CHAPTER-3

FUNGI

Fungi are a diverse group of multicellular organisms with an incredible array of vegetative and reproductive morphologies and diverse life cycles. They are more abundant (on mass basis) in soils than any other group of microorganisms and their biomass ranges from 500 to 5000 wet kg ha^{-1}. Fungi are active participants in ecosystems as

- Degraders of organic matter
- Agents of disease
- Beneficial symbionts
- Agents of soil aggregation and
- An important food source for humans and many other organisms

In many cases, fungi are vital component of ecosystem functionality and vitality. Humans depend considerably on fungi for metabolic by-products in food additives and medicines. At least 70,000 species have been described, but at least 20 times that number is estimated to exist worldwide.

CELL STRUCTURE

Unlike prokaryotic bacteria, fungal organisms are eukaryotic. The multitude of membrane bound organelles present in each cell is similar to those of insects, plants, and animals, but with some important differences:

- The membrane surrounding the nucleus in fungi constricts during formation of two daughter nuclei, whereas it degenerates and reforms in plant and animal cells.
- Vacuoles in the cell cytoplasm of fungi usually are smaller than those in plants.
- The network of endoplasmic reticulum is not as extensive, and it has fewer connections with the cytoplasmic membrane compared to that in plants and animals.
- An extensive membrane system generates many secretory vesicles that substitute for Golgi bodies found in other eukaryotic organisms. These vesicles are uniquely important in filamentous fungi for cellular function and growth because they transport structural and enzymatic molecules to active metabolic regions. These occur at the tips of tubular strands or filaments called hyphae that elongate and branch indefinitely.
- Even though fungi do not contain chlorophyll and thus cannot carry out photosynthesis, they are considered plantlike because they have cell walls, are generally nonmotile, and reproduce

by means of spores (microscopic parts that resemble seeds in functioning as vehicles of dissemination and formation of new individuals, but they lack embryos). However, other morphological characters and new molecular evidence suggest that fungi are more closely related to animals.

We now believe that they originated about one billion years ago as a group equal in rank to plants and animals.

GROWTH AND REPRODUCTION

Fungi can be single-celled, but the majority is multicellular organisms with a filamentous vegetative body. In contrast to the relative simplicity of the vegetative body, reproductive structures include various kinds of single-celled spores produced alone or in visible and complex fruiting bodies. Diverse reproductive structures produced during sexual and asexual phases of fungal life cycle provide many of the morphological characters defining species and other groups at higher taxonomic ranks. As technology advances, more characters are becoming available to improve taxonomies based on evolutionary relationships, such as those in sequence of nucleotides and amino acids of DNA and proteins, respectively, and in the chemistry of cell walls, storage carbohydrates, and lipids. Analyses of these characters now indicate that organisms traditionally considered to be fungi evolved from separate ancestors; they are now classified in at least two separate kingdoms i.e. Kingdom *Fungi* and Kingdom *Stramenopil.a.* Four Phyla (*Chytridiomycota, Zygomycota, Ascomycota, Basidiomycota*) from kingdom Fungi and one phylum (*Oomycota*) from kingdom Straminopila share many common morphological, nutritional, and ecological properties.

The vegetative body of a fungus is called a thallus, and it generally exists as one of the following three forms;

Chytrid cells:

Solitary globose cells with or without specialized root like filaments called *rhizoids.* They are unique to organisms of the phylum Chytridiomycota in the kingdom Fungi and the phylum Hyphochytridiomycota in the kingdom Straminopila.

Yeast Cells:

Spherical to ovoid cells are formed by fungi in the phyla Ascomycota and Basidiomycota. They divide by budding or fission. Some fungi are dimorphic and can change from a mycelium to a yeast form under conditions where penetration of a substrate is not needed to obtain nutrients, such as in aqueous environments or insect cavities.

Mycelia:

A filamentous network of hyphae that branch and grow only by apical extension. This vegetative growth form is the most common of organisms in the kingdom Fungi, and in soil fungi especially.

A mycelium is incredibly resilient (flexible) because growth is open-ended for as long as a nutrient source is available. The result is that size is highly variable, ranging from a pinpoint to a mass covering hectares of land. Soil is more heterogeneous, consisting of zones and microsites that can either benefit or hinder fungal growth. A mycelial strand or hypha, provides an ideal mechanism to penetrate soil pores and branch in all directions. As a network, hyphal interconnections establish a three-dimensional physical continuum capable of spanning nutrient-deficient zones, navigating around physical barriers or gas pockets in the soil matrix, and penetrating herbaceous and woody organic matter. The branching of hyphae around and within soil particles of all sizes influences soil structure by improving soil aggregation and aeration.

A hyphae is essentially one long tube with rigid crosswalls known as *septa* (singular, *septum)*. These septa are not the same as cell walls because they contain open pores for continuous flow of cytoplasm and cellular organelles and nuclei. They provide structural support along the length of a hypha and regulate cytoplasmic flow between hyphal compartments. In some fungal groups such as Zygomycota and Oomycota, the hyphae are coenocytic in that they rarely form septa in actively growing regions and nuclei are free floating. When septa are formed, they are located between cytoplasmically active regions of hyphae and aged or dead regions devoid of cytoplasm. In other fungal groups such as Ascomycota and Basidiomycota, septa are integral part of hyphae.

Many soil fungi in the Ascomycota and Basidiomycota can organize hyphae into specialized organs such as *mycelial strands*, *rhizomorphs,* and *sclerotia*.

Mycelial strands:

These are aggregates of parallel hyphae cemented together by sticky exudates and by fusion of hyphal branches. The cell walls of surface hyphae are thickened and often melanized (containing dark pigments). Mycelial strands of some fungi consist of modified hyphae functioning similar to plant tissues.

Rhizomorphs:

Rhizomorphs are more complex versions of mycelial strands, with a greater degree of tissue differentiation. They are highly resistant to environmental changes, provide stronger means than hyphae to penetrate soil or organic material, supply oxygen to leading tips, and transport nutrients throughout the fungal organism. The rhizomorphs resemble gray to black shoestrings, although they are much longer in length. They branch in the soil as much as eight times faster than a typical mycelium and then persist for years. The demise of many orchards started in forests cleared of diseased trees can be attributed to abundant and viable rhizomorph networks in soil even after fumigation.

Sclerotia (singular, sclerotium) :

These are spherical to oblong aggregates of interwoven hyphae that resemble plant parenchyma tissue in the center but are thick-walled and melanized on the periphery to form a hard outer rind. . Sclerotia often are separated from the parent mycelium and therefore are more readily dispersed than other forms of mycelial aggregates. They usually are 1 to 3 mm in diameter, and dark brown to black in colour. Sclerotia are better able to resist drought and temperature fluctuations and, therefore, are much longer-lived than mycelia. They also store lipids, carbohydrates, and proteins until soil conditions are favourable enough to germinate and form either a new mycelium or fruiting body.

Fungi reproduce by forming distinctive spores or fruiting bodies as a result of either sexual or asexual states designated as telomorphs and anamorphs, respectively. Spores are important in life cycles because they not only encapsulate different nuclei and cytoplasmic factors into numerous discrete packets for survival under stress or for dispersal, but they also increase the probability of contact between opposite mating types.

In general, the sexual cycle requires four essential processes:

1. **Plasmogamy:**

   It is the fusion of cytoplasm of two compatible haploid (1N) sexual organs or *gametangia* (singular, *gametangium*).

2. **Karyogamy:**

   It is the fusion of two haploid nuclei to establish a diploid (2N) condition.

3. **Meiosis:**

It is the reduction of diploid nucleus to haploid daughter nuclei, during which genes undergo recombination.

4. **Telomorph:**

   It is the encapsulation of each haploid nucleus and its associated cytoplasm in a discrete cell that differentiates into a spore.

Some fungi form morphologically distinctive “male” and “female” gametangia. Aside from hormonal signals, sexuality in fungi is regulated by physical origin of gametangia and their mating type. A mycelium on which both gametangia develop is called *homothallic* which is self-compatible. When each gametangium develops on separate mycelium, then it is *heterothallic* and plasmogamy in this case occurs only when gametangia are sexually compatible, which requires opposite mating types. The gametangia of most fungi are morphologically indistinguishable and, therefore, require chemical methods to recognize each of them. In lower fungi (i.e., Chytridiomycota and Zygomycota) and fungus like organisms (i.e., Oomycota), the telomorph usually consists of solitary, thick-walled resting spores. In higher fungi (i.e., Ascomycota and Basidiomycota), spores often are borne on a variety of fruiting bodies like the highly visible mushrooms found in the forest floor, lawns, and flower beds.

The asexual cycle is much simpler than the sexual cycle because it does not require interactions between nuclei or different mating types. The nuclei of one hypha divide repeatedly by mitosis and are partitioned into large number of spores called *conidia* (singluar, *conidium*). Conidia are formed in a variety of ways, but essentially they arise from hyphal tips. Most soil fungi tend to form conidia freely on a single hypha or hyphae in various degrees of aggregation; a low proportion develops fruiting bodies. Some soil fungi can transform hyphal compartments into thick-walled, darkly pigmented *chlamydospores*.

The diversity of life cycles found in fungi and fungus like organisms is far too great to be discussed in detail. Instead, salient features of some important phyla of fungi are being discussed in the proceeding section.

Oomycota:

These organisms differ from true fungi in that the mycelium cannot translocate the carbohydrate, hyphal walls are composed of cellulose instead of chitin, and the nuclei in the mycelium are diploid rather haploid. These fungus like organisms are found most commonly in aquatic habitat and moist soils. Many species are saprophytes degrading organic matter but some, such *as*

Phytophthora infestans (causing late blight of potato) and *Plasmopara viticola* (causing downy mildew of grapes) produce devastating plant diseases. The phylum is characterized by organisms that produce a coenocytic mycelium, thick-walled resting sexual spores called *oospores*, and nonmotile asexual spores called *sporangia*. Both oospores and sporangia can partition their cytoplasmic contents into clusters of motile *zoospores* under cool, moist conditions. Each zoospore is capable of directional movement in an aqueous environment by the whiplike action of two flagella.

Chytridiomycota:

These fungi are found **mostly** in aquatic environments, including wet soils. Many are saprophytes and can be baited from steams and ponds using pollen grains, hemp seed, and other plant parts. Others are parasites of algae and insects. A few are symptomless plant pathogens or produce wart damage on potato tubers.

These fungi reproduce by forming zoospores with a single whiplash flagellum within a sac-like cell called a sporangium. Most organisms are made up of single cells that vary considerably in how they colonize host cells. Some species form rudimentary coenocytic hyphae, called *rhizomycelia*, which serve as a channel for migration of nuclei much like true hyphae. Some develop completely within the host cell; others develop on the cell surface and are anchored by anucleate rootlike *rhizoids*.

Zygomycota:

These fungi are terrestrial, with many growing as saprophytes in soil and dung. Some are parasites of insects, and others form beneficial endomychorrhizal associations with many land plants. Most are characterized by coenocytic mycelium, sexual *zygospores* and asexual sporangia. Others, such as endomycorrhizal fungi produce asexual spores that share few (if any) morphological similarities with other zygomycetes. None of the spores produced by zygomycete fungi are motile.

Ascomycota:

Most of these fungi are terrestrial. Life cycles and morphologies of spores and fruiting bodies are more diverse than in any phylum of the kingdom Fungi. Many are saprophytes, but some preferentially colonize specific plant tissues or hosts. Notorious plant pathogens include those causing powder mildew diseases, ergot, chestnut blight and Dutch elm disease. Ascomycete yeasts are important in bread and alcohol production. Morels and truffles are prized delicacies.

Many wide-spread soil inhabitants are asexually reproducing species, such as those in the genera *Aspergillus*, *Fusarium*, and *Penicillium*.

Hyphae are partitioned by septa perforated with one to many small pores. The number of nuclei per compartment is variable, since the movement of nuclei is not tightly controlled. The main features of the sexual stage in fungi of this phylum are formation of *ascospores* enclosed within thin colourless sacs called *asci* (singular, *ascus*), which often are clustered within fruiting structures called *ascomata* (singular, *ascoma*). Ascomata vary considerably in shape and organization to accommodate inventive mechanisms for active and passive discharge of ascospores. They include closed spherical *cleistothecia*, flask-shaped *perithecia*, and saucer-shaped *apothecia*. Asexual conidia of various sizes, shapes, and colors are produced on vegetative hyphae. They also can form inside fruiting bodies of similar shapes to those producing asci. Some species, such as *Sordaria fimicola*, have only a sexual phase whereas many others, especially soil fungi are strictly asexual.

Basidiomycota:

These fungi generally are saprophytes in soil, leaf litter, and decaying wood (including standing trees). Some are plant pathogens that cause tree root and crown rots and destructive flower and foliar diseases, such as smuts and rusts. Others are important beneficial ectomycorrhizal symbionts of tree species. Many of the mushrooms produced by these fungi are prized for their excellent flavour or notorious for their deadly poison.

In the sexual stage, *basidiospores* are produced on the surface of a club-shaped *basidium* (plural, *basidia*) situated within diverse and often complex fruiting bodies such as mushrooms, conks, puffballs, or jellylike masses. Most soil basidiomycete fungi reproduce asexually by producing spores, such as chlamydospore, from the mycelium instead of expending energy on conidia formation. Asexual sclerotia and fragments of mycelia, rhizomorphs, and mycelial strands function somewhat like spores in dispersal, although for much shorter distances.

NUTRITIONAL PATTERN

Fungi are heterotrophs, in that they must obtain carbon and other nutrients from organic matter in the external environment. Nutrients are acquired by absorption through chytrid, yeast, or hyphal cell walls. Fungi which degrade nonliving organic matter are called saprophytes, and they are important agents in soil mineralization processes such as ammonification and carbon cycling. In saturated soils or aqueous environments, chytrids and oomycete fungi are the most common saprophytes, whereas fungi in other phyla are more abundant in drier soils. In general, fungi are obligate aerobes in that they cannot grow without an oxygen supply. They tend to be more abundant in acidic soils, where there is less competition from bacteria.

Saprophytic fungi, together with bacteria, are primary agents in the decomposition of cellulose, hemicellulose, and pectin in plant cell walls. Lignin, generally the third most common component of plant residues, can be degraded by fungi, especially some species that decay wood. Enzymes that decompose lignin also contribute to the degradation of organic pollutants and pesticides in soil.

Saprophytic fungi are capable of producing many other enzymes as well, including xylanases (to break down xylan, a hemicellulose polymer of plant cell walls) and cutinases (to break down cutin on leaf surfaces). Many of these enzymes are excreted into synthetic media when fungi are cultured in the laboratory. As a result, they are excellent candidates for industrial applications such as food processing, waste treatment, and production of alcoholic beverages.

The important role of fungi in decomposition of organic matter is unequivocal. Their contribution in transformation of inorganic compounds (e.g., heterotrophic nitrification) is more poorly understood, especially as compared to bacterial populations. Much of the definitive results obtained to date have been derived from *in vitro* experiments of fungi on selective substrates, and studies in the field where conditions are more variable and complex have been less conclusive.

The role of fungi in nitrification has been hard to document. Reduction of nitrification rates by antifungal compounds such as cycloheximide in acid soils indicates a fungal contribution, although it is not high. Fungi are able to oxidize a diverse range of compounds, such as ammonium ions, pyruvic oxime, benzohydroxmic acid, cycloserine, and 3-nitroproprionate. It is unclear if nitrification processes are linked to energy generation or provide energy to maintain the mycelial network.

Some fungi can produce nitrate reductase under low oxygen conditions in culture, suggesting their possible involvement in denitrification. Most studies, however, show that nitrate is reduced only as far as nitrate or N_2O rather than to dinitrogen gas. These processes, therefore, may have less to do with respiration than with modifying the environment around hyphae such as transformation of nitrate to less toxic compounds.

Bacteria, especially *Thiobacillus s*pecies have received considerable attention as major players in the oxidation of elemental sulfur, thiosulfate, tetrathionate, and sulfate in soil. Common fungal saprophytes such as *Aspergillus*, *Fusarium*, and *Penicillum* were contaminants in the first isolations of *Thiobacillus thiooxidans*, indicating they could grow under high sulfur conditions. Another soil saprophyte, *Trichoderma harzianum*, was able to oxidize elemental sulfur to tetrathionate and sulfate. Some yeasts, such as *Rhodotorula*, produce sulfite oxidase and thiosulfate oxidase enzymes much like *Thiobacillus*.

Fungi have the potential to increase solubilization of phosphorus compounds in soil by releasing organic acids from hyphae, such as citrate and 2-ketogluconate. Some evidence suggests that the reduced pH causes decomposition of rock phosphate, thus bringing phosphorus into solution. Chelation of calcium in phosphates also increases phosphorus solubility.

Fungi also oxidize and reduce other inorganic compounds such as manganese and iron. White-rot fungi can produce manganese peroxidase, which oxidizes manganous to manganic ions. These ions, in turn, oxidize lignin and facilitate its degradation.

Numerous fungi are capable of forming specialized positive (symbiotic) or negative (pathogenic) associations with other living organisms. The relationship is *necrotrophic* when a fungus produces abundant enzymes to degrade and kill tissues of its host. *Biotrophic* relationships are more complex because the fungus establishes close contact with host cell contents via specialized absorptive structures and then induces hormonal changes to channel carbon flow in the host towards those contact sites. Most necrotrophic relationships are facultative because the fungi can produce the degradative enzymes to live freely as a saprophyte for a part of its life cycle. Duration of contact between a fungus and its host is highly variable, often depending on the physiology of each associate and environmental condition. Facultative biotrophic associations are unique in that the fungal partners are saprophytic. For example, some of these fungi digest wood for ingestion by ants, which in turn forage for substrate and provide a competition-free home for the fungus. All other known biotrophic associations are obligate in that fungi need their hosts to grow and reproduce. Some symbiotic fungi, such as ectomycorrhizal are obligate symbionts under natural conditions but they still can grow slowly on a synthetic medium containing essential nutrients that can be absorbed by their mycelia.

Some species in Cytridiomycota and Oomycota produce motile zoospores that exhibit chemotaxis and swim along a gradient toward increasing concentrations of simple sugars, amino acids, or organic acids exuded by roots of the target host. The Ascomycota and Basidiomycota do not produce motile spores, but some species have evolved other effective methods to incapacitate their hosts. Some set traps for their hosts e.g. specialized hyphal structures to capture their nematode prey, such as adhesive knobs, branches or nets. Other species have constricting and nonconstricting rings as hyphal outgrowths. The mycelia of some species in Basidiomycota release toxins into the soil to paralyze nematodes long enough to allow hyphal penetration through the tough host cuticle.

The filamentous nature of the mycelium and the three-dimensional branch architecture of component hyphae afford fungi with a reliable and efficient mechanism to obtain nonuniformly distributed nutrients in soils and translocate those nutrients to distant parts of the organisms or wherever they are most needed for continued growth or reproduction. When nutrient depletion extends over a large area, fungal species often survive by storing available endogenous nutrients in spores and dispersing them to more favourable conditions.

FUNGISTASIS:

This is a phenomenon in which natural soils suppress germination of fungal spores or other resting structures. It is an important mechanism, by which fungi maintain viability when nutrients become limiting. This suppression remains in effect until exogenous nutrients again become available and sometimes even until other environmental conditions are optimal for growth. Fungistasis is usually broken after threshold levels of sugars and nitrogenous compounds are reached in proximity to the dormant spore or resting structure. Plant species-specific stimulants in the rhizosphere also may be involved.

The longevity of fungal organisms has not been measured in many species, but their genetic plasticity and open-ended growth habit offers unlimited potential. Fossil evidence, together with measurements of the rate of change in DNA nucleotide sequence, suggests that many fungi have a longevity measured in the millions of years.

ECOLOGY:

Fungal hyphae must be in close contact with a living or dead organic substrate to absorb nutrients, so they rarely grow alone, especially in soils. They compete aggressively among themselves and with other microorganisms for scarce nutrients. Competition and change in available nutrients usually leads to succession, or a directional change in composition and abundance of a species. In contrast to plant succession, a stable climax change is rarely reached with fungi because exploitation of nutrients results in continuous change in nutrient status. Competitive interactions among fungi are complex because many species can coexist in substrates constantly in a state of flux.

Directional taxonomic changes in fungal community structure occur as a plant substrate or a soil undergoes shifts in nutrient availability. In general, fungi in the Oomycota, Chytridiomycota, and Zygomycota tend to precede those in the Ascomycota which in turn precede those in Basidiomycota. Initial colonizers use simple soluble sugars, amino acids, and vitamins in plant protoplasm or plant parts such as fruits, seeds, and vegetables. These “sugar fungi” produce a rapidly spreading mycelium and abundant asexual structures. The dominance of these fungi is short-lived because waste by-products and other conditions cause growth to cease and resting survival structures to be produced.

Cellulose degraders appear next in succession, although sugar fungi may grow concurrently by using some of the by-products. Cellulose degraders constitute the most diverse and competitive group because of the heterogeneity of substrates present. Degradation of straw, which has a high C/N ratio of 80/1, requires that fungi and parasitize or decompose the mycelium of other fungi to obtain nitrogen for growth and enzyme production. Abundant colonizers of straw in no-till crops include many saprophytes or pathogens that reproduce mainly by asexual conidia, such as species of *Chaetomium*, *Fusarium* and *Trichoderma*. Lignin is the main substrate remaining after cellulose depletion. The number of fungal species capable of degrading lignin is low, so competition is greatly reduced. Basidiomycete fungi that produce the necessary enzyme tend to grow slowly because they expend considerable biomass in producing rhizomorphs and fruiting bodies. Because of the variables involved, numerous exceptions to these successional trends exist.

Different environments lead to different successions of saprophytic fungi. In compost, for example, successive colonists are increasingly thermophilic initially, followed by a decrease in tolerance over time. In frozen environments, increasingly psychrophilic fungi are selected.

Antagonism between soil microorganisms also is a common community interaction. Many soil fauns, especially soil animals such as collembola, are *mycophagous* (feed on fungal mycelia). Fungi also produce antibiotics. Antibiotic production by fungal species in ascomycete genera such as *Aspergillus*, *Fusarium*, and *penicillium* provide an ecological advantage in sustaining occupation of a nutrient resource.

Figure 3-1: General structure and organellar composition of a fungal cell at the apex of a hyphae. ER= endoplasmic reticulum, G=glycogen granules, L= lomasome, M= mitochondrion, N= nucleus, Nu= Nucleolus, CM= cytoplasmic membrane, R= ribosome, V= vacuole, Ve= vesicles

Figure 3-2: Types of cells that make up the vegetative body (thallus) of fungal organism: a) Chytrid cells, b) yeast cells, c) a mycelium containing compartments separated by cross walls.

Figure 3-3: Life cycles of fungi in the kingdom Stramenopila and true fungi in the kingdom Fungi: a) Oomycota, b) Chytridiomycota, c) Zygomycota, d) Ascomycota, e) Basidiomycota. Filled arrows indicate meiosis and initiation of a sexual (telomorph) phase; open arrows indicate mitotic divisions of nuclei and asexual (anamorph) phase. The dotted line separates phases where nuclei are haploid (iN) or diploid (2N).