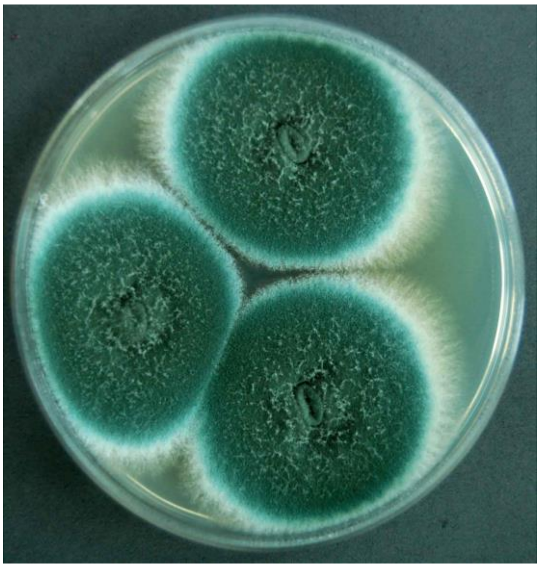
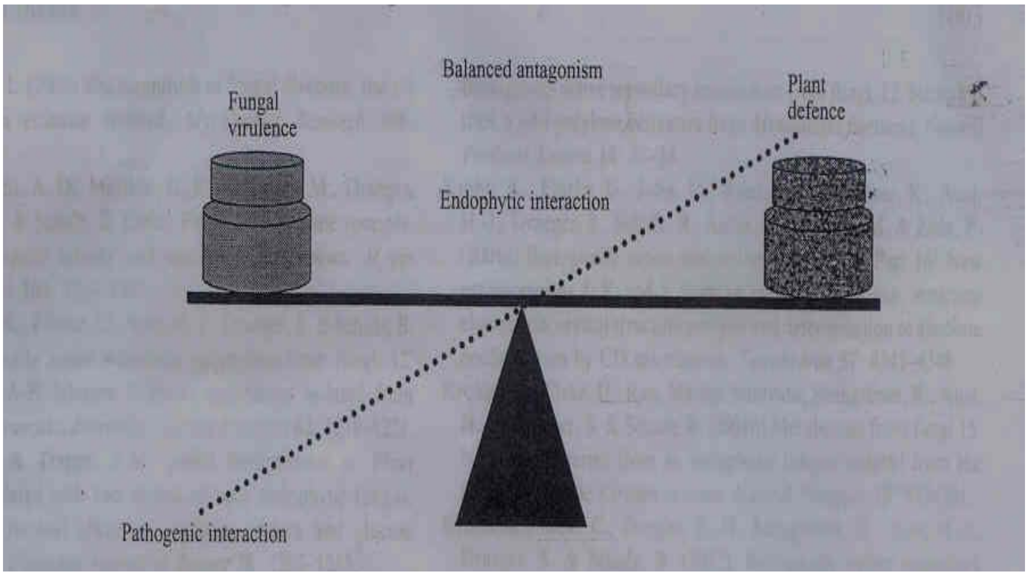
Advanced Fungi

For Diploma students





Dr. Mohamed A. Hussein

2023-2024

GENERAL INTRODUCTION

Mycology is the branch of biology concerned with the systematic study of fungi, including their genetic and biochemical properties, their taxonomy, and their use to humans as a source of medicine, food, and psychotropic substances consumed for religious purposes, as well as their dangers, such as poisoning or infection.

Fungi comprise a vast variety of microorganisms and are numerically among the most abundant eukaryotes on Earth's biosphere. Before the introduction of molecular methods for phylogenetic analysis, taxonomists considered fungi to be members of the plant kingdom because of similarities in lifestyle: both fungi and plants are mainly immobile, and have similarities in general morphology and growth habitat. Like plants, fungi often grow in soil and, in the case of mushrooms, form conspicuous fruit bodies, which sometimes resemble plants such as mosses. The fungi are now considered a separate kingdom, distinct from both plants and animals, from which they appear to have diverged around one billion years ago. Some morphological, biochemical, and genetic features are shared with other organisms, while others are unique to the fungi, clearly separating them from the other kingdoms.

Diversity

Fungi have a worldwide distribution, and grow in a wide range of habitats, including extreme environments such as deserts or areas with high salt concentrations or ionizing radiation, as well as in deep sea sediments. Some can survive the intense UV and cosmic radiation encountered during space travel. Most grow in terrestrial environments, though several species live partly or solely in aquatic habitats, such as the chytrid fungus *Batrachochytrium dendrobatidis*, a parasite that has been responsible for a worldwide decline in amphibian populations. This organism spends part of its life cycle as a motile zoospore, enabling it to propel itself through water and enter its amphibian host. Other examples of aquatic fungi include those living in hydrothermal areas of the ocean.

Around 100,000 species of fungi have been formally described by taxonomists, but the global biodiversity of the fungus kingdom is not fully understood. Based on observations of the ratio of the number of fungal species to the number of plant species in selected environments, the fungal kingdom has been estimated to contain about 1.5 million species. Recently, may be over 5 million species in the fungal kingdom.

Ecology

Although often inconspicuous, fungi occur in every environment on Earth and play very important roles in most ecosystems. Along with bacteria, fungi are the major decomposers in most terrestrial (and some aquatic) ecosystems, and therefore play a critical role in biogeochemical cycles and in many food webs. As decomposers, they play an essential role in nutrient cycling, especially as saprotrophs and symbionts, degrading organic matter to inorganic molecules, which can then re-enter anabolic metabolic pathways in plants or other organisms.

A. As pathogens and parasites

Many fungi are parasites on plants, animals (including humans), and other fungi. Serious pathogens of many cultivated plants causing extensive damage and losses to agriculture and forestry include the rice blast fungus *Magnaporthe oryzae*, tree pathogens such as *Ophiostoma ulmi* and *Ophiostoma novo-ulmi* causing Dutch elm disease and *Cryphonectria parasitica* responsible for chestnut blight, and plant pathogens in the genera

Fusarium, *Ustilago*, *Alternaria*, and *Cochliobolus*. Some carnivorous fungi, like *Paecilomyces lilacinus*, are predators of nematodes, which they capture using an array of specialized structures such as constricting rings or adhesive nets.

Some fungi can cause serious diseases in humans, several of which may be fatal if untreated. These include aspergillosis, candidiasis, coccidioidomycosis, cryptococcosis, histoplasmosis, mycetomas, and paracoccidioidomycosis. Furthermore, persons with immuno-deficiencies are particularly susceptible to disease by genera such as *Aspergillus*, *Candida*, *Cryptoccocus*, *Histoplasma*, and *Pneumocystis*. Other fungi can attack eyes, nails, hair, and especially skin, the so-called dermatophytic and keratinophilic fungi, and cause local infections such as ringworm and athlete's foot. Fungal spores are also a cause of allergies, and fungi from different taxonomic groups can evoke allergic reactions.

The plant pathogen *Aecidium magellanicum* causes calafate rust, seen here on a *Berberis* shrub in Chile.

B. As symbiosis

Many fungi have important symbiotic relationships with organisms from most if not all Kingdoms. These interactions can be mutualistic or antagonistic in nature, or in the case of commensal fungi are of no apparent benefit or detriment to the host.

With plants: Mycorrhizal symbiosis between plants and fungi is one of the most well-known plant–fungus associations and is of significant importance for plant growth and persistence in many ecosystems; over 90% of all plant species engage in mycorrhizal relationships with fungi and are dependent upon this relationship for survival. The mycorrhizal symbiosis is ancient, dating to at least 400 million years ago. It often increases the plant's uptake of inorganic compounds, such as nitrate and phosphate from soils having low concentrations of these key plant nutrients. The fungal partners may also mediate plant-to-plant transfer of carbohydrates and other nutrients. Such mycorrhizal communities are called "common mycorrhizal networks". A special case of mycorrhiza is myco-heterotrophy, whereby the plant parasitizes the fungus, obtaining all its nutrients from its fungal symbiont. Some fungal species inhabit the tissues inside roots, stems, and leaves, in which case they are called endophytes. Like mycorrhiza, endophytic colonization by fungi may benefit both symbionts; for example, endophytes of grasses impart to their host increased resistance to herbivores and other environmental stresses and receive food and shelter from the plant in return.

With Algae and cyanobacteria: Lichens are a symbiotic relationship between fungi and photosynthetic algae or cyanobacteria. The photosynthetic partner in the relationship is referred to in lichen terminology as a "photobiont". The fungal part of the relationship is composed mostly of various species of ascomycetes and a few basidiomycetes. Lichens occur in every ecosystem on all continents, play a key role in soil formation and the initiation of biological succession, and are prominent in some extreme environments, including polar, alpine, and semiarid desert regions. They can grow on inhospitable surfaces, including bare soil, rocks, tree bark, wood, shells, barnacles and leaves. As in mycorrhizas, the photobiont provides sugars and other carbohydrates via photosynthesis to the fungus, while the fungus provides minerals and water to the photobiont. The functions of both symbiotic organisms are so closely intertwined that they function almost as a single organism; in most cases the resulting organism differs greatly from the individual components. Lichenization is a common mode of nutrition for fungi; around 20% of fungi between 17,500 and 20,000 described species are lichenized. Characteristics common to most lichens include obtaining organic carbon by photosynthesis, slow growth, small size, long life, long-lasting (seasonal) vegetative reproductive structures, mineral nutrition obtained largely from airborne sources, and greater tolerance of desiccation than most other photosynthetic organisms in the same habitat.

The lichen *Lobaria pulmonaria*, a symbiosis of fungal, algal, and cyanobacterial species

With insects: Many insects also engage in mutualistic relationships with fungi. Several groups of ants cultivate fungi in the order Agaricales as their primary food source, while ambrosia beetles cultivate various species of fungi in the bark of trees that they infest. Likewise, females of several wood wasp species (genus *Sirex*) inject their eggs together with spores of the wood-rotting fungus *Amylostereum areolatum* into the sapwood of pine trees; the growth of the fungus provides ideal nutritional conditions for the development of the wasp larvae. At least one species of stingless bee has a relationship with a fungus in the genus *Monascus*, where the larvae consume and depend on fungus transferred from old to new nests. Termites on the African savannah are also known to cultivate fungi, and yeasts of the genera *Candida* and *Lachancea* inhabit the gut of a wide range of insects, including neuropterans, beetles, and cockroaches; it is not known whether these fungi benefit their hosts. Fungi in growing dead wood are essential for xylophagous insects (e.g. woodboring beetles). They deliver nutrients needed by xylophages to nutritionally scarce dead wood. Thanks to this nutritional enrichment the larvae of woodboring insect is able to grow and develop to adulthood. The larvae of many families of fungicolous flies, particularly those within the superfamily Sciaroidea such as the Mycetophilidae and some Keroplatidae feed on fungal fruiting bodies and sterile mycorrhizae.

DERMATOPHYTES

Is a group of three types of fungus that commonly causes skin disease in animal and humans? These anamorphic (asexual or imperfect fungi) genera are: *Microsporum*, *Epidermophyton* and *Trichophyton*. There are about 40 species in these three genera. Species capable of reproducing sexually belong in the teleomorphic genus Arthroderma. Dermatophytes cause infections of the skin, hair and nails, obtaining nutrients from keratinized material. The organisms colonize the keratin tissues causing inflammation as the host responds to metabolic by-products. Colonies of dematophytes are usually restricted to the nonliving cornified layer of the epidermis because of their inability to penetrate viable tissue of an immunocompetent host. Invasion does elicit a host response ranging from mild to severe. Acid proteinases, elastase, keratinases, and other proteinases reportedly act as virulence factors. The development of cell-mediated immunity correlated with delayed hypersensitivity and an inflammatory response is associated with clinical cure, whereas the lack of or a defective cell-mediated immunity predisposes the host to chronic or recurrent dermatophyte infection.

Some of these skin infections are known as ringworm or tinea. Toenail and fingernail infections are referred to as onychomycosis. Dermatophytes usually do not invade living tissues but colonize the outer layer of the skin. Occasionally the organisms do invade subcutaneous tissues, resulting in kerion development.

Diagnosis and Identification

Rapid in office testing can be done with scraping of the nail, skin, or scalp. Characteristic hyphae can be seen interspersed among the epithelial cells. *Trichophyton tonsurans*, the causative agent of tinea capitis (scalp infection) be solidly packed arthrospores within the broken hair shafts scraped from the plugged black dots of the scalp.

Fungal culture medium is used for positive identification of the species. Usually fungal growth is noted in 5 to 14 days. Microscopic morphology of the micro- and macroconidia is the most reliable identification character, but a good slide preparation is needed, and also needed is the stimulation of sporulation in some strains. Culture characteristics such as surface texture, topography and pigmentation are variable so they are the least reliable criteria for identification. Clinical information such as the appearance of the lesion, site, geographic location, travel history, animal contacts and race is also important, especially in identifying rare non-sporulating species like *Trichophyton concentricum*, *Microsporum audouinii* and *Trichophyton schoenleinii*.

A special agar called Dermatophyte Test Medium (DTM) has been formulated to grow and identify dermatophytes. Without having to look at the colony, the hyphae, or macroconidia - one can identify the dermatophyte by a simple color test. The specimen (scraping from skin, nail, or hair) is embedded in the DTM culture medium. It is incubated at room temperature for 10 to 14 days. If the fungus is a dermatophyte, the medium will turn bright red. If the fungus is not a dermatophyte, no color change will be noted. If kept beyond 14 days, false positive can result even with non-dermatophytes. Specimen from the DTM can be sent for species identification if desired.

Transmission

Dermatophytes are transmitted by direct contact with infected host (human or animal) or by direct or indirect contact with infected exfoliated skin or hair in clothing, combs, hair brushes, theatre seats, caps, furniture, bed linens, shoes, socks, towels, hotel rugs, sauna, bathhouse, and locker room floors. Depending on the species the organism may be viable in the environment for up to 15 months. There is an increased susceptibility to infection when there is a preexisting injury to the skin such as scars, burns, excessive temperature and humidity. Adaptation to growth on humans by most geophilic species resulted in diminished loss of sporulation, sexuality, and other soil-associated characteristics.

Classification

Dermatophytes are classified as anthropophilic (humans), zoophilic (animals) or geophilic (soil) according to their normal habitat.

- Anthropophilic dermatophytes are restricted to human hosts and produce a mild, chronic inflammation.
- Zoophilic organisms are found primarily in animals and cause marked inflammatory reactions in humans who have contact with infected cats, dogs, cattle, horses, birds, or other animals. This is followed by a rapid termination of the infection.
- Geophilic species are usually recovered from the soil but occasionally infect humans and animals. They cause a marked inflammatory reaction, which limits the spread of the infection and may lead to a spontaneous cure but may also leave scars.

Frequency of species

- About 76% of the dermatophyte species isolated from humans are *Trichophyton rubrum*.
- 27% are *Trichophyton mentagrophytes*
- 7% are *Trichophyton verrucosum*

- 3% are *Trichophyton tonsurans*
- Infrequently isolated (less than 1%) are *Epidermophyton floccosum*, *Microsporum audouinii*, *Microsporum canis*, *Microsporum equinum*, *Microsporum nanum*, *Microsporum versicolor*, *Trichophyton equinum*, *Trichophyton kanei*, *Trichophyton raubitschekii*, and *Trichophyton violaceum*.

Trichophyton

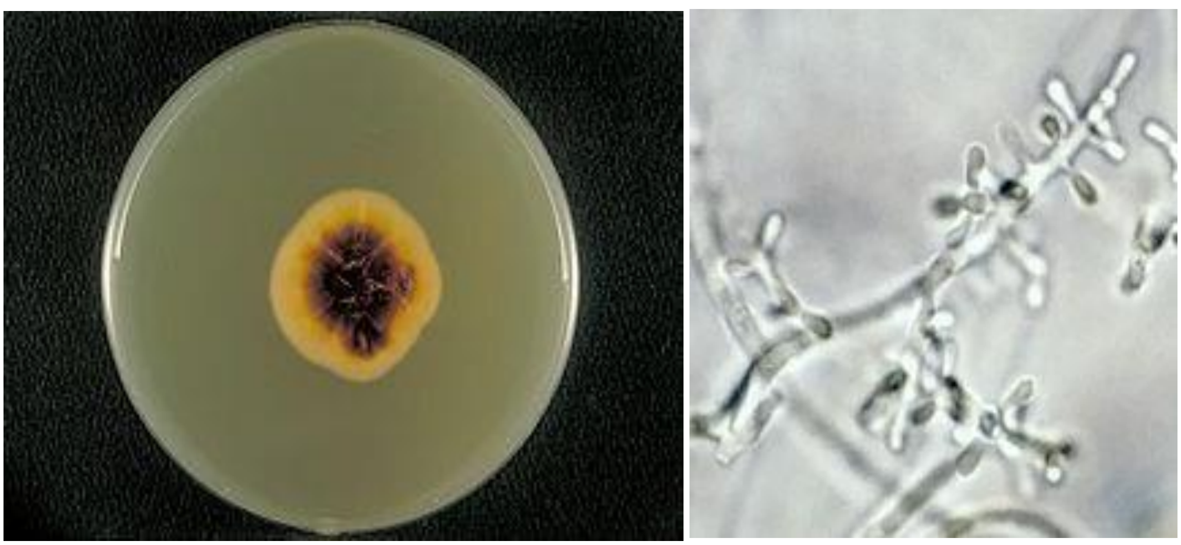
Is a genus of fungi belonging to Ascomycota division, which includes the parasitic varieties that cause tinea, including athlete's foot, ringworm, jock itch, and similar infections of the nail, beard, skin and scalp. *Trichophyton* fungi are molds characterized by the development of both smooth-walled macro- and microconidia. Macroconidia are mostly borne laterally directly on the hyphae or on short pedicels, and are thin - or thick-walled, clavate to fusiform, and range from 4 to 8 by 8 to 50 µm in size. Macroconidia are few or absent in many species. Microconidia are spherical, pyriform to clavate or of irregular shape, and range from 2 to 3 by 2 to 4 µm in size

Trichophyton rubrum

This species was first described by Malmsten in 1845 and is currently considered to be a complex of species that comprises multiple, geographically patterned morphotypes, several of which have been formally described as distinct taxa, including *T. raubitschekii*, *T. gourvilii*, *T. megninii* and *T. soudanense*.

Growth and morphology

Typical isolates of *T. rubrum* are white and cottony on the surface. The colony underside is usually red, although some isolates appear more yellowish and others more brownish. *Trichophyton rubrum* grows slowly in culture with sparse production of teardrop or peg-shaped microconidia laterally on fertile hyphae. Macroconidia, when present, are smooth-walled and narrowly club-shaped, although most isolates lack macroconidia. Growth is inhibited in the presence of certain sulfur, nitrogen and phosphorus-containing compounds. Isolates of *T. rubrum* are known to produce penicillin *in vitro* and *in vivo*.



Bottom view of a Sabouraud agar plate with a colony of *Trichophyton rubrum* var. *rodhainii* and Microconidia of *T. rubrum*.

Variants

- Strains of *T. rubrum* form two distinct biogeographical subpopulations. One is largely restricted to parts of Africa and southern Asia, while the other consists of a population that has spread around the world.

- Isolates of the Afro-Asiatic subpopulation most commonly manifest clinically as tinea corporis and tinea capitis.
- In contrast, the globally-distributed subpopulation manifests predominantly in tinea pedis and tinea unguium.
- Different members of the *T. rubrum* complex are endemic to different regions; isolates previously referred to *T. megninii* originate from Portugal, while *T. soudanense* and *T. gourvilii* are found in Sub-Saharan Africa.
- *Trichophyton raubitschekii*, which is common from northwestern India and southeast Asia as well as parts of West Africa, is characterized by strongly granular colonies and is the only variant in the complex that reliably produces urease.

Microsporum

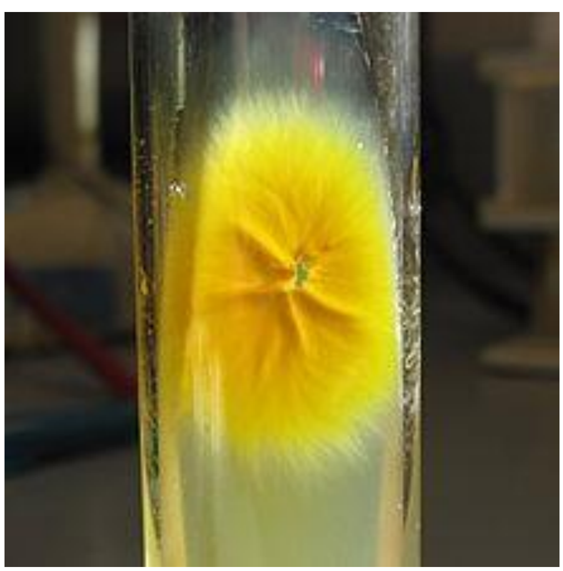
Microsporum is a genus of fungi that causes tinea capitis, tinea corporis, ringworm, and other dermatophytoses (fungal infections of the skin). *Microsporum* forms both macroconidia (large asexual reproductive structures) and microconidia (smaller asexual reproductive structures) on short conidiophores. Macroconidia are hyaline, multiseptated, variable in form, fusiform, spindle-shaped to obovate, 7-20 by 30-160 um in size, with thin or thick echinulate to verrucose cell walls. Their shape, size and cell wall features are important characteristics for species identification. Microconidia are hyaline, single-celled, pyriform to clavate, smooth-walled, 2.5-3.5 by 4-7 um in size and are not diagnostic for any one species. The separation of this genus from *Trichophyton* is essentially based on the roughness of the macroconidia cell wall, although in practice this may sometimes be difficult to observe. Seventeen species of *Microsporum* have been described; however, only the more common species are included in these descriptions.

Microsporum canis

Microsporum canis is a pathogenic, asexual fungus in the phylum Ascomycota that infects the upper, dead layers of skin on domesticated cats, and occasionally dogs and humans. The species has a worldwide distribution.

Colony morphology

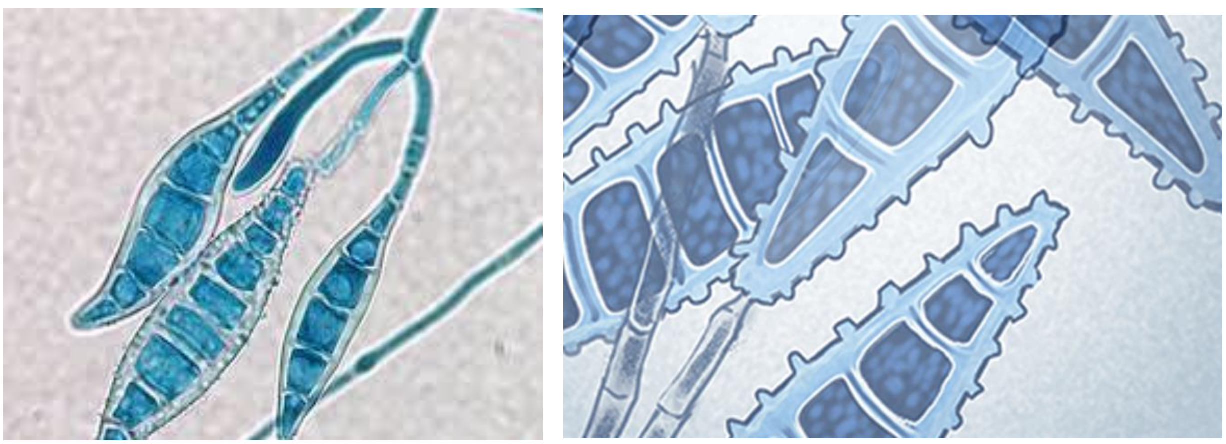
Microsporum canis forms a white, coarsely fluffy spreading colony with a distinctive "hairy" or "feathery" texture. On the underside of the growth medium, a characteristic deep yellow pigment develops due to the metabolites secreted by the fungus. The intensity of this yellow pigmentation peak on the 6th day of colony growth and fades gradually making the identification of older colonies difficult. Some strains of *M. canis* fail to produce yellow pigment altogether, exhibit abnormally slow colony growth and form undeveloped macroconidia. Cultivation on polished rice tends to reestablish the typical growth morphology and is helpful for identification.



Characteristic yellow, feathery colony of *Microsporum canis* photographed on a slope of Modified Leonian's agar after 7 days growth.

Microscopic morphology

Microsporum canis reproduces asexually by forming macroconidia that are asymmetrical, spherically shaped and have cell walls that are thick and coarsely roughened. The interior portion of each macroconidium is typically divided into six or more compartments separated by broad cross-walls. *Microsporum canis* also produces microconidia that resemble those of many other dermatophytes and thus are not a useful diagnostic feature



The conidia of *Microsporum canis*

Epidermophyton

Epidermophyton is a genus of fungus causing superficial and cutaneous mycoses, including *E. floccosum*, and causes tinea corporis (ringworm), tinea cruris (jock itch), tinea pedis (athlete’s foot), and tinea unguium (fungal infection of the nail bed).

Epidermophyton floccosum

Epidermophyton floccosum is an anthropophilic dermatophyte (preferring humans to other hosts) which can be found worldwide.

Description

The fungus is a moderate grower that reaches maturity after 10 days. The colonies are usually grainy, have a suede-like texture, and may be olive, yellow, or yellow-brown in color. The central region is raised slightly. Fluffy white sterile mycelia cover the colonies after several weeks. *Epidermophyton floccosum* contains an unusual lipid of unknown function, 1(3), 2-diacylglyceryl-3(1)-O-4′- (N, N, N-trimethyl) homoserine. Microconidia are usually absent.

Colony and macroconidia of *Epidermophyton floccosum*

Pathology

The fungus is the only pathogen of the two species comprising genus *Epidermophyton*. Hosts of the fungi are humans, wild animals, and domestic animals. The fungi can cause tinea pedis, tinea cruris, tinea corporis, and onychomycosis. The infection spreads by contact, especially in gyms and showers. The infection can be stopped by bathing with soap and water and applying an appropriate fungicide. A study of 900 patients afflicted with *E. floccosum* infection investigating the contagious aspects of the fungus was conducted in Korea, from 1976 to 1997. The study found that fewer people were infected by *E. floccosum* than by other dermatophytes. The fungus may be transmitted between humans and squirrels. The fungi can usually only infect the nonliving cornified layers of epidermis. An invasive infection has, however, been recorded in an immunocompromised patient with Behçet's syndrome.

Candidiasis

Candidiasis is a fungal infection due to any type of *Candida* (a type of yeast). When it affects the mouth, it is commonly called **thrush**. Signs and symptoms include white patches on the tongue or other areas of the mouth and throat. Other symptoms may include soreness and problems swallowing.

When it affects the vagina, it is commonly called a **yeast infection**. Signs and symptoms include genital itching, burning, and sometimes a white "cottage cheese-like" discharge from the vagina. Less commonly the penis may be affected, resulting in itchiness. Very rarely, the infection may become invasive, spreading to other parts of the body. This may result in fevers along with other symptoms depending on the parts involved.

More than 20 types of *Candida* can cause infection with *Candida albicans* being the most common. Infections of the mouth are most common among children less than one month old, the elderly, and those with weak immune systems. Conditions that result in a weak immune system include HIV/AIDS, the medications used after organ transplantation, diabetes, and the use of corticosteroids. Other risks include dentures and following antibiotic therapy. Vaginal infections occur more commonly during pregnancy, in those with weak immune systems, and following antibiotic use. Risk factors for invasive candidiasis include being in an intensive care unit, following surgery, low birth weight infants, and those with weak immune systems.

Causes

Candida yeasts are generally present in healthy humans, frequently part of the human body's normal oral and intestinal flora, and particularly on the skin;

however, their growth is normally limited by the human immune system and by competition of other microorganisms, such as bacteria occupying the same locations in the human body.

Candida requires moisture for growth, notably on the skin. For example, wearing wet swimwear for long periods of time is believed to be a risk factor. In extreme cases, superficial infections of the skin or mucous membranes may enter the bloodstream and cause systemic *Candida* infections.

Factors that increase the risk of candidiasis include: -

1- HIV/AIDS and cancer treatments.
2- Steroids and stress.
3- Antibiotic usage and diabetes.
4- Nutrient deficiency.

Hormone replacement therapy and infertility treatments may also be predisposing factors. Treatment with antibiotics can lead to eliminating the yeast's natural competitors for resources in the oral and intestinal flora; thereby increasing the severity of the condition. A weakened or undeveloped immune system or metabolic illnesses are significant predisposing factors of candidiasis. Almost 15% of people with weakened immune systems develop a systemic illness caused by *Candida* species. Diets high in simple carbohydrates have been found to affect rates of oral candidiases.

C. albicans was isolated from the vaginas of 19% of apparently healthy women, i.e., those who experienced few or no symptoms of infection. External use of detergents or douches or internal disturbances (hormonal or physiological) can perturb the normal vaginal flora, consisting of lactic acid bacteria, such as lactobacilli, and result in an overgrowth of *Candida* cells, causing symptoms of infection, such as local inflammation. Pregnancy and the use of oral contraceptives have been reported as risk factors. Diabetes mellitus and the use of antibiotics are also linked to increased rates of yeast infections.

In penile candidiasis, the causes include sexual intercourse with an infected individual, low immunity, antibiotics, and diabetes. Male genital yeast infections are less common, but a yeast infection on the penis caused from direct contact via sexual intercourse with an infected partner is not uncommon

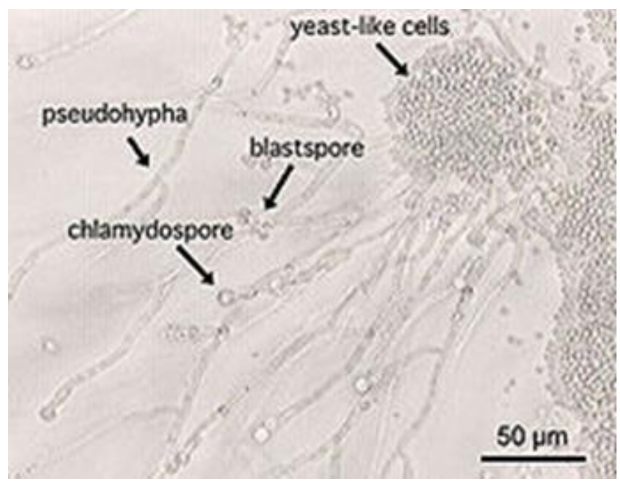
Candida albicans

Candida albicans commonly referred to as a dimorphic fungus since It grows both as yeast and filamentous cells. However, it has several different morphological phenotypes. It is a common member of human gut flora. It is detectable in the gastrointestinal tract and mouth in 40-60% of healthy adults. It is usually a commensal organism, but can become pathogenic in immunocompromised individuals under a variety of conditions. It is one of the few species of the *Candida* genus that cause the infection candidiasis in humans. Overgrowth of the fungus results in candidiasis (candidosis). Candidiasis is for example often observed in HIV-infected patients. *C. albicans* is responsible for 50-90% of all cases of candidiasis in humans. Together with *C. tropicalis*, *C. parapsilosis* and *C. glabrata* it is responsible for approximately 90% of *Candida* infections.

Morphology

Although often referred to as **dimorphic**, *C. albicans* is in fact polyphenic (often also referred to as pleomorphic). When cultured in standard yeast laboratory medium, *C. albicans* grows as ovoid "yeast" cells. However, mild environmental changes in temperature, CO_2, nutrients and pH can result in a morphological shift to filamentous growth. Filamentous cells share many similarities with yeast cells. Both cell types seem to play a specific, distinctive role in the survival and pathogenicity of *C. albicans*. Yeast cells seem to be better suited for the dissemination in the bloodstream while hyphal cells have been proposed as a virulence factor. Hyphal cells are invasive and speculated to be important for tissue penetration, colonization of organs and surviving plus escaping macrophages. The transition from yeast to hyphal cells is termed to be one of the key factors in the virulence of *C. albicans*, however it is not deemed necessary. When *C. albicans* cells are grown in a medium that mimics the physiological environment of a human host, they grow as filamentous cells (both true hyphae and pseudohyphae). *Candida albicans* can also form Chlamydospores, the function of which remains unknown, but it is speculated they play a role in surviving harsh environments as they are most often formed under unfavorable conditions.

Candida albicans growing on Sabouraud agar



Different morphological forms of *Candida albicans*

Methods of transmission include: -

1- Mother to infant through childbirth.
2- People to people acquired infections that most commonly occur in hospital settings where immunocompromised patients acquire the yeast from healthcare workers and has a 40% incident rate.
3- Men can become infected after having sex with a woman that has an existing vaginal yeast infection.

Parts of the body that are commonly infected include the skin, genitals, throat, mouth, and blood. Some distinguishing features also include, discharge, dry and, red appearance of vaginal mucosa or skin. *Candida* continues to be the fourth most commonly isolated organism in bloodstream infections.

Superficial and local infections

It commonly occurs, as a superficial infection, on mucous membranes in the mouth or vagina. Once in their life around 75% of women will suffer from vulvovaginal candidiasis (VVC) and about 90% of these infections are caused by *C. albicans*. It however may also affect a number of other regions. For example, higher prevalence of colonization of *C. albicans* was reported in young individuals with tongue piercing, in comparison to unpierced matched individuals. To infect host tissue, the usual unicellular yeast-like form of *C. albicans* reacts to environmental cues and switches into an invasive, multicellular filamentous form, a phenomenon called dimorphism. In addition, an overgrowth infection is considered superinfection, usually applied when an infection become opportunistic and very resistant to antifungals. It then becomes suppressed by antibiotics. The infection is prolonged when the original sensitive strain is replaced by the antibiotic-resistant strain.

Systemic infections

Systemic fungal infections (fungemias) including those by *C. albicans* have emerged as important causes of morbidity and mortality in immunocompromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation). Especially once candida cells are introduced in the bloodstream a high mortality can occur. *C. albicans* biofilms may form on the surface of implantable medical devices. In addition, hospital-acquired infections by *C. albicans* have become a cause of major health concerns.

Although *Candida albicans* is the most common cause of candidemia, there has been a decrease in the incidence and an increases isolation of non-albicans species of *Candida* in recent years. Preventive measures include keeping a healthy lifestyle including good nutrition, proper nutrition, and careful antibiotic use.

Candida glabrata

Known as "*Turolopsis glabrata*, the second most virulent yeast after *Candida albicans*, *Candida glabrata* is haploid yeast of the genus *Candida*. This species of yeast is non-dimorphic. Until recently, *C. glabrata* was notion to be a primarily non-pathogenic organism. However, with the ever-increasing population of immunocompromised individuals, trends have shown *C. glabrata* to be a highly opportunistic pathogen of the urogenital tract, and of the bloodstream (*Candidemia*). It is especially predominant in HIV positive people, and the elderly.

Candida glabrata is non-dimorphic yeast that occurs as small blastoconidia under all environmental conditions as a pathogen. In fact, *C. glabrata* is the only *Candida* species that does not form pseudohyphae at temperatures above 37 °C. *Candida glabrata* forms glistening, smooth, cream-colored colonies which are relatively indiscernible from those of other *Candida* species except for their relative size, which is quite small.

A critical differentiate characteristic of *C. glabrata* is its haploid genome, in contrast to the diploid genome of *C. albicans* and several other non-*albicans Candida* species. *Candida glabrata* ferments and assimilates only glucose and trehalose.

Candida tropicalis

Candida tropicalis is a species of yeast in the genus *Candida*. It is easily known as a common medical yeast pathogen, occurring as part of the normal human flora.

In tropical countries, *C. tropicalis* is one of the most common colonizer and pathogen causing human disease, especially found on human skin, in the gastrointestinal tract and also in female genitourinary tract. It can be transmitted between health-care workers and patients, especially in environments such as hospitals. *C. tropicalis* can survive for up to 24 hours therefore be cross-transmitted to a second hand with a probability of 69% and to a third hand with 38 % probability. It is the cause responsible for approximately half of the beyond-surface candida infections. *C. tropicalis* is the second most virulent *Candida* species that can significantly affect by spreading through the weakened immune system host and can occupy the gastrointestinal tract within 30 minutes of inoculation, all this resulting in increased mortality. Impact of candidiasis, infections cause by *C. tropicalis*, have increased globally. *C. tropicalis* is virulent due to its ability to produce biofilm, secrete lytic enzymes, adhere to epithelial and endothelial cells, and undergo transition of bud to hyphae.

Pichia Kudriavzevii

Pichia kudriavzevii is anamorph *Candida krusei*. This yeast was prime described by Kudryavtsev in 1960 as *Issatchenkia orientalis* but was classified as *P. orientalis* in 1964 and to *P. kudriavzevii* in 1965. *Pichia kudriavzevii* is very abundant yeast in the environment and can be found in soil, fruits and various fermented beverages. So far, *P. kudriavzevii* is mainly connected with food spoilage to cause surface biofilms in low pH products.

Pichia kudriavzevii (*Candida krusei*) is a species of clinical importance. In fact, *Candida krusei* is the 5th most popular cause of candidemia. A kind of fungi infection which can affect immunocompromised patients (such as AIDS patients).

Aspergillus fumigatus

Is one of the most common *Aspergillus* species to cause disease in individuals with an immunodeficiency. *A. fumigatus*, an opportunistic fungus and widespread in nature, is typically found in soil and decaying organic matter, such as compost heaps, where it plays an essential role in carbon and nitrogen recycling. Colonies of the fungus produce from conidiophores thousands of minute grey-green conidia (2-3 μm) that readily become airborne. For many years, *A. fumigatus* was thought to only reproduce asexually, as neither mating nor meiosis had ever been observed. In 2008, however, *A. fumigatus* was shown to possess a fully functional sexual reproductive cycle, 145 years after its original description by Fresenius. Although *A. fumigatus* occurs in areas with widely different climates and environments, it displays low genetic variation and lack of population genetic differentiation on a global scale. Thus, the capability for sex is maintained even though little genetic variation is produced.

The fungus is capable of growth at 37 °C or 99 °F (normal human body temperature), and can grow at temperatures up to 50 °C or 122 °F, with conidia surviving at 70 °C or 158 °F conditions it regularly encounters in self-heating compost heaps. Its spores are ubiquitous in the atmosphere, and it is estimated that everybody inhales several hundred spores each day; typically, these are quickly eliminated by the immune system in healthy individuals. In immunocompromised individuals, such as organ transplant recipients and people with AIDS or leukemia, the fungus is more likely to become pathogenic, over-running the host's weakened defenses and causing a range of diseases generally termed aspergillosis.

The shape of colony and conidiophore of *A. fumigatus*

Pathogenesis

Aspergillus fumigatus is the most frequent cause of invasive fungal infection in immunosuppressed individuals, which include patients receiving immunosuppressive therapy for autoimmune or neoplastic disease, organ transplant recipients, and AIDS patients. *A. fumigatus* primarily causes invasive infection in the lung and represents a major cause of morbidity and mortality in these individuals. Additionally, *A. fumigatus* can cause chronic pulmonary infections, allergic bronchopulmonary aspergillosis, or allergic disease in immunocompetent hosts

Proteinase

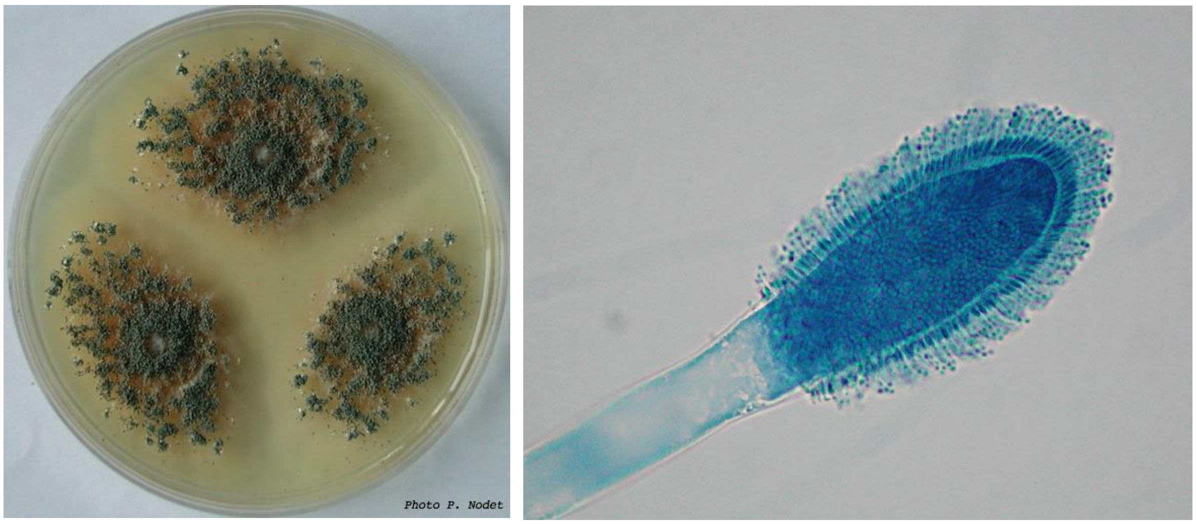
The human lung contains large quantities of collagen and elastin, proteins that allow for tissue flexibility. *Aspergillus fumigatus* produces and secretes elastases, proteases that cleave elastin to break down these macromolecular polymers for uptake. A significant correlation between the amount of elastase production and tissue invasion was first discovered in 1984. Clinical isolates have also been found to have greater elastase activity than environmental strains of *A. fumigatus*. A number of elastases have been characterized, including those from the serine protease, aspartic protease, and metalloprotease families. Yet, the large redundancy of these elastases has hindered the identification of specific effects on virulence.

Aspergillus clavatus

is a species of *Aspergillus* and is characterized by elongated club-shaped vesicles, and blue-green uniseriate conidia. The fungus was first described scientifically in 1834 by the French mycologist John Baptiste. It belongs to the *Aspergillus* section *Clavati*, alongside two species, *Aspergillus clavatus* and *Aspergillus giganteus*. In the succeeding years, four more species were discovered belonging to the *Aspergillus section Clavati*, which included *Aspergillus rhizopodus*, *Aspergillus longivesica*, *Neocarpenteles acanthosporus* and *Aspergillus clavatonanicus*.

Growth and morphology

A.clavatus undergoes rapid growth, resulting in the formation of a velvety and fairly dense felt that is observed to be bluish-grey green in colour. The emerging conidial heads are large and clavate when very young. The conidia bearing conidiophores are generally coarse, smooth walled, uncoloured, hyaline and can grow to be very long. The sterigmata are usually found to be uniseriate, numerous and crowded. Conidia formed in them are elliptical, smooth and comparatively thick-walled. *A. clavatus* usually express conidiophores 1.5-3.00 mm in length, which arises from specialized and widened hyphal cells that eventually become the branching foot cells. The conidia on *A. clavatus* has been measured up to 3.0 - 4.5 X 2.5 - 3.5 µm.



The shape of colony and conidiophore of *A. clavatus*

Pathogenicity

Aspergillus clavatus: -

1- known as agent of allergic aspergillosis and has been implicated in multiple pulmonary infections. It has also been labelled as an opportunistic fungus, as it is responsible for causing aspergillosis in compromised patients. In Scotland and elsewhere, *A. clavatus* is reported for causing the mould allergy malster's lung.

2- Cause neurotoxicosis in sheep and otomycosis.

Medical uses

Weisner (1942) first noted the production of an antibiotic by strains of *A. clavatus*, and the active substance was known as *clavatin.* Later the antibiotic was named *clavacin* in 1942. Clavacin is also known as patulin. Patulin is receiving significant attention in the world today because of its manifestations in apple juices. Clavacin was noted to be valuable in the treatment of common-cold and applies a fungistatic or fungicidal effect on certain dermatophytes

Alternaria

Alternaria is a genus of ascomycete fungi. *Alternaria* species are known as major plant pathogens. They are also common allergens in humans, growing indoors and causing hay fever or hypersensitivity reactions that sometimes lead to asthma. They readily cause opportunistic infections in immunocompromised people such as AIDS patients.

There are 299 species in the genus; they are ubiquitous in the environment and are a natural part of fungal flora almost everywhere. They are normal agents of decay and decomposition. The spores are airborne and found in the soil and water, as well as indoors and on objects. The club-shaped spores are single or form long chains. They can grow thick colonies which are usually green, black, or gray.

At least 20% of agricultural spoilage is caused by *Alternaria* species; most severe losses may reach up to 80% of yield, though. Many human health disorders can be caused by these fungi, which grow on skin and mucous membranes, including on the eyeballs and within the respiratory tract. Allergies are common, but serious infections are rare, except in people with compromised immune systems. However, species of this fungal genus are often prolific producers of a variety of toxic compounds. The effects most of these compounds have on animal and plant health are not well known. Many species of *Alternaria* modify their secondary metabolites by sulfoconjugation, however the role of this process is not yet understood. The terms *alternariosis* and *alternariatoxicosis* are used for disorders in humans and animals caused by a fungus in this genus.

Not all *Alternaria* species are pests and pathogens; some have shown promise as biocontrol agents against invasive plant species. Some species have also been reported as endophytic microorganisms with highly bioactive metabolites. *Alternaria* are cosmopolitan, predominately isolated from plants, either as pathogens or as saprobes, and from soil.

The shape of colony and conidia of *Alternaria*

Alternaria solani

Is a fungal pathogen, that produces a disease in tomato and potato plants called **early blight**. The pathogen produces distinctive "bullseye" patterned leaf spots and can also cause stem lesions and fruit rot on tomato and tuber blight on potato. Despite the name "early," foliar symptoms usually occur on older leaves. If uncontrolled, early blight can cause significant yield reductions. Primary methods of controlling this disease include preventing long periods of wetness on leaf surfaces and applying fungicides.

Geographically, *A. solani* is problematic in tomato production areas east of the Rocky Mountains and is generally not an issue in the less humid Pacific or inter-mountain regions. *A. solani* is also present in most potato production regions every year but has a significant effect on yield only when frequent wetting of foliage favors symptom development.

Hosts and symptoms

Alternaria solani infects stems, leaves and fruits of tomato (*Solanum lycopersicum* L.), potato (*S. tuberosum*), eggplant (*S. melongena* L.), bell pepper and hot pepper (*Capsicum* spp.), and other members of the *Solanum* family. Distinguishing symptoms of *A. solani* include leaf spot and defoliation, which are most pronounced in the lower canopy. In some cases, *A. solani* may also cause damping off.

On tomatoes

On tomato, foliar symptoms of *A. solani* generally occur on the oldest leaves and start as small lesions that are brown to black in color. These leaf spots resemble concentric rings a distinguishing characteristic of the pathogen and measure up to 1.3 cm (0.51 inches) in diameter. Both the area around the leaf spot and the entire leaf may become yellow or chlorotic. Under favorable conditions (e.g., warm weather with short or abundant dews), significant defoliation of lower leaves may occur, leading to sunscald of the fruit. As the disease progresses, symptoms may migrate to the plant stem and fruit. Stem lesions are dark, slightly sunken and concentric in shape. Basal girdling and death of seedlings may occur, a symptom known as collar rot. In fruit, *A. solani* invades at the point of attachment to the stem as well as through growth cracks and wounds made by insects, infecting large areas of the fruit. Fruit spots are similar in appearance to those on leaves brown with dark concentric circles. Mature lesions are typically covered by a black, velvety mass of fungal spores that may be visible under proper light conditions.

On potatoes

In potato, primary damage by *A. solani* is attributed to premature defoliation of potato plants, which results in tuber yield reduction. Initial infection occurs on older leaves, with concentric dark brown spots developing mainly in the leaf center. The disease progresses during the period of potato vegetation, and infected leaves turn yellow and either dry out or fall off the stem. On stems, spots are gaunt with no clear contours (as compared to leaf spots). Tuber lesions are dry, dark and pressed into the tuber surface, with the underlying flesh turning dry, leathery and brown. During storage, tuber lesions may enlarge, and tubers may become shriveled. Disease severity due to *A. solani* is highest when potato plants are injured, under stress or lack proper nutrition. High levels of nitrogen, moderate potassium and low phosphorus in the soil can reduce susceptibility of infection by the pathogen.

Leaf lesion of *Alternaria solani*

Life cycle

Life cycle starts with the fungus overwintering in crop residues or wild members of the Solanaceae family, such as black nightshade. In the spring, conidia are produced. Multicellular conidia are splashed by water or by wind onto an uninfected plant. The conidia infect the plant by entering through small wounds, stomata, or direct penetration. Infections usually start on older leaves close to the ground. The fungus takes time to grow and eventually forms a lesion. From this lesion, more conidia are created and released. These conidia infect other plants or other parts of the same plant within the same growing season. Every part of the plant can be infected and form lesions. This is especially important when fruit or tubers are infected as they can be used to spread the disease.

In general, development of the pathogen can be aggravated by an increase in inoculum from alternative hosts such as weeds or other solanaceous species. Disease severity and prevalence are highest when plants are mature.

Environment

Alternaria solani spores are universally present in fields where host plants have been grown. Free water is required for *Alternaria* spores to germinate; spores will be unable to infect a perfectly dry leaf. *Alternaria* spores germinate within 2 hours over a wide range of temperatures but at 26.6-29.4°C (80-85°F) may only take 1/2 hour. Another 3 to 12 hours are required for the fungus to penetrate the plant depending on temperature. After penetration, lesions may form within 2–3 days or the infection can remain dormant awaiting proper conditions [15.5°C (60°F) and extended periods of wetness]. *Alternaria* sporulates best at about 26.6°C (80°F) when abundant moisture (as provided by rain, mist, fog, dew, irrigation) is present. Infections are most prevalent on poorly nourished or otherwise stressed plants.

Types of Alternaria toxins

Most species also produce phytotoxins (plant toxins) which act in different sites causing cell death in plant tissues. Based on their effect on plants, these toxins are divided into non-host specific toxins and host specific toxins.

1- Generally, **non-host specific toxins** such as tenuazonic acid, tentoxin and zinniol, affect a broad range of plant species and are considered as virulence factors rather than key pathogenicity determinants.

   Some non-host-specific toxins such as alternariol, alternariol monomethyl ether, tenuazonic acid and altertoxins have been also described to induce potentially harmful effects in mammals.

2- On the other hand, **host-specific toxins** such as AAL-toxins have a limited host range and play a critical role in plant pathogenicity.

ENDOPHYTIC FUNGI

Endophytic fungi include all organisms that grow inside plant tissues without causing any immediate, negative effects and are not mycorrhizal. Endophytic fungi may also include a wide range of fungi characterized by the ability of living for a certain period of their life-cycle internally and asymptomatically in plants. They are believed to have evolved from pathogenic fungi that either have extended latent periods or have lost their virulence.

Endophytic fungi have attracted great attention in the past few decades for two reasons: **First,** growing evidence indicates that endophytes are found in all plants, are extremely abundant and are often very diverse. Most of these endophytes form internal localized infection in foliage, roots, stem, and bark and are horizontally transmitted via spores. A much smaller fraction, mostly found in poor grasses, form systemic infection in above-ground tissues. Some of these are vertically transmitted via hyphae growing into seeds. **Second,** endophytes may produce mycotoxins, or otherwise alter host physiology and morphology. Endophytic mycotoxins are thought to benefit their woody plant host as "inducible defenses" against insect herbivores and their grass host as "acquired plant defense" against both vertebrate and invertebrate herbivores. Endophytic may also alter other physiological, developmental or morphological properties of host plants such that competitive abilities are enhanced, especially in stressful environments.

Types of Endophytic Fungi

Fungal endophytes can be divided into two groups according to (Wang *et al*., 2007).

1) The balansiaceous group: this group specifically colonize grasses and usually belong to the Ascomycetous genera *Epicholä* and *Balansia* (anamorphs *Neotyphodium* and *Ephelis*).
2) The non-balansiaceous group: this group occur in almost all plant species and usually belong to the Ascomycota.

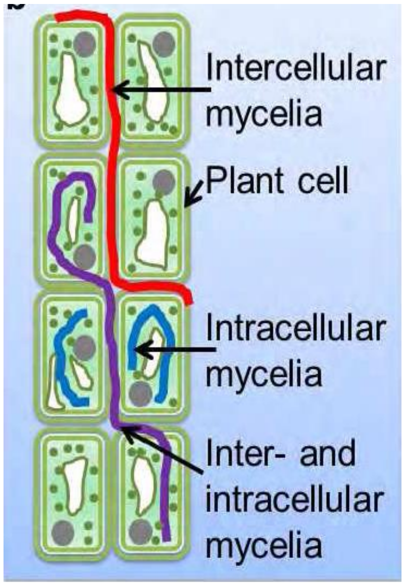


Figure. A diagrammatic representation of different localization patterns of fungal endophytes within plant tissues.

Diversity of Fungal Endophytes

Fungal endophytes are generally from the phylum Ascomycota including *Colletotrichum*, *Curvularia*, *Fusarium*, *Mycosphaerella* and *Neotyphodium*. Also, other phyla are represented. Some specific examples of which are found in orders Hypocreales and Xylariales of the Sordariomycetes (Pyrenomycetes) class. Additionally, the class of Loculoascomycetes includes endophytes. Although endophytes may be diverse taxonomically, broader ecological categories or functional classes have been proposed.

Distribution and Occurrence of Endophytic Fungi

Fungal surveys of various host during the past 20 years have demonstrated that endophytic colonization of land plants by fungi is ubiquitous. Endophytes are known from plants growing in tropical, temperate and boreal forests; from herbaceous plants, from various habitats, including extreme arctic, alpine and xeric environments and from mesic temperate and tropical forests. Endophytic fungi have been isolated from phanerogams in alpine, temperate and tropical regions, although the plants of the Coniferae, Ericaceae and Gramineae have been most intensively sampled.

Members of the Ascomycotina, Basidiomycotina, Deutromycotina, and Oomycetes have been isolated as endophytes, but the most fungal endophytes belong to Ascomycetes and fungi imperfecti.

Most of the researches on endophytes have been done in the northern hemisphere. However, results have shown that tropical plants present greater diversity of endophytes species than those from temperate zones. Partly because of increasing fungal knowledge by local mycologists and the higher number of plant species compared to temperate regions.

It is believed that the environment has an important role on endophyte biodiversity and species diversity is dependent upon the nature of the host plant and their ecological location. For example, endophytic fungi in woody plants are highly abundant and diverse, particulary in the tropical areas. Today endophytes have been isolated from all groups of plants range from large trees, palms and even from lichens.

The numbers of strains and species of endophytes vary considerably and generally depend on the intensity of the study. Few scientists conclude that,

a) The numbers of endophytes [strains and species] depends on how much care, time and Petri-dishes are used in a study; a meticulous researcher will laboriously isolate thousands of strains and consequently more species; a lackadaisical researcher will achieve the opposite.
b) Temperate plants yield different communities of endophytes as those from tropical plants.
c) Different tissues may yield diverse endophyte communities.

Mutualistic Relationship

The major features of mutualistic symbiosis include the lack of destruction of most cells or tissues, nutrient or chemical cycling between the fungus host, enhanced longevity and photosynthetic capacity of cells and tissues under the influence of infection, enhanced survival of the fungus, and a tendency toward greater host specificity than seen in nectrophic infection.

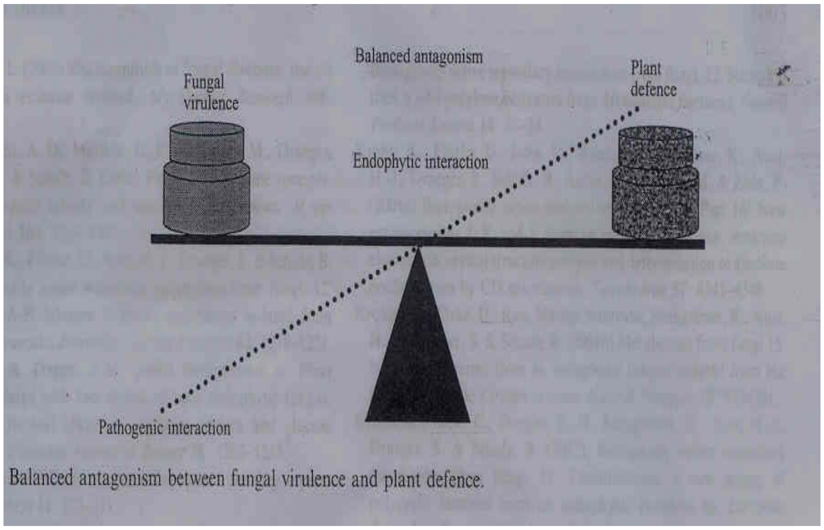


Fig: Balanced antagonism between fungal virulence and plant defence.

The Importance of Endophytic Fungi

1- Medicinal uses

Some species of endophytic fungi have been identified as sources of anticancer, antidiabetic, insecticidal and immunosuppr-essive compounds. There are many reports demonstrating that many bioactive compounds could be produced by endophytic microorganisms. Strains of the endophytic *Pezicula* species (and its anamorph *Cryptosporiopsis*) from several deciduous and coniferous tree hosts produce an ensemble of bioactive secondary metabolites in culture. Endophytic species of the Xylariaceae frequently produce compounds with high biological activity, including cytochalasins and indole diterpenes, although diverse endophytes produce toxins in culture, such compounds have been difficult to detect in plant host tissue.

Notgrass endophytes produce antifungal or antibacterial substances, as well as insecticidal compounds, *in vitro*. We do not know, however, whether these metabolites are produced

1) In plants during the periods of quiescent occupation of host tissue by endophytes or
2) In sufficient concentrations to benefit the host in a protective mutualism (e.g., by deterring insect herbivory).

Several reviews have discussed the products of endophytic microbes are promise for use in medicine, agriculture and industry.

2- Other uses

These endophytic microorganisms are ubiquitous, and many increase the plant fitness by improving tolerance to heavy metals and drought, reducing the herbivory or phytopathogen settling. The endophytic microorganisms are not considered as saprophytes since they are associated with living tissues and may in some way contribute to the well being of the plant. That is, the plant is thought to provide nutrients to the microbe, while the microbe may produce factors that protect the host plant from attack by animals, insects or microbes. There are many reports demonstrating that many bioactive compounds could be produce by endophytic microorganisms.

Many of these fungal endophytes (Ascomycota; Clavicipitaceae) form symbiotic relationships with cool-season grasses. Systemic fungal endophytes of the genus *Neotyphodium* remain asexual for long periods of time, cause no harm to the plant host and propagate vertically through the seeds of the host plant. *Neotyphodium* endophytes produce mycotoxins, notably alkaloides, by which increased resistance to herbivores, microbial pathogens and pathogenic nematodes is provided to the host plant in exchange for nutrients, shelter and safe transmission via the plant's seeds. Symbiotic fungal endophytes may thus take a key role for the regulation of herbivore populations.

Endophytes may survive in the plant as a symbiont by providing protective substances to the plant that may inhibit or kill tissue invading pathogens.

Endophyte Reproduction

Endophyte reproduction is related to the effect of the endophyte on its host's reproductive system. Two main modes of reproduction are recognized.

1) Reproduction via vegetative growth; this group of endophytic fungi grows completely inside the plant tissues and never produces the external structure or fruiting bodies on host plant. The reproduction of this group of

endophytes is completely internal. Their reproduction occurs through vegetative growth of hyphae into the developing ovules of the host which cause infection of seeds, and these are transmitted via seeds. The viability of hyphe is less than the viability of seeds.

2) Reproduction via spores; this group of fungi produces the stromatic tissue around the developing inflorescence. This inflorescence is deformed by the infecting endophytes. Mycologist has given the name of this symptom 'choke'. The conidia are formed on the stromatic tissues and it is reported that the rate of external manifestation of infection depends upon the host.

The differences between fungal endophytes and pathogenic fungi

Penetration, colonization and growth were monitored microscopically, revealing differences between endophytes and pathogens. Endophytic penetrated plant tissues through natural cracks, wounds, lenticels, the stomata and along the anticlinal epidermal cells due to air current or rain water flowing down, or through the agency of insects, beetles, mites and other animals which live and bread in the plants and trees; the pathogen, in contrast, was also able to penetrate directly through the cell wall. Colonization was limited, localized and intercellular, that of the pathogen also intracellular.

Summarizing the nature of the interaction of fungal endophytes with plant hosts, it can be said that:

1) Fungal endophytes produced the enzymes necessary to penetrate and colonize their hosts, colonizing the above ground organs locally and intercellularly, the root in contrast extensively, systemically, and inter and intracellularly.
2) They grew well using only the apoplastic washing fluid as a growth medium.

3) The association of fungal endophytes and plant host may be mutualistic.
4) Pathogenic infection resulted in lower concentrations of phenolic defense metabolites than endophytic colonization did.
5) Some biologically active fungal metabolites inhibit photosynthesis of the host plant.

Balanced Antagonism Between Fungal Virulence and Plant Defence

The fungal endophyte-plant host interaction is characterized by a finely defence. If this balance is disturbed by either a decrease in plant defence or an increase in fungal virulence, disease develops. Not only must the endophyte synthesize metabolites to compete first with epiphytes and then with pathogens to colonize the host, but presumably also to regulate metabolism of the host in their delicately balanced association.

Transmission Mode

1) Horizontal transmission: transmission of the fungus by sexual or asexual spore.
2) Vertical transmission: transmission of the systemic fungus from plant to plant via host seeds.

Vertical transmission in systematic endophytes appears to reduce concomitantly the ability of the fungus to infect new uninfected hosts horizontally by either asexual or sexual spores. In its extreme form, for example, *Neotyphodium* endophytes in grasses, the fungus has entirely lost its ability for contagious spread. The fungus has presumably entirely lost the independent phase in its life cycle, and the symbiosis is essential for survival and reproduction of the fungus but remain only conditional for the host.

However, asexual endophytes can retain control over host plant reproduction by

1- Increasing the proportional allocation to female functions.
2- Increasing vegetative propagation by tillering.
3- Inducing vivipary and pseudovivipary of the host.

Although for control of host functions by asexual endophytes is still unavailable, similar mechanisms of host manipulation are found in other vertically transmitted microbes, such as Wolbachia.

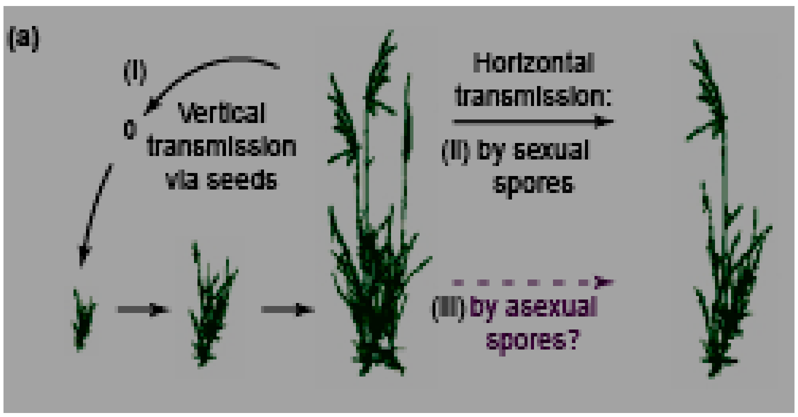


Fig: Transmission mode of endophytic fungi.

Trichoderma

Trichoderma is a genus of fungi that is present in all soils, where they are the most prevalent culturable fungi. Many species in this genus can be characterized as opportunistic a virulent plant symbiont. This refers to the ability of several *Trichoderma* species to form mutualistic endophytic relationships with several plant species.

Taxonomy

The genus was described by Christiaan Hendrik Persoon in 1794, but the taxonomy has remained difficult to resolve. For a long time it was considered to consist of only one species, *Trichoderma viride*, named for producing green mold.

The genus was divided into five sections in 1991 by Bissett, partly based on the aggregate species

- *Pachybasium* (20 species)
- *Longibrachiatum* (10 species)
- *Trichoderma*
- *Saturnisporum* (2 species)
- *Hypocreanum*

With the advent of molecular markers from 1995 onwards, Bissett's scheme was largely confirmed but *Saturnisporum* was merged with *Longibrachiatum*.

Characteristics

Cultures are typically fast growing at 25–30 °C, but some species of *Trichoderma* will grow at 45 °C. Colonies are transparent at first on media such as cornmeal dextrose agar (CMD) or white on richer media such as potato dextrose agar (PDA). Mycelium are not typically obvious on CMD, conidia typically form within one week in compact or loose tufts in shades of green or yellow or less frequently white. A yellow pigment may be secreted into the agar, especially on PDA. Some species produce a characteristic sweet or 'coconut' odor.

Different color and shape of *Trichoderma* spp. cultures

Conidiophores are highly branched and thus difficult to define or measure, loosely or compactly tufted, often formed in distinct concentric rings or borne along the scant aerial hyphae. Main branches of the conidiophores produce lateral side branches that may be paired or not, the longest branches distant from the tip and often phialides arising directly from the main axis near the tip. The branches may rebranch, with the secondary branches often paired and longest secondary branches being closest to the main axis. All primary and secondary branches arise at or near 90° with respect to the main axis. The typical *Trichoderma* conidiophore, with paired branches assumes a pyramidal aspect. Typically, the conidiophore terminates in one or a few phialides. In some species (e.g. *T. polysporum*) the main branches are terminated by long, simple or branched, hooked, straight or sinuous, septate, thin-walled, sterile or terminally fertile elongations. The main axis may be the same width as the base of the phialide or it may be much wider.

Phialides are typically enlarged in the middle but may be cylindrical or nearly subglobose. Conidia typically appear dry but in some species they may be held in drops of clear green or yellow liquid (e.g. *T. virens*, *T. flavofuscum*). Conidia of most species are ellipsoidal, 3-5 x 2-4 µm (L/W > 1.3); globose conidia (L/W < 1.3) are rare. Conidia are typically smooth but tuberculate to finely warted conidia are known in a few species.

Synanamorphs are formed by some species that also have typical *Trichoderma* pustules. Synanamorphs are recognized by their solitary conidiophores that are verticillately branched and that bear conidia in a drop of clear green liquid at the tip of each phialide.

Chlamydospores may be produced by all species, but not all species produce chlamydospores on CMD at 20 °C within 10 days. Chlamydospores are typically

Conidiophore of *Trichoderma* bearing phialides and conidia unicellular subglobose and terminate short hyphae; they may also be formed within hyphal cells. Chlamydospores of some species are multicellular (e.g. *T. stromaticum*).

Trichoderma spp. as opportunistic plant symbionts

Several plant-associated microbes are free-living and strongly beneficial to plants. Fungi in the genus *Trichoderma* and rhizobacteria in the genera *Pseudomonas*, *Bacillus*, *Streptomyces*, *Enterobacter*, and others have evolved multiple mechanisms that result in improvements in plant resistance to disease and plant growth and productivity.

Mention has already been made of the ability of root symbiotic *Trichoderma* strains to increase plant growth. This effect has been studied for some time with maize as the system. Among the positive effects on maize that have been noted over the past 5 to 10 years in work include: -

1- Control of root and foliar pathogens
2- Changes in the microfloral composition on roots
3- Enhanced nutrient uptake, including but not limited to nitrogen
4- Enhanced solubilization of soil nutrients
5- Enhanced root development
6- Increased root hair formation
7- Deeper rooting

Biocontrol agent

Several strains of *Trichoderma* have been developed as biocontrol agents against fungal diseases of plants. The various mechanisms include antibiosis, parasitism, inducing host-plant resistance, and competition. Most biocontrol agents are from the species *T. harzianum*, *T. viride* and *T. hamatum*. The biocontrol agent generally grows in its natural habitat on the root surface, and so affects root disease in particular, but can also be effective against foliar diseases.

A new set of models of the mechanisms of action of *Trichoderma* spp. has recently been proposed. These new models do not replace others, which may include: -

1- Inhibition of enzymes necessary for pathogens to penetrate plant surfaces.
2- Competition for nutrients including those necessary for pathogen propagules to germinate near planted seeds.
3- Induced resistance.
4- Biological control of diseases by direct attack of plant-pathogenic fungi.

It has been known for many years that *Trichoderma* can sense the presence of target fungi and appeared to grow tropically toward them. More recently, the gene encoding green fluorescent protein was inserted downstream of the regulatory regions of genes encoding an endo and an exochitinase that have biocontrol abilities. When paired with a target fungus, the endochitinase gene is activated before the fungi come into contact, while the activation of the exochitinase occurs only after contact is made. Different strains may follow different patterns of induction, but the fungi apparently always produce low levels of an extracellular exochitinase. Diffusion of this enzyme catalyzes release of cell wall fragments from target fungi and this, in turn, induces expression of fungi toxic cell wall degrading enzymes that also diffuse and begin the attack on the target fungi before contact is actually made. These cell wall fragments are highly potent inducers of enzymes and induce a cascade of physiological changes within the fungus, including an enhancement in *Trichoderma* growth.

MYCORRHIZA

A stable, usually mutualistic association between a fungus and the root (or rhizoid) of a plant. Mycorrhizas occur in most of plants, including vascular and some nonvascular species (e.g. liverworts). The fungi involved (e.g. basidiomycetes, ascomycetes and deuteromycetes) are always associated with the primary cortex of the root, and many appear never to occur as free-living saprotrophs. The formation of mycorrhizas leads to improved uptake of nutrients by the host plant; nutrients are apparently absorbed by hyphae (which may extend some distance from the root) and are transported back to the root to be released into the host tissue. Mycorrhiza formation and efficacy is greatest in nutrient-poor soils and may be reduced or eliminated by application of soil fertilizers. Three major types of mycorrhiza are recognized.

Ectomycorrhizas: (ectotrophic mycorrhizas) occur mainly in temperate forest trees; the fungi involved include basidiomycetes (e.g. agarics, boletes), ascomycetes (e.g. *Tuber* spp) and zygomycetes (Endogone). A given tree may associate with more than one species of fungus. In an ectomycorrhiza the fungal hyphae occur on the root surface and may penetrate between the cortical cells of the root, but the cortical cells themselves are not penetrated. Typically, the host root becomes completely enclosed by a sheath of pseudo parenchymal fungal tissue (the mantle); hyphae from the mantle may penetrate the soil surrounding the root and penetrate between the cortical cells of the root to enmesh individual cortical cells in a network of hyphae. The root is morphologically distinct from an uninfected root: e.g., it lacks root hairs and a root cap; it is thicker than an uninfected root and may be a different color; it may branch extensively and characteristically e.g. pinnately (in *Fagus* spp) or dichotomously (in *Pinus* spp) or not at all (e.g. in *Quercus* spp). In certain cases, an ectomycorrhiza may develop in the form of nodules, each consisting of a rounded, dense mass of mycorrhizal roots.

Ectomycorrhizal fungi appear to have only limited (or no) ability to use complex carbohydrates (e.g. cellulose); they obtain simple sugars (e.g. glucose, fructose, sucrose) from the plant and store them (e.g. as mannitol, trehalose or glycogen) in the mantle. Benefits to the plant, in addition to improved uptake of nutrients (particularly phosphate), include increased protection from certain pathogenus e.g., the fungus may produce antibiotic(s), or the mantle may function as a mechanical barrier to infection. Many ectomycorrhizal fungi produce phytohormones, but the significance of this to the plant is unknown. In certain cases, a mycorrhizal association appears to be essential for the normal development of the plant (e.g. in certain *Pinus* spp).

Endomycorrhizas: (endotrophic mycorrhizas) involve the development of the fungus within the cells of the root cortex; there is usually little or no change in root morphology, and an external fungal sheath is usually not formed. Typically, the fungal hyphae penetrate the cortical cells of the root and develop intracellularly; subsequently, the hyphae are digested by the root cell, leaving a knot of undigested hyphal wall material in the cell. As the root grows, the fungus invades new cells behind the root meristem; thus, a balance is set up between fungal invasion of the plant and plant digestion of the fungus.

There are three main types of endomycorrhiza.

a) **The vesicular-arbuscular (VA) type,** found in a very wide range of plants, arbuscular mycorrhizal (AM) fungi comprise the most common mycorrhizal association and form mutualistic relationships with over 80% of all vascular plants. AM fungi are obligate mutualists belonging to the phylum Glomeromycota and have a ubiquitous distribution in global ecosystems, in which the (aseptate) fungal hyphae spread through the primary cortex of unsuberized roots and penetrate the cortical cells. A characteristic, much-divided haustorium (the arbuscule) is formed, and both intracellular and extracellular hyphae usually develop spherical, lipid-rich, intercalary or terminal swellings (vesicles). VA mycorrhizal fungi belong to the genera *Acaulospora*, *Gigaspora*, *Glomus* and *Sclerocystis* (all formerly *Endogone* spp); they have not yet been grown in pure culture. VA mycorrhizas improve uptake of nutrients (particularly phosphate) by the host plant.

b) The *ericoid* type, found in members of the Ericaceae, in which the fungi colonize the fine terminal roots of the host plant and form coils or loops within the host cells. The fungi involved all belong to, or are closely related to, the species *Pezizella ericae*.

c) The *orchid* type, found in embryos and roots in members of the Orchidaceae, in which the fungi penetrate the host cells and form intracellular hyphal coils. All known orchid mycorrhizal fungi are also normal soil saprotrophs or parasites of other plants; they are usually basidiomycetes (e.g. *Armillaria*, *Ceratobasidium*, *Marasmius*, *Thanatephorus*, *Tulasnella*). Mycorrhizal associations appear to be essential to the orchid – at least for germination and seedling growth; orchid seeds are very small and have little or no food reserve, so that (under natural conditions) nutrients must be supplied by an invading fungus for successful germination to occur. Saprotrophic orchids may depend on mycorrhizal fungi throughout their lives, associating with fungi (e.g. *Armillaria mellea*) which can degrade substrates such as cellulose and pectin to simple compounds which the orchid can assimilate. Green orchids generally associate with *Rhizoctonia* spp, but may lose their mycorrhizal fungi when mature.

Ectendomycorrhizas: (ectendotrophic mycorrhizas) are intermediate in form between ecto- and endomycorrhizas; an organized fungal sheath is formed, and inter- and intracellular penetration of the root cortex occurs. This type of mycorrhiza is formed by only a limited number of plants, including certain members of the Ericaceae - e.g. *Monotropa* (a genus of achlorophyllous herbaceous plants) and *Arbutus* - and the seedlings of certain conifers. The fungi involved are apparently basidiomycetes; some fungi which form ectendomycorrhizas in plants of the Arbutae and Monotropaceae may form ectomycorrhizas in other plants.

Development of AM Fungi

The AM fungi are the most complex group of mycorrhizas which forms interradical structures:

1- Intracellular hyphae forming coils, often found in the outer layers of cortical parenchyma.
2- The intercellular hyphae.
3- The intracellular hyphae with numerous ramifications, i.e. the arbuscules,
4- The inter or intracellular hypertrophied hyphae, i.e. the vesicles.

The mycelial network surrounding the roots is dimorphic: -

1- with coarse thick walled irregular non-septate hyphae, and
2- smaller, thin walled ephemeral lateral branches.

The thick-walled hyphae penetrate the host root and cause internal infection. At the entry point, the penetrating hyphae form appressoria in the host plants. The penetrating, infecting hyphae spread inter- and intracellularly in the host root cortex. The highly-branched structures called arbuscules are usually formed in the inner cortex. These are ephemeral structures formed by the repeated dichotomous branching and complete their development in 4 or 5 days.

Arbuscules are the key sites for nutrient exchange and remain active only for 4-15 days. Many but not all endomycorrhizal fungi which form arbuscules later also form terminal or intercalary vesicles in the root cortex. These are expanded; thin-walled structures which are not delimited by a septum but often contain a large quantity of lipids called as vesicles. They may be spherical, oval or lobed and may become thick-walled and resemble resting spores. They serve as the endophytic storage organs and are rich in lipids.

Beneficial Effects of AM Fungi

1- **Nutrient transport**

In most mycorrhizal types, carbohydrates produced by photosynthesis move from the autotroph (host plant) to the heterotroph (fungal symbiont); while nutrients acquired from the soil solution pass in the opposite direction. The contribution of AMF to plant nutrient uptake is mainly through the acquisition of nutrients (especially P) from the soil by the extraradical fungal hyphae, especially from root-distant soil not depleted of nutrients by the root.

Mechanism of nutrient translocation to the host and the carbon drain (photosynthates) of the fungus on the host has significant effects on plant growth. Fungal hyphae are functionally analogous to fine root hairs as both are nutrient uptake organs. Diameters of fine root hairs, 5–20 µm and hyphae, 3-7 µm, are comparable, but hyphal length densities of AMF in soil of chamber and field experiments range from 10- to 100-fold greater than root length densities in the corresponding studies. Fungal hyphae extend the plant's effective absorption surfaces beyond the nutrient depleted zone that develops around the root caused by direct root uptake processes. However, greater hyphal density is not of equal significance for uptake of all ions in soil. Evidence suggests AMF produce extracellular phosphatase that mineralize organic P for uptake, but the activity of AMF phosphatase is relatively small in comparison to the activity of other soil microbes and autolysis.

2- Micronutrient uptake

Micronutrients are needed by the plant in small quantities but are very important for proper growth and development, as they are parts of various enzymes, pigments and other biological molecules essential for plant life. These elements are copper, zinc, magnesium, manganese and cobalt. AM fungi help the plant in two ways:

Firstly, they help in the uptake of these elements which are relatively immobile.

Secondly, they take up these elements and store them to prevent their concentrations to reach toxic levels. AM fungi could act as a sink for copper, cobalt and zinc. Most benefits of mycorrhiza have been traced to phosphorus uptake. Other elements like zinc also probably play a key role in increase of growth and yield of the plant. Uptake of micronutrients is usually limited by the rate of diffusion, if these elements move through soil to the plant root leading to quick formation of depletion zones around actively growing plant roots. Hyphae of AM fungi extend beyond these zones and help in acquisition and mobilization of these elements.

Increased uptake of iron by mycorrhizal fungi may be in part due to production of siderophores that specifically chelate iron. Elements like potassium, nitrate and sulphate in soil solutions have higher mobilities and it is unlikely that depletion zones would be formed around plant roots and that these nutrients would move more rapidly through hyphae than soil. AM fungi can increase the sulphate-absorbing power of roots, but this appears to be a secondary effect brought about by improved phosphorus nutrition.

3- AM and nitrogen fixation

Nitrogen is a non-metallic element needed for formation of amino acids, purines and pyrimidines, and thus indirectly involved in protein and nucleic acid synthesis. It is also a part of porphyrins and many coenzymes of the plant system. Deficiencies of this element lead to spindly growth of the plant and yellowing of leaves. Plants usually take up nitrogen in the form of nitrate, before its incorporation into any of the biological compounds. The process of symbiotic nitrogen fixation carried out by the root nodule bacteria and the ability of many non-symbiotic rhizosphere microbes to fix atmospheric nitrogen are well-recognized aspects of soil microbial activity.

Studies have revealed that nodulation by indigenous rhizobia is greatly improved by AM fungi. Specific root exudates in mycorrhizal legumes may act as chemotactic attractants to rhizobia. Thus, AM fungal colonization and spread within the root somehow predisposes the legume host to form more nodules resulting in higher nitrogen fixation. AM fungi help in utilization of superphosphate especially in an acid soil. Application of rock phosphate together with AM fungi can improve nodule mass leading to improved nitrogen fixation. Interaction between rock phosphate, AM and symbiotic nitrogen fixation has been studied, and it has been shown that legumes inoculated in most phosphorus deficient soils nodulated only in the presence of AM.

4- AM and phytohormones

Hormones accumulation in host tissues is affected by mycorrhizal colonization, with changes in the levels of cytokinin, abscisic acid and gibberlin-like substances. It is unclear if this is linked to improved nutrient status of the host, and it seems unlikely that phytohormone synthesis by the fungus could account for the magnitude of the increase. To what extent altered hormone production in mycorrhizal plants is due to improved nutrient status remains unclear. One AM fungus has been shown to synthesize phytohormones, but whether these can pass into the plant and affect its growth and physiology or its importance in the colonization process has yet to be determined.

5- Water stress

Many studies have reported enhanced survival of mycorrhizal plants over nonmycorrhizal plants under water-stressed situations. One may hypothesize a mechanism of direct uptake and translocation of water via hyphal network like the manner of hyphae-mediated nutrient uptake. The likely effect of mycorrhizal colonization on plant drought tolerance is related to nutrient acquisition. As the soil dries, nutrients become less available because the tortuosity of the diffusion path increases. It was shown that the higher hyphal length density of mycorrhiza decreases the diffusion distance for nutrients to reach an absorptive surface. Under drought conditions, the contribution of hyphae to nutrient uptake is advantageous to mycorrhizal plants.

6- AM and soil structure

Soil structure determines characteristics such as water inflow rate, biogeochemical cycling processes, erosion resistance and C storage. Soil organic matter plays a major role in aggregation, and organic matter accumulation is a function of biotic activity. Mechanistically, the role of fungal hyphae and plant roots in soil aggregation can be viewed as a “sticky-string bag”. The hyphae of AMF entangle enmesh soil particles to form aggregates in a hierarchal fashion, with the smaller aggregates held together by stronger forces than the larger aggregates. The glycoprotein glomalin is secreted onto hyphal surfaces in copious amounts. In terms of fungal physiology, glomalin is a recalcitrant hydrophobic molecule that enables aerial growth beyond the gas-water interface. Its concentration in soil has a strong correlation to water stability of aggregates. The hydrophobicity of this molecule may reduce macro aggregate disruption during wetting and drying cycles by retarding water movement into the pores, thereby allowing the non-disruptive escape of displaced gases from the pores.

7- AM in natural ecosystems

Natural ecosystems are disturbed habitats. Here, the diversity of the species (AM) is maintained based on the natural phenomenon of ecosystem survival of the fittest. Mycorrhizal fungi can, to an extent, regulate the communities in which they occur. Today, the biosphere is in danger in two senses: directly through outright destruction for productive use, and indirectly through the burden of waste disposal.

Therefore, there is a need to protect ecosystems. Mycorrhizae are believed to protect the environmental quality by enhancing beneficial biological interactions. A matured ecosystem is characterized by a nutrient conservative system in which nutrient is rapidly cycled between the biotic parts and is not available to leach out of the system. Mycorrhizae are critical in that these fungi permeate the soil, picking up nutrients and channeling them to the host plant. Mycorrhizae regulate the composition and functioning of plant communities by regulating the resource allocation and growth characteristics of interacting plants.

Several workers examined the role of mycorrhizal fungi in sand dune succession and found that the highest colonization of the host plant occurred within open communities, particularly within fixed, non-mobile dunes, and the colonization increased as the communities became closed.

The management of populations of AM fungi in acid infertile soils of Savannah ecosystem was studied and concluded that different plant hosts can caused the build-up of different populations of AM fungi in the soil around the root system. This indicates that, although species of AM fungi may be effective on a wide range of plants when introduced individually into sterile soil, under natural soil conditions, different plants are likely to become infected by several different AM fungi.

8- AM in forestation of arid lands

Arid regions comprise approximately one-fifth of the earth's land area and contain a large fraction of the known energy and mineral reserves. Restoration of forest land devastated for resource extraction is an immediate priority and a challenging task for arid land ecologists. In such areas, to cope with stress situations, plants have developed several strategies such as:

1- Changes in root absorption capacity.
2- Modification of root to shoot ratio.
3- Rhizosphere interactions, etc.

AM plays a role in all these adaptations. AM fungi are widespread in forest trees and this symbiosis can be manipulated to enhance productivity in forestation programs. The AM fungi change the supply of mineral nutrients from soil thereby modifying soil fertility, mycorrhizosphere and aggregation of soil particles. AM increases the growth rate of plants and influences the partitioning of phytomass between the root and shoot. The root/shoot ratio is usually lower in AM plants than in their non-mycorrhizal counterparts. The AM inoculated plants are not only large but also usually have an increased concentration and/or content of phosphorus. The enhancement of growth is primarily a result of increased mineral nutrition of plants. The non-nutritional effects of mycorrhizas in reducing the severity of some plants diseases, and in modifying water relations and soil structure are also potentially important. The benefits of AM may also extend to alleviation of effects of mineral excesses.

9- Mycorrhiza as biological agents

Mycorrhizae interact with plant-associated bacteria, fungi and other organisms and influence the interactions between the host and associated microorganisms. If the association between the host and the collective AMF community was not compatible, the association can lead to serious losses in crop yields. In contrast, a compatible association results in enhanced plant productivity, through enhanced P nutrition, prevention or control of plant diseases caused by soil-borne pathogens and enhancement of phytohormonal activity.

Several workers have successfully demonstrated the potential of mycorrhizal fungi to control plant pathogenic fungi. Successful disease control is reported to range from suppression or elimination of disease to disease tolerance by the host plant. However, most these reports only note whether the disease symptoms are alleviated by the mycorrhizal fungi treatment. Several studies investigating the potential of AMF for biological control of *Fusarium oxysporum* f. sp. *lycopersici*. Significant reductions in wilting by the pathogen were attributed, in part, to increased lignin deposition in the plant cell walls because of mycorrhizal colonization, which may have restricted the spread of the pathogen.

Weeds are major problems in agricultural systems causing yield reductions and diminishing the quality of crops. Herbicides are still considered the best means of weed control. Some workers have identified the potential of mycorrhizal fungi as agents for the inhibition or reduction of grass weeds such as *Poa annua* and *Lolium perenne*. Usually of weed inhibition using mycorrhizae, the competing crop or non-weed plant was either inoculated with an AMF species or plant roots were colonized by AMF propagules that were abundant in soil.

Reasons for Reduced Damage in Mycorrhizal Plants

1- **Change in root growth and morphology**

The colonization by AM fungi results in morphological changes to the root, leading to an increased surface area of root. Roots offer structural support to the plants and function in absorption of water and supply mineral nutrients for a wide range of microorganisms. Changes in root morphology will ultimately affect the plant's responses to other organisms. AM fungal-colonized roots being more highly branched, i.e., the root system contains shorter, more branched, adventitious roots of larger diameters and lower specific root lengths. The AM inoculated plants possess a strong vascular system, which imparts greater mechanical strength to diminish the effects of pathogens.

2- **Histopathological changes**

Histopathological studies on galls caused by the root-knot nematode *M. incognita* showed that galls in mycorrhizal plants had fewer giant cells, which are needed for the development of nematode larvae than did nonmycorrhizal plants. Nematodes in mycorrhizal plants were smaller and took longer times to mature to the adult form. Smaller syncytia and fewer giant cells were reported to confer resistance against nematodes on the host plant.

Histochemical and immunocytochemical studies provided evidence that decreased pathogen development in mycorrhizal root systems both in parts with or without mycorrhiza were associated with modifications in the host cells, together with the accumulation of defense-related molecules.

3- **Physiological and biochemical changes**

The physiological and biochemical changes caused by mycorrhizal fungi in the host plant generally reduce the severity of nematode diseases. Phenolic compounds have been shown to be formed after mycorrhizal colonization and are thought to play a role in disease resistance. Production of phytoalexin was greater on mycorrrhizal roots than on non-mycorrhizal roots and phytoalexins are believed to play a major role in the host defense system against pathogens.

An increase in lignin and phenols in mycorrhizal plants was observed and was associated with reduced nematode reproduction. Increased phenylalanine and serine concentrations in tomato roots due to inoculation with AM fungi have been observed. These two amino acids are known to be inhibitory to root-knot nematodes. Suresh and Bagyaraj (1984) reported that AM inoculation increased the quantities of sugars and amino acids in plant tissue which may be responsible for the reduction of nematode infestation. However, inferences based on the absence of galling on segments of roots and split root experiments argue for a more localized effect. Various evidence indicates structural and biochemical changes in the cell walls of plants colonized by AM fungi.

4- **Changes in host nutrition**

Mycorrhizal plants contain higher concentrations of phosphorus than do non-mycorrhizal plants. Improvement of phosphorus nutrition following AM colonization of phosphorus-deficient roots results in a decrease in membrane permeability and reduction in root exudation. Mycorrhizal-induced decreases in root exudation have been correlated with reduction of soil-borne disease, while improved nutritional status of the host brought about by AM fungus-root colonization may affect quantitative changes in root exudates.

The severity of nematode damage of cotton was greater on P-fertilized, non-mycorrhizal plants than non-mycorrhizal plants at a P level deemed adequate to high for cotton. This effect was attributed to zinc deficiency induced by nematode infection at high soil P levels. High levels of **P** fertilization inhibit zinc uptake but apparently AM fungi can alleviate this P-induced zinc deficiency and thus increase host tolerance to nematode parasitism. AM fungi have been shown to induce responses caused by environmental stress in root growth, root exudation, nutrient absorption and host physiology. Changes in exudation due to P nutrition alter the chemotaxic attraction of the nematodes to the roots and affects exclusion of nematode species that require a hatching stimulus. In general, AM fungi infection in P-deficient plants affects membrane permeability and exudation patterns in a fashion like that caused by P-fertilization in non-mycorrhizal plants. The obvious contribution to reduction of root diseases is increased nutrient uptake, particularly of P and other minerals, because AM symbiosis results in more vigorous plants, which thus become more resistant or tolerant to pathogen attacks.

In addition to P, AM fungi can enhance the uptake of Ca, Cu, Mn, S and Zn. Host susceptibility to pathogens and tolerance to disease can be influenced by the nutritional status of the host and the fertility status of the soil. Increase in plant growth after root colonization by AM fungi is due to improvement in the mineral nutrient status of host plant. Depending on the host plant and AM fungus isolate, colonization of the root system can increase phosphorus nutrition and other mineral nutrients. Host susceptibility to infection can also be influenced by nutritional status of the host and fertility status of the soil. However, in some cases enhanced mineral nutrition of mycorrhizal plants has no affect against pathogens.

5- **Mycorrhizosphere effect**

AM fungal colonized plants differing from non-mycorrhizal roots in terms of microbial community composition of the rhizosphere. These differences have been attributed to alterations in root respiration rate and quality and quantity of exudates. Plant root systems colonized by AM fungi differ in their effect on the bacterial community

composition within the rhizosphere and rhizoplane. The number of facultative anaerobic bacteria, fluorescent pseudomonads, *Streptomyces* species and chitinase producing actinomycetes differ depending on the host plant and the isolate of AM fungus.

In addition, extra radical hyphae of AM fungi provide a physical or nutritional substrate for bacteria. AM symbiosis can also cause qualitative and quantitative changes in rhizospheric microbial populations; the resulting microbial equilibria could influence the growth and health of plants. These changes may result from AM fungus-induced changes in root exudation patterns.

Changes in microbial populations induced by AM formation may lead to stimulation of the microbiota which may be antagonistic to root pathogens. AM establishment can change both total microbial populations and specific functional groups of microorganisms in the rhizoplane or the rhizosphere soil. Numbers of pathogenantagonistic actinomycetes were greater in the rhizosphere of AM plants than in nonmycorrhizal controls.

6- **Competition of colonization sites and photosynthates**

AM fungi and soil-borne plant pathogens occupy similar root tissues and there may be direct competition for space if colonization is occurring at the same time. If AM fungi and plant pathogens are colonizing the same host tissues, there may be competition for space because both usually develop within different cortical cells of roots.

Studies reported, localized competition between AM fungi and *Phytophthora*. They observed reduced development of *Phytophthora* in AM-colonized and adjacent uncolonized root systems, and pathogens never penetrated arbuscule-containing cells. Similarly, *Aphanomyces* was suppressed on pea roots by AM fungionly when the two organisms were present on the same root, also the number of infection sites was reduced within mycorrhizal root systems and colonization by the AM fungus had no effect on the spread of necrosis. AM fungi are dependent on the host as a carbon source and 4-20% of the host net photosynthate is transferred to the AM fungus. There is much information to support the competition for host photosynthates and this phenomenon may have an important role in interactions with endoparasitic nematodes because of the obligate nature of both organisms for host-derived compounds.

7- **Activation of defense mechanism**

The activation of specific plant defense mechanisms as a response to AM colonization is an obvious basis for the protective behavior of AM fungi.

The elicitation, via an AM symbiosis of specific plant defense reactions, could predispose the plant to an early response to attack by a root pathogen.

In relation to plant defense relevant compounds include phytoalexins, enzymes of the phenylpropanoid pathway, chitinases, -1,3-glucanases, peroxidases, pathogenesis-related (PR) proteins, callose, and phenolics. Phytoalexins are low-molecular-weight, toxic compounds usually accumulating with pathogen attack and are released at the sites of infection. Both phenylalanine ammonium-lyase (PAL), the first enzyme of the phenylpropanoid pathway, and chalcone isomerase, the second enzyme specific for flavonoid/ isoflavonoid biosynthesis, increased in amount and activity during early colonization of plant roots by AM fungi.

Chitinases are little or only transiently induced by AM colonization, it has been reported that increased levels of chitinase activity are only detected in AM roots at the beginning of colonization. A decrease in 1,3 endoglucanase activities has also been reported at specific stages during mycorrhiza development.

The increased lignification of root endodermal cells induced by AM colonization has been suggested to play an important in the plant defense mechanism. However, these compounds could sensitize the root to pathogens and enhance mechanisms of defense to subsequent pathogen infection.

8- **Nematode parasitism by AM fungi**

In some studies, parasitism of nematodes eggs with AM fungus has been demonstrated, but the level of parasitism was not considered sufficient to negatively affect nematode activities. AM fungal chlamydospores have been reported to occupy seeds and dead insects in soil and have limited saprophytic capabilities. It seems likely that the AM fungi colonize only stressed or weakened nematode eggs. The nematode parasitism by AM fungi is opportunistic and depends on carbon nutrition from autotrophic symbionts, rather than being representative of a true host-parasite relationship.

Factors Affecting the Symbiosis

A- Biotic Factors

There are many biotic factors affect the symbiosis including the influence of the host genetic factor is very significant in initiating the AM to colonize the root, the structure and morphology of roots also play a decisive role in mycorrhization and the microflora in soil and around the roots also influences the formation of AM.

1- **Host genotype and the type of fungus**

While the VA fungi are known to vary in their ability to colonize and transfer P to the plant and confer other beneficial effects, very little is known about the exact role of the host genotype in the expression of AM. The efficiency of the same VA fungus can vary very markedly between different species of host plant, so that certain host fungus associations are more effective than others. Response to a VA mycorrhiza can also vary within a plant species and cultivar irrespective of infection levels. The specific cultivar–fungus response may be dependent on soil pH. Many investigators attributed these variations in mycorrhizal dependency to the differing ability of plants to absorb phosphorus from low P soils, but other characteristics inherent to plants may also be determinant which include both physiological and anatomical features. Plant species or cultivars which are highly P-dependent tend to be strongly susceptible to AM.

Root anatomy is also said to influence mycorrhizal colonization. Mycotrophy is largely a feature of woody to herbaceous plants lacking root hairs. Mycorrhizal colonization shows a significant correlation with this type of root anatomy.

2- Mycorrhizal host specificity and seasonality

Arbuscular mycorrhizal fungi are considered to have low specificities of association with host species, but this conclusion is mostly based on experiments in which individual isolates of fungal species are grown separately, apart from competitive interactions. When fungi are examined as a community, evidence suggests fungal growth rates are highly host-specific. In an experiment in which AMF were trapped on different plant hosts, isolates of different fungal species sporulated differentially, with the relative dominance of fungal species being reversed, depending on the plant species with which they were associated. As this pattern of host specificity of growth rates in this “non-specific” association has been observed in other systems, including tall grass prairie and agricultural fields, this appears to be a general property of this interaction. This specificity of fungal response could contribute to the maintenance of diversity within the AMF community. Evidence showed that fungal spore density differs seasonally, with some fungi sporulating in late spring and others sporulating at the end of summer. As the spores represent the dormant state of the fungus, the physiologically active state is most likely the mirror image of the seasonal spore counts. For example, *Gigaspora gigantea*, which sporulates most abundantly in the fall and appears over winter as spores, is likely to be physiologically active during the warm season.

However, the competitive balance between AM fungal species in terms of their ability to colonize roots may be affected by environmental conditions. The symbiotic performance of AM fungi and the final mycorrhizal phenotype can be considered as resulting from the interaction of two main factors: infectivity and effectivity, both of which depend on fungal and plant determinants. Although plant mechanisms regulating mycorrhizal infection is complex, it is interesting that plant resistance to arbuscular mycorrhizal fungi can be induced by a simple mutation at one locus.

3- Interaction with free-living N2-fixing microorganisms

There are reports of positive interaction between free-living N2-fixing bacteria and AM associations. AM colonization favorably affects bacterial populations of *Azotobacter* in the rhizosphere of the plant they colonize. Growth stimulation of plants is better under inoculations with both than either microbe alone.

4- Interaction with symbiotic actinomycetes–*Frankia*

Many AM fungi have been observed in close association with different non-legume nitrogen fixing plant species. Preliminary experiments in case of *Casuarina* sp. have shown that double inoculation with AM and *Frankia* significantly improved plant growth and nodulation. Investigators reported an increase in plant dry weight, number and weight of nodules and N and P content in dual inoculated plants as compared to those inoculated with actinomycete alone.

5- Interaction with phytohormone-producing bacteria

Phytohormones synthesis by certain bacteria like *Azotobacter, Rhizobium, Pseudomonas* can significantly increase AM colonization. Investigators suggested that application of Indol acetic acid (IAA) could influence arbuscule formation in AM. A large proportion of rhizosphere bacteria can produce phytohormones, however, though how and to what extent they affect AM colonization needs to be investigated further.

B- Environmental Factors

Climatic and physico-chemical features of soil in which the host plant is growing influence AM development and establishment.

1- Light

The mycosymbiont obtain their energy source from the plant and hence rely on both the photosynthetic ability of the plant and the translocation of photosynthates to the root. For such systems, light is understandably a limiting factor. Light has been shown to stimulate development of AM. Shading not only reduces root colonization and spore production but also the plant response to the mycorrhiza, probably because of reduced spread of internal hyphae within root tissues and a consequent restricted growth of extrametrical hyphae in the soil.

2- Temperature

Soil temperature also influences all three stages of mycorrhizal development, i.e. spore germination, hyphal penetration of root and proliferation within the cortical cells of root. For spore germination, there is an optimum temperature which varies with the fungal species. Fungal penetration and development in roots is also sensitive to variations in soil temperature. Survival of fungal spores on soil after the death or harvest of the host plant is also dependent upon soil temperature. It is thought that its effect may increase or decrease depending upon the texture of the soil.

3- Soil pH

The endophyte efficiency is determined by AM adaptation to soil pH. Soil pH affects both spore germination as well as its development. Many researches, obtained colonization and growth stimulation in soils of pH 5.6 and 7 but not in acid soils of pH 3.3–4.4. The relationship between soil pH and mycorrhizal effect is complex, depends on the plant species and on soil type, forms of phosphorus and fungal species involved.

4- Salinity

There are few studies dealing with AM effects on plant growth under saline conditions or on the effects of salinity on mycorrhizal colonization. AM fungi may have the ability to protect plants from salt stress, but the mechanism is not very clear. In general, there is a decline in propagule production and AM colonization under high salinity conditions. However, the little data available do indicate that AM fungi have a potential to enhance the benefits derived from the salt-tolerant crop species if plant–endophyte combinations are properly selected.

PRACTICAL PART

(A) Endophytic Fungi

Isolation

Probably no other step is as critical to obtain good results as thorough, but non-penetrating surface sterilization. The possibility that isolates have been initiated from propagules on the surface must be minimized. The choice of sterilization times, concentration and volumes will be dictated be the thickness of sample, the relative permeability of its surface, and the texture of its surface.

1) Ten healthy leaves (5 old and 5 young) from each sample were used.

2) Serial washing in running tap water often was carried out to remove surface contamination in cases where a non-toxic method is desired.

3) Three 1cm diameter discs were cut from each leaf, from distal, central and proximal parts of the blade, surface sterilized by sequential immersion in 75% ethanol for 1min (act as a surfactant), 0.93-1.3 M solution of sodium hypochlorite (3%-5% available chlorine) for 3 min (actual sterilizing agent) and 75% ethanol for 0.5 min, and rinsed in sterilized distilled water, then they were thoroughly dried between sterilized filter paper.

4) The three parts from each leaf were inserted in sterilized Petri dishes containing glucose-Czapek's agar medium. The plates (5 plates) were incubated at $28 \pm 2^{\circ}$ C for 2-3 weeks and the developing fungi were counted

Medium used for isolation of plant-borne endophytic fungi: -

Non-selective and routine mycological medium is suitable for primary isolation of endophytic fungi and for subculturing, Glucose-Czapek's agar medium (glucose, 10g; $NaNO_3$, 2g; KH_2PO_4, 1g; $MgSO_4.7H_2O$, 0.5g; KCl, 0.5g; $FeSO_4.7H_2O$, 0.01g and agar, 15-20g per liter of distilled water) was used for isolation of endophytic fungi. Rose bengal (1/30000) and chloramphenicol (1/15000) were used as bacteriostatic and bacteriocidial agents.

Identification of fungal genera and species

Several references were used for the identification of fungal genera and species based on macro-and microscopic characteristics.

Antimicrobial activity of endophytic fungi

1- **Obtainment of the fungal crude extracts**

Firstly, the endophytic fungal strains were grown in GPY medium at 28±2˚C for 3-5 days. After that, small discs (10 mm) of the growth culture were introduced into 250 ml Erlenmeyer flasks containing 50 ml of GPY broth and incubated at 28±2˚C on a rotary shaker at 160 rpm with normal daily light and dark periods for 10 days. The culture broth was separated from the mycelium by filtration through Whatman filter paper and the filtrate was extracted with chloroform (1:1, v/v) under constant shaking. The organic phase was concentrated under reduced pressure using a rotary evaporator at 45˚C and, finally, the concentrated extract was stored in a vacuum desiccator until constant weight.

2- **Test microorganisms**

For the antimicrobial susceptibility test the several bacteria and fungi were used including gram positive, negative bacterial strains and unicellular and filamentous fungi.

3- Antimicrobial Assay

The antimicrobial activity test was carried out by disk diffusion method. The crude extracts (0.001g) dissolved with 1000µl of dimethylsulfoxide (DMSO) and sterile paper disks (7mm) were impregnated with 10µl of these extracts and placed on the Petri dishes surface containing Luria Bertani (LB) agar medium (g/L; Tryptone 10.0, Yeast extract 5.0, NaCl 5.0, agar-agar 15.0) previously spread with bacterial suspension. Subsequently, the Petri were incubated at 37±2˚C and the diameter of the inhibition zones was measured after 24 h. for antibacterial test. The fungal species were employed with GPY agar medium and the plates were incubated at 28±2˚C up to 5-7 days.

Gas Chromatography-Mass Spectrometry (GC/MS) analysis

The extracts of endophytic fungi (which have high antimicrobial activity) were analyzed using the Thermo-Scientific TRACE GC UltraTM gas chromatograph to know its ingredients.

(B) Medically Important Fungi

Medium used in isolation

Sabouraud's dextrose (Moss & McQuown 1969) agar medium (g/ litter; peptone from meat, 10.0; glucose, 40; agar, 15) was used for isolation of pathogenic fungi. To this medium two antibiotics were added to inhibit the growth of bacteria: chloramphenicol (0.5 mg/ml medium) and cycloheximide (actidione) (0.5 mg/ml medium). Before adding to the agar medium, the first antibiotic was dissolved separately in sterile distilled water, while the second was dissolved in methanol.

Identification methods for *Candida* species

Germ Tube test:

Germ tube is quick check of *Candida albicans* and *Candida dubliniensis*. germ tube appeared like projections extending from the yeast cells by inoculation one or two colonies of culture suspected *Candida* to with 0.5 ml of human serum, which contains 0.5% of glucose in the Eppendorf tube and incubated at 37 ºC for 2-3 hours. After a period of required incubation has been a complete loop of the culture on a glass slide and overlaid with a sliding lid and examined microscopically for the presence or absence of the formation of germ tube.

Growth on HiCrome *Candida* differential agar:

HiCrome Candida Differential Agar is a differential and selective medium, for rapid isolation methods of yeasts from mix cultures and facilitates differentiation of Candida species namely *C. albicans, C. krusei, C. tropicalis and C. glabrata* according to coloration and colony morphology.

On this medium incubate for 48 hours. Yeast extract and peptone special supply nitrogen and carbon source and other essential growth nutrients. Phosphate consider buffer or organizer for the medium. Chloramphenicol inhibits the bacterial flora.

According to the manufacturer *C. albicans* appear as light green colored smooth colonies, *C. tropicalis* appear as blue to metallic blue colored raised colonies. *C. glabrata* colonies appear as cream to white smooth colonies, while *C. krusei* appear as purple fuzzy colonies.