Chromoblastomycosis Mycetoma (bones of hands and feet)	*Fonsecaeapedrosoi* *Acremonium spp.*
Systemic	Blastomycosis Histoplasmosis Coccidioidomycosis Paracoccidioidmycosis	*Blastomycesdermatitidis* *Histoplasmacapsulatum* *Coccidioidesimmitis* *Paracoccidioidesbrasiliensis*
Opportunistic	Candidosis (superficial/systemic)	*Candida albicans* *Candida glabrata* *Candida parapsilosis*
	Aspergillosis Pneumonia	*Aspergillusfumigatus* *Pneumocytisjirovecii (carinii)*

ANTIFUNGAL THERAPY

These are therapies that aim at treating fungal infections. The choice and dose of an antifungal will depend upon the nature of the condition, whether there are any underlying diseases, the health of the patient and whether antifungal resistance has been identified as compromising therapy. Part of the difficulty in designing effective antifungal agents lies in the fact that fungi are eukaryotic organisms so agents that will kill fungi may also have a deleterious effect on

human tissue. The ideal antifungal drug should target a pathway or process specific to the fungal cell, so reducing the possibility of damaging tissue and inducing unwanted side effects. There are basically four classes of antifungal therapy. Each class has some examples of antifungal drugs that are used to combat these fungal infections. Most of these anti-fungal agents elicit their action by inhibiting the biosynthesis of ergosterol (ergosterol is a sterol found in the membrane of fungi and protozoa. it is a provitamin form of vitamin D2). Without ergosterol, fungi cannot survive), even though some of the antifungal therapy elicit their action by inhibiting protein synthesis or inhibiting the synthesis of β-1, 3 –glucan (one of the major polymer of fungal cell wall)

CLASSES OF ANTIFUNGAL THERAPY

1. Polyene Antifungal

Mechanism of action: it binds to ergosterol, which is the dorminant sterol in fungal cell membranes, and consequently increases membrane permeability by the formation of pores. Thus the action of polyenes eg amphotericin B relies on formation of pores through which intracellular contents can escape from the cell.

examples

i) Amphotericin B

ii) Nystatin

i) **Amphotericin B:** it is antifungal agent used for the treatment of severe cases of systemic fungal diseases though prolonged use can lead to renal damage. It is active against a broad range of fungal pathogens and is considered the " gold standard" against which the activity of other antifungal agents is measured. The renal toxicity associated with amphotericin B has led to its use reserved for severe cases of systemic fungal diseases but recent formulation in which the drug is encapsulated in liposomes have been shown to have a reduced toxicity.

ii) **Nystatin:** This drug has the same mode of action of Amphotericin B but it is less soluble than the former, thus its use has been restricted to topical infections because of its poor solubility.

2. Azole Antifungals

The azoles are still the most widely used group of antifungal agents.

Mechanism of action: The azoles function by interfering with ergosterol biosynthesis by binding to the cytochrome P-450 mediated enzyme known as 14-α-demethylase ($P\text{-}450_{DM}$). This blocks the formation of ergosterol by preventing the methylation of lanosterol (a precursor of ergosterol). This results in a reduction in the amount of ergosterol in the fungal cell membrane which leads to membrane instability, growth inhibition and cell death.

examples:

i) Clotrimazole
ii) Miconazole
iii) Econazole
iv) Voriconazole

(v) Ketoconazole
(vi) Itraconazole
(vii) Fluconazole

Itraconazole, voricon ale and fluconazole are newer drugs in this class.

These newer examples of the Azoles also have important applications in the treatment of systemic infections.

3. **Echinocandins;** The echinocandins are the relatively new group of antifungal drug.

Mechanism of action: The echinocandins target the synthesis of β-1,3-glucan, the major polymer of the fungi cell wall. Thus the inhibition of β-1,3- glucan synthesis disrupts the structure of the growing cell wall, resulting in osmotic instability and ballooning out of the intracellular contents as a reult of high osmotic pressure, and ultimately ends in cell lysis.

Examples;

i) Caspofungin
ii) Micafungin
iii) Anidulafungin

The unique characteristic of the Echinocandins is that they offer a safer alternative to the conventional antifungal therapies (i.e., polyenes and azoles)

4. **Synthetic Antifungal Agents**

Mechanism of action: its activity is elicited by the disruption of protein synthesis by inhibiting DNA synthesis while the other possible mode of

action is by the depletion of the amino acid pools within the cell as a result of inhibition of protein synthesis.

examples

i) Flucytosine

This is used as an oral antifungal agent, because of proven and known resistance issues, flucytosine has been limited in use and it is generally combined with another antifungal agent, e.g. Amphotericin β which can potentiate the effect of the second agent.

EFFECTS OF FUNGI

There are so many effects of this class of microbes called fungi; it involves both the beneficial effects and the harmful effects.

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. Fungi cause a no of Plant diseases
5. Spoilage of agriculture produce such as vegetables and cereals.
6. Damage the products such as magnetic tapes and disks, glass lenses, marble statues, bones and wax

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. Fungi are important sources of antibiotics, such as Penicillin
4. Fungi are used as model organisms for biochemical and genetic studies. E.g. *Neurosporacrassa*
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. Penicilium is used to flavour Roquefort and Camembert cheeses
9. Ergot produced by Clavicepspurpurea contains medically important alkaloids that help in inducing uterine contractions, controlling bleeding and treating migraine
10. Fungi (*Leptolegnia caudate* and *Aphanomyceslaevis*) are used to trap mosquito larvae in paddy fields and thus help in malaria control

Good luck and God bless you