

PATH 172 - INTRODUCTORY NEMATOLOGY (1+1)

Theory

Introduction. History of phytonematology. Economic importance. General characteristics of plant pathogenic nematodes. Nematode general morphology and biology. Classification of nematodes up to family level with emphasis on groups containing economically important genera. Classification of nematodes by habitat. Identification of economically important plant nematodes up to generic level with the help of keys and description. Symptoms caused by nematodes with examples. Interaction between plant parasitic nematodes and disease causing fungi, bacteria and viruses. Different methods of nematode management. Cultural methods (crop rotation, fallowing, soil amendments, other land management techniques), physical methods (soil solarisation, hot water treatment) Biological methods. Chemical methods (fumigants, non fumigants). Resistant varieties. IDM.

References

1. Dropkin, V.H. 1980. Introduction to plant nematology. John Wiley and sons, INC. New York.
2. Singh, R.S and Sitaramaiah, K. 1994. Plant pathogens. The plant parasitic nematodes. Oxford & IBH Pub. Co. Pvt. Ltd. New Delhi.
3. Parvata Reddy, P. 1983. Plant nematology. Agricole Pub. Co., New Delhi.
4. Southey, J. F. Laboratory methods for work with plant and soil nematodes Tech. Bull. Min. Agric. Fish. Food. Her Majesty's Stationary Office, London.

Lecture 01 - 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 obligate parasites have been estimated to be about \$ 78 billion worldwide 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’s “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.

1873 - Butschli – Descriptions of the morphology of free –living nematodes.

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

1889 – Atkinson and Neal – Publication about the root-knot nematodes in the United States.

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**

1914 – N.A.Cobb – Contributions to the Science of Nematology.

1918 – N.A. Cobb – Development of methods and apparatus used in Nematology.

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

1934 – Filipjev – Book on “Nematodes that are importance for Agriculture” translated from Russian to English in 1941 by S.Stekhoven under the title “A Manual of Agricultural Helminthology”.

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.

1948 – Allen – Taught the World’s first formal university course in Nematology at the University of California, Berkeley.

1950 – Oostenbrink – Wrote a Book of on “The Potato Nematode, A dangerous parasite to Potato Monoculture”.

1951 – Christie and Perry – Role of ectoparasites as plant pathogens. T.Goodey – Wrote a book on “Soil and fresh water nematodes”. Food and Agriculture Organisation of the United Nations organized the first International Nematology course and Symposium held at Rothamstead Experiment Station, England.

1955 – European Society of Nematologists founded.

1956 – Nematologica – The first journal published entirely for Nematology papers from The Netherlands.

1961 – Society of Nematologists founded in the United States.

1967 – Organization of Tropical American Nematologists founded.

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

1973 – Nematologa Mediterranea – published from Italy.

1978 – Revue de Nematologie published from France

1930s – 1990s – Barron, Duddington, Mankau, Linford, Sayre and Zuckerman – they provided an insight on the Biological control of plant – parasitic nematodes. Enhanced understanding of antagonists and related biology enhancing the potential for practical biocontrol.

1940-s – 1990s – Triantaphyllou – Provided advancement in Cytogenetics, modes of reproduction/sexually – and information data base for genetics/molecular research. Enhanced understanding of evolution and taxa interrelationships.

1950s – 1990s – Caveness, Jones, Oostenbrink, Sasser and Seinhorst – International programme such as International *Meloidogyne* project – They expanded educational base of nematologists world wide and provided ecological – taxonomic data base.

1960s- 1990s – Nickle, Poinar and Steiner – Biological control of insects with nematodes.

1960s- 1990s – Brenner, Dougherty and Nicholas – *Caenorhabditis elegans* developmental biology and genetics – model system – provided fundamental information on cell lineage, behavior, gene function ageing and overall genome for this model biological system.

In addition to the above, now the research advancement are in progress in the following areas in USA from the year 1990.

Molecular markers for resistance genes, which provide efficiency of breeding for resistance. Cloning of resistance genes- Elucidation of the molecular fundamental knowledge on mechanisms of pathogenesis. Cloning of resistance genes- Elucidation of the molecular

mechanism of resistance. Transgenic host resistance to plant parasitic nematodes – Great potential, but limited model system to date.

Lecture 02 - History of Nematology in India

Nematology as a separate branch of Agriculture Science in India has been recognized only about 37 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 laboratory established at Agricultural College and Research Institute, Coimbatore, with the assistance of Rockefeller Foundation and Indian Council of Agricultural Research.

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

1963 – Laboratory for potato cyst nematode research established at Uthagamandalam with the assistance of Indian Council of Agriculture Research

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

1968 – First South – East Asian Post – Graduate Nematology course held in India.

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

1969 – 1970 – Third South – East Asian Nematology course conducted at New Delhi.

1969 – 1970 – Third South – East Asian Nematology course conducted at New Delhi.

1971 – Indian Journal of Nematology published

1971 – Fourth South – East Asian Nematology course at New Delhi.

- 1972 – First All India Nematology Workshop held at IARI, New Delhi
- 1973 – Fifth South – East Asian Nematology Course at New Delhi.
- 1975 – Sixth South – East Asian Nematology Course at New Delhi.
- 1976 – Summer Institute in Phytonematology held at Allahabad.
- 1977 – Department of Nematology established at Haryana Agriculture University, Hisar.
- 1977 – All India Co-ordinated Research Project (AICRP) on nematode pests of crops and their control started functioning in 14 centres in India with its Project Co-ordinator at IARI, New Delhi.
- 1979 – M.Sc. (Ag.) Plant Nematology course started at Tamil Nadu Agricultural University, Coimbatore.
- 1979- All India Nematology Workshop and Symposium held at Orissa University of Agricultural University, Coimbatore
- 1979 – All India Nematology Workshop and Symposium held at Orissa University of Agriculture and Technology, Bhubaneswar
- 1979 – Seventh South – East Asian Nematology course at New Delhi.
- 1981 – Department of Nematology established at Tamil Nadu Agricultural University, Coimbatore.
- 1981 – All India Nematology Workshop and Symposium held at Tamil Nadu Agricultural University, Coimbatore.
- 1982 – Department of Nematology established at Rajendra Agriculture University, PUSA, Bihar
- 1983 – All India Nematology Workshop and Symposium held at Solan, Himachal Pradesh.
- 1985 – All India Nematology Workshop and Symposium held at Udaipur, Rajasthan.
- 1986 – National Conference on Nematology held at IARI, New Delhi
- 1987 – All India Nematology Workshop at Govt. Agriculture College, Pune.
- 1987 – Group Discussion on Nematological problems of Plantation crops held at Sugarcane Breeding Institute, Coimbatore.
- 1992 – Silver Jubilee Celebration of Division of Nematology, IARI, New Delhi.
- 1992 – Summer Institute on “Management of Plant Parasitic nematodes in different crops” organized by ICAR at Haryana Agricultural University, Hisar.
- 1995 – All India Nematology Workshop and National Symposium on Nematode problems of India held at IARI, New Delhi.

1997 – Summer School on “Problems and Progress in Nematology during the past one decade” was organized by ICAR at IARI, New Delhi.

1998 – Afro – Asian Nematology Conference held during April 1998 at Coimbatore.

1999 – National seminar on “Nematological Research in India: Challenges and preparedness for the new millennium” at C.S. Azad University of Agriculture and Technology, Kanpur.

2000 – National Nematology Symposium on “Integrated Nematode Management” held at OUAT, Bhubaneshwar, Orissa.

2001 – National Congress on “Centenary of Nematology in India: Appraisal and Future plans” at IARI, New Delhi.

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	Number of estimates per crop	Food and Agriculture Organization production estimates (1000 MT)	Estimated yield losses due to Nematodes (%)
Banana	78	2 097	19.7
Barley	49	171 635	6.3
Cassava	25	129 020	8.4
Citrus	102	56 100	14.2
Cocoa	13	1 660	10.5

Coffee	36	5 210	15.0
Corn	125	449 255	10.2
Cotton (lint)	85	17 794	10.7
Field bean	70	19 508	10.9
Oat	37	43 355	4.2
Peanut	69	20 611	12.0
Potato	141	312 209	12.2
Rice	64	469 959	10.0
Sorghum	53	71 698	6.9
Soybean	91	89 893	10.6
Sugar beet	51	293 478	10.9
Sugarcane	65	935 769	15.3
Sweet potato	67	117 337	10.2
Tea	16	2 218	8.2
Tobacco	92	6 205	14.7
Wheat	89	521 682	7.0

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.

Lecture 03 - 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*) to about 11.0mm (*Paralongidorus maximus*). Their body width vary from 0.01 to 0.05 mm. In few genera, the females on maturity assume pear shape (Meloidogyne), globular shape (Globodera), reniform (*Rotylenchulus reniformis*) or saccate (*Tylenchulus semipenetrans*). 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.

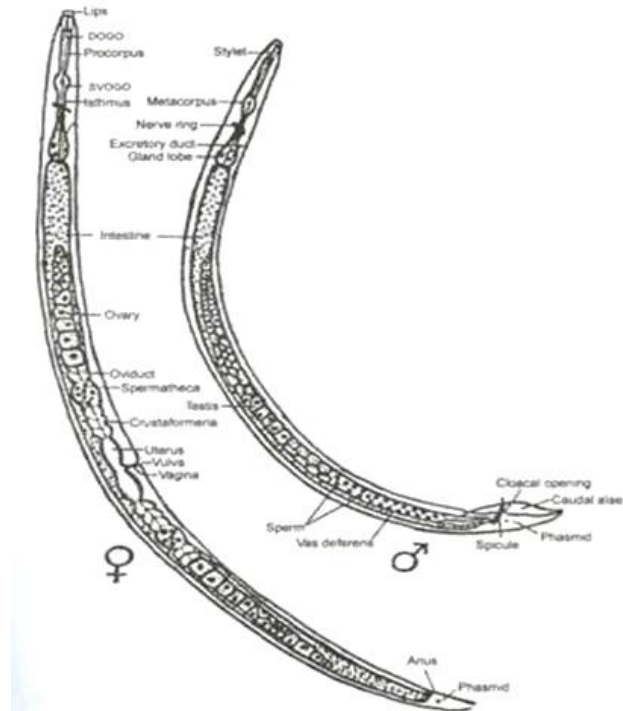


Fig. 1- Body morphology of a typical vermiform plant parasitic nematode
DOGO - dorsal oesophageal gland orifice, SVOGO - subventral oesophageal gland orifice

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 bancrofti*
2. Guinea worm - *Dracunculus medinensis*
3. Round worm - *Ascaris lumbricoides*
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 possess 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 pseudocoelom and by simple diffusion across membranes.

The following are the characteristics of members of the phylum Nemata.

1. Inhabit marine, freshwater and terrestrial environments as free – lived and parasites.
2. Bilaterally 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.*

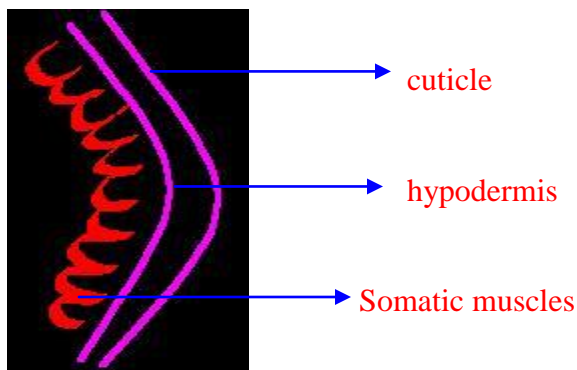
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|----------------------------|---|
| 3. Foliar nematode | <i>Aphelenchoides</i> spp. |
| 4. Lance nematode | <i>Hoplolaimus</i> spp. |
| 5. Lesion nematode | <i>Pratylenchus</i> spp. |
| 6. Needle nematode | <i>Longidorus</i> spp. |
| 7. Pin nematode | <i>Paratylenchus</i> spp. |
| 8. Reniform nematode | <i>Rotylenchulus</i> spp. |
| 9. Ring nematode | <i>Criconemella</i> spp. |
| 10. Root – knot nematode | <i>Meloidogyne</i> spp. |
| 11. Sheath nematode | <i>Hemicycliophora</i> spp. |
| 12. Spiral nematode | <i>Helicotylenchus</i> spp. |
| 13. Sting nematode | <i>Belonolaimus</i> |
| 14. Stubby – root nematode | <i>Paratrichodorus</i> spp and
<i>Trichodorus</i> spp. |
| 15. Stunt nematode | <i>Tylenchorhynchus</i> 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

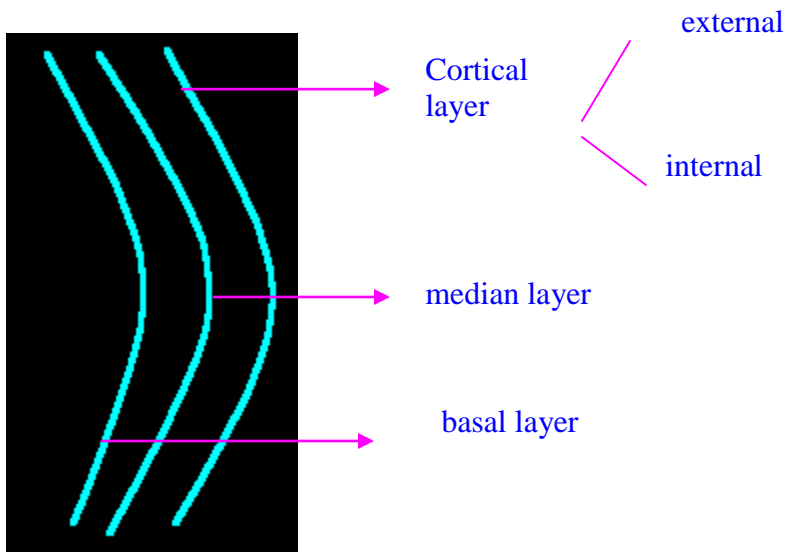
The cuticle is a non living, non cellular, triple – layers covering that is secreted by the underlying hypodermis. The cuticle is flexible. It covers the entire body and lines the oesophagus, vulva, anus, cloaca, excretory pore and sensory organs. The feeding stylet and copulatory spicules are formed 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.

Cuticle



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

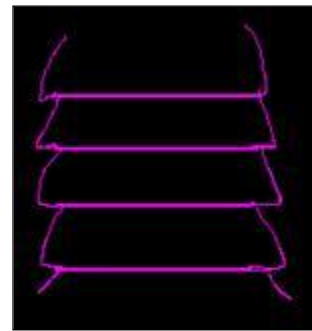
Perineal pattern

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 rugose wall pattern of *Heterodera* cysts, are considered to be the modifications of transverse markings.



Transverse marking



Criconematids- Annulations

iii. Longitudinal markings

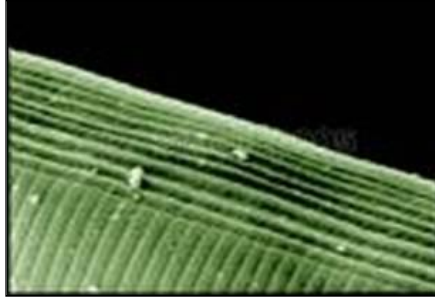
These markings are the lines on the cuticle, which runs longitudinally throughout the length of the nematode body. These markings are divided into **a) lateral lines or incisures** and **b) longitudinal ridges**.

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 and iii. Longitudinal alae.

i. Caudal alae

These are found in the posterior region and restricted to males as copulatory bursa.



Eg. *Hoplolaimus*

ii. Cervical alae

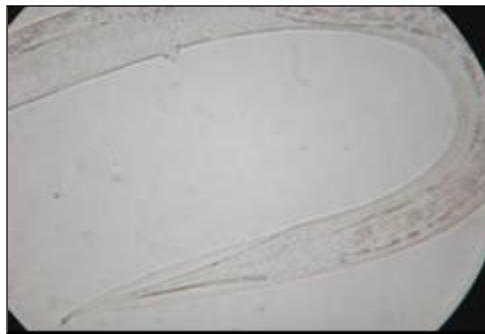
These are confined to the anterior part of the nematode body. Cervical alae are found in some species of marine nematodes.



Eg. *Toxocara*

iii. Longitudinal alae

The longitudinal alae delimit the lateral 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.



Eg. *Tripyla*

The functions of cuticle

Cuticle gives definite shape and size to the body, acts as an exoskeleton, helps in movement, being semipermeable, 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

This type 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.

Lecture 04 - Anatomy: Inner Body Tube

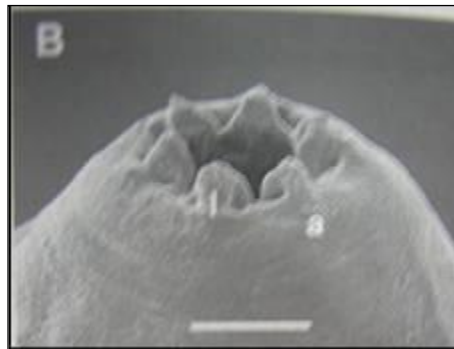
Digestive system

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

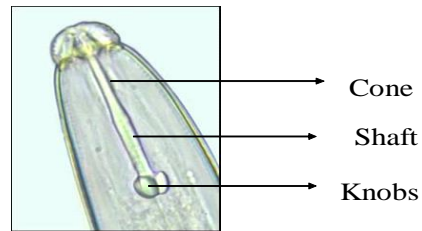
Stoma is the portion of the inner body tube lying between the oral opening and oesophagus. The stomatal opening is small and slit like and is surrounded by six lips.



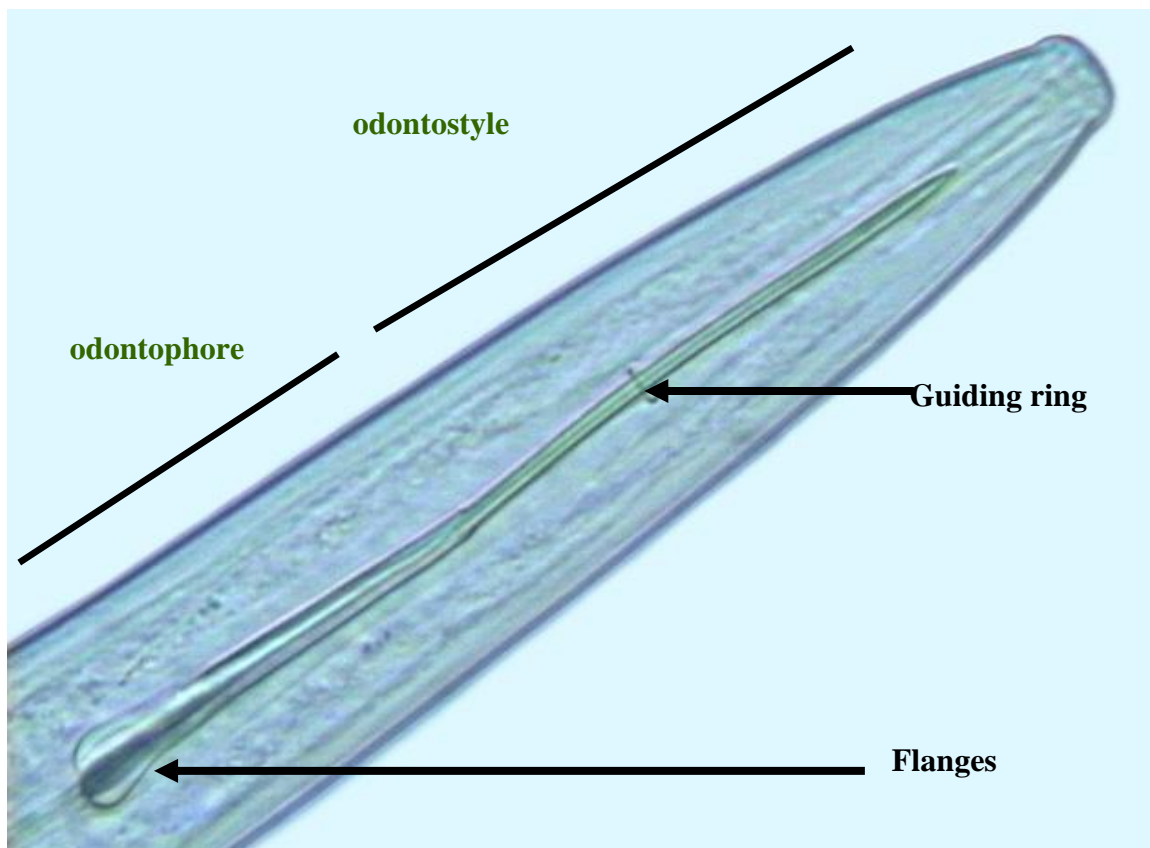
Stomatal opening

Two subdorsal, two subventral and two lateral. Plant parasitic nematodes are armed with a protrusible stylet which is usually hollow and functions like a hypodermic needle. In Secernentea, the stylet is thought to be derived from fusion of the stomatal lining and therefore called as stomatostylet. The stomatostylet consists of an 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 serve as points of attachment for the stylet protractor muscles. In some plant parasitic nematodes like Trichodorus and Paratrichodorus the odontostylet is distinctly curved ventrally, lacks flanges and it is not hollow. Its function is to pierce the cell wall of the root. The nematode secretes a hollow tube out of its stoma that connects it with the plant. This feeding tube serves as the interface between the nematode and the plant.

Stomatostylet- Eg. *Hoplolaimus*



Odontostylet – Eg. *Xiphinema*

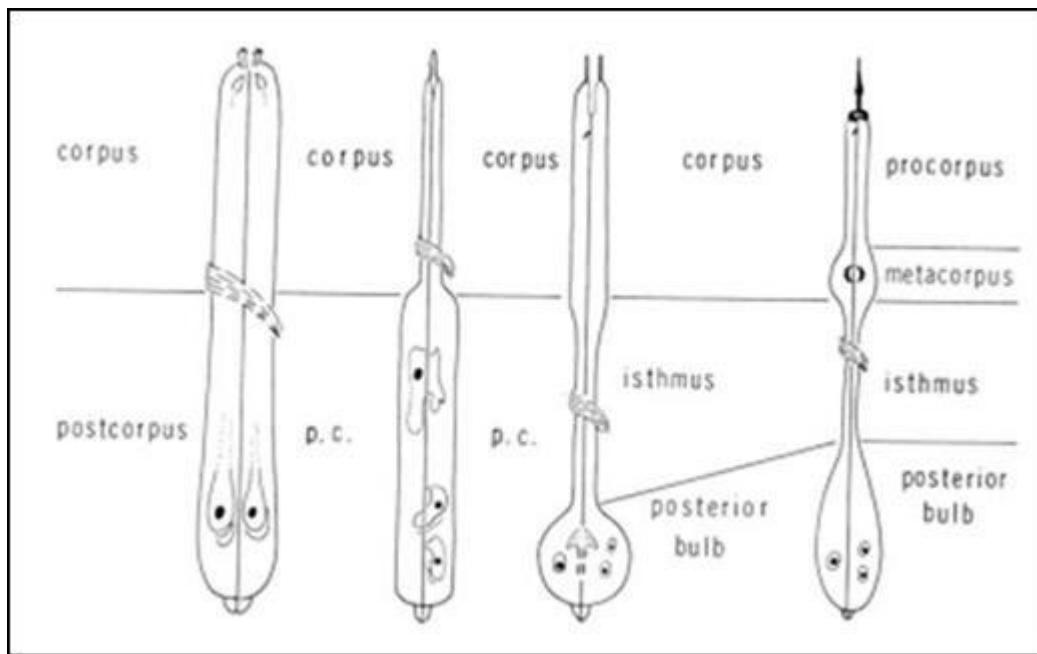


Oesophagus or pharynx

The oesophagus is a muscular pumping organ attached to the posterior portion of the stylet and lined 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 subventral 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, hollow, straight tube consisting of a single layer of epithelial cells. The intestine is generally divided into 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

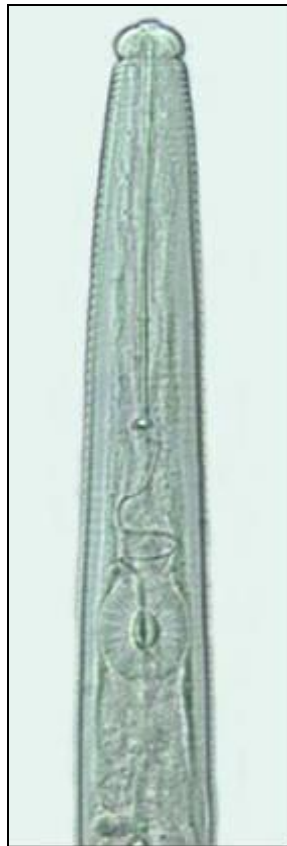
surface area of the intestine and are both secretory and absorptive in function. The whole intestine is separated from the pseudocoelom by a basement membrane.

The food moves in the intestine by the ingestion of more food and also by locomotory activity of the nematode.

Dorylaimid



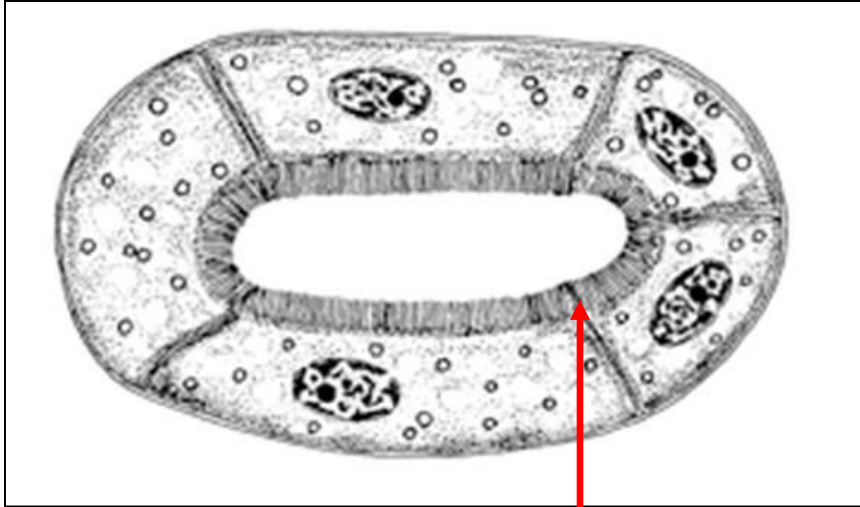
Tylenchid



Aphelenchid



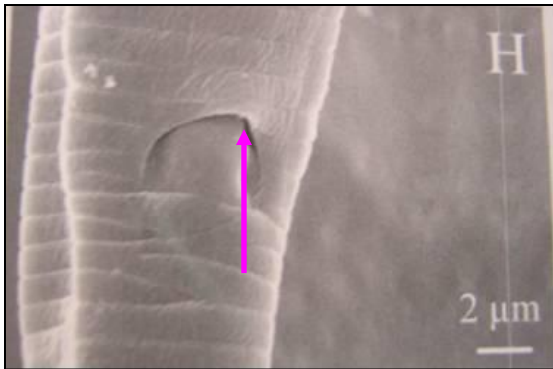
C.S. of intestine



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.

Lecture 05 - Anatomy: Oesophageal glands

Three uninucleated oesophageal glands. One gland on dorsal and other two 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 an important role in hatching host penetration and also establishment of host parasitic relationship.

Rectal glands

Are responsible for the copious production of gelatinous mucopolysaccharide 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 develops around the feeding site in the host cell. There are two feeding phases. 1. Injection phase or salivation phase and 2. Ingestion phase.

Injection phase or Salivation phase

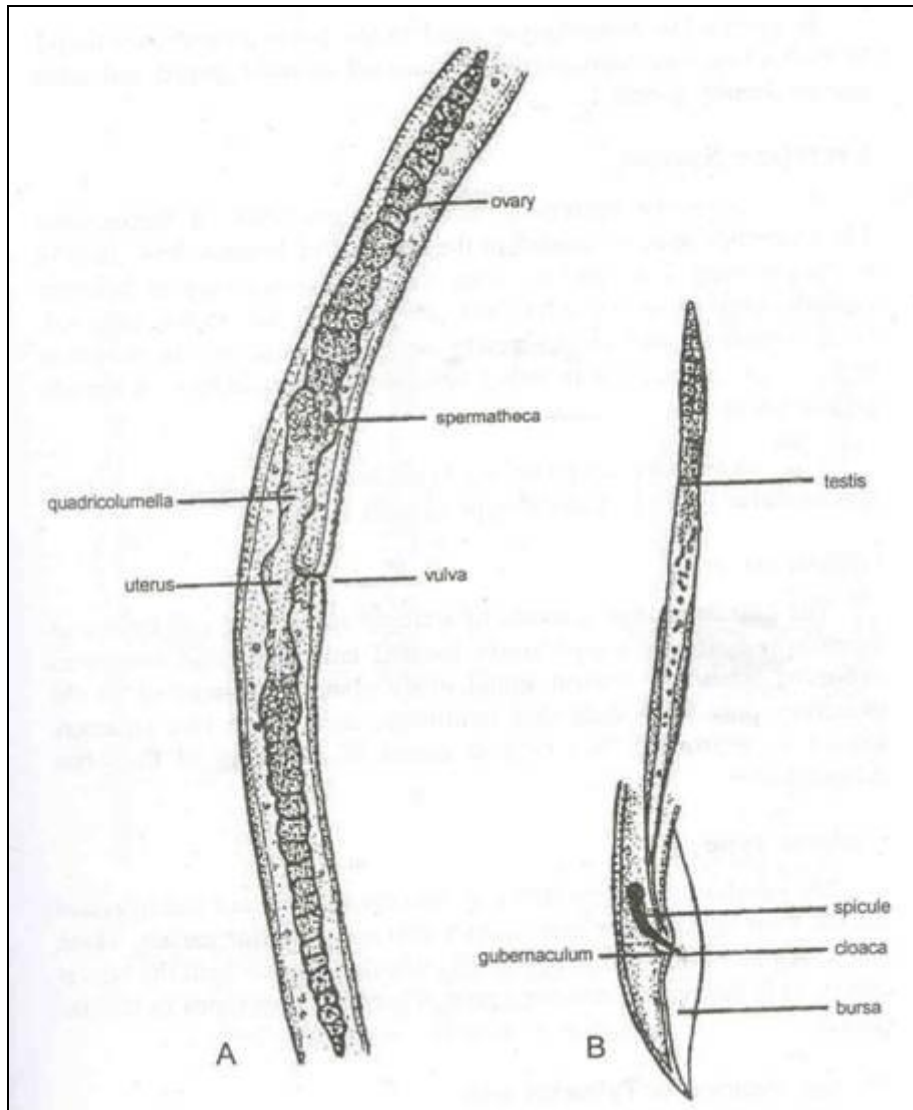
During this phase, the flow of salivary juices into the host cell occurs due to contraction of lateral muscle of the median bulb.

Ingestion phase

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

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

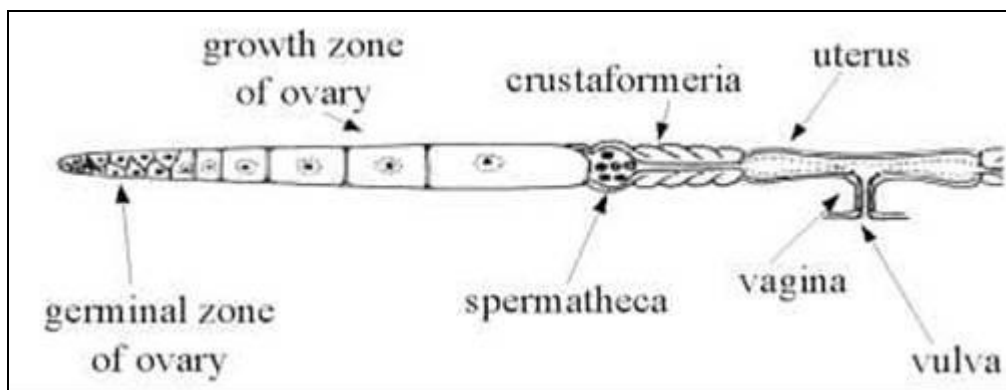
Present in nematodes having single ovary as observed in the genera *Pratylenchus* and *Ditylenchus*. The uterus opens outside to a ventrally located vulval opening through a tube known as vagina, which is a cuticularised structure. In plant parasitic nematodes the number of ovary may be one or two. When 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 some 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 transverse 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, an ovary is called hologenic if it produces oocytes throughout its length and telogenic if producing oocytes only at its distal end.

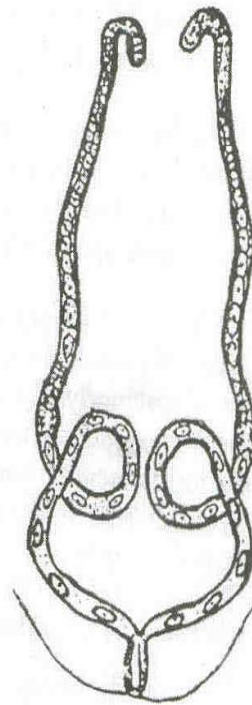




**Monodelphic
prodeplhic**



**Didelphic
amphidelphic**

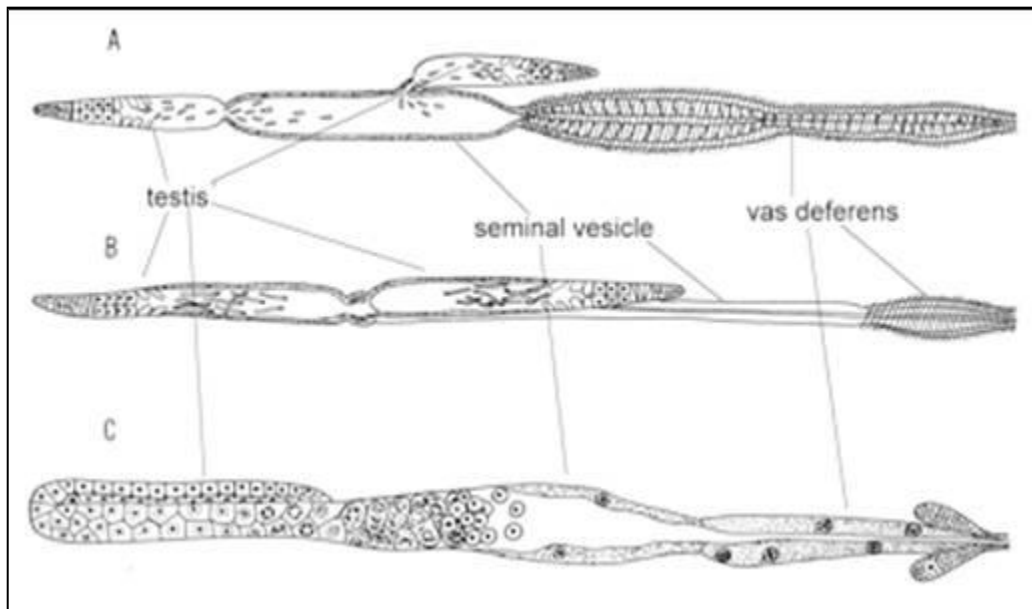


**Didelphic
prodeplhic ovaries**

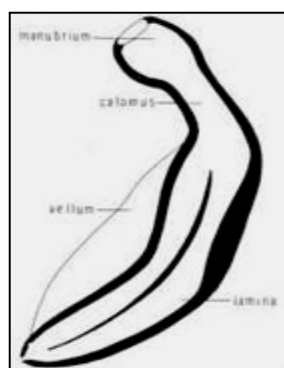
Lecture 06 - Male Reproductive System

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 two in number, the condition is known as dichogamy. The gubernaculum is a structure 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 gubernaculum 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 which helps to hold the female during copulation. Plant parasitic nematodes can reproduce sexually where male and female copulate and give rise to offspring. Sexual reproduction is also called as amphimictic reproduction. Parthenogenetic reproduction is also a common phenomenon in *Meloidogyne* and *Tylenchulus semipenetrans*.

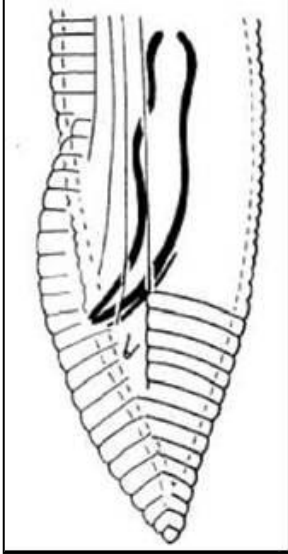
Male reproductive system



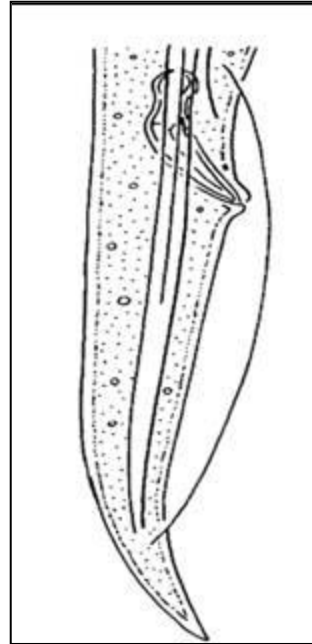
Spicule



Caudal alae



Peloderan
Eg. *Hoplolaimus*



Leptoderan
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.

Excretory System

The excretory system is not well developed in nematodes. The excretory pore is located in the anterior midventral line close to the nerve ring. The position of excretory pore may vary in different genera and even in different stages of the same species. In *T. semipentrans* the excretory. It secretes gelatinous matrix. The excretory system in nematodes are of two types. 1. Glandular type 2. Tubular type.

Glandular type

The glandular type consists of a single specialised cell known as renette cell. It has a posteriorly located enlarged gland known as excretory gland or ventral gland. This gland is connected to the excretory pore by a duct that terminates in a pouch like structure known as ampulla. This type is found in members of the class Adenophorea.(Fig.7)

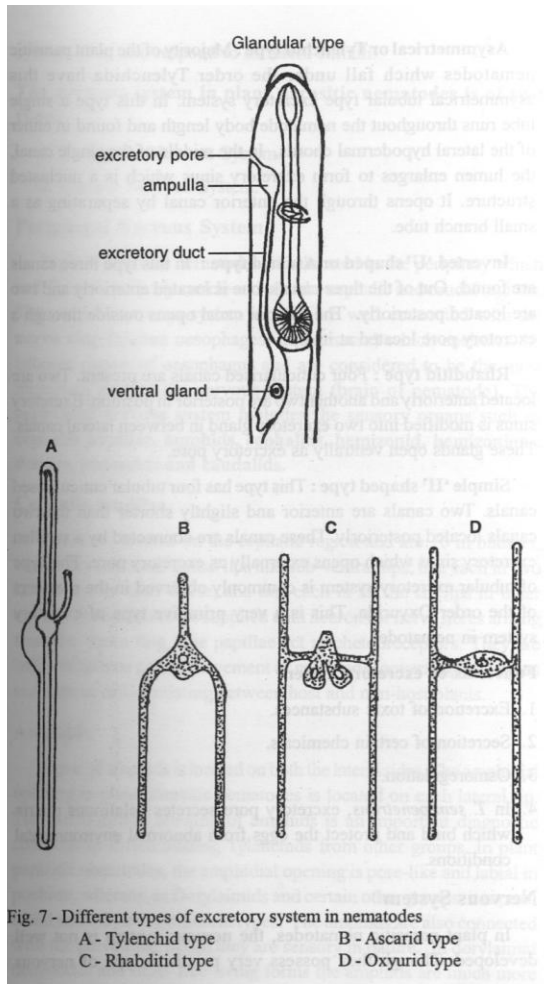


Fig. 7 - Different types of excretory system in nematodes

A - Tylenchid type B - Ascarid type
C - Rhabditid type D - Oxyurid type

Tubular type

The tubular type of excretory system consists of four – cuticularised canals. Two are anterior and another two are posterior canals. There is a pouch like structure in the middle which connects both the lateral canals. It is known as excretory pore. There are four types in tubular system.

1. Asymmetrical or Tylenchid type

2. Inverted 'U' shaped or Ascarid type
3. Rhabditid type
4. Simple 'H' shaped or Oxyurid type.

Asymmetrictrical or Tylenchid type : Majority of the plant parasitic nematodes which fall under the order *Tylenchida* have this asymmetrical tubular type excretory system. In this type a single tube runs throughout the nematode body length and found in either of the lateral hypodermal chords. In the middle of the single canal, the lumen enlarges to form excretory sinus which is a nucleated structure. It opens through the anterior canal by separating as a small branch tube.

Inverted 'U' shaped or Ascarid type

In this type three canals are found. Out of the three canals, one is located anteriorly and two are located posteriorly. The anterior canal opens outside through an excretory pore located at its tip.

Rhabditid type

Four cuticularised canals are present. Two are located anteriorly and another two are posterior in position. Excretory sinus is modified into two excretory glands in between lateral canals. These glands open ventrally as excretory pore.

Simple 'H' shaped type

This type has four tubular cuticularised canals. Two canals are anterior and slightly shorter than the two canals located posteriorly. These canals are connected by a swollen excretory sinus which opens externally as excretory pore. This type of tubular excretory system is commonly observed in the members of the order Oxyurida. This is a very primitive type of excretory system in nematodes.

Functions of excretory system

1. Excretion of toxic substances. 2. Secretion of certain chemicals. 3. Osmoregulation 4. In *T. semipenetrans* excretory pore secretes gelatinous matrix which binds and protects the eggs from abnormal environmental conditions.

The Nervous System

In plant parasitic nematodes, the nervous system is not well developed. Though they possess very primitive type of nervous system, they also respond to different stimuli.

The nervous system in plant parasitic nematodes is of two types

1. Peripheral Nervous System
2. Central Nervous System

Peripheral Nervous System

Located in the periphery which mainly includes body cuticle and also the cephalic and caudal regions. The parts of nervous system located are well connected with the nerve ring (circum oesophageal commissure) which encircles the thymus region of esophagus and are considered to be the most important part of the nervous system (brain of nematode). The peripheral nervous system includes the sensory organs such as cephalic papillae; amphids, cephalids, hemizonid, hemizonions, deirids, phasmids and caudalids.

Cephalic papillae

Located on the cephalic region and are 16 in number, two each in – two sub – dorsal lips and sub – ventral lips; one each in two lateral lips in outer circle ; and one each in all the six lips in inner circle. These papillae are supplied with neurons or nerve fibres arising from the nerve ring. The papillae act as chemoreceptors. They are believed to take part in movement of nematode governing directions and also in differentiating between host and non – host plants

Amphids

A pair of amphids is located on both the lateral sides. The amphidial opening is pore – like and labial in position. The amphids are also connected with the nerve ring, hence they are sensory in nature.

Cephalids

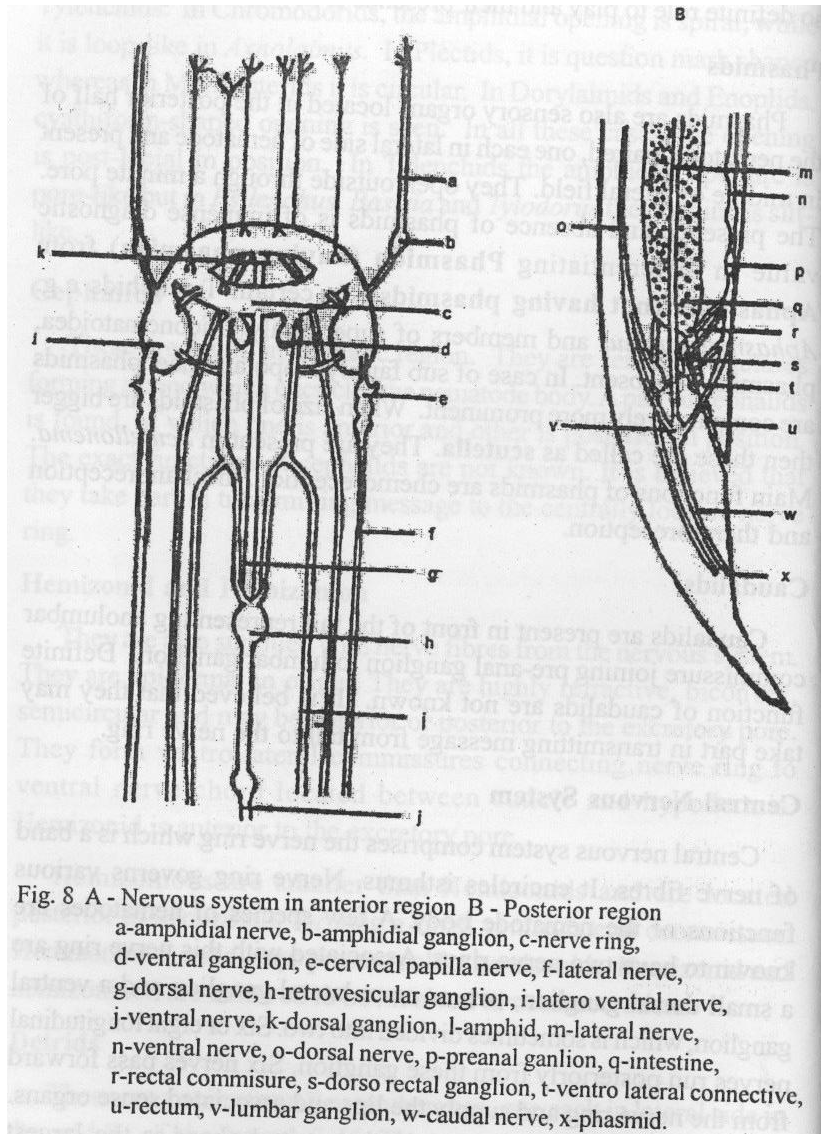
These are found in cephalic region. A pair of cephalids is found, of which one is anterior and another is posterior in position. The exact functions of cephalids are not known. It is believed that they take part in transmitting messages to the centrally located nerve ring.

Hemizonid and Hemizonion

They are highly refractive, biconvex, semi – circular and may be anterior or posterior to the excretory pore. Hemizonids is anterior to the excretory pore. Hemizonions are smaller than Hemizonids and are located posterior to hemizonid. Hemizonoid and hemizonion are believed to be involved in neurosecretion.

Deirids

These are a pair of small protuberance, one each in lateral side in the middle of the lateral field. These are located in the region of oesophagus at the region of excretory pore. They are also sensory organs.



Phasmids

Phasmids are also sensory organs located in the posterior half of the nematode paired one each in lateral side of nematode and present in middle of lateral field. They open outside through a minute pore. The presence and absence of phasmids is of immense diagnostic value having phasmids. When size of phasmids are bigger then these are called as scutella. They are present

in *Scutellonema*. Main functions of phasmids are chemoreception, mechanoreception and thermoreception.

Caudalids

Caudalids are present in front of the tail. It is believed that they may take part in transmitting messages from tail to the nerve ring.

Central Nervous System

Central nervous system comprises the nerve ring. Associated with this nerve ring are a small dorsal ganglion. Six or eight longitudinal nerves run posteriorly from these ganglion. Six nerves pass forward from the nerve ring and supply the lips and associated sense organs.

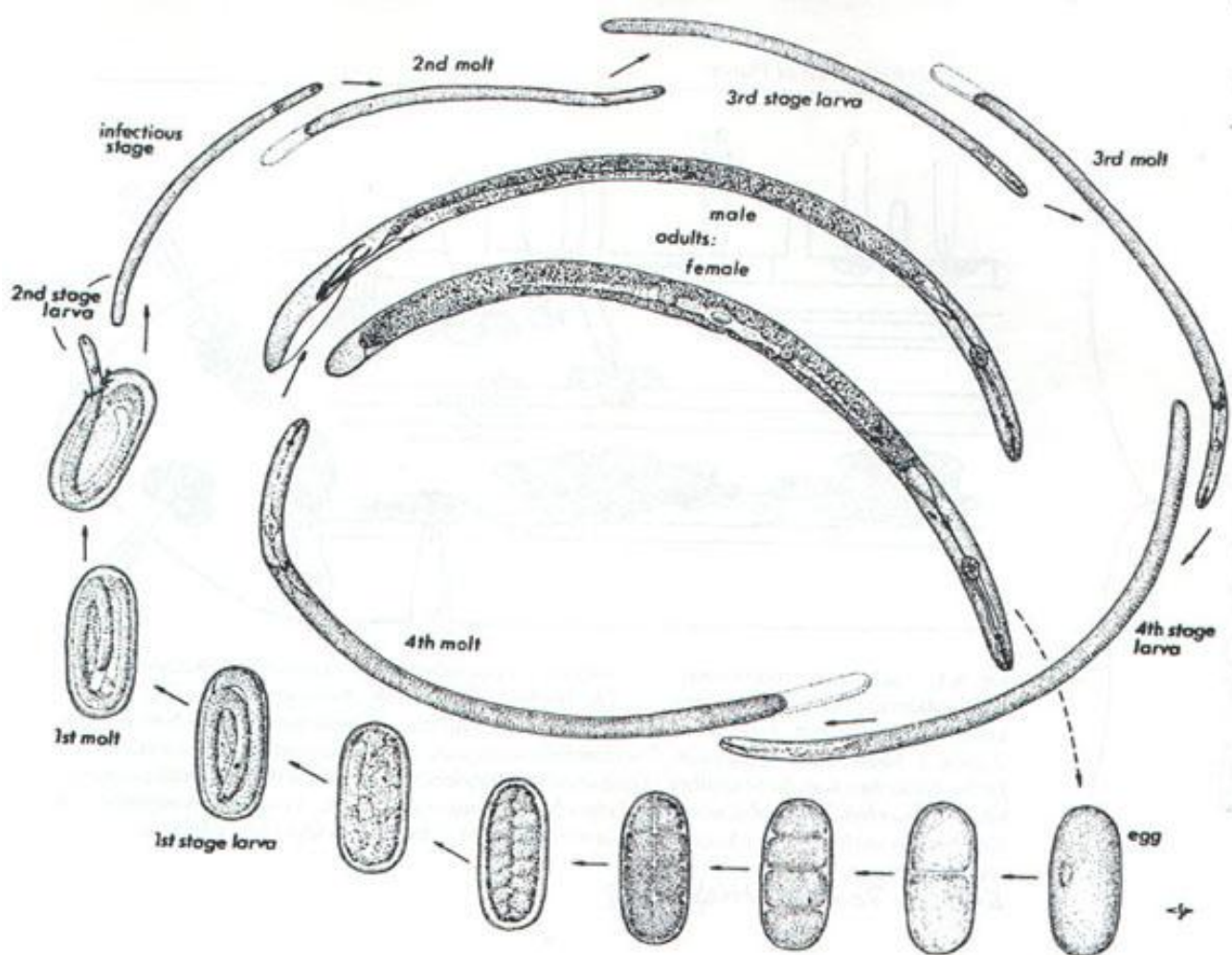
A pair of nerves runs forward from the lateral ganglion to the amphids at the anterior end of the nematode. The dorsal nerve is said to be chiefly motor and lateral nerves mainly sensory in function. The sub – median and the ventral nerves are partly motor and partly sensory. There is a system of three nerves in the pharynx, one in each sector, which are connected with one another by commissures and also with nerve ring. Nematodes are unique in that the muscle cells of the body are innervated by processes which pass from the muscle. It is claimed that the nerve muscle junction is similar to those found in other animals.(Fig.8)

Transmission along nerves

Nothing is known about the processes involved in the conduction of an impulse along nerves in nematodes. In *Ascaris* it is known that the pseudocoelomic fluid contains more sodium as compared with potassium ions. It is thus possible to speculate that the nerve axons in this species function as that of other animals in which the action current arises from an influx of external sodium ions.

Acetylcholine is apparently involved in nervous transmission in nematodes. Acetylcholine like substances have been detected in *Ascaris*. The head region of *Ascaris* was found to contain fifteen times more acetylcholine than that of the remaining body. Cholinesterase activity is observed to be more in the nerve ring, amphids, phasmids and other sense organs in nematodes.

Lecture 07 - Biology of Plant Parasitic Nematodes



The life cycle of nematode has six stages. The egg state, J1 or first stage larva, J2 or second stage larva, J3 or third stage larva, J4 or 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 vary in different species of nematodes.

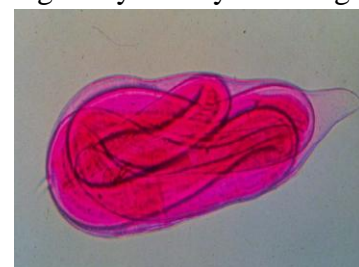
Embryonic Development

The adult female lays the eggs. The egg starts dividing by cleavage of their protoplasms 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 blastomere P1 dividing transversely by P2 and S2. At last these cells get arranged in a rhomboidal shape. The transverse and longitudinal mitotic divisions 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 endodermal tissue produced from the products of cell E1 and P1 divides into P5 and S5. The descendants of S5 give covers to the gonads and their ducts, while the products of P5, G1, G2 and their descendants proliferate into germ cells only.

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 embryo and are taken within the embryo by process of gastrulation. In further development the dorso ventrally



the

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 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



stage

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

Pratylenchus, *Paratylenchus*, *Nacobbus* and *Meloidogyne*. 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 with in 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 gelatinous 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.

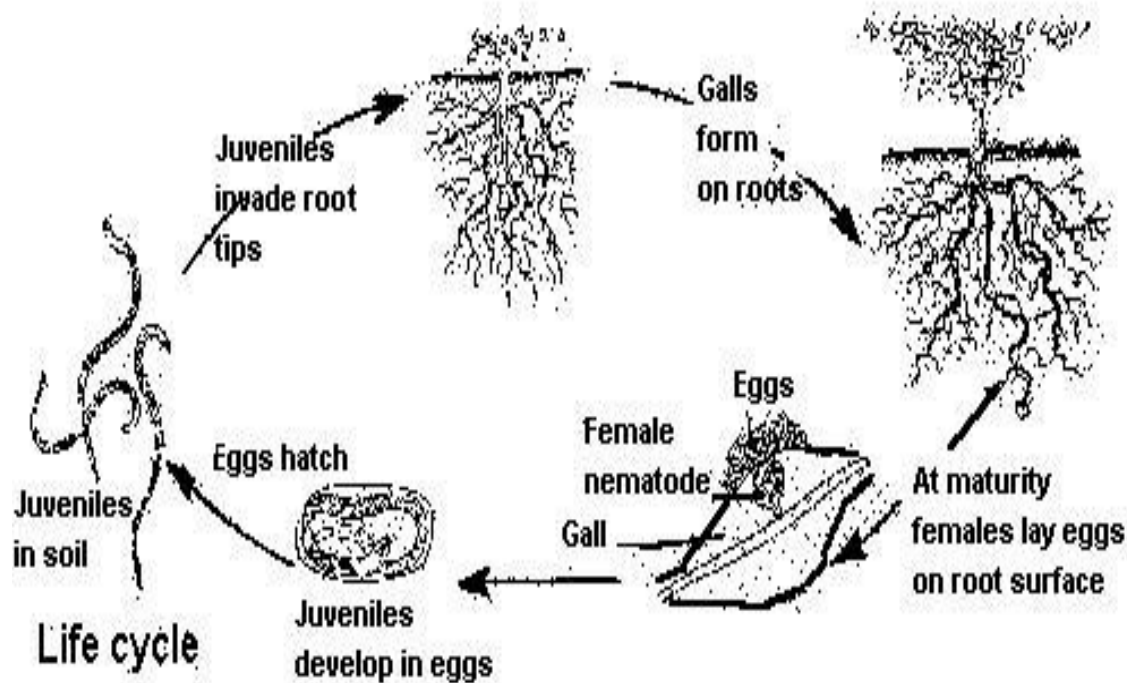
The life cycle starts from the egg usually in the one-celled stage deposited by the female. Development of the embryo starts within hour of deposition, resulting in two, four, eight cells, etc., The embryo and the first stage larva move with in the egg but not very active.

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 mitochondria 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 form 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 (*Globodera* spp. and *Heterodera* spp.)

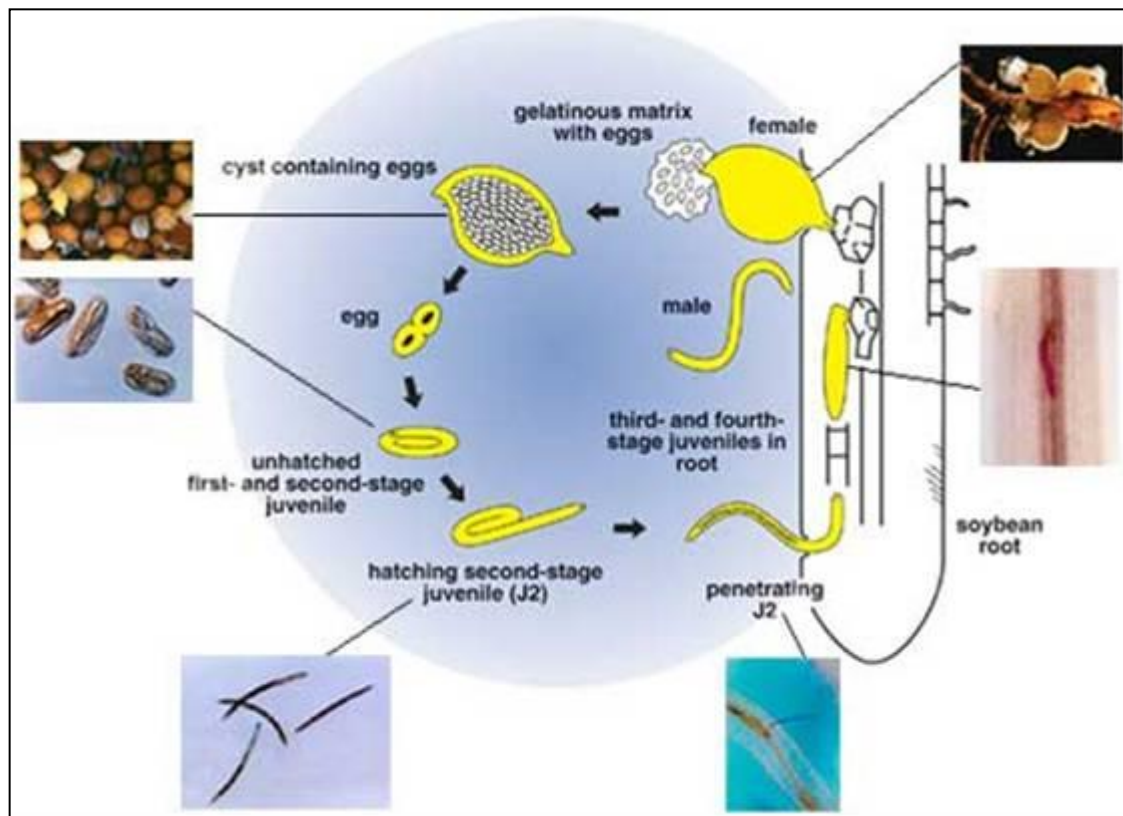
Second stages larvae usually penetrate the root just behind the growing point. These larvae grow rapidly and three moults occur in the host. In about 5 –6 weeks after penetration, the white cysts are clearly visible which protrude from the root surface. These young cysts are packed with eggs and upon 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.

Although cyst nematodes induce giant cell formation, gall formation is not distinct. Each syncytium is associated with only one nematode in the case of cyst nematodes unlike the root – knot nematodes where one or more nematodes are associated with a syncytium. Nuclei is enlarged in the syncytium caused by the root – knot nematode but in cyst nematode nuclei is relatively small. The syncytia are bound by the vascular elements especially xylem which develops specific wall in growth. There is enlarged nucleoli and irregular nuclei. Abundant mitochondria, golgi bodies, protoplasts and dense endoplasmic reticulum are also found in the syncytia.

Life cycle of cyst nematode- *Heterodera*



Citrus nematode (*Tylenchulus semipenetrans*)

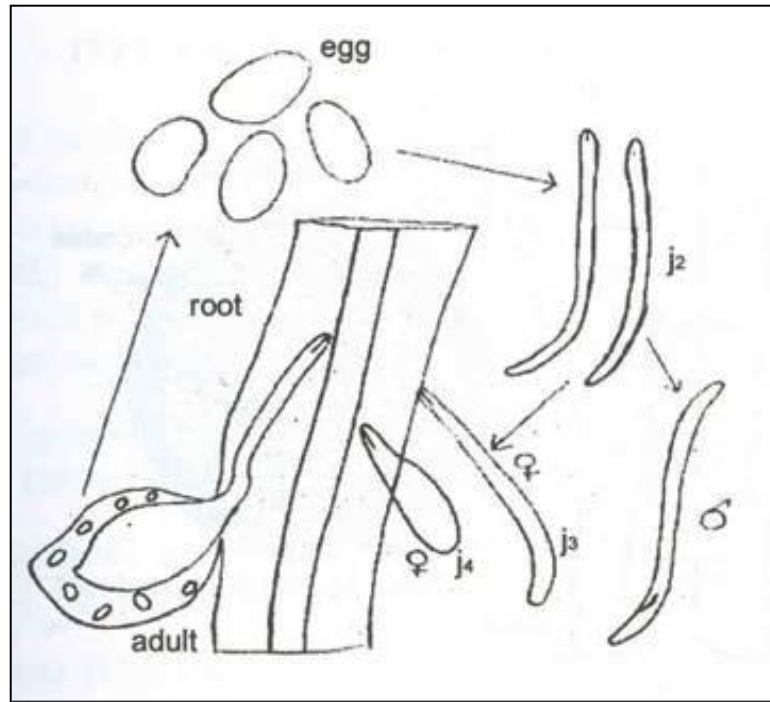
Citrus nematode is a sedentary semi – endoparasite of the Citrus root. Females are most commonly found on thick and stunted rootlets to which a layer of soil particle is clinging. These particles are held in place by a gelatinous mucus secreted by the female. The mucus and adhering soil particles protect 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 results in necrosis. 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



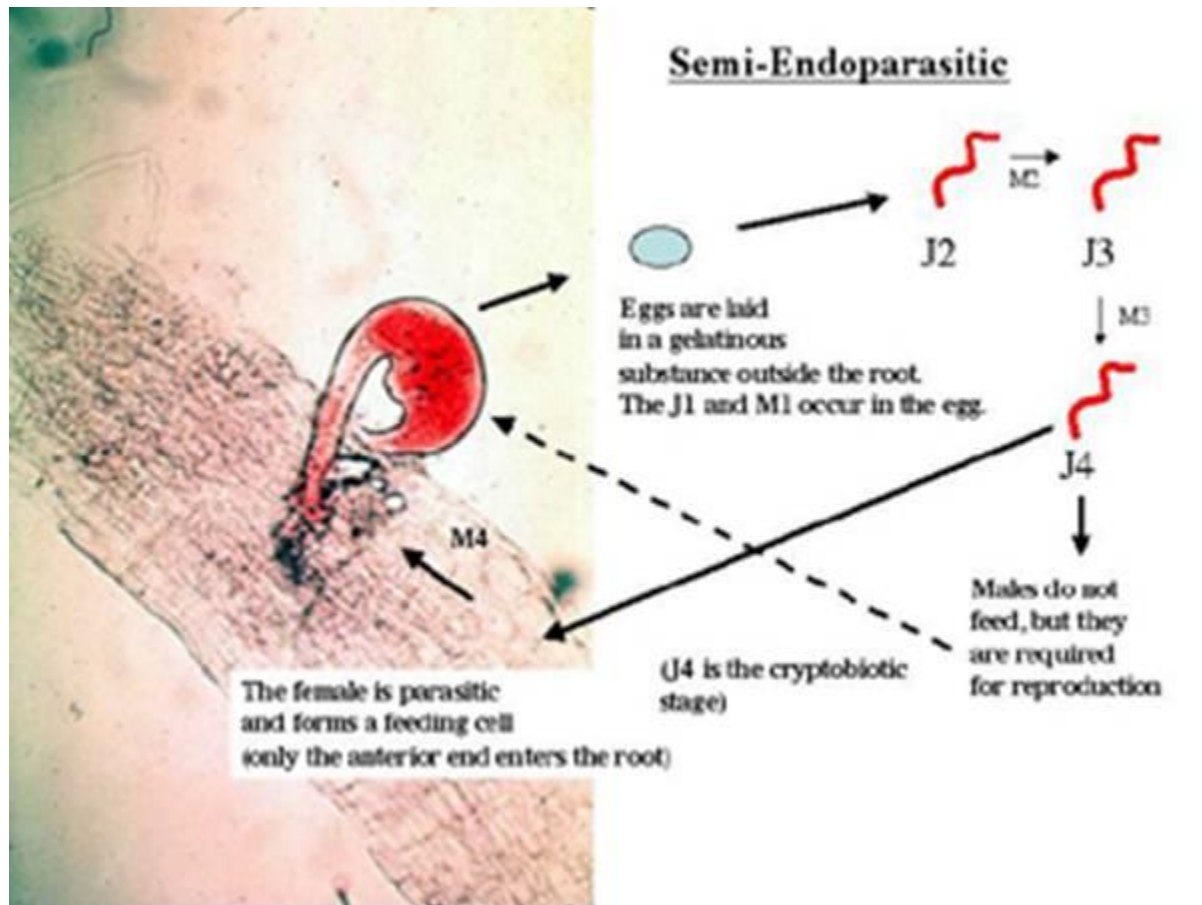
Reniform Nematode (*Rotylenchulus reniformis*)

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

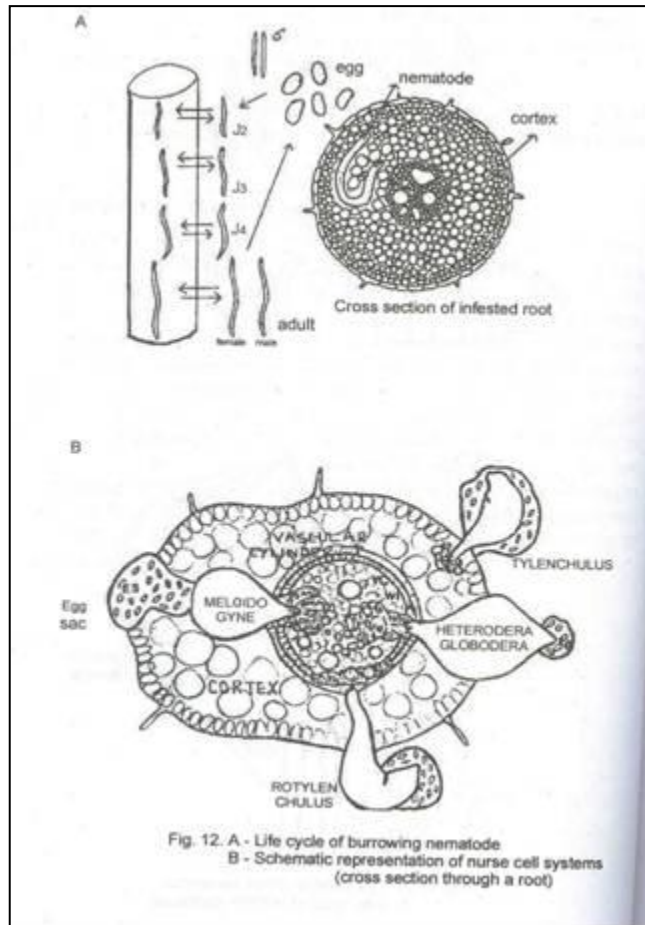
The species has an unusual life cycle. Although a newly hatched second stage larva have well developed stylet, they do not feed. They soon pass through three super imposed moults to become young females and adult males. The young females force their way through cells of root cortex until they partially 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 seen 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 semi-endoparasite 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 of roots and the damage increases when the nematode moves towards the phloem.

Life cycle of Reniform Nematode



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.



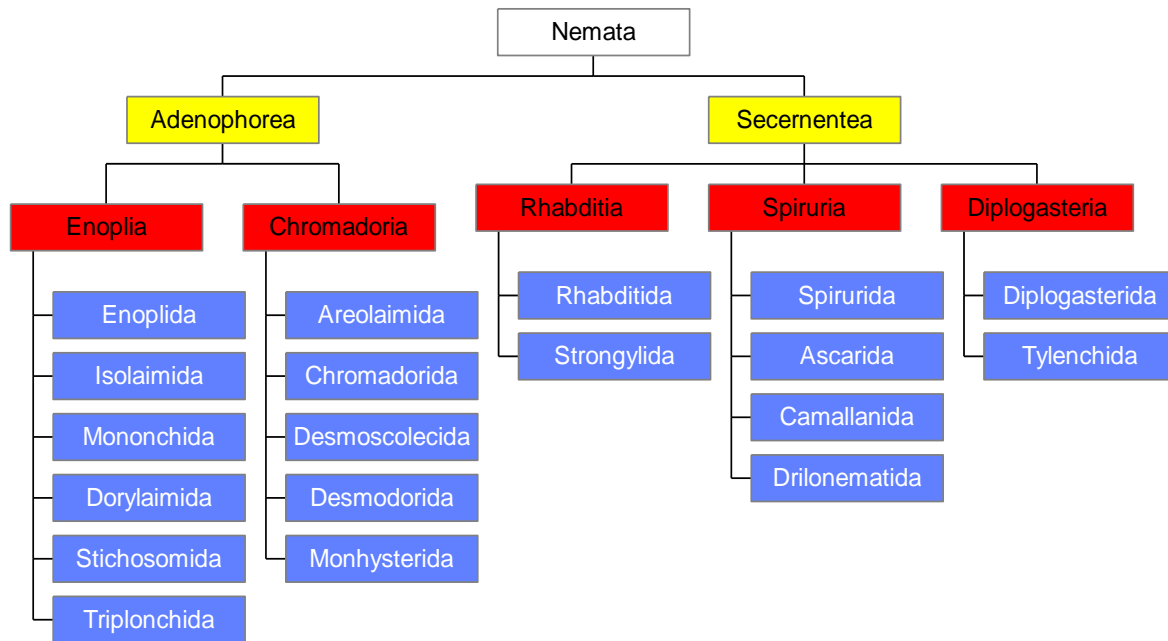
Life cycle of burrowing nematode



Lecture 08 - Taxonomy of Plant Parasitic Nematodes

Nematodes are placed in the group invertebrata, Kingdom Animalia. Under separate phylum, Nemata / Nematoda, which consist of two classes, Secernentea and Adenophorea.

Classification of Nematodes



Diagnostic characters of class Secernentea and Adenophorea

Secernentea (Phasmida)

Amphidial opening is on the head near the lip region.

Lateral canals open into the excretory duct

Oesophagus is divided into procorpus, median bulb, isthmus and basal bulb.

Male tail with bursa (Caudal alae)

Glands are absent Phasmids are present

Adenophoreea (Aphasmda)

Amphids open behind the head i.e. post labial

Lateral canals and excretory duct end in a cell

Oesophagus is cylindrical with an enlarges glandular base

Male tail lacks bursa but possess genital paillae.

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 sclerotized 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	<i>Tylenchoidea</i>	<i>Criconematoidea</i>
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 valve, 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; post uterine 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

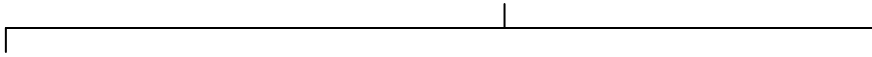
Difference between *Tylenchina* and *Aphelenchina*

Character	<i>Tylenchina</i>	<i>Aphelenchina</i>
Lip	Varying in shape	Set- off
Annules	Faint to strong annules	Faint annules
Stylet	Well developed; one dorsal and two sub ventral knobs	Weakly 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 procorpus	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 gubernaculum	Rose thorn 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 hollow 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, gubernaculum rare. The order is divided into three sub orders namely *Dorylaimina*, *Diptherophodrina* and *Nygolaimina*. The former two suborders 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.

Lecture 09 - Ecological classification of plant parasitic nematodes

I. Above ground feeders

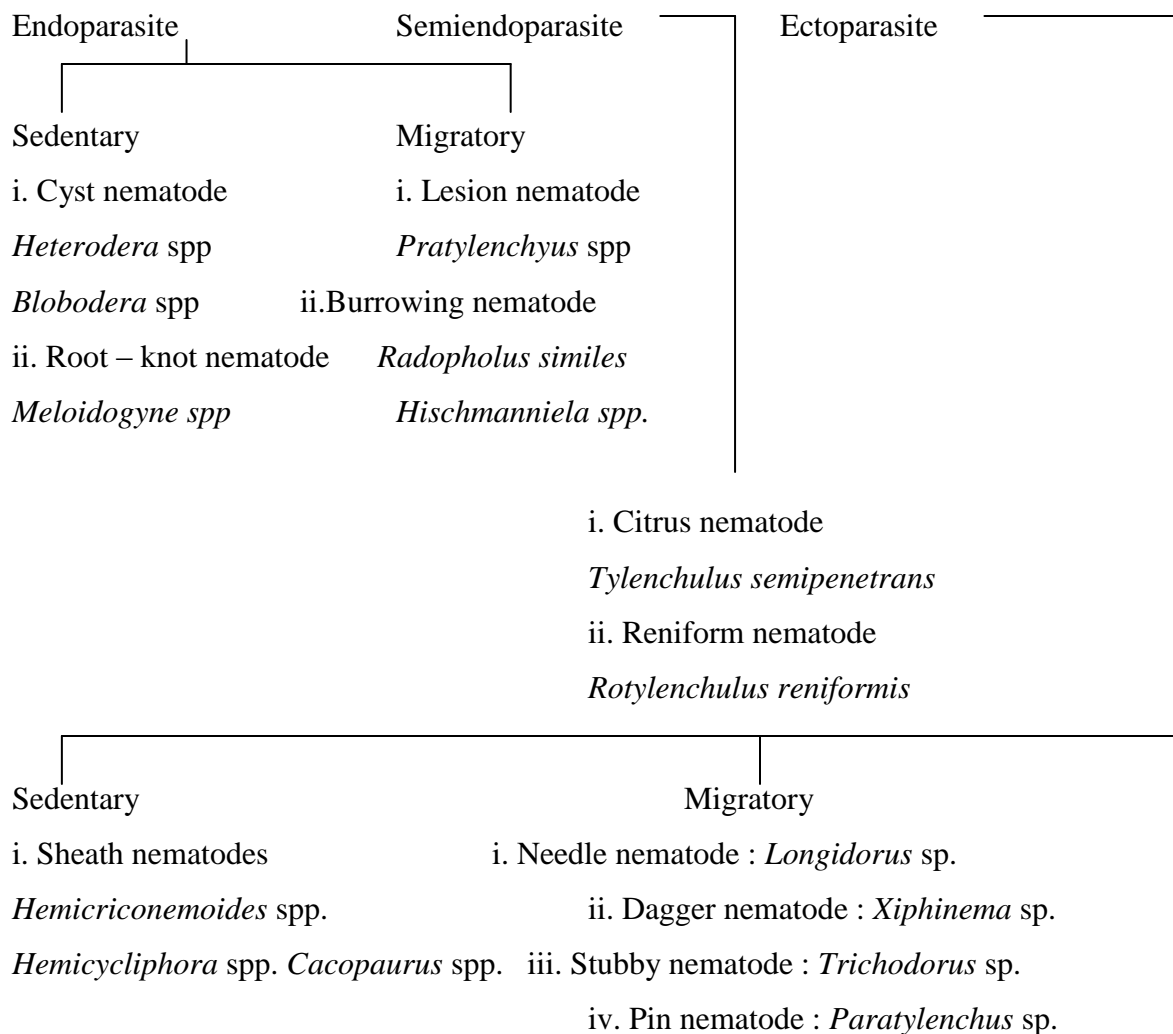
a. Feeding on flower buds, leaves and bulbs.

- i. Seed gall nematode : *Anguina tritici*
- ii. Leaf and bud nematode : *Aphelenchoides*
- iii. Stem and bulb nematode : *Ditylenchus*

b. Feeding on tree trunk

- i. Red ring nematode : *Rhadinaphelenchus cocophilus*
- ii. Pine wilt nematode : *Bursaphelenchus xylophilus*

II. Below ground feeders

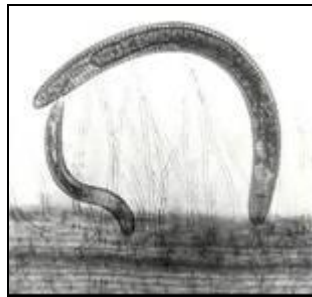


According to feeding habits, the nematodes can be divide into

1. Ectoparasitic nematodes,
2. Semi endoparasitic nematodes and
3. Endoparasitic nematodes

1. Ectoparasitic nematodes : These nematode live freely in the soil and move closely or on the root surface, feed intermittently on the epidermis and root hairs near the root tip.

A. Migratory ectoparasite : (e.g.) *Criconemoides* spp. *Paratylenchus* spp., and *Trichodorus* sp., etc., These nematodes spend their entire life cycle free in the soil . When the roots are disturbed they detach themselves.



B. Sedentary ectoparasites : (eg.) *Hemicycliphora arenaria* and *Cacopaurus pestis* etc., In this type of parasitism the attachment of nematode to the root system is permanent but for this, it is similar to the previous one.

2. Semi – endoparasitic nematodes : (e.g.) *Rotylenchulus reniformis* and *Tylenchulus semipenetrans*. The anterior part of the nematode, head and neck being permanently fixed in the cortex and the posterior part extents free into the soil.



Eg. *Tylenchulus semipenetrans*

3. Endoparasitic nematodes : The entire nematode is found inside the root and the major portion of nematode body found inside the plant tissue.

a. Migratory endoparasite : (eg.) *Hirschmanniella* spp., *Pratylenchus* spp and *Radopholus similis* etc., These nematodes move in the cortical parenchyma of host root. While migrating they feed on cells, multiply and cause necrotic lesions.



Eg. *Pratylenchus*

b. Sedentary endoparasite : (eg.) *Heterodera* spp and *Meloidogyne* spp. The second stage larvae penetrate the root lets and become sedentary through out the life cycle, inside the root cortex.



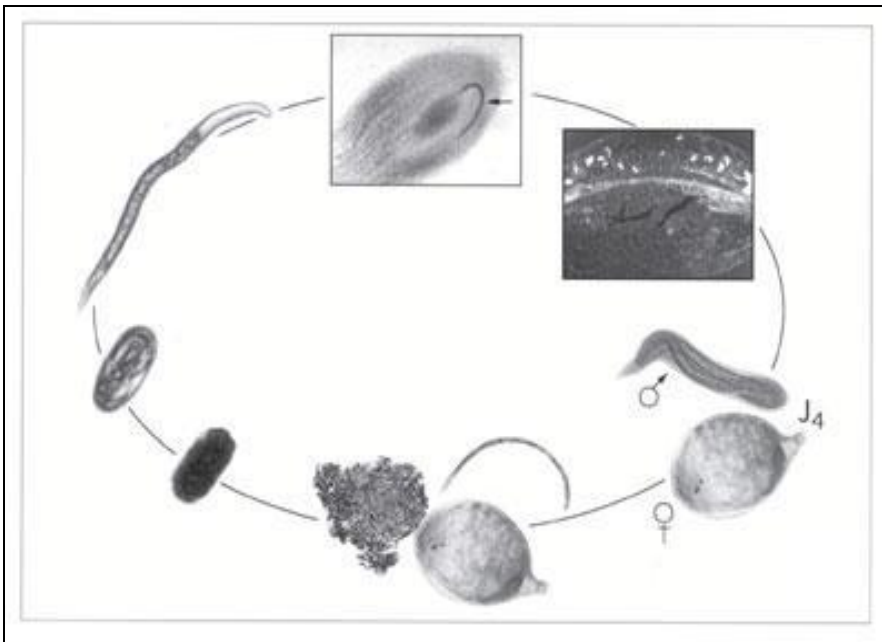
Eg. *Meloidogyne*

Lecture 10 - Identification of economically important plant nematodes

Root knot nematode (*Meloidogyne incognita*, *M. javanica*, *M. arenaria*, *M. halpa*)

Parasitism and habitat

Females as well as third and fourth stage juvenile are sedentary endoparasites on many plants. Males and second stage juvenile are migratory and can be located in soil also. The root-knot nematodes are widely distributed in nurseries and uplands or in well-drained soils all over India.



Main Morphological characters

- | | |
|-----------------------|---|
| Body | : Elongate juvenile (0.5mm) and males (1.0- 2.0mm); typically saccate, spheroid with a distinct neck in females (0.8mm long and 0.5mm wide) |
| Stylet | : Strong and rounded knobs in males; in females more slender than males or juveniles but with strong basal knobs. |
| Oesophagous | : With very large median bulb followed by a short isthmus |
| Excretory pore | : Often seen with part of excretory tube in the area between posterior part of stylet knobs and opposite to median bulb. |

- Vulva and annus** : In females, typically opposite to neck and surrounded by a pattern of fine lines resembling human finger prints. (These are used for identification of species in this genus)
- Spicules** : Very near the terminus of males; bursa absent
- Resembling genus** : *Heterodera*

Root-Lesion Nematode (*Pratylenchus spp*)

Parasitism and habitat

Migratory endo parasite, feeding in root cortex of many plants. All stages are found in root or soil. Males common in many species, unknown or less common in others.

Main Morphological characters

- Body length** : 0.4-0.8mm
- Lip region** : slightly set off from body
- Stylet** : Typically short, strong with massive knobs
- Ovary** : Typically one and posterior ovary rudimentary to form a post uterine sac.
- Vulva** : Typically on the posterior fourth of the body (75-80%)
- Tail** : Nearly round to pointed and in the case of male, tail has bursa
- Resembling genus** : *Radopholus* (Two ovaries present instead of one and great morphological difference between male and females observed in *Radopholus*).

Spiral nematode (*Helicotylenchus multicinctus*)

Parasitism and habitat

Endoparasitic and ectoparasitic on many plants; all are found in soil and root.

Main Morphological characters



stages

Body	: Typically arcuate	or
spiral in shape	when dead or relaxed. Length from 0.5-1.2mm	
Stylet	: Moderately long	
Dorsal oesophageal gland orifice	: Typically located more than one – half	stylet
	length posterior to stylet knobs.	
Ovaries	: Two	
Vulva	: posterior to middle of body (60-70%)	
Tail	: In females, round to nearly pointed; often with short projection on ventral side and in males the tail is short with bursa.	
Resembling genus	: <i>Hoplolaimus</i> , <i>Scutellonema</i> and <i>Rotylenchus</i>	

Cyst nematode (*Heterodera*)

Parasitism and habitat

Parasitic on many plants mostly in temperate zone (notably potato, sugarbeet, oat and other grains, clover, soyabean and various crucifers). Adult female with neck embedded in plant roots and the body exposed. Juveniles, males and cysts found in soil.

Main Morphological characters

Body	: Slender in males (1.0-2.0mm) and juveniles (0.3-0.6mm); in females, typically swollen lemon shaped (0.5-0.8mm in length), white or yellow in colour. Cysts dark brown, lemon shaped (0.8mm long and 0.5mm wide) or nearly the same shape as that of <i>Meloidogyne</i> female
Stylet	: Short in males with rounded basal knobs and in juveniles, more than 0.02mm long.
Oesophagous	: With well developed median bulb and lobe extending back and overlapping the intestine
Spicules	: Near the posterior end of females

Resembling genus : *Meloidogyne* (stylet of juveniles only 0.01-0.014mm long; adult females fully embedded in roots in case of *Meloidogyne*)

Potato cyst Nematode : (*Globodera*): The adult females are globular in shape and hence, the genus is named as *Globodera*.

Lance nematode (*Hoplolaimus spp.*)

Parasitism and habitat

Ectoparasitic and endoparasitic on many plants. All stages are found in soil or root.

Main Morphological characters

Body: Length of males and females ranges from 1.0-2.0 mm

Lip: Typically set off with annules divided into small segments (visible under oil immersion)

Stylet: Strongly developed with typically elongated and closely arranged basal knobs.

Oesophagus: With median bulb; oesophageal glands with short lobe overlapping dorsally to the anterior end of intestine.

Ovaries: Two

Vulva: Centrally located

Bursa: Present

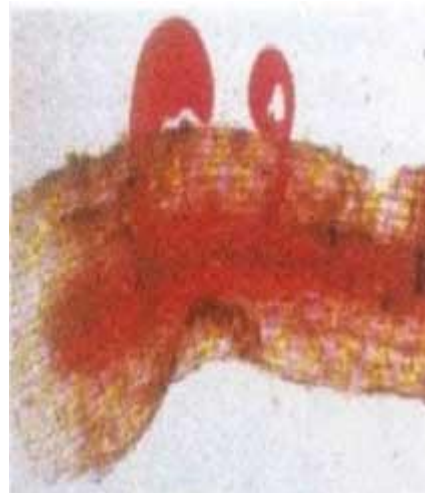
Resembling Genus: *Scutellonema* and *Rotylenchus* (Stylet knobs broader and male tail shorter in these two genera)

Helicotylenchus (Dorsal gland orifice located at least one half of the stylet length posterior to stylet)

Reniform nematode (*Rotylenchulus*)

Parasitism and habitat

Parasitic on many plants. Mature females with only their neck embedded in roots as semiendoparasites



(difficult to see because covered with egg masses and soil particles); juveniles, males and immature females are found in soil.

Main Morphological characters

Body: Slender and small in males (0.3-0.50mm), immature females (0.3-0.45mm); typically reniform (kidney shaped) in adult females (0.6-0.9mm)

Oesophagus: Dorsal oesophageal gland typically open about one stylet length posterior to stylet knobs.

Resembling genus: *Tylenchulus*

Stubby Root nematode (*Trichodorus*)

Parasitism and habitat

Ectoparasitic on many plants. All stages are found in soil.

Main Morphological characters

Body: 0.4-1.5 mm

Stylet: Solid, typically curved dorsally, without knobs

Oesophagus: With typical pyriform basal bulb

Ovaries: Usually Two

Vulva: Near middle of the body

Tail: Bluntly rounded in both female and male

Anus: Near posterior end

Stunt Nematode (*Tylenchorhynchus spp.*)

Parasitism and habitat

Ectoparasitic on many plants and rarely endoparasitic. All stages are found in the rhizosphere.

Main Morphological characters

Body : 0.6-1.4 mm in length

Lip : Typically continuous with body or slightly set off

Stylet : Usually strong with large basal knobs

Oesophagus : Typically with procarpus, meta carpus and well developed posterior bulb without overlapping the intestine

Ovaries	: Two
Vulva	: Almost near the middle of the body
Tail	: In female it is tapering to rounded, usually one or more times as long as anal body diameter
Resembling genera	: Tylenchus (In this genera only one ovary is present and vulva located in the posterior region and Psilenchus (the tail is slender and longer)

Lecture 11 - Symptoms Caused by Nematodes

Most of the plant parasitic nematodes affect the root portion of plants except *Anguina* spp., *Aphelenchus* spp., *Aphelenchoides* spp., *Ditylenchus* spp., *Rhadinaphelenchus cocophilus* and *Bursaphelenchus xylophilus*. Nematodes suck the sap of the plants with the help of stylet and causes leaf discolouration, stunted growth, reduced leaf size and fruits and lesions on roots, galls, reduced root system and finally wilting.

Symptoms of nematode diseases can be classified as

- I. Symptoms produced by above ground feeding nematodes
- II. Symptoms produced by below ground feeding nematodes

I. Symptoms produced by above ground feeding nematodes

Leaf discolouration: The leaf tip become white in rice due to rice white tip nematode *Aphelenchoides besseyi*, yellowing of leaves in Chrysanthemum due to Chrysanthemum foliar nematodes, *A. ritzemabosi*.



White tip symptom in rice



Leaf discoloration in Chrysanthemum

Dead or devitalized buds: In case of straw berry plants infected with *A. fragariae*, the nematodes affect the growing point and kill the plants and result in blind plant.

Seed galls: In wheat, *Anguina tritici* larva enter into the flower primordium and develops into a gall. The nematodes can survive for longer period (even upto 28 years) inside the cockled wheat grain.

Twisting of leaves and stem: In onion, the basal leaves become twisted when infested with *D. angustus*.

Crinkled or distorted stem and foliage: The wheat seed gall nematode. *A. tritici* infests the growing point as a result distortions in stem and leaves take place.

Necrosis and discoloration:

The red ring disease on coconut caused by *Rahadinaphelenchus cocophilus*. Due to the infestation, red coloured circular area appears in the trunk of the infested palm.



Red colored ring on coconut trunk

Lesions on leaves and stem: Small yellowish spots are produced on onion stem and leaves due *D.dipsaci*, and the leaf lesion caused by *A. ritzemabosi* on Chrysanthemum.

II. Symptoms produced by below ground feeding nematodes

The nematodes infest and feed on the root portion and exhibit symptoms on below ground plant parts as well as on the above ground plants parts and they are classified as

- a. Above ground symptoms
- b. Below ground symptoms

a. Above ground symptoms

Stunting: Reduced plant growth, and the plants can not able to withstand adverse conditions. Patches of stunted plants appears in the field. (eg.) in potato due to *Globodera rostochiensis*, in gingelly, due to *Heterodera cajani* and in wheat by *Heterodera avenae*.

Discolouration of foliage: Patchy yellow appearance in coffee due to *Pratylenchus coffeae*, *G. rostochiensis* infested potato plants show light green foliage. *Tylenchulus semipenetrans* induce fine mottling on the leaves of orange and lemon trees.

Wilting: Day wilting due to *Meloidogyne* spp. i.e. In hot weather the root – knot infested plants tend to droop or wilt even in the presence of enough moisture in the soil. Severe damage to the root system due to nematode infestation leads to day wilting of plants.

B. Below ground symptoms

Root galls or knots: The characteristic root galls are produced by root – knot nematode, *Meloidogyne* spp. false root galls are produced by *Nacobbus batatiformis* on sugar beet and tomato. Small galls are produced by *Hemicycliophora arenaria* on lemon roots. *Ditylenchus*

radicicola cause root galls on wheat and oats. *Xiphinema diversicaudatum* cause galls on rose roots.



Root galls

Root lesion: The penetration and movement of nematodes in the root causes typical root lesions eg. Necrotic lesions induced by *Pratylenchus* spp on crossandra; the burrowing nematode, *Radopholus similis* in banana. Similarly *Pratylenchus coffeae* and *Helicotylenchus multicinctus* cause reddish brown lesion on banana root and corm. The rice root nematode also cause brown lesions on rice root.



Root lesion

Reduced root system: Due to nematode feeding the root tip growth is arrested and the root produce branches. This may be of various kinds such as coarse root, stubby root and curly tip.



Coarse root: *Paratrichodorus* spp. infestations arrest the growth of lateral roots, and leads to a open root system with only main roots without lateral roots.

Stubby roots : The lateral roots produce excessive rootless (eg.*P.christei*)



Stubby roots

Curly tip: In the injury caused by *Xiphinema* spp. the nematode retard the elongation of roots and cause curling of roots known as “Fish hook” symptom.

Root proliferation: Increase in the root growth or excessive branching due to nematode infestation. The infested plant root produced excessive root hair at the point of nematode infestation.

(eg.) *Trichodorus christei*, *Nacobbus* spp., *Heterodera* spp. *Meloidogyne hapla* and *Pratylenchus* spp. etc.



Root – rot: The nematodes feeds on the fleshly structure and resulting in rotting of tissues (eg.) Yam nematode *Scutellonema bradys* and in potato *Ditylenchus destructor* cause root rot.



Root surface necrosis: The severe injury caused by *T. semipenetrans* on citrus leads to complete decortications of roots and results in root necrosis.

Cluster of sprouts on tubers: On the tubers, clusters of short and swollen sprouts are formed due to *D. dipsaci* infestation in many tuber plants.

Lecture 12 - Interaction of Nematodes with Micro-Organisms

Plant parasitic nematodes favour the establishment of secondary pathogens viz., fungi, bacteria, virus etc. The nematodes alter the host in such a way that the host tissue becomes suitable for colonization by the secondary pathogens. Even though the nematodes themselves are capable of causing considerable damage to the crops, their association with other organisms aggravate the disease. The nematodes cause mechanical wound which favours the entry of micro organisms. In some cases, the association of nematode and pathogen breaks the disease resistance in resistant cultivators of crop plants.

Nematode – fungus Interaction

Nematode – fungus interaction was first observed by Atkinson (1892) in cotton. *Fusarium* wilt was more severe in the presence of *Meloidogyne* spp. Since then the nematode – fungus interaction had received considerable attention on important crop like banana, cotton, cowpea, brinjal, tobacco and tomato. Some examples of nematode – fungus interaction are given in the following table.

Crop	Name of the disease	Nematode	Fungus	Role of nematode
Cotton	Damping off	<i>Meloidogyne incognita</i>	<i>Rhizoctonia solani</i>	Assists
		<i>acrita</i>	<i>Pythium</i>	
		<i>M. incognita acrita</i>		<i>Pythium</i>
	Vascular wilt	<i>debaryanum</i>		
		<i>M. incognita acrita</i>	<i>Fusarium</i>	Assists
			<i>Osyporum</i>	
			<i>F. vasingectum</i>	

<i>Rotylenchulus</i>	<i>F. oxysporium</i> f.sp	Assists
<i>Reniformis</i>	<i>Vasinfestum</i>	
<i>Belonolaimus gracilis</i>	<i>F.oxysporum</i> f.sp	

<i>B. longicaudatus</i>		Assists
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Blank shank (vascular wilt)	<i>M. incognita</i> <i>acrita</i>	<i>Pytophthora</i> <i>Parasitica</i> var <i>nicotianae</i>	Assists
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Nematode – bacterium interactions

Nematode – bacterium interactions are comparatively fewer than the nematode – fungal interactions. Some examples of nematode – bacteria association are presented.

Crop	Name of the disease	Nematode	Bacterium	Role of nematode
Wheat	Tundu	<i>A. tritici</i>	<i>C. tritici</i>	Essential Assists
	Vascular wilt	<i>M. incognita</i>	<i>Pseudomonas solanacearum</i>	

Nematode – Virus Interaction

In nematode – virus complex, nematode serves as a vector. Numerous virus – nematode complexes have been identified after the pioneer work by Hewit, Raski and Goheen (1958) who found that *Xiphinema* index was the vector of grapevine fan leaf virus. *Xiphinema* spp., *Longidorus* spp., and *Paralongodorus* spp. transmit the ring spot viruses which are called “NEPO” derived from Nematode transmitted polyhedral shaped particles. *Teichodorus* spp. and *Paratrachodorus* spp. transmit the rattle viruses and called “NETU” derived from Nematode transmitted tubular shaped particles. All these nematodes have modified bottle shaped oesophagus with glands connected by short ducts directly to the lumen of the oesophagus. This

actually of nematodes. Certain examples of the viral diseases and the nematode vectors are given in the following table.

Viruses	Nematode
NEPO – viruses	
Arabis mosaic	<i>Xiphinema diversicaudatum</i>
Arabis mosaic, grapevine fan leaf Arabis mosaic, Grapevine	<i>X. paraelongatum</i>

NETU Viruses

Tabacco rattle	<i>Paratrichodorus pachydermus</i> <i>P.allius,P.nanus,</i> <i>P.porosus,P.teres</i> <i>Trichodorus christei</i> <i>T.Primitivus, T.cylindricus,</i> <i>T. hooperi</i>
Pea early browning	<i>P.amenous, P.pachydermus,</i> <i>P.teres,T.viruliferus.</i>

Nematodes acquire and transmit the virus by feeding, which requires as little as one day. Once acquired, the virus persists for longer period in the nematode body than in vitro. For example, the grapevine fan leaf virus will exist for as many as 60 days in *X. index*. Two types of mechanisms are observed in virus transmission (i) retention through close biological association between virus and vector as in *Xiphinema*; (ii) retention of virus mechanically as in *Logidours*. Virus is retained in the inner surface of the guiding sheath of *Longidorus*, cuticle lining of the lumen of *oesophagus* in *Trichodorus* and *Paratrichodrous*, cuticle lining of style t extension and *oesophagus* in *Xiphinema*. The virus particles are released into plant cell with the help of *oesophagus*.

Lecture 13 - Different methods of Nematode Control

Plant parasitic nematodes can be controlled by several methods. The nematode control aims to improve growth, quality and yield by keeping the nematode population below the economical threshold level. The control measures to be adopted should be profitable and cost effective. It is essential to calculate the cost benefit ratio before adopting control measures.

The nematode control methods are

1. Regulatory (Legal) control
2. Cultural control
3. Physical control
4. Biological control
5. Chemical control.

Regulatory control

Regulatory control of pests and diseases is the legal enforcement of measures to prevent them from spreading or having spread, from multiplying sufficiently to become intolerably troublesome. The principle involved in enacting quarantine is exclusion of nematodes from entering into an area which is not infested, in order to avoid spread of the nematode

Quarantine principles are traditionally employed to restrict the movement of infected plant materials and contaminated soil into a state or country. Many countries maintain elaborate organizations to intercept plant shipments containing nematodes and other pests. Diseased and contaminated plant material may be treated to kill the nematodes or their entry may be avoided. Quarantine also prevent the movement of infected plant and soil to move out to other nematodes free areas.

Plant Quarantine in India

The Destructive Insects and Pests Act, 1914 (DIP) was passed by the Government of India which restricts introduction of exotic pests and disease into the country from abroad.

The agricultural pests and disease acts of the various states prevent interstate spread of pests within the country. The rules permits the plant protection advisor to the government of India or any authorizes officer to undertake inspection and treatments.

Strict regulations have been made against *G. rostochiensis*, the potato cyst nematode and *Rhadinaphelenchus cocophilus*, the red ring nematode of coconut. Domestic quarantine

regulations have also been imposed to restrict the movement of potato both for seed and table purposes in order to prevent the spread of potato cyst nematode from Tamil Nadu to other states in India.

Cultural Control

Cultural nematode control methods are agronomical practices employed in order to minimize nematode problem in the crops.

Selection of healthy seed material

In plants, propagated by vegetative means we can eliminate nematodes by selecting the vegetative part from healthy plants. The golden nematode of potato, the burrowing, spiral and lesion nematodes of banana can be eliminated by selecting nematode free plant materials. The wheat seed gall nematode and rice white tip nematode can be controlled by using nematode free seeds.

Adjusting the time of planting

Nematode life cycle depends on the climatic factors. Adjusting the time of planting helps to avoid nematode damage. In some cases crops may be planted in winter when soil temperature is low and at that time the nematodes cannot be active at low temperature. Early potatoes and sugar beets grow in soil during cold season and escape cyst nematode damage since the nematodes are not that much active, to cause damage to the crop during cold season.

Fallowing

Leaving the field without cultivation, preferably after ploughing helps to expose the nematodes to sunlight and the nematodes die due to starvation without host plant. This method is not economical.



Deep summer ploughing

During the onset of summer, the infested field is ploughed with disc plough and exposed to hot sun, which in turn enhances the soil temperature and kills the nematodes.

For raising small nursery beds for vegetable crops like tomato and brinjal seed beds can be prepared during summer, covered with polythene sheets which enhances soil temperature by 5

to 10°C which kills the nematodes in the seed bed. This method is very effective and nematode free seedling can be raised by soil solarization using polythene sheets.



Summer ploughing

Manuring

Raising green manure crops and addition of more amount of farm yard manure, oil cakes of neem and castor, pressmud and poultry manure etc enriches the soil and further encourages the development of predacious nematodes like *mononchus* spp. and also other nematode antagonistic microbes in the soil which checks the parasitic nematodes in the field.

Flooding

Flooding can be adopted where there is an enormous availability of water. Under submerged conditions, anaerobic condition develops in the which kills the nematodes by asphyxiation. Chemicals lethal to nematodes such as hydrogen sulphide and ammonia are released in flooded condition which kills the nematodes.



soil

Trap cropping

Two crops are grown in the field, out of which one crop is highly susceptible to the nematode. The nematode attacks the susceptible crop. By careful planning, the susceptible crop can be grown first and then removed and burnt. Thus the main crop escapes from the nematode damage. Cowpea is highly susceptible crop can



Cowpea

be grown first and then removed and burnt. Cowpea is highly susceptible to root – knot nematode and the crop can be destroyed before the nematodes mature.

Antagonistic crops

- ✓ Certain crops like mustard, marigold and neem etc have chemicals or alkaloids as root exudates which repel or suppress the plant parasitic nematodes.
- ✓ In marigold (*Tagetes* spp.) plants the α – terthiynyl and bithiynyl compounds are present throughout the plant from root to shoot tips. This chemical kills the nematodes.
- ✓ In mustard allyl isothiocyanate and in pangola grase pryrocaterchol are present which kills the nematodes.
- ✓ Such enemy plants can be grown along with main crop or included in crop rotation.



Marigold

Removal and destruction of infected plants

Early detection of infested plants and removal helps to educe nematode spread. After harvest the stubbles of infested plants are to be removed. In tobacco, the root system is left in the field after harvest. This will serve as a inoculum or the next season crops. Similarly in *D. angstus* the nematode remains in the left out stubbles in the field after harvest of rice grains. Such stubbles are to be removed and destroyed and land needs to be ploughed to expose the soil.

Use of resistant varieties

Nematode resistant varieties have been reported from time to time in different crops. Use of resistant varieties is a very effective method to avoid nematode damage. Nemared, Nematex,

Hisar Lalit and Atkinson are tomato varieties resistant to *M. incognita* . The potato variety Kufri swarna is resistant to *G. rostochiensis*.

Physical control

It is very easy to kill the nematodes in laboratory by exposing the nematodes to heat, irradiation and osmotic pressure etc., but it is extremely difficult to adopt these methods in field conditions. These physical treatments maybe hazardous to plant or the men working with the treatments and the radiation treatments may have residual effects

Heat treatment of soil

Sterilization of soil by allowing steam is a practice in soil used in green house, seed beds and also for small area cultivation. Insects, weed seeds, nematodes, bacteria and fungi are killed by steam sterilization. In such cases steam is introduced into the lower level of soil by means of perforated iron pipes buried in the soil. The soil surface needs to be covered during steaming operation. Plastic sheets are used for covering. In the laboratory and for pot culture experiments autoclaves are used to sterilize the soil.



Soil solarisation

Hot water treatment of planting material

Hot water treatment is commonly used for controlling nematodes. Prior to planting the seed materials such as banana corms, onion bulbs, tubers seeds and roots of seedlings can be dipped in hot water at 50 – 55 °C for 10 minutes and then planted.

Irradiation

Irradiation also kills the nematode. Cysts of *G. rostochiensis* exposed to 20,000 Y contained only dead eggs and at 40, 000 Y exposure, the eggs lost their contents. *Ditylenchus*

myceliphagus in mushroom compost exposed to γ rays between 48,000 to 96,000 γ inactivated the nematodes. UV light also kill the nematodes. But these irradiation is not practically feasible under field conditions.

Osmotic pressure

Feder (1960) reported 100% nematode mortality when sucrose or dextrose were added to nematode infested soil @ 1 to 5% by weight. But these methods are not practical and economical.

Washing process

Plant parasitic nematodes are often spread by soil adhering to potato tubers, bulbs and other planting materials. Careful washing of such planting material helps to avoid the nematodes in spreading in new planting field. Washing apparatus for cleaning potato and sugarbeet tubers are commercially developed and are being used in many countries.

Seed cleaning

Modern mechanical seed cleaning methods have been developed remove the seed galls from normal healthy wheat seeds.

Ultrasonics

Ultrasonic have little effect on *Heterodera* spp. The use of this ultrasonics is not practically feasible.

Lecture 14 - Biological Control

Biological control aims to manipulate the parasites, predators and pathogens of nematodes in the rhizosphere in order to control the plantparasitic nematodes. Addition of organic amendments such as farm yard manure, oil cakes, green manure and pressmud etc encourages the multiplication of nematode antagonistic microbes which inturn checks the plant parasitic nematodes.

The addition of organic amendments acts in several ways against the plant parasitic nematodes. Organic acid such as formic, acetic propionic and butric acids are released in soil during microbial decomposition of organic amendments. Ammonia and hydrogen sulphide gases are also released in soil during decomposition. These organic acids and gases are toxic to nematodes.

Nematode antagonistic microbes multiply rapidly due to addition of organic matter. Organic amendements improve soil conditions and helps the plants to grow. The organic matter also provides nutrition for the crps plants.

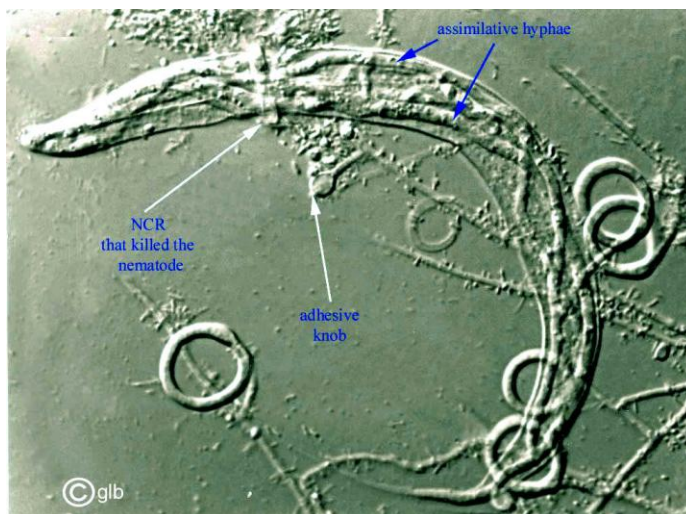
Predacious fungi

Most of the predacious fungi comes under the order Moniliales and Phycomcetes. There are two types of predacious activities among these fungi. They are nematode trapping fungi and endozoic fungi.

Non-constricting rings

The trap is formed similar to the constricting ring. It is a non – adhesive trap. The reing becomes an infective structure kills the nematode eg. *Daclylaria candida*.

In addition to formation traps and adhesive secretions, the predacious fungi may also produce toxin which kills the nematodes.



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Endozoic fungi

The endozoic fungi usually enter the nematode by a germ tuber that penetrates the cuticle from a sticky spore. The fungal hyphae ramify the nematode body, absorb the contents and multiply. The hyphae then emerge from dead nematode. *Catenaria vermicola* often attacks sugarcane nematodes.

Pasteuria penetrans was found to be very effective against the root –knot nematodes in many crops. The *P. penetrans* infested J2 of root knot nematodes can be seen attached with spores throughout the cuticle.

History of chemical control

Kuhn (1881) first tested CS₂ to control sugarbeet nematode in Germany and he could not get encouraging results. In South Carolina State, U.S.A. Bessey (1911) treated CS₂ for the control of root – knot nematodes but the method proved impractical. Later on the chemicals like formaldehyde, cyanide and quick lime were observed to have nematicidal properties, but all these chemicals were found to be highly expensive.

Mathews (1919) observed the effect of chloropicrin (tear gas) against plant parasitic nematodes in England. Carter (1943) an entomologist of Hawaii, Pineapple Research Institute, reported the efficacy of 1,3 dichloropropene 1,2 dichloropropane (DD) mixture @ 250 lb/acre, against the plant parasitic nematodes. In 1944, scientists from California and Florida states of USA reported the efficacy of ethylene dibromide (EDB). In the same year the Dow Chemical company, USA introduced the chemical as a soil fumigant for the management of nematodes. The introduction of these two nematicides viz., DD and EDB paved way for the chemical control of nematodes.

Description of some important nematicides

Ethylene dibromide (EDB) : 1,2 Dibromomethane. It is a colourless liquid and the gas is non-inflammable. It is available as 83% liquid formulation containing 1.2 kg active ingredient per litre and as 35% granules. It is injected or dibbled into the soil for the control of nematodes at 60 to 120 l or 200 kg ai/ha but it is not very effective against cyst nematodes. *Heterodera* spp. and soil fungi. Crops like onion, garlic and other bulbs should not be planted after soil treatment with EDB. It is available as Bromofume and Dowfume.

Dibromochloropropane : (DBCP) 1,2 – dibromo – 3 – chloropropane. It is a straw coloured liquid, a litre of it weighing 1.7 kg. It can be used as soil treatment before planting, at the time of

planting or as post when the soil temperature is above 20°C. It is applied as a sprinkle depending upon the crop and stage. Certain crops like tobacco and potato are sensitive due to high bromine content in the chemical. It functions more efficiently than other fumigant at high soil temperature due to its high boiling point (195.6°C). trade names are Nemagon and fumazone.

DD mixture It is the trade name of the mixture of compounds, chief of them contain the cis and the trans isomers in equal quantities of 1,3 – dichloropropene 30.35%, and a few other chlorinated compounds up to about 5%. Of these, dichloropropene is the most toxic compound and among its two isomers, the trans isomer is twice as toxic as the cis-isomer. It is a black liquid of 100% formulation and a litre of it contains approximately 1kg of technical compounds. It is used in the control of soil insects and nematodes and injected into the soil at a depth of 15 -20 cm at 25 x 30 cm spacing. It is a fungicide a very high diseases. Since it is highly phytotoxic, it is used for preplant soil application at least 2 -3 weeks before planting. It is used for preplant soil application at least 2 -3 weeks before planting. It is used as such at 225 – 280 l/ha, but in clay and peaty soils a higher dosage is required. It taints potato tubers and carrots grown in treated soil. Dichloropropene is available under the trade name Telone and in mixture with dibromoethane under the name Dorlane.

Methylbromide or Bromomethane It boils at 4.5°C. At ordinary temperatures it is a gas and therefore, confined in containers under pressure as a liquid. The gas is 1.5 times as heavy as air. Its insecticidal properties were described by Le Goupil in 19 -32. Its power of penetration into packed foodstuffs such as flour is remarkable. As it kills insects slowly a longer period of exposure to gas may be required. For control of stored grain pests it is used at 24 – 32 g.m³, exposure period being 48 h. In tent fumigation for the control of termites and powder post beetles, the dosage recommended is 32 0-64 g/ m³. For fumigating live plants, the dosage is 16 - 32. Some plants are likely to be injured. In soil application for the control of nematodes.

Phorate : 0,0 – diethyl S – (ethylthiomethyl) phosphorodithioate. Trade name is Thimet. It is systemic insecticide cum nematicide, available as 10% granule. It has got both contact and fumigant action. It does not persist for a longer period and gets metabolically oxidized yielding for rat: oral 16 – 3.7; dermal 2.5 to 6.2.

Aldicarb : 2 – methyl – 2 (methylthio) propionaldehyde O (methylcarbonyl) oxime. Trade name is Temik. The sulphur atom in the molecule is oxidized to sulfoxide and then to sulfone. It is a

systemic 10% granule. The residues remain in plants for 30 -35 days as a lethal dose. It also acts as repellent, contact nematicide and interferes with reproduction of the nematodes by way of sex reversal.

Carbofuran: C₁₂ H₁₅ N₃ O₃. It is 2,3 – dihydro – 2, 2, dimethyl 7 benzofuranyl methyl carbamate. Trade name Furadon. It is a systemic insecticide cum nematicide. It is formulated as 3% granule and also as 40F. The residual effect last for 30 – 60 days. It has also got phytotonic effect. This systemic chemical has got acropetal action and applied @ 1 -2 kg ai. /ha.

Resistant varieties

The use of resistant varieties provide an effective, economical and friendly means of nematode control.

Crop varieties identified/developed resistant to plant parasitic nematodes

Crop	Nematode	Resistant varieties
Tomato	Root-knot nematodes (<i>Meloidogyne javanica</i> / <i>M. incognita</i>)	PNR-7, NT-3, NT-12, Hisar Lalit
Chilli	Root-knot nematodes (<i>Meloidogyne javanica</i> / <i>M. incognita</i>)	NP-46A, Pusa Jwala, Mohini
Cowpea	Root-knot nematodes (<i>Meloidogyne javanica</i> / <i>M. incognita</i>)	GAU-1
Mungbean	Root-knot nematodes (<i>Meloidogyne javanica</i> / <i>M. incognita</i>)	ML-30 and ML-62
Cotton	<i>Meloidogyne incognita</i>	Bikaneri nerma, Sharda, Paymaster
Grapevine	Root-knot nematodes (<i>Meloidogyne javanica</i> / <i>Meloidogyne incognita</i>)	Khalili, Kishmish Beli, Banquabad, Cardinal, Early Muscat, Loose Perlett
Potato	Potato cyst nematode (<i>Globodera rostochiensis</i>)	Kufri Swarna, Kufri Giriraj

Lecture 15 - Integrated Nematode Management

Definition

INM can be defined as a systems approach to reduce nematode to tolerable levels through a variety of techniques including predators and parasites, genetically resistant hosts, natural environmental modifications and when necessary and appropriate chemical nematicides.

Integrated Nematode Management

The grower combines several measures to prevent the growth of damaging nematode populations. Where there is an advisory service equipped to monitor field populations, these control measures can be utilized with minimum amounts of nematicides. Without advisory services, integrated control of nematode is hardly possible and relatively heavy dosages of chemical nematicides are employed.

Decisions to implement nematode management programmes are based on the perceived value of the potential crop loss and the cost of the management procedure. Theoretically this involves determining an economic threshold. Management procedures should usually be implemented when the marginal revenue derived from the management input is equal to or excess the marginal cost.

The design development and implementation of INM systems for plant protection requires extensive interaction of nematologists with scientists in complementary disciplines. INM includes following components.

a. Exclusion

Crop losses caused by plant parasitic nematodes can be avoided through preventing the introduction of specific nematodes or nematode problems in areas where the species do not exist. The focal point of exclusion is the target nematode species. Exclusion procedures should be used as first order defenses to prevent dissemination and establishment.

Exclusion procedures include sanitation, certified plant material, nematode free soil or planting media, population reduction or eradication procedures and regulatory activities. Quarantines are used to prevent or slow the spread of plant parasitic nematodes. Certified plant material and nematode free planting media or equipment are

used for nematode exclusion. All available exclusion procedures should be considered in the system evaluation and design of the INM programme.

b. Population Reduction

Crop losses caused by plant parasitic nematodes can be alleviated by procedures designed to decrease population densities of the target species to an acceptable level. Whenever possible population reduction strategies should stabilize nematode population densities below the damage threshold. The focal point of population reduction is the target species. It is assumed that the species of concern is present and eradication is not feasible.

Established nematode populations are usually managed through population reduction by cultural, physical, biological and chemical procedures. Cultural procedures include the use of fallowing, flooding, cover crops, crop rotation, planting date, rouging, trap crops or weed management for population reduction. Heat is the physical factor most widely used in nematode population reduction. Nematode biocontrol resources include prokaryotes (viruses, rickettsias and bacteria), nematophagous fungi and predacious, parasitic or environmental modifying invertebrates (nematodes, protozoa, mites etc) and antagonistic plants.

Nematicides can directly or indirectly reduce populations associated with nematode infested materials and can be used for soil application, root tips, foliar applications or seed treatments.

c. Tolerance

Crop losses caused by plant parasitic nematodes can be reduced by manipulating the host in relation to its environment. The objective of tolerance is to elevate the damage threshold. The focal point is the host crop.

Procedures that protect or increase plant tolerance have excellent potential for INM. These include cultural manipulation, chemical application, mycorrhizal colonization and resistant cultivars.