FUNDAMENTALS OF PLANT PATHOLOGY

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Chapter 1
History of Plant Pathology

S.N.	Year	Scientist	Contribution
1	300BC	Theophrastus	Referred to plant diseases in the book *Historia plantarum* and *De causis plantarum*. Known as "Father of Botony"
2	1000AD	Surapal	Discussed plant diseases in his book *Vrikshayurveda*
3	1665	Robert Hooke	Observed cell wall of cork cells, illustrated teliospores of *Phragmidium disciflorum* under microscope. Written book "*Micrographia*"
4	1674	Anton von Leeuwenhoek	Observed bacteria – microbes named them "animalcules"
5	1729	Pier Antonio Micheli	Grew fungi on pieces of water melon and observed fungal spores. Known as "Father of Mycology". Written "*Nova Plantarum Genera*"
6	1755	Mathieu Tillet	Seed borne nature of wheat bunt. "Great grandfather of plant pathology"
7	1807	Benedict Prevost	Proved fungal pathogenicity in plant disease and demonstrated fungitoxic value of copper compounds for the first time.
8	1845-1849	Irish famine	Potato late blight epidemic in Ireland
9	1853	Anton de Bary	Established the role of fungi as a plant pathogen. Identified causal organism of late blight of potato. Known as "Father of Plant Pathology". Discovered heteroecism in rust.
10	1858	Julius Kuhn	Published the first book of plant pathology "Diseases of cultivated crops: their causes and their control"
11	1868-1882	Marshal Ward	Studies on coffee rust (*Hemilea vastatrix*) epidemics and etiology. "Father of Tropical Plant Pathology"
12	1876	Robert Koch	Koch's Postulates for ascertaining pathogenesis of microorganisms associated with plant disease
13	1878	Thomas J. Burrill	Described first bacterial disease – Fire blight of apple and pear
14	1882	Pierrie Alexis Millardet	Discovery of Bordeaux mixture for control of downy mildew of grapes
15	1885	Frank	Discovery of Mycorrhizal fungi
16	1886	Adolf Edward Mayer	Demonstrated the sap transmission of Tobacco mosaic virus (TMV)
17	1887	Erwin Frink	Major work on plant bacterial diseases and final proof of

		Smith	bacterial etiology of plant diseases. Father of Plant Bacteriology/Phytobacteriology
18	1892	Dimitri Ivanowski	Observed tobacco mosaic causing entities could pass through bacterial filters
19	1898	Martinus W. Beijerinck	*"Contagium vivum fluidum"* for TMV and coined the term virus. Father of Plant Virology
20	1905	Biffen	Described inheritance of resistance to yellow rust of wheat follow Mendelian laws of inheritance
21	1905	E.J. Butler	The work in the field of plant pathology was initiated at Pusa, Bihar with the establishment of Mycology section at the Imperial Agricultural Research Institute. "Father of Indian Plant Pathology"
22	1931-32	Knoll and Ruska	Invention of electron microscope
23	1935	W.M. Stanley	Crystallization of TMV and reported its as infectious protein by use of Ammonium sulphate
24	1943	S.A. Waksman	Discovered Streptomycin as a broad spectrum antibiotic
25	1943	Bengal famine	Brown spot of rice (*Helminthosporium oryzae*)
26	1946	H.H. Flor	Gene for gene hypothesis in flax rust system
27	1948	B.B. Mundkur	Establishment of Indian Phytopathological society
28	1968	Vanderplank	Concept of horizontal and vertical resistance. "Father of Plant Epidemiology"
29	1977	Clark and Adams	Development of ELISA assay for detection plant viruses
30	1984	Kary Mullis and Smith	Development of Polymerase Chain Reaction (PCR)
31	1986	Beachy et al.	Development of transgenic tobacco expressing coat protein gene of TMV showing resistance to the virus

Chapter 2
Plant Pathology – Concept, Terms and Definitions

1. **Plant Pathology**

   Plant pathology or Phytopathology (Phyton-plant: Pathos-ailments: logus-knowledge). This is the branch of agricultural, botanical or biological science which deals with the cause; etiology; resulting losses and management of plant diseases. The science of plant pathology has four main objectives:

   - To study the causes that induce disease
   - To study the mechanism of disease development by pathogens
   - To study the interactions between the plants and the pathogen
   - To develop strategies for managing the diseases and reduce the losses caused by them.

2. **Plant Disease**

   - Abnormal changes in physiological processes which disturb the normal activity of plant organs (Kuhn 1858).
   - Disease is a deviation from normal functioning of physiological processes of sufficient duration or intensity to cause disturbance or cessation of vital activity (American Phytopathological Society 1940).
   - Mal-functioning process in the plant body due to continuous irritation, which results in some suffering (Horsfall and Diamond).
   - Any malfunctioning of host cells and tissues those results from continuous irritation by a pathogenic agent that leads to symptom development.

   The pathogens bring about the irritation process resulting in a diseased condition of the plant through different but interrelated pathology:

   - By utilizing the host cell contents
   - By causing death of cells or by interfering with their metabolic activities through enzymes, toxins and growth regulators.
   - By weakening of tissues due to continuous loss of nutrients
   - By interfering with translocation of food, minerals and water.

3. **Disorders**

   The non infectious plant diseases due to abiotic causes such as adverse soil conditions, deficiency of nutrients etc. are termed as disorders.

4. **Symptom**

   External or internal reactions or alterations of a plant as a result of a disease.

5. **Pathogen**

   Any agent that can cause disease is called as pathogen. It includes all living organisms that can cause disease fungi, bacteria, fastidious bacteria, nematodes, phytoplasma, spiroplasma, virus, viroids, prions etc.

6. **Parasite**

   Organisms which derive nutrients they required for growth from living plants (susceptible host).

7. **Obligate parasites**
   The organisms which always obtain their food in nature from living tissues on they complete their life cycle. Eg. Rusts, powdery mildews, downy mildews, viruses etc.
8. **Saprophytes**
   Organisms that derive their nutrition from dead organic matter.
9. **Facultative saprophytes**
   It will attack living tissue in same way as that of biotrophs but will continue to to grow and reproduce after the tissue is dead. eg. *Phytophthora*
10. **Necrotroph**
    A pathogen which kills the host tissue in advance of penetration and then lives saprophytically. eg. *Sclerotium*
11. **Facultative parasite**
    Organism which lives as saprophyte but under favourable condition attack living host and become parasite. eg. *Pythium*
12. **Pathogenicity**
    The ability of a pathogen to cause disease.
13. **Pathogenesis**
    The chain of events (steps) which lead to development of disease in the host.
14. **Virulence**
    Degree of pathogenicity of an isolate or race of a pathogen
15. **Infection**
    Establishment of parasitic relationship between two organisms following entry or penetration.
16. **Immune**
    Denotes the condition where pathogen cannot establish parasitic relationship with the host.
17. **Resistance**
    The ability of an organism to exclude or overcome, completely or in some degree, the effect of a pathogen or other damaging factors.
    - **Vertical**/differential/Monogenic/Complete/Environment independent resistance: Complete resistance to one or few races of a pathogen but not to all races of the same pathogen.
    - **Horizontal**/Uniform/Polygenic/General/Partial/Field/Dilatory/Slow rusting/Environment dependent: Partial resistance equally effective against all races of a pathogen.
18. **Tolerance**
    The ability of a plant to sustain the effects of a disease without dying or suffering serious injury or crop loss.
19. **Disease escape**
    The ability of an otherwise susceptible plant to avoid damaging disease stress because of the way it grows.
20. **Incubation Period**

The time lapse between inoculation and appearance of symptoms.

21. **Inoculum**
    Part of pathogen that can initiate infection
22. **Primary Inoculum**
    An inoculum that survives dormant in the winter or summer and causes original infection in the spring or autumn.
23. **Secondary Inoculum**
    An inoculum produced from primary inoculum.
24. **Epidemic disease**
    Occurs widely, in severe and virulent form
25. **Endemic disease**
    Constantly present in moderate to severe form in a locality.
26. **Pandemic disease**
    Occurs all over the world and cause mass mortality.
27. **Sporadic disease**
    Occurs at very irregular interval and location.
28. **Hypersensitivity**
    Excessive sensitivity of plant tissues to certain pathogens. Affected cells are killed quickly, blocking the advance of obligate parasites.
29. **Wilt**
    Loss of rigidity and drooping of plant parts, generally caused by insufficient water in the plants.
30. **Virulent**
    Capable of causing a severe disease, strongly pathogenic.
31. **Virus**
    A submicroscopic obligate parasite consisting of nucleic acid and protein.
32. **Viroids**
    Small, low molecular weight RNA that can infect plant cells, replicate themselves, and cause disease.
33. **Vector**
    An organism that is able to transmit a pathogen.
34. **Systemic**
    Spreading internally throughout the plant body, said of a pathogen or a chemical.
35. **Susceptible**
    Lacking the inherent ability to resist disease or attack by a pathogen.
36. **Spore**
    Reproductive unit of a fungi consisting of one or more cells.
37. **Sign**
    The pathogen or its parts or products seen on a host plant.
38. **Rust**

A disease giving a "rusty" appearance to a plant and caused by one of the Uredinales rust fungi.

39. Smut

A disease caused by smut fungi (Ustilaginales) characterized by masses of dark, powdery and sometimes odorous spores.

40. Rot

Softening, discoloration and often disintegration of a succulent plant tissues as a result of fungal or bacterial infection.

41. Sanitation

The removal and burning of infected plant parts, disintegration of succulent plant tissues as a result fungal or bacterial infection.

42. Quarantine

Control of import and export of plants to prevent spread of diseases and pests.

43. Polymerase chain reaction

A technique that allows an almost infinite amplification (multiplication) of a segment of DNA for which a primer (short piece of that DNA) is available.

44. Perfect stage

The sexual stage in the life cycle of a fungus, the Teleomorph.

45. Polycyclic disease

Pathogen completes more than one (many) generation per growth season in one year or crop season and with each cycle the amount of inoculum is multiplied manyfold.

46. Monocyclic disease

Pathogen completes single disease cycle or single generation in a crop season.

47. Integrated disease management

The attempt to prevent pathogens from causing economic losses by using a variety of management methods that are cost effective and cause the least damage to the environment.

48. Host range

The various kinds of host plants that may be attacked by a parasite.

49. Biological control

Total or partial destruction inhibition or destruction of pathogen populations by other organisms.

50. Disease triangle

Susceptible Host + Virulent pathogen + Congenial environment = Disease

51. Race

A genetically and often geographically distinct mating group within a species, also a group of pathogens which can infect a given set of plant varieties.

52. Isolate

A single spore or culture and the sub cultures derived from it. Also used to indicate collections of a pathogen made at different times.

53. Forma specialis (f.sp)

A group of races or biotype of a pathogen species that can infect only plants within a certain host genus or species.

54. Biotype

A subgroup within a species or race, usually characterized by common possession of a single or a few new characters.

55. Pathovar

In bacteria, a subspecies or a group of strains that can infect only plants within a certain genus or species.

56. Strain

The descendents of a single isolation in pure culture, an isolate. Also a group of similar isolates, a race. In plant viruses, a group of virus isolates having most of their antigens in common.

Chapter 3
Important Plant Pathogenic Organisms

A. Fungi

History

- Anton de Bary is considered father of plant pathology for – identifying late blight pathogen, worked on *Sclerotinia* diseases with biochemical studies, given term heteroecism. He is also known as Founder of Modern Mycology.
- P.A. Micheli is considered as father of Mycology (study of fungus).
- Toadstool is derived from German word 'Todestuhl' means – death chair.
- Roman emperor, Cladius Caesar, was murdered by his wife in 54 AD with decoction of *Amanita phalloides.*
- A festival 'Robigalia' was celebrated to owe the wheat rust god 'Robigus'.
- Late blight epidemics of potato occurred in 1845 in Ireland and Europe. Mass migration of human population took place from Europe to northern America and Canada.
- Alexander Fleming discovered Penicillin from *Penicillium notatum.*
- Bakane disease or foolish seedling of rice was discovered in Japan. This disease is caused by *Gibberella fujikuroi* (imperfect stage: *Fusarium moniliforme*).
- IAA was first time isolated *Rhizopus niger*.
- Heterothallism was discovered in *Mucor tenuis* by Blakeslee in 1904.
- Parasexual reproduction was discovered by Pontecorvo and Roper (1952) in *Aspergillus nidulans.*
- LSD (d-lysergic acid dimethylamide) is isolated from *Claviceps purpurea*.
- 'Five Kingdom Classification' was given by R.H. Whittakar in 1969.
- Bordeaux mixture was discovered by P.A. Millardet in 1882.
- Kernal bunt disease of wheat was discovered by M. Mitra in 1931 from Kernal of Haryana.
- Solar heat treatment was given by Luthra and Sattar (1934) for loose smut of wheat.

Classification

Ainsworth's Classification

Classification was done on the basis of sub-division.

1. Mastigomycotina (lower fungi, downy mildew causing fungi)
2. Zygomycotina (Mucror, Rhizopus etc.)
3. Ascomycotina (powdery mildew causing fungi)
4. Basidiomycotina (rust, smut and bunt causing fungi)
5. Deuteromycotina (*fungi-imperfecti*: fungi do not reproduce sexually)

Modern Classification

1. Funga-like organism
    a. Kingdom Protozoa
        i. Phylum – Myxomycota e.g. *Physarales*
        ii. Phylum – Plasmodiophoromycota e.g. *Plasmodiophorales*

    b. Kingdom Chromista
        i. Phylum – Oomycota e.g. *Saprolegniales, Peronosporales*

2. True fungi
    a. Kingdom Fungi
        i. Phylum – Chytridiomycota
        ii. Phylum – Zygomycota
        iii. Phylum – Ascomycota
        iv. Phylum – Basidiomycota

3. Imperfect fungi
        i. Phylum – Deuteromycota

Reproduction

a. Vegetative Reproduction

i. Fragmentation

In this process, the mycelium breaks into two or more similar fragments either accidentally or due to some external force. Each fragment grows into a new mycelium.

ii. Budding

The parent cell produces one or more projections called buds, which later develop necessary structures and detach to grow into new individuals. Budding is common in unicellular forms like yeast.

iii. Fission

In this process, the parent cell splits into two equal halves, each of which develops into a new individual. Fission is also common in yeast.

iv. Sclerotia

In some cases, as in *Claviceps*, the hyphae become interwoven to form a compact mass and get surrounded by a hard covering or rind. Such structures are called sclerotia. They remain dormant under unfavourable conditions and germinate into new mycelia on the return of favourable conditions.

v. Rhizomorphs

In some higher fungi, several hyphae may become interwoven to form rope-like structures called rhizomorphs. Under favourable conditions, they resume growth to give rise to new mycelia.

b. Asexual Reproduction

It is a type of reproduction in which special reproductive structures called spores or propagates are formed. The fungal spores always result from mitosis and hence are described as mitospores. Following are the types of spores produced in different groups of fungi:

i. Zoospores

They are flagellated, motile spores produced inside structures called zoosporangia. These spores do not have a cell wall. Such spores are produced in lower fungi such as *Achyla* and *Saprolegnia*.

ii. Sporagiospores

These are non-motile spores produced inside structures called sporangia in fungi such as *Rhizopus* and *Mucor*. These spores are dispersed by wind.

iii. Chlamydospores

These are thick walled resting spores which arise directly from hyphal cells. They store reserve food.

iv. Oidia

These are spore like structures formed by the breaking up of hypha cells. They do not store reserve food and hence cannot survive under unfavourable conditions. Such spores are produced in *Rhizopus*.

v. Conidia

These are non-motile spores produced singly or in chains at the tip of the hypha branches that are called conidiophores. Such spores are produced in fungi like *Aspergillus* and *Penicillium*.

c. Sexual Reproduction

Sexual reproduction is known to occur in all groups of fungi except the *fungi-imperfecti* or Dueteromycetes. It may involve fusion of gametes, gametangia or hyphae. The process may involve only fusion of cytoplasm (plasmogamy) or fusion of nuclei (karyogamy) or production of meiotic spores (meiospores). In most of the lower fungi plasmogamy is immediately followed by karyogamy and meiosis. In higher fungi karyogamy is often delayed so that the hyphae remain dikaryotic. This phase of fungal life cycle is called dikaryophase. Such fungi complete their life cycle in three phases a haplophase, a dikaryophase and a diplophase. Sexual fusion in fungi is of different types, as follows:

vi. Planogametic copulation

Here motile gametes called planogametes undergo fusion. When both the gametes are motile and morphologically similar, the fusion process is called isogamy.
e.g.: *Synchytrium*: when both the gametes are motile but differ in their size, the fusion process is called anisogamy.
e.g.: *Allomyces*: when one gamete (male) is smaller and motile and the other (female) gamete is larger and non motile, the fusion process is called heterogamy.

vii. Gametangial contact

Here, gamete bearing structures called gametangia come closer to each other and develop a fertilisation tube through which the male gamete migrates into the female gametangium.
e.g.: *Phytophthora, Albugo*.

viii. Gametangial copulation

Here, the gametangia fuse with each other, lose their identity and develop into a zygospore.
e.g.: *Mucor, Rhizopus*.

ix. Spermatisation

In some fungi like Puccinia, tiny unicellular spore like structures called spermatia are formed. They get transferred to female gametangia through various agencies.

x. Somatogamy

In examples like *Agaricus*, fusion occurs between two somatic cells and involves only plasmogamy. This results in the formation of dikaryotic hyphae. Hence, the process is called dikaryotization.

xi. Heterothallism

Based on the compatibility in sexual reproduction the fungal hyphae can be distinguished into two types homothallic and heterothallic. In homothallic forms, fusion occurs between the genetically similar strains or mating types.

Reproduction between morphologically similar but genetically or physiologically dissimilar thalli is known as heterothallism. It was proved in *Mucor tenuis* by Blakeslee (1904). Heterothallism means having male and female reproductive organs on different thalli, in which sexual reproduction occurs only between two self-sterile mycelia.

B. Bacteria

History

- Father of Plant Bacteriology is E.F. Smith. He established the role of bacteria as plant pathogen. He worked on cucumber and solanaceous crops, black rot of crucifers. He was the first person who showed crown gall disease caused by *Agrobacterium tumefaciens*.

- Father of bacteriology is Louis Pasteur.
- Father of bacteriological techniques is Robert Koch – given Koch's Postulates.
- Anton von Leeuwenhoek first time seen bacteria and protozoa under a self-made microscope and regard them as 'animalcules'.
- T.J. Burrill first time reported a bacterial disease, fire blight of pear and apple, caused by a bacterium *Erwinia amylovora*.
- Se'dillot coined the term 'microbe'.
- F.J. Cohn coined the term 'bacteria'.
- Woronin reported that root nodules of legumes contain bacteria.

Morphology of bacteria

- Coccus - spherical shaped
- Diplococcus - cocci from paired cells
- Tetracoccus - four cells arranged in a cube
- Streptococcus - chain of cocci
- Sarcina - division in three planes produces eight cells arranged in a cube
- Staphylococcus - grape-like cluster
- Bacillus - rod or capsule
- Vibrio - comma shaped
- Spirillum - rigid, wavy shaped
- Spirochaete - cork-screw shaped
- Star-like - star-shaped bacteria

Types of bacteria

1. Stalked bacteria: e.g. *Caulobacter*, *Gallionella*
2. Budding bacteria: e.g. football shaped, network of cells
3. Myxobacteria: e.g. gliding bacteria, lack rigid cell wall and flagella, cigar shaped
4. Spirochaetes: e.g. *Spirochaeta*, *Cristispira* – large, helical or spiral, flagella is absent
5. Actinomycetes: e.g. *Streptomyces* – thread-like mycelial bacteria

Structure of bacterial cell

1. Surface appendages – flagella, fimbriae and pilli.
2. Surface adherents – capsule and slime layers.
3. Cell wall – made up of mucopeptide.
4. Cytoplasm and organelles – lack organized nucleus, nucleoid is circular, genome is not organized into chromosomes, ribosomes are free.
5. Special structures – endospores, stalks, mesosome.

Flagellation in bacteria

1. Atrichous – e.g. *Lactobacillus*
2. Monotrichous – e.g. *Xanthomonas*
3. Lophotrichous – e.g. *Spirilla*
4. Amphitrichous – e.g. *Pseudomonas*

5. Cephalotrichous – e.g. *Spirilla*
6. Peritrichous – e.g. *Erwinia*

Difference between Gram positive and Gram negative bacteria

Gram staining was given by Christian Gram in 1884

Gram positive	Gram negative
Homogenous layer	3-layered
Techoic acid present	Techoic acid absent
Outer membrane absent	Outer membrane present
Periplasmic space absent	Periplasmic space present

Classification of bacteria

On the basis of division:

1. Fermicutes: Gram$^+$: e.g. *Clavibacter*
2. Gracillicutes: Gram$^-$: e.g. *Agrobacterium*, *Pseudomonas*, *Acetobacter*
3. Tenericutes: mollicutes e.g. spiroplasma, phytoplasma, *Streptomyces*
4. Mendosicutes: e.g. *Archea*

Important bacterial diseases of plant

S.N.	Bacterium	Disease
1	*Erwinia amylovora*	Fire blight of pear and apple
2	*Erwinia carotovora* pv. *carotovora*	Soft rot of vegetables
3	*Agrobacterium tumefaciens*	Crown gall of trees
4	*Streptomyces scabies*	Common scab of potato
5	*Xanthomonas oryzae* pv. *oryzae*	Bacterial leaf blight of paddy
6	*Xanthomonas campestris* pv. *malvacearum*	Angular leaf spot of cotton
7	*Xanthomonas campestris* pv. *citri*	Citrus canker
8	*Pseudomonas solanacearum*	Bacterial wilt (Moko disease) of banana
9	*Pseudomonas syringae* pv. *phaseolicola*	Halo blight of beans
10	*Pseudomonas syringae* pv. *glycinea*	Bacterial blight of soybean
11	*Pseudomonas syringae* pv. *tabacci*	Wild fire of tobacco
12	*Clavibacter michiganensis*	Ring rot of potato
13	*Clavibacter xyli*	Ratoon stunting of sugarcane
14	*Xylella fastidiosa*	Pierce's disease of grapes

C. Fastidious Vascular Bacteria or Mollicutes

History

- L. Pasteur claimed that the incitant of 'bovine pleuropneumonia' could neither be seen nor grown in culture.
- Nocard and Roux (1898) succeeded in growing the organism in artificial culture medium, what they regard as *Mycoplasma mycoides*.
- Doi and co-workers in 1967 observed mycoplasma-like bodies in phloem cells of mulberry, and regard as phytoplasma.
- Ishiie and co-workers in 1967 observed mycoplasma-like bodies could be temporarily disappeared when plants were treated with tetracycline.

- Davis and Worley in 1972 discovered spiroplasma in corn stunt disease.
- Rickettsia was discovered by Windsor and Black (1972) in clover club disease.

Characteristics of phytoplasma

- Pleomorphic: bound by 'unit membrane'.
- They are surrounded by 'triple-layered membrane'.
- Lack cell wall.
- Filterable in Millipore Filter (0.45µ).
- Ribosomes are randomly distributed.
- All enzymatic activities are found.
- Transmission: mostly by leaf hopper.

Characteristics of spiroplasma

- Helical shaped.
- Lack true cell wall.
- Fried-egg appearance.
- Sensitive to tetracycline.

Characteristics of Rickettsia

- Cell wall present.
- Double-layered membrane.
- Habitat: Xylem – e.g. *Xylella fastidiosa* (G^-), *Clavibacter xyli* (G^+)
  Phloem – e.g. *Candidatus liberobacter* (G^-)
- Sensitive to both tetracycline and penicillin.

D. Viruses

History

Adolph Mayer (1886):

- Inoculated healthy tobacco plant with extraction of yellow tobacco.
- Yellow mosaic symptom appeared.

Ivanowski (1892):

- Filtered the infected tobacco extraction through a filter that retains bacteria.
- Inoculated the decoction on a healthy tobacco plant.
- Symptom developed.
- Conclusion: toxin that produced similar symptom from the juice.

Beijirinck (1898):

- Repeated the experiment of Ivanowski.
- Conclusion: disease was not caused by a microorganism.
- Coined the term: '*contagium vivum fluidum*'.
- Considered father of plant virology.

Hashimoto and co-workers (1894) first time reported a virus (dwarf disease of rice) can be transmitted by insect.
Twort and d'Herelle (1917) discovered bacteriophage.
Stanley (1935) crystallize virus for the first time by using ammonium sulphate.
Bawden and Pierie (1936) explained that virus is made up of protein and RNA.

Kausche co-workers (1939) first time seen virus (tobacco mosaic virus) under electron microscope.
Gierrer and Schramm (1956) concluded that protein doesn't carry genetic information, thus protein could be removed to cause infection.

Virus is a nucleoprotein that multiplies only in living cells and has the ability to cause disease. Viruses are neither cell nor do they consist of cells.

Classification
94% viruses are RNA virus. Among them 70% are ssRNA.

ssRNA	TMV, Poty virus (PV-Y) – papaya ring spot virus, Closteroviridae – citrus tristeza, Siquiviridae (RTSV – rice tungro spherical virus)
dsRNA	Reoviridae (Fiji disease of sugarcane), rice ragged stunt virus
ssDNA	Geminiviridae (bean golden mosaic virus, tomato leaf curl virus)
dsDNA	Caulimoviridae (rice tungro bacilliform virus)

Transmission

1. Mechanical e.g. TMV
2. Seed e.g. TMV, sunflower necrosis virus (pollen transmitted)
3. Propagation e.g. citrus tristeza, banana bunchy top virus
4. Vector e.g.
   a. Nematode: *Trichodorus*, *Paratrichodorus*, *Xiphinema*, *Longidorus*
   b. Mite: *Aceria cajani* (pigeonpea sterility mosaic virus)
   c. Fungus: *Spongospora subterranea* (potato mop top virus)
   d. Insect: Hemiptera (aphid, whitefly)
      Thysanoptera (thrips)
      Coleoptera (beetle)

E. Viroid

Viroids are small, naked, single-stranded, circular molecule of infectious RNA. Viroid lack protein coat.
e.g. cadang-cadang disease of coconut, citrus exocortis.
Viroid was first time discovered by T.O. Deiner in 1971 in potato spindle tuber disease.

F. Phanerogamic plant parasite

Loranthus was first time recognized by Albertus Magnus around 1200 AD.

Stem parasite

Cuscuta	Total stem parasite
Loranthus	Semi stem parasite

Root parasite

Orobanche	Total root parasite
Striga	Semi root parasite

G. Algae

Plant pathogenic alga causes red rust.

It has disc-like thallus, single-celled organism. *Cephaleuros virescens* causes spots in leaf and stem of tea, coffee, cacao, citrus, mango etc.

H. Protozoa

Lafont in 1909 observed latex bearing cells of plants in family Euphorbiaceae.

Stahel in 1931 observed flagellate trypanosomatid, which is phloem-restricted.

Phytomonas causes empty root of cassava, hartrot of coconut, phloem necrosis of coffee.

Chapter 4

Survival of Plant Pathogens

I. Fungal pathogens

i. Infected host as a reservoir of primary inoculum

Cultivated host: The crops which are grown year round

eg. Red rot of sugarcane *Colletotrichum falcatum* (Infected cuttings)

Wheat rusts in India: uredospores are source of primary inoculum from Nilgiri, Palni and Karnataka hills for stem rust since alternate host of *Puccinia graminis tritici* that is *Barberry* is absent in India. Himalayan foot hills for yellow rust and both the above given cases for brown rust of wheat.

Wild host of same family as that of the host (Collateral host).

Rice blast (*Pyricularia oryzae*) Seed and soil transmission but in India the primary inoculum comes from early sown rice or relative wild species and grasses (*Poa pratense*).

Wild host of other family (Alternate host).

Stem rust of wheat: Alternate host (*Barberry vulgaris*) all over the world but in India the presence of alternate host is still not known.

ii. Saprophytic survival

Facultative parasites are capable of surviving as saprophytes

Fungi with high saprophytic ability:

eg. *Pythium*: Foot rot of Papaya (*P. aphanidermatum*), Damping off of potato (*P. debaryanum*).

Rhizoctonia: Sheath blight of rice

Fungi with low saprophytic ability:

eg. *Fusarium* and *Verticillium*: Wilt of different crops

Root inhabiting fungi are having low saprophytic ability, remain active in saprophytic phase as long as host tissue in which they were living as parasites are not completely decomposed.

iii. Dormant structures of fungi

Produces spores and other resting structures for their inactive survival.

Soil borne: Conidia, oospore, perithecia, thickened hyphae.

Seed borne: Dormant spores on seed coat (Externally seed borne: Covered smut of bajra), Dormant mycelium and spores under seed coat on embryo (Internally seed borne: Loose smut of wheat)

Dormant fungal structures on dead plant remains: Club root of crucifers (*Plasmodiophora brassiacae* as resting spores on crop debris to an extent of 10 years)

II. Survival of Phytopathogenic Bacteria

1. Seed

e.g. bacterial blight of rice (*Xanthomonas oryzae pv. oryzae*), bacterial leaf streak of rice (*X. oryzae pv. oryzicola*), bacterial blight of cotton (*X. axonopodis pv. malvacearum*), canker of tomato (*Clacibacter michiganensis subsp. michiganensis*) 3 years and bacterial speck of tomato (*Psedomonas syringae pv. tomato*) 20 years.

2. Plant residue

Decomposing debris of the host plants: *Xanthomonas* (citrus canker: *X. axonopodis pv. citri*, black rot of crucifer (*Xanthomonas campestris pv. campestris*) 1 year.

3. Soil

Transient visitors

Consist of bacterial species whose populations are developed almost exclusively on host plant where maximum number of generations is produced. When such bacteria reach the soil with rain water or with debris the population decline rapidly and don't remain important source of primary inoculum for next season.

e.g. *Xanthomonas, Erwinia, Clavibacter*

Resident visitor

These bacteria also have maximum generation in the host but their number only gradually decline in soil.

e.g. *Agrobacterium tumefaciens* (crown gall of stone fruits)
Ralstonia solanacearum race 1 (wilt of solonaceous vegetables)
Streptomyces scabies (potato scab)

4. **Perennial host**

Citrus canker survives on fallen leaves (*X. axonopodis pv. citri*)
Fire blight of apple and pear (*Erwinia amylovora*) on dead twigs and buds.

5. **Insect Association**:

Corn wilt: *Pantoea stewartii* subsp. *stewartii* overwinters in corn flea beetle *Chaetocnema pullicaria*.

III. Plant Viruses

No dormant stage and maintains a continuous infection chain on crop host and reservoirs host.

Insect association:

- Whitefly-leaf curl (tomato, chilli, etc)
- Thrips-spotted wilt (tomato), bud necrosis (groundnut)
- Mite-Sterility mosaic (Pigeon pea)
- Leafhopper-Tungro (rice)
- Aphid-Bunchy top (Banana), Ring spot (Papaya), mosaic (Soyabean, Cauliflower)
- Mealy bug- Swollen shoot (Cocoa)

Chapter 5

Dispersal of Plant Pathogens

The dispersal of pathogens is accomplished in the following manner:

I. Direct (active or autonomous) dispersal, such as dispersal in and by soil and by seed and planting material.

II. Indirect (passive) dispersal involving the role of man, insects, nematodes and other animals, water and air.

1. Soil

The dispersal through soil can take place by following ways:

i) Movement of pathogens in soil. e.g. Nematodes, flagellated zoospores of *Pythium* and *Phytophthora* can swim on film of water present in soil.

ii) Dispersal in soil through growth of pathogen. eg. *Armillaria mellea* (Root rot of citrus and other tress) produces strands of interwoven hyphae (Rhizomorph) to reach roots of plants.

iii) Movement of contaminated soil. e.g. Bacteria: *Streptomyces scabies* (Potato scab), facultative parasites and facultative saprophytes.

2. Seed

The dispersal through seed can take place by following ways:

i) Seed admixture: By fixing of fungal and bacterial structures in seeds during harvesting Eg. Ear cockle of wheat (*Anguina tritici*), Bajra smut (*Tolyposporium penicillaria*) and Ergot of bajra (*Claviceps fusiformis*).

ii) Internally seed borne: Loose smut of wheat (*Ustilago nuda tritici*), Pea seed borne mosaic virus and Bean common mosaic virus.

iii) Externally seed borne: Covered smut of barley (*Ustilago hordei*)

3. Vegetative propagation

Virus and viroids are generally spread from infected mother plant to progeny through vegetative propagation. Citrus canker (*Xanthomonas axonopodis* pv *citri*), Red rot of sugarcane (*Colletotrichum falcatum*) and ratoon stunting of sugarcane (*Clavibacter xyli* or *Leifsonia xyli*).

4. Mechanical or human practices

Various cultivation practices like, cultural operations, weeding, pruning, cutting, grafting, budding by the use of contaminated implements, cutting knives (*Potato spindle tuber Viroid*-PSTVd). The spread of spores, cysts and other fungal and bacterial dormant structures can take place from one area to other.

Sap transmitted viruses: *Tobacco mosaic virus* (TMV), *Potato virus X* (PVX), *Cucumber mosaic virus* (CMV) and *Cucumber green mottle mosaic virus* (CGMMV).

5. Nematode

Yellow ear rot/ Sehun disease of wheat: *Rathayaibacter tritici* and *Anguina tritici.* Where the nematode Anguina helps in spread of the bacteria and its movement from root to upper portion of plant.

Xiphinema: Grape vine fan leaf virus

6. Insect Association

Various bacteria, virus and phytoplasma are spread through the insect vectors.

S.N.	Vector	Pathogen	Disease	Crop
1	Spotted cucumber beetle (*Diabrotica didecipunctata*)	*Erwinia tracheiphila*	Cucurbit wilt	Cucurbits
2	Corn flea beetle (*Chaetocnema pullicaria*)	*Pantoea stewartii* subsp. *stewartii*	Corn wilt	Maize
3	Aphid (*Myzus persicae*, *Aphis craccivora*)	*Potato virus Y, Papayya ring spot virus, Banana bunchy top virus, Soyabean mosaic virus*	Papaya ring spot disease, Cauliflower mosaic disease	Various fruit and vegetable diseases
4	Whitefly (*Bemisia tabaci*)	*Leaf curl viruses* (*Geminivirus/Begomovirus*)	Leaf curl disease	Vegetables and fruits
5	Thrips (*Thrips tabaci* & *Thrips palmi*)	*Tospoviruses*	Spotted wilt disease and Bud necrosis disease	Tomato and Groundnut
6	Leaf hopper	*Tungro virus* (RTBV) & *Waika virus* (RTSV)	Tungro disease	Rice
7	Mites	*Pigeon pea sterility mosaic virus*	PPSMD	Pigeon pea
8	Mealy bug	*Badnaviruses*	Cocoa swollen shoot disease	Cocoa

7. Dispersal by fungi

Olpidium brassicae: Lettuce big vein virus

Spongospora subterranean: Potato mop top virus

8. Dispersal by phaenerogamic or angiospermic parasites

Cuscuta: Cucumber mosaic virus, Citrus exocortis Viroid and potato leaf roll virus.

9. Dispersal through air

Uredospores of rust fungi, spore balls of smut fungi and other fungal and bacterial structures are spread through air.

10. Dispersal by water

Water can move the infected plant debris and dormant pathogen structures from one field to other.

Splash dispersal: rain drops or water drops falling with force from sprinkler irrigation spreads the bacterial ooze, spores of fungal and bacterial pathogens from infected plants to healthy ones.

e.g. Red rot of sugarcane (*Colletotrichum falcatum*), *Fusarium, Pythium and Phytophthora.*

Chapter 6
Plant Disease Epidemiology

Vanderplank (1963) stated the science of disease in population.
Agrios (2005) mentioned the study of factors affecting the outbreak and spread of infectious diseases. It is the dynamics of change in plant disease in time and space.

Factors affecting plant disease epidemiology

1. Pathogen factor
   - Virulence and aggressiveness
   - Inoculum potential
   - Inoculum density-disease relationship
   - Growth and reproduction
2. Host factor
   - Types of plant community
   - Genetic make-up
   - Host age and nutrition
3. Environment factor
   - Micro-environment
   - Meso-environment
   - Macro-environment
4. Time factor
   - $X = X_0 (1 + rt)$ for monocyclic diseases
   - $X = X_0 e^{rt}$ for polycyclic diseases

Plant Disease Forecasting

Forecasting involves all the activities in ascertaining and notifying the growers of community that condition is sufficiently favourable for certain diseases, that application of control measures will result in economic gain.

1. **Positive forecast:** employs need-based chemical sprays – provides adequate protection to crop and reduce damage to environment.
2. **Negative forecast:** avoids unnecessary sprays – no risk to the crop health and no disruption of environment.

Disease forecasting models

1. **Empirical models:** based on experience of growers or scientist or both e.g. Dutch Rules.
2. **Fundamental models:** involves research and experimentation. Time and amount of disease is involved.

Remote sensing

Remote sensing (RS) is the collection of information about an object or phenomenon without making physical contact with the object. RS makes it possible to collect data on dangerous or inaccessible areas, RS application also include monitoring of deforestation.

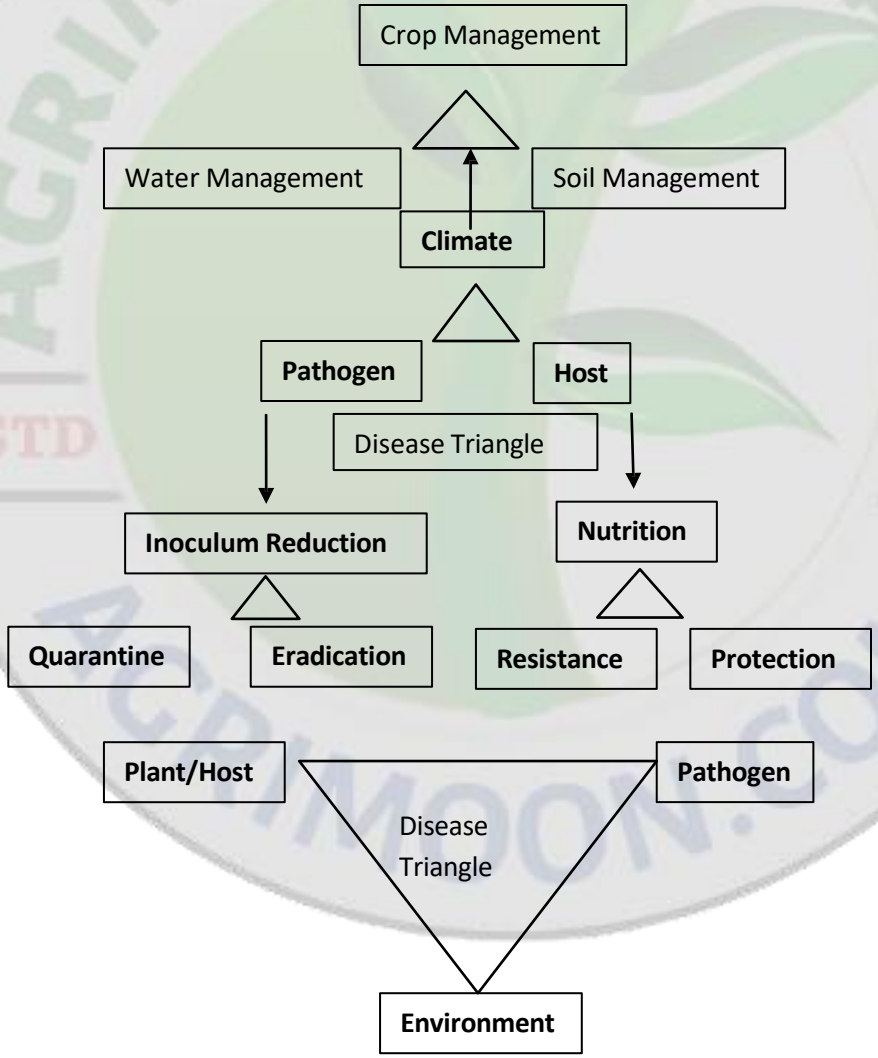
Chapter 7
Disease Management

Pre-requisite of development of disease management strategy:

1. Identification of the cause of disease
2. Mode of survival and dissemination of the infectious agent of the disease
3. Host parasite relationship and means of secondary spread
4. Effect of environment on pathogenesis in the plant and spread of the disease in the plant population

In polycyclic diseases in which many cycles of spore production occur in the standing crop, the strategy for management involves the prevention of entry of the pathogen in the crop and prevention of spore formation. Once spore formation starts, the control becomes costly and difficult.

If primary inoculum is stopped from becoming active, there should be no disease in the crop. In other word, if seed and soil are free from inoculum and entry of the pathogen is prevented in the standing crop there should be no disease.



Disease management – Principles

I. Exclusion of the Pathogen:

The aim is to prevent entry of a pathogen in a field or area (state, country) supposedly free from that pathogen.

1. Quarantine:

Plant quarantine aims at preventing entry of the pathogens from infested areas into non-infested areas at international or national level. If in a particular area some disease is present in serious form and is likely to be disseminated by propagating materials, the government passes necessary regulations to stop entry of such materials from the infested area.

For implementation of these regulations at the international level, proper check is maintained at points of entry (airports and seaports). Suspected materials are kept under quarantine for s specific period and if found infected it is either destroyed or effectively treated.

2. Inspection and Certification:

The crops grown exclusively for seed are periodically inspected for presence of diseases that are disseminated by seed. Necessary precautions are taken to remove the diseased plants. The method is supposed to prevent regional and inter-regional spread of the pathogen.

3. Seed Treatment:

Seeds, tubers, grafts, bulbs and other propagative materials can be given heat, gas or chemical treatments to exclude the pathogen present in or over them. Seed treatment is mandatory for seed agencies supplying certified seed.

4. Eradication of Insect vectors:

Since the flight of insects cannot be checked so the crop should be given preventive sprays at the time of increase in insect vector population.

II. Avoidance of the Pathogen

It involves the tactics that prevent contact between the host and the pathogen, presuming that pathogen has crossed the barriers placed by exclusion or it is already present in the area and can attack the host.

It includes following strategies:

1. Choice of Geographic Area:

Selection of geographic area for any crop is made on the basis of suitability of climate for the crop. Many fungal and bacterial diseases are common in wet areas.

2. Selection of field:

Management of drainage is one of the important aspect of disease management. Fields free from soil borne diseases such as wilt, ear cockle and other fungal bacterial and nematode diseases should be taken for cropping.

3. Choice of time of Planting:

The disease is severe or devastating when the susceptible stage of the plant growth coincides with the favorable condition of the pathogen.

4. Disease escaping varieties:

Pea varieties that mature early (January) generally escape much damage from powdery mildew pathogen which becomes serious in Jan or later.

5. Selection of seed and planting material:

A disease which is carried by seed or planting material and spread the infection in the field requires proper selection of healthy seeds to avoid multiplication of the pathogen in field and contaminate the healthy crops.

III. Eradication of the pathogen:

The principle involves the removal of inoculum already present in the field or the crop.

1. **Biological control of Pathogens**:
   The biological control aims at eradication and reduction of inoculum and protection of plant surfaces through the activity of microorganisms.
2. **Crop rotation**:
   It is one of the oldest method of fighting soil sickness and root diseases. The method is restricted against the pathogens which have limited host range and restricted survival ability in the soil.
3. **Removal or destruction of diseased plants or plant organs**:
   The presence of diseased plants in fields or in orchards is a source of continuous release of inoculum. Removal of alternate and collateral hosts is also recommended.
4. **Heat and chemical treatment of diseased plants**:
   The pathogen present in plants or its special organs can be inactivated or killed by heat or chemical treatments. Root dip in fungicides is a method of sanitizing the seedlings before transplanting.
5. **Soil treatments**:
   To inactivate or eradicate the pathogens present in the soil. It involves use of chemicals, heat and cultural practices such as flooding or fallowing.
6. **Soil solarization**:
   It is a novel method of soil treatment to destroy most fungal, bacterial and nematode propagules on as well as weed seeds. It is a system of raising temperature of wet soil kept covered with polyethylene sheets which traps the solar heat. The system is highly useful in sanitizing the nursery soils and small fields.

IV. Protective measures:

These measures include use of chemical sprays and dusts to create a toxic barrier between the host surface and the propagules of the pathogen and necessary modification of the environment to make it unfavorable for development of the disease.

1. **Chemical treatment**:
   The aim of most chemical sprays, dusts and seed treatment is to form a protective toxic layer on the host surface so that when the pathogen comes in contact with the surface it is killed or prevented from growth.
2. **Control of Insect vectors**:
   Those chemicals which kill the insect within few seconds are most effective in control of insect transmitted diseases.
3. **Modification of the environment**:
   Mixed cultivation of crops, intercropping, trap crops, antagonistic crops, root diseases favored by high temperature are often controlled by irrigation. Post harvest decay of fruits and vegetables is reduced or prevented by cold storage.

4. **Modification of host nutrition**:

High nitrogen dose increases susceptibility of the plants. High potassium renders plants resistance to many diseases such as rice blast. High calcium increases the resistance towards wilt and soft rot diseases.

V. <u>Development of resistance in the host</u>:

1. **Selection and hybridization**:

Selection of resistant plant and hybridize them with commercial high yielding but susceptible cultivars, to produce new resistant plants with high commercial yield.

2. **Genetic manipulation through biotechnology**:

Creation of transgenic plants in which resistance genes from sources other than the particular plant species is done, through genetic engineering.

3. **Induction of acquired resistance**:

There are numerous methods through which plants acquire localized or systemic resistance during their life time via use of chemicals or microorganisms. PGPR induces systemic resistance. Salicylic acid induces systemic acquired resistance.

4. **Resistance through chemotherapy**:

Systemic fungicides or antibiotics when applied to plants in low concentration results in development of resistance in plants

5. **Resistance through host nutrition**:

By making available major and micronutrients through sprays, seed treatment or soil application is reported to strengthen the tissues that can defend the invasion of the pathogen.

VI. <u>Therapy of diseased plants</u>:

In many crops and particularly fruit trees chemical and physical therapy has been applied to cure diseases.

1. **Chemotherapy**:

Chemical treatments are applied to eradicate pathogens from diseased to avoid its spread to other healthy parts of plants. Use of systemic fungicides, antibiotics, Bordeaux pastes etc.

2. **Thermotherapy**:

These treatments are usually used for seed, tuber, bulb, grafts etc. Ratoon stunting disease of sugarcane and many viruses of sugarcane are eradicated by hot water, air or moist heat treatment of seeds.

3. **Tree surgery**:

Large sized fruit trees are cleaned of infection by cutting or scrapping of the diseased part and covering the wound with a fungicidal paste.

<u>Disease management – Practices or Methods</u>

I. <u>Cultural methods</u>:

Adjustment of crop management procedures has been an age old practice with the farmers for prevention of losses of crops due to diseases and other causes.

1. **Production and use of disease free propagating material**:

i) Dry Climate for seed production: Bean anthracnose, bacterial blight of legumes, black rot of cabbage etc.

ii) Isolation distance for seed plots: Separation of seed plots (for production of certified seeds) from source of inoculum of neighboring field.
iii) Inspection of seed plots: Negative selection
iv) Drying and ageing of seeds: Long term storage inactivates many seed borne diseases.
v) Cleaning of seeds: For removal of spores and sclerotia.
vi) Thermal and chemical treatment of seeds: Wheat seeds with carboxin (Smuts) and root dip treatments.
vii) Site and treatments of nursery beds: Soil solarization, removal of crop debris etc.
viii) Adjustment of harvesting time of crop: Delayed harvesting provides more time for pathogen to attack and contaminate seeds.

2. **Adjustment of crop culture to minimize disease incidence**:
i) Crop rotation
ii) Avoid Monoculture
iii) Mixed cropping (sorghum – smoother crop produces HCN in root exudates against *Fusarium* wilt)
iv) Green manuring
v) Adjustment of date of sowing: Early planting saves crop from white rust in mustard, blast in rice, karnal bunt and stem rust in wheat, bacterial blight of cotton etc.
vi) Adjustment of depth of seedlings: deep placement of seed gives pathogen more time to invade the seed and seedlings.
vii) Plant spacing, rate of sowing and density of stand: Fungi and bacteria are generally more severe in dense planting due to development of congenial micro-climate while viral diseases are reduced due reduction in vector movement.
viii) Management of irrigation: The disadvantages of excess irrigation and prolonged stay of water around the root zone are many. It makes plants susceptible to damping off and other root diseases.
ix) Management of host nutrition
x) Management of soil acidity and alkalinity: Common scab of potato (*Sterptomyces scabies*) is sensitive to acidity. The disease is reduced when pH is brought down below 5.2. The disease increases from pH 5.2 to 8. Lowering the pH by use of sulphur is effective method. Club root of crucifer (*Plasmodiophora brassicae*) can be controlled by increasing soil pH by adding lime and gypsum.
xi) Organic amendments of soil: Compost. FYM, Green manure etc. *Fusarium oxysporium* fsp. *cubense*: Sugarcane bagasse
xii) Management of top soil: Mulching – covering of top soil with organic residues often help in reduction of diseases. Soil solarization.
xiii) Minimizing influx of inoculum from neighboring crops
xiv) Choice of crop variety

3. **Field and plant sanitation**:
i) Management of crop debris: Deep ploughing, summer ploughing etc.
ii) Management of diseased plants: roguing
iii) Management of irrigation water

iv) Crop free period or crop free zone: narrow host range pathogens

v) Creating barrier by non hosts

vi) Decoy crops, trap crops and antagonistic crops: Decoy crops are non host or cover crops, trap crops are highly susceptible host crops (linseed as trap crop of *Orobanche*), antagonistic crops produce toxic chemicals that directly destroy the pathogen in soil, *Marigold*, *Crotalaria*, *Asparagus* etc. These are non hosts of pathogen.

vii) Management of weed, collateral and volunteer host

viii) Management of insect vectors

ix) Harvesting time and practices: Improper harvesting spreads: cysts of nematodes, spores of fungus (smut), seeds of phaenerogamic parasites.

II. Biological methods:

The reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state by one or more organisms except man, accomplished naturally or through manipulation of the environment, host or antagonists or by mass introduction of one more antagonists.

1. Destruction of surviving propagules: eg. *Trichoderma viridae* against *Rhizoctonia solani* while *Pseudomonas fluoroscenes* against *Plasmodiophora brassicae.* *Trichoderma* is one of the successful biocontrol agent.
2. Prevention of inoculum formation: *Ampelomyces quisqualis* against powdery mildew fungi.
3. Reduction of virulence of pathogen (Hypovirulence): Mycoviruses (dsRNA) are present in fungi which reduce their surviving ability as well as virulence. Eg. *Rhizoctonia solani, Magnaporthe oryzae.*
4. Cross protection: When a mild or avirulent strain of virus is introduced into the host may induce resistance to a virulent strain of the same or serologically related viruses.

III. Host resistance for disease management

Resistant varieties can be the most simple, practical, effective and economical method of plant disease management.

1. True resistance
   - **i)** Vertical resistance
   - **ii)** Horizontal resistance
2. Apparent resistance
   - **i)** Disease escape
   - **ii)** Tolerance
3. Development of resistant varieties:
   - **i)** Selection
   - **ii)** Mutation
   - **iii)** Hybridization
4. Breeding for resistance using biotechnology:
   - **i)** Transgenic plants
   - **ii)** Recombinant DNA technology

IV. Physical Methods

Depends on physical factors like heat, cold, light wavelengths and radiation to reduce or eradicate pathogen inoculum.

1. Heat Treatment: Generally used for soil sterilization, disinfection of propagative materials, freeing plants from viruses and treating storage organs with hot air.
2. Refrigeration: Used to control post harvest diseases. The principle behind it is that low temperatures at or slightly above the freezing point inhibit or greatly reduce the growth activities of pathogens.
3. Radiation: Such as UV light, X-rays etc. have used to manage post harvest diseases of many fruits and vegetables.

Physical Methods – Heat

1. Hot Water Treatment
2. Hot Air Treatment
3. Aerated Steam Therapy
4. Moist Hot Air Treatment
5. Solar Heat Treatment
6. Soil Solarisation
7. Steam Sterilization
8. Hot Air Sterilization
9. Hot Water Treatment
10. Refrigeration

V. <u>Disease management through chemicals</u>:

1. **Aim of use of chemicals in plant disease management:**
   - **i)** To create a toxic barrier between the host surface or tissue and the pathogen
   - **ii)** To eradicate the pathogen present at a particular site on or in the host including seed, foliage and roots.
2. **Fungicides are classified on the basis of functions:**
   - **i)** Protectants: contact fungicides as spray, dust or paste.
   - **ii)** Eradicant: Destroy the dormant or active pathogens from host.
   - **iii)** Chemo-therapeuants: Specifically remove pathogen from host after infection. Systemic fungicides.
3. **Contact fungicides:**
   a. Sulphur fungicides:
   lime sulphur: Effective against Powdery mildew fungi.
   Dithiocarbamates: Organic compounds of sulphur. Thiram (Commonly used for seed treatments), Mancozeb (Dithane M-45).
   b. Copper fungicides:
   Bordeaux mixture: Copper sulphate + slaked lime + water. Effective against downy mildew fungi.
   Copper oxychloride: Blitox 50. False smut of rice
4. **Systemic fungicides:**
   a. Oxathiins:

The first systemic fungicide developed in 1966 by Von Schemuling and Kulka was Carboxin (Vitavax) – Smut. Oxycarboxin – Rust.

b. Benzimidazoles:
Carbendazim (Bavistin), effective against wide range of fungi except oomycetes and dark colored fungi such as *Alternaria, Helminthosporium* etc.

c. Acylalanine: effective against oomycetes.
Metalaxyl (Ridomil and Apron) – *Pythium*, *Phytophthora* and downy mildew fungi.

d. Sterol biosynthesis inhibitor:
   i) Triazoles: Propiconazole (Tilt) – Rust, smut, blast
   Tricyclazole (Beam) – Rice blast

Antibiotics:
Metabolites of microorganisms which, in very dilute concentration have the capacity to inhibit the growth of, or even destroy other microorganisms.

a. **Streptomycin** – First antibiotic used in plant disease fire blight of apple and pear in 1953.
b. Tertracycline (Acromycin), Oxytetracycline (Terramycin) and Chlorotetracycline (Aureomycin) – against fastidious bacteria, phytoplasma, MLO's.

Methods of application of fungicides

1. Seed treatment
2. Soil treatment
3. Foliar application
4. Postharvest application
5. Special method of application

Chapter 8
Nematotes

INTRODUCTION

Nematology is an important branch of biological science, which deals with a complex, diverse group of round worms known as Nematodes that occur worldwide in essentially all environments. Nematodes are also known as eelworms in Europe, nemas in the United States and round worms by zoologists. Many species are important parasites of plants and animals, whereas others are beneficial to agriculture and the environment. Nematodes that are parasites of man and animals are called helminthes and the study is known as Helminthology. The plant parasitic forms are called nematodes and the study is known as Plant Nematology. The name nematode was derived from Greek words nema (thread) and oides (resembling). Annual crop losses due to these bligate parasites have been estimated to be about $ 78 billion wordwide and $ 8 billion for U.S. growers. The estimated annual crop loss in Tamil Nadu is around Rs. 200 crores. The soils in a hectare of all agro ecosystem typically contain billions of plant parasitic as well as beneficial nematodes. The damage to plants caused by nematodes is often overlooked because the associated symptoms, including slow growth, stunting and yellowing, can also be attributed to nutritional and water related disorders.

History of Plant Nematology

In light of the high population numbers of nematodes. N.A. Cobb (1915) who is considered to be the father of American Nematology, provided a dramatic description of the abundance of nematodes. He stated, “If all the matter in the universe except the nematodes were swept away, our world still would be dimly recognizable we would find is mountaintops, valleys, rivers, lakes and oceans represented by a film of nematodes. The statement “sowed cockle, reaped no corn” in Shakespeare’2 “Love’s Labour’s List”’ act4, scene 3, as suggested by Throne (1961) possibly the first record of plant parasitic nematodes in 1549. The nematode that Throne suspected to be in that reference actually was described by Needham in 1743. Subsequently, discovery of microscope and developments in various disciplines of science led to the discovery of plant parasitic nematodes and the disease caused by them. Some of the important milestones on the history of plant nematology are listed below in chronological order.

1743 – Needham – Discovery of wheat seed gall nematode *Anguina tritici*, the first plant parasitic nematode to come to the attention of the early investigators.

1855 - Berkeley – Determination of root-knot nematode, *Meloidogyne* spp. To cause root galls on cucumber plants in greenhouse in England.

1857 - Kuhn – Reported the stem and bulb nematode, *Ditylenchus dipsaci* infesting the heads of teasel.

1859 - Schacht - Report of sugarbeet cyst nematode, *Heterodera schachtii* from Germany.

1884 - deMan – Taxonomic monograph of soil and fresh water nematodes of the Netherlands.

1892 – Atkinson-Report of root-knot nematode and *Fusarium* complex in vascular wilt of cotton.

1907 - N.A.Cobb – joined the USDA and considered to be the **Father of American Nematology**

1933 – T. Goodey – Book on "Plant parasitic nematodes and the diseases they cause"

1943 – Carter-Description of nematicidal value of D-D which is used in the era of soil fumigation.

1945 – Christie – Description of the nematicidal value of EDB.

1961 – Society of Nematologists founded in the United States.

1969 – Journal of Nematology was first published by the Society of Nematologists, USA.

BRIEF HISTORY OF NEMATOLOGY IN INDIA

Nematology as a separate branch of Agriculture Science in India has been recognized only few years back. The history and development of Nematology in India have been listed below in chronological order.

1901 –Barber reported root – knot nematode on tea in Devala Estate, Tamil Nadu, South India.

1906 – Butler reported root – knot nematode on black pepper in Kerala.

1913, 1919 – Butler reported Ufra disease on rice in Bengal due to the infestation of *Ditylenchus angustus.*

1926, 1933 – Ayyar reported root – knot nematode infestation on vegetable and other crops in India.

1934, 1936 – Dastur reported white tip disease of rice caused by *Aphelenchoides besseyi* in Central India.

1959 – Prasad, Mathur and Sehgal – reported cereal cyst nematode for the first time from India.

1961 – Nematology unit established at the Central Patato Research Institute, Simla.

1964 – First International Nematology course held at IARI., NEW Delhi.

1966 – Nair, Dass and Menon reported the burrowing nematode on banana for the first time from Kerala.

1966 – Division of Nematology established at IARI, New Delhi

1969 – Nematological Society of India founded and first All India Nematology Symposium held at IARI, New Delhi.

1971 – Indian Journal of Nematology published

Importance of Nematodes in Agriculture

In the United States, the nematodes are known to cause six per cent loss in field crops, ($ 100 million / year), 12 per cent loss in fruits and nuts ($ 225 million / year), 11 per cent loss in vegetables ($ 267 million / year) and 10 per cent loss in ornamental ($ 60 million / year). In India, the cereal cyst nematode, *Heterodera avenae* causes the „molya" disease of wheat and barley in Rajasthan, Punjab, Haryana, Himachal Pradesh and Jammu and Kashmir. The loss due to this nematode is about 32 million rupees in wheat and 25 million rupees for barley in Rajasthan State alone.

Economic annual losses due to nematodes for selected world crops

Crops	**Estimated yield losses due to Nematodes (%)**	**Crops**	**Estimated yield losses due to Nematodes (%)**
Banana	19.7	Potato	12.20
Barley	6.3	Rice	10.00
Cassava	8.4	Sorghum	6.90
Citrus	14.2	Soybean	10.60
Cocoa	10.5	Sugar beet	10.9
Coffee	15.0	Sugarcane	15.3
Corn	10.2	Sweet potato	10.2
Cotton (lint)	10.7	Tea	8.2
Field Bean	10.9	Tobacco	14.7
Oat	4.2	Wheat	7.0
Peanut	12.0		

The examples are only a small portion of nematode problem in India. Besides this direct damage, they also associate with bacteria, fungi and viruses to cause complex diseases.

MORPHOLOGY AND ANATOMY OF NEMATODES

Even though nematodes occupy nearly every habitat on earth, they are remarkably similar in morphology and life stages. Despite their structural complexity, certain basic principles are common to all nematodes. Nematodes are triploblastic, bilaterally symmetrical, unsegmented, Pseudocoelomate, vermiform and colourless animals. The plant parasitic nematodes are slender elongate, spindle shaped or fusiform, tapering towards both ends and circular in cross section. **The length of the nematode may vary from 0.2 mm (*Paratylenchus*)** (*Paralongidorus maximus*). Their body width varies from 0.01 to 0.05 mm. In few genera, to about 11.0mm the females on maturity assume pear shape (Meloidogyne), globular shape (Globodera), reniform (*Rotylenchulus reniformis*) or saccate (*Tylenchulus semipenetrans*).

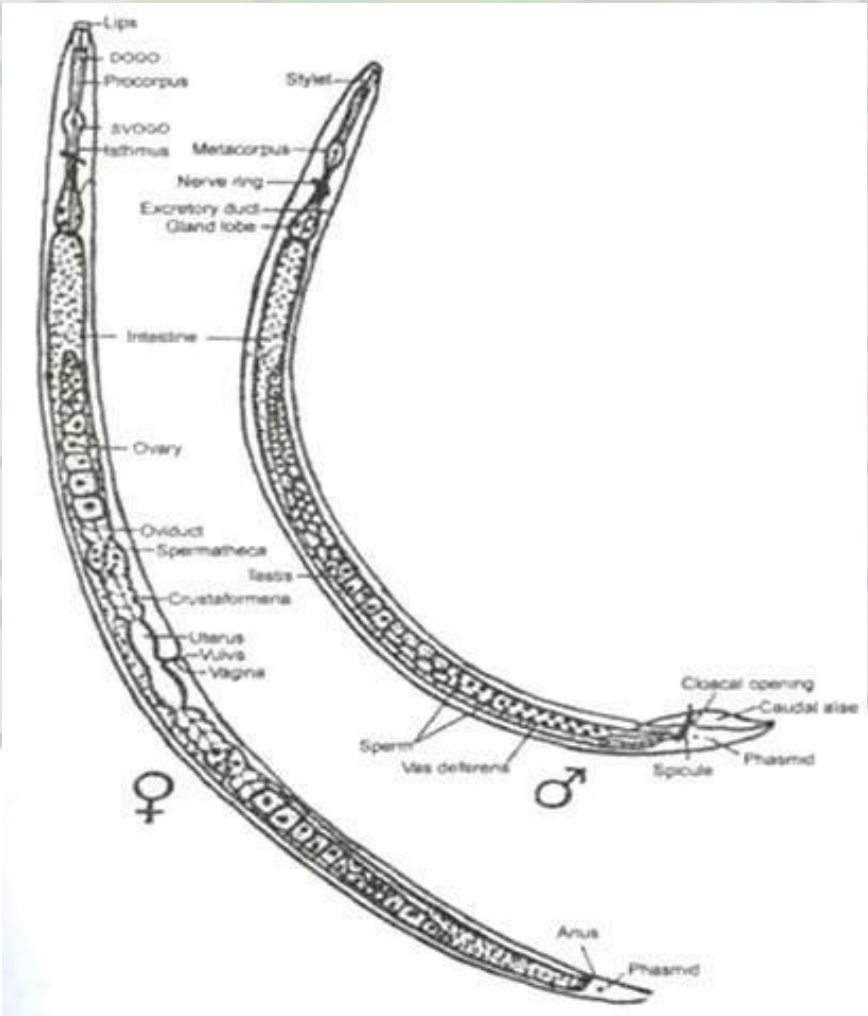


Fig. 1- Body morphology of a typical vermiform plant parasitic nematode DOGO - dorsal oesophageal gland orifice, SVOGO - subventral oesophageal gland orifice

The swelling increases the reproductive potential of the organism. Radially symmetric traits (triradiate, tetraradiate and hexaradiate) exist in the anterior region. The regions of intestine, excretory and reproductive systems show tendencies towards asymmetry. The nematodes have one or two tubular gonads which open separately in the female and into the rectum in the male which also have the copulatory spicules.

The free living saprophytic nematodes are generally larger in size. The animal and human parasitic helminthes may have length of few centimeters to even a meter or more. The helminth parasitising whale fish is about 27 feet long. The study on these animal and human parasites are known as Helminthology.

The following are some examples of Helminths

1. Filarial worm - *Wucheria bacrofti*
2. Guinea worm - *Dracunculus medinesis*
3. Round worm - *Ascaris lumricoides*
4. Tape worm - *Taenia solium*

The nematode body is not divided into definite parts, but certain sub – divisions are given for convenience. The anterior end starts with the head, which consists of mouth and pharynx bearing the cephalic papillae or setae. The portion between the head and the oesophagus is known as the neck. Beginning at the anus and extending to the posterior terminus is the tail.

Longitudinally the body is divided into four regions as dorsal, right lateral, left and ventral. All the natural openings like vulva, excretory pore and anus are located in the ventral region. The nematode body is made up of several distinct body systems. They are the body wall, nervous system, secretory – excretory system, and digestive system and reproductive system. Nematodes do not posses a specialized circulatory or respiratory system. The exchange of gases is thought to occur through the cuticle and circulation proceeds through the movement of fluids within the pseudocolelom and by simple diffusion across membranes.

T**he following are the characteristics of members of the phylum Nemata.**

1. Inhabit marine, freshwater and terrestrial environments as free – livers and parasites.
2. Bilateraly symmetrical, triploblastic, unsegmented and pseudocoelomates.
3. Vermiform, round in cross – section, covered with a three – layered cuticle.
4. Growth accompanied by molting of juvenile stages, usually four juvenile stages.
5. Oral opening surrounded by 6 lips and 16 sensory structures.
6. Possess unique cephalic sense organs called amphids.
7. Body wall contains only longitudinal muscles connected to longitudinal nerve chords by processes extending from each muscle.
8. Unique excretory system containing gland cells or a set of collecting tubes.
9. Longitudinal nerve cords housed within the thickening of the hypodermis.

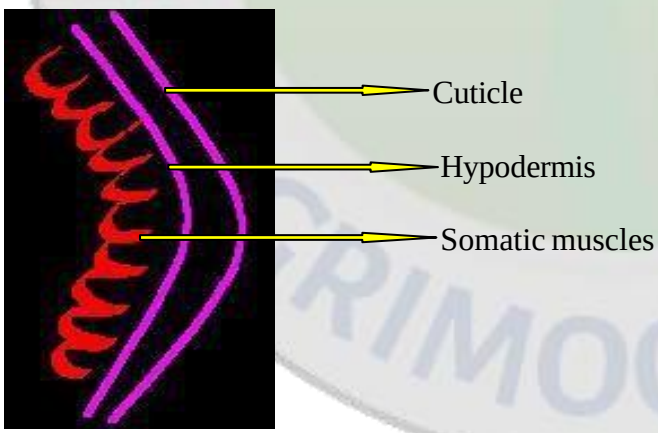
Genera of the most common plant parasitic nematodes

1. Cyst nematode *Globodera spp*. and *Heterodera spp.*
2. Dagger nematode *Xiphinema* spp.
3. Foliar nematode *Aphelechoides* spp.
4. Lance nematode *Hoplolaimus* spp.
5. Lesion nematode *Pratylenchus* spp.
6. Needle nematode *Longidorus* spp.
7. Pin nematode *Paratylenchus* spp.
8. Reniform nematode *Rotylenchulus* spp.
9. Ring nematode *Criconemella* spp.
10. Root – knot nematode *Meloidogyne* spp.
11. Sheath nematode *Hemicycliophora* spp.
12. Spiral nematode *Helicotylenchus* spp.
13. Sting nematode *Belonolaimus*
14. Stubby – root nematode *Paratrichodorus* spp and *Trichodorus* spp.
15. Stunt nematode *Tylenchorhynchus* spp.

The nematode body is divided into three regions. They are the outer body tube or body wall, inner body tube and body cavity or pseudocoelome.

The outer body tube

The outer body tube or body wall includes the cuticle, hypodermis, and somatic muscles. The body wall protects the nematode from the harsh external environment, serves as the exoskeleton and provides the mechanism for movement of the organism through the soil and plant tissue. The body wall also contains much of the nervous and secretory – excretory systems, and it plays a role in the exchange of gases.



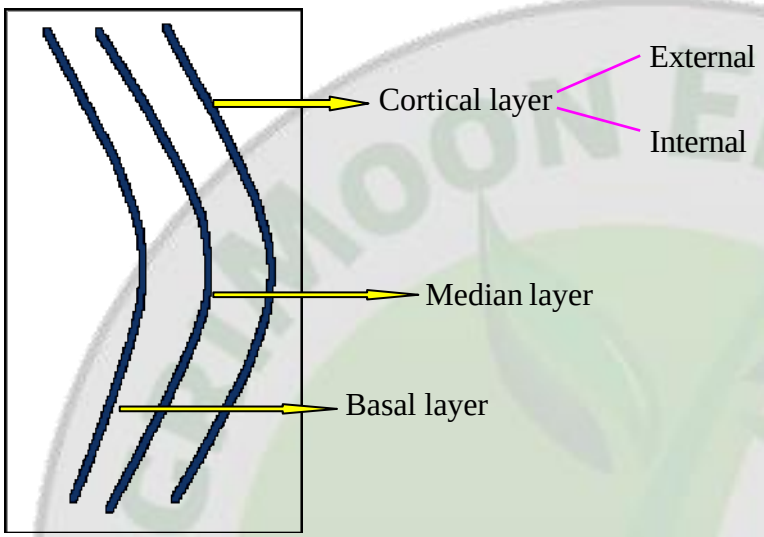
Outer body tube

The cuticle or exoskeleton

The cuticle is a non living, non cellular, triple – layers covering that is secreted by the underlying hypodermis. The cuticle is flexible. It covers the entire body and lines the oesophagus, vulva,

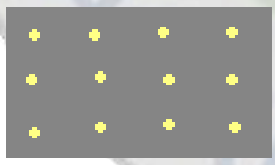
anus, cloaca, excretory pore and sensory organs. The feeding stylet and copulatory spicules are formed from cuticle.

The composition and from of the cuticle is highly variable. In general, the cuticle is composed of three primary zones viz., the cortical layer, median layer and basal layer. The cuticle of may nematodes have markings on the surface. They are varied and complex and have been often used by taxonomists to assist in the identification of various species. The cuticular markings are categorized into different types. I. Punctuation ii. Transverse marking or striations and iii Longitudinal markings.



i. Punctuations:

These are minute, round dots arranged in a pattern. They act as structures for strengthening the cuticle rather than as pore canals through which cuticular proteins may be transported. In the perineal pattern of *Meloidogyne hapla* these punctuations can be seen.



Punctuations

Cut RKN

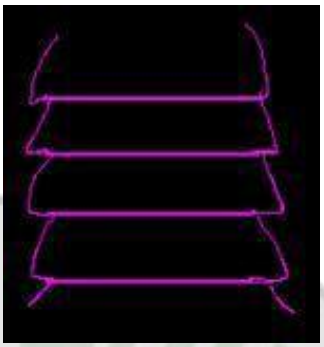
Perineal pattern

ii. Transverse marking or Striations:

There are transverse lines present on the surface of the cuticle. These markings exhibit distinct variations among the plant parasitic nematodes and often used by the taxonomists for identification. The transverse markings cause a pattern of ridges and furrows right from head to tail and these markings gives the false appearance as if the nematode is segmented. These markings are well pronounced in some families such as *Criconematidae, Tylenchidae* and *Heteroderidae*. In *Criconematids*, the annulations are clearly visible and known as scales and

spines. The perineal pattern in the posterior body region of *Meloidogyne* females, as well as rugse wall pattern of *Heterodera* cysts, are considered to be the modifications of transverse markings.

Transverse marking



Criconematids- Annulations

iii. Longitudinal markings:

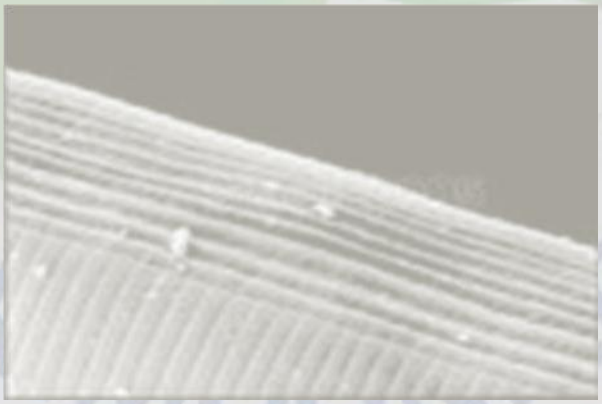
These markings are the lines on the cuticle, which runs longitudinally throughout the length of the nematode body. These markings are divided into

a) Lateral lines or incisures:

These are lines running longitudinal to the body axis of nematode but they are confined to the lateral field in area just on top of lateral hypodermal chords on either side of the nematode body running throughout the length. The number of lateral lines or incisures is an important taxonomic character as it shows stability within the genus.

b) Longitudinal ridges:

Longitudinal ridges are raised lines present on cuticle running longitudinal to nematode body axis but are confined in the area other than lateral field. The number of these ridges is used by taxonomists for species identification.



Longitudinal ridges

Apart from this, alae also present. They are thickening or projections of the cuticle which occur in the lateral or sublateral region. There are 3 types of alae.

i. Caudal alae ii. Cervical alae iii. Longitudinal alae.

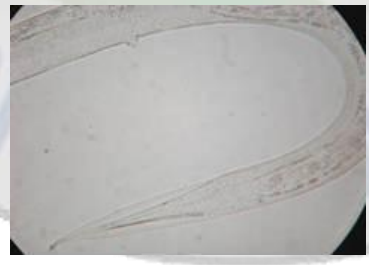
i. Caudal alae: These are found in the posterior region and restricted to males as copulatory bursa.

Example: *Hoplolaimus*

ii. Cervical alae: These are confined to the anterior part of the nematode body. Cervical alae are found in some species of marine nematodes.

Example: *Toxcara*

iii. Longitudinal alae: The longitudinal alae delimit the later fields and are known as lateral alae. Their form varies in different species. They are transversed by striations or furrows varying in number from 1 to 12. Functionally, they probably assist in locomotion and may permit slight changes in the width of nematodes.



Example: *Tripyla*

The functions of cuticle:

Cuticle gives definite shape and size to the body, acts as an exoskeleton, helps in movement, being semi-permeable, it regulates permeability and provides important taxonomic characters for identification of nematodes.

Somatic musculature:

Platymyrian: A flat type of cell with contractile elements limited in places to the base lying close to the epidermis.

Coleomyarian: 'U' shaped cells in which muscle fibre are adjacent and perpendicular to the hypodermis and extend along the sides of the muscle cell of varying distances.

Circomyarian: These types of muscle cells are almost round and the muscle fibres completely surround the cytoplasm.

The platymyarian muscle cell is considered primitive which might have modified into coelomyarian type of narrowing and upward elongation of the fibrillar zone. Muscle cells are connected to each other by means of cytoplasmic bridges and have nerve connections.

Inner Body Tube

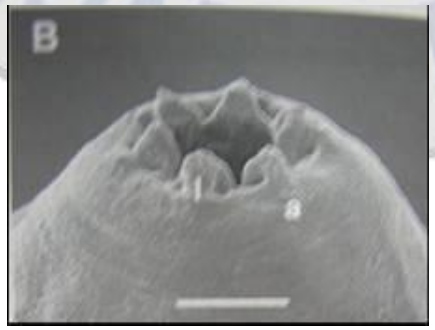
Digestive system

The digestive system of nematodes includes the stoma, oesophagus, intestine and posterior gut. The inner body tube is divided into 3 main regions.

1. Stomodeum : which constitute the stoma, oesophagus and cardia
2. Mesenteron : which constitute the intestine
3. Proctodeum : which is the posterior –most region comprising rectum and anal opening.

1. Stomodeum

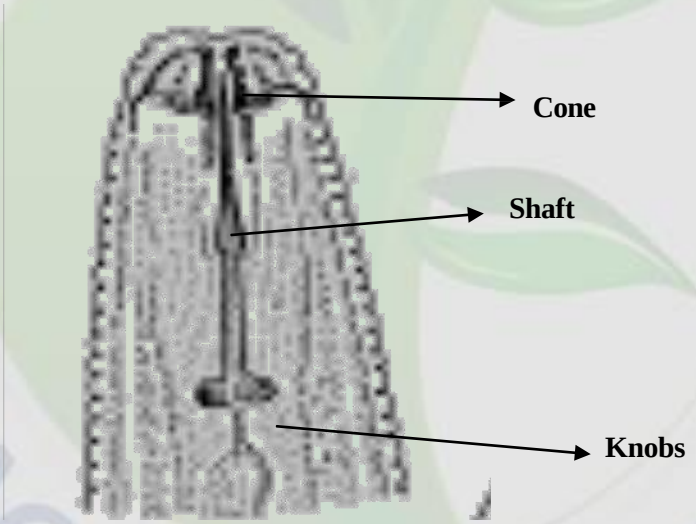
Stoma is the portion of the inner body tube lying between the oral opening and oesophagus. The stomatal opening is small and slit like and is surrounded by six lips.



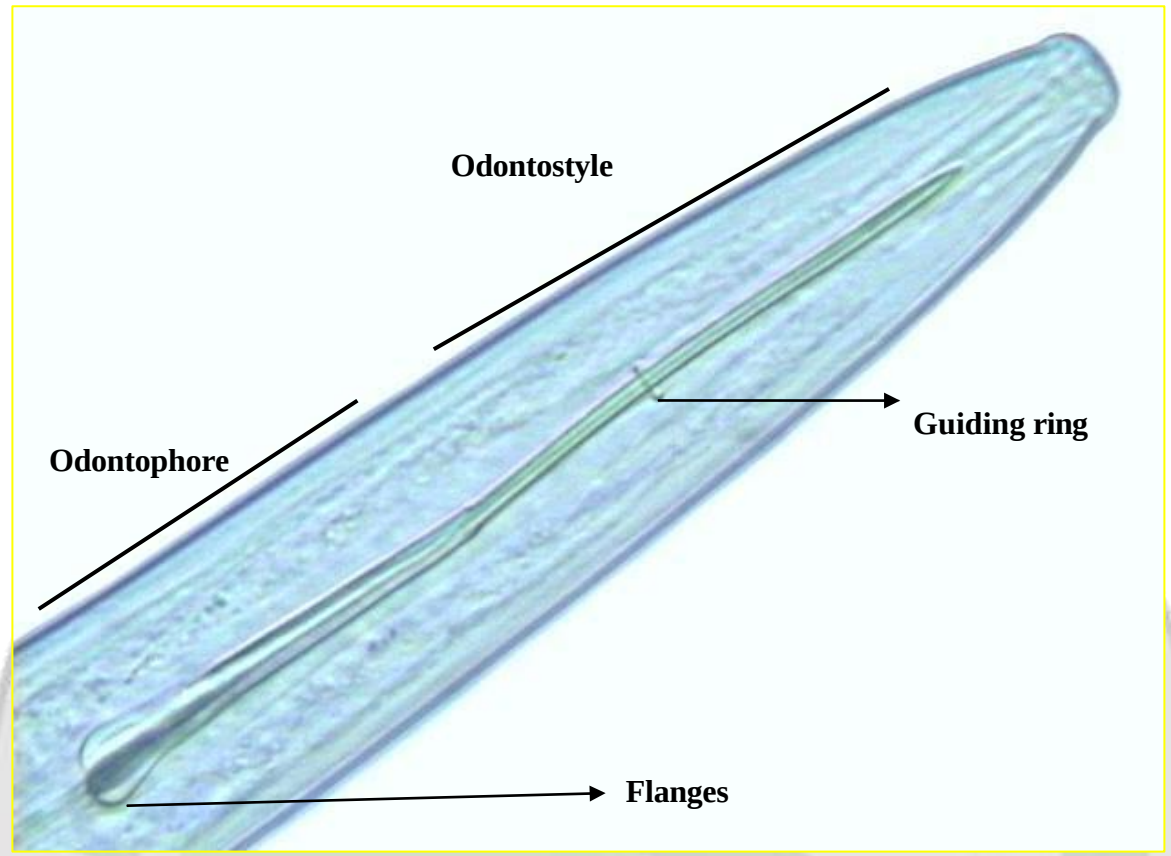
Stomatal opening

Two subdorsal, two subventral and tow lateral. Plant parasitic nematodes are armed with a protrusible stylet which is usually hallow and functions like a hypodermic needlw. In, Secernentea, the stylet is thought to be derived from fusion of the stomatal lining and therefore called as stomatostylet. The stamatostylet consists of a anterior cone, a cylindrical shaft and three rounded basal knobs. In Adenophrea, the stylet is thought to be derived from a tooth and, therefore, it is called as odontostylet. The flanges, that serves as points of attachment for the stylet protractor muscles. In some plant parasitic nematodes like Trichodorous and Paratrichodorus the odontostylet is dinstinctly curved ventrally, lacks flanges and it is not hallow, in functions to pierce the cell wall of the root. The nematode secrets a hallow tube out of its stoma that connect it with the plant. This feeding tube serves as the interface between the nematode and the plant.

Stomatostylet- Eg.*Hoplolaimus*



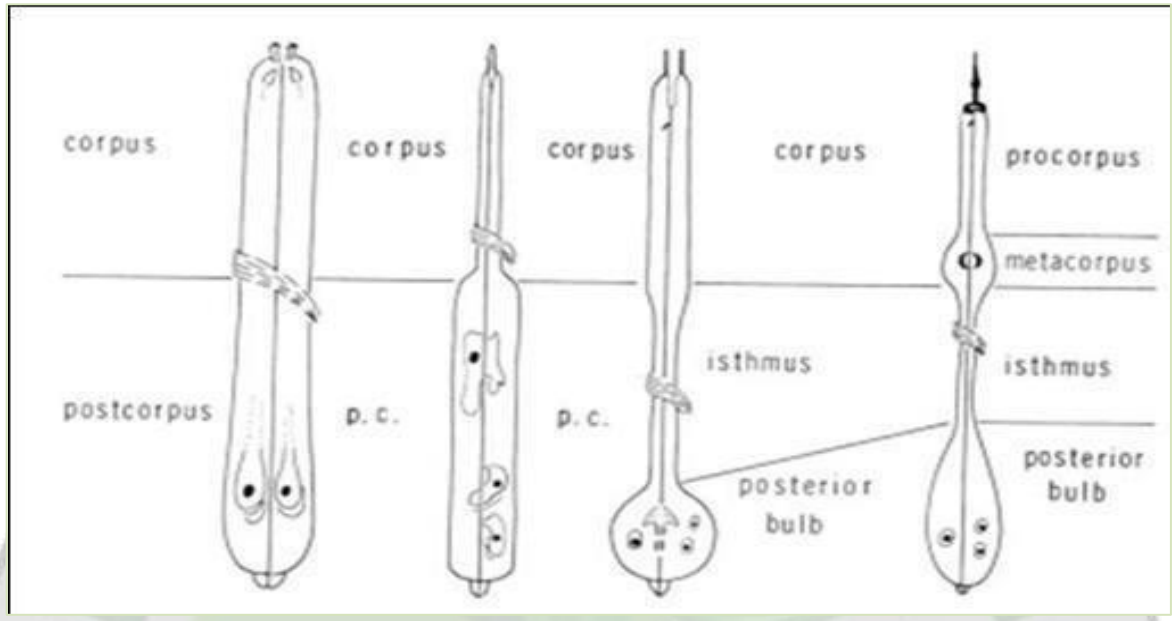
Odontostylet – Eg. *Xiphinema*



Oesophagus or pharynx:

The oesophagus is a muscular pumping organ attached to the posterior portion of the stylet and lines with cuticle. In Adenophorea, the oesophagus is divided into a narrow anterior procorpus and a broad posterior corpus. Three to five oesophageal gland cell empty into the lumen (one dorsal and two to four sub-ventral) in position in Secernentea the oesophagus is divided into distinct regions, such as narrow procorpus, followed by a broad muscular median bulb or pump, a narrow isthmus and gland lobe. The gland lobe may overlap the intestine in some genera and contain three to six gland cells (One dorsal and two sub–ventral). The oesophagus has valve (cordia) at the posterior end which prevents the regurgitation of food.

Types of Oesophagus

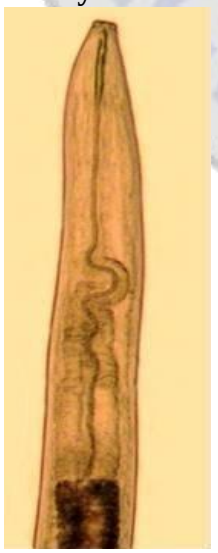


Enoplid Dorylaimid Rhabditid Tylenchid

Mesenteron or intestine:

The nematode intestine is a simple, hallow, straight tube consisting of a single layer of epitherlial cells. The intestine is generally divided anterior or ventricular region, the midintestinal region and the posterior or prerectal region. The microvilli are finger like projection of the plasma membrane projecting in to the intestinal regions. They increase the surface area of the intestine and are both secretary and absorbtive in funtion. The whole intestine is separated from the pesducoelom by a basement membrane. The food moves in the intestine by the ingestion of more food and also by locmotory activity of the nematode.

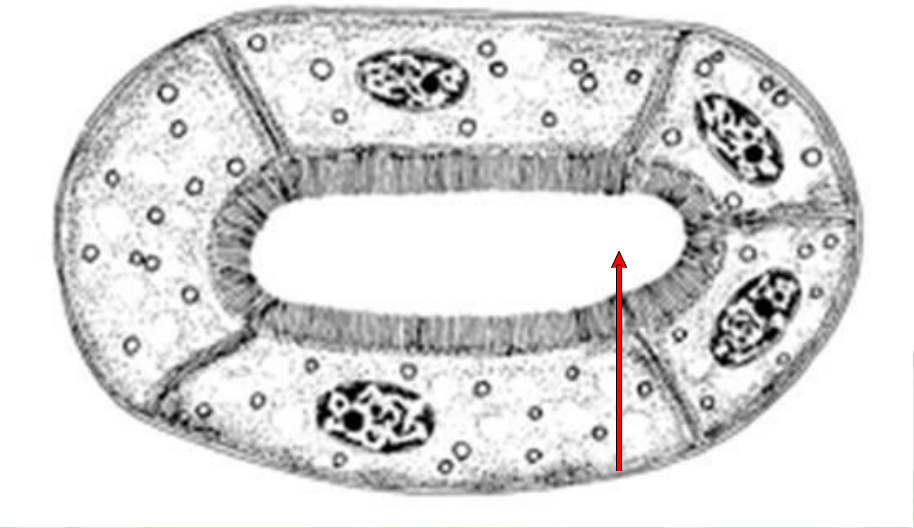
Dorylaimid



Tylenchid

Aphelenchid

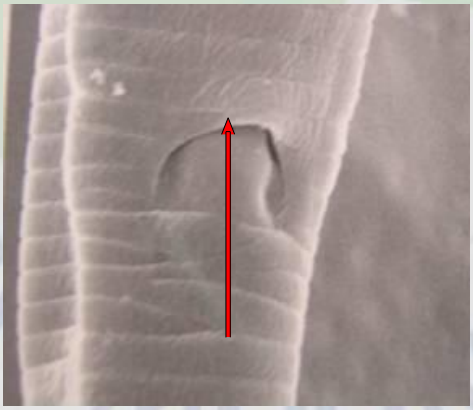
C.S. of intestine



Microvilli seen on the inner lining

Proctodeum:

Proctodeum comprises rectum and anus. The intestinal tube is connected with a narrow small tube at the posterior end, through a valve known as rectum. It regulates the flow of undigested food material which is to be passed outside the nematode body through a ventrally located aperture known as anus.



Anal opening

In male nematode, the rectum joins with the hind part of the testis forming a common opening known as cloaca. In female, there is a separate opening.

Glands:

Oesophageal and rectal glands are present in nematodes. The oesophageal gland enter the stomodeum and rectal gland enter proctodeum.

Oesophageal glands

Three uninucleated oesophageal glands, One gland on dorsal and other tow ventro lateral or sub ventral in position. These glands connect with the lumen of the oesophagus by means of ducts, often by means of a terminal swelling or ampulla.

The oesophageal glands have important role in hatching host penetration and also establishment of host parasitic relationship.

Rectal glands;

Are responsible for the copious production of gelatinuous mucopolysacccharide matrix in which eggs are deposited as a mass. It protects the eggs from adverse environmental conditions.

Function of digestive system:

Digestive juices secreted from dorsal oesophageal glands are injected into the host plant cell by means of the stylet. During feeding a distinct zone develop around the feeding site in the host cell. There are two feeding phases. 1. Injection phase or saliavation phase and 2. Ingestion phase.

Injection phase or Salivation phase:

During this phase, the flow of salivary juices into the host cell occurs due to contraction of lateral muscle of the median bulb.

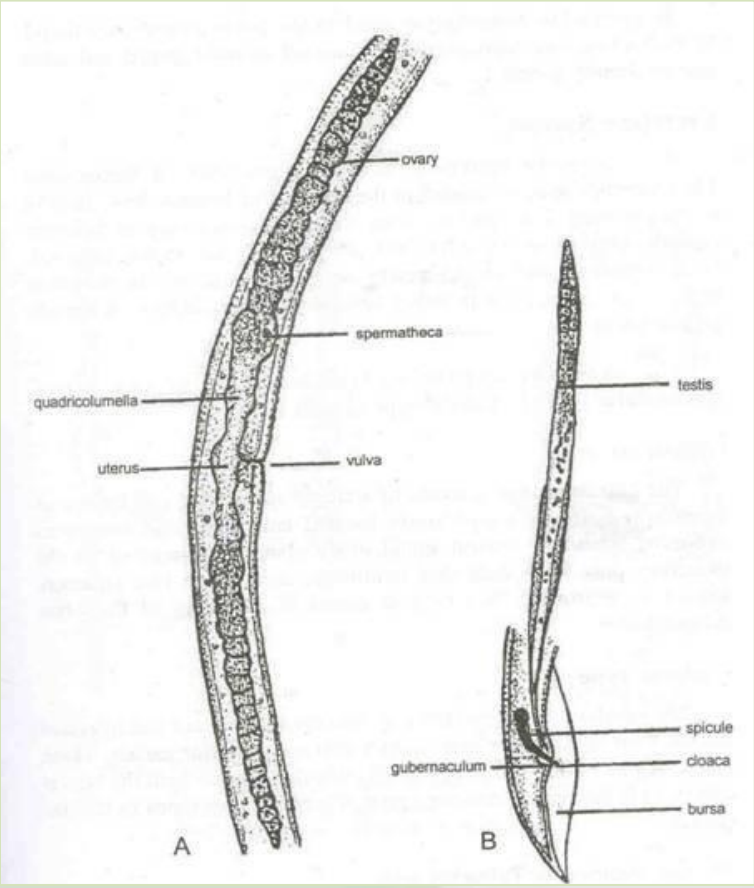
Ingestion phase:

During this phase rhythmical contraction of the posterior part of the oesophagus associated with the median bulb occurs.

Lecture-03

Reproductive System:

The nematodes are generally dioecious. Majority of plant parasitic nematodes do not exhibit any differences as far as body shape. Both sexes are vermiform. However, sexual dimorphism is observed in some genera *viz.*, *Meloidogyne, Heterodera, Globodera, Rotylenchulus, Tylenchulus* and *Nacobbus*. The females of these genera become enlarged and assume different shapes after attaining maturity.



Female **Male**

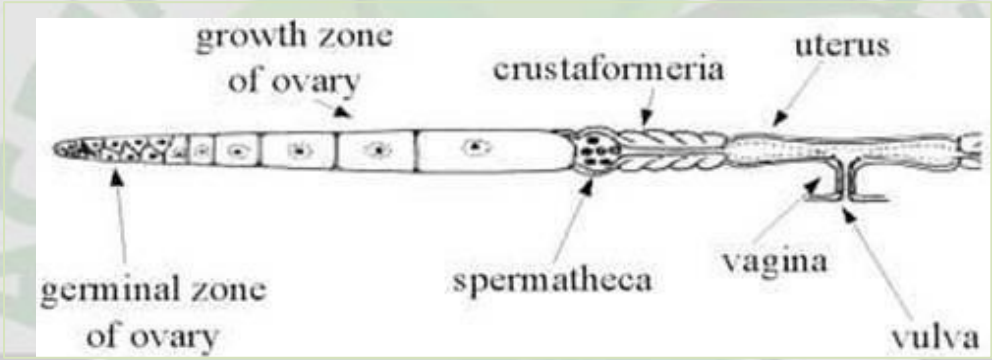
Female Reproductive System:

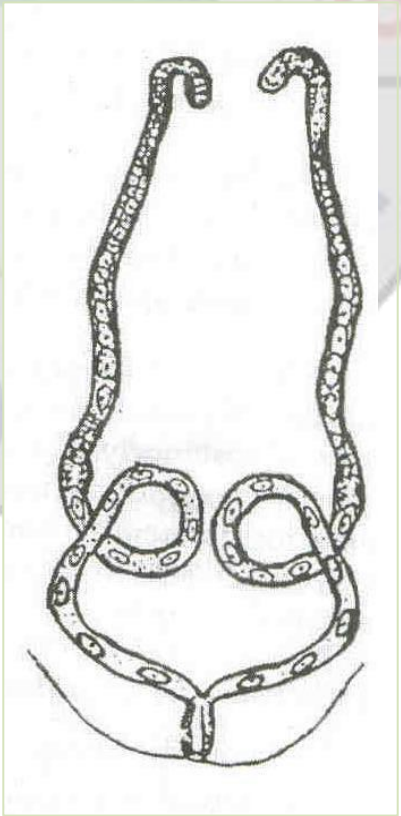
Present in nematodes having single ovary as observed in the genera *Pratylenchus* and *Ditylenchus*. The uterus opens outside to a ventrally located vulval opening through a tube known as vagina, which is a cuticularised structure. In plant parasitic nematodes the number of ovary may be one or two. When there is one ovary that condition is known as monodelphic and when the number is two, the condition is called as didelphic.

In monodelphic condition, the ovary is always, anteriorly directed, i.e Prodelphic. In case of didelphic ovaries, if both the ovaries are anteriorly directed and vulva is terminal in position then the condition is known as didelphic prodelphic as found in the case of *Meloidogyne*, *Heterodera* and *Globodera*. In som nematodes, two ovaries are opposite to one another, such that one is anteriorly directed and the condition, as found in the case of *Tylenchorhynchus*, *Hoplolaimus* and *Helicotylenchus* etc.

The vulval opening is a trasverse slit and not covered with any flap, but in *Agelenchus* and *Coslenchus* vulva is covered with a membranous flap known as vulval flap. The vaginal tube in *Hoplolaimus* and *Cosaglenchus* are provided with a cuticular sclerotised structure encircling the tube known as epiptygma. Ovary in most of the plant parasitic nematodes is always straight and does not curve back. Such ovaries are called as outstretched ovaries as in the case of *Tylenchorhynchus, Radopholus* and *Hirschmanniella* etc. In Dorylaimid nematodes, the tip of the ovary is curved back. It is known as reflexed ovary. If the ovary is single and posteriorly directed, then it is known as monodelphic ophisthodelphic condition and such conditions are rarely seen. (eg.*Xiphinema* spp.)

Further, and ovary is called hologenic if it produced oocytes throughout its length and telogenic if producing oocytes only at its distal end.



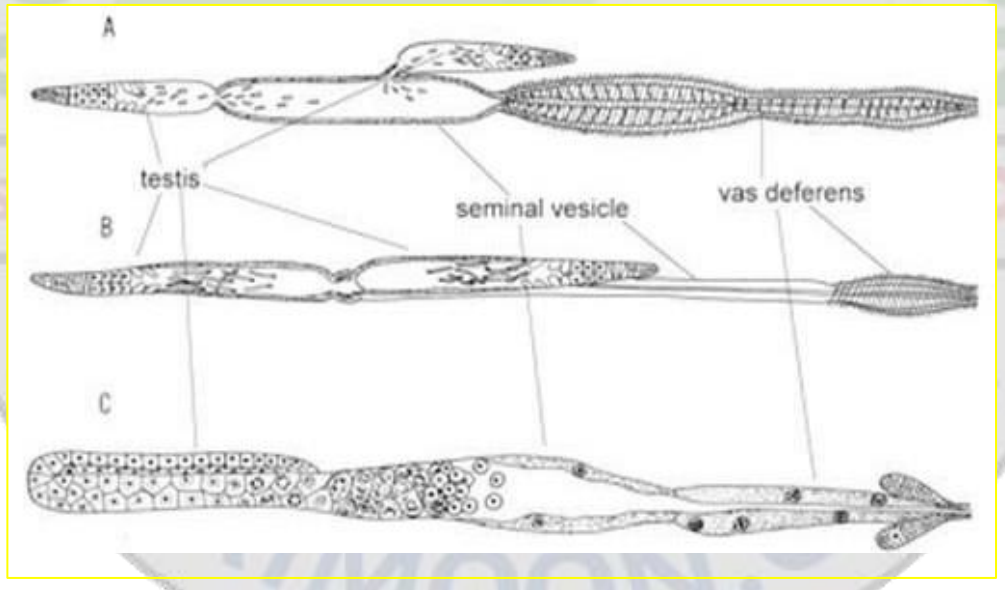


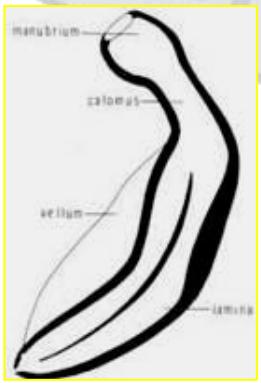
Monodelphic Didelphic Didelphic
Prodeplphic Amphidelphic Prodelphic ovaries

- Male Reproductive System

The production of sperms takes place in testis. In nematodes, whenever the number of testis is one, it is known as monarchic conditions and when they are tow in number, the condition is known which moves forward and backward with help of specialized muscles attached with its head region. Spicule is narrower at its tip. A cuticularised structure lying beneath the pair of spicule is known as guberrnaculum which helps and gives support in movement of the spicule. At the tail end, two filamentous cuticular expansions are found and they are known as bursa helps to hold the female during copulation. Plant parasitic nematodes can reproduce sexually where male and female copulate and give rise to off – springs. Sexual reproduction is also called as amphimetic reproduction. Parthenogenetic reproduction is also common phenomenon in *Meloidogyne* and *Tyelchulus semipenetrans*.

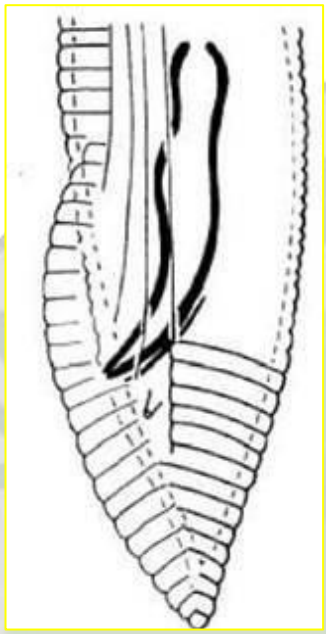
Male reproductive system:

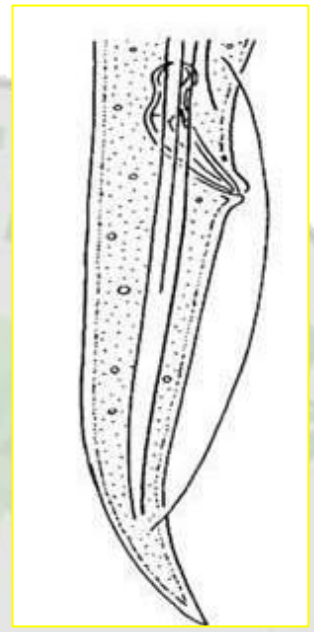




Spicule

Caudal alae





Peloderan

Leptoderan

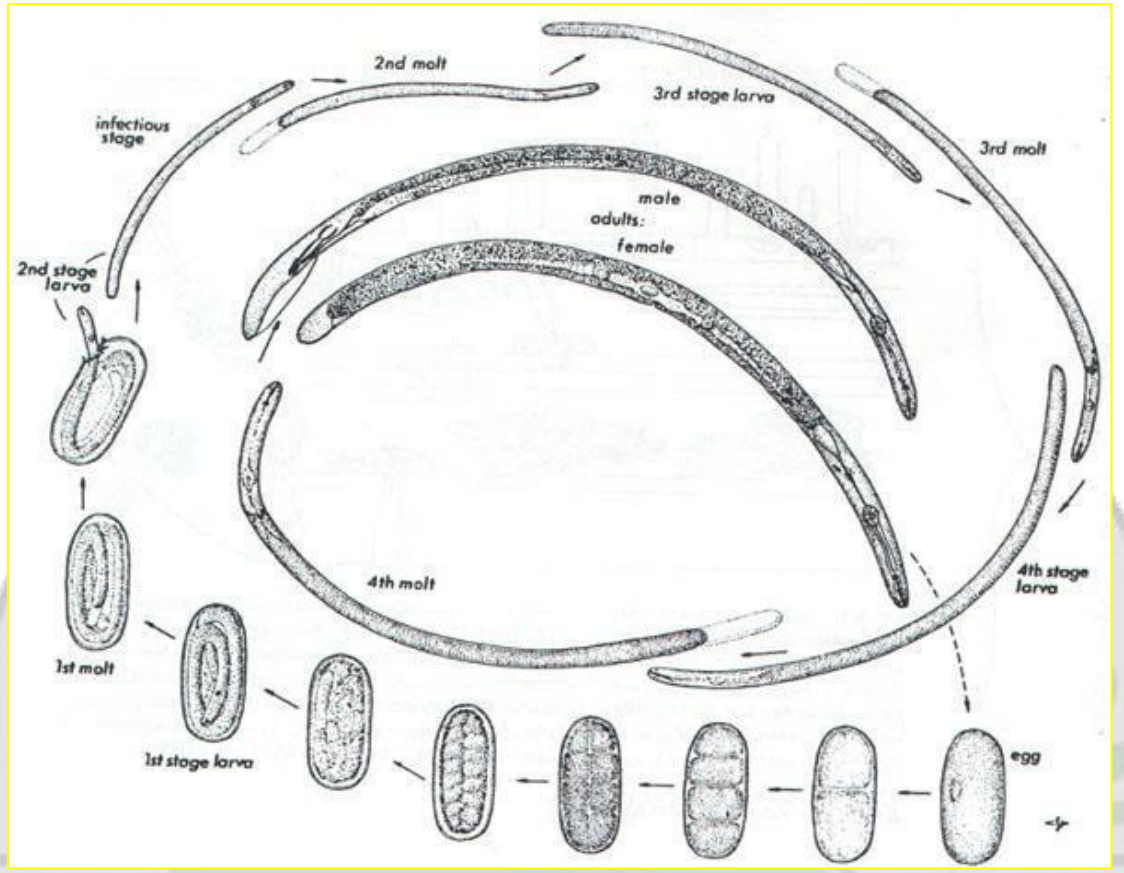
Eg. *Hoplolaimus* **.** **Eg. *Hirschmanniella***

Inter sexes:

In genera like *Meloidogyne* and *Ditylenchulus* inter sexes are found. In such cases one reproductive system act as male gonad and other one as female gonad.

Lecture 04 - Biology of Plant Parasitic Nematodes

The life cycle of nematode has six stages. The egg stage, J1; first stage larva, J2; second



stage larva, J3; third stage larva, J4; fourth stage larva and the adult stage. The first four stages are the immature stages and are known as juvenile stages. The female lays eggs in soil or in plant tissues, singly or in groups as egg mass that hatch out into larvae which are almost similar to adults in appearance. The first moult occurs within the egg shell and the second stage juvenile comes out by rupturing the egg shell as J2. In case of *Xiphinema index*, the larvae are reported to emerge from the egg before the first moult. The larval cuticle is shed after each moult.

The Egg:

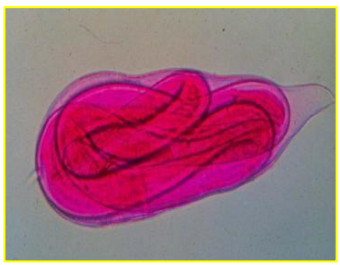
The nematode eggs are oval in shape. The eggs are covered by three membranes, the external protein layer which is the secretion of uterus wall, the middle chitinous layer or the true shell secreted by egg itself and the inner lipid layer. The chitin content in the egg shell varies in different species of nematodes.

Embryonic Development:

The adult female lays the eggs. The egg starts dividing by cleavage of their protoplasams to form cells. The first cleavage occur transverse to the longitudinal axis and gives two equal cells or blastomeres which are the first somatic cell (S1) and the parental germinal cell (P1). The second cleavage results in four cells which are first arranged in a T shape. This shape is achieved by the blastomere S1 dividing longitudinally and the blatomere P1 dividing transversely by P2 and S2. At last these cells get arranged in a rhomboidal shape. The transverse and longitudinal mitotic divisons of daughter cell continue. The S1 blastomere is the primary somatic cell and its two products (A & B) produce most of the nematode's ectodermal cells. The S2 blastomere produce somatic tissue and give rise to ectoderm (E), mesoderm (M) and stomodeum (St) tissues. The gonads of nematode are derived from P1. In the blastula stage the cells are so arranged as to form a fluid filled sphere bound by a single layer of the cells, while in the gastrula stage, the early embryo consists of an open mouthed sac like body with a wall of two layers of cells.

The cells A and B further divide to produce a, b, and P2 divides to give P3 and S3. The dorsal cells produced by A and B continue to divide and finally give rise to most of the hypodermis, excretory cells and nervous system. The daughter cell P2 divides into P4 and S4. These S3 and S4 are ectodermal and produce the hypodermis in the posterior region of the nematode body.

The primary mesodermal cell M gives rise the nematode's body wall musculature and its pseudocoelomic cells, while the pharynx from St cells. During early embryonic stage these primary cells St, M and E present on the ventral surface of the embryo and are taken within the embryo by process of gastrulation. In further development the dorso ventrally flattened embryo is changed to a cylindrical shape. The embryo starts to become worm shaped and a coiled juvenile is recognized inside the egg membrane. At last the cell constancy is reached and further cell multiplication stops in all organs except the reproductive system. The first moult take place within the egg and J2 ruptures the egg shell and hatch out. Before hatching the J1 can be seen riggling inside the egg shell.



First moult take place within the egg.

The post embryonic development in plant parasitic nematodes take

place within the egg leading to the formation of juvenile which is ready to undergo first moult. In the process of post embryonic development, hatching and moulting are the important stages.

Hatching (Ecdysis):

The term hatching is used for the emergence of the juvenile from the egg. It occurs either in response of a stimulus or stimuli from the host or take place under normal environment. In cyst forming nematodes the release of juvenile from cyst is an emergence and not hatching. Eggs have hatched within the cyst. The eggs of *Globodera rostochiensis* generally hatch in response to root exudates provided by solanaceous crops Viz., potato and tomato. After embryonic development the first stage juvenile undergoes the first moult within the egg and thus second stage juveniles are found within the egg.

After reaching a particular stage of growth and favourable hatching conditions are present, the juvenile shows vigorous movement, often causing bulging of the egg membrane as seen in case of *Pratylenchus*, *Paratylenchus*, *Nacobbus* and *Meloidogyne*.

2nd stage juvenile cyst nematode has just hatches from an egg

After that the juvenile makes a series of thrusts with the help of stylet on the egg shell @40-90 per minute and finally juvenile emerge out by breaking the egg shell at perforated places.

Moulting (Eclosion):

The hatched juvenile resembles the adult except for body size and gonad development. The juvenile undergoes some changes in form, particularly at the anterior and posterior ends and formation of gonads. Growth in nematodes is associated with moulting which usually occurs four times and there are five stages. After the fourth moult the nematode becomes fully grown adult. During moulting the entire cuticle including the cuticular lining of the stoma, stylet, oesophagus, vulva, cloaca, rectum, amphids, phasmids and excretory pore are shed. In most of the plant parasitic nematodes greatest growth occurs after the last moult and moulting tends to occur in the earlier half of the growth curve.

Stimulus:

It is reported that the neurosecretory cells of nematodes are stimulated to produce some secretions which activate glands that produce enzymes or hormones which initiate moulting. In

some cases root exudates act as a stimulus for moulting as in the case of *Paratylenchus nanus* and it acts as a stimulus to the fourth stage juvenile moult. In endoparasitic nematode, the stimulus may be more complex and may be closely associated with a increase in size of nematode, because in these nematode moulting does not occur until some growth has completed within the host. The stimulus may depend on the host, temperature, pH and the salt content of the soil. When these factors are optimal, the stimulus acts after a short exposure. Juvenile once stimulated it release the exsheathing fluid into space between the new and old cuticle which then digest the area of the sheath near the excretory pore ultimately releasing the juvenile.

The receptor:

In all cases the receptor may be cuticular and hypodermal structure eg. Hemizonid. It seems to be associate with neuro secretory activity which leads to the production of an enzyme which is responsible for moulting. The juvenile becomes sluggish inactive and feed vigorously just before moulting. The old cuticle is discarded by abrasion against soil particles or any rough material. The cuticle may be shed in one piece or the anterior part may be shed separately as a cap.

Growth and development:

In plant parasitic nematodes, there are four juvenile stages and an adult stage. The immature stage of the nematode called as juvenile. In case of endoparasitic nematodes, three moults occur within the host plant. The duration of the different juvenile stages is highly variable. Gonad development starts in the first juvenile stage before hatching but the growth of the organs is slow. The development starts with the formation of genital primordial which consists of two control germinal cells or one large cell which are bordered by two smaller somatic cells. External environment affect the structural development and physiology of the host which may influence the development of the nematode. The plant parasitic nematode fixes its feeding site in different regions of the root. *Meloidogyne* goes even up to stellar region, *Heterodera* and *R. reniformis* mostly confine to pericycle and *T. semipenetrans* penetrates cortex region.

Root – knot nematodes (*Meloidogyne* spp.):

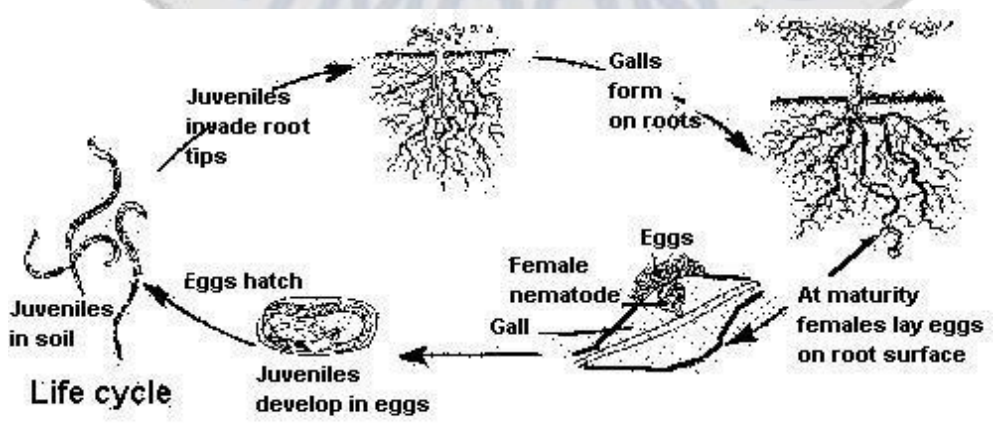
The root – knot nematodes are sedentary endoparasites of underground plant parts. The eggs are retained in a gelantinous matrix, which normally protrudes out of the host tissues. About 200 to 300 oval eggs are found in a single egg mass which makes its size larger than the female body.

The life cycle starts from the egg usually in the one-celled stage deposited by the female. Development of the embryo starts within hour of deposition, resulting in two, four, eight cells, etc., The embryo and the first stage larva move within the egg but not very active.

After the first moult, the second stage infective juvenile is formed within the egg. Larval hatch occurs under suitable physical condition but not depending on host root exudates or hatching factor. The emerging second stage larvae are found free in the soil. They attack new host root tissue in the region behind the root tip (meristamatic zone). The larvae which develop into females establishes feeding site in the pericycle region and become sedentary. Subsequently three moults occur and the larvae develop into females with spherical body embedded in the host tissue. The neck region is unaltered.

During feeding the larvae pierce the cell wall with secretions cause enlargement of cells in the vascular cylinder and increased cell division in the pericycle. The nematode feeding stimulates the development of a typical nurse cell system called ‘Syncytium’ or ‘Giant cell’. These cells are multinucleate which contain dense cytoplasm and enlarged nuclei with several mitochindrial and golgi bodies and are metabolically active.

The larval which develop into adult males are initially parasitic. After moulting three times they leave the host as a worm like from and come closer to the females for copulation. Parthenogenesis is reported to be common in *Meloidogyne*. For development of a mature female, it takes around 30 days which may vary depending upon the species of the host and parasite and environmental factors like temperature and soil type.



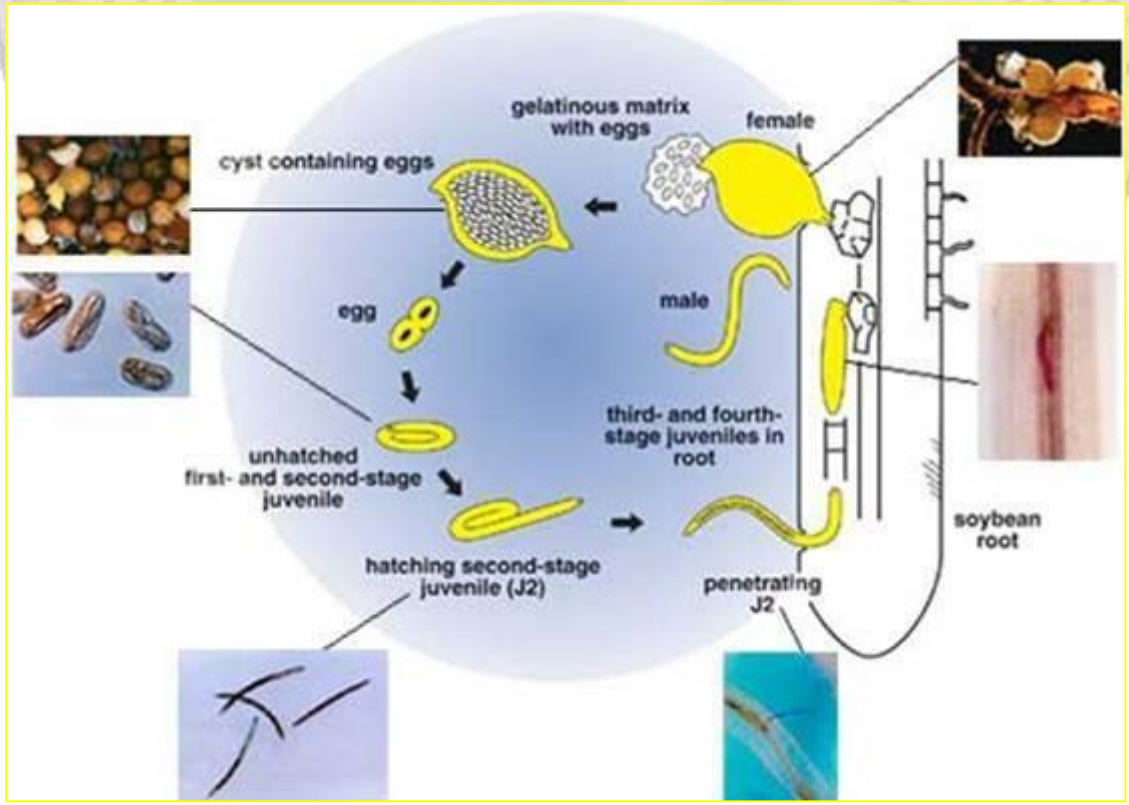
The life cycle of the root knot nematode

Cyst nematodes (*Heterodera* spp. and *Globodera* spp.):

Second stages larvae usually penetrate the root just behind the growing point. These larvae grow rapidly and thee moults occur in the host. In about 5 –6 weeks after penetration, the white cysts are clearly visible which protrude from the root surface. These young cysts are packed with eggs and upon death the body wall hardens due to quinone tanning into a tough resistant brown covering known as cysts. The cysts get separated from the root and fall into soil.

Larval emergence from cysts is often in response to root exudates from a host plant. The best emergence of juveniles occurs as a result of a rise in temperature after a period of low temperature. Maximum emergence of larvae from cysts under Indian condition takes place at a temperature of 20 -22°C. The cysts continue to release eggs over a period of 3 –4 years at the rate of 50 per cent viable eggs per year. There is only one generation of the nematode in a year. Multiplication of nematode is favoured by soil texture. Migration of second stage juveniles is favoured by light textured soils. The host cells close to the head region of the sedentary female being to modify and finally enlarge to form multinucleate syncytium with a thick outer boundary. The female feeds from this nurse cell system and grows. The swollen adult female protrudes out of the root tissues and later changes into brown cysts.

Life cycle of cyst nematode- *Heterodera*



Citrus nematode (*Tylenchulus semipenetrans*):

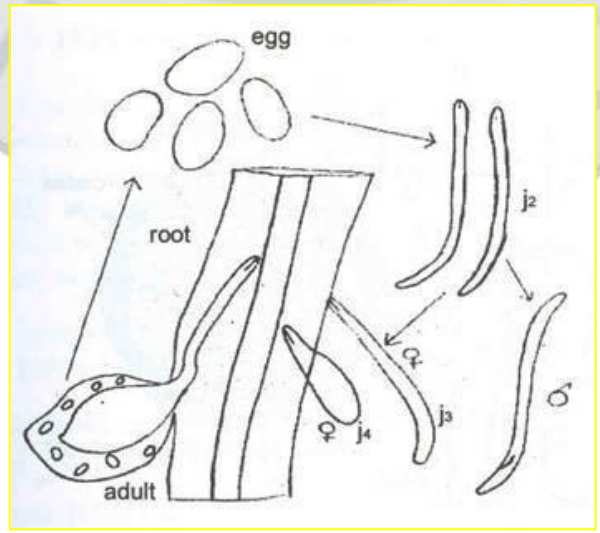
Citrus nematode is a sedentary semi – endoparasite of the Citrus root. Females are most commonly found on thick and stunted rootlets to which a layer of soil particle is clinging. These particles are held in place by a gelatinous mucus secreted by the female. The mucus and adhering soil particles protect the females and eggs deposited by them from their natural enemies. The egg laying young females can be seen in groups clinging to rootlets with their head and neck buried in the root cortex, whereas the posterior body region found outside the root surface.

Larva hatches from egg in 12 – 14 days. Mature males develop within a week after 3 moults and one moult having occurred within the egg. The long slender individuals fail to develop unless they feed on a root. The second stage female larva requires about 14 days to locate the host root and feed on epidermal cells until ready for moulting. Fourth stage females and young females are seen in about 21 days after the entry into roots. At maturity the females secrete the gelations matrix in which eggs are deposited. Egg laying occurs in about 40 days. The complete cycle from egg requires six to eight weeks at 25°C. Reproduction occurs without the help of males.

The feeding zone developed by this nematode is termed as nurse cell, which consists of uninucleate but not enlarged discrete parenchyma cells which are located in the cortex. Syncytium is not formed. This type of nurse cells system is characteristic for this nematode. Feeding of the citrus nematode in cortical cells causes necrosis in plants. The injury does not extend to the stellar region of the root.

The population of the citrus nematode is closely related to the stage of decline of the trees. The nematode infestation is severe in sandy loam soil.

Life cycle of citrus nematode

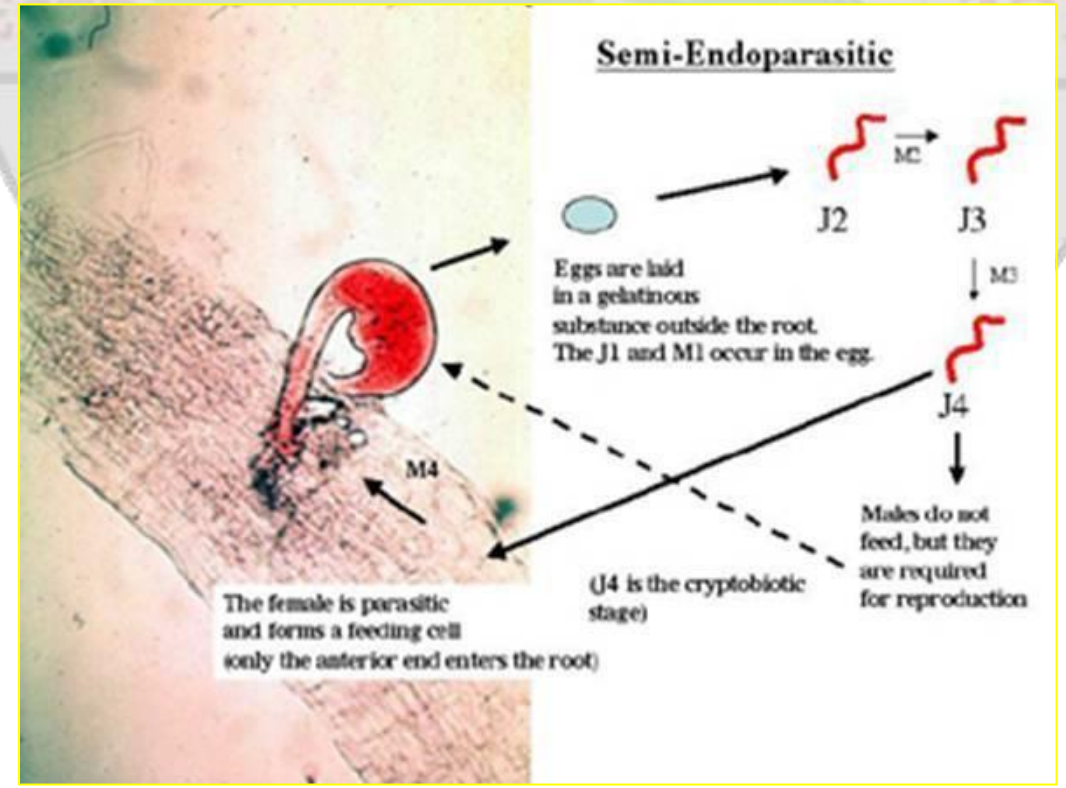


Reniform Nematode (*Rotylenchulus reniformis*)

The adult female is an obligate, sendentary, semi-endoparasite of roots while the males are non – parasitic. The species is bisexual and reproduction is by amphimixis.

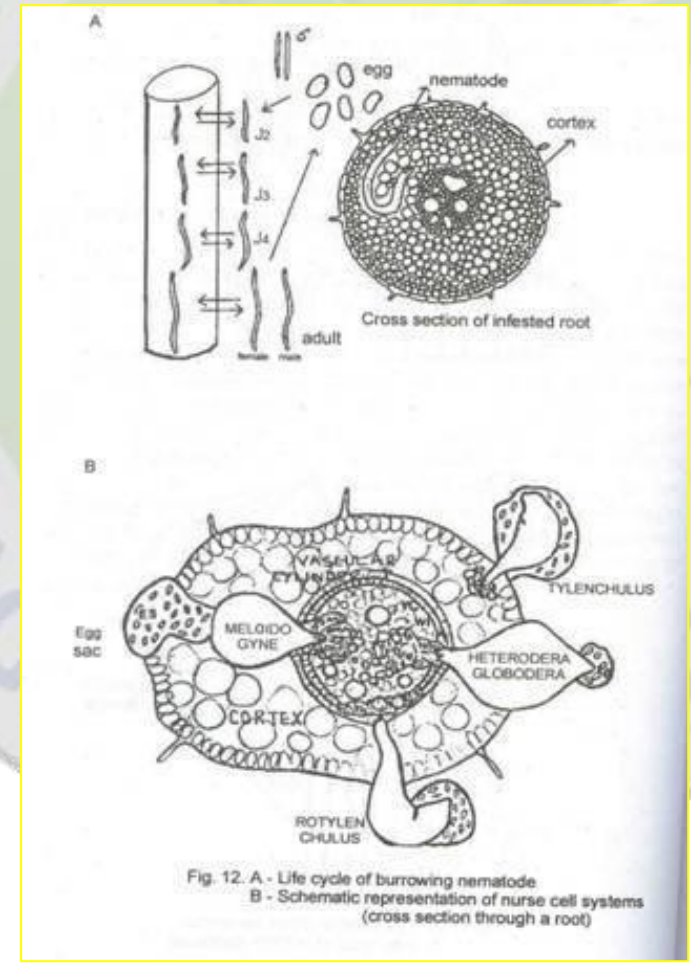
The species has an unusual life cycle. Although a newly hatched second stage larva have well developed stylet, they do not feed. They soon pass through three super imposed moults to become young females and adult males. The young females force their way through cells of root cortex until they partially or completely become embedded in this tissues. During the process they feed on cortical cells. Three days after feeding, a slight swelling of the posterior body is seed and eight days later eggs are deposited in a gelatinous matrix outside the root tissue. When these eggs are placed in water they promptly begin to hatch. The life cycle is completed in about 25 days provided the young females have found the host immediately. The nematode as a semiendoparasite of sedentary nature induces a specialized nurse cell systems for continuous food supply. The system involves wall expansion of several cells at the feeding site, partial wall dissolution, fusion of neighboring cell protoplasts and finally establishment of a multinucleate syncytium. These syncytia are mostly confined to the pericycle. Other pericycle cells are metabolically stimulated but they remain discrete and uninucleate.

The young infective females destroy the exterior cortical cells o roots and the damage increase when the nematode moves towards the phloem.



Burrowing Nematode (*Radopholus similis*):

Females and all juvenile stages are infective. Males are non-parasitic and morphologically degenerate (without stylet). Penetration occurs mostly near the root tip. The nematode penetrates within 24 hours and the cells around the site of penetration becomes brown. After entering the roots, the nematodes occupy intercellular position in the cortical parenchyma where they feed on the cytoplasm of nearby cells causing cavities which coalesce to form tunnels. Nematodes do not enter the stellar portions of the root. The nematode completes its life cycle within 24 – 30 days at a temperature range of 21 - 32°C. Females lay eggs within infested tissues with an average of 4 –5 eggs for two weeks. Eggs hatch after 8 – 10 days and the juvenile stages are completed in 10 –13 days. A low soil temperature, adequate soil moisture and availability of fresh tender roots help in the build up of population.



Life cycle of burrowing nematode

Lecture-05

Taxonomy of Plant Parasitic Nematodes

Theory and practice of classification is known as Taxonomy , Nematodes are placed in the group invertebrate, Kingdon Animalia. Under separate phylum, Nemata / Nematoda, which consist of two classes, Secernentea and Adenophorea.

International Code of Zoological Nomenclature (ICZN):-

The rules and regulations regarding scientific names are provided by ICZN and adopted by 15th International congress of Zoology held at London on 6th July 1958 and published in 1961

The objective of ICZN is to ensure that each name is unique and distinct.

Classification:

The grouping and ordering of and organism in to various sets or categories on the basis of their similarities is known as classification

In the classification two types of practice are generally followed

(i) Monothetic type/ Artificial classification

Grouping of an organism on the basis of one or two distinct characters possessed by them.

(ii) Polythetic type/ Natural classification:

Grouping of an organism on the basis of majority of shared characters in between them.

Here equal weight is given to all morphological characters possessed by individual

Thus it is also known as natural classification

Example:

Aphasmatylenchus which does not have phasmid was kept in the class Phasmida Whereas *Teratocephalus* having phasmid was kept in Aphasmida

Hierarchy :

The different levels in classificatory scheme for systematic position of any individual is called hierarchy

Kingdom - Animal
Phylum - Nematoda
Class - Secernentea
Order - Tylenchida (ida)
Sub-order - Tylenchina (ina)
Super-family - Tylenchoidea (oidea)
Family - Tylenchidae (idae)
Sub family - Tylenchinae (inae)
Genus - Tylenchus sp.

Classification of Nematodes

Phylum Nematoda (Poinar 1983)				
Class Secernentea (Phasmidia)			**Adenophorea** (Aphasmidia)	
Sub Class			**Sub Class**	
Rhabditia	Diplogastria	Tylenchia	Chromadoria	Enoplia
Order	**Order**	**Order**	**Order**	**Order**
Rhabditida	Diplogastrida	**Tylenchida**	Araiolamida	Enoplida
Oxiurida	Drylenematida	**Aphilenchida**	Menohysterida	**Dorylaimida**
Stronglida			Desmodarida	Monochida
			Chromodorida	Mermithida
			Desmoscolaicida	

Diagnostic characters of class Secernentea and Adenophorea:

Secernentea (Phasmida)	**Adenophoreea (Aphasmida)**
Amphidial opening is on the head near the lip region.	Amphids open behind the head i.e. post labial
Lateral canals open into the excretory duct	Lateral canals and excretory duct end in a cell
Oesophagus is divided into procorpous, median bulb, isthmus and basal bulb.	Oesophagus is cylindrical with an enlarges glandular base
Male tail with bursa (Caudal alae)	Male tail lacks bursa but possess genital paillae.
Glands are absent Phasmids are present	Caudal glands are present Phasmids are absent
The mesenterial tissues are less developed	The mesenterial tissues are well developed

The plant parasitic nematodes are included in the orders *Tylenchida* of class *Secernentea* and *Dorylaimida* of class *Adenophorea*.

Order: *Tylenchida*:

Stoma armed with a protrusible spear or stomatodtylet. *Oesophagus* consists of a procarpus, media bulb with selerotized valvular apparatus, nerve ring encloses the narrow isthmus and with a basal bulb. It consists of two super families namely *Tylenchoidea* with *Tylenchida* and *Aphelenchina* as suborders and *Criconematoidea.*

Differences between *Tylenchoidea* and *Criconematoidea*:

Character	Tylenchoidea	Criconematoidea
Labial region	Lips are hexaradiate, Labial frame work present	Labial region is poorly developed, labial plate is present
Stylet	Conus, shaft and knobs are variable in shape and size	'Criconematoid' type stylet long and anchor shape knob which lies in base of metacarpus
Oesophagus	Narrow procarpus, round metacarpus with value, isthmus followed by glandular basal bulb	Pro and metacarpus amalgamated to a single unit, short isthmus, the post carpus reduced, appears as 'set-off' smaller than pro and metacarpus
Deirids	Present (2 pair)	Absent
Female gonad	Single or two ovary; postuterine sac (PUS) is present	Single ovary with posterior vulva; PUC absent
Male gonad	Single testis, caudal alae is present	Single testis; caudal alae rare
Phasmid	Erratically present in tail region	Not known

Difference between *Tylenchina* and *Aphelenchina*:

Characters	*Tylenchina*	*Aphelenchina*
Lip	Varying in shape	Set- off
Annules	Faint to strong annules	Faint annules
Stylet	Well developed; one dorsal and two sub ventral knobs	Weekly developed; no stylet knobs
Oesophagus	Three parted	Three parted with square shaped median bulb
Gland bulb	Abutting, dorsal, ventral or dorso – ventral overlapping on intestine	Only dorsal overlapping
Gland opening	Behind the stylet knob in procorpous	Opens in the median bulb
Female	One or two; vulval position vary	Single ovary; vulva posterior
Male	Bursa present	Bursa rare
Spicule	Weak to strong sclerotization is seen with gubernaculums	Rose thorne shape spicule present

Order : *Dorylaimida*:

The labial region is set off from body contour. The stoma is armed with a movable mural tooth or a hallow axial spear. *Oesophagus* is divided into a slender, muscular anterior region and an elongated or pyriform glandular posterior region. Females have one or two reflexed ovaries;

males have paired equal spicules, gubernaculums rare. The order is divided into three sub orders namely *Dorylaimina*, *Diptherophodrina* and *Nygolaimina.* The former two sub-orders containing the plant parasitic nematodes.

Sub order

Dorylaimina	**Diptherophodrina**
Stylet with flangers or guiding ring, long and straight	Teeth like spear, solid, short and ventrally curved.

Family: Longidoride

Genus : ***Longidorus****:* Amphids pouch like, slit like opening, spear extension without flanges, guiding ring located near the spear tip.

Genus : ***Xiphinema*** : Amphids funnel shaped wide opening, spear extension with flanges, guiding ring located near the spear base.

Family : Trichodoridae

Genus : ***Trichodorus***: Long curved onchiostylet, female rectum runs parallel to the longitudinal body axis and the anus lies sub terminally. Male tail curved bursa absent, vaginal sclerotization strong, lateral pores present near vulva.

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