Lectures in Botany 8

Fourth year- Biology & Geology students

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Plant Diseases

For 4 th year students

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INTRODUCTION

Plant pathology has four objectives:

1. To study the structure and life cycle of the etiologic organism.
2. To study the pathogenesis and the disease symptoms.
3. To study the epidemiology and the conditions that favor spread of the pathogen.
4. To know the methods of disease control or management to reduce losses in crop yield.

Terminology of Plant Pathology

1-Disease: pathological process involving harmful physiological changes in the living plant after infection by a living organism.

2-Disorders: Physiological changes due to non parasitic agents.

3-Pathogens: living organisms (fungi, bacteria, etc.) which cause damage to the host plants

4- Parasites: pathogens deriving nutrients for growth from a living plant. They are:

* Obligate (biotrophs), restricted to living tissues.
* Facultative colonize living or dead tissues.
* Necrotrophs: grow on dead tissue; they kill in advance, thus more dangerous than biotrophs.

5- Pathogenicity: the ability to cause disease.

6- Virulence: degree of pathogenicity in a qualitative sense. Some strains of a pathogen may be avirulent

7- Aggressiveness: capacity of a parasite to invade and grow in its host plant and to reproduce on or in it.

8- Inoculum: portion of a pathogen capable of infecting a host.

9- Inoculum potential: a measure of the biological energy available for the colonization of a host. It is a function of: i - inoculum density,
ii - nutrients available to the infectious units for germination or growth,
iii - virulence of the pathogen, iv - susceptibility of the host.

10 - Immune: exempt from infection.

11 – Resistance& susceptibility: the extent to which the plant is able to prevent the entry or subsequent growth of the pathogen within it. High resistance means low susceptibility that approaches immunity. Low resistance means high susceptibility.

12 – Hypersensitivity: development of necrotic spots resulting from rapid death of cells in the vicinity of invading pathogen (confers high resistance to host plant).

13 – Entry (Penetration): direct or indirect.

14 – Infection: Establishment of nutritional relationship between the pathogen and the host.

15– Colonization: the pathogen advances through the tissues of host to varying extent.

16 – Symptoms: visible external alterations on the host by which a disease can be recognized.

General categories of symptoms are:

a- Necrosis: (death of infected tissue).

b- Hyperplasia: (increased cell division) and / or hypertrophy (increase in cell size) leading to galls, tumors, and witches ' brooms

c- Hypoplasia (reduced growth or stunting of infected plant).

Significance of Plant Diseases

1. Reduction of quality and quantity of plant products (flowers, fruits, fibers, wood, latex, etc.).
2. Limitation the kinds of plants and industries in an area.
3. Contamination of plant products with poisonous substances.
4. Responsible for direct/indirect financial losses (costs of control).

Stages in the Development of a Disease (Disease Cycle)

1- Inoculation: pathogen in contact with plant.

2- Prepenetration: germination of spores, attachment to host and recognition // host &pathogen

3- Penetration: a- Direct through cuticle.
b- Indirect through natural openings (stomata, hydathodes)
c- Indirect through wounds caused by nematodes or farming tools.

4- Infection (includes invasion): pathogen establishes contact with host cells & tissues, absorbs nutrients.

5- Colonization: growth and reproduction of the pathogen on host surface, within the plant or its vascular elements.

6- Dissemination of the pathogen: transfer of inoculum from the site of its production to the susceptible host surface either actively or passively by air, water, human, animal, insects, agricultural practice, seeds transplants etc.

7- Seasonal carryover (overwintering or over summering): survival of the pathogen in the form of hyphae, resting spores, sclerotia, chlamydospores, etc.

Classification of Plant Diseases

A- <u>According to mode of primary infection:</u>

1- Soil-borne diseases: due to soil-borne pathogens e.g. damping-off of seedlings, vascular wilt, root rots etc.

2- Air-borne disease: fungal spores are disseminated by wind and infect the shoot of plant e.g. rusts, downy mildews, powdery mildews, etc.

3- Seed-borne diseases: some pathogens survive as dormant mycelium in the seeds or other propagative structures of host plants e.g. many smuts.

B- <u>According to extent of occurrence and geographic distribution:</u>

1- Endemic diseases: constantly present in a particular country or part of the earth.

2- Epidemic (epiphytotic) diseases: occur periodically but in a severe form under favorable environmental conditions

3- Sporadic: occur at very irregular intervals and locations in few instances A disease may be endemic in one region and epidemic in another.

<u>C- According to disease symptoms:</u>

I- Necrosis (death of cells & tissues)

1- Rusts 2- Smuts 3- Mildews 4- Root-rots 5- Blights

6- Leaf spots 7- Wilts 8- Cankers 9- Fruit rots 10- Dieback

11- Chlorosis 12- Bloch 13- Damping-off 14 - Scab

15- Streaks or stripes 16- Burn, scald or scorch 17- White blisters or pustules

II- Hypertrophy and hyperplasia

1- Elongated internodes: rice infected with *Gibberella fujikuroi*; *Euphorbia* with *Uromyces pisi*; sugarcane with *Sclerospora sacchari.*

2- Galls and tumors: globose, elongated or irregular large sized outgrowths formed on attacked part e.g. Club root of Crucifers;

3- Witche's broom: upright cluster of small shoots contrasting with horizontal growth habit of normal shoot.

4- Curls: leaves are arched, twisted and distorted eg. peach leaf curl,

5- Floral abnormalities: enlargement of infected inflorescence which become green and fleshy with stamens converted into leafy structures.

III- Hypoplasia

1- Chlorosis: reduced development of chlorophyll (mosaic, vein clearing yellowing).

2- Reduction of individual organ: e.g. leaves, flowers, internodes as in dwarf bunt of wheat by *Tilletia contraversa.*

3-Floral abnormalities: in anther smut of Caryophyllaceae caused by *Ustilago violacea,* stamens become sterile.

<u>D- According to major Phyla of fungi:</u>

1- Diseases caused by Myxomycota

2- Diseases caused by Oomycota

3- Diseases caused by Chytridiomycota

4- Diseases caused by Ascomycota

5- Diseases caused by Basidiomycota

6- Diseases caused by Deuteromycota

I- Diseases caused by Myxomycota

Class: Plasmodiophoromycetes
Order: Plasmodiophorales
Family: Plasmodiophoraceae
Plasmodiophora brassicae

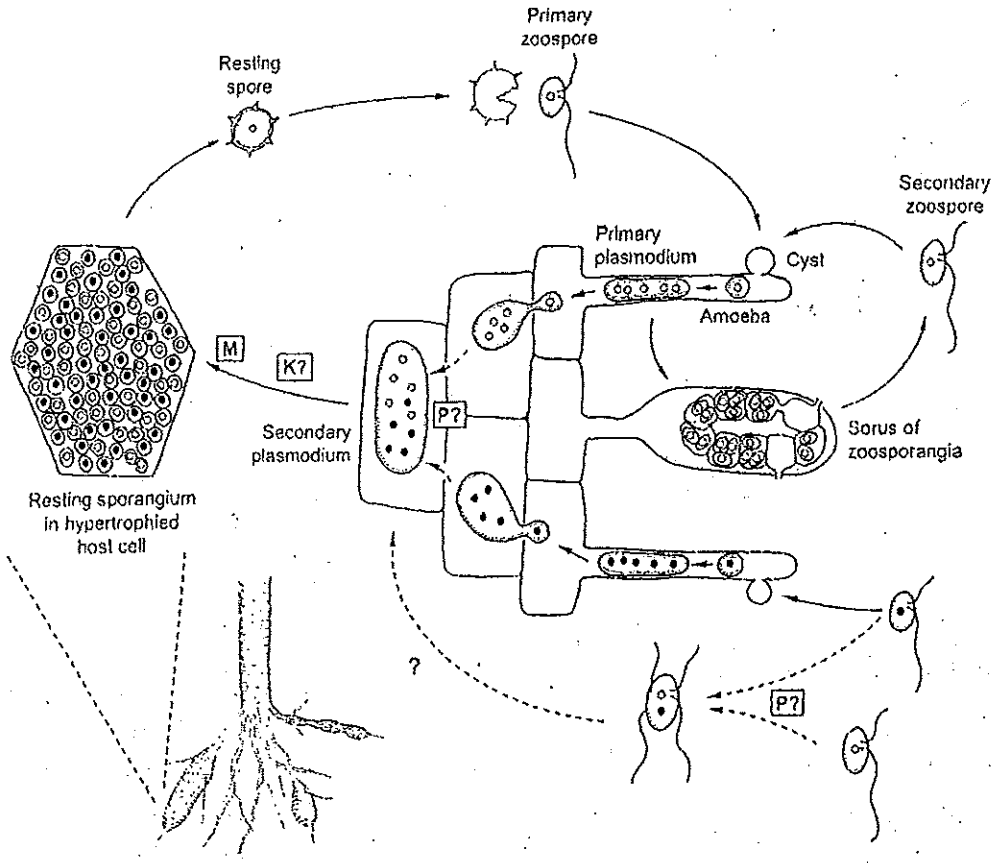
A- Club Root of Crucifers (finger and toe disease)

- Causal agent: *Plasmodiophora brassicae*
- Host plants: Cruciferous vegetables such as cabbage, cauliflower, radishes, and turnips; and field crops such as mustard.
- Symptoms:

Roots show malformation and enlargement due to spindle or club shaped swellings resulting from hypertrophy and hyperplasia of infected cells. Inside root cells, plasmodia followed by resting spores are formed. Leaves show yellowing and wilting.

- Disease cycle:

Plasmodiophora brassicae infects susceptible host plants through root hairs. It stimulates abnormal growth of affected parts, resulting in a swollen clubs. Infection is favored by excess soil moisture and low pH. Numerous resistant spores of the fungus are produced in the "clubbed" tissues. As tissues decay, spores are released into the soil where they can remain infectious for at least 10 years.



- Disease management:
  1. Eradication of cruciferous weeds.
  2. Use of well drained, pathogen free pots.
  3. Use of seedlings raised in pathogen free soil.
  4. Very long crop rotation with non cruciferous crops.
  5. Soil fumigation with volatile chemicals such as vapam, methyl dibromide etc.
  6. Alteration of soil pH to 7 or above by adding lime.
  7- Soil treatment with fungicides (e.g. PCNB)

B- Powdery scab of potatoes

- Causal agent: *Spongospora subterranea*

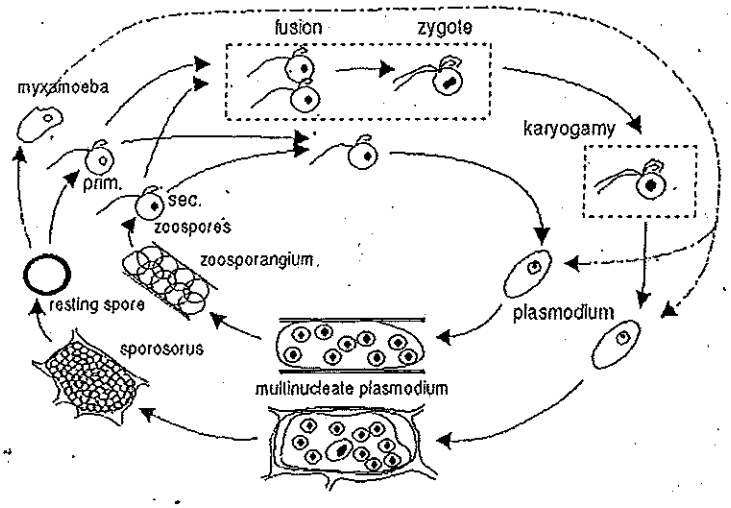
  It is generally found in wet, badly drained soils. The spores remain in the soil for several years.

Class: Plasmodiophoromycetes
Order: Plasmodiophorales
Family: Plasmodiophoraceae
Spongospora subterranea

- Symptoms:

  On tubers, irregular brown depressions with raised papery margins (scabs) are formed. These scabs are filled with dusty brown spongy masses of spore balls. Infected young tubers show distortion and swollen outgrowths.

- Control:
  1. Healthy, powdery scab-free seeds are only planted.
  2. Infected tubers should be disposed correctly not composted.
  3. Crop rotation is useful where replanting potatoes in the same position is avoided for three years.
  4. Improved soil aeration.
  5. There are no fungicides that can be used.



II- Diseases caused by Chytridiomycota

Class: Chytridiomycetes
Order: Chytridiales
Family: *Synchytriaceae*
Synchytrium endobioticum

1- Black Wart of Potatoes

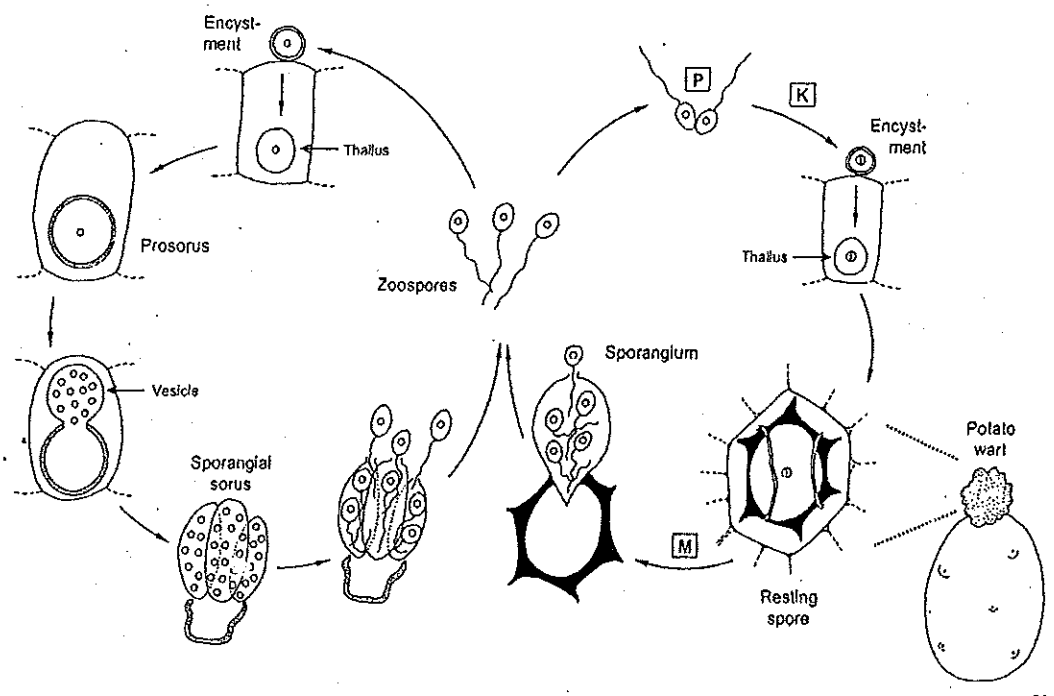
Caused by: *Synchytrium endobioticum*. It is a non mycelial, unicellular, holocarpic biotrophic chytrid fungus.

Disease Cycle:

- Infected host cells contain spherical (2n) resting sporangia (RS) of dark brown walls
- RS are released by the decay of warts and they may remain viable in soil for up to 40 years. They germinate producing prosporangia (vesicles) in which uniflagellate zoospores (n) are produced.
- Meiosis occurs during germination.
- Zoospores encyst on host epidermis before infection
- Inside the host cell the small fungal cell enlarges and the host is stimulated to enlarge.
- Zoosporangia (n) are formed producing up to 600 zoospores per spoangium.
- At later stages zoospores behave as gametes to give resting sporangia

Symptoms:

- Large irregular cauliflower-like warts or galls develop on all underground parts except roots.
- Warts at first greenish-white, becoming dark or black
- Warts develop due to hyperplasia and hypertrophy.
- The disease causes losses by reducing the quantity and quality of tubers.



Class: Oomycetes
Order: Saprolegniales
Family: *Saprolegniaceae*
Aphanomyces euteiches

III- Diseases caused by Oomycota

1- Aphanomyces root rot

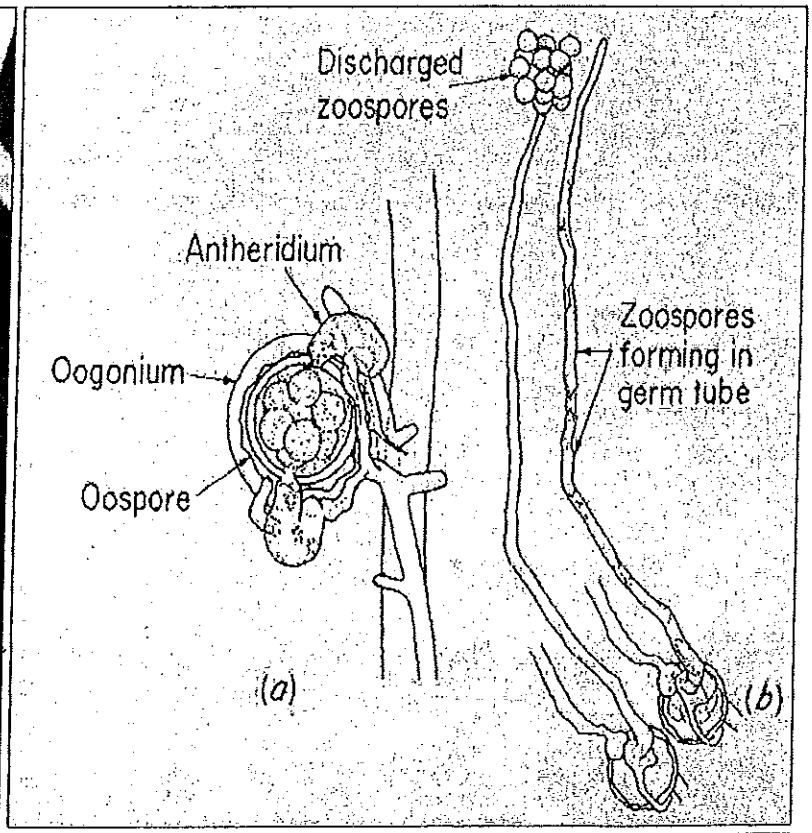
Caused by *Aphanomyces* one of the zoosporic fungi belonging to the Family Saprolegniaceae

Hosts: Sugar beet, wheat, pea, etc

Symptoms:

- Soft decay of the root cortex.
- Vascular core of root tends to come out when roots are pulled up.
- Progressive death of the leaves from the base of the stem.
- General check in growth.
- Infected plants may survive but produce poorly filled pods.
- Oospores of the fungus are present in decaying tissues.
- Disease is most serious in soils with high moisture content and at 15° – 30° c.

Aphanomyces root rot of Sugar beet



Aphanomyces: asexual and sexual reproductive units

2- Damping off and seedling blight

Caused mainly by *Pythium* species, Family Pythiaceae

- Other fungal species belonging to *Phytophthora, Rhizoctonia, Fusarium, Helminthosporium and Botrytis* could be associated with damping off and seedling blight

Class: Oomycetes
Order: Peronosporales
Family: *Pythiaceae*
Pythium aphanidermatum
Pythium oligandrum

Symptoms:

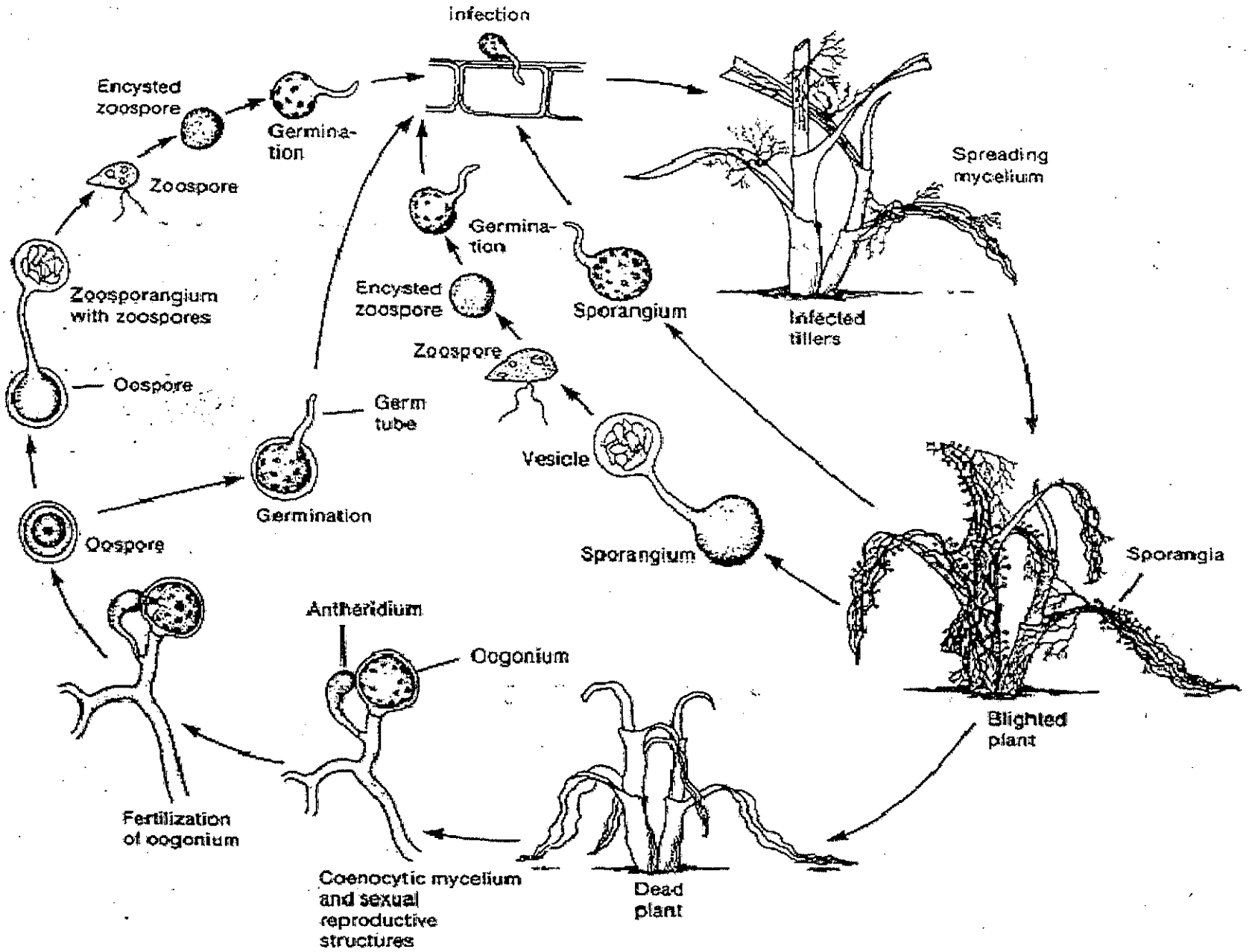
- Pre-emergence: Emergence of seedlings is poor even with seeds of high germinative capacity
- There are patches with no seedlings at all.
- Post- emergence: Seedlings that have emerged often show water soaking, browning or shriveling of the stem tissues at soil level and die.
- When plants are pulled up they show browning and rotting of the smaller roots or stem, and stem lesions at soil level.
- Plants are stunted.
- Plants wilt at midday and may recover at night.
- Plants show yellowing and die.
- Brown tissue on the outer portion of the root easily pulls off leaving a bare strand of vascular tissue exposed.
- Root tips are brown and dead
- The cells of roots contain round, microscopic, thick-walled oospores of *Pythium*

Control:

The most favorable environments for *Pythium* disease are soil treated with high-nitrogen fertilizers, alkaline soil and soil with low calcium levels.

To prevent or minimize contamination it is recommended to:

a- Utilize balanced fertilizers and keep soil pH neutral or slightly acid.
b- Prune trees and shrubs to improve air circulation.
c- Correct drainage problems, avoid over-watering,
d- Preventive fungicide treatment programs using metalaxyl, terrazole, coban, etc


Infection
Encysted zoospore
Germination
Zoospore
Spreading mycelium
Germination
Zoosporangium with zoospores
Encysted zoospore
Sporangium
Infected tillers
Oospore
Zoospore
Germ tube
Vesicle
Germination
Oospore
Sporangium
Sporangia
Antheridium
Oogonium
Blighted plant
Fertilization of oogonium
Coenocytic mycelium and sexual reproductive structures
Dead plant

3- Late blight of potato and tomato

Caused by: *Phytophthora infestans*

Class: Oomycetes
Order: Peronosporales
Family: *Pythiaceae*
Phytophthora infestans

- Late blight epidemics in the 1840s led to the Irish Potato Famine, in which over a million people died and a million emigrated to other countries.
- Even today, *Phytophthora infestans* poses a major threat to potato agriculture.

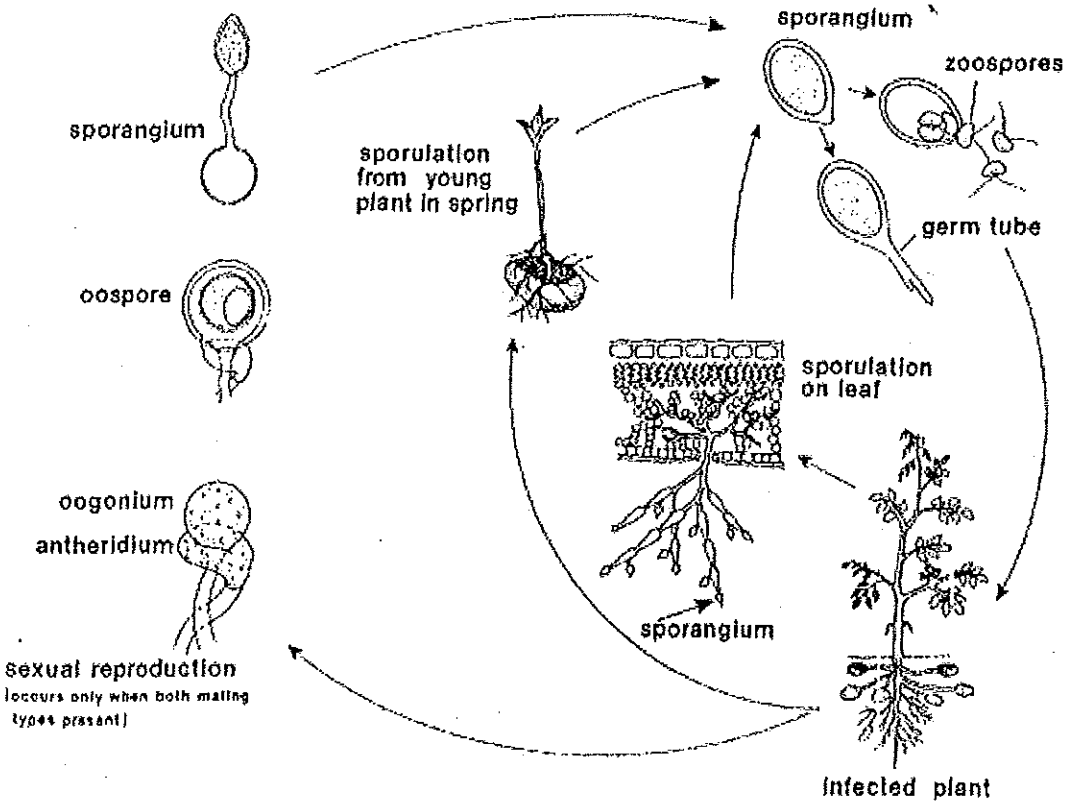
Symptoms:

On leaves and stems:

- Dark brown lesions of varying sizes and shapes.
- Under moist conditions a mass of sporangiophores
- (White bloom) develop on the lower leaf surfaces.
- Lesions increase rapidly and coalesce.
- Potato shoots are killed within 3-4 weeks.

On tubers:

- Tubers show irregular dark and sunken areas associated with brownish rot.
- Rotting often increase during storage due to further invasion by bacteria.



This is a simplified disease cycle for late blight of potato.

4- White rust Diseases

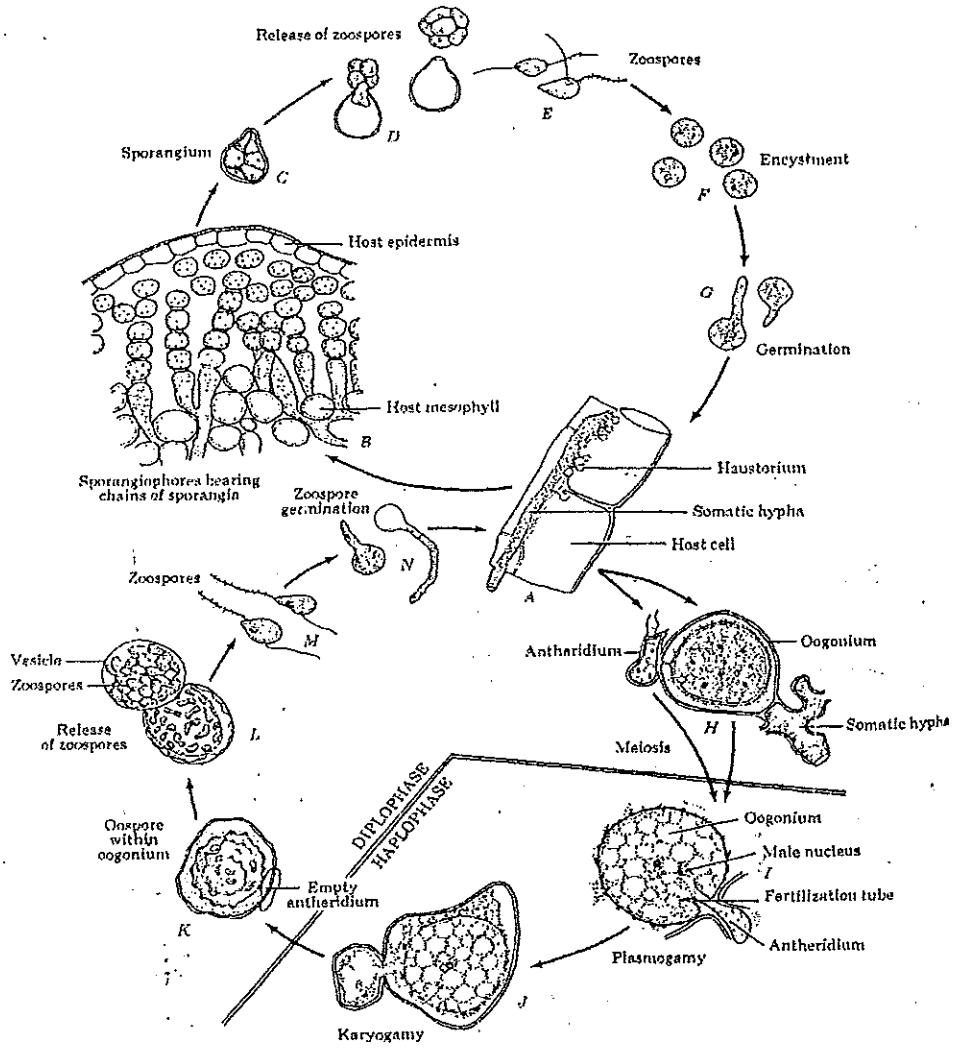
A- White rust of Crucifers by *Albugo candida* of the Hosts include radish, turnip, cabbage, cauliflower, mustard,

B- White rust of *Portulaca* by *Albugo portulacae*

Class: Oomycetes
Order: Peronosporales
Family: Albuginaceae
Albugo candida
Albugo portulacae

Symptoms:

- White pustules or sori develop on leaves and stems.
- Host epidermis ruptures exposing a white powdery mass of spores (chains of sporangia on clavate sporangiophores).
- The fungus grows inside the whole plant tissue stimulating various types of deformities.
- Inflorescences and flowers become thickened due to hypertophy and hyperplasia of affected cells.
- The swollen parts are full of oospores



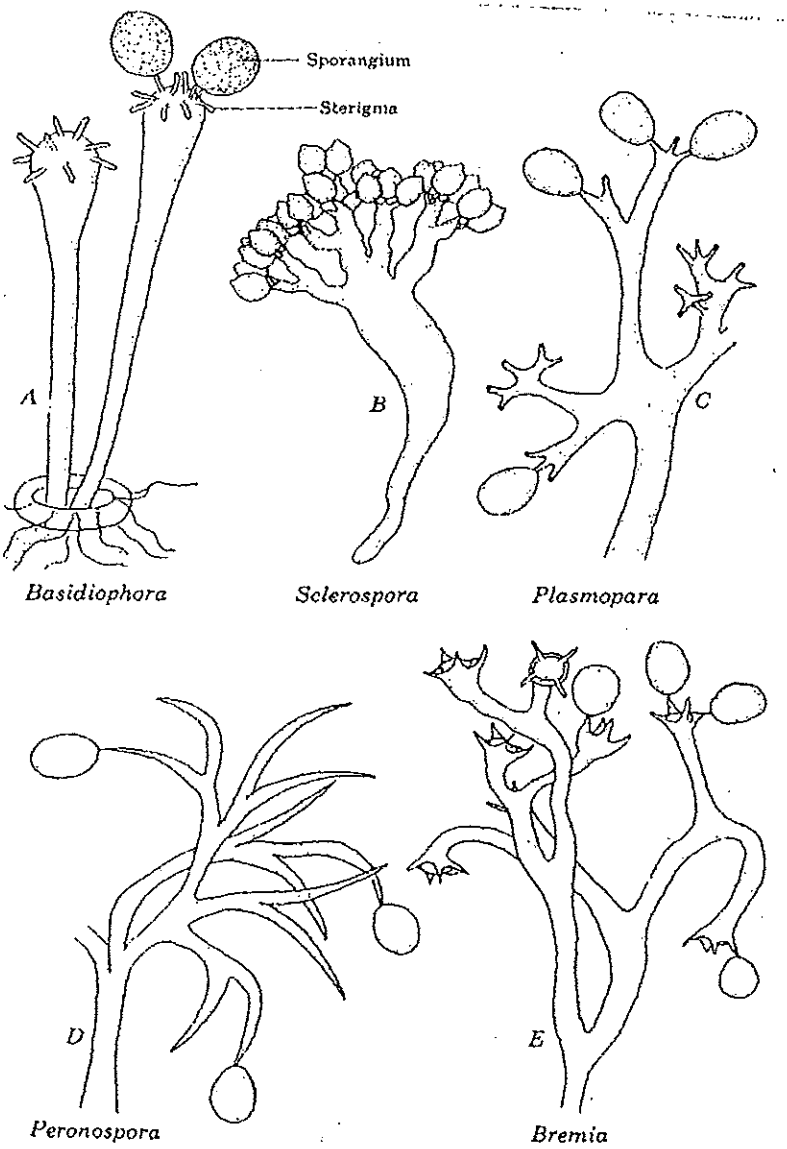
Life cycle of *Albugo candida*

5- Downy mildew diseases

Caused by members of the Family Peronosporaceae, Order Peronosporales, Class Oomycetes. Fungal species, their hosts are shown in the following table.

Pathogens	Hosts
Plasmopara viticola	grapevine
Bremia lactucae	lettuce
Peronospora destructor	onion
Peronospora pisi	peas
Peronospora parasitica	Cauliflower, Cabbage
Pseudoperonospora cubensis	Cucurbits
Sclerospora graminicola	cereals (wheat, sorghum, corn)
Basidiophora sp	Compositae (*Sonchus, Helianthus*)

The sporangiophores of downy mildew fungi are illustrated in the following figure



A- Downy Mildew of Grapevine by *Plasmopara viticola*

Class: Oomycetes
Order: Peronosporales
Family: *Peronosporaceae*
Plasmopara viticola

Signifcance of disease:

- The fungus causes direct yield losses by rotting inflorescences, clusters and shoots.
- Indirect losses can result from premature defoliation of infected vines.
- Premature defoliation predisposes the vine to winter injury.

Symptoms:

a- On leaves:

- Aappearance on the upper leaf surface of irregular pale-yellow to greenish-yellow spots up to 1/4 inch or more in diameter.
- On the underside of the leaf, the fungus mycelium (the "downy mildew") can be seen within the border of the lesion as a delicate, dense, and white to grayish, cotton-like growth.
- Infected tissue gradually becomes dark brown, irregular, and brittle.
- Severely infected leaves eventually turn brown, wither, curl, and drop.

b- On fruits:

- young berries turn light brown and soft, and under humid conditions are often covered with the downy-like growth of the fungus .
- Berries infected at late summer do not turn soft or become covered with the downy growth. Instead, they turn dull green, then dark brown to brownish-purple.
- They may wrinkle and will never mature normally

c- On shoots and tendrils:

- Early symptoms appear as water-soaked, shiny depressions on which the dense downy mildew growth appears.
- Young shoots usually are stunted and become thickened and distorted.
- Severely infected shoots and tendrils usually die.

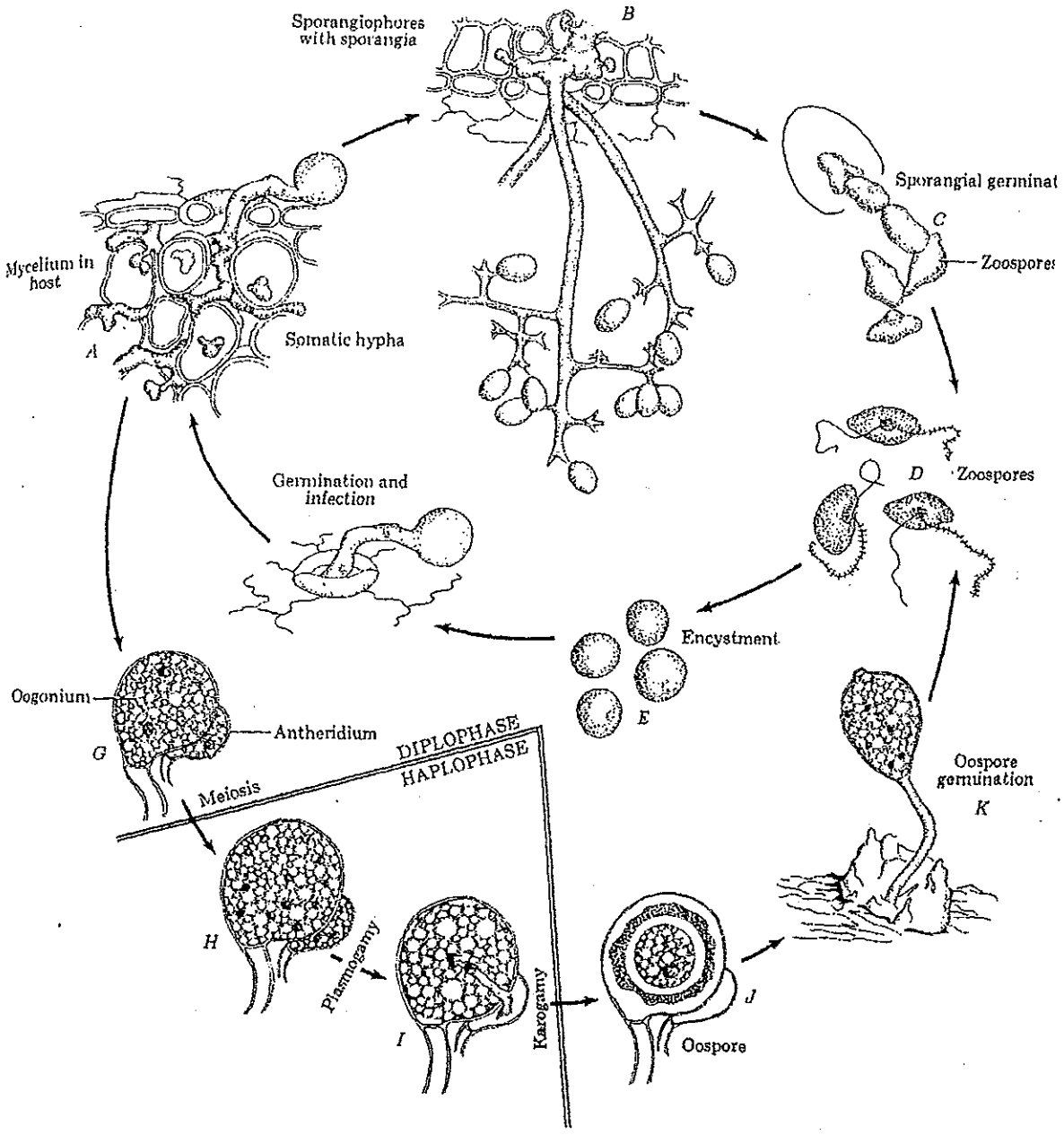
Disease cycle:

- The overwintering oospore germinates in the spring and produces a sporangium. Sporangia are spread by wind and splashing rain.
- When plant parts are covered with a film of moisture, the sporangia release small swimming spores, called zoospores.
- Zoospores, germinate by producing a germ tube that enters the leaf through stomata on the lower leaf surface.
- The optimum temperature for disease development is 18 to 25 C.
- Once inside the plant, the fungus grows and spreads through tissues.
- Infections are usually visible as lesions in about 7-12 days.
- At night during periods of high humidity and temperatures above 13 degrees C, the fungus grows out through the stomata of infected tissue and produces microscopic, branched, tree-like sporangiophores on the lower leaf surface.

- The small sporangiophores and sporangia make up the downy mildew growth.
- Sporangia cause secondary infections and are spread by rain

Control:

- Select a planting site where vines are exposed to all-day sun, with good air circulation and soil drainage.
- Proper spacing and orientation of vines in the rows to maximize air movement
- Removal of dead leaves and berries from vines and the ground after leaf drop.
- Downy mildew can be effectively controlled by properly timed and effective fungicides.



Life cycle of *Plasmopara viticola*

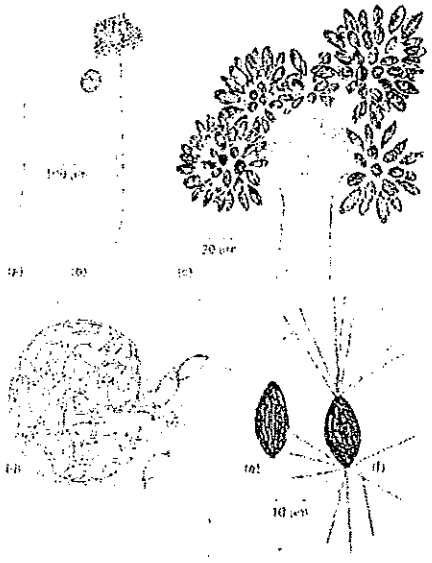
IV- Diseases caused by Zygomycota

Class: Zygomycetes
Order: Mucorales
Family: *Choanephoraceae*
Choanephora cucurbitarum

A- Choanephora rot

Symptoms: Fruits rot rapidly and white fungal mold appears on the infected area. With time, fruit look like a pin cushion with numerous small, black-headed pins stuck in it. Initially, the heads are white to brown but turn purplish-black within a few days. Affected flowers, pedicels, and immature fruit become water-soaked, and a soft, wet-rot develops. An entire fruit can rot in a 24 to 48 hour period. Symptoms usually begin on the blossom end of the fruit.

PERSISTENCE AND TRANSMISSION. The fungus overwinters as a saprophyte (living on dead plant tissue) and/or in a dormant spore form (such as a chlamydospore or zygospore). In spring, fungal spores are spread to squash flowers by wind and by insects such as bees and cucumber beetles. Infection occurs through the blossom, into the fruit and stem. Development of wet rot is favored by high relative humidity and excessive rainfall.



Choanephora cucurbitarum

Choanephora rot of squash by *Choanephora cucurbitarum*.

Other diseases by Zygomycetes:

a- Mucor rot of vegetables and fruits e.g. Guava, cucumber, grape, etc.
b- Rhizopus rot of vegetables and fruits e.g. Tomatoes, cantaloupe, mandarine, sweet potato, strawberry, etc.

V- Diseases caused by Ascomycota

Disease	Causal agent
Peach leaf curl	*Taphrina deformans*
Powdery mildews	Members of the Family Erysiphaceae
Apple scab	*Venturia inaequalis*
Duch elm disease	*Ophiostoma ulmi*
Ergot disease	*Claviceps purpurea*
Canker of trees	*Nectria galligena*
Sclerotinia soft rot of vegetables	*Sclerotinia sclrotiorum*

1- Peach Leaf Curl

Caused by *Taphrina (Exoascus) deformans*

Class: Ascomycetes
Order: Exoascales
Family: Exoascaceae
Taphrina deformans

Taphrina has four unique features:

(A) The assimilative mycelium is dikaryotic.

(B) It produces an exposed layer of asci on the surface of the host leaf .

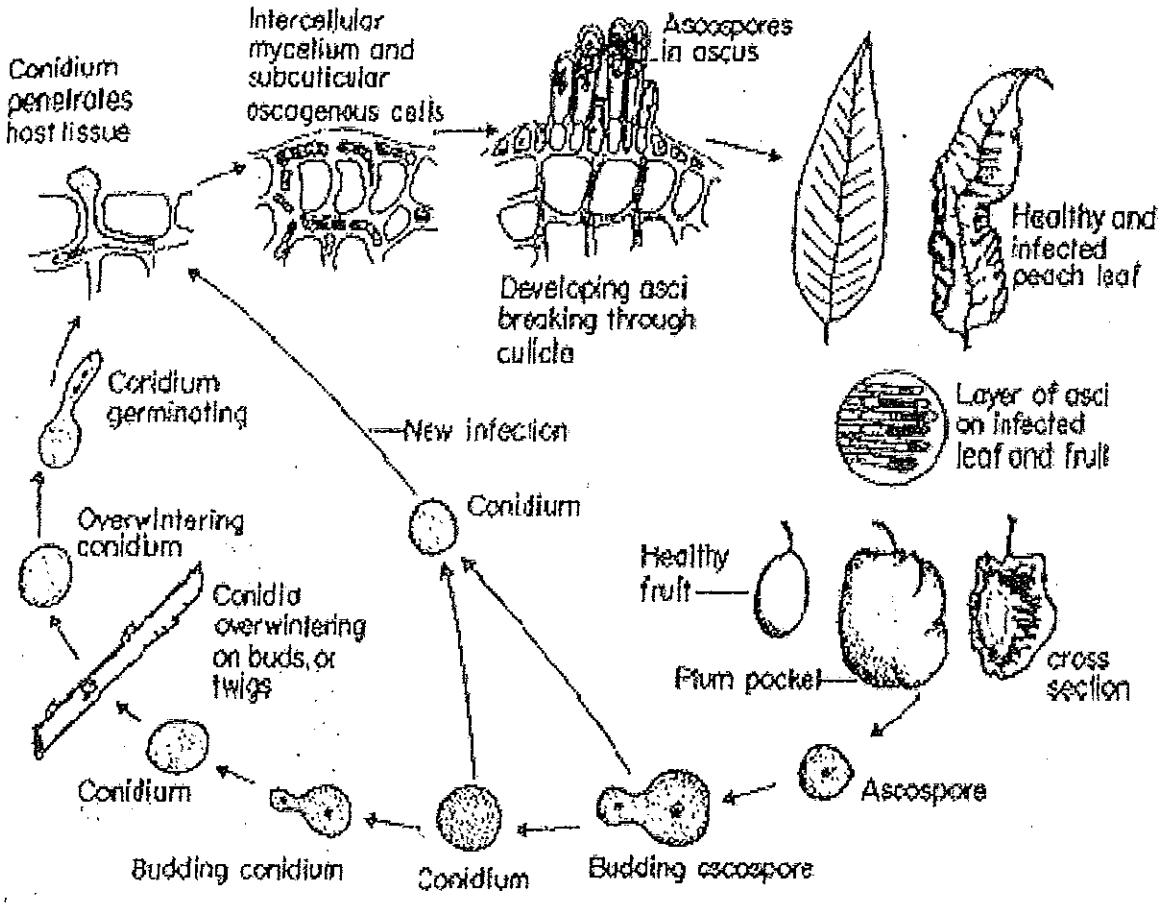
(C) Ascospores often bud in a yeast-like manner, even while still inside asci.

(D) When the asci open to release their spores, they tend to split across the tip.

(E) The anamorph of *Taphrina*, the phase in which it grows in culture, is single- celled budding yeast named *Lalaria*.

Symptoms:.

- Infected leaves are severely deformed and often display a variety of colors ranging from light green and yellow to shades of red and purple.
- The fungus causes the meristematic cells at leaf margins to proliferate quickly and randomly, which results in the leaves becoming variously wrinkled, puckered, and curled.
- As infected leaves mature, naked asci containing ascospores of the pathogen are produced on the surface giving them a dusty appearance, after which the leaves turn brown, shrivel, and drop from the tree.
- Many infected fruits drop early while others develop reddish to purple, wart-like deformities on the fruit surface.
- Infections on young peach leave occur at temperatures of 10-21 C.



Disease cycle of Peach leaf curl caused by *Taphrina deformans*

Control of peach leaf curl

1- Treat trees with a fungicide in late fall using:
   a- Copper ammonium complex
   b- 90 % tribasic copper sulfate
   c- Potassium resinate and potassium oleate
   d- Bordeaux Mixture
   e- Lime Sulfur
2- Select resistant Varieties.

2- Powdery mildews

- Caused by members of the Family Erysiphaceae of the Order Erysiphales, Class Pyrenomycetes, Phylum Ascomycota
- General characteristics of Powdery mildew fungi:

1- They are obligate, biotrophic parasites

2- They tend to grow superficially, or epiphytically, on plant surfaces producing whitish, powdery asexual structures (hyphae and conidia) on upper and lower leaf surfaces.

3- Few genera produce endophytic hyphae.

4- Infections can also occur on stems, flowers, or fruit.

5- Specialized absorption cells (haustoria) extend into the plant epidermal cells to obtain nutrients.

6- Conidia develop either singly or in chains on specialized conidiophores

7- Conidiophores arise from the epiphytic hyphae, or in the case of endophytic hyphae, the conidiophores emerge through stomata.

8- Tiny, dark sexual structures (ascomata) are produced later on infected shoots

9- Infection by these fungus is favored by high humidity but not by free water.

10- Individual species typically have a very narrow host range.

Powdery mildew fungi and their hosts

Fungal species	Hosts
Erysiphe graminis	Wheat, barley
E. cichoracearum	Cucurbits, *Sonchus,*
E. polygoni	Beans, peas
Leveillula taurica	artichoke
Sphaerotheca macularis	strawberry
Sphaerotheca pannosa	Rose, peach
Uncinula necator	Grape vine
Microsphaera alphitoides	Oak, lilac
Podsphaera leucotricha	apple
Phyllactinea corylea	Oak, elm, tulip,
Oidium mangiferae	mango

Types of conidiophores of Powdery mildew fungi

1- The oidium type: short stipe of one or more cells, conidiogenous cell and a chain of maturing conidia, e.g. ***Erysiphe, Uncinula, Microsphaera, Podosphaera and Sphaerotheca***

2- The ovulariopsis type: with clavate conidia e.g. ***Phyllactinia.***

3- The oidiopsis type: conidiophores branched arising from stomata. e.g. ***Leveillula***

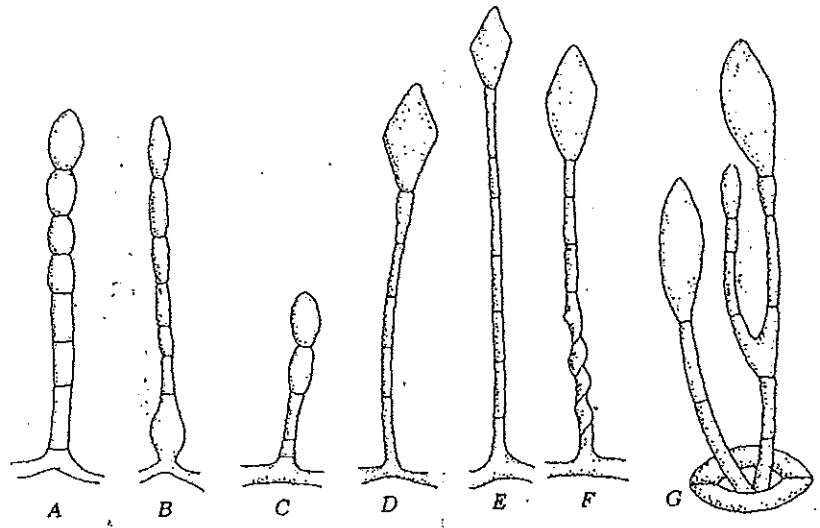


Figure 15-6 . Conidiophore types. (A) *Erysiphe cichoracearum*. (B) *Erysiphe graminis*. (C) *Erysiphe polygoni*. (D) *Phyllactinia suffulta*. (E) *Phyllactinia rigida*. (F) *Phyllactinia subspiralis*. (G) *Leveillula taurica*. (Redrawn from Blumer. 1933. By R. W. Scheetz.)

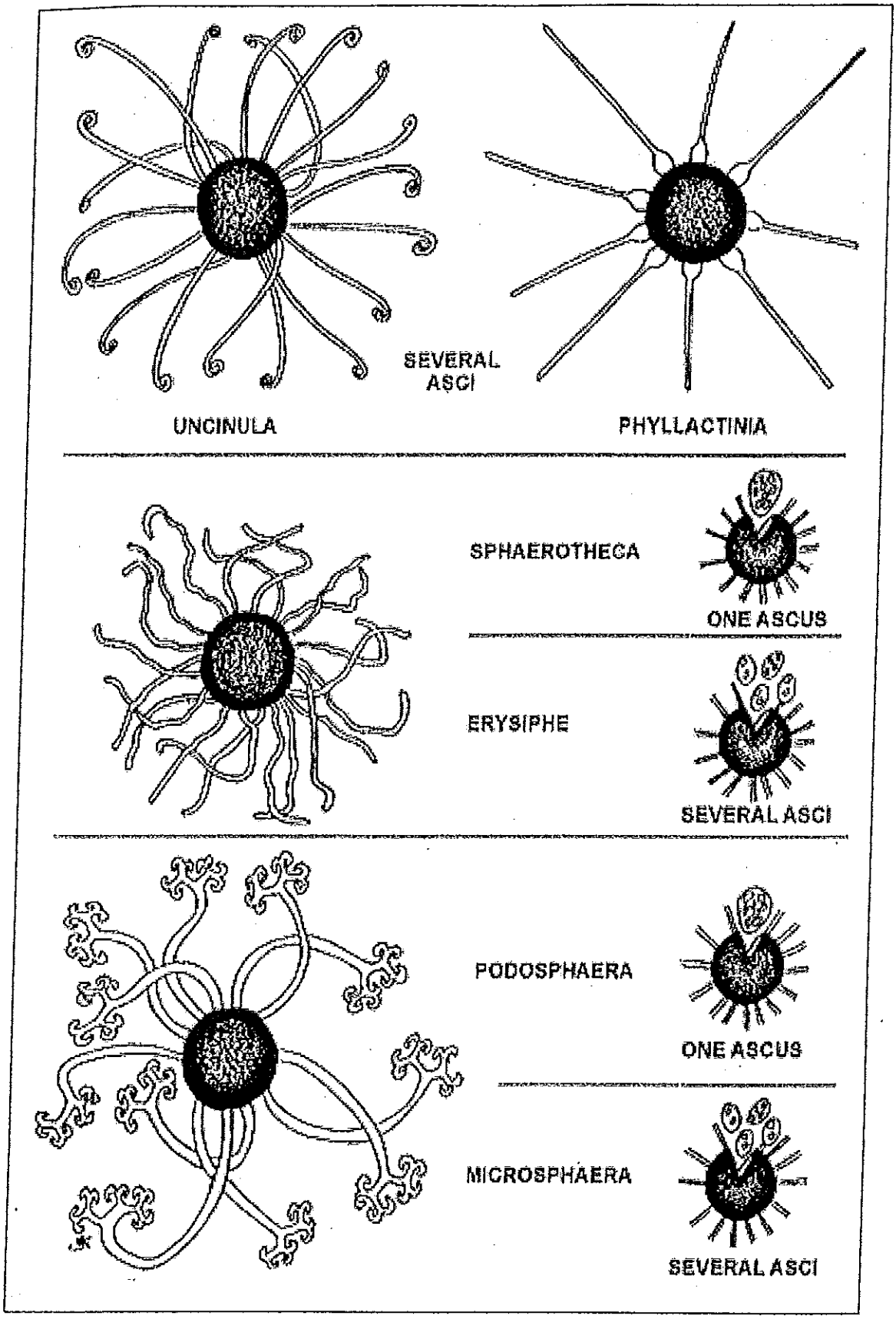
Key to Genera of Powdery Mildew Fungi (based on ascomatal appendages and number of asci)

- Appendages coiled or hooked at tip ----------*Uncinula*
- Appendages simple and straight with bulb-like base ----*Phyllactinia*

- Appendages simple or irregularly branched, often enterwined
  - # Cleistothecium contains a single ascus ----------*Sphaerotheca*
  - # Cleistothecium contains several asci ----------*Erysiphe*

  (endophytic mycelium-----*Leveillula*)

- Appendages branching dichotomously at tip, Cleistothecium contains a single ascus ----------*Podosphaera*
  - # Cleistothecium contains several asci ----------*Microsphaera*



Types of Ascomata of powdery mildew fungi

Disease cycle of powdery mildew fungi

1- The fungus can overwinter as dormant mycelium or resting ascospores (in dark cleistothecia) on infected stems or leaves.

2- In spring, dormant mycelia becomes active producing asexual conidia while the cleistothecia produce ascospores.

3- Conidia and ascospores are then carried by wind to susceptible young plant parts.

4- when a conidium germinates on host leaf surface it produces a germ tube which gives an appressorium.

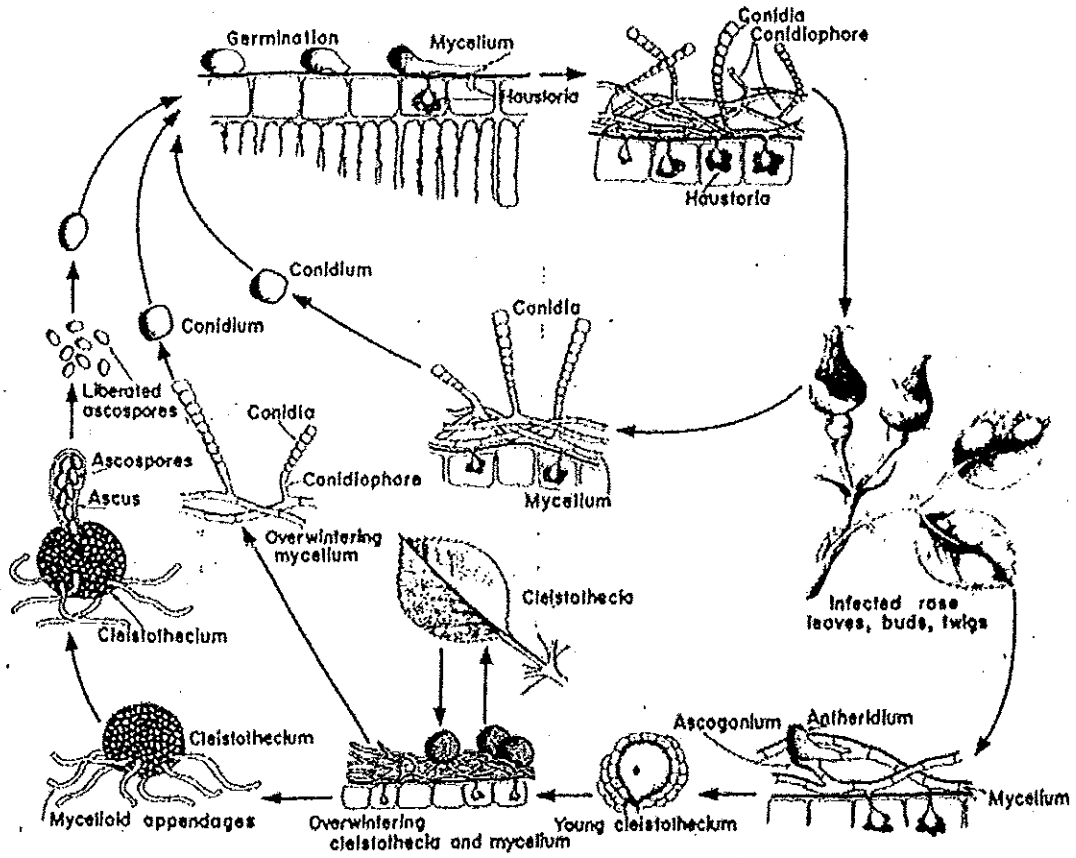
5- From the appressorium a penetration hypha grows through the cuticle and cell wall then swells out in the epidermal cell to form a haustorium (globular or finger-like in shape). The haustorium is a fungus structure that takes the nutrients from the plant

6-Further germ tubes, appressoria and haustoria are produced and the fungus grows out radially from the point of inoculums.

7- About 4 days after inoculation, sporulation starts extending outwards giving conidial chains

8- Dark pin point cleistothecia develop superficially in the same mycelial felt.

9- Cleistothecia overwinter and provide inoculums for infection of next season's crop.



Disease cycle of powdery mildew of rose by *Sphaerotheca pannosa*

Factors influencing disease development

1- Moisture: Powdery mildews are most severe in dry weather. germination of conidia is poor in free water. Spore maturation and release usually occurs during the day when relative humidity is low, at night an increase in relative humidity favors spore germination and penetration of the fungus.

2- Temperature: 11-28 C is favorable for infection. Cool damp nights and warm sunny days favor the development of Powdery Mildew.

3- Light: Higher incidence of powdery mildew on shaded than on exposed leaves. Effects of light include increased conidial germination, negative phototro-pism of germ tubes to white light (+ve to green).

4- Soil fertility: mineral nutrition (K, N, P) affects susceptibility. K- deficiency increases susceptibility.

5- Others: Closely planted gardens with some air movement are ideal conditions for spread of this disease.

Symptoms of powdery mildews:

- Slightly raised blister like areas on the upper leaf surfaces.
- Later, the young expanding leaves become twisted, distorted and covered with a white powdery mass of mycelium and spores.
- Young peduncles, sepals, petals and stems may also show distortion while growing tips and buds may be killed.
- Infected older leaves and stems may remain symptomless

Control of powdery mildew

1- Separation of new plantings from old ones.

2- Application of crop rotation of at least 1 year.

3- Control of weeds especially those related to host plants.

4- Fungicide sprays: e.g. with sulfur, karathane (0.1%), benlate (0.1%), calixin. Seed treatment with bayleton (0.1- 0.2%) or its spray on leaves (200- 500 ug /ml)

5- Breeding of resistant varieties:

3- Apple scab by *Venturia inaequalis*

Ascomycota
Dothideomycetes
Pleosporales
Venturiaceae
Venturia inaequalis

Symptoms:

Dull black or grey-brown lesions on the surface of tree leaves, buds or fruits.
Lesions may also appear less frequently on the woody tissues of the tree.
The disease rarely kills its host, but can significantly reduce fruit yields and fruit quality.
Affected fruits are less marketable due to the presence of the black fungal lesions.

Life cycle

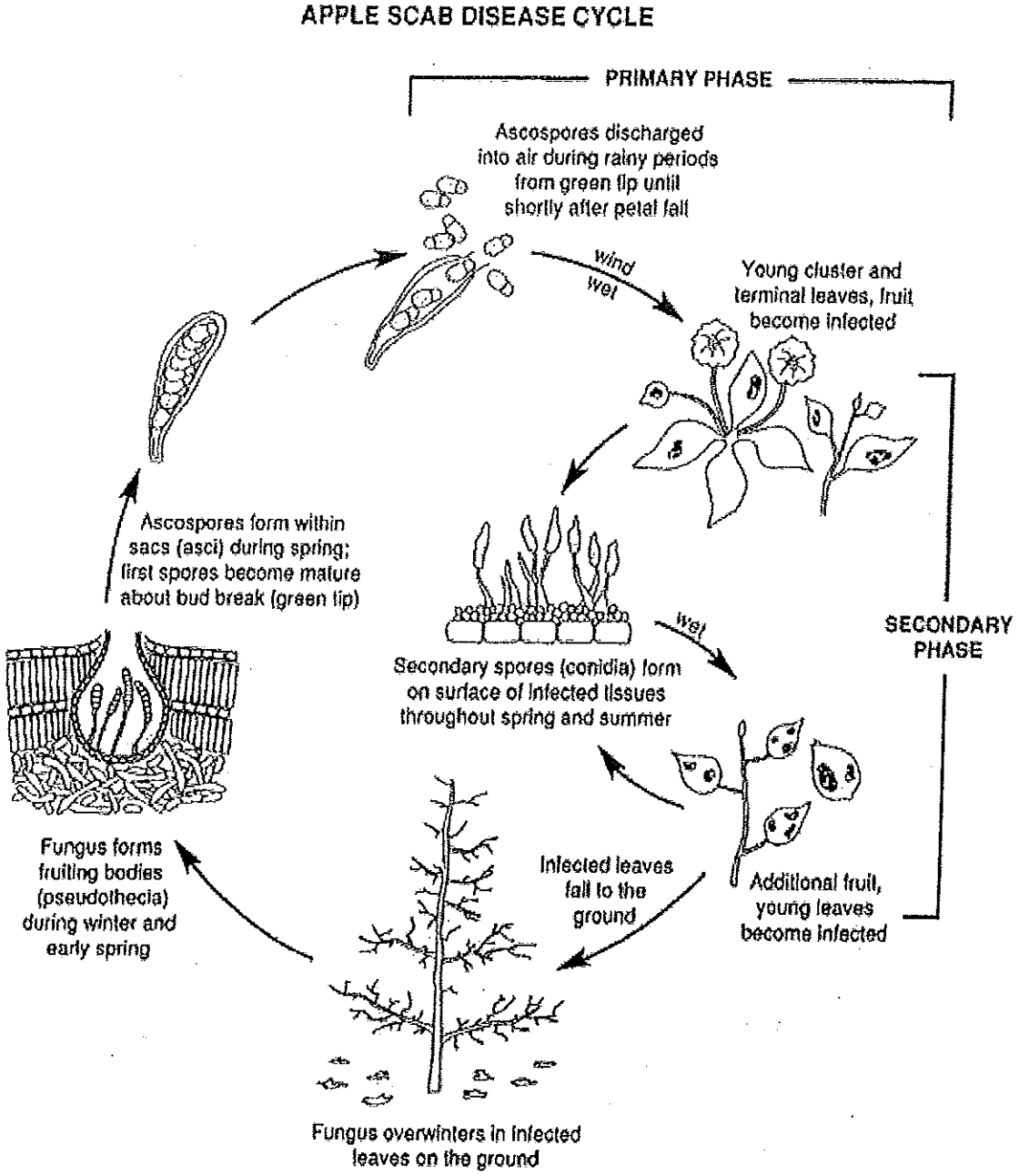
1. The infection cycle begins in the springtime, when suitable temperatures and moisture promote the release of *V. inaequalis* ascospores from leaf litter around the base of previously infected trees.
2. These spores rise into the air and land on the surface of a susceptible tree, where they germinate and form a germ tube that can directly penetrate the plant's waxy cuticle.
3. A fungal mycelium forms between the cuticle and underlying epidermal tissue, starting as a yellow spot that grows and ruptures to reveal a black lesion bearing the asexually as the conidia are released and germinate on fresh areas of the host tree, which in turn produce another generation of conidial spores.
4. This cycle of secondary infections continues throughout the summer, until the leaves and fruit fall from the tree at the onset of winter.
5. Over the winter, *V. inaequalis* undergoes sexual reproduction in the leaf litter around the base of the tree, producing a new generation of ascospores that are released the following spring.
6. Scab lesions located on the woody tissues may also overwinter in place, but will not undergo a sexual reproduction cycle; these lesions can still produce infective conidial spores in the spring.

Control:

a- Resistant cultivars: Breeding programs to develop high quality disease-resistant apple cultivars

b- Sanitation: Prevention of pseudothcial formation in overwintering apple leaves would probably eliminate scab as a serious threat to apple production. Leaf pickup and destruction in late autumn can be employed. Applications of 5% urea to foliage in autumn can hasten leaf decomposition, thus reducing formation of pseudothecia.

c- Chemical treatment: Protectant fungicides prevent the spores from germinating or penetrating leaf tissue. Postinfection fungicides control the scab fungus inside leaves and fruit. These chemicals can penetrate plant tissues to eliminate or inhibit lesion development. Several fungicides are available for controlling apple and pear scab. These include fixed copper, Bordeaux mixtures, copper soaps (copper octanoate), sulfur, mineral or neem oils, and myclobutanil. All these products except myclobutanil are considered organically acceptable



4- Dutch elm disease (DED) by *Ophiostoma ulmi*

Fungi
Pezizomycotina
Sordariomycetes
Ophiostomatales
Ophiostomataceae
Ophiostoma ulmi

Symptoms:

Dutch elm disease results in the blockage of the water-conducting tissue within the tree.

Initial symptoms include discoloration and wilting of foliage.

This insect (*Scolytus scolytus)* feeds primarily on small branches high in the tree crown.

Foliage on diseased branches turns yellow. .

Wilt symptoms continue to progress on other branches in the tree crown over successive weeks or months.

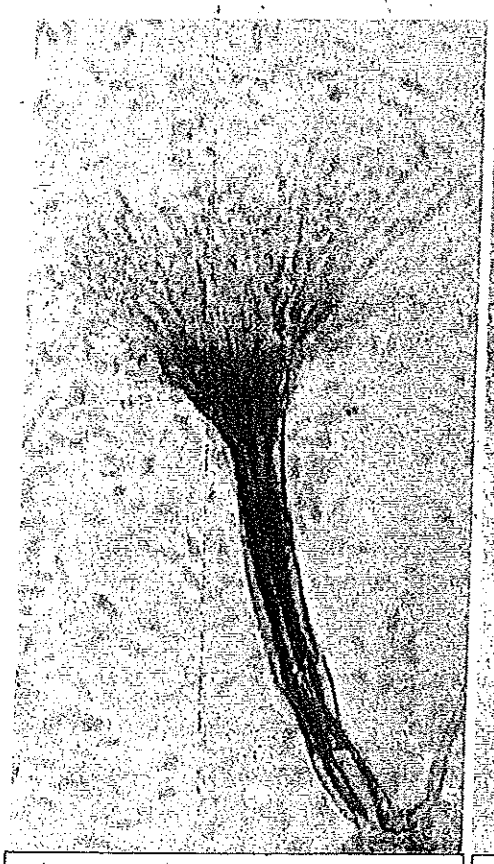
Foliage throughout the crown wilts and the tree dies.

Another diagnostic feature is the formation of brown streaks in infected sapwood.

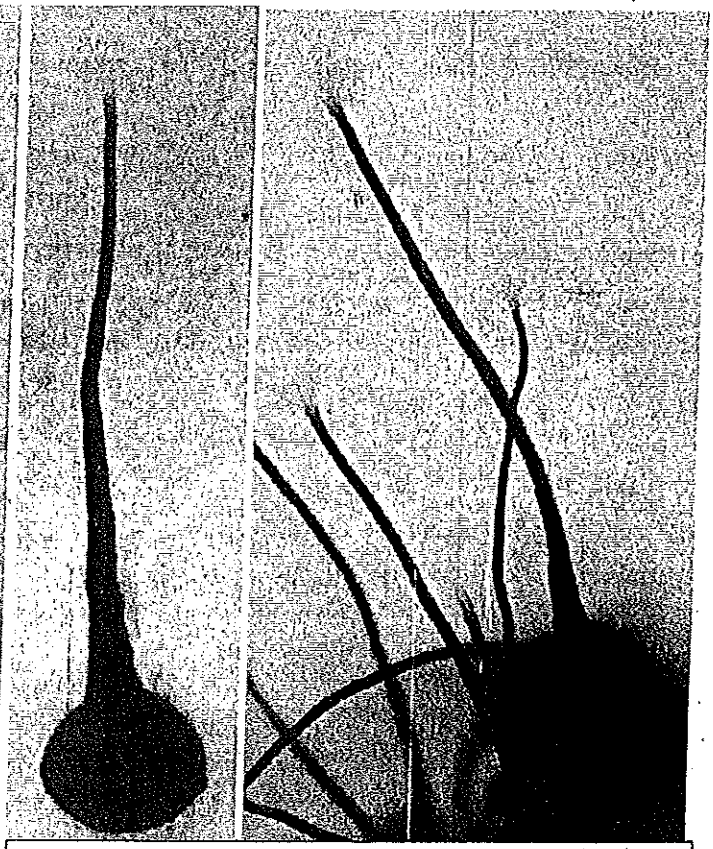
This is common in trees where infections started by beetle transmission.

Discoloration may occur in the main trunk on trees rapidly killed by root graft infection.

Branches infected with the fungus typically have long brownish red streaks running the length of a branch section.



Graphium state of *O. ulmi*



Ascoma of Ophiostoma ulmi

Control:

Dutch elm disease control involves two different but related programs: (1) community-wide sanitation programs designed to reduce the level of elm bark beetles (principal carriers of the Dutch elm disease fungus); and (2) prevention of the spread of the disease through natural root grafts from infected trees to adjacent healthy trees.

Insecticides: Dursban insecticide spray of tree bases as part of their regular DED control program

Sanitation: destruction of all dead or dying elm wood present in the community.

The only way to prevent transmission through the roots is to create a barrier between diseased and healthy trees by severing or killing those roots between the trees

Chemical Treatment: Systemic fungicides (Arbotect) can be injected into the trunk or root-collar of the affected tree

Therapeutic tree injection is generally only effective where less than 5 percent of the crown of the tree shows symptoms.

Protective Treatment of Healthy Elms: The most effective chemical currently available is Arbotect.

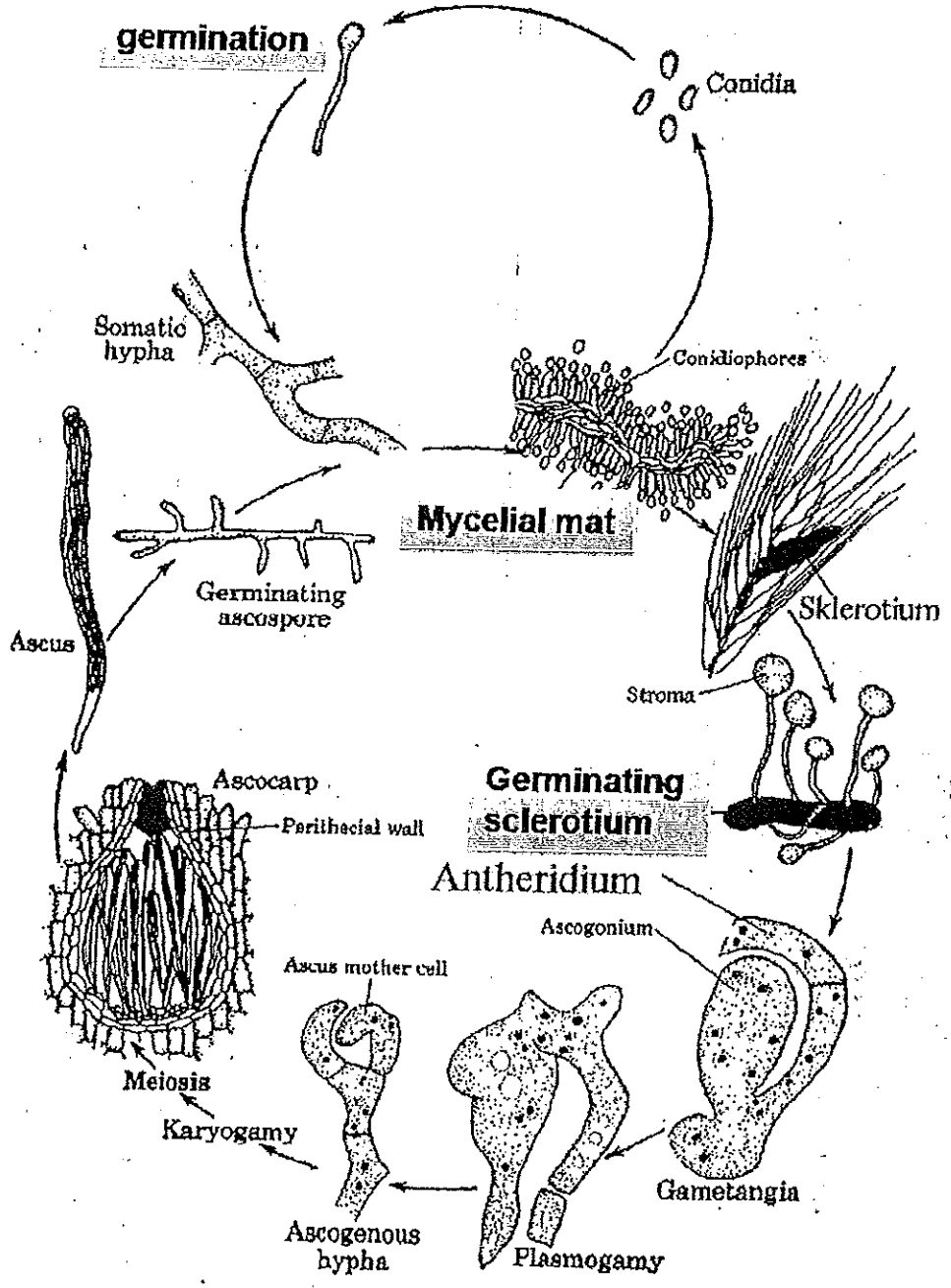
5- Ergot Disease of cereals by *Claviceps purpurea*

Symptoms and Signs

- Dark purple to black sclerotia (ergot bodies) found replacing the grain in the heads of cereals and grasses just prior to harvest.
- The ergot bodies consist of a mass of vegetative strands of the fungus. The interior of the sclerotia is white or tannish-white.
- In some grains, ergot bodies are larger than the normal grain kernels, while in other grains, such as wheat, grain kernels and the ergot bodies may be similar in size .
- Prior to development of the sclerotia bodies, the fungus develops a honey dew stage in the open floret.
- The "honey dew" consists of sticky, yellowish, sugary excretions of the fungus.

- Disease Cycle of *Claviceps purpurea*
- Sclerotia produced in small grain fields or grassy areas fall to the ground and survive on the surface of the soil.
- In the spring and early summer, the sclerotia germinate to produce tiny mushroom-like bodies (stroma) approximately the size of a pin .
- Spores (ascospores) formed by a sexual process in these bodies are shot into the air, and wind currents may carry them to grain heads.
- The first infections are from these wind-borne ascospores which invade the embryo of the developing kernel
- Soon a yellow-white, sweet, sticky fluid ("honey-dew") exudes from the infected flowers. The fluid contains a large number of asexually produced fungus conidia.

- Many species of insects visit the "honey-dew" and become contaminated with the fungus spores.
- These insects visit other grass flowers and spread the fungus.
- Spores may be transferred to other grain heads by rain-splash and direct contact, as well.
- Once the fungus becomes established in the florets, it grows throughout the embryos and replaces them, later producing the dark sclerotia.
- Many sclerotia fall to the ground before harvest and overwinter on the soil surface, serving as potential sources of spores the following year.



Life cycle of *Claviceps purpurea*

6- Nectria canker of hardwoods

Causal agent: *Nectria galligena* Bres.

Host: beech, white and yellow birch, red and sugar maple, poplar, and willow.

Symptoms:

A depressed or flattened area of bark near small wounds or at the base of dead twigs or branches is the first indication of the disease.

These areas may have a darker color and a water-soaked appearance.

The older and larger cankers may be concentric or target-shaped with callous ridges evident and the bark completely sloughed off or irregular in shape and lacking evidence of callous tissue.

The tiny, red, balloon-like fruiting bodies may be evident on the canker margin.

Cankered area is partially or completely covered by a roll of callus, (the tree is overcoming the infection.

The resulting deformation reduces the value of the tree

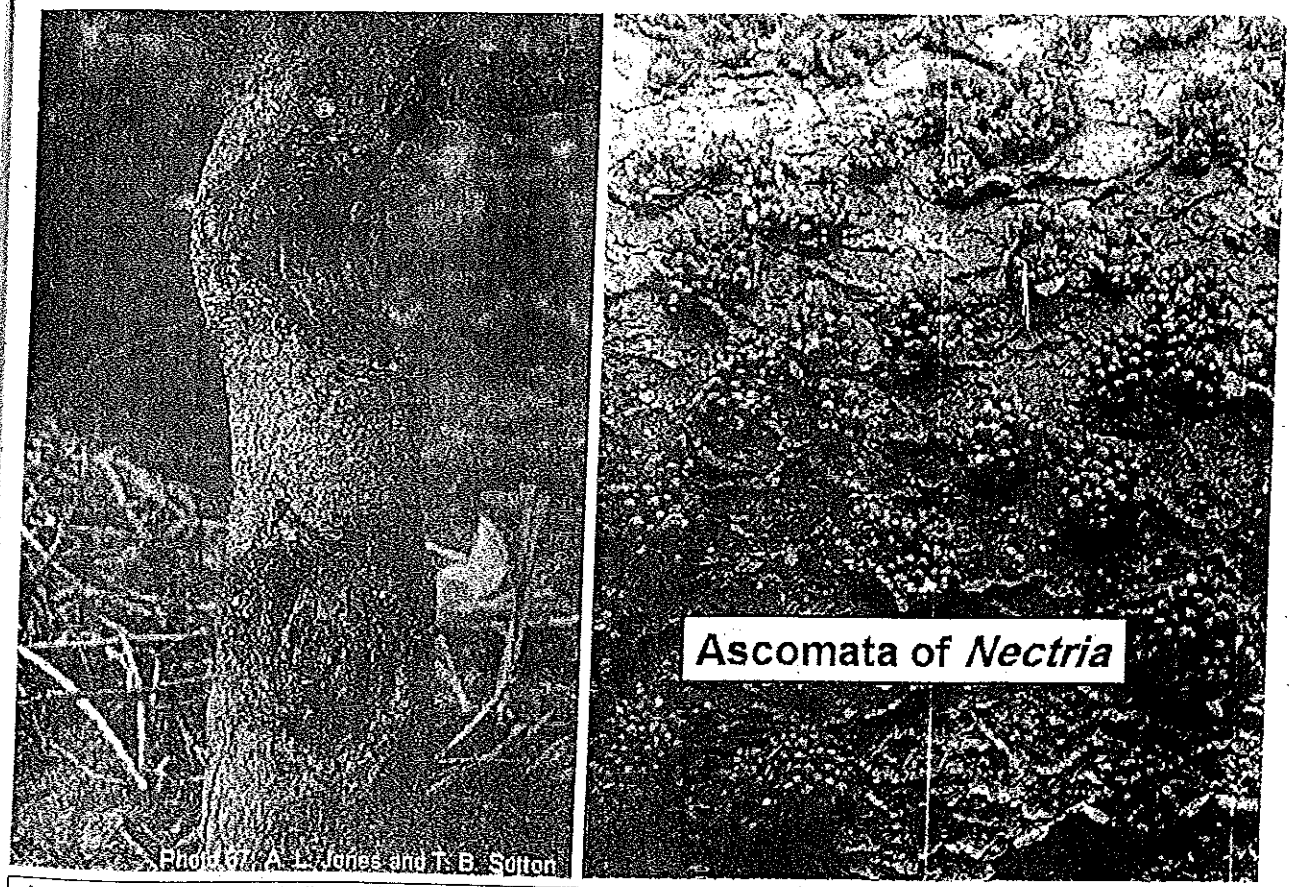


Photo: A. L. Jones and T. B. Sutton

Nectria canker on apple tree caused by *Nectria galligena*

7- Soft rot Diseases by *Sclerotinia sclerotiorum*

Hosts: Cabbage, bean, citrus, celery, coriander, melon, squash, soybean, tomato, lettuce, carrots,, onions, peas, pumpkins and cucumber.

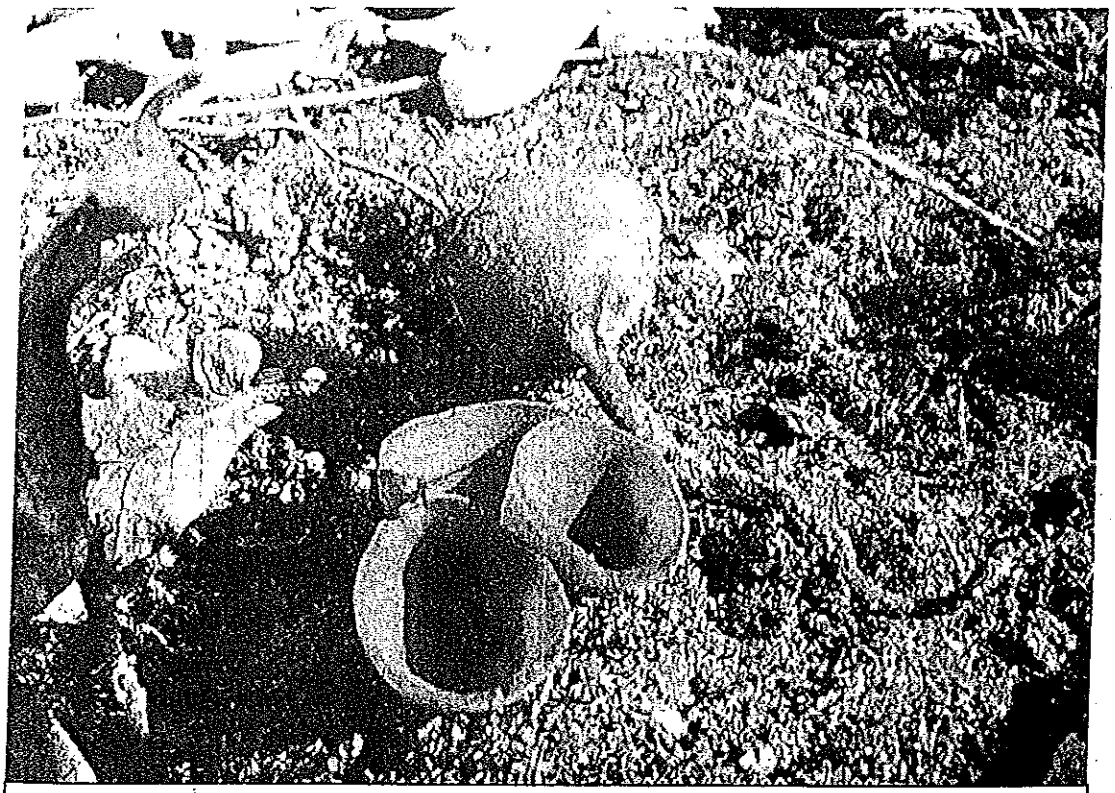
Symtoms:

Water-soaked spots on fruits, stems, leaves, or petioles which usually have an irregular shape.
These spots enlarge and a cottony mycelium covers the affected area.
The fungus spreads and the plant becomes a soft, slimy, water-soaked mass.
The cottony mycelium usually produces numerous sclerotia, black seed-like reproductive structures, a reliable diagnostic sign of *Sclerotinia* (these usually do not form until after host death).
In contrast to the water-soaked symptoms, the host may exhibit "dry" lesions on the stalk, stems, or branches, with an obvious definition between healthy and diseased tissues.
The lesions enlarge and girdle the plant part.

Distal portions of the plant become yellow, then brown, then die.

The girdled portion is often the base of the plant which causes the plant to die.

Sclerotia form within the stem pith cavities, fruit cavities, or between tissues (i.e., bark and xylem).



Apothecia of ***Sclertiorum***

VI- Diseases caused by Basidiomycota

- These are the most structurally complex fungi, and include what we commonly call mushrooms, toadstools and bracket fungi. Rust and smut fungi are plant parasitic basidiomycetes.
- Basidiomycetes are characterized by a septate mycelium.
- The septa are highly complex and are pierced by a particular kind of pore termed a dolipore.
- The dolipore does not allow nuclei to pass through the septum.
- Consequently, hooked outgrowths called clamp connections are formed to ensure the proper distribution of nuclei as the hyphae grow.

The Basidiomycota have three classes:

a) Hymenomycetes

- Mushrooms and toadstools, composed of highly complex fruiting bodies (basidioma) and networks of dikaryotic mycelia.
- Basidioma have pores or gills, which are lined with basidia.
- Mushrooms as *Armillaria mellea* attack roots and trunks of many trees.
- Bracket fungi as *Ganoderma* grow on solid substrates such as tree trunks.

b) Uredinomycetes

- These are highly specialized plant pathogens which can only grow and reproduce on their host species or closely related species.
- Over 6000 members of the Uredinomycetes (commonly known as rusts) are important members of these sub-phyla.
- Wheat and bean rusts are economically important diseases.

Rust Diseases caused by Order: Uredinales

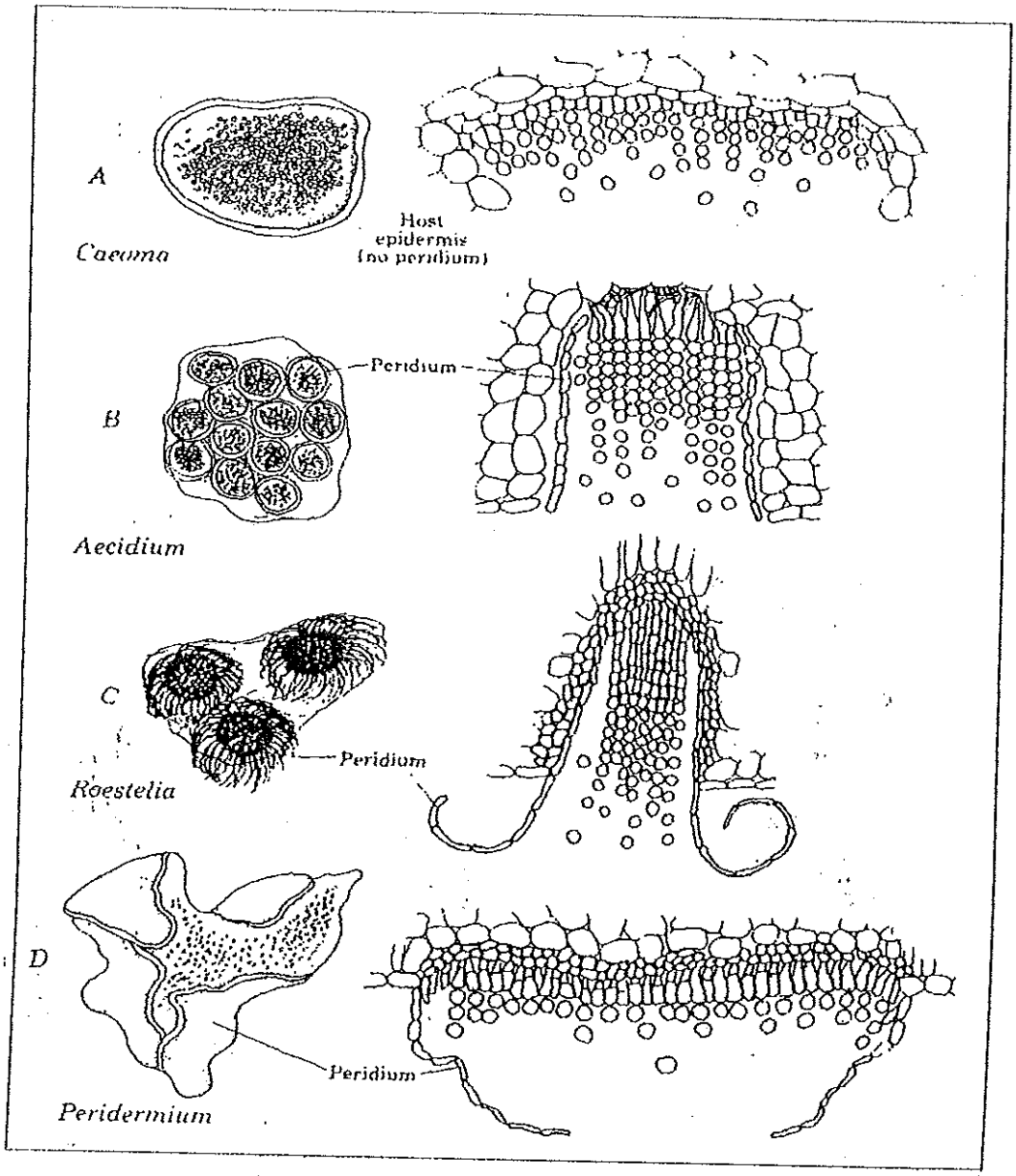
1- *Family Pucciniaceae*: (teliospores stalked)
   a. *Uromyces fabae, U. appendiculatus*
   b. *Puccinia graminis tritici*
   c. *Hemilea vastatrix*
   d. *Gymnosporangium junperi- virginianae*
   e. *Phragmidium mucronatum*

2- *Family Melampsoraceae*: (teliospores sessile)
   a. *Melampsora lini*
   b. *Cronartium ribicola*

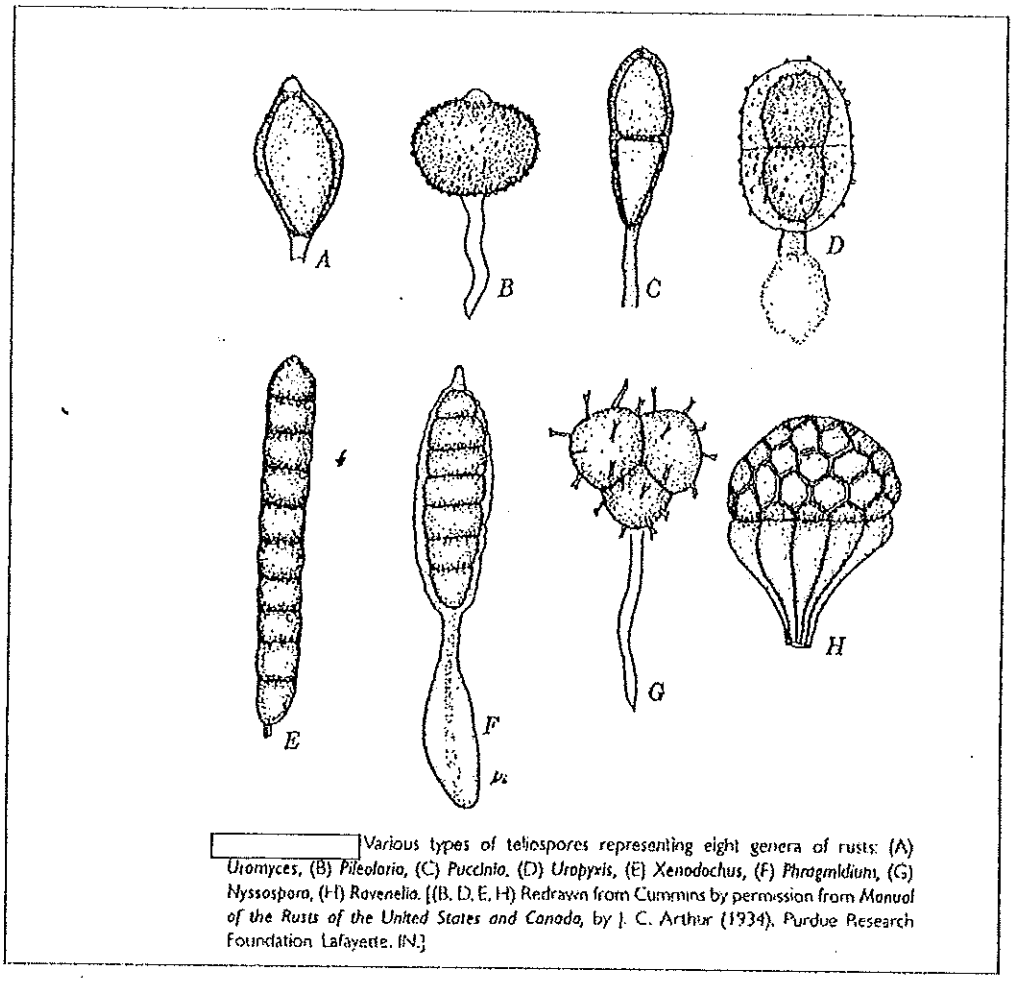
The life cycle of a typical rust species is among the most complex found anywhere in nature, consisting of five different spore stages (macrocyclic) on two plant hosts which are taxonomically entirely unrelated to each other.

The macrocyclic life cycle is consisting of:

A- Spermogonium or pycnial stage
B- Aecial stage
C- Uredial stage
D- Telial stage
E- Basidial stage (in soil)

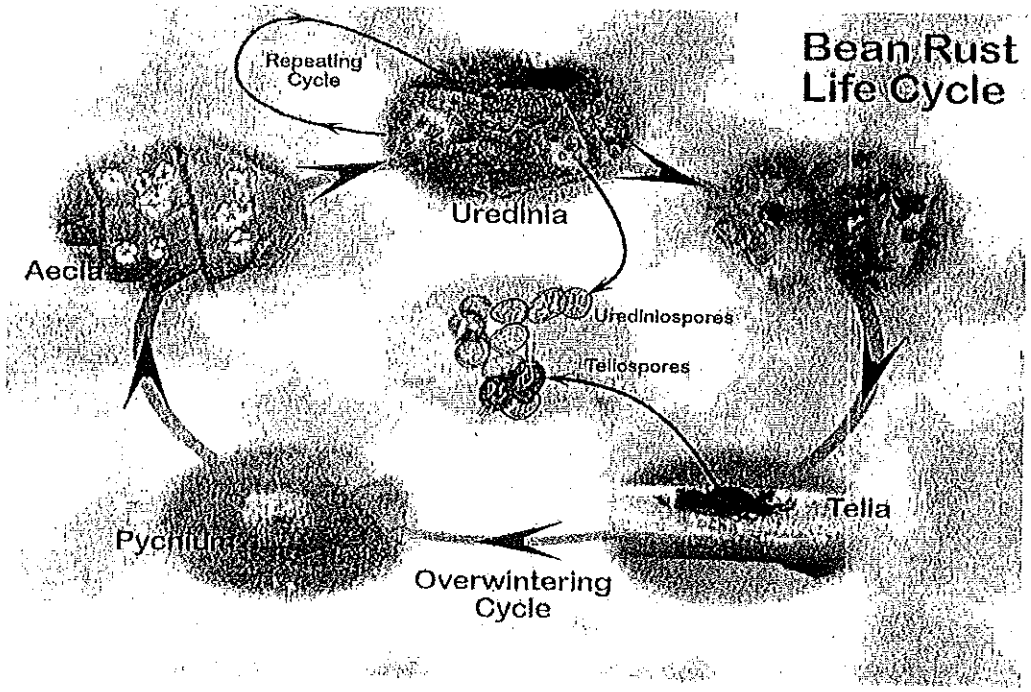


Types of aecia of rust fungi



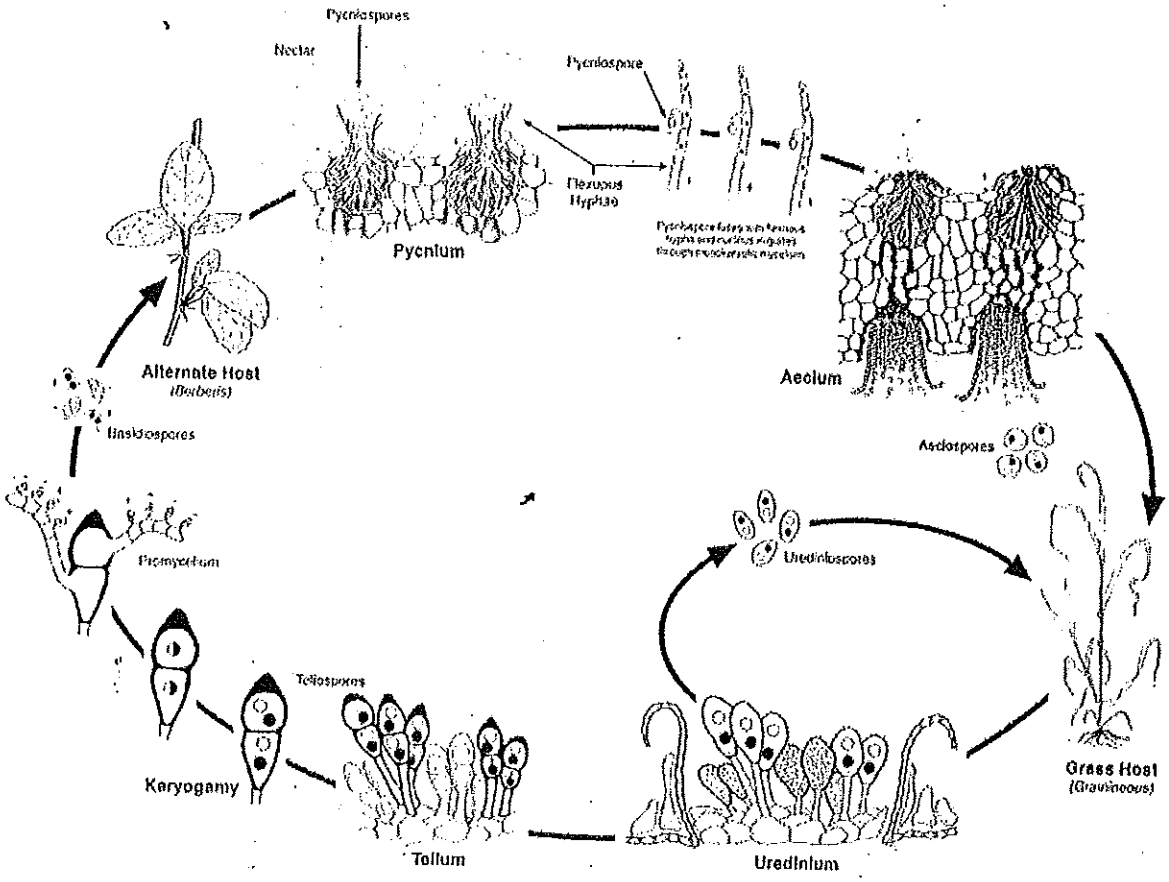
Various types of teliospores representing eight genera of rusts: (A) *Uromyces*, (B) *Pileolaria*, (C) *Puccinia*, (D) *Uropyxis*, (E) *Xenodochus*, (F) *Phragmidium*, (G) *Nyssospora*, (H) *Ravenelia*. [(B, D, E, H) Redrawn from Cummins by permission from *Manual of the Rusts of the United States and Canada*, by J. C. Arthur (1934), Purdue Research Foundation Lafayette, IN.]

A- Rust of broad bean by *Uromyces fabae*



B- Black stem rust of wheat caused by *Puccinia graminis*

- Life cycle: (macrocyclic, 5 stages)
  - A- Spermogonium or pycnial stage: on upper srface of *Berberis vulgaris* leaves
  - B- Aecial stage :(on lower surface of *Berberis* leaves)
  - C- Uredial stage (early on wheat stem)
  - D- Telial stage (late on wheat stem)
  - E- Basidial stage (in soil)



Disease cycle of black stem rust of wheat caused by *Puccinia graminis tritici*

C- Yellow (stripe) rust of wheat by *Puccinia striiformis*
D- Orange rust on wheat leaves by *Puccinia recondite*
E- Rust of garlic by *Puccinia allii*
F- Peanut rust by *Puccinia arachidis* on the underside of leaves
G- Aple rust by *Gymnosporangium clavariaeforme*
H- Rust of rose by *Phragmidium mucronatum*
I- White pine rust by *Cronartium ribicola*
J- Rust of flax by *Melampsora lini*

c) Ustilagomycetes

They are commonly known as smuts, and over 1000 members of this class live in a similar manner to the rusts, as obligate biotrophic fungi – they can only grow on living plants.

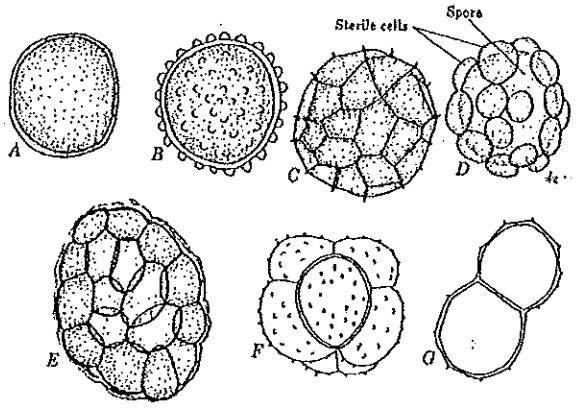
Maize Smut, caused by *Ustilago maydis* is an economically important disease

Smut Diseases caused by Order: Ustilaginales

1- *Family Ustilaginaceae*
Ustilago maydis, Ustilago tritici,
Ustilago hordei, Ustilago avenae,
Sphacelotheca sorghi, S. reliana
Tolyposporium ehrenbergii

2- *Family tilletiaceae*
Tilletia caris
Urocystis cepulae

3- *Family: Graphiolaceae*
Graphiola phoenicis



Modes of infection by smut fungi

1- Embryo infection:
- Loose smut of wheat by *U. nuda*

2- Seedling infection:
- Loose smut of oats by *U. avenae*
- Covered smut of barley by *U.hordei*
- Stripe smut of grasses by *U. striiformis*
- Dwarf bunt of wheat by *Tilletia contraversa*
- Onion smut by *Urocystis cepulae*

3- Shoot or local infection:
- Smut of anthers of *Melandrium album* by *U. violaceae.*
- Sugarcane smut by *U. scitaminae*
- Long smut of sorghum by *Tolyposporium ehrenbergii*
- Common smut of corn by *U. maydis*
- Rice bunt by *Tilletia barclayana*

Examples of smut diseases

1- Loose smut of wheat and barley by *U. nuda*

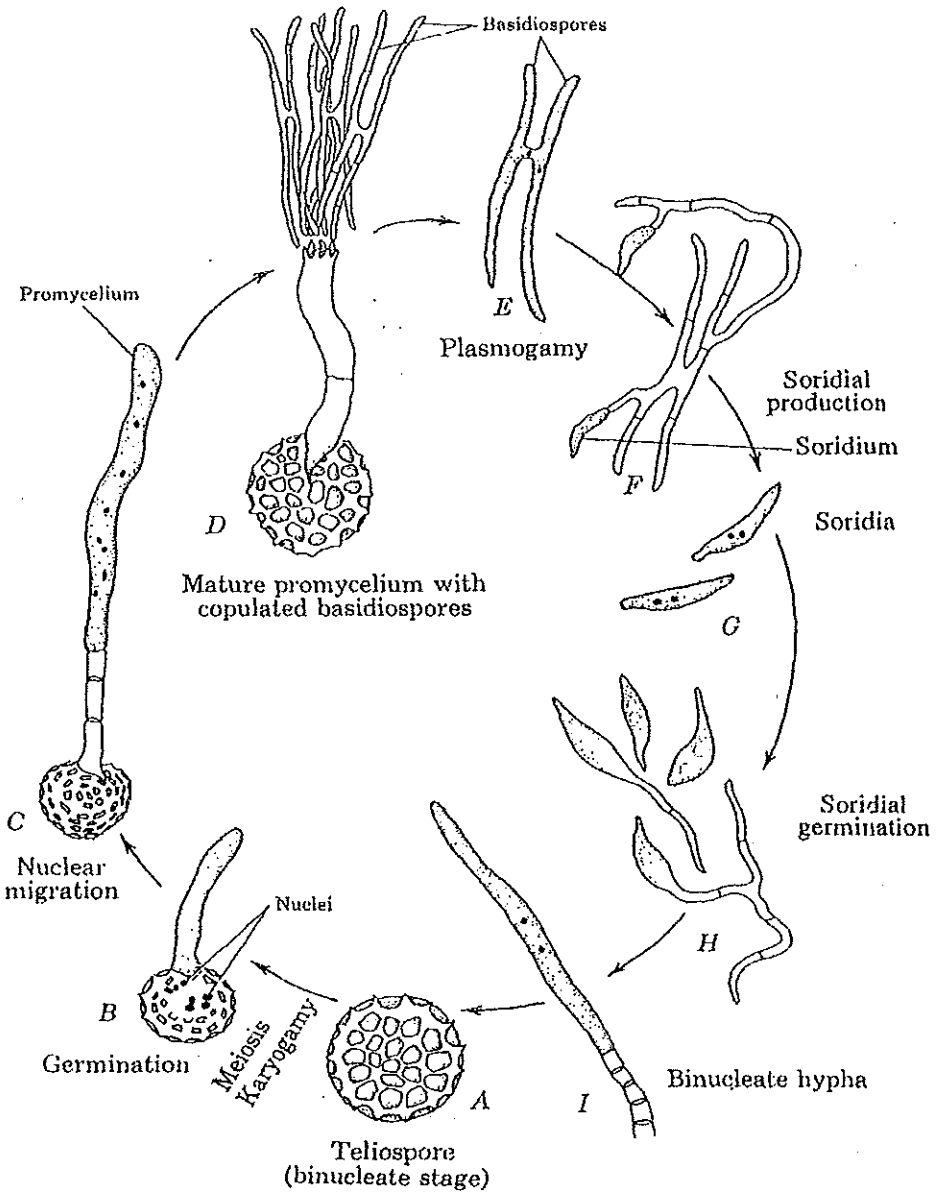
- Embryonic infection.
- It is not possible to determine whether a plant is diseased or not until the ears emerge when in infected plants the inflorescence is replaced by a mass of black, smut spores.
- Flag leaf may be infected in highly susceptible plants.
- Only glumes are affected in resistant plants
- Once spores are exposed they are blown by wind to flowers of healthy plants.
- Infection by *U. nuda* occurs through ovary walls, hyphae cross pericarp, enter the testa (intracellular), move towards the embryo (intercellular).
- Infected grains appear as healthy.
- When grains germinate, fungal mycelium becomes active, passes into the crown node of the seedling and is carried up during growth to the inflorescence primordea.
- Spore formation begins some weeks before the ears emerge and is complete at emergence.

2- Loose smut of oat:

- caused by *Ustilago avenae*
- Seedling infection.
- Spores are dispersed at flowering.
- On germination, the mycelium becomes established in glumes and pericarp.
- Embryo not invaded.
- When seeds are planted the dormant mycelium becomes active and invades the young seedlings.
- Subsequent development of the fungus is similar to that of *U. nuda.*

3- Bunt of wheat (stinking smut)

- caused by *Tilletia caries.* Seedling infection.
- Spores have an odor of bad fish (trimethylamine).
- All parts of the grain except the coat are replaced by smut spores.
- Infected grains (Bunt balls) are shorter and plumper than healthy.
- Broken bunt balls release millions of spores which contaminate healthy grains
- When contaminated grains are sown, spores on the grain coat germinate and the binucleate hyphae formed by fusion of sporedial cells infect the young coleoptile.
- Subsequent events are similar to those in U. nuda

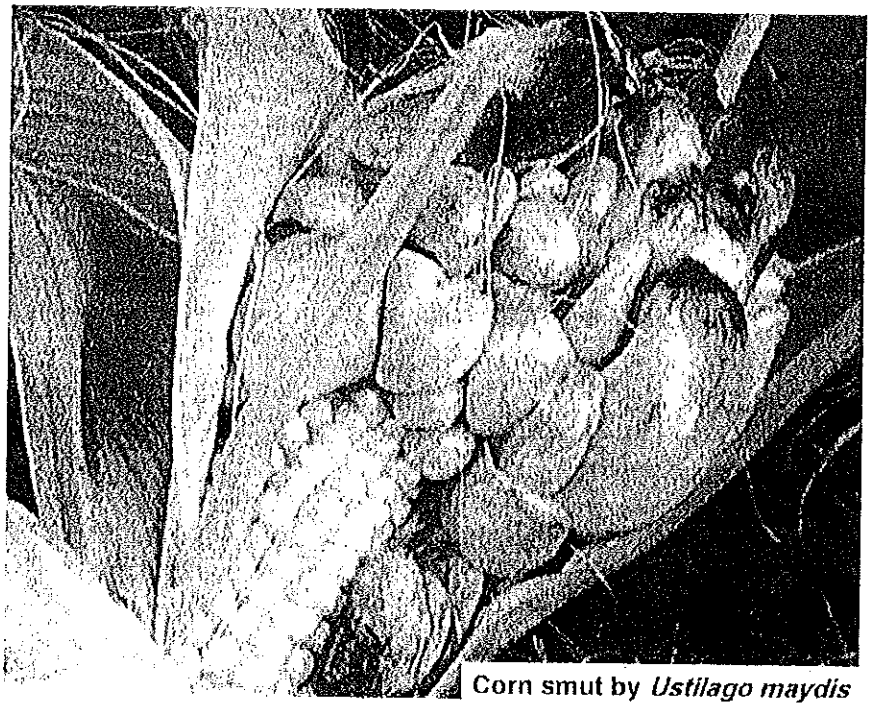


Life cycle of *Tilletia carie* cause of bunt of wheat

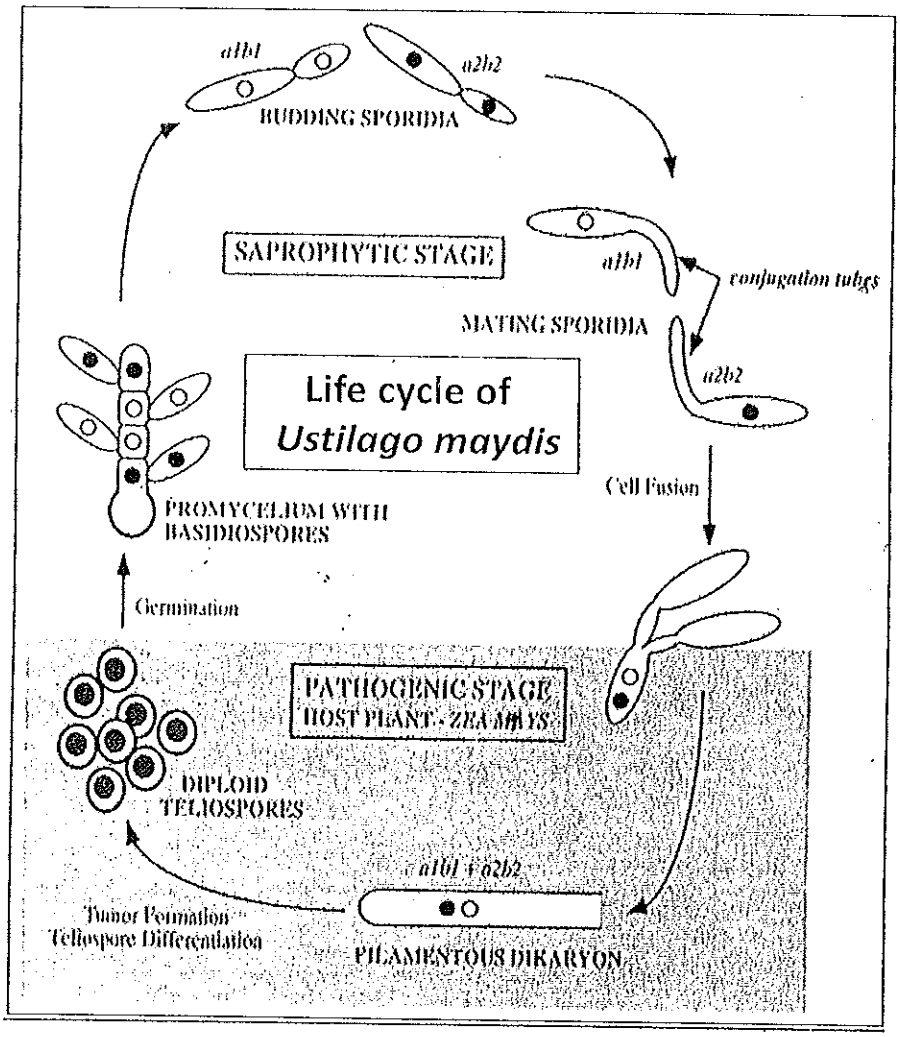
4- Sugarcane smut: Black, whiplike sorus arising from the terminal meristem of a stalk infected by *Ustilago scitaminea.*

5- - Onion smut by *Urocystis cepulae*

6- Common smut of corn by *Ustilago maydis*



Corn smut by *Ustilago maydis*



VII- Diseases caused by Deuteromycetes

1- Fusarium and Verticillium wilts

Fungus	Host
F. oxysporum f.sp. lycopersici	Tomato, potato, eggplant
F. oxysporum f.sp. vasinfectum	Cotton
F. oxysporum f.sp. conglutinans	Cabbage
F. oxysporum f.sp. Cubense	banana
F. oxysporum f.sp. betae	sugarbeet
Verticillium alboatrum (dark resting mycelium)	Tomato, alfalfa
Verticillium dahliae (microsclerotia)	*Potato, tomato,*
Verticillium nigrescens (chlamydospores)	Tomato

Mode of infection and survival:

- Wilt inducing forms of *Fusarium* and *Verticillium* enter their hosts through uninjured young roots or injured old roots.
- Plants infested by nematodes are severely attacked by wilt fungi.
- Fungi invade root cortex but do not damage it to a great extent. They become established in xylem vessels.
- Fungal enzymes disintegrate walls of xylem vessels.
- Fungal toxins are considered as a cause of wilt.
- Survival: by resting mycelium, chlamydospores or microsclerotia.

Disease symptoms:

- Lower leaf-petioles bend downwards (epinasty)
- Slight vein clearing and yellowing of the lower leaves.
- Chlorosis and death of leaves.
- Similar symptoms develop on younger leaves.
- During hot days, leaves wilt ,then recover at night.
- Wilt becomes permanent and plants die.
- Browning of vascular system.
- Water supply to leaves is plugged with fungal mycelium, conidia, tyloses and gums
- Some collapse of the vessels and disintegration of adjoining parenchyma.

4- Bean Anthracnose by *Colletotrichum lindemuthianum*

- Symptoms and Signs
- Seedlings grown from infected seeds often have dark brown to black sunken lesions on the cotyledons and stems.
- Severely infected cotyledons senesce prematurely, and growth of the plants is stunted. Diseased areas may girdle the stem and kill the seedling.
- Under moist conditions, small, pink masses of spores are produced in the lesions. Spores produced on cotyledon and stem lesions may spread to the leaves.
- Symptoms generally occur on the underside of the leaves as linear, dark brick-red to black lesions on the leaf veins. As the disease progresses, the discoloration appears on the upper leaf surface.
- On pods. Small, reddish brown to black lesions.
- Mature lesions are surrounded by a circular, reddish brown to black border.
- During moist periods, the interior of the lesion may exude pink masses of spores.
- Severely infected pods may shrivel, and the seeds they carry are usually infected.
- Infected seeds have brown to black sunken lesions.

5- Post harvest diseases

Disease	Causal agent
Spoilage of corn grains	*Aspergillus flavus,*
Rot on peanut kernels	*Aspergillus flavus*
Blue rot of apple fruits	*Penicillium expansum*
Blue rot of Citrus fruits	*Penicillium italicum*
Green rot of Citrus fruits	*Penicillium digitatum*
Cladosporium rot of corn	*Cladosporium cladosporioides*
Penicillium rot of corn	*Penicillium oxalicum*
Fusarium rot of corn	*Fusarium graminearum*

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Part 3: Plant Flora

Lectures in Botany 8

Fourth year- Biology & Geology students

Professor

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Physiology of fungi

Sterilization can be defined as any process that effectively kills or eliminates transmissible agents (such as fungi, bacteria, viruses and prions) from a surface, equipment, foods, medications, or biological culture medium. In practice sterility is achieved by exposure of the object to be sterilized to chemical or physical agent for a specified time. Various agents used as steriliants are: elevated temperature, ionizing radiation, chemical liquids or gases etc. The success of the process depends upon the choice of the method adopted for sterilization.

These apply to all methods of control of microbial growth:

1. Agent used must be able to affect the micro-organisms directly.
2. The item must be cleaned so as to remove extraneous soil.
3. Moisture is essential for the action of chemical agents.
4. Killing of micro-organisms is not instantaneous, and the time required depends upon:
    a. The nature of the organism.

b. The nature of the agent used.
c. The numbers of organisms present.
d. The temperature.

Methods of Sterilization:

The various methods of sterilization are:

A- Physical Methods.
1- Thermal (Heat) methods.
2- Radiation methods.
3- Filtration methods.

B- Chemical Methods.

Physical Methods

1- Heat.

Heat sterilization is the most widely used and reliable method of sterilization, involving destruction of enzymes and other essential cell constituents. The process is more effective in hydrated state where under conditions of high humidity, hydrolysis and denaturation occur, thus lower heat input is required. Under dry state, oxidative changes take place, and higher heat input is required.

This is rapid and penetrates objects as well as clumps of micro-organisms, and all types of organism can be destroyed. It can be used in either a dry or moist form.

In it's dry form is kills by the oxidation effect, denaturing the cell's proteins. In it's moist form because the presence of the water allows the hydrogen bonds in the proteins to be broken down, denaturing the protein by a different method. With moist heat spores and bacteria are killed more rapidly at a given temperature for two reasons. Chemically the proteins are denatured due to the breaking of the hydrogen bonds, whilst physically hot water has greater heat content than air at the same temperature.

Dry Heat Sterilization: Examples of Dry heat sterilization are:

1. Incineration.
2. Red heat (Flamming).
4. Hot air oven.

Flaming: is 100% effective, done to loops and straight-wires in microbiology labs. Leaving the loop in the flame of a **Bunsen burner** or alcohol lamp until it glows red ensures that any infectious agent gets inactivated. This is commonly used for small metal or glass objects, but not for large objects. However, during the initial heating infectious material may be "sprayed" from the wire surface before it is killed,

contaminating nearby surfaces and objects. Therefore, special heaters have been developed that surround the inoculating loop with a heated cage, ensuring that such sprayed material does not further contaminate the area. Another problem is that gas flames may leave residues on the object, e.g. carbon, if the object is not heated enough.

Incineration:

Rapidly kills micro-organisms and is reliable, will also burn any organism to ash. It is used to sanitize medical and other biohazardous waste before it is discarded with non-hazardous waste.

Hot air oven:

The use of hot ovens is not as efficient as moist heat, as higher temperatures are required for longer periods of time to ensure cell death, e.g. 120 ^{o}C of dry heat will take 8 hours to achieve sterilization. This is reduced to 2 hours minutes at 180 ^{o}C. Sterilization by this method is also effective for enclosed containers, and items made from glass or metal, but not cloth or rubber.

Moist Heat Sterilization:

Moist heat may be used in three forms to achieve microbial inactivation

1. Dry saturated steam – Autoclaving.
2. Boiling water/ steam at atmospheric pressure.
3. Hot water below boiling point.

Moist heat sterilization involves the use of steam in the range of 121-134°C. Steam under pressure is used to generate high temperature needed for sterilization. Saturated steam (steam in thermal equilibrium with water from which it is derived) acts as an effective sterilizing agent. Steam for sterilization can be either wet saturated steam (containing entrained water droplets) or dry saturated steam (no entrained water droplets).

Autoclaves:

Use pressurized steam to destroy microorganisms and are the most dependable systems available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents. For efficient heat transfer, steam must flush the air out of the autoclave chamber. Before using the autoclave, check the drain screen at the bottom of the chamber and clean if blocked. If the sieve is blocked with debris, a layer of air may form at the bottom of

the autoclave, preventing efficient operation. Autoclaves should be tested periodically with biological indicators like cultures of *Bacillus stearothermophilus* to ensure proper function. This method of sterilization works well for many metal and glass items but is not acceptable for rubber, plastics, and equipment that would be damaged by high temperatures (Figure: 1).

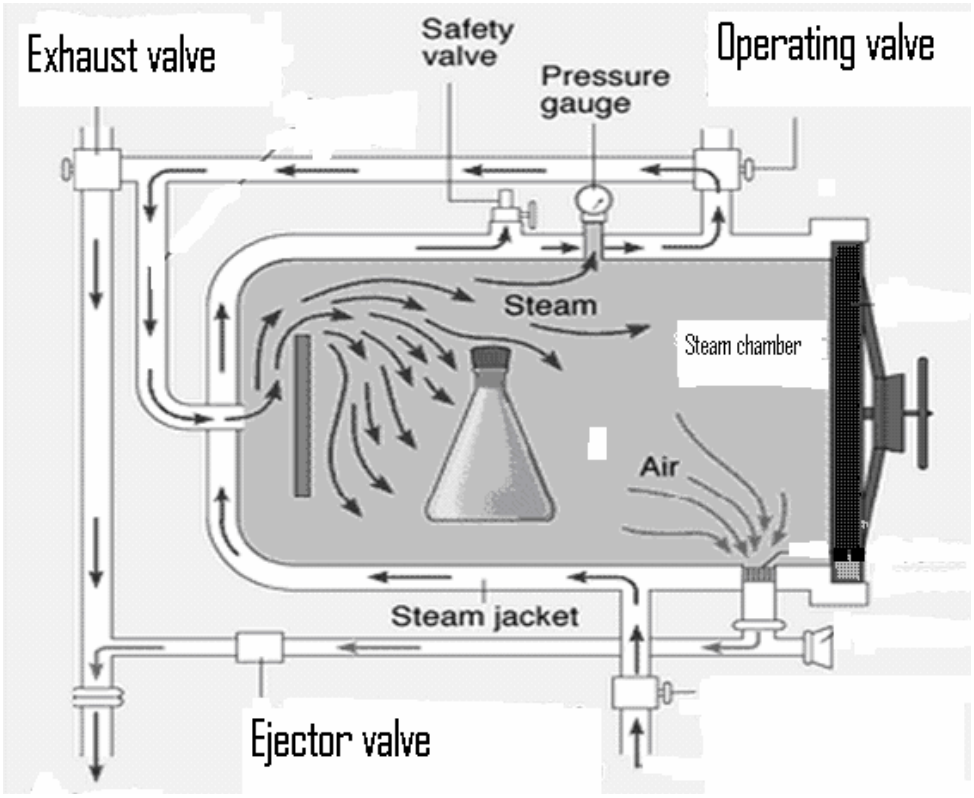


Fig.(1): An Autoclave

Boiling in water:

Boiling in water for fifteen minutes will kill most vegetative bacteria and inactivate viruses, but boiling is ineffective against prions and many bacterial and fungal spores; therefore boiling is unsuitable for sterilization. However, since boiling does kill most vegetative microbes and viruses, it is useful for reducing viable levels if no better method is available. Boiling is a simple process, and is an option available to most people, requiring only water, enough heat, and a container that can withstand the heat; however, boiling can be hazardous and cumbersome.

Tyndallization:

Named after John Tyndall is a lengthy process designed to reduce the level of activity of sporulating bacteria that are left by a simple boiling water method. The process involves boiling for a period (typically 20 minutes) at atmospheric pressure, cooling, incubating for a day, boiling, cooling, incubating for a day, boiling, cooling, incubating for a day, and finally boiling again. The three incubation periods are to allow heat-resistant spores surviving the previous boiling period to germinate to form the heat-sensitive

vegetative (growing) stage, which can be killed by the next boiling step. This is effective because many spores are stimulated to grow by the heat shock. The procedure only works for media that can support bacterial growth it will not sterilize plain water. Tyndallization is ineffective against prions.

2- Radiation Sterilization

Many types of radiation are used for sterilization like:

1- Electromagnetic radiation (e.g. gamma rays and UV light).
2- Particulate radiation (e.g. accelerated electrons).

The major target for this radiation is microbial DNA. Gamma rays and electrons cause ionization and free radical production while UV light causes excitation.

Radiation sterilization with high energy gamma rays or accelerated electrons has proven to be a useful method for the industrial sterilization of heat sensitive products. But some undesirable changes occur in irradiated products, an example is aqueous solution where radiolysis of water occurs.

Radiation sterilization is generally applied to articles in the dry state; including surgical instruments, sutures,

prostheses, unit dose ointments, plastic syringes and dry pharmaceutical products.

UV light, with its much lower energy, and poor penetrability finds uses in the sterilization of air, for surface sterilization of aseptic work areas, for treatment of manufacturing grade water, but is not suitable for sterilization of pharmaceutical dosage forms.

3- Filtration Sterilization:

Filtration process does not destroy but removes the microorganisms. It is used for both the clarification and sterilization of liquids and gases as it can prevent the passage of both viable and non-viable particles.

The major mechanisms of filtration are sieving, adsorption and trapping within the matrix of the filter material. Sterilizing grade filters are used in the treatment of heat sensitive injections and ophthalmic solutions, biological products and air and other gases for supply to aseptic areas. They are also used in industry as part of the venting systems on fermentors, centrifuges, autoclaves and freeze driers. Membrane filters are used for sterility testing.

Application of filtration for sterilization of gases:

HEPA (High efficiency particulate air) filters can remove up to 99.97% of particles >0.3 micrometer in diameter. Air is first passed through prefilters to remove larger particles and then passed through HEPA filters. The performance of HEPA filter is monitored by pressure differential and airflow rate measurements.

Chemical Methods

Gaseous Sterilization:

The chemically reactive gases such as formaldehyde, (methanol, H.CHO) and ethylene oxide $(CH_2)_2O$ possess biocidal activity. Ethylene oxide is a colorless, odorless, and flammable gas.

The mechanism of antimicrobial action of the two gases is assumed to be through alkylations of sulphydryl, amino, hydroxyl and carboxyl groups on proteins and amino groups of nucleic acids. The concentration ranges (weight of gas per unit chamber volume) are usually in range of 800-1200 mg/L for ethylene oxide and 15-100 mg/L for formaldehyde

with operating temperatures of 45-63 °C and 70-75 °C respectively.

Both gases being alkylating agents are potentially mutagenic and carcinogenic. They also produce acute toxicity including irritation of the skin, conjunctiva and nasal mucosa.

a. Ethylene oxide sterilizer:

An ethylene oxide sterilizer consists of a chamber of 100-300-Litre capacity and surrounded by a water jacket. Air is removed from sterilizer by evacuation, humidification and conditioning of the load is done by passing sub-atmospheric pressure steam, then evacuation is done again and preheated vaporized ethylene oxide is passed. After treatment, the gases are evacuated either directly to the outside atmosphere or through a special exhaust system. Ethylene oxide gas has been used widely to process heat-sensitive devices, but the aeration times needed at the end of the cycle to eliminate the gas made this method slow.

b. Low temperature steam formaldehyde (LTSF) sterilizer:

An LTSF sterilizer operates with sub atmospheric pressure steam. At first, air is removed by evacuation and steam is admitted to the chamber.

Media

All the living organisms require energy for maintaining their life activities. Based on the sources from which this energy is derived by organisms, two distinct groups of living beings have been recognized.

1- **Chlorophyllous plants:** are categorized as phototrophs because they possess the mechanism to trap the radiant energy of the sun directly, which is stored as chemical potential energy in the assimilated organic compounds.
2- **Non-chlorophyllous plants:** Such organisms which lack the capacity to use solar energy directly are designated as the chemotrophs.

Both these groups of organisms have, however, certain basic requirements of raw material for their nutrition. Carbon, nitrogen, sulphur, phosphorus, sodium, potassium, calcium, magnesium, iron, manganese, zinc, copper, cobalt, molybdenum and vitamins are some of the important substances.

Among these, carbon occupies a unique position, because compounds with carbon to carbon linkage are the characteristic features of the animate world as a whole. Green plants can utilize inorganic carbon in the form of carbon dioxide, which is converted to carbohydrates, through photosynthesis. The photosynthetic organisms thus, do not depend upon any other form of life, but are self reliant, as far as the carbon assimilation is concerned, and hence are designated as the autotrophs. The non-chlorophyllous organisms, including fungi, on the contrary, depend entirely upon the autotrophs for meeting their carbon-requirement and are, therefore, categorized as the heterotrophs. This is because the heterotrophs are unable to harness the inorganic sources of carbon.

Nitrogen, a major constituent of proteins, nucleic acids and other cell-contents, is the next most needed element. For its utilization, it is the heterotrophs which seem to be better equipped. Fungi and bacteria exhibit considerable versatility with regard to utilization of nitrogen sources. Besides their capacity to obtain nitrogen from organic, inorganic, or

ammonium compounds, some of these micro-organisms can utilize atmospheric nitrogen also.

Sulphur and phosphorus are also well distributed in all the living beings. While sulphur forms an essential constituent of some amino acid, vitamins and hormones, phosphorus occurs as a component of phospholipids, nucleic acid, etc. Phosphorus plays a significant role in phosphorylation reactions, and through the energy-rich phosphate bonds, this element helps in the transference of energy in every living phosphate system. Utilization of these two elements also differs. While phosphorus is principally obtained in the form of phosphate, the utilization of sulphur varies in different organisms. Higher plants utilize inorganic sulphur only, while fungi and bacteria can use both the inorganic as well as organic sources of this element. Some bacteria posses the capacity to utilize the elemental sulphur as well.

All living organisms require vitamins and other growth factors though in minute quantity. Green plants and some bacteria can synthesize most of the vitamins required by them. So are many of the fungi also but a few of them exhibit their inability to synthesize some of these compounds and require

preformed vitamins or their precursors in the culture media.

The mineral requirements of all the organisms are generally met by the soil, which contains this element as inorganic salts. However, these compounds must be available in the form of solution before they can enter into a living system. This is accomplished through the agency of water, which as a solvent not only helps in the absorption and translocation of nutrients in general but is also essential for all metabolic processes of every living entity of this biosphere.

Classification:

Some of the common criteria for classifying media are their chemical composition, physical state and their empirical use. In fact, every medium is designed for a definite use and hence its physical and chemical characteristics must conform to its application and function.

A- According to their use: media may be categorized into the following types:

1) Routine laboratory media: These are media with certain complex raw materials of plant or

animal origin such as yeast extract, malt extract, peptone etc., and are employed for routine cultivation and maintenance of a wide variety of fungi.

2) Enriched media: these media are prepared by supplementing the routine laboratory media with some specific substances to meet the nutritional requirements of more fastidious and are employed for their cultivation.
3) Selective media: these media facilitate the isolation of a particular group of organisms of species from a mixed inoculum. Such media contain substances which inhibit all except the desired organisms.
4) Differential media: supplemented with certain reagents or chemicals, these media aid in differentiating between various kinds of organisms on the basis of visible differences in their growth patterns. However, such type of media is used more often in bacteriological aboratories.
5) Assay media: this type of medium is specifically employed for the assay of vitamins, amino

acids, antibiotics, disinfectants etc., and are of definite composition.

6) Biochemical media: such media are generally used for the differentiation of micro-organisms on the basis of their biochemical activities and are helpful in the study of their metabolic processes.

B- <u>According to chemical composition</u>: media are classified into the following types:

1) Natural media: a natural medium comprises entirely complex natural products of unknown composition. The raw material of a natural medium may be of plant or animal origin, and some of the common ingredients employed for this purpose include extracts of plant and animal tissues, e.g., fruits, vegetables, egg, milk, blood, body fluids, yeast, malt and manure extracts etc. Obviously, the chemical composition and concentration of a natural medium is not well defined. Because of their complex nature, these media can support a variety of organisms, and hence are quite useful for routine laboratory cultures of fungi.

2) Semisynthetic media: these media are so designed that some of their constituents are of known chemical composition, while others are derived from some natural sources with unknown composition. The chemical makes up of a semisynthetic medium is, thus, only partly known. Consequently, only a limited amount of control may be exercised on the composition and concentration of a semisynthetic medium, by making necessary changes in chemically known faction. Semisynthetic media have also limited application in physiological studies on fungi and can best serve as a routine medium. Potato dextrose agar is one of such accepted and popular media.
3) Synthetic media: these are chemically defined media of known composition and concentration and are exclusively composed of pure chemical substances. However, absolute purity of the ingredients is seldom achieved, although substances of only analytical reagent quality are used for such purposes. One account of their known composition as well as being in solution, these media are quite useful for nutritional and

metabolic studies of fungi. The composition of these media may be amended as per requirement and as such they may be simple or complex in make –up. A simple synthetic medium contains a single carbon and energy source, a nitrogen source, generally as ammonium salt, some sulphur and phosphorus sources and various minerals. All these ingredients are dissolved in a buffered aqueous base. However, for more fastidious organisms, a complex synthetic medium is designed by incorporating some additional factors such as certain vitamins, amino-acids, purines, pyrimidines etc., or by employing a multitude of carbon and nitrogen sources together.

C- <u>According to their physical states</u>: media are classified into the following types:

1) Solid media: Media in solid state are in use since the beginning of studies on fungi in the laboratory. The first laboratory culture of a fungus was obtained on a solid medium, viz. fruit slices. Some common examples of such

media are nutrient imprignated slices of potato, carrot, sugar-beet etc. and coagulated egg or serum. However, with the advent of agar-agar as a solidifying agent, such media have largely been replaced by agar media. Use of fruits and vegetable slices in the cultivation of fungi is now more or less restricted to the baiting technique employed for isolation of some specific organisms.

2) Solid- reversible to liquid media: Such reversible media were first introduced by Koch (1881) who observed that addition of 2 to 5 per cent of gelatin to the commonly employed media rendered them a semi-solid consistency. However, gelatin could not find a wide application because of its low melting point (37 °C), and also because it is hydrolized by many proteolytic bacteria at ordinary temperature. The use of agar-agar for solidifying culture media was also initiated the same year and in the same laboratory.

3) Semi-solid media: These are media with gelatinous consistency and are employed for specific purpose. They contain a small amount

of agar or some other solidifying agent like corn meal. These media are sometimes used for the study of motile reproductive structures of fungi.

4) Liquid media: These are media without any solidifying agent and are indispensable for most of the quantitative studies. Nutritional and metabolic studies on fungi, as well as microbiological assays are invariably carried on liquid media. Some of the advantage of liquid media is that they permit the cultures to be aerated, the mycelium to be weighed and the metabolic products to be analysed easily. However, with respect to routine studies, liquid media have some distinct disadvantages. Growth in liquid media does not manifest the morphological characteristics of organisms. They are also difficult to handle without disturbing the culture. Moreover, liquid media are least helpful in the purification of organisms from a mixed culture. For an even distribution of nutrients and for providing uniform aeration to growing fungus, the liquid cultures are sometimes put to constant mechanical shaking.

Fungal nutrition and cellular biosyntheses

Chemical requirements for growth

Yeasts and fungi have relatively simple nutritional needs and most species would be able to survive quite well in aerobic conditions if supplied with glucose, ammonium salts, inorganic ions and a few growth factors.

1- Macronutrients, supplied at millimolar concentrations, comprise sources of carbon, nitrogen, oxygen, sulphur, phosphorus, potassium and magnesium.
2- Micronutrients, supplied at micromolar concentrations, comprise trace elements such as calcium, copper, iron, manganese and zinc that would be required for fungal cell growth.

Some fungi are oligotrophic, apparently growing with very limited nutrient supply, surviving by scavenging minute quantities of volatile organic compounds from the atmosphere.

Being chemo-organotrophs, fungi need fixed forms of organic compounds for their carbon and energy supply. Sugars are widely utilized for fungal growth and can range from simple hexoses such as

glucose to polysaccharides such as starch, cellulose and aromatic hydrocarbons (inc. lignin). Table (3) outlines the variety of carbon sources that can be utilized by yeasts and filamentous fungi for growth.

Fungi are non-diazotrophic (cannot fix nitrogen) and need to be supplied with nitrogen-containing compounds, either in inorganic form such as ammonium salts, or in organic form such as amino acids. Ammonium sulphate is a commonly used nitrogen source in fungal growth media since it also provides a source of utilizable sulphur. Many fungi (but not the yeast *S. cerevisiae*) can also grow on nitrate, and if able to do so may also utilize nitrite. Nitrate reductase, followed by nitrite reductase, are the enzymes responsible for converting nitrate to ammonia.

Table (2): Elemental requirements of fungal cells

Element	**Common sources**	**Cellular functions**
Carbon	Sugars	Structural element of fungal cells in combination with hydrogen, oxygen and nitrogen. Energy source
Hydrogen	Protons from	Transmembrane proton motive

	acidic Environments	force environments vital for fungal nutrition. Intracellular acidic pH (around 5–6) necessary for fungal metabolism
Oxygen	Air, O2	Substrate for respiratory and other mixed-function oxidative enzymes. Essential for ergosterol and unsaturated fatty acid synthesis
Nitrogen	$NH4^+$ salts, urea, amino acids	Structurally and functionally as organic amino nitrogen in proteins and enzymes
Phosphorus	Phosphates	Energy transduction, nucleic acid and membrane structure
Potassium	K^+ salts	Ionic balance, enzyme activity
Magnesium	Mg^{2+} salts	Enzyme activity, cell and organelle structure
Sulphur	Sulphates, methionine	Sulphydryl amino acids and vitamins
Calcium	Ca^{2+} salts	Possible second messenger in signal transduction
Copper	Cupric salts	Redox pigments

Iron	Ferric salts: Fe^{3+} is chelated by siderophores and released as Fe^{2+} within the cell	Haem proteins, cytochromes
Manganese	Mn^{2+} salts	Enzyme activity
Zinc	Zn^{2+} salts	Enzyme activity
Nickel	Ni^{2+} salts	Urease activity
Molybdenum	Na_2MoO_4	Nitrate metabolism, vitamin B12

Most fungi can assimilate amino acids, amines and amides as nitrogen sources. Urea utilization is common in fungi and some basidiomycetous yeasts are classed as urease positive (able to utilize urea) whilst most ascomycetous yeasts are urease negative.

In terms of oxygen requirements, most fungi are aerobes. Although yeasts such as *S. cerevisiae* are sometimes referred to as facultative anaerobes, they cannot grow in strictly anaerobic conditions unless supplied with certain fatty acids and sterols (which they cannot synthesize without molecular oxygen). For aerobically respiring yeasts and fungi,

oxygen is required as the terminal electron acceptor. Different fungal species respond to oxygen availability in diverse ways and Table (4) categorizes fungi into different groups on this basis.

Sulphur sources for fungal growth include sulphate, sulphite, thiosulphate, methionine and glutathione with inorganic sulphate and the sulphur amino acid methionine being effectively utilized. Virtually all yeasts can synthesize sulphur amino acids from sulphate, the most oxidized form of inorganic sulphur.

Phosphorus is essential for biosynthesis of fungal nucleic acids, phospholipids, ATP and glycophosphates. Hence, the phosphate content of fungi is considerable (e.g. in yeast cells it accounts for around three to five percent of dry weight; the major part of this is in the form of orthophosphate ($H_2PO_4^-$), which acts as a substrate and enzyme effector). The fungal vacuole can serve as a storage site for phosphate in the form of polyphosphates. Both nitrogen and phosphorus availability may be growth limiting in nature. Filamentous fungi have evolved a number of biochemical and morphological strategies allowing capture of often poorly available phosphorus

within the natural environment. Plants exploit such efficiency during symbioses between their roots and certain mycorrhizal fungi.

Concerning requirements for **minerals**, potassium, magnesium and several trace elements are necessary for fungal growth. K and Mg are macroelements required in millimolar concentrations whereas other microelements (trace elements) are generally required in the micromolar range. These include Mn, Ca**,** Fe, Zn, Cu, Ni, Co and Mo. Toxic minerals (e.g. Ag, As, Ba, Cs, Cd, Hg, Li and Pb) adversely affect fungal growth at concentrations greater than 100 mM.

Fungal growth factors are organic compounds occasionally needed in very low concentrations for specific enzymatic or structural roles, but not as energy sources. These include vitamins (e.g. thiamin, biotin), purines, pyrimidines, nucleosides, nucleotides, amino acids, fatty acids and sterols. For fungi to have a growth factor requirement indicates that cells cannot synthesize the factor, resulting in the curtailment of growth without its provision in culture media. Some fungi (e.g. *Aspergillus niger* and *Penicillium*

chrysogenum) have very simple nutritional needs and can synthesize their own growth factors from glucose.

Physical requirements for growth

Most yeast and fungal species thrive in warm, sugary, acidic and aerobic conditions.

Temperature

The range of temperature for fungal growth is quite wide, but most species grow very well around 25 °C. Low-temperature psychrophilic fungi and high-temperature thermophilic fungi do, however, exist in nature. Fungal growth at various temperatures depends not only on the genetic background of the species but also on other prevailing physical growth parameters and nutrient availability. About high-temperature stress on fungal cells, **thermal damage can disrupt hydrogen bonding and hydrophobic interactions, leading to general denaturation of proteins and nucleic acids**.

Fungi, of course, have no means of regulating their internal temperature, and the higher the temperature the greater the cellular damage, with cell viability declining when temperatures increases beyond growth-optimal levels. Temperature optima

vary greatly in fungi, with those termed ‘thermotolerant’ growing well above 40°C. Thermotolerance relates to the transient ability of cells subjected to high temperatures to survive subsequent lethal exposures to elevated temperatures, such that *intrinsic* thermotolerance is observed following a sudden heat shock (e.g. to 50 °C), whereas induced thermotolerance occurs when cells are pre-conditioned by exposure to a mild heat shock (e.g. 30 minutes at 37 °C) prior to a more severe heat shock. Heat-shock responses in fungi occur when cells are rapidly shifted to elevated temperature and, if this is sub-lethal, induced synthesis of a specific set of proteins, the highly conserved ‘heat-shock proteins’ (Hsps), occurs. **Hsps play numerous physiological roles, including thermoprotection**.

Water activity (*a*w)

High water activity is required for growth of most fungi, with a minimum *a*w of around 0.65. Water is essential for fungal metabolism, and any external conditions that result in reduced water availability to cells (i.e. water stress’) will adversely affect cell physiology.

The term water potential refers to the potential energy of water and closely relates to the osmotic pressure of fungal growth media. Species such as *Zygosaccharomyces rouxii* and some *Aspergillus* species able to grow in low-water-potential conditions (i.e. high sugar or salt concentrations) are referred to as osmotolerant or zerotolerant. *S. cerevisiae* is described as non-osmotolerant yeast. Mild water stress, or **hypersomotic shock**, occurs in fungi when cells are placed in a medium with low water potential brought about by increasing the solute (e.g. salt, sugar) concentration. Conversely, cells experience **a hypo-osmotic shock** when introduced to a medium of higher osmotic potential (due to reducing the solute concentration).

Fungi are generally able to survive such short-term shocks by **altering their internal osmotic potential** (e.g. by reducing intracellular levels of K+ or glycerol). Glycerol is an example of a compatible solute that is synthesized to maintain low cytosolic water activity when the external solute concentration is high. Glycerol can effectively:

1. Replace cellular water.
2. Restore cell volume.

3. Enable fungal metabolism to continue.

Trehalose, arabitol and mannitol can similarly protect against osmotic stress. Evidence suggests that the accumulation of compatible solutes is attributed not only to their synthesis but also to control of membrane fluidity, thus preventing leakage of these to the external environment.

As for pH

Most fungi are acidiophilic and grow well between pH 4 and 6, but many species can grow, albeit to a lesser extent, in more acidic or alkaline conditions (around pH 3 or pH 8, respectively). Fungal cultivation media acidified with organic acids (e.g. acetic, lactic acids) are more inhibitory to yeast growth compared with those acidified with mineral acids (e.g. hydrochloric, phosphoric acids) because organic acids can lower intracellular pH (following their translocation across fungal plasma membranes). This forms the basis of action of weak acid preservatives in inhibiting growth of food spoilage fungi.

Many filamentous fungi can alter their local external pH by selective uptake and exchange of ions (NO_3^- or NH_4^+/ H^+), or by excretion of organic acids such as oxalic acid.

Other physical parameters

There are many parameters influencing fungal physiology include radiation (light or UV may elicit mycelial differentiation and sporulation in some fungi that produce airborne spores), aeration, pressure, centrifugal force and mechanical shear stress.

Nutrient uptake and assimilation:

Fungal cells utilize a diverse range of nutrients and employ equally diverse nutrient acquisition strategies. Fungi are non-motile, saprophytic (and sometimes parasitic), chemo-organotrophic organisms. Fungi exhibit dynamic interactions with their nutritional environment that may be exemplified by certain morphological changes depending on nutrient availability. For example, the filamentous mode of growth observed at the periphery of yeast colonies growing in agar is akin to a foraging for nutrients as observed in certain eucarpic fungi. Metabolic dynamism is also evident in yeasts, which, although not avid secretors of hydrolytic enzymes like higher filamentous fungi, are nevertheless able to secrete enzymes to degrade polymers such as starch

(amylolytic yeasts such as *Schwanniomyces occidentalis*).

Several cellular envelope barriers to nutrient uptake by fungal cells exist, namely the capsule, the cell wall, the periplasm and the cell membrane. Although not considered as freely porous structures, fungal cell walls are relatively porous to molecules up to an average molecular mass of around 300Da, and will generally retain molecules greater than around 700 Da. Generally, fungi can absorb only small soluble nutrients (monosaccharides, amino acids or small peptides).

The plasma membrane is the major selectively permeable barrier that dictates nutrient entry and metabolite exit from the fungal cell. Membrane transport mechanisms are important in fungal physiology since they govern the rates at which cells metabolize, grow and divide. Fungi possess different modes of active and passive uptake at the plasma membrane: -

1- Active transport: is the movement of a substance across a plasma membrane against its concentration gradient (from low to high concentration) Active transport of nutrients such

as sugars, amino acids, nitrate, ammonium, sulphate and phosphate in filamentous fungi involves spatial separation of the ion pumps mostly behind the apex, whereas the symport proteins are active close to the tip. Thus, nutrient uptake occurs at the hyphal tip as it continuously drives into fresh resource, and the mitochondria localized behind the apex supply ATP to support the ion pump.

2- Passive transport, unlike active transport, it does not require chemical energy. The rate of passive transport depends on the permeability of the cell membrane which, in turn depends on the organization and characteristics of the membrane lipids and proteins. free diffusion, facilitated diffusion, diffusion channels are consider kinds from passive transport.

Fungal cell wall growth

The structural polysaccharides in fungal cell walls include mannans, glucans and chitin and are synthesized from sugar nucleotide substrates formed by pyrophosphorylase enzymes. For example:

Glucose 1-phosphate + UTP → UDF-glucose + PPi

Mannose 1-phosphate + GTP → GDP-mannose + PPi

1- Glucan synthesis involves plasma-membrane-associated glucan synthetases for assembly of β -1, 3 linkages and β-1,6 branches of cell wall glucan.
2- Chitin (a polymer of N-acetylglucosamine) is an important fungal cell wall structural component and is involved in the yeast budding process and in dimorphic transitions from yeast to filamentous forms. Chitin synthetases catalyse the transfer of N-acetylglucosamine from UDP-N-acetylglucosamine to a growing chitin polymer within the cell wall.
3- Mannoproteins pre-assembled within the Golgi are delivered to the cell wall via vesicles from the vesicle supply center.

Various vesicles, containing:

a- Wall-synthetic enzymes.

b- Wall-lytic enzymes.

c- Enzyme activators

d- Certain pre-formed wall components.

Vesicles are transported to the growing hyphal tip, where they fuse with the plasma membrane and release their contents, which together with substrates delivered from the cytosol synthesize the growing cell wall.

Fungal metabolism

Carbon catabolism

Being chemo-organotrophs, fungi derive their energy from the breakdown of organic compounds. Generally speaking, fungi, but few yeast species, extracellularly break down polymeric compounds by secreted enzymes prior to utilization of monomers as carbon and energy sources. Due to their relatively large size (20–60 kDa), enzymes assembled by the Golgi are transported in vesicles to be secreted from sites of cell growth, essentially from extending hyphal tips. Enzymes may either:

1- Become linked to the cell wall as wall-bound enzymes

2- Or may diffuse externally to decay substrates within the local environment.

Some examples follow of hydrolytic, oxidative, peroxidative and free-radicalgenerating enzyme systems produced by fungi for the degradation of polymeric compounds.

Several lipolytic yeasts are known (e.g. *Candida rugosa, Yarrowia lipolytica*) that secrete lipases to degrade triacylgycerol substrates to fatty acids and glycerol.

In wood, the cellulose and hemicellulose components are embedded within a heteropolymeric 3D lignin matrix, thus forming a complex lignocellulose material. Only certain filamentous basidiomycete or ascomycete fungi are able to degrade the recalcitrant lignin component, making available the cellulose or hemicellulose components. These are known as white-rot fungi due to resultant colouration of the delignified wood. Such fungi employ a cocktail of oxidative (including laccase) and peroxidative enzymes, together with hydrogenperoxide- generating

enzyme systems, to attack the at least 15 different interunit bond types extant within the lignin polymer.

The manganese and lignin peroxidase enzyme systems operate by releasing highly reactive oxygen free radicals, which react with parts of the lignin molecule, generating a chain of chemical oxidations and producing a range of mainly phenolic end products.

White-rot fungi have applications in, for example:

1- Upgrading lignocellulose waste for animal feed.
2- Paper production and bleaching.
3- Bioremediation of contaminated land and water.

Brown-rot and soft-rot (in wet wood) fungi are only able to degrade the cellulose and hemicellulose components of wood. Cellulose decomposition involves the synergistic activity of:

A- Endoglucanases (hydrolyse the internal bonds of cellulose).
B- Exoglucanases (cleave cellobiose units from the end of the cellulose chain).
C- Glucosidase (hydrolyse cellobiose to glucose). Initial attack of cellulose microfibrills within the

cell wall may involve the generation of hydrogen peroxide.

The sequence of enzyme-catalysed reactions that convert glucose to pyruvic acid is known as **glycolysis**, and this pathway provides fungal cells with energy, together with precursor molecules and reducing power (in the form of NADH) for biosynthetic pathways. In serving both catabolic and anabolic functions, glycolysis is sometimes referred to as being an amphibolic pathway. Glycolysis may be summarized as follows:

Glucose → 2pyruvate + 2ATP + 2NADH + H^+

During glycolysis, glucose is phosphorylated using ATP to eventually produce fructose 1,6-biphosphate, which is then split by aldolase to form two triose phosphate compounds. Further phosphorylation occurs, forming two triose diphosphates, from which four H atoms are accepted by two molecules of NAD. In the latter stages of glycolysis, four molecules of ATP are formed (by transfer of phosphate from the triose diphosphates to ADP), and this results in the formation of two molecules of pyruvic acid. ATP

production during glycolysis is referred to as substrate-level phosphorylation.

Nitrogen metabolism

Fungi assimilate simple nitrogenous sources for the biosynthesis of amino acids and proteins. For example:

1- Ammonium ions are readily translocated and can be directly assimilated into the amino acids glutamate and glutamine, which serve as precursors for the biosynthesis of other amino acids.

   Glutamate is a key compound in both nitrogen and carbon metabolism and glutamine synthetase is important as it catalyses the first step in pathways leading to the synthesis of many important cellular macromolecules. Other important enzymes of fungal nitrogen metabolism include glutamate dehydrogenase and glutamate synthase (glutamine amide: 2-oxoglutarate-aminotransferase, or GOGAT), the latter requiring ATP. When glutamine synthetase is coupled with glutamate synthase this represents a highly efficient ‘nitrogen-scavenging’ process for fungi to assimilate ammonia into amino acids and citric

acid cycle intermediates. The particular route(s) of ammonium assimilation adopted by fungi depend on the concentration of available ammonium ions and the intracellular amino acid pools.

2- Proteins can also be utilized as nitrogen source following release of extracellular protease enzymes.

3- Some yeasts and fungi can use nitrate as a sole source of nitrogen through the activities of nitrate reductase,

$$NO_3^- \rightarrow NO_2^-$$

and nitrite reductase,

$$NO_2^- \rightarrow NH_4^+$$

The resulting ammonium ions can then be assimilated into glutamate and glutamine, which represent end products of nitrate assimilation by yeasts.

4- Urea can also be utilized following its conversion to ammonium by urea aminohydrolase (urea carboxylase plus allophanate hydrolase):

$$NH_2CONH_2 + ATP + HCO_3^- \rightarrow NH_2CONHCOO^- \rightarrow 2NH_4^+ + 2HCO_3^-$$

5- Amino acids can either be assimilated into proteins or dissimilated by decarboxylation, deamination, transamination and fermentation. Amino-acid degradation by yeasts and fungi yields both ammonium and glutamate. During fermentation, yeasts may produce higher alcohols or fusel oils such as isobutanol and isopentanol following amino-acid deamination and decarboxylation. These represent important flavour constituents in fermented beverages.

Cellular reproduction

Fungal growth involves transport and assimilation of nutrients, followed by their integration into cellular components, followed by biomass increase and eventual cell division. The physiology of vegetative reproduction and its control in fungi has been most widely studied in two model eukaryotes, the budding yeast, *Saccharomyces cerevisiae*, and the fission yeast, *Schizosaccharomyces pombe*.

1- Budding is the most common mode of vegetative reproduction in yeasts and multilateral budding is typical in ascomycetous

yeasts (see Table 1.11). In *S. cerevisiae*, buds are initiated when mother cells attain a critical cell size and this coincides with the onset of DNA synthesis. The budding processes results from localized weakening of the cell wall and this, together with tension exerted by turgor pressure, allows extrusion of cytoplasm in an area bounded by a new cell wall. Cell wall polysaccharides are mainly synthesized by glucan and chitin synthetases. Chitin is a polymer of N-acetylglucosamine and this material forms a ring between the mother cell and the bud that will eventually form the characteristic *bud scar* after cell division.

2- Fission yeasts, typified by *Schizosaccharomyces* spp., divide exclusively by forming a cell septum, which constricts the cell into two equal-sized daughters. In *Schiz. pombe*, newly divided daughter cells grow in length until mitosis is initiated when cells reach a constant cell length (about 14mm). The cell septumin *Schiz. pombe* forms by lateral growth of the inner cell wall (the primary septum) and proceeds inwardly followed by deposition of

secondary septa. Cellular fission, or transverse cleavage, is completed in a manner resembling the closure of an iris diaphragm.

In certain yeast species, the presence or absence of pseudohyphae and true hyphae can be used as taxonomic criteria (e.g. the ultrastructure of hyphal septa may discriminate between certain ascomycetous yeasts). Some yeasts grow with true hyphae initiated from **germ tubes** (e.g. *Candida albicans*), but others (including *S. cerevisiae*) may grow in a pseudohyphal fashion when starved of nutrients. Filamentous growth of yeasts by hyphal or pseudohyphal extension represents a different developmental pathway that is generally reversible. In other words, cells can revert to yeast unicellular growth in more conducive growth conditions, indicating that a filamentous mode of growth represents an adaptation by yeast to foraging when nutrients are scarce.

What constitutes a cell in filamentous fungi is ambiguous. The apical compartments of higher filamentous fungi are often multinucleate, and so the process of nuclear replication and segregation into a

newly extended septated hyphal compartment is known as the duplication cycle. Thus, *Aspergillus nidulans* apical compartments contain approximately 50 nuclei per compartment produced during a 2 hour duplication cycle period. Continued septation results in the formation of sub-apical compartments containing fewer nuclei. Hyphae also commonly branch, usually at some distance behind the leading growing hyphal tip and often just behind a septum in higher fungi. The processes that control branching are not fully elucidated but branch initiation is associated with the appearance of a Spitzenkörper at the site of tip emergence and extension. Branching allows filamentous fungi to fill space in an efficient and appropriate way, according to local environmental circumstances. So, fungi colonizing nutrient-rich substrata branch frequently, producing dense mycelia for resource exploitation, whereas hyphae colonizing nutrient-poor substrata branch less frequently producing effuse mycelia appropriate for resource exploration.

Rates of branching and tip growth are related to the cytoplasmic volume. Thus, the hyphal growth unit is a measure of the average length of hypha required to

support hyphal tip growth. It can be calculated from microscopic preparations growing on agar media as the ratio between the total lengths of mycelium and the total number of tips. The ratio becomes constant after the initial stages of growth, and is characteristic of each fungal species or strain.

Fungal cell death:

An understanding of the death of fungal cells is important from a fundamental viewpoint because fungi, especially yeasts, represent valuable model systems for the study of cellular ageing and apoptosis (programmed cell death). From a practical perspective, cell death in fungi is pertinent in relation to the following situations: industrial fermentation biotechnology (where high culture viabilities are desired), food preservation (regarding inhibition of spoilage fungal growth), food production (promotion of cellular autolysis for yeast extracts) and clinical mycology (where fungal death is the goal in treatment of human mycoses).

Numerous physical, chemical and biological factors influence fungal cell death, which may be defined as an inability of cells to reproduce.

1- **Physical factor**: fungi will die if confronted with excessive heat, extreme cold, high voltage electricity, ionizing radiation or high hydrostatic or osmotic pressures. When the cells' physiological protection responses are insufficient to counteract the cellular damage caused by such physical stress, cells will die. In industrial situations, physical treatments can be used to eradicate contaminant fungi. For example, exposure of yeasts to elevated temperatures may lead to their thermal death, and this is exploited in the pasteurization of foods and beverages to kill spoilage yeasts.

2- **Chemical factors can be classified into:**

a- **External chemical agents** are fungicidal, including toxic organic compounds, oxygen free radicals and heavy metals. Chemical preservatives are commonly employed as antifungal agents in foodstuffs, including weak acids such as sorbic, benzoic and acetic acids. These agents, which are

generally fungistatic rather than fungicidal, act by dissipating plasma membrane proton gradients and depressing cell pH when they dissociate into ions in the yeast cytoplasm. Similarly, sulphur dioxide, which has long been used to eliminate undesirable yeasts (and bacteria) from wine, dissociates within the yeast cell to SO_3^{2-} and HSO_3^-, resulting in a decline in intracellular pH, and this forms the basis of its antizymotic action. Fungicidal acids include medium-chain fatty acids (e.g. decanoic acid), which may cause the rapid fungal cell death of yeast by disruption of cell membrane integrity.

b- **Endogenous chemical factors** such as ethanol and other toxic metabolites (e.g. acetaldehyde) produced by fermentative activity, excessive intracellular acidity or alkalinity, inability to protect against oxidative damage or sequester toxic metals, may also prove lethal to fungi. If fungal cells are unable to detoxify or counteract detrimental effects of chemicals, they may die.

Commercial fungal products:

Role of yeast in bread making

Yeast plays a central role in the manufacture of bread. In this case the dough is infused with *Saccharomyces cerevisiae* and aerated. The dough is left to stand for a short period in a warm environment, during which time yeast cell respiration occurs and carbon dioxide is produced. Respiration can be summarized by the following equation:

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ ΔG^o(respiration) = -686kcal/mol

The production of carbon dioxide gives bread its light, airy quality.

While baking is essentially an aerobic process, some semi-anaerobic conditions develop in the dough and ethanol can be produced in small amounts. This, together with the carbon dioxide, is burnt off during the baking process and gives rise to the "**fruity smell**" often associated with bakeries. In some parts of Japan bread dough is left to stand for a few days, during which the oxygen is used up in respiration and fermentation commences. The dough soon contains appreciable levels of ethanol and is subsequently eaten

Antibiotics, Enzymes and Chemical Commodities from Fungi

The economic significance of fungal biotechnology cannot be understated; indeed, as this chapter will outline, fungi have been exploited to yield a range of important products, some of which have proved invaluable to mankind. Since the time of the Pharaohs, fungi have been utilized for simple food processing; however, the last century has seen the development of fungal biotechnology for the subsequent production of valuable commodities such as antibiotics, enzymes, vitamins, pharmaceutical compounds, fungicides, plant growth regulators, hormones and proteins. As we move into the 21st century, this list will expand further, but it is beyond the scope of this chapter to fully appreciate the enormous benefits and economic impact of fungi in the area of biotechnology.

Instead, we will concentrate on a number of the more economically significant production processes, which have been developed through the utilization of fungi. The diverse nature of some of the economically important products produced by fungi is demonstrated in Table.

Fungal metabolism:

A common link between all fungi is their heterotrophic nature: they cannot manufacture their own food and depend on the organic material in other organisms for their survival. In a broad sense, however, it is possible to ascribe fungi into two main groups depending on how they obtain and assimilate nutrients.

1- One group, the parasitic and mutualistic symbionts, obtains its nutrients in an effective manner from living organisms.

2- The second group, saprotrophs, has the ability to convert organic matter from dead organisms into the essential nutrients required to support growth. It is this second group that we are particularly interested in, as this group of organisms gives rise to the production of the main bulk of the commodities commonly associated with fungi. However, regardless of this division, within the fungal life cycle one can clearly delineate the production of certain products or metabolites into two phases, namely primary and secondary metabolism.

Table ( ): Fungal products of economic importance

Class of product	Typical example	Industrial/commercial application	Common production organism
Enzymes	Amylase	Starch processing Fermentation application	*Aspergillus niger* *Rhizopus oryzae*
	Cellulase	Animal feed industry Brewing	*Trichoderma longibrachiatum*
	Protease	Meat/leather industry, Cheese manufacture	*Aspergillus oryzae*, *Rhizopus oligosporus*
Organic acid	Citric acid Itaconic acid Malic acid Fumaric acid	Soft drinks industry Chemical industry Beverage/food industry Food industry	*Aspergillus niger* *Candida/Rhodoturula* *Candida* *Candida*
Vitamins	Riboflavin Pyridoxine D-erythro-ascorbic acid	Health industry Health industry Health industry	*Candida* *Pichia* *Candida*

Antibiotics	Penicillin Cephalosporin	Human/animal health Human/animal health	*Penicillium chrysogenum* *Cephalosporium acremonium*
Fatty acids	Stearic Dicarboxylic	Food industry Chemical industry	*Cryptococcus* *Candida*
Alcohol	Industrial alcohol Beverage alcohol	Fuel industry Beverage industry	*Saccharomyces* *Saccharomyces*
Pharmaceuticals	Lovastatin Cyclosporin	Human health Human health	*Monascus rubber* *Tolypocladium inflatum*
Amino acids	Lysine Tryptophan Phenylalanine	Health industry Health industry Health industry	*Saccharomyces* *Hansenula* *Rhodoturula*
Recombinant proteins	Insulin Phytase Hepatitis B surface antigen	Treatment of diabetes Phosphate liberation Vaccine preparation	*Saccharomyces cerevisiae* *Aspergillus niger* *Saccharomyces cerevisiae*

1- Primary metabolites are those that are essential for growth to occur and include proteins, carbohydrates, nucleic acids and lipids. Indeed, the precursors of these primary products must be synthesized if they cannot be obtained from the growth medium. These primary metabolites have essential and obvious roles to play in the growth of the fungus.

Typically, primary metabolites are associated with the **rapid initial growth phase** of the organism and maximal production occurs near the end of this phase. Once the fungus enters the stationary phase of growth, however, primary metabolites may be further metabolized.

Examples of primary metabolites produced in abundance include enzymes, fats, alcohol and organic acids. Economically speaking, primary metabolites are easily exploited as the biochemical pathways involved in their production are widespread throughout the fungal kingdom. This allows for the rapid screening of classes of fungi for such products and the rapid development of production processes for their utilization.

Primary metabolic processes have also been extensively usurped through the use of recombinant DNA technologies, to the extent that heterologous proteins can be routinely produced by the host fungus as part of its primary metabolic phase.

2- Secondary metabolites are not essential for vegetative growth and indeed may have little or no primary function within the organism. Secondary metabolites are produced when the organism enters the **stationary phase**, once the initial phase of rapid growth has declined. The metabolites produced in this phase are often associated with differentiation and sporulation and can have profound biological activities, which in some instances have been exploited economically.

A number of distinct differences are apparent between primary and secondary metabolites. **In the first** instance they have been shown to possess an enormous variety of biosynthetic origins and structures that are not, in general, found among the primary metabolites.

Secondly, their occurrence tends to be restricted to a small number of organisms and indeed can vary between individual strains of the same species.

Finally, their production is characterized by the generation of groups of closely related compounds, which may have very different biological properties. Important examples of secondary metabolites include medically important compounds such as antibiotics, statins, cyclosporins and ergot alkaloids. Agriculturally important secondary metabolites include strobilirubin, an antifungal compound, and plant hormones such as giberellic acid. Fungal biotechnology has developed, to allow the utilization of the metabolic processes inherent to the organisms, in a commercially viable manner.

Table ( ): Example of primary and secondary metabolites

Metabolites	**Example**	**Production organism**
Primary metabolites	Enzymes Industrial alcohol Organic acids Fats Polymers	*Aspergillus* sp. *Saccharomyces cerevisiae* *Aspergillus/Candida* *Candida* *Yarrowia*
Secondary metabolites	**Antibiotics:** Penicillin Fusidic acid	*Penicillium* *Fusidium coccineum*
	Cholesterol lowering agents: Lovastatin Mevastatin	*Monascus rubber* *Penecillium citrinum*
	Immunosuppressing drugs: Cyclosporin A	*Tolypocladium inflatum*
	Plant hormones: Giberellic acid	*Gibberella fujikuroi*

Antibiotic production:

Overview

The most studied secondary metabolites are a class of compounds known as antibiotics. These low-molecular-mass compounds are so called because at low concentrations they inhibit the growth of other micro-organisms. While many thousands of antibiotics have been discovered, their use has been limited to perhaps 60 at most due to the toxic properties they exhibit towards humans. Clinically speaking the majority of antibiotics are produced by the Actinomycetes, a bacterial order, and will not be dealt with here. Whilst several fungal genera produce antibiotics, only two do so to a commercially viable extent, *Aspergillus* and *Penicillium*. The b-lactams, of which penicillin is the most famous, not least because of its fortuitous discovery by Fleming in 1928, comprise a very large group of antibiotics and include both the cephalosporins and penicillins. In 2000 the estimated world market for antibiotics was $28 billion, which underlies the importance both medically and economically of these metabolites.

The word penicillin can be regarded as a generic term used to describe a large group of natural and

semi-synthetic antibiotics that differ only by the structure of the side chains on the core aminopenicillanic acid ring. As a rule the basic penicillin molecule consists of a b-lactam ring, a five-membered thiazolidine ring and a side chain. b-lactams with non-polar side chains such as phenylacetate and phenoxyacetate are hydrophobic in nature and include penicillin G (benzylpenicillin) and penicillin V (methylpenicillin). The non-polar penicillins are synthesized only by filamentous fungi.

At their core they all possess a b-lactam (four-atom cyclic amide) ring on which side-chain substitutions and differences give rise to a series of antibiotics each with differing anti-bacterial activity. In addition to the so-called classical b-lactams, semi-synthetic varieties can be manufactured by the removal of the naturally occurring side chains and the subsequent chemical derivitization of the core b-lactam ring. Figure (2): illustrates the core b-lactam ring and the basic structures of penicillin and cephalosporin.

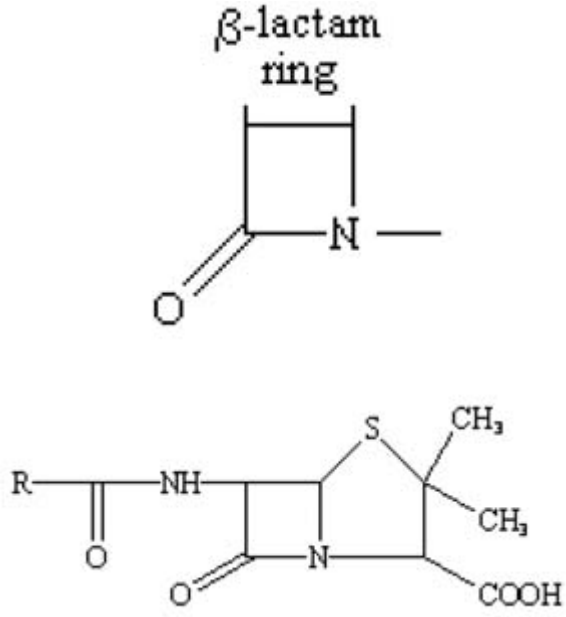
Gram positive bacteria possess on the outer aspect of the cell wall a layer that is composed of characteristic groupings of proteins and carbohydrates that comprise the antigenic determinants responsible for

generating an immune response. Inside this outermost layer there is a polymeric structural layer known as peptidoglycan which is composed of repeating units of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM). Associated with this cell wall structure are a number of proteins known as penicillin-binding proteins (PBP); the function of some is unclear. During cell wall biosynthesis, a cross-linking process occurs, whereby peptidoglycan strands become linked, leading to the structural stability of the wall. It is this cross-linking that is extremely sensitive to b-lactam antibiotics. For instance, various penicillins bind to the PBPs through their different side-chains, leading to a variety of effects. Reaction with PBP-1 (a transpeptidase) produces cell lysis, while binding to PBP-2 (also a transpeptidase) leads to the generation of oval cells, which are unable to replicate. Cephalosporins act in a very similar fashion to the penicillins and are also able to react with the PBPs by forming covalent bonds, thus leading to cellular lysis.

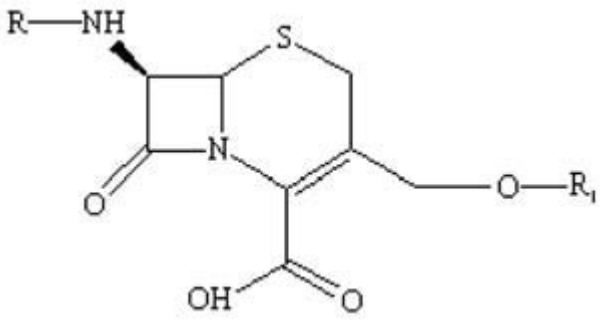
Gram negative cells have a more complex cell wall structure and usually contain an outer membrane and a complex periplasm containing lipopolysaccharides.

Whilst the gram negative cell wall also contains a peptidoglycan layer, it is not as extensive as that of gram positive bacteria but is sensitive to b-lactam antibiotics due to the presence of PBPs.

Penicillins with polar side chains, such as D-a-aminoadipate, include penicillin N and possess hydrophilic characteristics. They are more widely synthesized by a range of micro-organisms, including fungi, actinomycetes and unicellular bacteria.



Basic penicillin structure



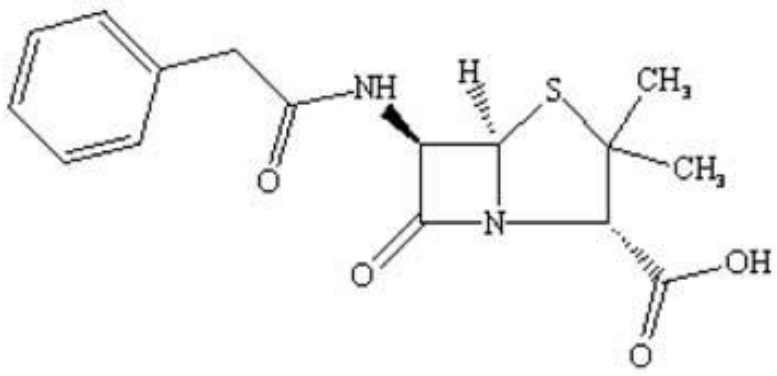
Basic cephalosporin structure
Fig. (2) : Structures of the core β-lactam ring and b-lactam antibiotics.

The production of semi-synthetic penicillins is quite easy and involves the removal of the side-chain from a naturally occurring penicillin and its subsequent replacement with a different side-chain to yield a novel b-lactam derivative. Examples of semi-synthetic varieties include methicillin and ampicillin. Figure (3): illustrates the structure of a natural and a semi-synthetic penicillin.

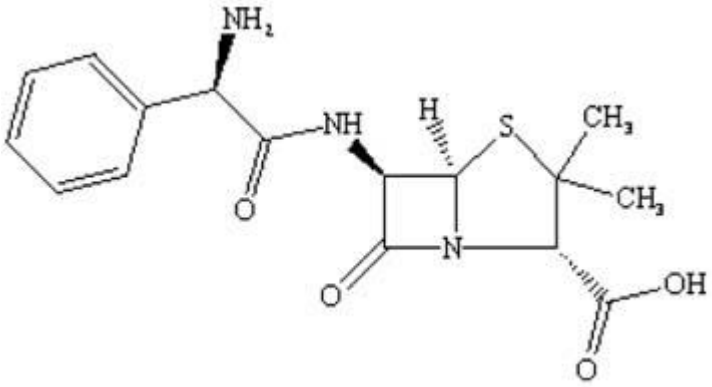
One serious problem with penicillins relates to the highly reactive nature of the β-lactam ring, which can result in their being susceptible to a variety of degradation processes. Factors that can affect their stability include: -

1- Their reactivity with hydroxide ions, which can result in the formation of inactive penicilloic acid, or their acid-sensitive nature, which can lead to their degradation at low pH. Acid sensitivity can be overcome clinically by use of the compounds in a buffering solution.

2- A more serious limitation to their use is their susceptibility to a group of enzymes known as **penicillinases**, which are produced by bacteria and can result in the development of antibiotic resistance. The most common of these enzymes is β-lactamase, which cleaves the β-lactam ring and thus inactivates the antibiotic. A variety of **acylases** have also been identified, whose mode of action is to



Penicillin G (natural)



Ampicillin (semi-synthetic)

Fig. (3): Natural and semi-synthetic penicillins

cleave the acylamino side-chain of the antibiotic, thus rendering it inactive.

To combat these enzymes many compounds such as clavulinic acid or the sublactams have been developed, which when given in combination with the susceptible antibiotic result in the permanent inactivation of the antibiotic-degrading enzymes. Of more importance, however, has been the development of the semi-synthetic penicillins, many of which are resistant to b-lactamase and other penicillinases. For instance, methicillin is completely resistant to these enzymes, though it does have the disadvantage that it is less effective. Almost all β-

lactamase-resistant penicillins are less potent than the parent molecules.

Cephalosporins are very structurally and chemically closely related to the penicillins, however, unlike the penicillins their use was limited for a long period until a clinically useful agent was found. Cephalasporin C is regarded as the prototypical cephalosporin and following its structural elucidation it was found to be a b-lactam with a six-membered dihydrothiazine ring instead of the five-membered thiazolidine ring characteristic of the penicillins. Chemical removal of the side chain of cephalosporin C results in the generation of 7-aminocephalosporonic acid (7-ACA), which can be used as a synthetic starting point for most of the cephalosporins available today. Indeed, it is more economically feasible to produce 7-ACA from penicillin G by a series of synthesis reactions rather than to incur the prohibitive costs of fermentation to produce the antibiotic.

One notable feature of the structure of the cephalosporins is their reduced chemical reactivity relative to the penicillins. However, some b-

lactamases are more efficient at cleaving cephalosporins than penicillins and this has led to the development of so-called second-, third- and fourth-generation cephalosporins.

These compounds all differ in their antimicrobial properties, susceptibility to microbial resistance, absorption, metabolism and side-effects. Examples of first generation, cephalosporins include cepalothin and cephazolin, second-generation cephalosporins include cefamandole and cefachlor, third-generation cephalosporins include cefotaxime and cefixime and fourth-generation examples include cefapime. The structures of the so-called first-, second-, third- and fourth-generation cephalosporins are illustrated in Figure (4).

Fungal production cycles

At a cellular level, the production pathways for the cephalosporins and penicillins share some similarities and indeed the first two steps are common to both classes of antibiotic.

1- Initially, a tri-peptide known as ACV is formed from the amino acids L-cysteine, L-a-aminoadipic acid and L-valine.

2- This key intermediate (ACV) is then converted to isopenicillin N (IPN) by the enzyme IPN synthase. It is this intermediate that gives rise to both the penicillins and the cephalosporins.

In the case of penicillin formation: IPN is hydrolysed to 6-amino penicillanic acid (6-APA), which can be used subsequently to give rise to specific penicillins.

Alternatively, in the formation of the cephalosporins: IPN is epimerized to penicillin N, which is further reacted enzymatically to yield deoxycephalosporin C. This last molecule can undergo further modification to give rise to cephalosporin C and cephamycin C.

As discussed earlier, the generation of semi-synthetic varieties of penicillins and cephalosporins is a simple process, by reacting the core penicillin compound 6-APA with a variety of organic acids, numerous penicillins can be formed. Indeed, the production of 6-APA is now carried out through the removal of the side-chain from penicillin G and then reacted to yield a range of antibiotics.

Similarly, the removal of the side-chain from cephalosporin C to yield 7-amino cephalosporonic acid (7-ACA), can lead to the generation of numerous cephalosporins through the reaction of this compound with a variety of acids.

Industrial production of antibiotics

Industrially speaking, penicillin production is a relatively inefficient process, where it is estimated that only 10 per cent of the carbon source utilized in the fermentation ends up as antibiotic. Production of b-lactam antibiotics occurs best under conditions of carbon, nitrogen and phosphorus limitation and at low growth rates. Each manufacturer uses a different production process, the details of which are closely guarded. Overall though, the basics of the production process are similar in nature.

1- Production starts with the inoculation of a primary culture from a preserved culture stock. Typically the culture stock can be in the form of lyophilized spores or spores preserved in liquid nitrogen.

A- Primary culturing can utilize either agar slants or liquid culture, with agar slants being the most common. The primary culture is used to inoculate a secondary culture.

B- Secondary culture is aimed in the case of antibiotic production to generate of spores. Secondary culturing can take place in agar-coated bottles or on particulate material, both of which result in the generation of a large quantity of spores.

2- A spore suspension prepared from the secondary culture is subsequently used to inoculate liquid media as part of an inoculum build-up process. It should be pointed out that stringent aseptic techniques are used throughout the process to prevent the contamination of the antibiotic producing culture with a more robust micro-organism. Practically speaking, industrial strains of antibioticproducing fungi are less robust than naturally occurring fungi due to the aggressive mutation and selection pressures placed on them when they were originally isolated.

3- Depending on the size of the process, the scale-up procedure can have as many as three or four stages. Typically, the initial seed culture produced from the secondary spore suspension is less than 10 L. Following a defined period of growth, this can be used to inoculate a culture of less than 20000L, which in the final stages of the production cycle will serve to start a culture of up to 300000 L.

4- One important point is the nature of the product that the production cycle is centred upon. Antibiotics are secondary metabolites, and in order to obtain the maximum productivity from the final stage culture it is necessary to ensure that growth of the organism is limited and the organism enters its secondary metabolism phase. This is usually achieved by designing the growth medium to ensure that a key nutrient becomes limiting at the right time to effect the change in metabolism necessary for antibiotic production. In the case of penicillin production, this is usually achieved by limiting the supply of glucose.

5- At the end of the fermentation it is necessary to separate the antibiotic material from the fungal mycelia, medium constituents and any other metabolites produced during the process. This is known as downstream processing, and the types of step involved will depend on the antibiotic in production and also on the production process. Typically it will involve some form of centrifugation or filtration to remove the fungal biomass and additional steps such as solvent extraction, ultrafiltration, chromatography and drying to produce a relatively, pure antibiotic, which can then be used for the manufacture of pharmaceutical preparations. It is estimated that over 10 000 tonnes of penicillin G are produced by fermentation each year.

Additional fungal antibiotics

Fungi also produce a number of other antibiotics, which are structurally unrelated to the b-lactams. Griseofulvin, a natural organic compound containing chlorine, is produced by *Penicillium griseofulvin*. This

compound is interesting as it inhibits the growth of fungi by preventing the assembly of fungal microtubules and thus mitosis. Another unrelated antibiotic is the steroidal compound fusidic acid, which is produced by *Fusidium coccineum*. This antibiotic is active against gram-positive bacteria and has clinical use against β-lactam-resistant strains of bacteria.

Enzymes

Overview

In contrast to the secondary metabolites we have discussed, primary metabolites such as enzymes have clearly defined roles in the fungal life cycle. In this instance, the production and secretion of enzymes serve as facilitators, enabling the fungus to obtain essential nutrients for its growth and reproduction.

However, by their very nature it has been possible to utilize these enzymes for numerous industrial processes. The industrial uses of enzymes are numerous and we will only concentrate on those of greatest economic significance. Some of the major fungal enzymes produced on an industrial scale, their

roles and the producer organisms are listed in below table

Table (12): Fungal enzymes of industrial importance

Enzyme	**Application**	**Source organism**
Amylase	Starch processing	*Aspergillus niger*
Cellulase	Cellulose and hemi-cellulose modification	*Trichoderma longibrachiatum*
Protease	Protein hydrolysis	*Aspergillus oryzae*, *Rhizopus oligosporus*
Lipase	Vegetable oil processing	*Rhizopus oryzae*
Phytase	Phosphorus release from phytic acid	*Aspergillus niger*
Glucoamylase	Soluble starch processing	*Aspergillus niger*
α-amylase	Production of high-maltose syrup	*Aspergillus niger*
Pectinase	Soft-drink manufacture	*Aspergillus niger*
Invertase	Confectionery	*Saccharomyces cerevisiae*
Lactase	Dairy	*Kluyveromyces*
Raffinase	Food processing	*Saccharomyces cerevisiae*

Applications of enzymes

Proteases have been utilized in food processing for centuries and their use today is very widespread, particularly those with acidic pH optima. **The biggest application for fungal protease** is in cheese manufacture where the enzyme rennet is used instead of the traditional preparation, calf chymosin, to hydrolyse a specific peptide linkage in the k-casein protein present in milk. This hydrolysis allows coagulation of the milk protein into curds, which can then be compressed and turned into cheese.

The reaction itself is dependent on:

1- The presence of $Ca2^{+}$ ions

2- Reaction temperature, thus leading to easy control of the process.

Proteases are widely used in the production of beer, where they are used to remove protein ‘hazes’. In general, proteolysis increases the solubility of proteins at their isoelectric points, a phenomenon that can be exploited in haze reduction. Proteases are also used extensively in the baking industry and can be used for wheat-gluten modification in biscuit preparation. Hydrolysis of soy protein using fungal protease can increase both the usage range and

value of this inexpensive protein source. Hydrolysed soy proteins can be added to cured meats or to impart flavour characteristics to soft drinks.

Starch is a polymeric structure made up entirely of glucose residues in either of two forms, α-amylose and amylopectin. In contrast to protein degradation, efficient starch hydrolysis relies on the combined actions of both bacterial and fungal enzymes.

Fungal cellulases have numerous industrial applications, for example in the brewing and fruit juice industries, where they are required for the removal of cellulose complexes, which have been found to interfere with the production process. These enzymes have also been utilized as digestive aids, particularly as supplements to animal feedstuffs. Cellulases are also widely employed in textile manufacturing, though problems can arise from the additional enzyme activities that may be present in industrial cellulase preparations. Commercially speaking, the main production organisms are strains of ***Trichoderma reesei***.

Fungal xylanases are used extensively as digestive aids, where they are effective in combating problems associated with arabinoxylan components of

plant based feed materials. Essentially, the enzyme breaks down the b-1,4 bonds between xylose residues and reduces the intestinal viscosity of wheat-based diets. As with cellulolytic enzyme preparations, industrial xylanase preparations are typically multi-component preparations made up of a number of endo- and exoacting xylanolytic enzymes. Xylanases are also widely used in paper manufacture, where they are used to degrade the lignin component in the pulp. In this instance there is a need for cellulase-free xylanolytic enzyme preparations.

Other fungal enzymes that are widely used include glucanases, lipases and hemicellulases, and the reader should refer to the reading list at the end of the chapter for further study material.

Antifungal Agents for Use in Human Therapy

Introduction

Despite the successes and achievements of modern medicine, competent immunity remains the best hope for effective and long-lasting protection against infection and disease. Inherited and acquired causes,

however, render the immune system deficient. The competence of the immune system can also be compromised by several disease states, chemotherapy and radiotherapy. Both immunocompromised and immunodeficient individuals are highly susceptible to viral, bacterial and fungal infections. The rising trends of viral, bacterial and fungal infections coupled with the appearance of new infective agents, the development of resistance against existing drugs and the toxic side-effects associated with current drugs necessitates a serious and continuous search for safer, more powerful and more selective anti-infective drugs.

With regard to fungal infections, potassium iodide was introduced in 1903 as an antifungal agent, followed by Whitefield's ointment in 1907 and then undecylenic acid in 1940. In the early 1950s, polyene antifungal drugs (nystatin and amphotericin B) were discovered and applied clinically. Since then, several classes of antifungal compounds have been introduced; however, serious problems continue to plague the use of antifungal compounds as therapeutic agents. Absence of selective toxicity of antifungal agents remains a major problem in the

clinical application of these drugs. Fungi, like mammalian cells, are eukaryotic cells and as such there is a great resemblance in cell structure and metabolism between them. Therefore, antifungal agents can indiscriminately disrupt the normal metabolic processes of both fungal and host cells. This is why the development of toxic side-effects is always a concern during or following chemotherapy with antifungal drugs. Insolubility or decreased solubility of many antifungal agents and the poor absorption through the gastrointestinal tract demands the use of parenteral routes, which increases the levels of toxicity associated with the use of antifungal agents. Fungi tend to infect poorly vascularized tissues; poor tissue penetration and distribution of systemic antifungals adversely affect the therapeutic efficacy of antifungal compounds.

Great efforts and large sums of money have been, and are being, spent on the development of new antifungal agents; nonetheless, the number of antifungal agents approved for clinical use is very limited. In the USA, only ten antifungal compounds are FDA approved to treat fungal infections. The limited number of molecular targets that fungal agents

can attack to kill or disrupt the metabolism of fungi makes the development of new drugs more challenging.

Antifungal agents currently available for clinical use belong to three major classes: polyenes, azoles and 5-fluorocytosine. Additional antifungal compounds such as allylamines, candines and nucleoside analogues plus some miscellaneous compounds with antifungal activity are also available. Optimally, antifungals should exhibit selective toxicity; they should be able to inhibit the growth of the fungus without adversely affecting the host. Based on the mechanism of action and the degree of toxicity to target fungal cells, antifungal agents can kill cells (fungicidal) or reversibly inhibit their growth (fungistatic). Antifungal agents may be naturally derived (antibiotic) or chemically synthesized (synthetic) compounds.

Polyene antifungal agents

The discovery of **nystatin (fungicidin)** by Rachel Brown and Elizabeth Hazen in the early 1950s has led to the isolation and characterization of numerous antibiotics. Amphotericin B (fungizone),

first isolated in the 1957 from *Streptomyces nodosus*, an actinomycete cultured from the soil of the Orinoco Valley in Venezuela, was the first commercially available systemic antifungal drug; so far, about 200 antifungal agents of this class exist.

However, problems associated with:

1- The stability
2- Solubility.
3- Toxicity
4- Absorption

of many such compounds reduced the number of polyenes approved for therapeutic use to only a few. In the UK and the USA, only amphotericin B and nystatin are approved for therapeutic use. While nystatin is useful for superficial mycoses, amphotericin B remains the drug of choice for the treatment of invasive and lifethreatening mycoses.

General properties of polyenes

Polyenes are characterized by a large macrolide ring of carbon atoms closed by the formation of an internal ester of lactone The macrolide ring contains

12–37 carbon atoms; the conjugated double-bond structure is contained exclusively within the cyclic lactone. A number of hydroxyl groups (6–14) are distributed along the macrolide ring on alternate carbon atoms. Amphotericin B has a free carboxyl group and a primary amine group that confer amphoteric properties on the compound, hence the drug's name. Being amphoteric, amphotericin B tends to form channels through the cell membrane causing cell leakage.

The amine group present in some of the polyene antibiotics is associated with an amine sugar connected to the macrolide ring through a glycosidic linkage Figure (6). The carbohydrate moiety in amphotericin B and nystatin is the mycosamine ($C_6H_{13}O_4N$; 3-amino-3, 6-dideoxymannose) sugar. Polyenes show limited solubility in water and non-polar organic solvents but they dissolve easily in polar organic solvents such as dimethyl sulphoxide or dimethyl formamide.

Although amphotericin B remains the preferred compound for treating systemic mycoses, problems associated with solubility in water, toxicity and ineffectiveness against mould diseases in

immunocompromised patients limit its therapeutic potential. Three lipid formulations of amphotericin B (amphotericin B lipid complex, amphotericin B cholesteryl sulfate and liposomal amphotericin B) have been developed and approved for use in the USA. These drug delivery systems offer several advantages over conventional amphotericin B. The parent drug can be introduced in much higher doses (up to ten-fold) compared with conventional amphotericin B. Clinical data suggest that all three formulations are indicated for patients with systemic mycoses who are either intolerant to conventional amphotericin B or have pre-existing renal dysfunction. However, there is debate regarding their use in immunocompromised patients with serious invasive mould infections (aspergillosis and zygomycoses). Amphotericin B cochleate, a novel lipid-based delivery vehicle formed by the precipitation of a negatively charged lipid and a cation (phosphatidylserine) and calcium has been developed. As cochleate lipid particles promote the absorption of amphotericin B from the gastrointestinal tract, the use of this route for therapeutic purposes can now be considered.

Mechanism of action of polyenes

Polyene antibiotics cause:

1- Increase cell membrane permeability, which causes leakage of cellular constituents (amino acids, sugars and other metabolites), leading to cell lysis and death.
2- Inhibition of aerobic and anaerobic respiration observed in cells treated with polyenes is thought to be a consequence of leakage of cellular constituents.
3- Polyenes could also cause oxidative damage to the fungal plasmalemma, which may contribute to the fungicidal activity of the drugs.

Inhibition of fungal growth by polyenes depends, to a large extent, **on the binding of the drug to the cell**; only cells that bind appreciable amounts of the drug are sensitive.

Polyene antifungals selectively bind to membrane sterols – ergosterol in fungal cells and cholesterol in mammalian cells. All organisms susceptible to polyenes (yeast, algae, protozoa and mammalian cells) contain sterols in the outer membrane, while those resistant do not. Sensitive

fungi can be protected from the inhibitory effects of polyenes by adding sterols to the growth medium, which compete with membrane sterol for the drug.

Resistance to polyenes

Despite four decades of clinical use, resistance to nystatin and amphotericin B remains rare. Although yeast is intrinsically capable of giving rise to polyeneresistant variants, neither primary nor acquired resistance develops. Polyene resistant variants of several *Candida* species are known to exist; all resistant isolates have low membrane ergosterol content. Mutant strains of *Aspergillus fennellial* that are resistant to polyenes have sterols other than ergosterol in the cell membrane. Nystatin-resistant strains of *Saccharomyces cerevisiae* have 5, 6-dihydroergosterol instead of ergosterol as the main membrane sterol component. Resistant organisms with altered sterol content bind smaller amounts of polyenes compared with susceptible ones. Decreased binding capacity of polyenes to mutant strains of *C. albicans* could be attributed to one of several mechanisms:

(a) Decreased total ergosterol content of the cell without concomitant changes in sterol composition.

(b) The presence of low-affinity polyene-binding sterols instead of ergosterol (3-hydroxysterol or 3-oxosterol).

(c) Reorientation or masking of existing ergosterols so that binding with polyenes is less favoured.

The majority of polyene-resistant *Candida* isolates belong to the less common species of *Candida* (*C. tropicalis*, *C. lusitaniae*, *C. glabrata* and *C. guilliermondii*).

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Part 2: Physiology of Fungi

Lectures in Botany 8

Fourth year- Biology & Geology students

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Definition of the word Flora

The name Flora is a Latin name meaning " the mythological Roman goddess of flowers and spring" الهة الرومان الاسطورية من الازهار والربيع.

Flora is the plant life occurring in a particular region or time.

Plants are grouped into floras based on region (floristic regions), period, special environment, or climate.

Regions can be geographically distinct habitats بيئات واضحة جغرافيا like mountain and flatland.

Definition of the word Flora

Floras can mean plant life of a historic era as in fossil flora.

Floras may be subdivided by special environments:

Native flora. The native and indigenous الاهلية flora of an area.

Agricultural and horticultural flora (garden flora). Plants are deliberately بتعمد grown by humans.

Weed flora. نباتات الأعشاب الضارة this classification was applied to plants regarded as undesirable, and studied in efforts to control or eradicate them.

Plant Flora concept:

Plant life on the earth can be distinguished from each other in several ways. The simplest is to divide on the basis of region. Plants that grow specifically in the mountains will be very different from those that grow in the desert. Similarly, the plants that have adapted to living underwater are treated as a unique form of flora. Scientists can also study ‘Fossil Flora’, which comprises of plant life that was found in pre-historic times. The current flora and fauna of the earth is also divided on the basis of the environment in which it is grown or seen naturally.

When we talk about ‘Native Flora’, we are referring to the plant life that is indigenous to a particular region. Cacti are the native flora of deserts all over the world. They can grow in most weather conditions but are native to the sandy dunes of deserts. When we refer to ‘Agricultural Flora’, we are talking about plant life that has been grown by humans for a certain purpose.

They may or may not be native but are used by humans for their own needs. Similarly, there is ‘Garden Flora’ or ‘Horticultural Flora’, which are plants grown for decorative purposes. And then we have the ‘Weed Flora’,

which are plants that are either undesirable in certain areas or invasive within the native plant life.

An Herbarium

1. History of Herbarium:

The art of herbarium was initiated by an Italian taxonomist from Bologna, Italy, named Luca Ghini (1490-1556), who collected plants, dried and affixed them on paper with gum, in the form of herbarium specimens. He had a collection of about 300 specimens in 1551.

Not much is currently known about his herbarium, which is now lost. Later, his student Gherards Cibo, continued this art, and his herbarium is still preserved in Rome. In the middle years of the sixteenth century, three Ghini students, namely Aldrovandi and Caesalpino from Italy and Turner from England, also made their own herbaria.

Caesalpinia's herbarium, currently in Firenze, is very important, as it can be compared with his book "De Plantis Libri XVI", which introduced a scientific approach to the study and classification of plants. An Englishman, John Falconer, who probably met Ghini in Italy, prepared an herbarium in 1553.

There are currently more than twenty herbaria created before 1600, preserved in various European cities. The first

recorded written published is that of the native of Brussel, Adrian Spieghel, in, “Isagoges”, a treatise of botany, which dates to 1606, dealing with how to dry plants and what kind of paper one should use, along with other accurate information.

2. Meaning of Herbarium:

The word herbarium (plural herbaria) was however, first applied by Pitton de Tournefort, in the book “Elemens”. Other herbaria were developed during the seventeenth century. A very good example is that of the Museum National d’histoire Naturelle in Paris. During this period, many collections of exotic plants were created, as a result of the many geographical explorations.

Some of these exotic herbaria have been of great importance for the advancement of scientific knowledge of some areas like Asia or Africa and can be currently seen in a few European museums.

It was Linnaeus who first started the current practice of mounting the plant specimens on separate sheets and storing them horizontally. Before Linnaeus, the actual practice was to sew dried plants on a sheet by thread and binding them into volumes.

With the simple beginning by Linnaeus, herbaria today have developed into facilities for storing millions of specimens. In

the earlier days the herbaria included plants of local or regional significance. But now, most of the herbaria include plants from different parts of the world and have developed into centers of advanced research in the field of taxonomy.

3. Functions of Herbarium:

A modern herbarium serves numerous valuable functions. Some of the important functions of herbaria are as follows:

1. An herbarium serves as an invaluable conservatory of plant material of flora. Collected from different parts of the world. Thus, they provide at one place, basic material for study of flora and vegetation of different places or regions.

2. Since it serves as a permanent record of flora of those regions, collections in the herbarium provide evidence of the vegetation of a region, which may be destroyed due to some natural catastrophes الكوارث الطبيعية.

3. The specimens in the herbarium carry valuable data on their labels. These include data on habitat, habit, local names, color of flowers or other characters of the plant, native uses of the plant, abundance or frequency of the species, associated plants, etc.

Such data provides valuable material for proper morphological description and range of variation of a similar plant collected from a different region, range of distribution and variation in its uses in different places. Thus, herbaria provide data for botanical, ethno-botanical and phytogeographical studies.

4. The herbarium serves as an aid in teaching botany to students in institutions where an herbarium is present, as it helps a teacher to show his students a plant specimen which may not be available fresh at the time of giving the course. It also helps students to identify local plants collected by them.

5. Preserved specimens of herbaria are used in almost all types of taxonomic research. It is believed to be an essential requirement for biosystematics research today, for correct identification and nomenclature of the plant under study.

6. The specimens in the herbaria are very often used as a source of material for anatomical, palynological and chemo-taxonomical studies.

7. The herbaria provide important data on actual places of occurrence, time of flowering and fruiting, associated

species and other data for research in embryology, cytology and ecology.

8. The herbaria have proved to be very valuable source of information for ethno-botanical research as many native uses of plants are recorded on the herbarium sheets.

4. Kinds of Herbarium:

Depending upon the interest of the organization or institution, the contents of holding and the labels and notes on the sheets in an herbarium vary accordingly.

(a) The herbaria of organizations like Botanical Survey contain all collections from any part of the world.

(b) Those institutions, which are interested in drugs and medicines, have herbaria, which include specimens of plants of known medicinal properties.

5. Important Herbarium of the World:

Many herbaria have been established in different parts of the world over a period of the last four hundred years.

Nearly from ten years ago, there are about one thousand two hundred recognized herbaria in the world, excluding

many smaller unrecorded herbaria of various universities and colleges.

The following tables give the name of the institution, location, approximate number of sheets, year of founding and the standard abbreviation of some important herbaria in different parts of the world:

Table 4.1 : Some important herbaria of the world

Sl. No.	Name of Herbarium	Place	Year of founding	Abbreviation	Total number of sheets (Approx.)
1.	Royal Botanic Garden, Kew	London, UK	1853	K	6,500,000
2.	V.L. Komarow Botanical Institute Meseum National d' Histoire	Leningrad, USSR	1823	LE	5,000,000
3.	Naturelle Laboratoire de Phanerogramme	Paris, France	1635	P	5,000,000
4.	Coservatoire Et Jardin Botaniques	Geneva, Switzerland	1817	G	4,000,000
5.	New York Botanic Garden	New York, USA	1891	NY	3,000,000
6.	U.S. National Museum	Washington, USA	1868	US	3,000,000
7.	Vienna Botanischer Gaertn	Vienna, Austria	1748	W	2,500,000
8.	National History Museum	Chicago, USA	1893	F	2,350,000
9.	Royal Botanic Garden	Edinburgh, UK	1893	E	2,500,000
10.	Missouri Botanic Garden	St. Louis, USA	1859	MO	1,700,000
11.	National Herbarium	Melbourne, Australia	1857	MEL	1,500,000
12.	Zurich Botanischer, Gaertn	Zurich, Germany	1834	Z	1,500,000
13.	Gray Herbarium, Harvard University	Cambridge, USA	1807	GH	1,485,000
14.	Philadelphia Academy of Sciences	Philadelphia, USA	1812	PH	1,000,000
15.	Arnold Arboretum	Boston, USA	1872	A	700,000
16.	Department of Agriculture	Peradeniya, Ceylon	1817	PDA	85,000
17.	Gordon College	Rawalpindi	1893	RAW	60,000
18.	Herbarium of the Rangoon University	Rangoon, Burma	1947	RANG	15,000
19.	Botanical Research Institute of Texas	Texas	1985	BRIT	500,000
20.	Fowler Herbarium – Queen's University, Canada	Canada	1987	—	—
21.	G.F. Leningham Herbarium – University	Regina	1989	—	50,000
22.	New Mexico State Range Science Herbarium	New Mexico	—	—	20,000
23.	University of Florida Herbarium (FLAS)	Florida	1990	FLAS	25,000
24.	University of Minnesota Herbarium	Venezuela	1992	—	830,000
25.	Washington State University — Marion Ownbey Herbarium	Marion	1995	—	350,000

Table 4.2 : Some important herbaria of India

Sl. No.	Name of Herbarium	Place	Year of founding	Abbreviation	Total number of sheets (Approx.)
1.	Central National Herbarium	Shibpur, Howrah	1793	CAL	2,500,000
2.	Botanical Survey of India, Southern Circle	Coimbatore	1874	MH	200,000
3.	Botanical Survey of India, Eastern Circle	Shillong	1956	ASSAM	100,000
4.	Botanical Survey of India, Western Circle	Poona	1956	BSI	125,000
5.	Botanical Survey of India, Northern Circle	Dehradun	1956	BSD	60,000
6.	Botanical Survey of India, Industrial section	Kolkata	1887	BSIS	50,000
7.	Botanical Survey of India, Central Circle	Allahabad	1955	BSA	40,000
8.	National Botanic Garden, Herbarium	Lucknow	1948	NGB	100,000
9.	Forest Research Institute Herbarium	Dehradun	1816	DD	300,000

Method of collection

Following is an outline مخطط of the method of collection and how to preserve these plants for future study.

1. A field-bag to carry smaller items such as notebook, polythene and paper bags, binoculars, camera, GPS, pens or pencils, knives/scissors, Laptop's computer……. etc.

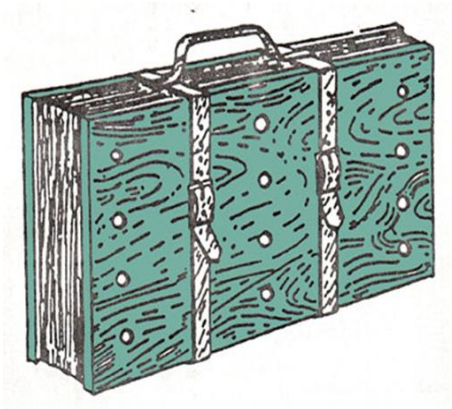
2. A field press consists of two end boards having 18 X 12 inches size and 1-inch thickness, two strong webbing straps, preferably 1.5 m long and non-slip buckles. Double faced cardboards have long axis (18X12 inches), enough quantity of old newspapers and blotting نشاف papers.

3. Number tags: Each tag بطاقةwill carry a number which enables the collector to find out the description of the specimen from the field notebook at a later stage.

4. Field book: Many features of the specimen cannot be detected from a dried specimen. The description in the field book will be a great help for the collector in identifying the plant. A usual field book contains information such as Name of the specimen, local name, locality, collectors name, collection number, date of collection, soil type and a brief description about the plant.

5. Cutting implements like pruners, showel مجرفة, axes فئوس, etc.

6. Polythene/paper bags of various sizes.



Collecting the specimens

- A botanical specimen may consist of one or more whole plants, complete with roots, stem, leaves, flowers and if possible, with fruits.
- If the plants are small, it is advisable to collect several specimens of the same plant from proximity.
- In the case of large herbs, shrubs or trees, it is necessary to collect a portion of a twig with leaves and flowers/fruits as a representative specimen.

The size of the specimen, if it is for preservation, depends upon the size of the sheet for mounting. Usual size of the herbarium sheet is 17X11 inches.

After collection, the specimen is tied with a tag with a number (same as the number given for the description of the plant written in the field book.). Most of the plants wilt very rapidly after being cut or dug out of the ground.

Previously, collectors used a metal box called vasculum into which the collected plants were placed for carriage to the field base or herbarium. Now the advent of polythene bags has become more convenient and can keep the plants fresh for more than 3 hrs.

Sometimes the flowers or fruits are too large to be pressed with the leaves. In such cases, flowers or fruits may be kept separately in small polythene or paper bags,

each containing the same number as the other specimen with leaves.

A minimum of two specimens should be collected for every species (depending upon the size of the populations). Once the collection is over, the next step is to press the specimens. This is the most difficult part of the collection and is to be done as early as possible.

In arid countries like Egypt, the specimens can be dried with the help of an ordinary press without using artificial heat.

- The ventilated drying press for this purpose can be prepared as follows:

1. Place the two straps horizontally on a table or the ground with buckles of the left.
2. Place one end board across the strap.
3. Then place one or two corrugated cardboards on the top of the end boards.
4. Take one of the specimens carefully from the polythene bag. - Make sure that the tag is still tied on to the specimen. The specimen is then placed in the newspaper folder.

While pressing, make sure that the leaves are upside down to show the ventral side.

The leaves which are too large to fit on the herbarium sheet need to be cut into parts or folded. Large, pinnately or

bipinnately compound leaves much be pruned to fit on the herbarium sheet.

While pruning, the base of the leafstalk should not be removed.

This may help taxonomists who examine the specimen at a later stage to get an idea of the position and number of leaflets.

If the flowers are too large or have deep tubular corolla, it is advisable to split the flower longitudinally so that their parts can be readily seen.

Many fruits are difficult to press satisfactorily because of their large size. Such fruits can be cut transversely and longitudinally and press one half or part of it.

5. Place a blotting paper or corrugated cardboard over the newspaper folder containing the specimen.

6. Repeat this method for the rest of the specimens.

When the press has sufficient number of specimens with blotting papers and corrugated cardboards alternating with the newspaper folders, the other end board can be placed over it and tighten the straps evenly.

The specimens have to examined periodically, preferably in every 24 hrs.

- The newspapers, if moist, need to be replaced with new ones. While changing the newspapers, make sure that the

number tag is still with the material and all the fragments such as flowers or fruits are also transferred to the new newspaper folder.

- This way moisture is absorbed from the material to absorbent by the simple process of diffusion.
- The used absorbents can be made ready for re-use by placing them outside during the daytime or in a drier.

During winter season an artificial drier will be helpful to speed up the removal of moisture from the specimens.

This can be done by making a wooden box with metal net or a perforated sheet on the top side and two or three electric bulbs connected at the bottom of the box.

The number of days that the materials remain in the press depend on the type of specimens.

As a rule, a plant is ready when it is crispy and not cool when touched.

In many tropical and sub-tropical countries, poisoning the specimens is another important step before mounting in order to prevent any possible fungal or bacterial attack in future.

The solution used for this purpose consists of Mercuric Chloride, Ammonium Chloride and Ethyl Alcohol.

The quantity of chemical used at a time depends upon the number of specimens to be poisoned.

As a rule, a plant is ready when it is crispy and not cool when touched.

Dissolve 150 grams of Mercuric Chloride and 350 gm of Ammonium Chloride in a little water as possible.

To This add 10 liters of 96% alcohol.

Applying the chemicals can be done by brushing it gently on the specimens.

After poisoning, the specimens may remain in press for another day or two in order not to get the leaves and flowers wrinkled. The next step is mounting.

It can be mounted to any stiff paper of 17x11 inches size. Specimens can be mounted by using any non-toxic white glue available in the market or can be done by using narrow strips of adhesive tape either cloth or paper backed.

Gluing, as the name implies, involves the attachment of the specimen to the mounting sheet by applying glue on the underside of leaves and twigs.

After gluing, the specimen is mounted in the middle of the sheet and place small weight over it for some time.

Fragments of the inflorescence, detached flowers, broken twigs or loose fruits can be put in a separate plastic or paper bag (5X5 cm) and pined or stick at the left-hand corner of the herbarium sheet.

This can be used for studying the specimen in the future. Finally comes the labeling.

Usually the label (14X14 cm) is fixed at the right-hand bottom corner of the sheet.

A label consists of collection number, Latin name, Family Name, Vernacular Name, Habit, Habitat, Locality, Date of Collection and Collector's name.

A small space may be provided at the bottom of the label to write notes of any special interest.

In some labels, map of the country is also included in order to mark the locality of the plant from where it is collected.

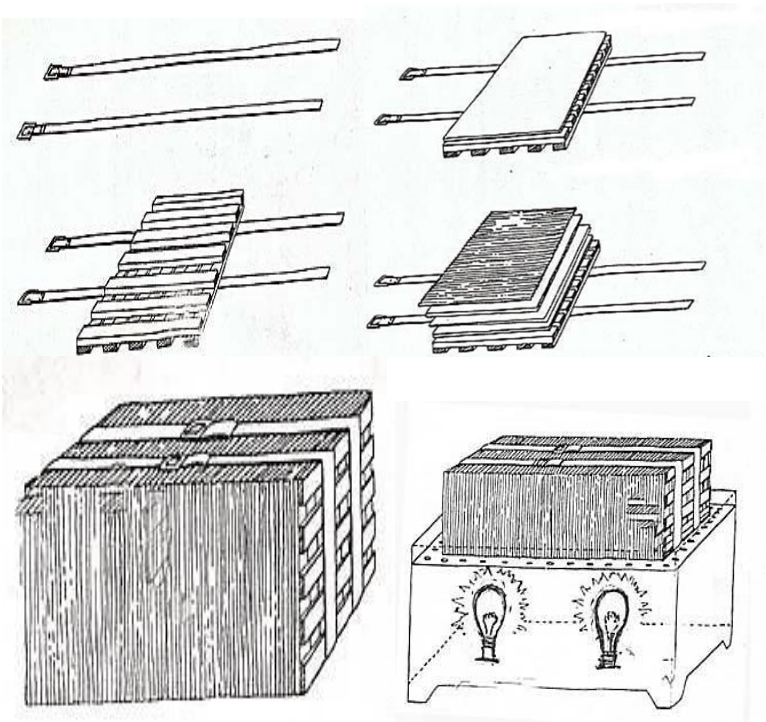
Storing the Herbarium sheets: Most of the genera have more than one species.

All these specimens can be brought together in a separate cover, called species cover.

This should be of a different color in order to differentiate it from the herbarium sheet.

In some herbaria species covers are grouped together in a genus cover.

The species covers should be clearly labeled with genus and species names and placed in the pigeon-holes of the cabinets in alphabetic order.



Plant Diversity in Egypt

Introduction

Egypt is one of the north African countries. It is occupying the northeastern corner of the Africa. It extends eastward in the Asia for approximately 61000 $km^{2.}$ (The area of the Sinai Peninsula. It is approximate roughly a square. It measures 1073 Kms in length, 1226Kms in width. It embraces a total area of a million sq. kms. Egypt is characterized by a warm and almost rainless climate. The air temperature rises to over 40 °C during summer, and seldom falls as 0 °C during the coldest nights. The average rainfall over the country from 200 mm in the coast to practically no rain at all in the south. With so scantly a rainfall, the greater part of Egypt consists of barren and inhospitable desert.

The Nile River within its Valleys, the Fayium depressions the Delta are fertile areas. It is true that Egypt fertile land is the Gift of the Allah. The desert area of Egypt is about 96% of the total area of the country. It consists of stony plateau in many places, dissected by valleys and in others, pitted with huge depressions and oases or covered with drifted sands. In some regions the desert is mountains. Ecologically, Egypt can be divided into 4 main

regions: **the Nile region, the Western Desert, the Eastern Desert and the Sinai Peninsula**.

1- <u>The Nile Region</u> comprises all the land formed and irrigated by the Nile River, and this include:

a. The Nile Valley or Upper Egypt, that extends from Wadi Halfa (350 km south of Aswan) in the Sudan-Egyptian border northward to Cairo for approximately 1530 km.

b. The Nile Delta or Lower Egypt, from Cairo northward to Rosetta and Damietta at the Mediterranean Coast and the Nile Fayium, a depression in the western desert and may comprised the northern lakes of the Nile Delta and the Deltaic Mediterranean Coast.

2- <u>The Western Desert comprises three subregions:</u>

- The western Mediterranean coast,
- The Oases and Depressions and
- Gebel Uweinat.

3- The Eastern Desert comprises:

- The Red Sea coastal land and
- The Inland Desert.

4- The Sinai Peninsula comprises three subregions:

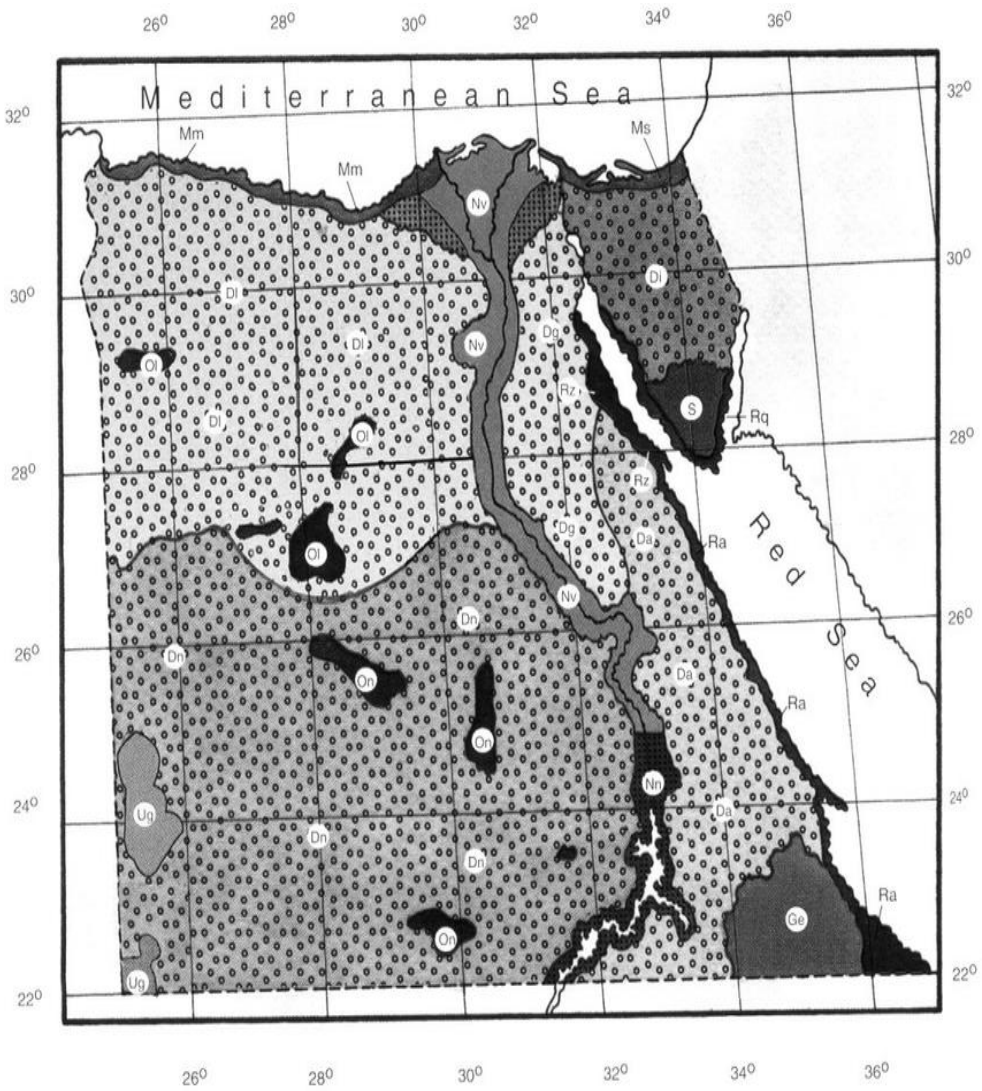
- The northern subregion that includes the eastern section of the Mediterranean coastal land of Egypt,

- The central and the Southern subregions with their coastal area along the Gulf of Aqaba and Gulf of Suez.

Each of the 4 main regions of Egypt is characterized by its environmental characterize which produce different floristic elements.

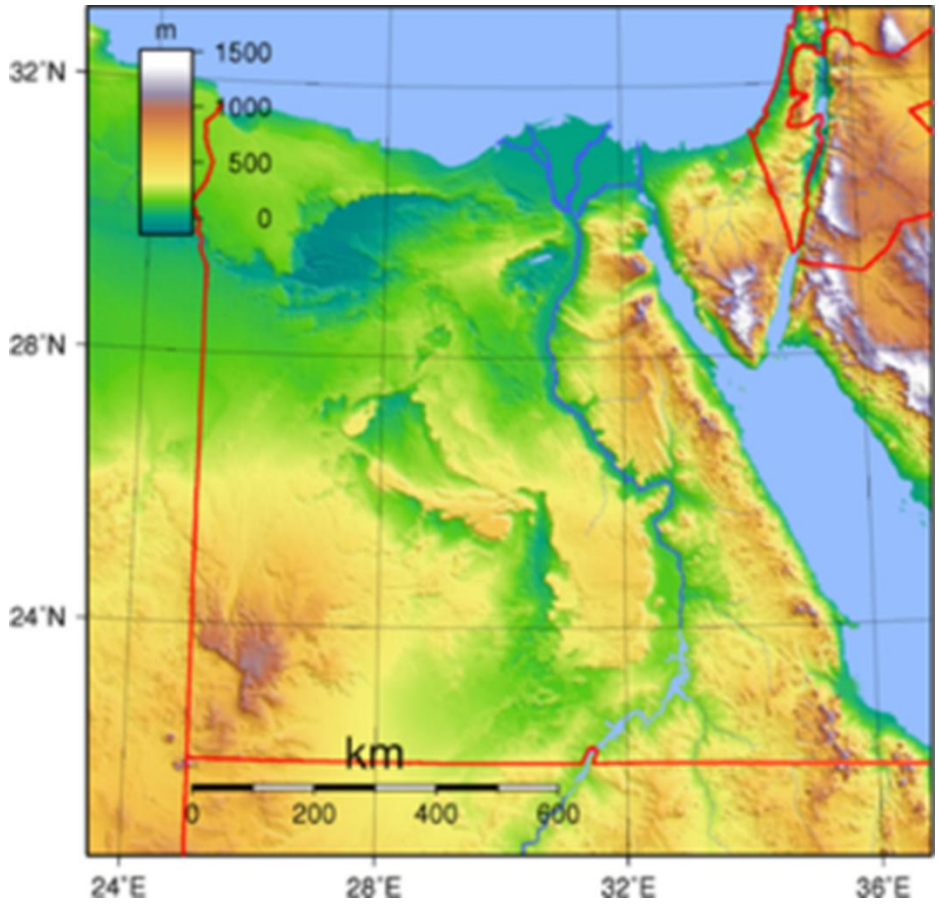
El Hadidi (2000) recognized the phytogeographical territories of Egypt (Map 1) as follows:

(**Da**) Arabian Desert, (**Dg**) Galala Desert, (**Di**) Isthmic Desert, (**Dl**) Libyan Desert, (**Dn**) Nubian Desert, (**Ge**) Gebel Elba district, (**Mm**) Mareotis sector of the Mediterranean coastal land, (**Ms**) Sinaitic sector of the Mediterranean coastal land, (**Nn**) Nubian sector of the Nile land, (**Nv**) Nile valley sector of the Nile land, (**Ol**) Oases of the Libyan Desert province, (**On**) Oases of the Nubian Desert province, (**Ra**) Arabian sector of the Red Sea coastal plains, (**Rq**) Aqaba Gulf sector of the Red Sea coastal plains, (**Rz**) Suez Gulf sector of the Red Sea coastal plains, (**S**) Mountainous southern Sinai, and (**Ug**) Gebel Uweinat



Map 1. The phytogeographical territories of Egypt (after El HADIDI, 2000).


Mediterranean Sea
Lebanon
Syria
Iraq
West Bank
Gaza Strip
Palestine
Jordan
Nile Delta
Nile River
Saudi Arabia
Egypt
Libya
Red Sea
Lake Nasser
Sudan
Kilometers



Indications of the wild plants

The natural resources الموارد الطبيعية, particularly the wild plants are regarded as a vital component عنصر حيويof Egypt's natural wealth الثروات الطبيعية. The plant diversity of Egypt is relatively not as simple as one would expect from a 'desert' country. The species are not evenly distributed in Egypt. Most species are seen in the Mediterranean and Nile regions. The average number of species occurring in a km^2 area is only a few, especially in the Western and eastern

regions. The Mediterranean region is densely vegetated, containing the maximum number of species.

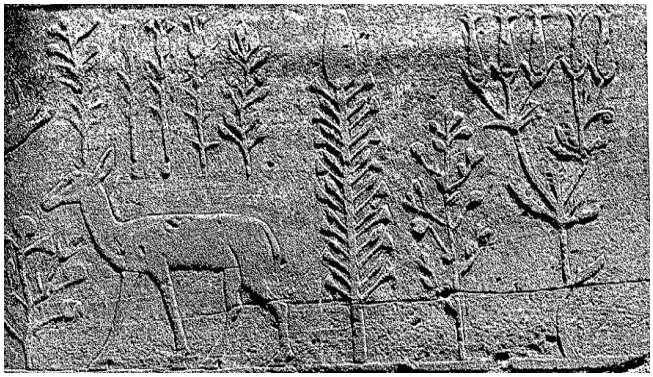
Approximately 50% of the flora are reported from the Mediterranean region. A total of **191** endemic and near-endemic taxa are recorded from Egypt and one or more neighboring countries. About **31.6%** of endemics are found in the Mountainous Southern Sinai subregion.

Most endemic and near-endemic taxa belong to Leguminosae, Labiatae, Caryophyllaceae and Compositeae which is the largest.

History of Botanical Studies in Egypt

Botany in Ancient Egypt

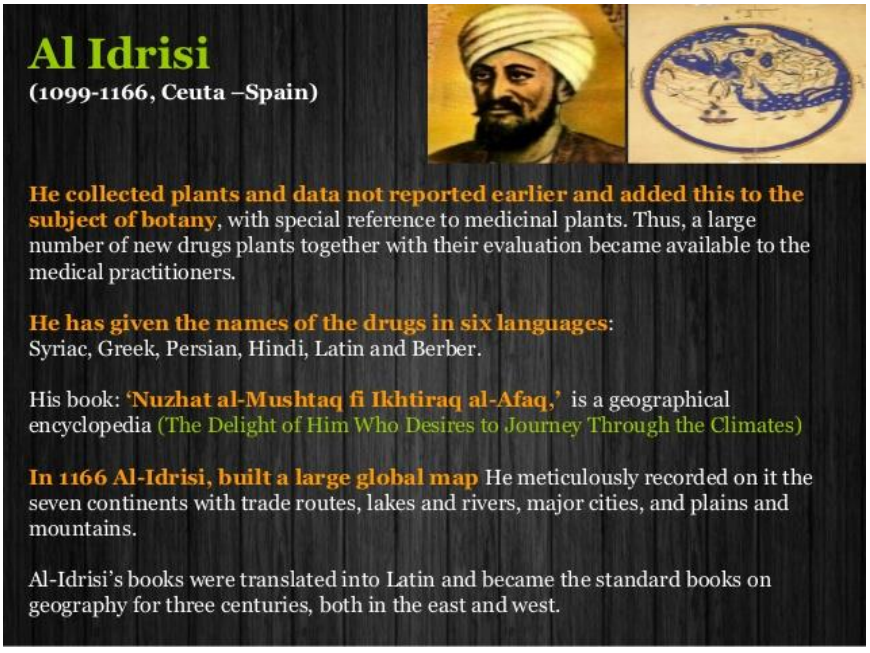
Plants featured heavily in Egyptian culture: in food, medicine, religion, perfumes and beyond. Early medicinal texts, such as the Ebers Papyrus from 1550 BCE, provide detailed insight into their extensive herbal knowledge.



Botanical Garden' was erected in the temple of Akh at Karnak. This 'garden' is a chamber whose walls depict carved representations of the plants and animals collected by Thutmose.

Contributions of earlier author

A.H. Dinawari (895 A.D.) in his book "Kitab al Nabat", had given a comprehensive knowledge of the agriculture and medicinal practices of the Bedouins. Other known collectors who lived from tenth to fourteenth centuries, such as **Idrisi** (1153 A.D.), **A. Al-Fida** (1331 A.D.) have also written about Arabian plants. Subsequently several Muslim travelers and plant collectors visited the country over a period of 500 years or so and studied the vegetation of Arabian countries, with special emphasis on the study of medicinal plants. Their findings have been depicted in various books and reports.



Historical Notes concerned with the Flora of Egypt

The botanists in modern times who studied in Egyptian flora were:

1- **Petter Forsskal**, Swedish (1732-1763),

- A pupil of Linnaeus.
- He herborized around Alexandria, Cairo and Suez and then continued to Arabia.
- Some of these plants were described as new in the posthumous publication "Flora Aegyptiaco-Arabica" by Niebuhr (1775).

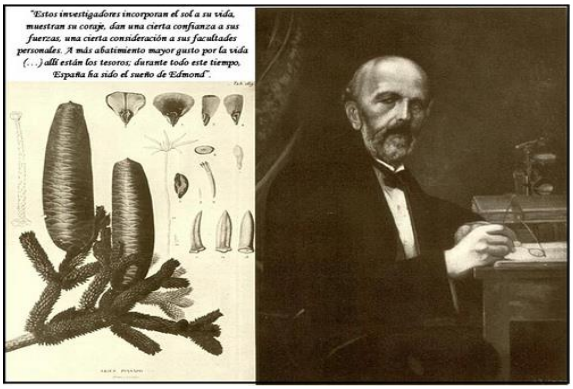
2- Alyre Reffenau Delile, French (1778 – 1850).

- He joined Napoleon’s expedition to Egypt as a member of the Scientific staff.

- He arived Egypt in 1798 and left again in 1800 in connection with the English occupation of the country.
- He succeeded with great difficulties to take with him all the notes on the Egyptian Flora, which appeared in print in 1813 in a beautifully illustrated volume “Flora Aegyptiacae illustratio” accompanied by text “Flora Egypte”.

3- Edmond Boissier, Swiss (1810 - 1850). A citizen of Geneva.

- He made many journeys to Spain and to the Orient.
- He spent his life writing a magnificent book in 5 volumes and one supplement, all in Latin, but with flora of all Middle east.



4- Gearge Schweinfurt, German (1836 – 1925),

He came to Egypt in 1863 and made a journey down the Red Sea coast. He made a journey to Africa to exploring and botanizing. His book “In the heart of Africa” has appeared in 9 languages. He devoted his time to the

exploration of Egypt. He made 10 great journeys the Arabian Desert. He visited Kharga and other parts of the Libyan desert. In 1875 he founded the Royal Geographical Society in Cairo and became the first Chairman.

5- Ibrahim Ramis, Egyptian (1896 – 1928),

He was a surgean with Botany as a hobby. He wrote a book "Bestimmungstahellen Zur Flora von Agyptien", which appeared after his death, 1929. Ramis collection are kept in the Agricultural Museum, Giza.

6- Vivi Tackholm, Swedish (1898 – 1987).

She came to Egypt with her husband Gunnar Tackholm (1891 – 1933). She was the first Botanist in the Egyptian University (now Cairo University). They established the Department of Botany and created its Herbarium. They joined several expeditions to various parts of Egypt and planes to write the flora of Egypt. The husband died 1933 and She came back to Egypt to realize that dream. She wrote 4 volumes of the flora of Egypt and two editions of the Students Flora of Egypt. Tell now the school of Vivi Tackholm is still active.

7- Loutfy Boulos, Egyptian, (1932-2017).

Born in Qena, received his B. Sc. & M. Sc. From Cairo Univ., and Dr. Sc. from Montpellier Univ. France. He introduced the Egyptian flora in four volumes of his book “Flora of Egypt”. The first three volumes covered the dicots, while the fourth volume concerned the monocots families. These books include more species than those of V. Tackholm and described with more critical and advanced characters.

Flora of Egypt

The components of the flora of Egypt are a vital for various ecosystems and play a key role in maintaining the region's environmental balance and stability.

It also helps in the protection of watersheds, stabilization of slopes, improvement of soils, moderation of climate and the provision تقديم of a habitat for much of our wild fauna.

The association of man and plants are well known and the basic needs required for man such as food, clothing, fuel, shelter مأوىand medicine are fulfilled by plants.

Egypt contains one of the diverse floras of this region.

- In addition to the endemic plants, the influences of the surrounding floristic regions can also be seen in many parts of the plant diversity hotspots النقاط الساخنة of this county.
- The flora of Egypt has about 2100 species in 755 genera and 129 families of vascular plants.
- 60 taxa are cited as endemics and 93 taxa are near endemics i.e. known from Egypt and one neighboring country.
- **The Western Desert:**
- The western desert of Egypt occurs on the west side of the River Nile.

- It extends from the Mediterranean coast in the north to the Egyptian- Sudano border in the south (± 1073).
- And from the Nile Valley in the east to the Egyptian-Libyan border in the west with width between 600-750 km. It covers about two thirds of Egypt (± 681,000 km^2).

Figure . Illustrates the Western desert

the Western desert comprises three main subregions, namely:

1. The western Mediterranean Coast that extends for about 575 between Sallum in the West to Abu Qir in the east.
2. The Oasis and Depressions.
3. Gebel Uweinat.

The Western Mediterranean desert or Mariut is arid desert but with increased rainfall. It may be named semi-arid desert. The vegetation of it is entirely dependent on the rainfall. The density of vegetation depends on the amount of rain. Mariut comprises the coastal area from Alexandria to Sallum (575 km long, 25 – 30 km board). During Predynastic periods the rainfall was richer and there was grass vegetation all over the desert. When the climate dried up, the cultivation got restricted to the coastal belt, and to the western delta. Mariut nowadays is a part of the arid desert, but with increased rainfall in the winter, maximum in December – January. The total amount of rainfall is 5-20 cm yearly. The humidity is rather uniform, 60 – 75%, and there is dew most part of the year. Temperature 20-35^{o} in summer, 10-25^{o} in winter.

Mariut is characterized by 4 main habit types. These are the Coastal sand dunes, Rocky ridges, salt marshes and barley fields.

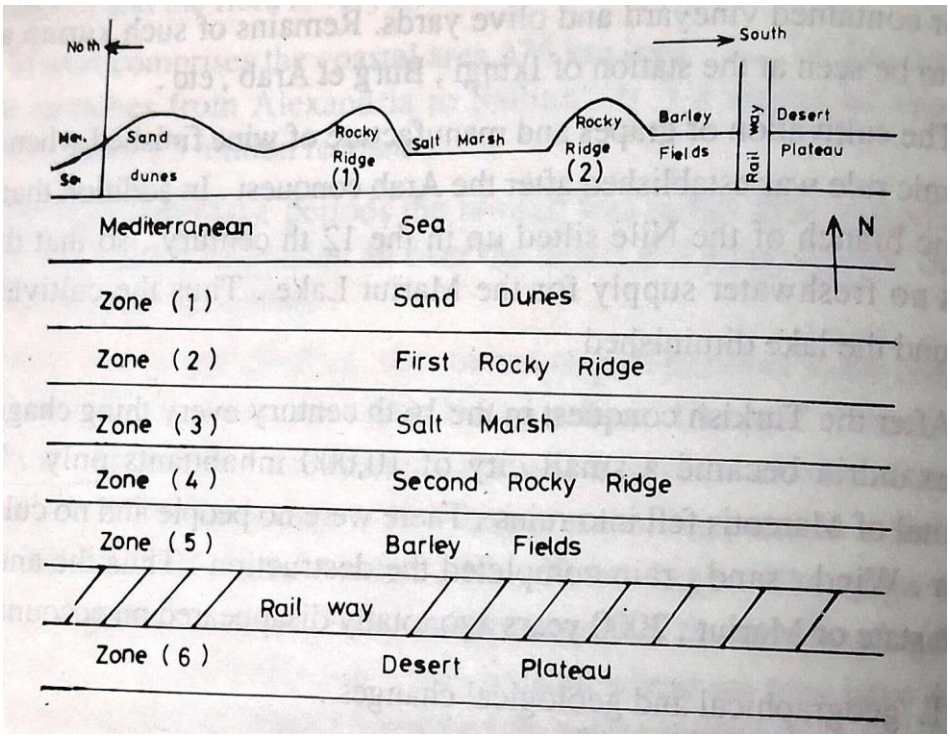


Figure showing a **View of Mariut coastal area from north to south.**

1- Dunes: Next to the seashore is a snow – white glittering sand dunes. This area is about one mile wide.

The sand is consisting of calcium-carbonate granules.

There are three types of dunes:

- **a.** Small of irregular shape,
- **b.** larger, of 10-20 sqm and 3-4 m high, moving,
- **c.** Fixed dunes, 10 m high of cemented carbonate of lime.

Flora on the seashore:

Number of dried algae washed by the waves. *Posidonia oceanica* (Potamogetonaceae) (spherical fiber balls “Pilae

marine"). *Ammophila arenaria* (sand binder grass in the moving sand). *Ononis vaginalis*, *Orlaya maritima*, *Lotus polyphyallos* (argenteus), Hyoseris lucida, Silene succulenta …….. etc. *Pancratium maritimium* (a bulbous plant "susan"). *Helianthemum sphaerocalyx* (rare plant, endemic to Mariut).

In cemented dunes we see:

Crucianella mritima, *Echium sericeum*, *Echiochilon fruticosum*…etc. *Echinops spinosissimus* (acquire a characteristic aspect when growing in maritime dunes).

Posidonia oceanica

Ammophila arenaria

Ononis vaginalis

Orlaya maritima

Lotus

Hyoseris

Silene

2- Rocky ridges:

They are south of the dune area, two ridges of calcareous hills running parallel to the sea share, covered with sand and clay, with an interesting flora of small rock species. Along the northern ridge that runs next to the sea there is an extensive fig cultivation.

Among rock plants should be noticed:

Helichrysum conglobatum, Thymus capitatus, *Globularia arabica, Phagnalon rupestre, Teucrium polium*, *Fumana thymifolia, Dactylis hispanica*

3- Salt Marshes: Between the two ridges, there is a large depression which is an extension of the Mariut Lake. It is covered with water during the winter and forms a brilliant white sheet of solid salt in summer. Soil is clayey with gypsum crystals. On the slopes of marshes is cultivation. In the middle there are vegetation of marshes plants.

The common flora is:

Halocnemum strobliaceum, Salicornia fruticose, *Frankenia revoluta, Suaeda fruticosa, Cressa cretica*, *Limonium pruinosum, Limoniastrum monopetalum, Sphenopus divaricatus*

4- Flora of the Barley fields:

The soil here is loose and cultivated by the Bedou

ins with Barely. These Barely fields have rich weed flora depend on rain.

5- The weed flora in Mariut dominated by 4 families:

- Compositae (c. 100 species).
- Leguminosae (c. 100 species).
- Gramineae (c. 100 species).
- Cruciferae (c. 50 species).

6. Fruit trees cultivated in Burg el Arab and suitable for Mariut. Some fruit trees that can be cultivated in Mariut and suited for the climate of this region:

Fig: It grows extremely in the Sandy dunes all along the Coast.

Kharroub: is resistant to drought and salt.

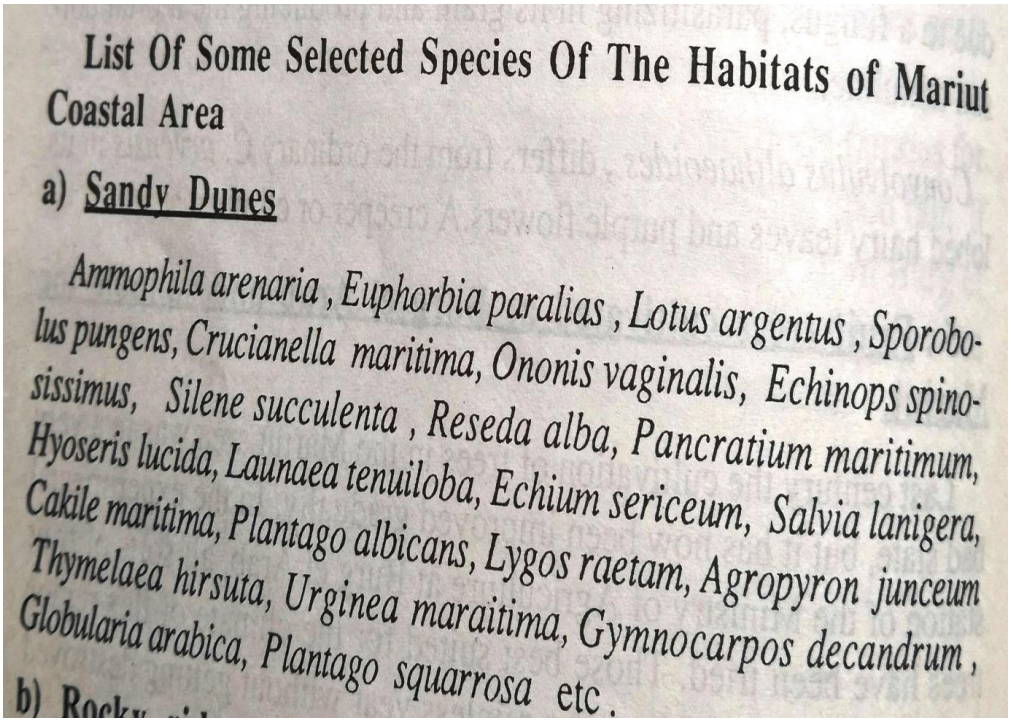
Almond: is also resistant to drought and salt.

Nabq (Ziziphus): produces heavy crops.

Olive: It is the best suited tree for cultivation along the coast. It is could grow without irrigation and could stand a rainless year.

As a result of these developments, large areas of virgin land in the mountainous regions and the range lands in the Northern, Eastern and Central regions are turned into urban and agricultural lands. In addition to this, the dramatic fluctuations in climate which resulted in periodic drought

have made it much more difficult for plants to survive in their habitats.



List Of Some Selected Species Of The Habitats of Mariut Coastal Area

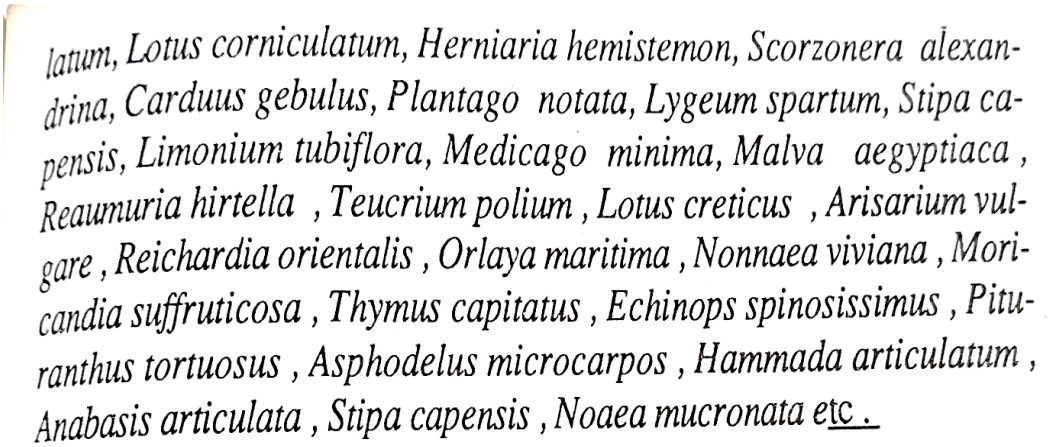
a) Sandy Dunes

Ammophila arenaria , Euphorbia paralias , Lotus argentus , Sporobolus pungens, Crucianella maritima, Ononis vaginalis, Echinops spinosissimus, Silene succulenta , Reseda alba, Pancratium maritimum, Hyoseris lucida, Launaea tenuiloba, Echium sericeum, Salvia lanigera, Cakile maritima, Plantago albicans, Lygos raetam, Agropyron junceum Thymelaea hirsuta, Urginea maraitima, Gymnocarpos decandrum , Globularia arabica, Plantago squarrosa etc .

b) Rocky

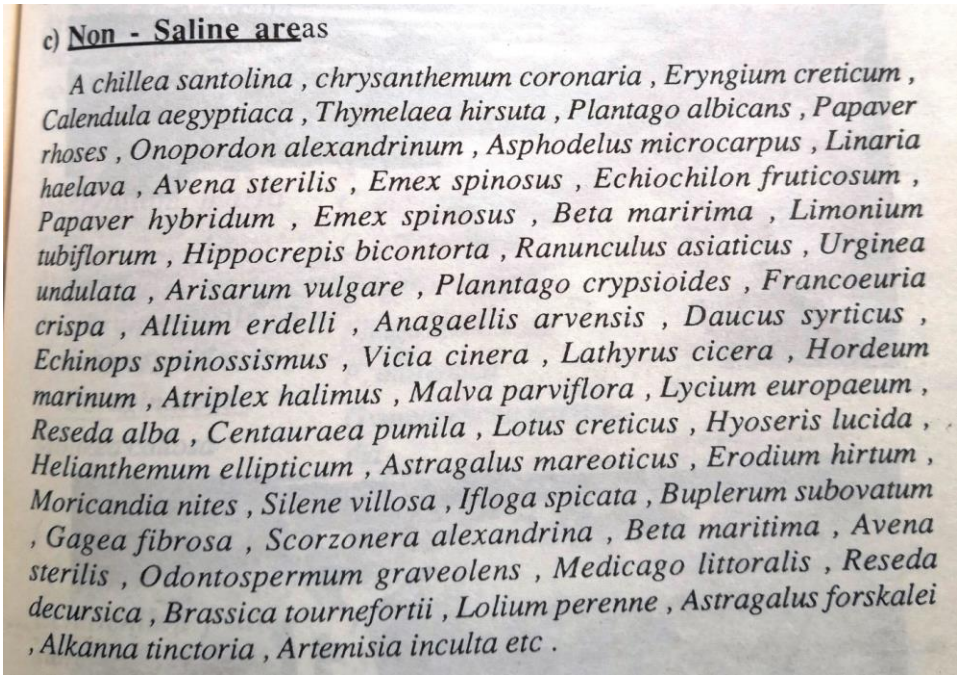
B- Rocky ridges plants:

Thymelaea hirsuta, Gymnocarpus decandrum, Helianthemum stipulatum



latum, Lotus corniculatum, Herniaria hemistemon, Scorzonera alexandrina, Carduus gebulus, Plantago notata, Lygeum spartum, Stipa capensis, Limonium tubiflora, Medicago minima, Malva aegyptiaca , Reaumuria hirtella , Teucrium polium , Lotus creticus , Arisarium vulgare , Reichardia orientalis , Orlaya maritima , Nonnaea viviana , Moricandia suffruticosa , Thymus capitatus , Echinops spinosissimus , Pituranthus tortuosus , Asphodelus microcarpos , Hammada articulatum , Anabasis articulata , Stipa capensis , Noaea mucronata etc .

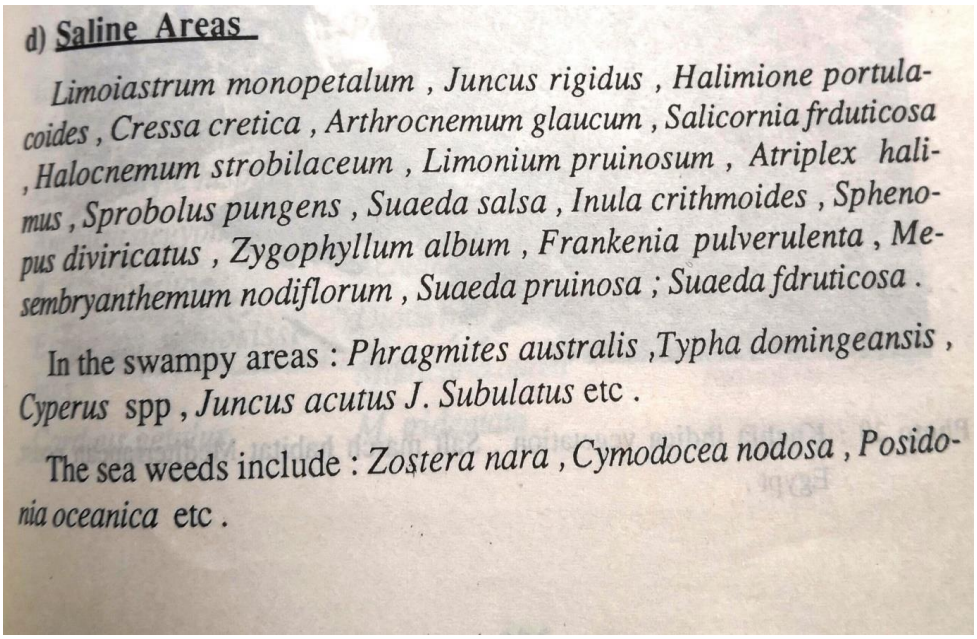
c- Non-Saline areas plants:



c) Non - Saline areas

A chillea santolina , chrysanthemum coronaria , Eryngium creticum , Calendula aegyptiaca , Thymelaea hirsuta , Plantago albicans , Papaver rhoses , Onopordon alexandrinum , Asphodelus microcarpus , Linaria haelava , Avena sterilis , Emex spinosus , Echiochilon fruticosum , Papaver hybridum , Emex spinosus , Beta maririma , Limonium tubiflorum , Hippocrepis bicontorta , Ranunculus asiaticus , Urginea undulata , Arisarum vulgare , Planntago crypsioides , Francoeuria crispa , Allium erdelli , Anagaellis arvensis , Daucus syrticus , Echinops spinossismus , Vicia cinera , Lathyrus cicera , Hordeum marinum , Atriplex halimus , Malva parviflora , Lycium europaeum , Reseda alba , Centauraea pumila , Lotus creticus , Hyoseris lucida , Helianthemum ellipticum , Astragalus mareoticus , Erodium hirtum , Moricandia nites , Silene villosa , Ifloga spicata , Buplerum subovatum , Gagea fibrosa , Scorzonera alexandrina , Beta maritima , Avena sterilis , Odontospermum graveolens , Medicago littoralis , Reseda decursica , Brassica tournefortii , Lolium perenne , Astragalus forskalei , Alkanna tinctoria , Artemisia inculta etc .

d- Saline Areas plants:



d) Saline Areas

Limoiastrum monopetalum , Juncus rigidus , Halimione portulacoides , Cressa cretica , Arthrocnemum glaucum , Salicornia frduticosa , Halocnemum strobilaceum , Limonium pruinosum , Atriplex halimus , Sprobolus pungens , Suaeda salsa , Inula crithmoides , Sphenopus diviricatus , Zygophyllum album , Frankenia pulverulenta , Mesembryanthemum nodiflorum , Suaeda pruinosa ; Suaeda fdruticosa .

In the swampy areas : *Phragmites australis ,Typha domingeansis , Cyperus* spp , *Juncus acutus J. Subulatus* etc .

The sea weeds include : *Zostera nara , Cymodocea nodosa , Posidonia oceanica* etc .

Flora of a Sector along the Western Desert

Along the western desert the plant life varies due to the variation of the climatic conditions. The amount of rainfall increases towards Alexandria from 24 mm/ year in Giza, 38 mm in Tahrir Proview, 138 mm in Ameriya, to about 170 mm in Alexandria.

Air temperature decreases north-word, relative humidity increases, and evaporation decreases north-words.

In the extreme southern part of the Sector the Plant cover is very poor.

Few scattered individuals of *Aristida plumosa, Calligonum comosum, Eremobium lineare* and *Zygophyllum coccineum*.

After 46 km north of Cairo the number of plants increases and the flora include: *Aristida plumosa, Pituranthus tortuosus, Artemisia monosperma, Helianthemum lippii* …… etc. This flora continues till km 157 of Cairo, then new elements start to appear eg. *Thymelaea hirsuta, Anabasis articulata, Salsola tetrandra* ……etc. The number of plants and the density of vegetation increases near the coast.

The Oasis and Depressions of the WD

The Western Desert of Egypt is characterized by several Oases and Depressions:

e. g. Siwa, Moghra, Baharia, Farafra, Dakhla, Kharga, KurKur and Dungul Oases; Qattara, Wadi El-Natrun and Wadi El-Rayan Depressions.

The climatic conditions of these Oases and Depressions are arid or extreme arid: high temperature, low humidity, high evaporation and rainfall is negligible.

Thus, the underground water is the main water resources of these depressions which can be obtained by digging wells or from natural springs.

Natural lakes are present in many of these Oases e.g. Siwa lake, Wadi El-Natrun Depression Lakes ……. etc.

Five vegetation's types can be recognized in these Oases and Depressions namely Hydrophytes, Helophytes, Halophytes, Psammophytes and Xerophytes.

A + b. Hydrophytes and Helophytes:

The swampy habitats are formed by the overflowing of the underground water to the depressed areas of these Oases. Hydrophytes grow in filled with water Lakes and Lagoons. Helophytes are present in shallow watered edges of these water bodies.

e.g. of Hydrophytes: *Typha domingensis*, *Phragmites australis*, *Typha elephantina* (restricted to Wadi El-Natrun). Also *Cyperus papyrus, Berula erecta, Samolus valerandii, Cyperus articulatus* ……etc.

The wet fringes of these lakes and lagoon are suitable for growth of : *Cyperus laevigatus*, *Juncus acutus*, *J. rigidus*, *Panicum repens* ……. etc.

The Halophytes (the flora of salt marsh habitat) are widely present in the oases and depressions of the WD of Egypt.

e.g. *Cladium mariscus* (present only in Siwa Oasis), *Cyperus laevigatus* (forms meadows around the lakes of Wadi El-Natrun), *Juncus rigidus*, *J. acutus*, (both are present in all Oases), *Nitraria retusa, Zygophyllum album, Cressa cretica, Alhagi maurorum, Tamarix nilotica, Saueda monoica*, ……etc.

- Psammophytes (these are the plants that inhabit the sand formations):- Sand bars, sand hillocks and sand dunes are usually associated with the lakes of Oases and Depressions.

In Wadi El-Natrun there are sand bars on **the eastern side** dominated by *Desmostachya bipinnata, Sporobolus spicatus, Panicum turgidum, Juncus acutus, Artemisia monisperma* and *Phoenix dactylifera*.

On the western side, there are sand hillocks with Tamarix nilotica, T. passerinoides, and Nitraria retusa.

On the south and southeastern sides of lake Siwa there are extensive area of huge sand dunes where, *Zygophyllum album, Aristida scoparia, and Cornulaca monacantha* gow.

Also *Populus euphraticus* (most interesting plant present only in Siwa Oasis).

- **Xerophytes:**

The lands surrounding the Oases and depressions of the WD of Egypt are characterized by perennials Xerophytes. These include: *Artemisia monosperma*, *Panicum turgidum*, *Pituranthus tortuosus*, *Aristida plumosa*, *Salsola baryosma*, *Zygophyllum simplex*, *Fagonia arabica*, *Acacia raddiana* …….. etc.

III. Gebel Uweinat:

It lies in the extreme southwestern portion of the WD where the boundaries of Egypt, Sudan and Libya meet.

The flora of Gebel Uweinat: *Phoenix dactylifera*, *Hyphaene thebaica, Tamarix nilotica* (grows near the springs and wells).

Under Palms, there are Halophytes like *Junicus rigidus, Sporobolus spicatus, Imperata cylinderica, Alhagi maurorum*……. etc.

In Swampy patches grow *Phragmites australis*.

Xerophytes include: *Cassia italica*, *Aerva javonica, Francoeuria crispa* …….. etc.

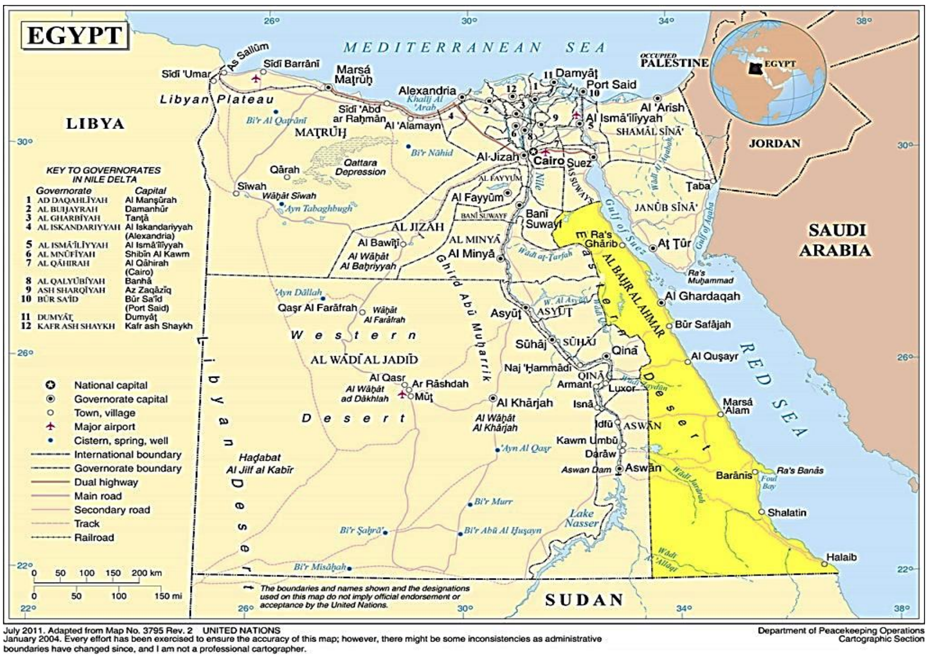
The Eastern Desert of Egypt

It occupies the area extends from the Nile Valley eastern to the Gulf of Suez and the Red Sea which is about 223,00 km^2 (21%).

It is higher than the WD and it consists essentially of a backbone of high and rugged mountains running parallel to the coast. E.g. Ataqa, Shayeb El-Banat, Elba ………etc.

The Mountains divide the ED into two main subregions:

- The Red Sea coastal land,
- The Inland Desert.



The Red Sea Coastal Land of Egypt (RSCL):

RSCL extends from Suez southwards to Marsa Halaib at the Sudano-Egyptian border for about 1100 km. It comprises the western coast of the Gulf of Suez (from Suez

to Hurghada, about 400 km) and the northern section of the western coast of the Red Sea (from Hurghada to Marsa Halaib, 700 km). This coastal area is situated within a region of arid climate with rainfall ranges between 3 – 25 mm.

In the mountains area, there is orographic rain which has its effect on its flora: richer flora than the coastal desert of the Red Sea and other parts of the Egyptian deserts.

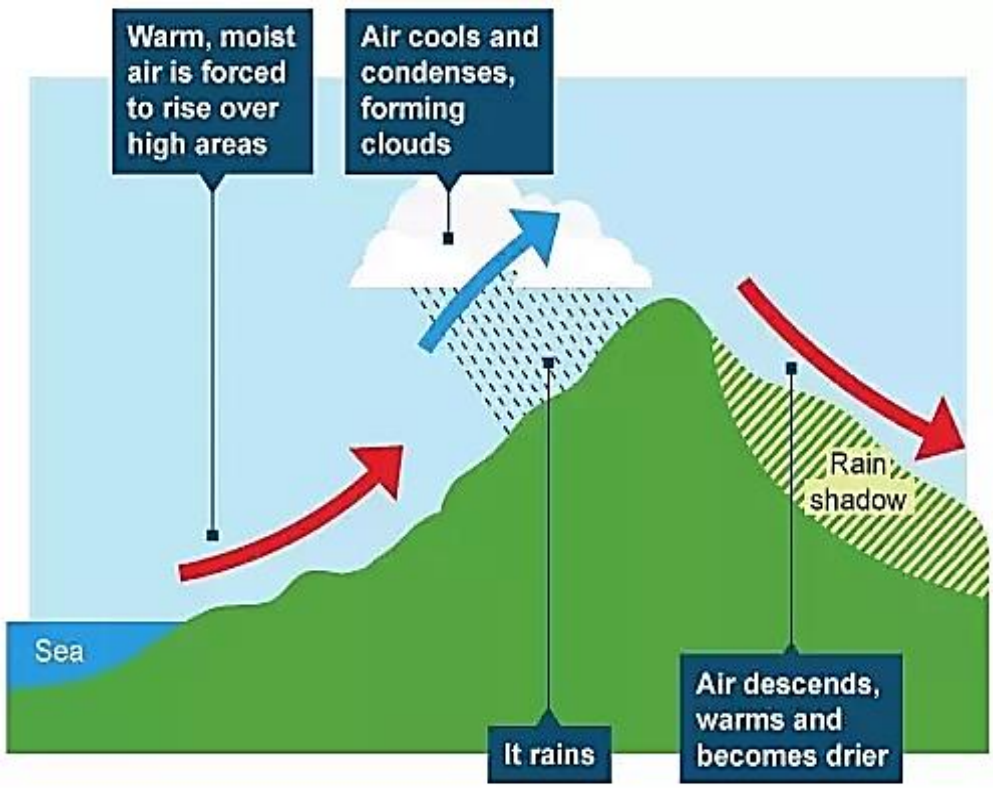
Four vegetation types have been recognized in RSCL:

A- Mangrove Vegetation,

B- Littoral Salt Marsh Vegetation,

C- Coastal Desert Vegetation,

D- Mountainous Vegetation.



The Mangrove vegetation is present only along the Red Sea coast. It is represented by common tree *Avicennia marina* (Avicenniaceae), that appears from Hurghada southwards, absent in the north. Also, *Rhizophora mucronata* (Rhizophoraceae) in the most southern part of the Red Sea Coast of Egypt.

Several Sea weeds are constantly washed up on the shores among which are: *Cymodocea ciliata, Cymodocea rotundata, C serrulata* (Cymodoceaceae),

Diplanthera uninervis (Cymodoceaceae), *Halophila stipulaceae, H. ovalis, Thalassia hemprichii* (Hydrocharitaceae).

Ain Sokhna is a hot water spring, characterized by a salt marsh vegetation (Halophytes), its flora includes: *Juncus rigidus, Cressa cretica, Arthrocnemum glaucum, Halocnemum strobilaceum, Nitraria retusa, Tamarix nilotica* and *Phragmites australis* (dominated in water creeks).

The salt marsh vegetation occupies the land parallel to sea water and comprises halophytes communities dominated by: *Halocnemum strobilaceum, Arthrocnemum glaucum, Salicornia fruticosa, Limonium pruinosum, Zygophyllum album*, ……. etc.

Also, *Nitraria retusa, Tamarix nilotica, Suaeda monoica, Juncus rigidus, Alhagi maurorum*.

The water creeks are suitable habitat for the growth of swamp plants e.g. *Typha domingensis* and *Phragmites australis*.

RSC desert is dissected by several Wadis run eastward towards the red sea. In these Wadis many Xerophytic plants grow like: *Cleome droserifolia, Hammada elegans, Anabasis articulata, Zygophyllum coccineum, Lindenbergia sinaica, Capparis decidua, Zilla spinosa*, etc.

The forest vegetation is present in the mountainous area of RSCL particularly in Gebel Elba area (height 1436 m).

Gebel Elba is the only mountainous area in Egypt with a forest vegetation mainly consisting of *Acacia* trees intermingled with *Moringa peregrina* and *Balanites aegyptiaca*.

In the shaded areas grow e.g. *Ficus pseudosycomorus, Olea chrysophylla, Delonix elata, Dodonaea viscosa*, etc.

The most interesting plant of Gebel Elba is *Dracaena ombet* (Liliaceae). The other with ecological interest plant is *Caralluma retrospiciens* (Asclepiadaceae).

Of the Acacia plants of Gebel Elba is the Liana *Loranthus acaciaea*, which live on these trees.

Ferns are represented with a number of small rock species e.g. *Adiantum* sp.

Loranthus acaciaea

Adiantum sp.

2-The Inland Desert

It is part of the ED of Egypt, located between the range of the coastal mountains and the Nile River. It is characterized by several Wadis (Hof, Qena, Allaqi), that run towards the River Nile and some northward cutting Cairo – Suez Desert Road bordering this inland desert from the north.

Cairo – Suez Desert Road:

It is a wide-open desert with low hills covered with pebbles. It is described as “Gravel Desert” which is sterile. The vegetation is located to smaller and larger water courses filled with wind-borne sand originating partly from the mobile dunes.

In the beginning, the sandy courses show a flora of small ephemerals: e.g. *Stipa capensis, Schismus barbatus*.

When the sand sheet gets a little thicker, Perennial grasses take place of the ephemerals like *Panicum turgidum* and *Lasiurus hirsuts*, where small sand hills are accumulating around them.

With increasing in the soil thickness, the shrubs appear like: *Hammada elegans* competes with *Panicum turgidum, Retama raetam, Convolvulus lanatus, Farsetia aegyptia, Zilla spinosa,* etc.

The next stage will be represented by a climax stage with a vegetation of Acacia trees.

In rainy years, a very rich flora along the Suez road of small ephemerals (annuals) appears, dominates with *Mesembryanthemum forsskaolii.*

Other very common ephemerals: *Matthiola livida, Neurada procumbens, Arnebia tinctoria, Centaurea pallescens, Filago spathulata, Plantago ovata* …….etc.

All of them are herbaceous, mesophytic and have a very rapid development which allows them to flower and produce seed before the hot season sets in.

Some bulbous plants along Suez Road: e.g. *Pancratium sickenbergeri, Dipcadi erythraeum, Allium desertorum, Urginea undulata*. *Ephedra alata* is a peculiar gymnosperm, quite different from *Ephedra alte* (= *E. aphylla* Forssk.).

Flora of Wadi Hof:

It is one of the big Wadis of the ED of Egypt located in Helwan Area to the south of Cairo. It runs westwards to debouch its water into the Nile River. The landform of it is of the “erosion pavemnent”. It has arid climate with light amount of rainfall (30 mm).

Wadi Qena (greatest Wadi in the ED with length about 300 km)**:** It is the most notable feature of the inland part of the ED. It runs north south to debouch into the Nile near the Qena City. It is running parallels to the Nile Valley, though its water flows in the opposite direction. Ecologically, it is

divided into two parts, **the deltaic plain and the main channel.**

The deltaic plain or the downstream section of the Wadi is characterized by vegetation comprising: *Zilla spinosa, Hammada elegans, Francoeuria crispa, Artemisia judaica, cleome droserifolia, Aerva javonica, Acacia ehrenbergiana*, ……etc. The downstream section of Wadi Qena is characterized by extensive hillocks of a relic growth of old *Tamarix* plants.

Wadi Allaqi: is one of the most extensive drainage systems in the Nubian section of the ED south of Aswan.

It is ecologically, divided into four main Sections:

1) A mountainous east (upstream) section
2) A middle hilly section
3) A low plateau section
4) A deltaic section.

The mountainous (upstream) section of Wadi Allaqi forms the natural divide between the inland and coastal parts of the ED. The Vegetation of this part may be described by a desert-forest with trees of *Acacia raddiana, Balanities aegyptiaca, Cocculus pendulus, Ochradenus baccatus, Salvadora persica, Acacia tortilis* and *Acacia ehrenbergiana* which widely distributed in this upstream part of Wadi Allaqi.

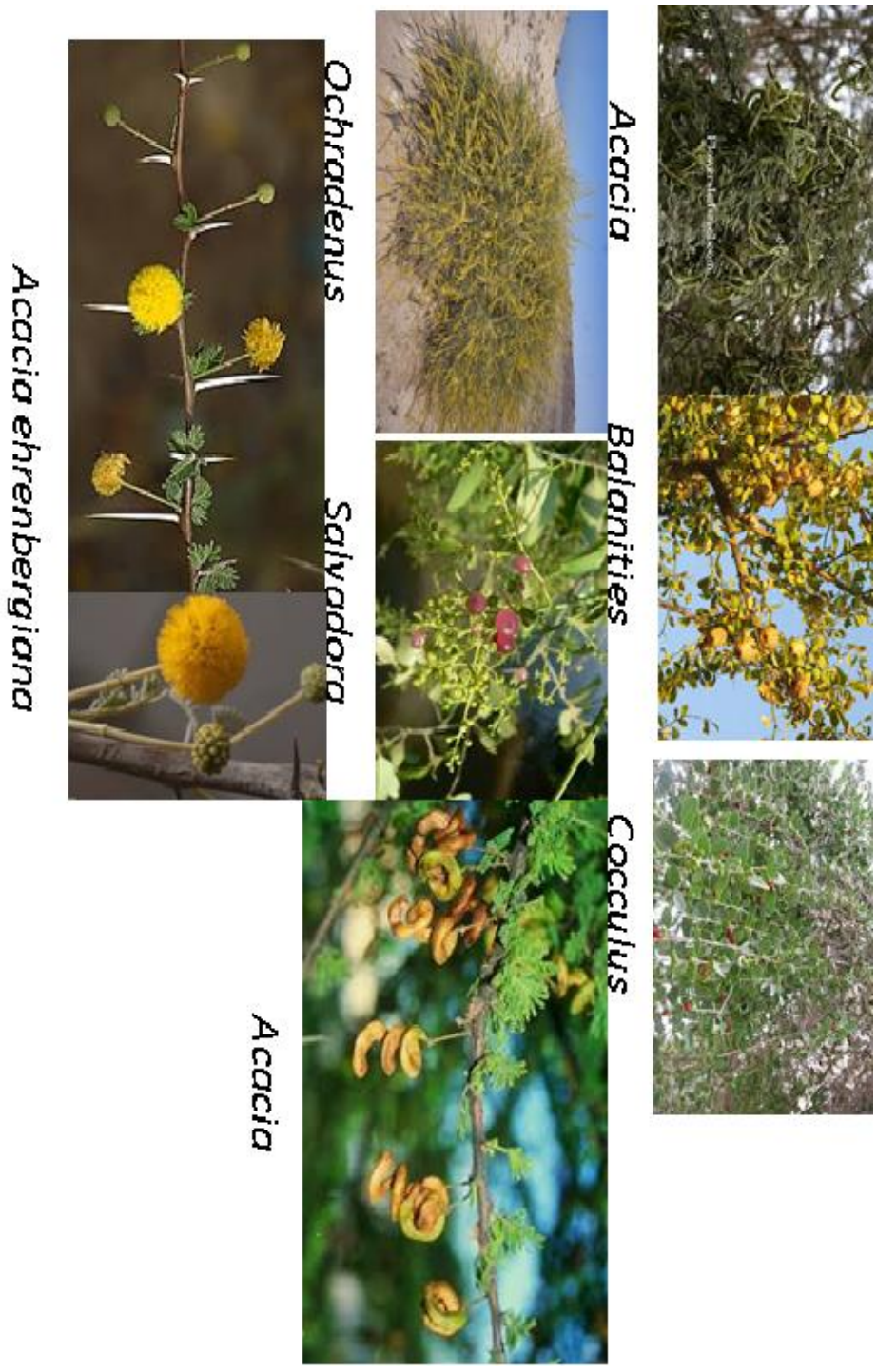
The vegetation of the middle section of Wadi Allaqi is open scrubland of *Acacia ehrenbergiana, Acacia raddiana, Salsola baryoma, Fagonia indica, Indigofera argentea, Morettia philaena, Cassia senna, Aerva javonica, Aristida plumosa* ….etc.

In this section, there are several fossil hillocks with dead remains of *Tamarix aphylla* and *Salvadora persica*.

In the low plateau section of Wadi Allaqi there is an open scrubland of *Acacia ehrenbergiana* with individuals of *Acacia raddiana*. Fossil hillocks of *Tamarix aphylla* are present, where *Salvadora persica* is absent.

Also, In the low plateau section, there are patches of green cover dominated by *Salsola baryosma* with: *Cassia senna, Citrullus colocynthis, Trianthema crystallina, Fracoeuria crisa, Cistache tictoria* (parasite on *Salsola terandra*), *Convolvulus prostratus, Fagonia indica, Morettia philaea*, ……etc.

A wide deltaic plain covered with a matrix of gravel and sand. In this section, no Acacia sp. plants are recognized. Indivduals of *Hyoscyamus muticus, Aerva javonica, Crotalaria aegyptiaca, Citrullus colocynthis, Echinochlon colonum, Trigonella hamosa, Beta vulgaris, sonchus oleraceaus, Imperata cylinderica,* ……. etc.


Acacia
Balanities
Cocculus
Ochradenus
Salvadora
Acacia
Acacia ehrenbergiana

The Sinai Peninsula

The Sinai Peninsula is a triangular plateau occupying the north – eastern corner of Egypt. The area of the Sinai Peninsula (61,000 km^2) is about 6% of the total area of Egypt and represents its Asian part. **SP** has 3 coastal areas:

1- in the north, the eastern section of the Mediterranean coastal land of Egypt that extends from Port Said to Rafah (240 km).

2- In the west, the eastern coast of the Gulf of Suez that extends from el-Shatt (facing Suez) to Ras Muhammed for about 400 km.

3- In the east, the western coast of the Gulf of Aqaba that extends from Aqaba to Ras Muhammed for about 235 km.

Sinai is characterized by high mountains in its southern section, the most important ones are:

- Gebel Saint Katherin (2641 m),
- Gebel Musa (2285 m),
- Gebel Halal (890 m),
- and Gebel Hammam Faraon.

Several Wadis are present in SP, some are running westward to the Gulf of Suez: e.g. Wadi Firan, Wadi Sidri, Wadi El – Tor …etc. Also, there are Wadis run eastward to the Gulf of Aqaba.

The climate affecting Sinai is semi-arid along the Mediterranean coast, arid along the coasts of The Gulfs of Suez and Aqaba.

In the southern region, there are high Mountains orographic rains occurs and snow is also common during winter at top of these Mountains.

The flora of Egypt comprises about 2300 species belonging to 130 families. Out of these 63 are endemics in the different regions of Egypt: WD, ED, SP and the Nile region.

In the SP there are about 1247 species belonging to 94 families as follows: 46 endemic species. 346 not endemics but are confined to the SP without being penetrated to the other regions of Egypt and 855 species are present in SP as well as in other regions of Egypt.

The total number of the flora of SP represents about 49.92% of the total number of the flora of Egypt. A summary introduces the Sinai Peninsula Flora as follows:

- Yet the endemics of Sinai make the main bulk (76.19%) of the total endemics of Egypt.
- Family Labiatae comprises, relatively, the highest number of endemics (6 species, *Origanum syriacum var aegyptiacum, Ballota kaiseri, Thymus decussata* …. etc.).
- Followed by Caryophyllaceae (5 species, e.g. *Dianthus sinaicus, Silene leucophylla* ……. etc.
- 4 species in Scrophulariaceae (e.g. *Verbascum schempericum, Kickxia macilenta* …. etc.).

- Compositae (e.g. *Phagnalon sinaicum, Scorzonera drarii* …. etc.).
- 3 plants in Leguminosae (e.g. *Vicia sinaica*) and Umbelliferae (e.g. *Ferula sinaica*), two plants in Cruciferae, Resedaceae and Dipsacaceae, and one species belong to Ranunculaceae, Liliaceae, Juncaceae and Gramineae ……etc.

The flora of the Mediterranean coast of Sinai comprises:

Halocnemum strobilaceum, Arthrocnemum glaucum, Suaeda vermiculata, Nitraria retusa, Tamarix nilotica, Limoniastrum monopetalum, Juncus rigidus, Zygophyllum album, Cressa cretica, ……etc. in the salt marshes.

The sand dunes habitat of the coastal area is characterized by the growth of: *Ammophila arenaria, Artemisia monosperma, Cornulaca monocantha, Panicum turgidum, Stipagrostis scoparia, Cyperus capitatus* ……. etc.

In the coastal desert grow: *Thymelaea hirsuta, Achillea fragrantissma, Lycium europaeum, Pituranthus tortuosus, Echinops galalensis, Tamarix aphylla, Fagonia arabica* …. etc.

Four vegetation types have been recognized in the southern section of Sinai namely: **Mangrove, Salt marsh, Desert and Mountains**:

1) **The mangrove vegetation** is present in Ras Muhamed only where Avicennia marina grow.
2) **The Salt marshes** are present along the coasts of the Gulfs of Suez and Aqaba where halophytes grow e.g. *Halocnemum strobilaceum, Arthrocnemum glaucum, Limonium pruinosum, Halopeplis perfoliata, Zygophyllum album, Nitraria retusa, Tamarix nilotica, Juncus rigidus*, ……. etc. The reed swamp vegetation is present in the swampy habitat where *Typha domingensis* and *Phragmites australis* grow.

Typha domingensis

Phragmites australis

In the Wadis of the southern region of Sinai the Xerophytic vegetation comprises the following flora: *Hammada elegans, Artemisia judaica, Achillea fragrantissima, Zygophyllum decumbens, Acacia*

raddiana, Capparis cartilaginea, Iphiona mucronata, Fagonia sinaica, Solanum nigrum, etc.

3) **The flora of the mountains of Sinai include**: *uniperus phoenicea*, Family Cupressaceae (plant grows only in the cold area of the world), as well as the following plants : *Moringa peregrina, Ficus pseudosycomorus, Origanum syriacum, Galium sinaicum, Cratagus sinaica, Stachys aegyptiaca, Gymnocarpus decandrum, Zilla spinosa, Capparis cartilaginea, Peganum harmala, Varthemia montana*, etc. *Cupressus semipervrens* (semi-wild) is commonly present also in these mountains

The Nile Region

The River Nile extends from Lake Tanganyika in Tanzania (Latitude 3°S) to the shore of the Mediterranean Sea (Latitude 31° 15'N) for a length of about 6625 km. In this long course the river flows generally a south to north path, both its source in Equatorial Africa and its mouth in the Mediterranean Sea lies within one degree of the same meridian of longitude. Of the total course of the River Nile only a terminal of 1530 km lies within the borders of Egypt. It enters Egypt

from the Sudan at Wadi Halfa, 350 km south of Aswan. The Nile Valley (the Upper Egypt, Nv) extends from Wadi Halfa southwards to Cairo for about 1285 Km. and comprises the lands irrigated by the River Nile on its both banks. The Nile Delta (the Lower Egypt, Nd) appears as a triangular in shape: about 170 km in length and 220 km in breadth. Its lands are irrigated by water of the Rosetta Branch (about 239 km) and Damietta Branch (about 245 km). Both branches start from the Delta Barrage (Muhammed Ali Barrage), 20 km north of Cairo. The northern coast of the Nile Delta close to the Mediterranean sea is characterized by three shallow lakes: Lake Manzala (in the east) lake Burullus (in the middle) and Lake Idku (in the west) . These lakes were formed by the River Nile during the past history and are receiving the main bulk of drainage water from the Nile Delta lands.

The northern coast of the Nile Region of Egypt: the deltaic Mediterranean coast extends from Abu Qir to Port Said for about 180 km. and landward in a NS direction for an average of 15 km from the Sea.

Fayium province is one of the depressions of the Western Desert of Egypt. Being the nearest to the Nile

Valley and after being connected with the River Nile with Bahr Yusuf Canal, Fayium Depression is considered as a part of the Nile Region (Nf) . Its lowest part is occupied by a shallow saline lake called birket Qatrun.

Formerly all the Nile lands were watered by the inundation (or basin) system. During the past century a number of great canals were constructed to allow perennials irrigation. This scheme was completed with the construction of several barrages towards the end of the last century for raising the water level for feeding the canals. In 1902 the Aswan Dam (old Dam) was ready and the High Dam (new Dam) was ready on 1965 (El - Sadd El - Aaly).

The cultivated strip of land around the Nile is largest at Cairo and diminishes as one proceeds south. South of Aswan it disappears completely, and, in most places, the naked desert borders the Nile directly.

The Nile Delta starts 20 km north of Cairo, is embraced by the Rosetta and Damietta Branches. Its area is about 22,000 Km2 while the area of the Nile Valley (cultivated lands) is 12000 km2 Thus, the, delta comprises about 63% of Egypt's fertile land.

The soil of the agricultural lands of the Nile Region of Egypt is classified into loam, clay and sand. Loamy soils are mainly composed of Nile silt and form most Egyptian soils. Clay soils occur in small amounts where sandy soils are principally found at the edges of the desert. Soil impregnated with salts is found in the Delta and Fayium. Alkaline land lies all around the lower edges of the Delta from Alexandria in the west to beyond the Suez Canal in the east and also in certain districts of Upper Egypt, where basin irrigation has been changed into perennial one . Seepages near the banks of the high-level canals in another cause of increasing soil salinity. Salt affected lands are also present around the northern lakes of the Nile Delta and along the deltaic Mediterranean coastal land.

FLORISTIC COMPOSITION OF THE NILE VALLEY

The floristic elements of the Nile Region of Egypt represent about 30% of the total number of the flora of Egypt. About 130 species of these plants never recorded elsewhere in Egypt. Of the total number of these floristic elements, 149 species are present in the Nile valley, 291 species in the Nile Delta and 64 species characterize e the Nile Fayium. Therophytes represent 59. 4% of the

total number followed by the Hydrophytes (9. 8%), Hemicryptophytes (9%), Chamaephytes (8. 6%), Geophytes (6.9%), Nanophanerophytes (2.9%), Parasites (2 .6 %) and Microphanerophytes (1.8%). Apart from *Opuntia ficus-indica* (L.) Mill. (family Cactaceae) which is usually cultivated as fence plant and for its edible fruit, no stem succulent is present in the Nile Region of Egypt. Also, megaphanerophytes, and epiphytes are absent The percentage of the hydropytes and helophytes (9. 8%) is, however, the relatively higher than in the floristic elements of the other regions of Egypt.

The Nile region of Egypt comprises a number of habitats that are formed and / or greatly influenced by the water of the River Nile, these are:

a) The Aquatic Habitat,

b) The Swampy Habitat,

c) The Canal Bank Habitat,

d) The Cultivated Lands,

e) The Northern Lakes,

f) The Man - Made Lakes,

and g) The Nile Islands.

The natural vegetation of these habitats is of ecological interest, they include certain floristic elements never recorded elsewhere in the other regions of Egypt.

The coastal area of the Nile Region of Egypt is a natural extension of the Mediterranean coastal land of Egypt. The following is a report about the flora of the different habitats of the Nile Region of Egypt.

I THE DELTAIC MEDITERREAN COAST

The deltaic Mediterranean coast of Egypt (the middle section of the Mediterranean coast) extends for about 180 km from Abu Qir to Port Said with a width in a NS direction for an average of 15 km from the sea. It is dotted with cities, villages and summer resorts such as Rashid, Baltim, Gamasah, Ezbit El - Burg, Kafr El-Batikh, Ras El-Bar, Damietta, etc.

The climate of this coast is a semi-arid one with total annual rainfall = 69 - 160 mm, mean maximum temperature = 27. 9° C mean minimum temperature = 14. 3oC, relative humidity = 60 - 74%, evaporation rate = 3.8 - 11. 1 mms / Piche.

Unlike the western and eastern sections, the middle section of the Mediterranean coast of Egypt is not only affected by the Sea water but it is also affected by the

waters of the northern lakes and the Damietta and Rosetta Branches of the River Nile. The plant cover of the deltaic Mediterranean coastal land is organized into sea landward zones (habitats) that vary in domination and floristic composition which will be studied in Baltim coast as sample area of this coast.

Baltim is a summer resort belongs to Kafr El-Sheikh Governorate. Its coastal area comprises 6 successive zones (habitats): beach zone, sand sheet zone, Gebel El - Nargis zone, zone of salt marshes, zone of palm trees - sand dunes and zone of the swamps.

The beach is a narrow strip of sand with a width varies between 100 - 200 m. It is practically barren of vegetation except of some dry remains of sea weeds and marine algae. The second zone is also narrow with sandy substratum dominated by *Silene succulenta* associate with *Cakile maritima , Cynodon dactylon , Polygonum equisetiforme , Alhagi maurorum , Melilotus indica , Erodium hirtum , Cyperus capitatus , Acacia saligna , Paspalidium geminatum , Dactyloctenium aegyptium , Lippia nodiflora , Ricinus communis (semi-wild) , Senecto desfontainei Mesembryanthemurn crystallinum , Parapholis marginata, Launaea angustifolia*

, Polypogon maritimus , Emex spinosus , Amaranthus sp ., Salsola kali, Malva parviflora, Ifloga spicata, Cutandia memphitica, Lotus halophilus, Euphorbia sp . etc. *Figs (Ficus carica) and watermelon (Citrullus vulgaris)* are cultivated by the natives in this zone.

Gebel El - Nargis sand dunes occupy the third zone of Baltim coast. They have two slopes: north facing and south facing, both are dominated by *Silene succulenta .* The floristic composion of these dunes include : *Lolium rigidum , Phoenix dactylifera* (semi - wild) , *Alhagi maurorum , Melilotus indica , Erodium hirtum , Lycopersicum esculentum , Cynodon dactylon Desmostachya bipinnata , Imperata cylindrica , Polygonum equisetiforme , Ipomaea stolonifera , Pancratium arabicum , Stipagrostis ciliaris , Echinops spinosissimus , Salsola kali , Ifloga spicata , Bromus rubens , Cakile maritima, Rumex pictus , Plantago indica , Malva parviflora , Ononis serrata , Pseudorlaya pumila , Carthamus glaucus , Polypogon monspeliensis , Daucus bicolor*

Cyperus capitatus and the cultivated plants Ficus carica, Vitis vinifera and Citrullus vulgaris. The fourth zone is a salt marsh habitat dominated by *Arthrocnemum glaucum*

with *Halocnemum strobilaceum* as abundant associate species. The other plants of this zone are: *Cressa cretica, Zygophyllum aegyptiurn, Frankenia revoluta, Sporobolus spicatus, Cyperus conglomeratus, Limonium pruinosum, Limoniastrum monopetalum, Cynodon dactylon, Polygonum equisetiform, Spergularia sp., Moltkiopsis ciliats, Aetheorhiza sp., Lippia nodiflora, Reichardia tingitana, Mesembryantemum crystallinum, Chenopodium sp.* etc.

The fifth zone is another zone of huge sand dune dominated by the semi - wild palm trees *Phoenix dactylifera (zone* of palms). In the depressed areas within these dunes the underground water is exposed forming local swampy habitat where *Typha domingensis* predominates. In the saline patches of the runnels within these dunes there are societies dominated by *Arthrocnemum glaucum, Schoenus nigricans, Sporobolus viginicus, Imperta cylindrica* and *Zygophyllum aegyptium.* The other societies of the palm dunes include: *Pancratium arabicum* (abundant), *Erodium hirtum Alhagi maurorum, Cyperus capitatus, Desnmostachya bipinnata, Rumex pictus, Ononis serrata, Pseudorlaya pumila, Launaea angustifolia,*

Malva parviflora, Sinapis arvensis, Adonis dentatus, Lobularia libyca, Plantago sp. etc.

The innermost zone is a depression which receives the drainage water seeped from the cultivated lands of Baltim villages and from Lake Burullus. *Typha domingensis* is the dominant reed of these swamps associated with: *Phragmites australis.* In the saline banks of these swamps grow: *Juncus rigidus, J acutus, Cyperus conglomeratus, Cressa cretica, Suaeda pruiosa , Tamarix tetragyna , Halimione portulacoides, Inula crithmoides, Mesembryanthemum crystallinum, Frankenia revoluta, Polygonum equisetiform* etc.

II THE AQUTIC HABITAT

In Egypt, where the climate is warm during most of the year the aquatic flora (the hydrophytes) of the River Nile and its irrigation and drainage canals are well developed. The establishment of Aswan High Dam (1965) controls to great extent the flow of water in the River Nile and its Damietta and Rosetta Branches which resulted in a better penetration of light (due to the great reduction in silt) and reduction in rate of water flow. Such new conditions enhance the growth of the hydrophytes of

both types: submerged and floating. Even, new water weeds e. g. *Myriophyllum spicatum* started to appear.

The aquatic plants of the Nile System of Egypt comprise 35 species belonging to 19 genera and 15 families as follows.

1. **Family Araceae**

Pistia stratiotes: Free floating weed present only in the calm and stagnant water canals of Faraskur (20 km south of Damitta) absence elsewhere in Egypt

2. **Family Ceratophyllaceae**

Ceratophyllum demersum is very common and dangerous submerged weed. *C. submersum* and *C. muricatum* are rare in Egypt.

3. **Family Haloragidadceae**

Myriophyllum spicatum has been recently recorded invading the River Nile System, never seen before the establishent of Aswan high Dam. It is a submerged weed.

4. **Family Hydrocharitaceae**

To this family belongs three submerged plants, namely: *Ottelia alismoides, Elodea canadensis and Vallisneria spiralis.*

5. Family Lemnaceae

This family comprises a group of very small floating water plants without distinct stem and leaves but with tiny leaf -like fronds forming green masses on the surface of stagnant waters. In the Nile system there are 6 species belonging to 3 genera namely: *Spirodela punctata, S. polyrrhiza. Lemna gibba, L. minor, L. perpusilla* and *Wollfia hyalina.*

6. Family Lentibulariaceae

Utricularia inflexa floating plant with finely dissected leaves carrying bladders in which small animals are caught (insectivorous water weed) It usually grows in the rice fields of the Nile Delta.

7. Family Marseliaceae

Marselia aegyptiaca is an aquatic fern common in all waters of the Nile System of Egypt. *Marselia capensis* is rare and present only in the Nile Delta.

8. Family Najadaceae

Najas spp. is submerged water plants. *N*. *pectinatus*, N. minor and *N.graminea* are very rare in the Nile Delta, absent from other parts of the Nile System. *N*. *armata* is common in the Nile delta and Fayium

9. Family Nymphaeaceae

Nymphaea coerulea (blue water Lily) and N. louts (White water Lily) are the sacred water lilies of the ancient Egyptians. They are floating plants common in the Nile Delta, rare or absent in the Nile valley.

10. Family Onangaraceae

Jussiaea repens very rare free-floating weed.

11. Family Pontederiaceae

The genus *Eichhornia* includes free floating plants that occur in Egypt in two species: *E. crassipes* and *E. azurea.* The second is very rare and grows (cultivated) in the gardens of Cairo, it causes no trouble. *Crassipes,* on the other hand, is the most dangerous water weed in Egypt (water Hyacinth or Ward El - Nil).

12. Family Potamogetonaceae

Potamogeton spp. are submerged weeds and include:

(i) *P. crispus* very common.

(ii) *P. pictinatus* very common

(iii) *P. nodosus* common

(iv) *P. peifoliatus* rare (in Nile Nubia only) All *Potamogeton* spp. are dangerous hydophytes.

13. Family Ranunculaceae

Ranunculus saniculifolius, R.rionii R,trichophyllus **and**

R. sphaerospermus is rare in the Nile Delta absent from Upper Egypt.

14. Family Ruppiaceae

Ruppia maritima var. *spiralis, R.maritima* var*. rostrata* **are** submerged hydrophytes.

15. Family Zannichelliaceae

Zannichellia Palustris is very common water weed in all water bodies of Nubia and Lake Nasser.

III THE SWAMPY HABITAT

The weeds of the swampy habitat are immersed plants with roots, rhizomes and lower parts of their aerial shoots are under water. These include *Phragnzites australis* the most serious and very common reed in Egypt. It belongs to grass family Gramineae. *Typha domingensis (Typhaceae*) is another dangerous reed very common in Egypt.

The other swampy plants comprise the following: *Echinochloa staginum, E.crusgalli, Paspalidium geminatum, Polypogon monspeliensis, Diplacahne fusca* etc. (Gramineae), *Polygonum salicifolium, P. senegalensis, (Polygonaceae)*, *Veronica anagallis - aquatica* (Schrophulariaceae), *Cyperus articulates C. longus, C. difformis, Scirpus litoralis,* (Cyperaceae)

Juncus subulatus (Juncaceae) etc. *Cyprus papyrus* was very common is the Nile Delta swamps during ancient time. Its culms were used is making papers. Nowadays it is eliminated from the swampy habitats of Egypt. Few individuals are growing in Chaman Garden of Cairo.

IV CANAL BANK HABITAT

These include cultivated and naturally growing trees, shrubs, under shrubs and herbs. The important species are:

a) Cultivated plants.

Ficus sycomors, Morus alba, M. nigra , Acacia nilotica , Melia azederach , Parkensonia aculeata , Salix safsaf , S. babylionica , Zizyphus spina - cristi , Casuarina equisetifolia , Dalbergia sisso , Eucalyptus rostrata , E. citriodora , Ricinus communis , Opuntia ficus - indica etc.

Wild Plants

Tamarix arborea, Conyza dioscoridis, Desmostachya bipinnata, Imperata cylindrica, Inula crithmoides, Suaeda vermiculata. Arthrocnemum glaucum, Arundo donax, Alhagi maurorum, Dichanthium annulatum, Panicum maximum, Kochia. indica , Mentha silvestris, Lippia nodiflora, Silybum marianum, Sphaeranthus suaveolens, Canna indica, Saccharum spontaneum, Cyperus

laevigatus, Trifolium resupinatum, Nitraria retusa, Ambrosia maritima, Andropogon annularis, Urospermum picroides, Halimione portulacoides , Glinus lotoides , Ethulia conyzoides , Verbena supina etc.

V. WEEDS OF THE CULTIVATED LANDS

Weed flora of the cultivated lands of Egypt are mainly ephemeral, and annual herbs. Perennial herbs, under shrubs and shrubs may also be present. These weeds are associated with the summer and winter crops. Weeds of common occurrence in winter crops are : *Melilotus indicus, Cynodon dactylon, Sonchus oleraaceus, Chenopodium murale, Trifolium resupinatum, Anagallis arvensis, Chenopodium album, Brassica nigra, Polypogon monspeliensis, Vicia calcarata, Malva parviflora, Emex spinosus, Solanum nigrum, Polygonum equisetiforme, Xanthium brasillicum, Urochloa reptans, Cichorium pumilum, Dactyloctenium aegypticum, Eragrostis pilosa* etc .

The weeds of summer crops include. *Echinochloa colonum , Cynodon dactylon, Portulaca oleracea , Convolvulus arvensis , Cyperus rotundus , Sonchus , oleraceus , Solanum nigrum , Xanthium spinosum , Silene rubelaa , Amaranthus chlorostachys , Beta*

vulgaris , Rumex dentatus , Ammi majus , Euphorbia peplus , P lantago lagopus , Lotus corniculatus and Reichardia orientalis

VI. THE NOTHERN LAKES

The northern lakes of the Nile Delta namely: Lake Manzala, Lake Burullus and Lake Idku are located very close to the Mediterranean Sea . They are separated from it by strip of land that are very narrow in several places and in the same time are connected with the sea through outlets (straits).

Lake Manzala is the largest (= 300,000 feddans). It lies between the Mediterranean Sea to the north, the Suez Canal to the east, the damietta Branch and the povinces of Sharkiya and Dakahlya to the west. Thus, Lake Manzala serves 5 provinces of Egypt namely: Ismaillia, Port Said, Damietta, Sharkiya and Dakhaliya. It is shallow lake with depth does not exceed one meter. It is characted by many Islands (about 1022).

The plant life of Lake Manzala comprises halophytic elements that grow mainly on the shores of the islands. These include 26 species belonging to all families as follows : *Artrocnemum glaucum, Atriplex fahnosa , Halimione portulacoides , Halocnemum strobilaceum ,*

Halopeplis perfoliata , Salicornia fruticosa , S.herbaces , Salsola kali , S.longifolia, Suaeda pruinosa , S.salsa S.vermiculata and S.vera (Chenopodiaceae), Arundo donax, Phragmites australis and Sporobolus spicatus (Gramieae) , *Cressa cretica (Convolvulaceae) Cistanche Phelypaea (* Orobanchaceae) *Cyperus Laevigatus (* Cyperaceae) , *Inula crithmoides (* Compositae) , *Juncus rigidus (* Juncaceae) , *Tamarix aphylla (Tamaricaceae*), *Typha domingensis (Typhaceae) and Zygophyllum album (* Zygophyllaceae) .

Fresh water hydrophytes namely: *Eichhornia crassipes. Potamogeton crispus, P.petinatus , Lemna spp., Ceratophyllum demersum* are present in the water of the lake .

VII MAN MADE LAKE

The construction of Aswan High Dam in the most southern part of Egypt resulted in the formation of a huge man-made lake: High Dam, Lake: mean depth - 24.8 m, mean width = 18 Km.

The shore - line vegetation comprises the following floristic elements: *Tamarix nilotica , Hyoscyamus muticus , Phragmites australis , Salsola baryosma , Francoeuria crispa , Citrullus colocynthis , Fagonia arabica , Glinus*

lotoides , Heliotropium supinum , Rumex dentatus, Echium raumolfii , Portulaca oleracea , Pulicaria undulata , Senecio aegyptus , Calotropis procera , Morettia philaena etc . The shallow waters along the shore line is the habitat of some water plants e.g *Potamogeton trichoides, Najas minor, N. armata, Portamogeton nodusus, Zanichellia palustris, Ceratophyllum demersum* etc .

VIII THE NILE ISLANDS

The Nile at Aswan north of the High Dam is interrupted by abotut 30 uninhabited granite islands e.g. Duns islans , Burbur Island, Gezel Island etc . The submerged land of these islands is usually occupied by aquatic flora e.g *Ceratophylum demersum* and *Potamogeton crispis.* In the partly submerged land *Phragmites australis, Polygonum senegalensis, Panicum repens* and *Cyperus* sp. *Typha domingensis, Veronica anagallis-aquatic* etc grow. The meadow-grass habitat of this island is co-dominated by *Cyperus longus* and panicum repens. The floristion composition: *Cyperus mundtii, Cynodon dactylon , Sesbania sesban , Lotus arabicus , Cyperus rotundus , Tamara- nilotica, Trigonella hamosa, Mimosa Pigra, Salix subserrata, Cajanus Cajan , Saccharum*

spontaneum , Senecio aegyptus , Gnaphalium luteo-album, Sonchus oleraceus , Plantago major , Trigonella , hamosa , Leptadenia pyrotechinca, Francoeuria crispa etc.

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