Applied Mycology

Fungi include hundreds of species which are of tremendous economic importance to man. In fact our lives are intimately linked with those of fungi. Hardly a day passes when we are not benefited or harmed directly or indirectly by these organisms.They play an important role in medicine yielding antibiotics, in agriculture by maintaining the fertility of the soil and causing crop and fruit diseases, forming basis of many industries and as important means of food. Some of the fungi are important research tools in the study of fundamental biological processes.

Some of the fungi particularly molds and yeasts play a negative role by causing spoilage of stored goods such as foodstuffs, textiles, leather, rubber, plastic, timber and even glass.

1. Role of Fungi in Medicine:

Some fungi produce substances which help to cure diseases caused by the pathogenic microorganisms. These substances are called the antibiotics.

The term antibiotic, therefore, denotes an organic substance, produced by a microorganism, which inhibits the growth of certain other microorganisms. The most important antibiotics are produced by the moulds, actinomycetes or bacteria.They are used to combat the evil effects of pathogenic bacteria and viruses. The use of antibiotics is not limited to disease treatment.

Name of the Medicine/Antibiotic	Source	Action	Application
Penicillin	*Penicillium sp*	Gram Positive Bacteria	Tonsilitis, Sore Throat, Gnonorrhea, Rheumatic Fever, some Pneumonia types.
Cephalosporin	*Cephalosporium acremonium*	Broad spectrum	respiratory tract infections, skin infections and urinary tract infections
Griseofulvin	*Penicillium griseofulvum*	Dermatophytic fungi	Antifungal, especially for Ringworm.
Gentamicin	*Micromonospora purpurea*	Broad spectrum	used to treat several types of bacterial infections like bone infections, endocarditis, pelvic inflammatory disease, meningitis, pneumonia, urinary tract infections, and sepsis
Rifamycin	*Streptomyces mediterranei*	Mycobacteria	Used to treat tuberculosis, leprosy, and mycobacterium avium complex (MAC) infections.
Riboflavin (Vitamin B_2)	*Ashbyagossypii Eremotheciumashbyi*		• Used as a vitamin supplement for human diet.

			• Added to animal feed for proper nutrition. • It is an antioxidant that controls free radicals. • Riboflavin plays a supportive role in the treatment of anemia • It minimizes the effects of cancer-producing carcinogens.

*****Claviceps purpurea* produces sclerotia in the ovaries of the flowers of grasses such as rye. The sclerotium is called the ergot of rye. Ergot is used in veterinary and human medicine.It contains a mixture of alkaloids which cause rapid and powerful contractions of the uterus. The medicine is thus used to control bleeding during child birth. Ergot is highly poisonous. A derivative of ergot known by the name of lysergic acid (LSD) is used in experimental psychiatry.

2. Role of Fungi in Industry:

The industrial uses of fungi are many and varied. In fact the fungi form the basis of many important industries. There are a number of industrial processes in which the biochemical activities of certain fungi are harnessed to good account.A brief account of some of the most important of these processes is given below:

Alcoholic fermentation:

It is the basis of two important industries in India or rather all over the world. These are brewing and baking. Both are dependent on the fact that the fermentation of sugar solutions by yeasts produces ethyl alcohol and carbon dioxide.

- In brewing or wine making industry alcohol is the important product. The other by-product which is carbon dioxide was formerly allowed to escape as a useless thing.

Now carbon dioxide is also considered a valuable by-product. It is collected, solidified and sold as “dry ice”. In the baking or bread- making industry CO_2 is the useful product.

It serves two purposes: (i) Causes the dough to rise.

(ii) Makes the bread light.

- The other by-product, which is alcohol, is incidental. The yeasts secrete the enzyme complex called zymase which brings about conversion of sugar into alcohol. Many excellent yeast strains are now available.
- The yeasts lack diastase. So they cannot break starch into sugar. There are a number of fungi popularly known as the moulds. They secrete a whole range of enzymes and thus bring about fermentation of complex carbohydrates.

- In producing industrial alcohol moulds are employed as starters to bring about scarification of the starch. At the second stage yeast is employed to act on the sugar.
- Although mould can complete the conversion to sugar but the yield is better if yeast is employed for the second stage. The moulds commonly used for purpose of scarification are *Mucorracemosus*. *M. rouxii*and some species of *Rhizopus*. *Aspergillusflavus* is used in the production of African native beer.

	Source	Uses
Baker's yeast	*Saccharomyces cerevisiae*	• Commonly used as a leavening agent in baking bread. • Baker's yeast determines the taste and texture of bread. • It is also used as leavening agent in other bakery products like crakers, rolls, doughnuts etc. • Used in wine industry.
Ethanol	*Saccharomyces cerevisiae*	• It is used in beer, wine and liquor industry. • Used in products like paints, coatings, adhesives and household product like nail polish remover. • Used as a solvent in the manufacture of varnishes and perfumes. • Also used in many medicines and drugs; as a disinfectant and in tinctures. • Used as a fuel and gasoline additive.

Enzyme preparations:

Takamine on the basis of his intensive study of the enzymes produced by Aspergillusflavus-oryzae series has introduced in the market a few products of high enzymic activity. These are Digestin, Polyzime, Taka diastase, etc. They are used for dextrinization of starch and desiring of textiles.

	Source	**Uses**
α-amylase **(Enzyme)**	*Aspergillus niger* *Aspergillus oryzae*	• Used in the fermentation of complex sugar, starch etc. • It is important in brewing beer and liquor made from sugars derived from starch.

		• Amylase is also used in clothing and dishwasher detergents to dissolve starches. • It is used in bread making. • Amylase plays an important role in the fermentation of alcohol.

Preparation of organic acids:

The important organic acids produced commercially as the result of the biochemical activities of moulds are oxalic acid, citric acid, gluconic acid, gallic acid, fumaric acid, etc.

Gluconic acid	*Aspergillus niger*	• Calcium gluconate, in the form of a gel, is used to treat burns from hydrofluoric acid; • Calcium gluconate injections may be used for more severe cases to avoid necrosis of deep tissues, as well as to treat hypocalcemia in hospitalized patients. • Quinine gluconate is a salt of gluconic acid and quinine, which is used for intramuscular injection in the treatment of malaria.
Fumaric acid	*Rhizopus sp*	• Fumaric acid has been used as a food acidulant. • As a food additive, it is used as an acidity regulator
Oxalic Acid	*Aspergillus niger*	• It is used in the making of wheat tortillas as a food preservative and as the acid in leavening.
Citric Acid	*Aspergillus niger*	• Citric acid is used as a flavouring agent in processed foods and soft drinks. • It is the most frequently used preservative. • It is used as a household cleaners in kitchen and bathroom sprays. • It is used in production of jam, jelly, ice cream candies etc. • Citric acid's ability to chelate metals makes it useful in soaps and laundry detergents. • Used in the production of cosmetics.

Mycoproteins

Mycoprotein is a form of single-cell protein, also known as fungal protein, is defined as "Protein derived from fungi, especially as produced for human consumption." "Myco" is from the Greek word for "fungus".

Synthesis: The fungus is grown in vats using glucose syrup as food. A fermentation vat is filled with the growth medium and then inoculated with the fungal spores. The *Fusarium venenatum* culture respires aerobically, so for it to grow at an optimum rate, it is supplied with oxygen, and carbon dioxide is drawn from the vat. To make protein, nitrogen (in the form of ammonia) is added and vitamins and minerals are needed to support growth. The vat is kept at a constant temperature, also optimized for growth; the fungus can double its mass every five hours. When the desired amount of mycoprotein has been created, the growth medium is drawn off from a tap at the bottom of the fermenter. The mycoprotein is separated and purified. It is a pale yellow solid with a faint taste of mushrooms. Different flavors and tastes can be added to the mycoprotein to add variety.

Nutrition potential: Mycoprotein is able to provide greater satiety than traditional protein sources like chicken while also being rich in protein and low in caloric content. Replacing two servings of meat protein with mycoprotein can result in a daily deficit of 80 kilocalories (330 kJ), whilst also extending the period of satiation, which is promising for weight management programs. Mycoprotein is rich in fiber and protein content but very low in fat, making it a desirable food source for consumers trying to limit fat intake but still participate in a high protein diet.

F. venenatum's high fiber content also has potential in managing blood sugar levels. The mechanism that links fiber content and *F. venenatum*'s effect on managing glycemia and insulenaemia is not completely understood, but it is known to decrease the rate of glucose absorption and insulin secretion and helps mitigate the maximum limit an amount of insulin can process glucose, known as insulin peak.Under optimum conditions *F. venenatum* biomass can be 42% protein while also functioning as a prebiotic material for the lower gut.

Mycotoxins

Mycotoxins are secondary metabolites produced by fungi that are capable of causing disease and death in humans and other animals. Because of their pharmacological activity, some mycotoxins or mycotoxin derivatives have found use as antibiotics, growth promotants, and other kinds of drugs; still others have been implicated as chemical warfare agents.

A. **Food Toxins:**These toxins are mainly responsible for poisoning of foods. The mycotoxin production occurs in many plant products, especially cereals and oilseeds. Others like peanuts, rice, corn and cotton seeds also get con-taminated and store mycotoxins. The mycotoxins are produced by more than 100 mold species, mainly by *Aspergillus*, *Penicillium* and *Fusarium*.
   a. **Aflatoxins:**Aflatoxins are potent toxic, carcinogenic, mutagenic, immuno-suppressive agents, produced as secondary metabolites by the fungi on variety of food products. Aflatoxins normally refers to the group of difuranocoumarins and are classified in two broad groups according to their chemical structure the difurocoumarocyclopentenone series and the difurocoumarolactone series. About 18 types of aflatoxins have been identified, the major members are aflatoxin B_1, B_2, G_1, G_2, while M_1, and M_2 are less significant.
   b. **Ochratoxins:**Ochratoxins are closely related derivatives of isocoumarin, linked to an amino acid, L-β-phenylalanine. Of the nine types of ochratoxins, ochratoxin A is the most effective one.
   c. **Zearalenone**:Zearalenone is a phenolic resorcyclic acid lactone, produced in maize and also in other cereals, infested with different members of Fusarium such as *F. moniliformae* and *F. graminearum*.
   d. **Trichothecenes**: Trichothecenes are produced by different species belongs to the genera Fusarium, Myrothecium, Trichoderma, Stachybotrys and Cephalosporium. This group of toxins shows sub-epidermal haemorrhage, several local irritations and gene-ral necrosis.

B. **Ergot Toxins:**These toxins are stored in the elongated sclerotia of Clavicepspurpurea, the pathogen of ergot disease of rye. These are the poi-sonous alkaloids such as ergotamine, ergocrystinine, ergometrimine, ergonovin and ergocrystine.Due to consumption of sclerotia along with rye straw, the domestic animals show the following symptoms:

(i) The hoofs, legs and tail become gangrenous,
(ii) Abortion in cows.

Due to consumption of powdered sclerotia along with rye flour, following symptoms become pronounced in human being:

(i) Diarrhea, abdominal pain and vomiting.

(ii) Effects on nerves and cause psychiatric disturbances, and

(iii) Decrease the diameter of terminal blood vessels.

C. **Mushroom Toxins:**Several mushrooms produce toxins and by consuming such mush-rooms as food, different abnormalities become visible in human beings like diarrhoea and vomi-ting within 2-3 hrs of consumption. At a later stage they cause liver damage, kidney failure, complete unconsciousness and even death.The following mushrooms are important in this respect:

(i) *Amanita phalloides* (the 'death cup'). Out of 10 toxins so far reported, the phalloidin and α-amanitin are important. The phalloidin affects the cell membrane of liver cells and α-amanitin causes lesions in stomach and intestine cells.
(ii) Different species of *Helvella* such as *H. infula*, *H. gigas*, *H. esculenta* and *H. underwoodii*, produce gyromitrin, a fatal toxin.
(iii) Inocybe and Clitocybe produce toxin muscarine that causes several abnor-malities.

Aflatoxin

Definition: Aflatoxins are mycotoxins, chemically oxygenated heterocyclic compounds produced by *Aspergillus flavus* and *A. parasiticus*, which are highly toxic and carcinogenic.

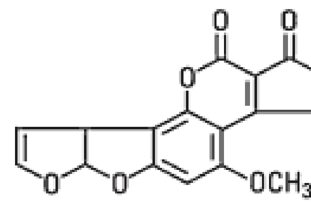
Introduction:

- Aflatoxin was discovered in the early 1960s, when the so-called "turkey X disease" appeared in farms of England and was shown to be caused by feed contaminated with aflatoxins (produced by *Aspergillus flavus*), causing death of more than one lakh turkey.
- These fungi (*Aspergillus flavus*) occur commonly in the soil as saprophytes and do not generally seem to invade grain prior to harvest causing extensive qualitative as well as quantitative loss as storage fungi.

- They are mainly produced on grains like wheat, corn, rice, barley, bran, flour, soybean, peanut, pulses and oil seeds.

Chemical nature and toxicity:

- In 1965, Asao et al. determined the structure of aflatoxin by X-ray analysis. These secondary metabolites comprise a unique group of oxygenated coumarin derivatives of heterocyclic compounds.



Aflatoxin B1

- At present there are eight characterized aflatoxins : B_1 , B_2 , G_1 , G_2 (named due to blue-green fluorescence in UV light) , M_1 , M_2 (present in milk of cows) and B_{2a} and G_{2a} (derivatives of B_2 and G_2)
- Different types of aflatoxin vary in their toxicity. The most common of them are aflatoxin B_1, which shows highest toxicity.
- The furofuran ring of aflatoxin is essential for the toxic and carcinogenic nature of them. The double-bond present in the terminal furan ring determines its toxicity.

Mode of Action:

- Effect of aflatoxin has been shown on the liver cells and was found that the initial site of action was the DNA of nucleus.
- Aflatoxin binds with the DNA and prevents transcription (formation of mRNA from DNA). As a result protein synthesis is inhibited.
- Degradation of endoplasmic reticulum has also been shown in electron microscope of aflatoxin treated cells.
- Aflatoxin B_1 is a genotoxic or mutagenic carcinogen. Carcinogenic property of aflatoxin B_1 is expressed when it is activated by Cytochrome P_{450} and other oxidative enzymes.
- Aflatoxin B_1 is then transformed to several products but the compound B_1-exo-8, 9-epoxide is responsible for carcinogenesis in liver. It then intercalates between DNA base pairs and produce N_7-guanilyl adduct.
- These adducts are resistant to DNA repair system and thus brings about mutation and carcinogenesis.

- It has been shown that patients infected with hepatitis B virus have higher chances of liver cancer due to aflatoxin.

Effects of Aflatoxin

➢ **Effect on animals:**

- **Hepatic-toxicity:** Acute toxicity is expressed by death of animals, in which the liver becomes enlarged, pale and discoloured. It also shows necrotic zones caused by the toxin.
- **Carcinogenicity:** Feeding of contaminated peanut meals initiates development of liver tumors. Besides liver, tumors have also been recorded in other parts of the animal body due to aflatoxin poisoning. Carcinoma of the colon has been reported by several workers.
- **Mutagenicity:** Aflatoxins are mutagenic to a wide variety of microorganisms and cells in culture. Studies have shown that aflatoxin induces chromosomal aberration in rats and kangaroo. Chromosomal abnormalities such as clamping, fragmentation, meiotic polyploidy and euploidy has also been reported in mice fed with aflatoxin-contaminated diets.
- **Effect on reproductive system:** Urogenital systems of swine cattle and poultry animals are known to be affected by aflatoxin. In male swine, it produces testicular atrophy and mammary gland enlargement. At high concentration it is associated with infertility, embryonic death and defective development in swine.

➢ **Effect on metabolism:**

- It was shown that aflatoxin B_1 inhibits replication by stopping incorporation of thymine.
- Aflatoxin B_1, G_1 and G_2 was shown to inhibit rat liver RNA polymerase activity.
- Aflatoxin B_1 also inhibits protein synthesis, especially inhibiting induction of tryptophan pyrrolase enzyme in rat liver cells.

➢ **Effect on human system:**

- Direct ingestion of aflatoxin occurs through consumption of contaminated food such as cereals, pulses, fruits, nuts and spices, whereas indirect consumption takes place through contaminated milk and milk products, meats and eggs causing human pulmonary edema, dilated heart, childhood cirrhosis etc.

- Aflatoxin B_1, G_1 and M_1 are carcinogenic mycotoxins and having genotoxic effects also. Aflatoxin B_1 causes liver cancer. It binds with DNA at the guanine base in liver cells, disrupting the genetic code that regulates cellular functions.

➢ **Effect on plants:**

- Aflatoxin inhibits plant growth by restricting seed germination and seedling formation. Inhibition of seed germination is associated with suppression of DNA, RNA and protein synthesis.

- Soaking of seeds of different crop-plants in filtrates of toxigenic strains of A. *flavus* greatly reduced germination and inhibition was up to 100% in *Phaseolus aureus* seeds.

- Chlorophyll deficiency has also been reported in aflatoxin treated plants.

- Cytological abnormalities has also been observed in aflatoxin treated root cells of *Lepidium sativum*, including occurrence of ring shaped nucleoli with nuclear caps; mitochondria with enlarged cisternae; degranulation of endoplasmic reticulum and irregular shaped nucleoli.

- In lower plants, inhibition of protonema development in ferns, inhibition of growth in *Chlorella* etc. has been observed after treatment with aflatoxin B_1.

Fungus as Bio-control Agent

Mycofungicide:

Microbial antagonists can suppress plant diseases and organisms that suppress pathogens can be referred to as biological control agents. Various fungal species can be used as biological control agents and may provide effective activity against various pathogenic microorganisms.An effective biological control agent should be genetically stable, effective at low concentrations, easy to mass produce in culture on inexpensive media, and be effective against a wide range of pathogens. Following are some examples of mycofungicides:

- *Trichoderma harzianum* - a species with biocontrol potential against *Botrytis cineria*, *Fusarium*, *Pythium* and *Rhizoctonia*.
- *Ampelomyces quisqualis*, - a hyperparasite of powdery mildew.
- *Chaetomium globosum* and *C. cupreum*, - having biocontrol activity against root rot disease caused by *Fusarium*, *Phytophthora* and *Pythium*
- The non-pathogenic strains of *Fusarium oxysporum* is widely used to control *Fusarium* wilt
- *Pythiu moligandrum* oospores have been applied as seed treatments which reduce damping-off disease caused by *P. ultimum* in sugarbeet.

Mycoinsecticide:

Myco-biocontrol offers an attractive alternative to the use of chemical pesticides. Myco-biocontrol agents are naturally occurring organisms which are perceived as less damaging to the environment. Their mode of action appears little complex which makes it highly unlikely that resistance could be developed to a biopesticide.

A substantial number of mycoinsecticides and mycoacaricides have been developed worldwide since the 1960s. Products based on *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria fumosorosea* and *B. brongniartii* are the most common. Approximately 75% of all listed products are currently registered, undergoing registration or commercially available, whereas 15% are no longer available. Insects in the orders Hemiptera, Coleoptera, Lepidoptera, Thysanoptera, and Orthoptera comprise most of the targets. Research has focused on the relatively easily produced asexual spores (conidia) of the hyphomycete genera *Metarhizium*, *Beauveria, Verticillium*, and *Paecilomyces*. These fungi have a wide host range although there is considerable genetic diversity within species and some clades show a high degree of specificity. For example, *Metarhizium anisopliae* var. *acridum* is only effective against insects like grasshoppers and locusts. The commercial *Beauveria bassiana*-based mycoinsecticides are relatively stable compared with other biological insect control agents for lepidopteran insect pests.

Mycoherbicide:

There have been many microbial agents under evaluation for their potential as bioherbicides with horticultural crops, turf, and forest trees, including obligate fungal parasites, soil-borne fungal pathogens, non-phytopathogenic fungi, pathogenic and non-pathogenic bacteria, and nematodes. One of the first bioherbicides registered was with the active ingredient *Phytophthora palmivora*, which was developed to control strangler vine (*Morrenia odorata*) on citrus in Florida.

Some fungal pathogens are toxic to a wide range of weed species. The early mycoherbicides had highly virulent fungal plant pathogens that could be mass-cultured to produce large quantities of inoculums for inundative application to the weed host. These fungi infect the aerial portion of weed hosts, resulting in visible disease symptoms.

- The rust fungus *Puccinia canaliculata* is a foliar pathogen of yellow nutsedge (*Cyperus esculentus*), and it can be mass-cultured on the weed host in small field plots or the greenhouse.
- Applying the fungal pathogen *Chonrotereum purpureum* to wounded branches or stumps of weedy tree species inhibited re-sprouting and decayed the woody tissues.

Myconematicide

Plant-parasitic nematodes (PPNs) are considered hidden enemy of the farmers as the nematodes are subterranean in habitats and growers are unaware of losses caused by them. The most studied and promising groups among the nematode-antagonistic organisms are the nematophagous fungi and bacteria. The two groups include many species. These bio-nematicides are frequently applied to sites and ecosystems that routinely receive other inputs including chemical pesticides, surfactants (e.g. wetting agents), fertilizers, mineral nutrition, and soil amendments which may interact with bio-active ingredients targeting PPNs.

Fungus	Integrated with	Nematode managed	Crop	Result
Trichoderma harzianum	Carbofuran	*Meloidogyne i ncognita*	French bean	*T. harzianum* in combination with carbofuran resulted in decreased root galling, egg masses, and nematode population in soil by 65.15% as compared to untreated control.
T. viride	*P. lilacinus* + carbofuran + mustard cake	*M. incognita*	Tomato	Integrated application of *T. viride* along with *P. lilacinus*, carbofuran, and mustard cake showed least nematode reproduction factor.
Paecilomyces lilacinus	Neem cake + NPK (nitrogen, phosphorus, potassium)	*M. incognita*	Tomato	The antagonistic potential of *P. lilacinus* against *M. incognita* infesting tomato seedlings under nursery conditions was enhanced (53.6%) when applied in combination with neem cake and NPK.
Pochonia chl amydosporia	Carbofuran	*M. incognita*	Tomato	*P. chlamydosporia*+ carbofuran resulted in maximum plant growth and minimum galls and egg masses.

Medical Mycology

Definition: Mycoses [sing. mycosis; Greek- mykes, fungus] may be defined as the growth and infection caused by pathogenic fungi inside the body of the host.

- Fungal toxicosis or mycotoxicosis may be defined as poisoning caused by toxic fungal metabolites (toxins). Eg. Aflatoxicosis caused by aflatoxins produced by the fungus *Aspergillus flavus* in infected food grains.
- These fungal diseases are typically divided into five groups according to their site of occurrence and route of infection: superficial, cutaneous, subcutaneous, systemic and opportunistic mycoses.
- Superficial, cutaneous, and subcutaneous mycoses are direct contact infections of the skin, hair, and nails. Systemic mycoses are fungal infections that have disseminated to visceral tissues. While opportunistic microorganisms are generally harmless in its normal environment but becomes pathogenic in a compromised host.

Group	Pathogen	Location	Disease	Treatment
Superficial mycoses	*Piedraia hortae* *Trichosporon beigelii* *Microsporum sp*	Scalp, Beard, mustache	Black piedra White piedra	Griseofulvin
Cutaneous mycoses	*Trichophyton mentagrophytes,* *T. verrucosum,* *T. rubrum*	Beard hair Scalp Bare skin Feet	**Trichophytosis** Tinea barbae Tinea capitis Tinea corporis Tinea pedis (athlete's foot)	Griseofulvin
Subcutaneous mycoses	*Phialophora verrucosa*	Legs, feet	Chromoblastomycosis	Amphotericin B
Systemic mycoses	*Blastomyces dermatitidis* *Cryptococcus neoformans*	Lungs, skin Lungs, skin, bones, viscera	Blastomycosis Cryptococcosis	Amphotericin B
Opportunistic mycoses	*Aspergillus fumigatus* *A. flavus* *Candida albicans*	Respiratory system Skin or mucous membranes	Aspergillosis Candidiasis	Amphotericin B Nystatin.