K. K. COLLEGE OF AGRICULTURE, NASHIK

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

THEORY NOTES

Course No.:- ENTO-364

Course Title: - Introductory Nematology

Credits: - 2 (1+1)

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TEACHING SCHEDULE

Semester : VI **Course No. : ENTO-364**
Course Title : Introductory Nematology **Credits : 2(1+1)**

Lecture No.	Topics	Rating
1	Introduction- History of phytonematology and economic importance.	4
2	General characteristics of plant parasitic nematodes.	2
3	Nematode- General morphology and biology.	4
4	Classification of nematode up to family level with emphasis on group of containing economical importance genera (Taxonomic).	4
5	Classification of nematode by habitat.	2
6	Identification of economically important plant nematodes up to generic level with the help of key and description.	4
7	Symptoms caused by nematodes with examples.	4
8	Interaction of nematodes with microorganism	4
9	Different methods of nematode management.	4
10	Cultural methods	4
11	Physical methods	2
12	Biological methods	4
13	Chemical methods	2
14	Entomophilic nematodes- Species Biology	2
15	Mode of action	2
16	Mass production techniques for EPN	2

Reference Books:

1) A Text Book of Plant Nematology – K. D. Upadhay & Kusum Dwivedi, Aman Publishing House

2) Fundamentals of Plant Nematology – E. J. Jonathan, S. Kumar, K. Deviranjan, G. Rajendran, Devi Publications, 8, Couvery Nagar, Karumanolapam, Trichirappalli, 620 001.

3) Plant Nematodes - Methodology, Morphology, Systematics, Biology & Ecology Majeebur Rahman Khan, Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, India. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.

4) Introductory Nematology (Theory Notes) – Dr. B. S. Shewale

LECTURE NO. : - 1
INTRODUCTION

Nematode is an important branch of biological science, which deals with complex, diverse group of roundworms known as nematodes that occur worldwide essentially in all environments.

Nematodes are triploblastic (having three layers), bilaterally symmetrical, multicellular, unsegmented, generally microscopic worms with single cavity (Pseudocoelomic).

Nematodes are generally found in all type of environment from the artic to the tropic sand, from the ocean depth to the tops of high mountains. They constitute the largest group of animal kingdom, comprising 80 to 90 percent at all multicellular animals. They are also mostly found in soil and most of the crops are attacked by them which are known as plant parasitic nematodes or phytonematodes.

Nematodes are also known as eelworm, *nemas* and roundworm. Many species are important parasites of plant and animals, whereas others are beneficial to agriculture and environment. Nematodes that are parasites of man and animals are called **Helminthes** and the study is known as **Helminthology**. The name nematode was derived from the Greek word *nemas* (thread) and adios (form or resembling).

The soil hector of all agro-ecosystem typically contain billions of plant parasitic nematodes. They feed on roots, buds, stems, crowns, leaves and developing seed of the plants. The damage caused by the nematodes on plant often overlooked because of association of symptoms like slow growth, stunting, yellowing and also could be attributed to the nutritional disorder.

Existence of Nematodes

- Sea water : 50%
- Free living : 25%
- Animal parasites : 15%
- Plant parasites : 10%

HISTORY OF NEMATOLOGY

The history of animal parasitic nematodes is almost as ancient as the history of man. The Guinea worms and round worms as parasites of man were known to Egyptians as early as 1553-1500 B.C. As far as phytonematology is concerned Needham (1743) discovered the ear cockle disease caused by wheat gall nematode *Anguina tritici* which is the first record of plant parasitic nematode.

I) History of Nematology in World

- **Early History (1743-1940):-**

Needham, T. (1743)- Wheat gall nematode, *Anguina tritici.* First record of plant parasitic nematode. Described associated diseases of wheat and other grains.

Berkeley, M. J. (1855)- Discovered root-knot nematode, *Meloidogyne spp* in greenhouse cucumber.

Goeldi, E. A. (1887), Neal, J.C. (1889), Atkinson, G.F. (1989), Bessey, E.A. (1901)- Contribution in *Meloidogyne spp.* Distribution, host ranges and disease complexes .

Schacht, H. (1859)- First reported cyst forming nematode in sugar beet in Germany

Schmidt, A. (1871)- Described sugar beet nematode, Central Europe *Heterodera schachtti* Carbon disulfide (CS2) – First report of effective chemical control of nematodes.

Kuhn, J. (1857)- Described stem nematode, *Ditylenchus dipsaci* – on teasel heads. Serious problem to alfalfa, garlic, oats, onion, red clover, rye.

Ritzema Bos, J. (1891)- Discovered foliar nematode, *Aphelenchoides fragariae* on straw berry. *A. ritzemabosi* on chrysanthemum *A. Besseyi* on rice.

Cobb, N. A. (1914 to 1932)- Father of Nematology (America)

- ❖ Developed techniques for sampling soil for nematodes.
- ❖ Wet screening for extraction of nemas from soil.
- ❖ Methods of preserving/sectioning/mounting nematodes.
- ❖ Demonstrated presence of amphids, cephalic papillae, phasmids and deirids on tylenchs.

Developed en face section techniques

Filipjev, I. N. (1930)- Russian Scientist published a book "Nematodes that are of importance to Agriculture".

Chitwood, B. G.(1937)- Published a book "Introduction to Nematology".

- **New Era in Nematology (1941-1990):-**

Cannon, O. S. (1941)- New York – Potato root eelworm (Golden Nematode) *Heterodera rostochiensis (Globodera rostochiensis)*

Carter, C. C. (1943)- Discovery of D.D. (1, 3 *Dicloropropane)* soil fumigant for control of golden nematode.

Christie, J. R. & Albin, F. E. (1944)- Discovery of races of root-knot nematodes.

Chitwood, B. G.(1949)- Discovered genus *Meloidogyne.* Described several species of root-knot nematodes which made possible to :

- ❖ Determine the host range of individual species.
- ❖ Design keys for their identification based on morphological differences.
- ❖ Develop crop cultivars resistant to certain species.

Study individual species cytologically and biochemically

Early (1950)- Declining citrus in Florida due to burrowing nematodes, *Radopholus similis.*

Christie, J. R. and Perry, V. G. (1953)- Demonstrated the importance of several ectoparasitic species (*Belonolaimus, Dolichodorus, Xiphinema, Trichodorus* etc.)

Moutain, W. B. (1955)- Culturing plant parasitic nematodes under sterile conditions.

Hewitt, W. B. (1958) - Discovery of transmission of virus diseases.

II) History of Nematology- India:-

1901 : Barber, C. A.- Root-knot nematode infesting tea in south India – First report of plant parasitic nematode in India.

1906 to 1919- Root-knot nematode – Black pepper in Kerala, Ufra disease of rice (*Ditylenchus angustus)*

1934: Ayyar, P. N. K.- Root-knot nematode infesting vegetables and other crops

1936: Dastur, J. F.- White tip nematode of rice (*Aphelenchoides spp.)*

1961: Jones, F. G. W.- First authentic report on potato cyst nematode, *Heterodera (Globodera) rostochiensis* from Nilgiris.

1965- First authentic report on *Radopholus similis* on banana from Kerala.

1966- Division of Nematology established at IARI, New Delhi.

1971- Indian Journal of Nematology

1977- AICRP on Nematode Pests of Crops and Their Control (14 centers).

III) History of Nematology – Maharashtra

Dhande & Sulaiman (1961)- Reported occurrence of root-knot nematode on betel vine from Vadner Bhairav (Nashik)

Manjrekar & Talgeri (1969)- Enumerated the problems of plant parasitic nematodes of Maharashtra in All India Nematology Symposium.

Manjrekar (1977)- Submitted M.Sc. (Agri.) thesis and reported : *M. incognita* – Banana, Grapevine, Betel vine & Vegetables. *T. semipenetrans* – Citrus, *R. reniformis* – Grapevine. Ectoparasites – *Helicotylenchus, Hoplolaimus, Xiphinema, Tylenchorhynchus*

January, 1978- AICRP on Nematodes at MPKV, Rahuri.

Economic Importance of Plant Parasitic Nematodes

The plant parasitic nematodes play a vital role in crop production as most of the crop including field, orchards, vegetables, kitchen garden; ornamental crops are attacked by various species of nematodes. More than 2000 species of phytonematodes belonging to about 200 genera have been described; while it is estimated that about 42000 species of phytonematodes may be present.

It has been estimated that on global basis 12 percent crop loss due to diseases, 7 percent due to insects, 3 percent due to weeds and 11 percent due to nematodes. The annual crop losses due to these obligate parasites have been estimated to be about $78 billion worldwide. Estimated overall average annual yield loss of the world's major crops due to plant parasitic nematodes was 12.3%. Estimated losses due to plant parasitic nematodes in developing countries were 14.6% and 8.8% for developed countries. On worldwide basis, the ten most important genera were reported to be *Meloidogyne, Pratylenchus, Heterodera, Ditylenchus, Globodera, Tylenchulus, Xiphinema, Radopholus, Rotylenchulus* and *Helicotylenchus.*

In India, the losses caused in different crops are due to the following major nematode species.

The seed gall nematode, *Anguina tritici* is responsible for ear cockle disease of wheat in Northern India. It also causes tundu or yellow slime disease with the association of a bacterium, *Clavibacter tritici.* The overall damage is one percent but sometimes it is as high as 80 percent.

The root-knot nematode, *Meloidogyne* spp. is one of the few nematodes known to the farmers due to the spectacular symptoms of root gall formation on vegetable, pulses, fruits and ornamental plants. The percent yield losses due to this nematodes has been estimated to the tune of 28-47 percent in tomato, 26.2-50 percent in brinjal, 19.7-33 percent in chillies, 6.0-90 percent in okra, 38-47.2 percent in bitter gourd and 18-33 percent in melons at different AICRP projects in India.

The cereal cyst nematode, *Heterodera avenae* causes **Molya** disease of wheat and barley in the states of Rajasthan, Haryana, Punjab, Delhi, U.P., Himachal Pradesh, Jammu & Kashmir. It may cause up to 50 percent or even a total loss of the crop.

The reniform nematode, *Rotylenchus reniformis* attacks a large number of plants and causes considerable losses to vegetables and pulses varying from 4.8 to 14.9 percent loss in yield in different crops.

Citrus nematode, *Tylenchulus semipenetrans* causes slow decline disease of citrus and also associated with 'die-back' in citrus.

The burrowing nematode, *Radopholus similis* causes severe damage to many fruit crops including banana, spice crop and other plantation crops. It is responsible for spreading decline of citrus, black head disease of banana and root-rot disease of crops.

The golden nematode of potato, *Globodera rostochinensis* is a serious problem in Nilgiri and Kodaikanal hills. An average loss of 9 percent is caused by this nematode.

The root lesion nematode, *Pratylenchus coffeae* is important pest of coffee in South India. It causes foot-rot of young plants and decline or die-back of older plants.

The above examples include only the major nematode pests. Besides infesting alone, they are also known to be associated with various bacteria, fungi and viruses in causing complex plant diseases which further increases the losses in yield of crops. The nematode problem is more important in developing countries, in tropical and sub-tropical regions.

LECTURE NO. : - 2

General Characteristics of Phylum Nemata/Nematoda/Nematodes

1. The nematodes possess elongate, unsegmented, cylindrical or worm like body tapering towards both ends, unciliated and circular in cross section.
2. Body is bilaterally symmetrical.
3. They are aquatic, terrestrial and parasitic or free living.
4. The body is covered by tough and resistant cuticle secreted by epidermal (hypodermal) cells.
5. Terminal oral aperture (mouth) surrounded with lips and papillae.
6. Digestive system consists of feeding apparatus, oesophagus, intestine and rectum.
7. Body consists of two tubes.
8. The nervous system consists of circum-oesophageal nerve ring and longitudinal nerves.
9. Primitive excretory system is devoid of protonephridial cilia or matanephridial funnel.
10. The circulatory and respiratory systems completely absent.
11. The females have separate genital pore and males have a common opening known cloaca and well developed copulatory apparatus consisting of spicules and gubernaculum.
12. Females are oviparous or ovoviviparous or viviparous. The cleavage is terminated and growth is accompanied by molting.
13. Life cycle is direct and there are four juvenile stages.

LECTURE NO. : - 3

Nematode - General Morphology and Biology

Nematode exhibits considerable variation in their external and internal structure. Despite this structural complexity, certain basic principle is common to all nematodes

General shape & size

Nematodes : Triploblastic, bilaterally symmetrical, unsegmented, colourless, pseudo coelomate, vermiform and circular in cross section animals.

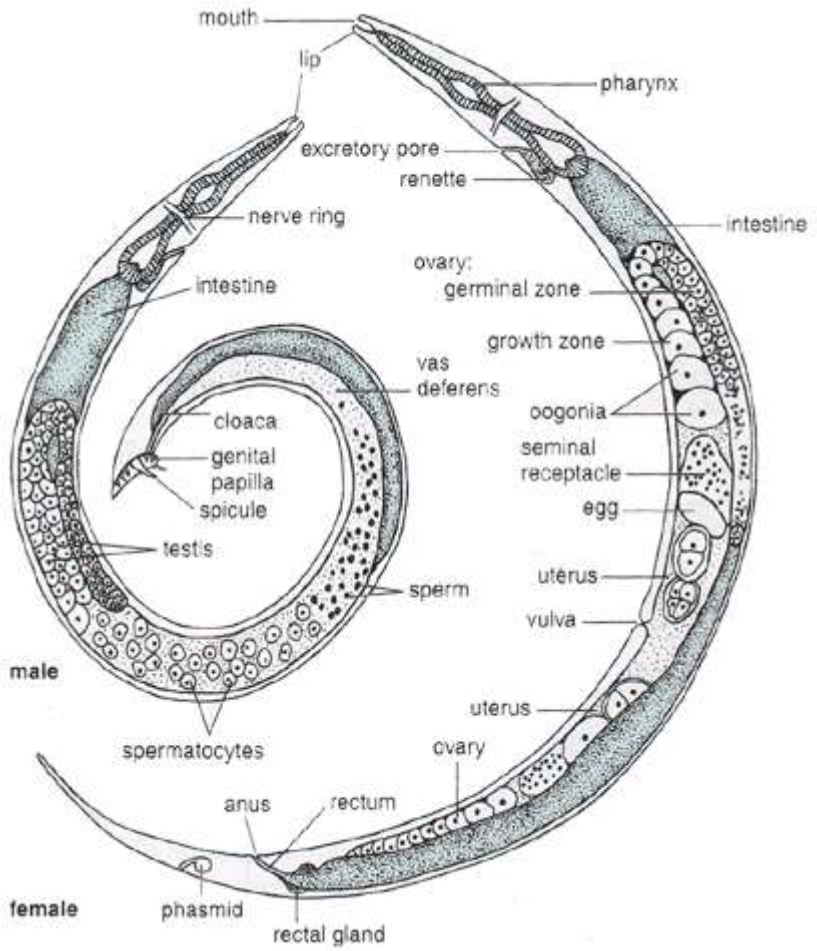


Fig: General Morphology of Nematode

Shape: Nematode show great variation in their morphological characters. The plant parasitic nematodes are slender, spindle shaped or fusiform organisms. The nematodes when relaxed with gental heat either lie straight (*Pratilenchus)* or slightly curved (*Hoplolaimus)* or curved in 'C' shaped (*Tylenchorynchus)* or form a spiral (*Helicotylenchus).* Sexual dimorphism in few species. Lemon, pear, kidney, saccate shape.

Size: Their size may vary from 0.2 mm to about 12 mm length with an average of 0.01 mm and 0.5 mm in breadth (1 to 15 % to length). Males are smaller than females

Body regions: The nematode body is not divisible into definite regions as that of insects, however there are certain subdivisions like anterior part of the body having the mouth, lips and stoma is called head and it is continuous with main body. The portion between the head and the base of oesophagus is the neck. The part of the body beginning from the anus or cloca and extending up to posterior extremity is the 'tail'. Longitudinally, the body can divide into four zones: the ventral which bears the natural openings like excretory pour, anus or cloaca and vulva; the side apposite to the ventral is dorasal. The other two sides are right and left laterals.

Lip region: The lip region is also called as head exhibits great variation which may be used taxonomic purpose.

Tail region: It is the post-anal elongation of the body present in all stages of nematodes.

General structure of nematode:

The body of nematode is tubular which may be divided into three regions

I) Outer body tube or body wall

II) Inner body tube –Digestive system

III) Body cavity– Reproductive system, Nervous system, Excretory system

I) Outer body tube

The outer body tube is comprises of

(A) Exoskeleton or cuticle,

(B) Hypodermis and

(C) Muscle layer.

(A) Exoskeleton or cuticle:

It is outermost covering of body wall which is non-cellular, semipermeable and tough layer secreted by the epidermal cells. It invades all natural opening of body including the mouth, rectum, cloaca, vagina, excretory pore, amhids and phasmids.

The cuticle of many nematode species has markings on the surface. They are varied and complex and often used by taxonomist in identification of nematode species. The cuticular lining/markings are categorized in different types are as follows.

Cuticular lining or markings:

1. Punctations – They are commonly appearing as minute or round areas which are arranged in pattern. It acts as a structure for strengthening cuticle and transport of proteins.

2. Transverse markings or Annules or Striations – There are several transverse lines present on the surface of cuticle. These markings are exhibit on most of the plant parasitic nematodes and often used for identification. Annulations give segmented appearance *e.g.* scales in Criconemoides & perineal pattern of root-knot nematodes. Necessary for dorsoventral undulatory movement.

3. Longitudinal markings – These markings are the lines on the cuticle, which runs longitudinally throughout the nematode body.

i) Ridges – These are raised areas, which run length of the body and occur on sub-median as well as lateral surface.

ii) Alae – These are thickening or projections occur in lateral or sub-lateral region. They assist in locomotion. There are three types of alae

- **Caudal alae –** These are found in the posterior region and restricted to males as copulatory bursa.
- **Cervical alae –** These are confined to anterior part of the nematode body. Cervical alae are found in some species of marine nematodes.
- **Longitudinal alae –** These are limits to the lateral fields. They are transverse by striations or furrows varying in number from one to twelve which provide locomotion and may permit slight change in the width of nematode.

Cuticular layering or covering:

The nematode cuticle is basically three layer structure and composed of (a) Cortical layer, (b) Median layer and (c) Basal layer.

(a) Cortical layer – It is often divided into external cortical layer and internal cortical layer. The surface of external cortical layer is exposed to the environment. This layer is very thin measuring about 25 to 40 mμ. The external layer has been considered to be kertatine (protein) chemically. In cyst nematode the cuticle of the female on maturity becomes tough and leathery to form cyst which protect eggs under dry conditions.

(b) Median layer – The average thickness of the median layer is 0.1 μ in the larva of *Meloidogyne* and *Heterodera.* Chemically the median layer consists of protein, which resembles collagen (Non osmophilic collagen protein).

(c) Basal layer – It consist of regularly arranged vertical rods or striations. It is composed of protein with very close linkage between the molecules, resulting in resistant layer which protect the nematode from outer environment. The thickness of basal layer varies from 125 to 500 mμ (Osmophilic protein close to keratine)

Functions of cuticle:

1) Protects the nematode from harsh environment.
2) Serves as exoskeleton
3) Provide mechanism of movement of the nematode through the soil and plant tissue.

(B) Hypodermis –

The hypodermis is cellular or partially cellular layer. It secretes the cuticle. It lies between cuticle and somatic muscle layer. It is important metabolic active part of the nematode. Forms 4 cords (dorsal, ventral and two laterals). Contains hypodermal glands

(C) Muscle layer -

It is arranged in a single layer. The muscle cells are spindal shaped and attached to the hypodermis throughout their length. It is well connected to the nervous system. The stimulation of the muscles by dorsal and ventral nerves cause contractions in the dorso-ventral plane and result in the characteristic scinusodial movement of nematode.

On the basis of arrangement of the basic cells, following three types are identified:

a. Holomyarian: Having two muscle cells in each zone.

b. Meromyarian: Two or five muscle cells in each interchordal zone.

c. Polymyarian: More than five muscle cells in each zone

Specialized muscles:

- ❖ Feeding, food movement and defecation
- ❖ Reproduction *e.g.* vulvar, spicular, gubernaculum, copulatory and bursal muscles.
- ❖ Chemically muscle layer is made up of myosin and actin.

II) Inner body tube or Digestive System:

The inner body tube of nematode forms the gut or alimentary canal in which some glands are open. It is distinguishable in to three regions as:

1. Stomodaeum (Forgut)
2. Mesenteron (Midgut)
3. Proctodeum (Hindgut)

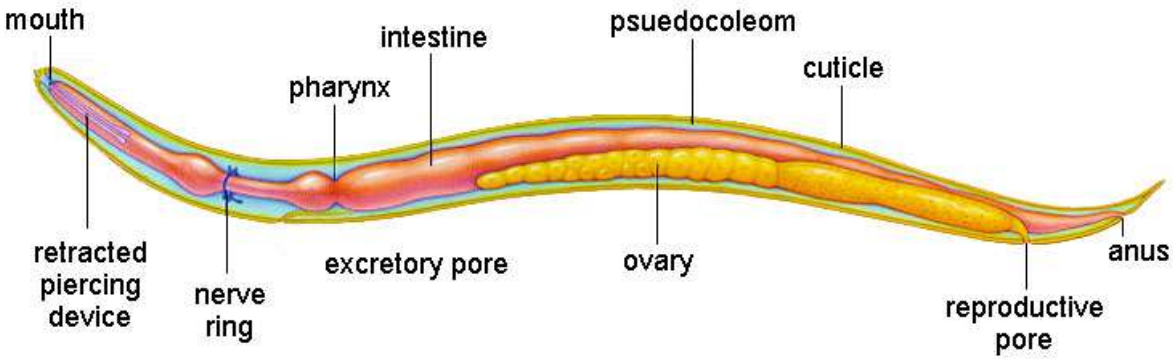


Fig. Digestive system of Nematode

1. Stomodaeum: It includes the mouth and lips, the stoma and the oesophagus.

Mouth and lips: The mouth and lips are also associated with the feeding activity of the nematode. Generally, there are 6 lips (two sub dorsal, two sub ventral and two lateral) which surround the mouth. In some cases they may be reduced by partial fusion to 3 or by complete fusion to form a united ring around the mouth.

Stoma or Buccal cavity: The stoma, which is also called as mouth cavity or buccal cavity forms the feeding apparatus and lies between the mouth and the oesophagus. The simple stoma is found in many bacterial feeding nematodes, takes the form of a cylindrical or triangular tube, terminating in a valve like glottoid apparatus, which may bear the minute teeth. The cuticular lining of stoma may form teeth. Plant parasitic nematodes are armed with a protrusible stylet which is usually hallow and functions like a hypodermic needle. Stylet with basal knob are called as Stomatostylet *e.g.Tylenchida* and the stylet without basal knob are called as odontostylet or ononiostyle *e.g. Dorylaimida.*

Oesophagus or pharynx: The oesophagus is a muscular pumping organ attached to the posterior portion of the stylet and lined with cuticle. It is the largest part of stomodaeum and found between stoma and intestine. Internally, pharynx lined with cuticle and externally by membrane (basal lamella). It contains radial muscles, oesophageal glands and valves, which prevents the regurgitation of food. In some nematodes median and posterior part of pharynx swollen to form muscular bulb. The cylindrical oesophagus has three well defined regions are as follows.

i) **Corpus-** The corpus may further divided to form pro and meta corpus, which is swollen contain muscle cells, supporting cells, nerve cells, gland cells (one dorsal and two sub ventral).
ii) **Isthumus**
iii) **Basal bulb**

2. Intestine or midgut: The midgut is endodermal in origin. It is simple, hallow, straight tube consisting of a single layer of epithelial cells. The intestine is generally divided in to three region which merge in to each other without any perceptible boundaries. They are anterior or ventricular region, the mid intestinal region and posterior pre-rectal region.

3. Proctodeum: The proctodeam or hind gut consist of Rectum and anus in female and cloaca in male.

Rectum is cuticular linings and invaginated in to rectal gland on par in nematodes. Female nematodes consist of simple tube leading to anus, whereas reproductive system opened in to it and form cloaca in male contain spicules and other copulatory structure.

Anus consists of slit structure on ventral side. The control of anus opening is by unicellular, H shaped depressor muscle, which acts by raising dorsal wall of the rectum and pulling posterior lip of anus to open it.

Glands:

1) Pharyngeal or Esophageal – There are three uninucleated glands are present. One is dorsal & other two ventro-lateral or sub ventral positions. The glands connected with lumen of oesophagus by means of terminal ampulla or swelling.
Function: Hatching, host penetration and digestion

2) Rectal - Rectal glands are varies from species to species or male and female of same species. Copious production of gelatinous mucopolusaccharide matrix in the eggs of deposited as mass. Which range to protect the eggs.
Function: Secretion of gelatinous matrix.

Function of Digestive system:

Digestive juices which are 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.

1) 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.
2) Ingestion phase: During this phase, rhythmical contraction of the posterior part of oesophagus associated with the median bulb occurs and in some forms, the oesophageo-intestinal valve or cardia is responsible for ingestion of material from the host.

Secretion- Various glands associated with digestive system. Active protein and mucopolysaccharide synthesis their product and shed through cuticle- either through cuticle lined either into stomodaeum or proctodeum.

Excretion- Intestine acts as an excretory organ and defection is mechanically controlled and it is an intense process.

III) Body cavity or Psuedocoelom:

The body cavity of nematode is different from animal. The true coelomic cavity is that completely fined with mesodermal in origin.

a) **Coelomic cavity:-** Lined externally with somatic muscle (Mesodermal in origin) and internally by alimentary canal (Ectodermal in origin).
b) **Pseudocoelomic cavity: -** Lined with tissues of Mesodermal in origin.
c) **Pseudocoelomic fluid: -** Baths all internal organs.
   Chemical composition of fluid- Protein, Glucose, Sodium, Phosphorus, Chloride, Potassium, Magnesium, Copper, Zinc, Iron, Hematin, Ascorbic acid with neutral pH.

Body cavity of nematodes comprises Reproductive system, Nervous system and Excretory system. The **Circulatory** and **Respiratory** systems are absent in nematodes.

1. **Reproductive system of nematode:-**
   - The males are generally slightly smaller than females.
   - The nematodes are dioecious or amphigonus having a separate male and female within a species.
   - Generally the males are lesser in number than females or even be completely absent. This indicates a tendency towards hermaphroditism and parthenogenesis.

A. Female Reproductive system:-

- **Monodelphic-** The nematodes may have a single ovary the female is called as monodelphic.
- **Didelphic-** The nematodes may have two ovaries then the female is called as didelphic.
- **Prodelphic-** When a single gonad is present, it may be either directed towards anterior to vulva then female is called as prodelphic.
- **Opisthodelphic-** The gonad either directed towards posterior to vulva then female is opisthodelphic.
- **Amphidelphic-** The two ovaries are opposite to one another, such as one is anteriorly directed and other posteriorly directed.

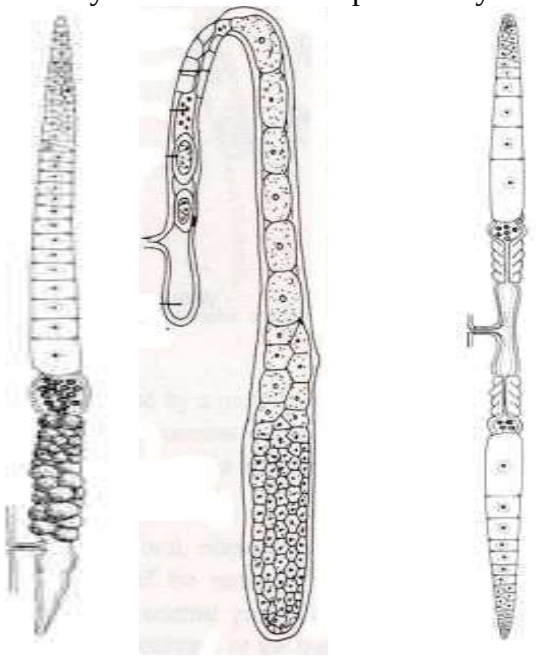


Fig. Prodelphic, Opisthodelphic and Amphidelphic

The female reproductive system typically consists of ovary, oviduct, uterus, vagina and vulva.

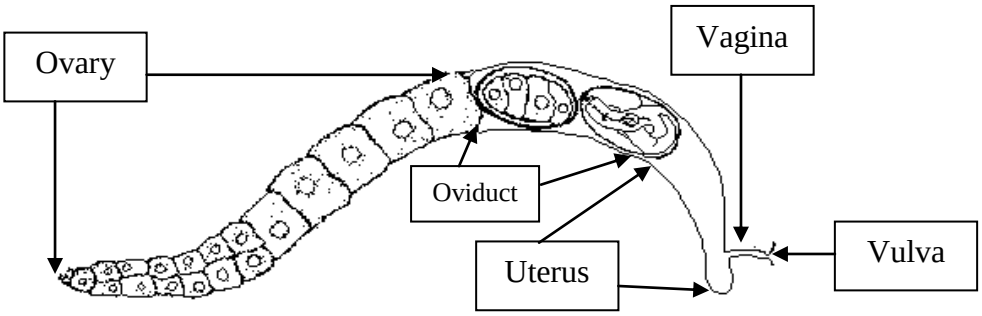


Fig. Female Reproductive System

(i) The ovary-

It is hollow elongate tube. The apical end of ovary has a cap cell at the tip which is called as germinal or zone of multiplication in which rapid cell division takes place to give rise germinal cells. This region is followed by growth zone which constitutes the greater part of ovary. The oocytes or germ cells in this zone become big and ripe which are generally arranged in single rows. After maturity they are called oogonia.

(ii) Oviduct-

Next to growth zone of ovary the gonad has oviduct. The oocytes when ripe they pass in to oviduct. Oviduct may serve as spermathica in some nematode. However in others, the spermathica is in the proximal part of uterus or in the post-vulvar sac at the distal end of gonad.

(iii) The uterus-

It is the largest and most complex part of the gonad, serves and function of fertilization, egg shell formation and laying of eggs. As started above, the upper part of uterus serves as spermathica in some nematodes.

(iv) Vagina-

The uterus entered in common vagina, which is a short, narrow and flattened tube lined with cuticle and provided with muscles.

(v) Vulva-

The vagina opens through female gonopore, the vulva. The eggs are expelled through a vulva which is normally situated middle of the body.

B. Male Reproductive system:-

- **Monarchic-** The nematode may have one testis are called monarchic.
- **Diarchic-** The nematode may have two testis are called diarchic.

The male reproductive system generally consists of three primordial parts: the testis, seminal vesicle, and vas deference.

(i) The Testis-

In the testis the germinal and growth zone can be easily distinguished. In germinal zone Spermatogonial division takes place, while in growth zone, spermatocytes increases in size. The spermatocytes are arranged in single or double rows.

(ii) Vas deference-

It consist of an anterior glandular region and posterior muscular region and containing the ejaculatory duct at the posterior end.

(iii) Ejaculatory duct-

The ejaculatory duct helps in the ejection of sperms during fertilization. It tapers gradually and opens ventrally into the cloca. The cloca is provided male copulatory structures such as spicules, gubernaculums etc.

2. Excretory system of Nematodes:-

The excretory system is not well developed in nematodes. The excretory pore is located in midventral line close to the nerve ring. The excretory system in nematodes are two types.

a. Glandular type
b. Tubular type

a. Glandular type:-

The glandular type consist of a single specialized cell known as renette cell. It has 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 found in members of the class Adenophorea.

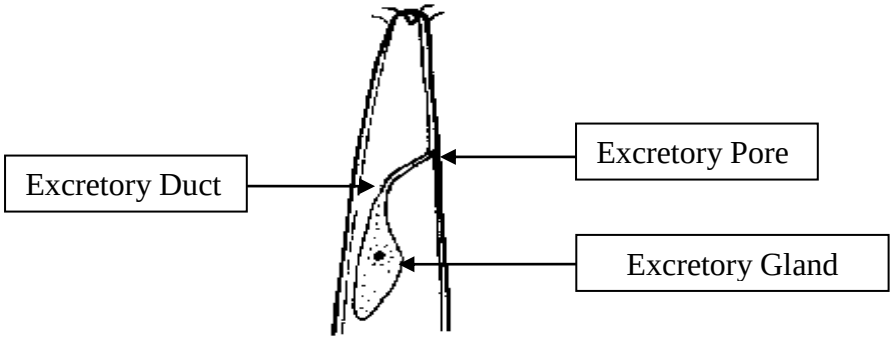


Fig. Glandular type

b. 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.

i) Asymmetrical or Tylenchid type
ii) Inverted 'U' shaped or Ascarid type
iii) Rhabditid type
iv) Simple 'H' shaped or Oxyrid type

i) Asymmetrical or Tylenchid type-

Majority of pant 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 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.

Fig: Asymmetrical or Tylenchid type

ii) 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.

Fig: Inverted 'U' shaped or Ascarid type

Fig.: Rhabditid type

iii) Rhabditid type-

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

Fig.: Simple 'H' shaped or Oxyrid type

v) Simple 'H' shaped or Oxyrid 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.

Functions of Excretory system:-

1. Excretions of toxic substances.
2. Secretion of certain chemicals.
3. Osmoregulation.
4. In *T. semipenetrans*, excretory pore secrets gelatinous matrix which bind and protect the eggs from abnormal environmental condition.

3. Nervous system of Nematodes:-

In nematodes, a central nervous system and a peripheral nervous system can be described.

Central nervous system-

It is also known as brain consist of nerve ring associated with ganglia and nerves. The nerve ring or circum-oesophageal commissure is belt which may be broad and flat. It is present around the oesophagus in majority of nematodes. In Tylenchida it encircles the isthumus while in Dorylaimida it is present around the narrow anterior part of oesophagus. The nerve ring is placed obliquetly with dorsal side most anterior. Towards the anterior end of nerve ring six ganglia are present (2 sub-dorsal, 2 sub-ventral and 2- lateral) known as papillary ganglia which are very small in size. Towards the posterior side of nerve ring nerves arise in the dorsal, lateral and ventral side of the body. Transverse commissure connecting the nerves are also present in different regions of the body.

Peripheral nervous system-

It includes somatic nerve, cephalic papillae nerve, amphidial nerve, amphids, phasmids, dierids, hemizonid, hemizonian and other associated structures.

1) Somatic nerve: The nerves which run longitudinally in the hypodermis are called as somatic nerves. The following are different types of somatic nerves-

a) Dorsal somatic nerve- Its originates from the dorsal ganglia of the posterior side of the nerve ring, goes through the dorsal chord up to anal region where it bifurgate and join the lumber ganglia.

b) Latero-dorsal nerve- It is paired structure originates from the nerve ring and extend towards the posterior side in sub median position. They also innervate the muscular layer.

c) Latero-ventral nerve- It originates from the nerve ring and extends posterior on sub median position.

d) Ventral nerve- It is the part of central nervous system.

e) Lateral nerve- It is in anal area and having lumber ganglion on each side.

f) Dorso lateral nerve- Paired nerve and joins the ventro lateral nerve in anal region.

2) Cephalic papillae nerve: These nerves go through the body cavity. These are nerve fibers arising from cephalic papillae ganglion from the cephalic nerve near the lips.

3) Amphidial nerve: In above the papillary ganglia are directly connected with nerve ring, while in this case the connection is indirect *i.e.* through sub-ventral trunk by lateral ventro commissure. Anteriorly each amphideal nerve enters to amphideal glands and their processes (nerves) breaks up in an elongate sac, which represent the neuron, are called terminals and pouch. Sensory elements which represent the neuron are called terminals and the group of such terminal is called as sensilla. The has an amphid aperture situated either on the lips (labial) or post labial and opening to the exterior. Internally the aperture is connected to a pouch (fovea) which leads to sensilla pouch or fusus through an amphid duct or canalis amphidianlis. The sensilla pouch is connected to the amphidial nerve through the nerve process.

4) Amphids: Amphids are paired lateral sensory organs probably chemoreceptors situated in cephalic region of the nematode. The amphid aperture exhibited various shapes *viz.*, pore like, circular hooked, stirrup-shaped, spiral *etc.* There may be sexual dimorphism in respect of amphids, they may be larger in males than females or may be more complex in males.

5) Phasmids: Phasmids are paired lateral sense organs, usually one on each side of tail at lateral fields. The phasmids open to the exterior through a minute pore. Internal, they possess a canal or pouch containing sensory receptors which are supplied by lateral caudal nerve. In a few nematode species they may enlarged and known as scutella.

6) Deirids: They are the paired papillae situated in the mid region of the body (oesophageal region) opposite to the excretory pore. These are sensory structures but do not have an opening to the exterior. They are also called as cervical papillae. They function as mechanoreceptors.

7) Hemizonid and Hemizonion: The hemizonid (belt or girdle) is highly refractive biconvex structure forming a semi-circle in the ventral side of the body and ending at the lateral fields, located either anterior or posterior to the excretory pore and situated between the cuticle and hypodermis.

Hemizonion is small nerve commissure which is structurally identical to hemizonid and situated posterior to it.

8) Cephalids: Like hemizonid and hemizonions cephalids are also highly refractive band like structures situated in the cuticle dorsally and ventrally, but they form a complete ring or round the body, anteriorly just behind the cephalic region. There are two pairs. Generally, the lateral epidermal chord originate at a level of posterior cephalids.

9) Caudalids: It is a small nerve commissure situated in caudal region slightly posterior to anus and linking the pre-anal ganglion to the lumber ganglion.

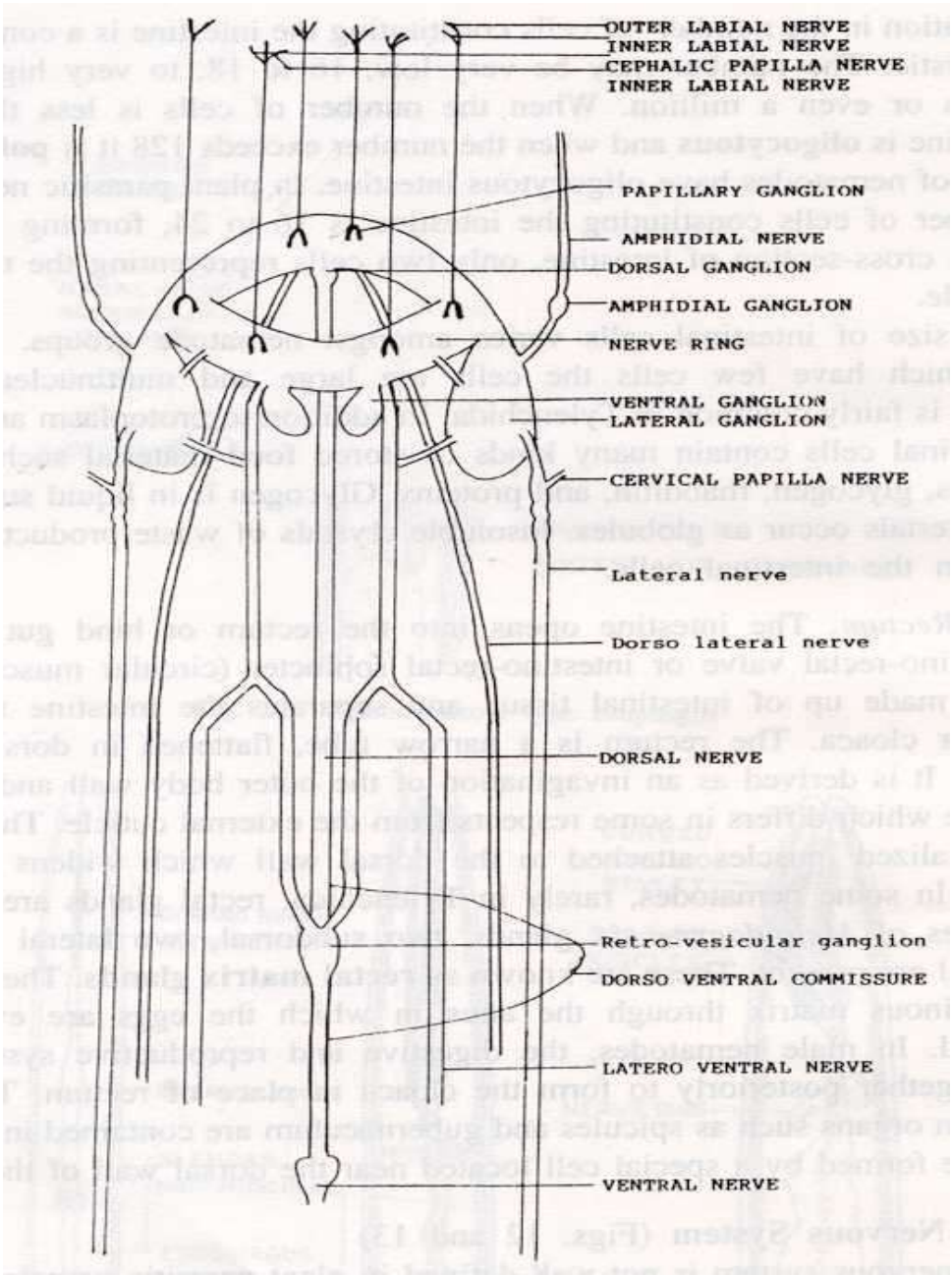


Fig. Anterior nervous system of nematode

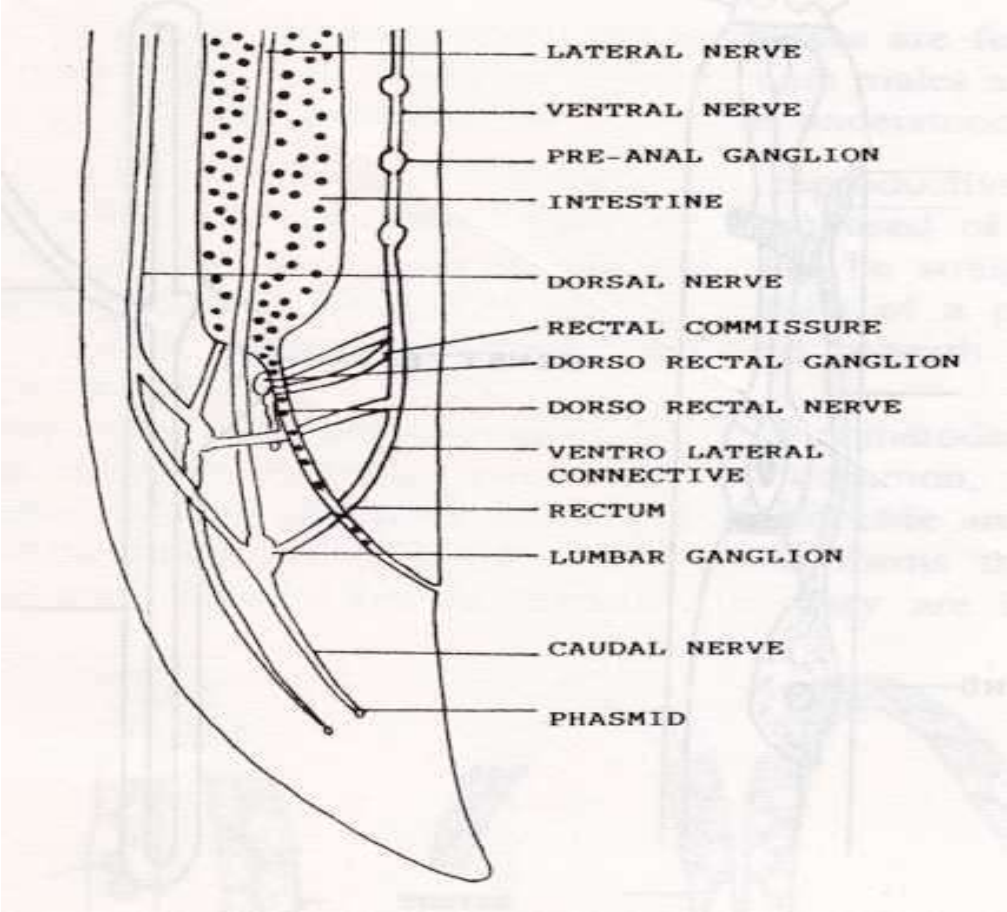


Fig. Posterior nervous system of nematode

Biology of Plant Parasitic Nematodes

Under biology, the life cycle of nematode is studied. The life history of most plant parasitic nematodes is simple and direct. The basic pattern of life cycle is given bellow:-

The following six stages or instars are found in the life cycle of a primitive nematode:

(i) The egg
(ii) The first stage larva or juvenile (L1)
(iii) The second stage larva or juvenile (L2)
(iv) The third stage larva or juvenile (L3)
(v) The fourth stage larva or juvenile (L4)
(vi) The adult

.

i) The Egg:

The egg of most of the nematodes is similar in shape (oval) and size irrespective of the adult nematode. The eggs are covered by three membranes,

a) The external protein layer- It secreted by uterus wall.

b) The middle chitinous layer or true shell- It secreted by egg itself.

c) The inner lipid layer- It is soluble in various dehydrating agents and made up of protein and lipid.

These three layers are not found in most of the Tylenchids. The amount of chitin in chitinous layers differs in different species of nematodes.

ii) Embryonic development:

After discharge of egg by female, it starts dividing by cleavage of their protoplasm's to form cells. The first cleavage occurs transverse to the longitudinal axis and gives two equal cells or blastomeres which are the first somatic (S_1) cell and the parental germinal (P_1) cells. The second cleavage results in four cells which are first arranged in a 'T' shape. This shape achieved by the blastomere S_1 dividing longitudinally and blastomere P_1 dividing transversally by P_2 and S_2. At last these cells get arranged in rhomboidal shape. The transverse and longitudinal mitotic division of daughter cells continues. The S_1 blastomere is the primary somatic cell and its two products (A & B) produce most of the nematodes ectodermal cells. The S_2 blastomere produces somatic tissues and gives rise to ectoderm (E), mesoderm (M) and stomoderm (St) tissues. The gonads of nematode are derived from P_1. 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 consist 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, band P_2 divide to give P_3 and S_3. The dorsal cells produced by A and B continue to divide and finally give to most of the hypodermis, excretory cells and nervous system. The daughter cells P_2 divides into P_4 and S_4. These S_3 and S_4 are ectodermal and produce the hypodermis in the posterior region of nematodes body.

The ectodermal tissue is produced from the products of cell E_1 and P_1 divides into P_5 and S_5. The descendants of S_5 give rise the epithelium which covers the gonads and their ducts while the products of P_5. G_1 and G_2 and their descendants proliferate germ cells only.

The primary mesodermal cells M gives rise the nematodes body wall musculative and its Pseudocoelomic cells, while the pharynx from St cells. During early embryonic stages, these primary cells St, M and E present on ventral surface of the embryo and are taken within the dorso-ventrally flattened and anterior-posteriorly directed embryo is changed to cylindrical shape. The embryo starts to become worm-shaped and coiled larva 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. In plant parasitic nematodes the stylet is also present.

ii) Post-embryonic development:

The post embryonic development in plant parasitic nematodes takes place within the egg leading to the formation of larva which is ready to undergo first moult. In the process of post embryonic development, the organ differentiation, hatching and moulting are the important stages.

iii) Hatching:

Hatching occurs in response to stimuli from host or it take place under favorable environment. In cyst forming nematodes, the release of larva from cyst is an emergence not hatching. Egg have hatched within the cyst. The egg of *Globodera rostochinensis* generally hatch in response to root exudates (stimuli) provided by the solanaceous crop *viz.*, Potato and tomato. After embryonic development the first stage larva are found within the egg. After reaching a particular stage of growth and hatching condition are present, the larva sows vigorous movement, often causing bulging of the egg membrane as seen in case of *Pratylenchus, Paratylenchus, Nacobbus* and *Meloidogyne.* After that the larva makes a series of thrusts with the help of stylet on the egg shell @ 40-90 per minute.

iv) Moulting (Exclusion):

The growth in nematode is associated with moulting which usually occurs four times and there are five stages. After the fourth moult the nematode become full grown adult. During moulting, the entire cuticle, including the cuticular lining of stoma, stylet, oesophagus, vulva, cloaca, rectum, amphids, phasmids and excretory pore is shed. In most plant parasitic nematodes greatest growth occurs after the last moult and moulting tends to occur in the earlier half of the growth curve.

v) The 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 exudations act as stimulus *e.g.* in case of *Pratylenchus nanus* root exudes will act stimulus during 4^{th} moult. In endoparsitic nematodes of plants, the stimulus may be more complex and may be closely associated with an increase in size of nematode, because in these nematodes moulting does not occur until some growth has completed within the host. The receptor may function as stretch receptor in this case.

It is well association with neirosecretory cell which leads to production of enzyme which initiates the moulting. The nematode cuticle may shed in one piece in *Pratylenchus* the lining of amphids; oesophagus, excretory duct phasmid and rectum are shed with moult.

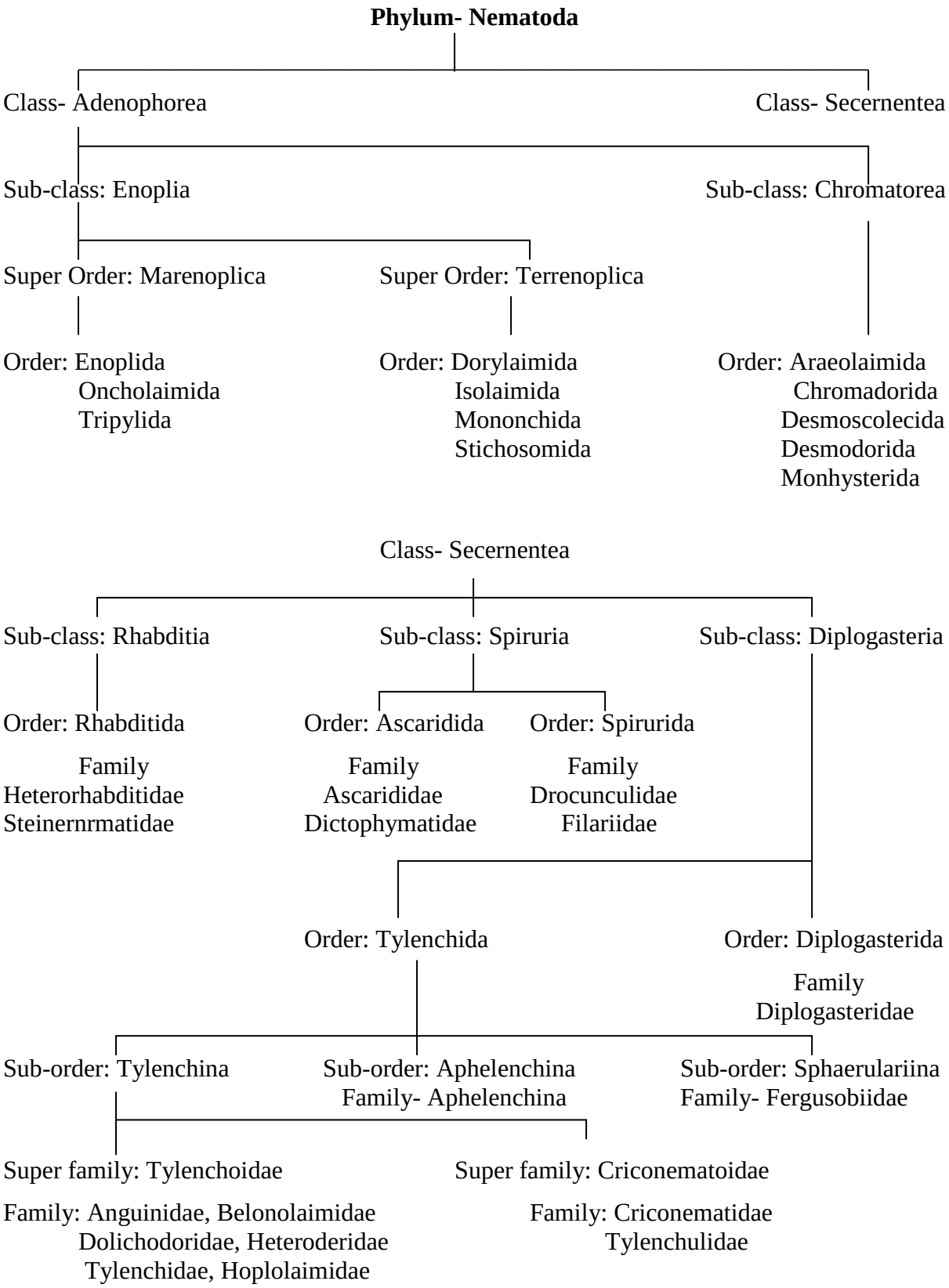
Prior to moulting the hypodermis increase in thickness due to accumulation of ribosome and protein.

Significance of Life History:

1. Life cycle of nematodes should be understood, when control measures are considered.
2. The wheat gall nematode may effectively control by crop rotation with non-host plant.
3. Emergence of larva from gall is virtually complete when soil moisture and temperature between favorable.
4. Larvae die when they are outside the gall in absence of host.

LECTURE NO. : - 4

CLASSIFICATION OF NEMATODE (Taxonomic)



LECTURE NO. : - 5

ECOLOGICAL CLASSIFICATION OF NEAMATODE

(BY HABITAT)

There are two major classes

I. Above ground feeder
II. Below ground feeder

I. Above ground feeder

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: *Dictylenchus*
b. Feeding on tree trunk
   i) Red ring nematode: *Rhadinaphelenchus cocophilus*
   ii) Pine wilt nematode: *Bursaphelenchus xylophilus*

II. Bellow ground feeder

It is again classified in to three classes

I) Endoparasitic nematodes
II) Semiendoparasitic nematodes
III) Ectoparasitic nematodes

a) Endoparasitic nematodes

The entire nematode is found inside the root and the major portion of nematode body found inside the plant tissues. They are two types

1) Migratory endoparasite: - These nematodes move in cortical parenchyma of host root. While migrating they feed on cells, multiply and cause necrotic lesion. Example, *Pratylenchus* spp., *Radopholus* spp. and *Hirschmanniella* spp.
2) Sedentory endoparasite: - the second stage larvae penetrate the root lets and become sedentary throughout the life cycle, inside the root cortex. Examples, *Heterodera* spp. and *Meloidogyne* spp.

b) Semiendoparasitic nematodes

The anterior part of nematode, head and neck being permanently fixed in the cortex and the posterior part extends free into the soil. Examples, *Rotylenchulus reniformis* and *Tylenchulus semipenetrans*.

c) Ectoparasitic nematodes

These nematodes live freely in the soil and move closely or on the root surface, feed intermittently on the epidermis and root hair near the root tip. They are two types,

1) Migratory ectoparasites:- These nematodes spend their entire life cycle free in the soil, feeding externally on the host plants, deposit eggs in soil. When the roots are disturbed they detach themselves. Examples, *Criconemoides* spp., *Paratylenchus* spp. and *s* spp., etc.
2) Sedentory ectoparasites:- 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. Examples, *Hemicycliophora arenaria* and *Trichodorus* spp., etc.

- **Diagnostic characters of class Secernentea and Class Adenophora**

Sr. No.	Class- Secernetea	Class- Adenophorea
1	Amphidial opening is on the head near the lip region.	Amphids open behind the head.
2	Lateral canals open into the excretory duct.	Lateral canals & excretory duct end in a cell.
3	Oesophagous is divided into procorpus, mediun bulb, isthumus & labial bulb.	Oesophagous is cylindrical with an enlarged base.
4	Male tail with bursa (caudal alae)	Male tail lacks bursa but possess genital papillae.
5	Caudal glands are absent.	Caudal glands are present.
6	The mesenterial tissues are less developed.	The mesenterial tissues are well developed.

- **Differences between sub-order Tylenchina and sub-order Aphelenchina**

Character	Tylenchina	Aphelenchina
Lip	Varying in shape	Set-off
Annules	Faint to strong annules.	Faint annules.
Stylet	Well developed; one dorsal & two sub ventral knob.	Weekly developed; no stylet knob.
Oesophagous	Three parted	Three parted with square shaped medium bulb.
Gland opening	Behind the stylet knob in procorpus	Open in the mediun bulb.
Female	One or two ovary, vulval position vary.	Single ovary; vulva posterior.
Male	Bursa present	Bursa rare
Spicule	Weak to strong sclerotization is seen with gubernaculums	Rose thorne shape spicule present.

- **Differences between family Tylenchoidea and family Criconematoidea**

Character	Tylenchoidea	Criconematoidea
Labial region	Lips are hexaradiate, labial frame work present.	Labial region is poorly developed, labial plate present.
Stylet	Conus, shaft and knobs are variable in shape	Criconematoid type stylet; long and anchor shape knob which lies in base of metacarpus.
Oesophagous	Narrow procarpus, round metacarpus with vaue, isthumus followed by glandular basal bulb.	Pro and metacarpus amulgameted to a single unit, short isthumus, the post carpus reduced, appears as ‘set-off’, smaller than pro & 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 preset.	Single testis; caudal alae rare.
Phasmid	Eratically present in tail region.	Not known.

LECTURE NO. : - 6

IMPORTANT PLANT PARASITIC NEMATODES

1) Root-knot Nematode, *Meloidogyne* spp.

Systematic Position:-

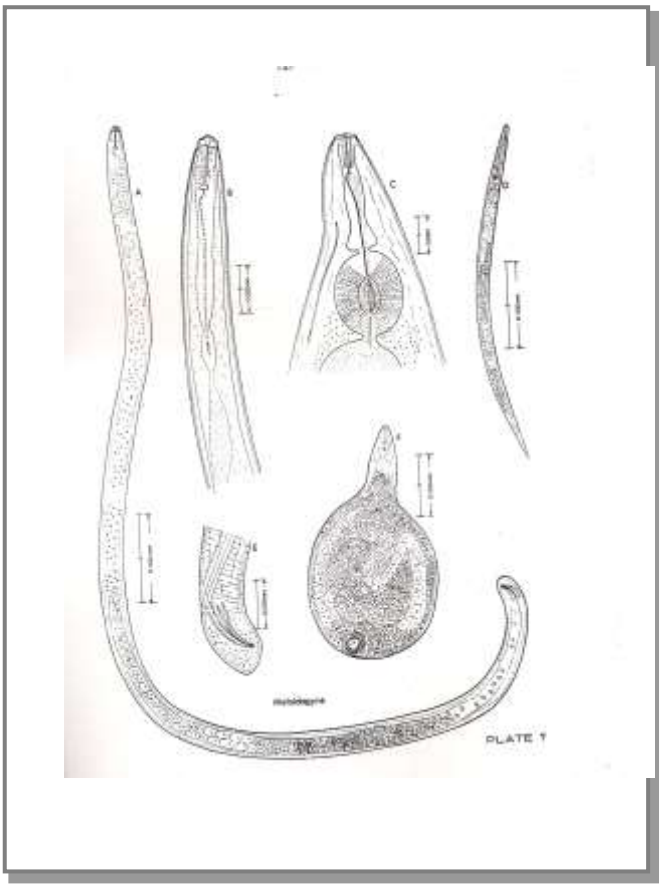
Order - Tylenchida
Sub order - Tylenchina
Super family - Tylenchoidea
Family - Heteroderidae
Sub family - Meloidogyninae
Genus - Meloidogyne
Species -
i) *incognita*
ii) *javanica*
iii) *arenaria*
iv) *hapla*

Parasitism & Habitat:-

i) Females and III & IV stage of larvae are Sedentory endoparasites.
ii) Males and II stage larvae are migratory.

Morphological characters:-

i) Body - Elongate larvae and male typically saccate, spheroid with a distinct neck in females.
ii) Stylet - In males, Strong with rounded knob & in females, more slender than males.
iii) Oesophagous - With large median bulb followed by short isthumus.
iv) Excretory pore - Often seen with part of excretory tube in the area between posterior part of stylet knobs and opposite to median bulb.
v) Vulvas & anus - Females typically opposite to neck and surrounded by a pattern of fine lines like human fingerprint.(Perennial pattern)
vi) Spicule - Very near the terminus of males Bursa is absent.



Symptoms:-

- Yellowing of leaves
- Stunted growth
- Reduced vigor
- Reduced size & number of fruits
- Gall formation

- **Multinucleate cell** – Giant cell (Nurse Cell)
- **Hypertrophy** – Enlargement of cell
- **Hyperplasia –** Multiplication of cell

Control:-

- Two to three deep Ploughing
- Rotation with cereal crops
- Apply carbofuron (Furdan 3G) @ 7 g/m^2
- Resistant varieties of Tomato eg. Hisar Lalit, PNR 7

2) Reniform Nematode, *Rotylenchulus reniformis*

Systematic Position:-

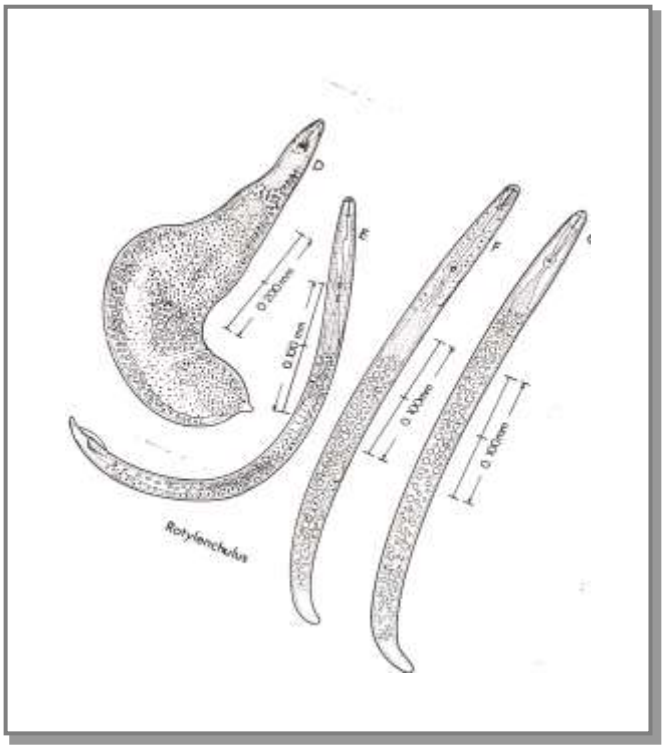
Order	- Tylenchida
Sub order	- Tylenchina
Super family	- Hoplolaimoidea
Family	- Hoplolaimidae
Sub family	- Hoplolaiminae
Genus	- *Rotylenchulus*
Species	- *reniformis*

Parasitism & Habitat:- Females are Semiendoparasitic on many plants.

Morphological characters:-

i) Body - Males and immature females are slender and small, adult females are kidney shaped

ii) Oesophagous - Dorsal oesophageal glands opens about one stylet length posterior to stylet knobs.



Symptoms:-

Yellowing of leaves, delayed germination, reduced plant growth and vigor, stunted growth, browning of roots due to penetration of nematode are the general symptoms of this nematode. Young and tender plants are more vulunerable to nematode attack.

3) Root-lesion Nematode, *Pratylenchus* spp.

Systematic Position:-

Order	- Tylenchida
Sub order	- Tylenchina
Super family	- Tylenchoidea
Family	- Pratylenchidae
Sub family	- Pratylenchinae
Genus	- *Pratylenchus*

Species - i) *coffeae* - Citrus, Banana & coffee
ii) *zeae* - Maize
iii) *thornei* - Pulses

Parasitism & Habitat:-
- Migratory endoparasites
- Feeding on root cortex of many crop/plant
- All stages found in root or soil.

Morphological characters:-
i) Body length - 0.4-0.8 mm.
ii) Lip region - Slightly set-off from body.
iii) Stylet - Typically short, strong with massive knob.
iv) Ovary - Monodelphic
v) Vulva - Posterior fourth of the body (75-80%).
vi) Tail - Nearly round to pointed and in males, the tail has bursa.

Symptoms:-

Late emergence of seedlings, less germination and stunted growth with necrotic lesions on the root surface which are initially small coalesce at the later stage and cause death of the rootlets. Root system is reduced

Control:-
- Raise nursery in nematode free soil
- Pull and burn infected plants

4) Spiral Nematode, *Helicotylenchus* spp.

Systematic Position:-

Order - Tylenchida
Sub order - Tylenchina
Super family - Tylenchoidea
Family - Hoplolaimidae
Sub family - Rotylenchoidinae
Genus - *Helicotylenchus*

Parasitism & Habitat:- Endoparasitic and ectoparasitic on many plants

Morphological characters:-
i) Body - Arcuate to 'C' shape when relaxed
ii) Stylet - Moderately long, typically located more than one half stylet length posterior to stylet knobs.
iii) Ovaries - Two (didelphic)
iv) Vulva - Posterior to middle of body (60-70%)
v) Tail - In females, rounded to nearly pointed often with short projection on ventral side and In males, tail is short with bursa.

Symptoms: - The nematodes attack root cortex and produce necrotic lesions.

5) Cyst Nematode, *Heterodera* spp & *Globodera* spp.

Cyst means any abnormal membranous sac or blister like pouch containing fluid.

Systematic Position:-

Order - Tylenchida
Sub order - Tylenchina
Super family - Tylenchoidea
Family - Heteroderidae
Sub family - Heteroderinae

Genus - i) *Heterodera*
ii) *Globodera*

Species of *Heterodera* -

i) *avenae* - Cereal cyst nematode(wheat & barley) found in north India
ii) *zeae* - Maize cyst nematode
iii) *cajani* - Pigeon pea cyst nematode (tur, mung, Udid & cowpea)
iv) *oryzicola* - Rice cyst nematode (rice & banana) found in Kerala, M.P., Orissa & West Bengal.

Species of *Globodera* -

i) *rostochinensis* - Potato cyst nematode or Golden nematode
ii) *pallida*

Host plants - Potato, Tomato & Brinjal

Parasitism & Habitat:-

Parasitic on many plants mostly in temperate zone (Notably potatoes, sugar beets, oats & other grains, clover, soybean & various cruciferous)

Morphological characters:-

i) Body - Slender in males (1.0-2.0 mm) and larvae (0.3-0.6 mm) In females, typically swollen, lemon shaped (0.5-0.8 mm)
ii) Colour - White or yellow, cyst dark brown, lemon shaped (0.8 mm long & 0.5 mm wide) or nearly same shape as that *Meloidogyne* female.
iii) Stylet - Short in males with rounded basal knobs & in larvae, more than 0.02 mm long.
iv) Oesophagous - With well-developed median bulb & lobe extending back & overlapping the intestine.
v) Spicule - Near the posterior end of males

Globodera - Similar to *Heterodera* spp. slight difference in adult females are globular (rounded) in shape and hence the genus is named as *Globodera*.

Symptoms:-

Heterodera - The diseased plants show yellowing of leaves, stunted growth, reduced tillering. Earheads if formed are very small known as **'Molya'** disease'

Globodera - Typical symptoms of heavy infestation are stunted plants with unhealthy foliage, premature yellowing, poor development of root system, reduction in size and number of tubers. Such plants exhibit temporary wilting during hotter part of the day.

Control:-

Heterodera

- Two- three summer ploughing at 10-15 days interval.
- Rotation with Mustard, chick pea
- Apply Carbofuron @ 1-2 kg a.i./ha.

Globodera

- Rotation with pea, cabbage, carrot, cauliflower during autumn season.
- Grow resistant varieties of potatoes - Kufri Swarna, Kufri Thenmalai

6) Daggar Nematode, *Xiphinema* spp.

Systematic Position:-

Order - Dorylaimida
Sub order - Dorylaimina
Super family - Dorylaimoidea
Family - Longidoridae
Sub family - Xiphineminae

Genus - *Xiphinema*

Parasitism & Habitat: - Migratory ectoparasites

Morphological characters:-

i) Body - Females elongate, cylindrical, forming open spiral with a greater curvature in posterior half.
ii) Stylet - Typically long..
iii) Ovaries - Monodelphic or didelphic.
iv) Vulva - Situated at middle of body.
v) Tail - Bluntly rounded or with projections on ventral side in both males and females.
vi) Males extremely rear, not essential for reproduction.

Symptoms: - Attacked roots show necrosis, lack of laterals, terminal swelling, root galling etc.

7) Rice stem Nematode, *Dictylenchus angustus*

Systematic Position:-

Order - Tylenchida
Sub order - Tylenchina
Super family - Tylenchoidea
Family - Anguinidae
Sub family - Anguininae
Genus - *Dictylenchus*
Species - *angustus*

Morphological characters:-

i) Body - Females swollen, when relaxed 'C' shaped.
ii) Stylet - Small with delicate knob.
iii) Oesophagous - Basal oesophageal bulb not overlapping the intestine, cardia absent.
iv) Vulva - Situated in posterior region of body.
v) Ovary - Single Prodelphic.
vi) Tail - Elongate.
vii) Males similar to females but more slender caudal alae subterminal.

Disease Caused:- Alfa disease of Rice.

Symptoms:-

At vegetative phase, yellowing or white splash pattern of leaf sheath where margins become concorted. Later splash patterns develop brownish stains and internodes and stems turn black.

At the reproductive phase, the nematode collects around the floral primordia and feed upon the developing earheads. Earheads emerges as crinkled or twisted with empty spiklets **(ripe ufra)** or does not emerge at all **(swollen ufra).**

8) Citrus Nematode, *Tylenchulus semipenetrans*

Systematic Position:-

Order - Tylenchida
Sub order - Tylenchina
Super family - Criconematoidea
Family - Tylenchulidae
Sub family - Tylenchulinae
Genus - *Tylenchulus*
Species - *semipenetrans*

Parasitism :- Endoparasitic on roots of citrus and other plants. Mature females are semiendoparasitic.

Morphological characters:-

i) Body - Small all stages. Mature females swollen.
ii) Stylet - Small in larvaes and males, well developed in mature females.
iii) Oesophagous - With distinct posterior bulb in larvae young males and immature females.
iv) Vulva - Prominent in posterior end of young and adult females.
v) Excretory pore - Typically situated posteriorly in protuberance just anterior to vulva.
vi) Anus - Absent or difficult to see in immature stages.
vii) Bursa - Absent.

Symptoms:-

The diseased trees show reduction in growth and vigor with yellowing of leaves. Such trees show gradual dieback symptoms starting from the uppermost portion.

Roots of infected trees appear larger in diameter and darker than the healthy trees mainly due to adherence of soil particles to the gelationous matrix excreted by the adult females. Cortex of highy infested feeder roots decays and gets sloughed off easily.

9) Burrowing Nematode, *Radopholus similis*

Systematic Position:-

Order	- Tylenchida
Sub order	- Tylenchina
Super family	- Tylenchoidea
Family	- Pratylenchidae
Sub family	- Pratylenchinae
Genus	- *Radopholus*
Species	- *similus*

Parasitism: - Endoparasitic on roots of Banana and citrus.

Morphological characters:-

i) Body - 0.4-0.9 mm in length.
ii) Lip - Rounded in females, set off and knob like in males.
iii) Stylet - Short and stout in females, slender and rudimentary in males.
iv) Oesophagous - Forming a lobe, dorsally overlaps to intestine.
v) Vulva - Located at middle of the body.
vi) Ovaries - Didelphic
vii) Tail - Blunt end in females and male long tail with bursa.

Symptoms:-

In banana, bearing plants show poor growth and small fruit size, prone to toppling over under high wind pressure. The nematode causes wounding of roots resulting in reddish brown cortical lesions which are clearly visible by splitting the affected roots longitudinally. Purplish streaks on the young roots. The lesions lead to the formation of tunnels and cavities in the roots. The infection spreads to young suckers also in which necrotic tissues develop.

LECTURE NO. : - 7

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.* Nematode 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 disease can be classified as

A) Symptoms produced by above ground feeder nematodes
B) Symptoms produced by below ground feeder nematodes

A) Symptoms produced by above ground feeder nematodes

i) Dead or devitalized buds – Nematode infection kills growing buds *e.g. Aphelenchoides fragariae* on strawberry.

ii) Crinkled stems and foliage - *e.g.* Wheat gall nematode, *Anguina tritici* Ulfa disease of rice, *Ditylenchus angustus.*

iii) Seed galls – *e.g.* Wheat gall nematode, *Anguina tritici* larva enter into the flower primordium and develops in to a gall.

iv) Necrosis & discolouration – *e.g.* Red ring disease of coconut, *Rhadinaphelenchus cocophilus*. Due to the infestation, red coloured circular area appear in the trunk of infested palm.

v) Leaf lesions - Symptom on broad-leafed foliage plants. *e.g.* Chrysanthemum foliar nematode, *Aphelenchoides ritzemabosi*

vi) Twisting of leaves and stem: *e.g.* In onion basal leaves become twisted when infested with *Ditylenchus dipsaci.*

vii) Leaf discolouration: The leaf tip become white in rice due to rice white tip nematode *Aphelenchoides besseyi.*

B) Symptoms produced by below ground feeder nematodes

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

I) Above ground symptoms
II) Below ground symptoms

I) <u>Above ground symptoms:-</u>

i. Stunting: Reduced plant growth and the plant cannot able to withstand in adverse conditions. Patches of stunted plants appears in the field. *e.g. Heterodera avenae* – Molya disease in wheat & barley. *Globodera rostochiensis* – Golden nematode in potato

ii. Discolouration of foliage: Also due to nutritional deficiency

e.g. Root lesion nematode, *Pratylenchus coffeae*
White tip nematode, *Aphelenchoids besseyi*
Citrus nematode, *Tylenchulus semipenetrans*

iii. Wilting: *e.g.* Root-knot nematodes, *Meloidogyne spp*

iv. Decline and die back: eg. In banana decline and die back are caused by *Radopholus similis.*

II) <u>Below ground symptoms:-</u>

i. Root galling: *e.g.* ***Meloidogyne spp***. – Characteristic galls on host roots
***Nacobbus spp* –** Larger galls on beet & tomato
***Ditylenchus radicicola* –** Small galls on cereals.
***Hemicycliophora arenaria* – G**alling on lemon roots
***Xiphinema diversicaudatum* - G**alling on roses

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

a) Stubby roots – Stubby branches or rootlets arranged in cluster eg. *Trichodorus christiei* on corn

b) Coarse root – Lateral roots stopped growth with no branches *e.g. Belonolaimus longicaudatus* on corn.

c) Curly root – The nematode retard the elongation of roots and cause curling of roots known as **'Fish hook'** symptom. Eg. Injury caused by *Xiphinema* spp.

iii) Root lesions – Necrotic lesions *e.g. Pratylenchus spp* (soybean), *Radopholus similis* (citrus & banana), *Helicotylenchus multicinctus* (banana)

iv) Rotting – Nematode + Micro-organisms. *e.g. Ditylenchus destructor – potato rot.*

v) Excessive root branching – *e.g. Meloidogyne hapla* in tomato

LECTURE NO. : - 8

INTERACTION OF NEMATODES WITH MICRO-ORGANISMS

Plant parasitic nematodes favor 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. The nematode cause mechanical injury which favors the entry of microorganisms. The association of nematode and pathogen break the resistant in resistant cultivar of crop plant.

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 crops 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	*Meloidogyne incognita* *Meloidogyne incognita*	*Rhizoctonia solani* *Pythium debarynum*	Assist
	Vascular wilt	*M. incognita*	*Fusarium oxysporrum* *F. vasinfectum*	Assist
		Rotylenchulus reniformis	*Fusarium oxysporrum* *F. vasinfectum*	Assist
Tobacco	Dampink off	*Meloidogyne incognita* *Meloidogyne incognita*	*Pythium debarynum* *Alternaria tenuis*	Assist
	Vascular wilt	*Meloidogyne incognita* *Meloidogyne incognita*	*Fusarium oxysporrum* *Fusarium parasitica*	Assist
Banana	Vascular wilt	*Radopholus similis*	*Fusarium oxysporrum*	Essential
Tomato	Cortical rot	*Globodera rostochinensis*	*Rhizoctonia solani*	Assist
	Vascular wilt	*Meloidogyne* spp.	*Fusarium oxysporrum*	Assist
Potato	Damping off	*Ditylenchus destructor*	*Ppytophthora infestans*	Assist
	Cortical rot	*Globodera rostochinensis* *Globodera rostochinensis*	*Rhizoctonia solani* *Verticillium dahliae*	Assist
Onion	Damping off	*Ditylenchus dispaci*	*Botrytis allii*	Assist
Brinjal	Vascular wilt	*Pratylenchus penetrans*	*Verticilllium dahliae*	Assist
Pea	Vascular wilt	*Pratylenchus* spp.	*Fusarium oxysporrum*	Assist
		Pratylenchus penetrans	*Fusarium pisi*	Assist
Soybean	Damping off	*Meloidogyne javanica*	*Rhizoctonia solani*	Assist
	Vascular wilt	*Heterodera glycines*	*Fusarium* spp.	Assist
Cow pea	Vascular wilt	*Meloidogyne javanica*	*Fusarium oxysporrum*	Assist
wheat	Stem rot	*Anguina tritici*	*Dilophospora alopecuri*	Essential
	Wheat rot	*Heterodera avenae*	*Rhizoctonia solani*	Assist

Nematode - Bacterium Interaction

Nematode - bacterium interaction comparatively fewer than the nematode - fungal interactions. Some examples of nematode - bacterium interactions are presented in the following table.

Crop	Name of the disease	Nematode	Bacteria	Role of nematode
Wheat	Tundu	*Anguina tritici*	*Clavibactor tritici*	Essential
Tobacco	Vascular wilt	*Meloidogyne incognita*	*Pseudomonas solanacearum*	Assist
Tomato	Vascular wilt	*Meloidogyne hapla* *Meloidogyne incognita*	*Pseudomonas solanacearum*	Assist
		Helicotylenchus nannus	*Pseudomonas solanacearum*	Assist
	Canker	*Meloidogyne incognita*	*Clavibactor michiganens*	Assist
Potato	Vascular wilt	*Meloidogyne* spp.	*Pseudomonas solanacearum*	Assist

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 a vector of grapevine fan virus. *Xiphinema, Longidorus, Paralongidorus* spp. transmits the ring spot viruses called NEPO derived from nematode transmitted polyhedral shaped particles. *Trichodorus* spp. and *Paratrichodorus* spp. transmitted rattle virus called NETU derived from nematode transmitted tubular shape virus particles. All these nematodes have modified bottle shape oesophagus.

NEPO Virus	Nematode
Arabis mosaic	*Xiphinema diversicaudatum* *X. paraelongatum*
Grapevine fan leaf	*X. index*
Grapevine yellow mosaic	*X. index*
Tobaco ring spot	*X. americanum*
Cowpea mosaic	*X. basiri*
Tomato black ring, beet ring spot	*L. elongates*
Tomato black ring, lettuce ring spot	*L. attenuatus*
NETU Virus	**Nematode**
Tobacco rattle	*Paratrichodorus* *P. allius, P. nanus* *P. porosus, P. teres* *Trichodorus christei* *T. primitivus, T. cylindricus* *T. hooperi* *T. minor, T. similes*
Pea early browning	*P. anemones, P. pachydermus* *P. teres, T. viruliferus*

The nematodes acquire and transmit the virus by feeding required little one day. Once acquired it persist for longer time in nematode body *e.g.* Grapevine fan leaf virus will exist upto 60 days in *X. index.*

LECTURE NO. : - 9

DIFFERENT METHODES OF NEMATODE MANAGEMENT

Plant parasitic nematodes can be controlled by several methods. In view to keep the nematode population below economic threshold level. The management tactics should be profitable and cost effective. It is essential to calculate the benefit ratio before adopting control measures.

The nematode control methods are

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

LECTURE NO. : - 10

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.

Adjusting the time of planting:

Nematode life cycle depends on the climatic factors. Adjusting the time of planting helps to avoid nematode damage. When the crops planted in winter the soil temperature is low and at that time the nematodes can not be active at low temperature.

Fallowing:

Leaving the field without cultivation, preferably after ploughing helps to expose the nematodes to sunlight and the nematode 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 intern 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 sheet which enhances the soil temperature by 5 to 10^0C which kill nematodes in seed bed.

Manuring:

Raising green manure crops and addition of more amounts of farm yard manure, oil cakes of neem and castor, press mud 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 soil which kills the nematodes by asphyxiation.

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.

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 – terthinyl and bithinyl compounds are present throughout the plant from root to shoot tips. These chemicals kill the nematodes.

Removal and destruction of infested plants:

Early detection of infested plants and removal helps to reduce 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 inoculation for the next season crops.

Use of resistant varieties:

Nematode resistant varieties have been reported from time to time in different crops. **Nemared, Nematex, Hisar Lalit** and **Atkinson** are tomato varieties resistant to *Meloidogyne incognita*. The potato variety **Kufri Swarna** is resistant to *Globodera rostochiensis.*

LECTURE NO. : - 11

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 method in field conditions. These physical treatment may be hazardous to plant or the man working with the treatments and the radiation treatments may have residual effects.

Heat:

a) 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.

b) 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, seed and roots of seedlings can be dipped in hot water at 50-55^{0}C for 10 minutes and then planted.

Irradiation:

Irradiation also kill the nematodes. Cyst of *Globodera rostochiensis* exposed to 20,000γ contained only dead eggs and at 40,000 γ exposure, the eggs lost their contents.

Osmotic pressure:

Feder (1960) reported 100% nematode mortality when sucrose or dextrose was added to nematode infested soil @ 1 to 5% by weight. But this method is 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 material helps to avoid the nematodes in spreading in new planting field.

Seed cleaning:

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

Ultrasonic:

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

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LECTURE NO. : - 12

BIOLOGICAL CONTROL

Biological control aims to manipulate the parasites, predators and pathogens of nematodes in the rhizosphere in order to control the plant parasitic 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 intern check the plant parasitic nematodes.

The addition of organic amendments acts in several ways against the plant parasitic nematodes. Organic acids such as formic, acetic, propionic and butyric 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.

Organic amendments improve soil conditions and help the plants to grow. The organic matter also provides nutrition for the crops plants.

Predacious Nematodes:

Predacious nematodes have specialized open stoma armed with teeth to catch and swallow the plant parasitic nematodes. Addition of organic amendments helps to encourage the multiplication of predacious nematodes such as *Mononchus* spp. Other genera like *Diplogaster* spp. and *Tripyla* spp. are also come under the group of predacious nematodes.

Predacious Fungi:

Most of the predacious fungi comes under Moniliales and Pjycomycetes. There are two types of predacious activities among these fungi. They are nematode

a) Trapping fungi and
b) Endozoic fungi

a) Trapping fungi:

The nematode trapping fungi have adhesive networks and sticky knobs produced by the mycelium to capture the plant parasitic nematodes. The nematode trappers are grouped as follows.

Sticky branches: The fungal mycelia have short lateral branches and they anastomose to form loops. The nematode trapped in this loops.

Sticky networks: The mycelium curls around and anastomoses with similar branches. These loops produce complex three dimensional structures. The adhesive surface of network helps to hold the nematode *e.g. Arthrobotrys* spp.

Sticky knob: Small spherical or sub spherical lobes are present on one or two celled lateral hyphae. Only the terminal knob is sticky to hold the nematodes *e.g. Monacrosporium ellipsospora.*

Constricting ring: The short hyphal branch curls back on itself and anastomoses and forming a ring. When the nematode enters the ring and contact the inner walls of the ring cells bulge inward filling the lumen of the ring and kills the nematode *e.g. M. bembicoidesd* and *Dactylaria brachophaga.*

Non-constricting rings: The trap is formed similar to the constricting ring. The ring becomes an infective structure and kills the nematode *e.g. Daclyaria candida.*

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

b) **Endozoic fungi:**

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

Parasitic fungi:

Paecilomyces lilacinus in an effective egg parasite on many nematodes. The parasitic fungus is particularly effective against *Meloidogyne, Heterodera, Rotylenchulus* and *Tylenchulus.* The fungus attacks the eggs as they are deposited in groups as a mass. The parasitic fungus was found to be effective against potato cyst nematode, root-knot nematodes in tomato, brinjal, betel vine and banana and *T. semipenetrans* in citrus.

Bacteria:

Recent studies have shown the influence of introduced microbial antagonist in controlling the plant parasitic nematodes. Seed treatment with *Pseudomonas fluroscens* has been found to reduce the cyst nematode, *H. cajani* in cowpea.

The rhizobacteria *viz., Bacillus cereus, Burkholderia cepacia* and *P. fluroscens* were found to be effective against *M. incognita* in tomato and banana.

LECTURE NO. : - 13

CHEMICAL CONTROL

Chemical which kill the nematodes are called as nematicides.

Nematicide:

Nematicide is defined as a substance or mixture of substances used for killing, repelling, or otherwise preventing the plant parasitic nematodes.

Kuhn (1881) first tested CS_2 to control sugar beet nematode in Germany and he could not get encouraging results.

Bessey (1911) test CS_2 for the control of root-knot nematodes later formaldehyde, cynide, quicklime.

Mathews (1919) chloropicrin (test gas) against plant parasitic nematode in England.

In 1944, scientists from California and Florida states of USA reported the efficacy of EDB, DD paved way for chemical control.

Classification of Chemicals/ pesticides:

The pesticides or chemicals can be classified on their mode of entry, mode of action and also by their chemical nature.

I) Classification based on mode of entry:

1) **Stomach poison:** The chemicals are applied on foliage and other parts of plant when ingested by the nematode, it act on digestive system and cause death. *E.g.* Lead Arsenate and Phosphomidon.
2) **Contact poison:** The toxicant which brings death of the pest species by means of contact and are directly absorb by the cuticle. *E.g.* Methyl Parathion.
3) **Fumigant:** The toxicant in its gaseous state penetrates the organism and kill them. CS_2, DD and EDB are example for fumigants.

II) Classification based on mode of action:

1) **Physical poison:** The toxicant which brings death of organism by exerting a physical effect is known as physical poison. The heavy oils like tar oil leads to asphyxiation and cause death.
2) **Protoplasmic poison:** A toxicant responsible for precipitation of protein, especially destruction of cellular protoplasm of intestine epithelium. *E.g.* Formaldehyde, Ethylene oxide, Nitro phenols *etc.*
3) **Respiratory poison:** A chemicals which blocks the cellular respiration or inactivate cellular respiratory enzymes are respiratory poisons. *E.g.* H_2S, DD and EDB.
4) **Nerve poison:** These chemicals are anti acetylcholinestrase activity which lead to constant excitation of nerves in the target organism. Due to this, the organism faces convulsions, tremors, muscle paralysis and lead to death. *E.g.* diazinon & aldicarb.

III) Classification based on chemical nature:

1) **Synthetic inorganic compounds:** These compounds are systemic inorganic salts which acts as stomach poison and kill the target organism. *E.g.* Calcium arsenate.
2) **Synthetic organic compounds:** These group are further classified as

i) **Halogenated hydrocarbon:** *e.g.* Chloropicrin, methyl bromide, DD, EDB and DBCP *etc.*

ii) **Organophosphorus compound:** The basic constituents of organophosphorus compound are carbon, hydrogen and chlorine and certain compounds may have oxygen and sulphur also. *e.g.* Parathion, Dichlorofenothion, Thionazin, Phorate *etc.*

iii) **Carbamates:** Carbamate compounds are derivatives of carbamic acid. *E.g.* Aldicarb, Carbofuron *etc.*

iv) **Substituted phenols:** In this compounds the phenol is substituted by any other group *e.g.* Binapacryl.

v) **Thiocyanates:** *e.g.* Lethane and Thanite

vi) **Flourine compound:** *e.g.* Flourine sodium flouroacetate.

vii) **Sulphur compounds:** *e.g.* CS_2. H_2S and Endosulphan.

IV) **Natural products:** Nicotine, Pyrethrin, Neem cakes. α terthinyl in Marigold, Catechol in Ergrostris, Mustard, Sesame, Bitter cucumber.

Important Nematicides:

1) **Ethylene dibromide (EDB):** 1,2-Dibromomethane, colourless liquid, gas in non-inflammable, 83% liquid formulation containing 1.2 kg a.i/lit and 35% granules. **Use:** It is injected/ dibbed into soil @ 60-120 liter or 200 kg ai/ha **Toxic**- Cyst nematode/fungi. Crop like onion, garlic and other bulb should not be planted after soil treatment with EDB. **Trade Name:** Bromofume and Dowfume.

2) **Dibromochloropropane (DBCP):** 1,2- Dibromo-3-Chloropropane, straw coloured liquid BP 195^0C, 1 liter of weighing 1.7 kg. **Use:** Soil treatment before planting, at the time of planting, post-plant treatment, effective when soil temperature is above 20^0C, sprinkled or mixed with irrigation water. **Recommended dose:** 10-60 lit/ha. **Trade Name:** Nemagone, Fumazone.

3) **DD mixture:** Trade name of the mixture of compound, cis and trans isomers of 1,3-Dichloropropane 30-35% + 1,2-dichloropropane other are few chlorinated 5%, black liquids of 100% formulation, 1 liter of weighing 1 kg technical, inject up to the depth of 15-20 cm at 25 X 30 cm spacing. **Use:** 225-280 lit/ha. **Trade Name:** Dibromomethane, Dorlone.

4) **Methyl bromide or Bromomethane:** Boil at 4.5^0C gas is heavy 1.5 time than air, insecticidal properties were described by Le Goupil in 1932. Store grain pest it is used 24-32 g/m^3 exposure period 48 hrs. Termite/wood beetle @ 32-64 g/m^3, fumigation of live plant @ 16-32 g/m^3. Application of nematode/ insect @ 4-7 ml/ft^2. It also kills the rodents, insects, fungi and weeds in the soil, highly dangerous to warm blooded animals.

5) **Chloropicrin or Trichloroditromethane:** It is the tear gas, non-inflamable, good penetrating effect. **Recommended dose:** 16-48 g/m3. It is in control of nematode/ insect in soil. **Trade Name:** Acquinite and Pic fume.

6) **Fensulfothion:** It is systemic nematicides, effective against golden nematode in Nilgiri hills. **Trade Name:** Dasanit, Terracur.

7) **Fenamiphos:** Systemic nematicide, effective on root-knot nematode and cyst nematodes, 1-5 % granules. **Trade Name:** Nemacur 40 EC.

8) **Ethoprop:** Systemic, effective against juvenile nematode. **Trade Name:** Mocap.

9) **Phorate:** Trade name Thimet 10% granules, fumigant action.

10) **Metham sodium:** Sodium N-methyldithiopcarbamate, **Trade Name:** Vapam, Sistan, Vitafume and Unifume, Recommended dose 100-200 ml/m^2 injected in to soil.

11) **Aldicarb:** 2-methyl-2 (methylthio) Trade name is Temik, the sulphur atom in the molecule is oxidized to sulfoxide and then to sulfone, it is a systemic, 10% granule, 30-35 days remain residual in plant.

12) **Carbofuran:** Trade name Furdan, it is systemic insecticide cum nematicide. It is formulated as 3% granule residual effect 30-60 days, got phototonic effect, acropetal action applied @ 1-2 kg ai/ha.

13) **Methomyl:** It is effective against insects, mites and nematodes, Trade name is Lannate

14) **Oxamyl:** (Carbomate) 40% EC systemic, effective against foliar nematode, Trade name Vydate.

INTEGRATED NEMATODE MANAGEMENT STRATEGIES

For Nursery (Transplanted crops):-

- Keep nursery area fallow (2-3 months)
- Deep summer ploughing (2-3) April-May
- Grow non-host or antagonistic crops
- Soil solarization- 100 gauge LLDPE for 20 days
- Follow rabbing at 7 kg(husk)/m3
- Use resistant varieties
- Green manuring with sunhemp
- Use bioagents like *P. lilacinus, T. viride,T. plus, P. fluorescens at 10 to 20 g/m2*
- Use effective nematicides like carbofuran 3 G or phorate 10 G at 1 to 2 kg a.i. /ha.

For Field Crops:-

- Keep the field fallow (2-3 months)
- Deep summer ploughing (2-3) April-May
- Flooding (2-3 months)
- Crop rotation
- Field sanitation
- Healthy planting material
- Resistant varieties
- Green manuring with sunhemp
- Grow trap crop, non-host or antagonistic crops
- Inter or mixed crops with marigold
- Use FYM or compost at 20-25 t/ha
- Use non-edible oil cakes at 1 to 2 t/ha
- Use bioagents like *P. lilacinus, P. fluoreascens, T. plus, T. viride at 5 kg/ha*
- Seed treatment with carbosulfan 25 DS at 3 % w/w
- Seedling root dip with carbolsulfan 25 EC at 0.05 % for 4 to 6 hours.
- Seed soaking with carbonsulfan 25 EC at 0.05 % for 12 hours.
- Soil application of carbolfuran 3G, phorate 10 G at 2 to 4 kg a.i./ha.

LECTURE NO. : - 14

ENTOMOPHILIC NEMATODES

Nematode associate with insects are referred as entomophilic, entomogenus and entomophagus nematodes. They belong to the super families Tylenchoidea, Rhabditoidea, Oxyuroidea and Mermithoidea of phylum nematoda.

Nature of parasitism:

Entomophilic nematodes are a group of parasites that cause debilitation, sterility or death of insect.

They are vary greatly in size, shape having insects as intermediate are as definite hosts. The earliest nematode parasite of insect is *Mermis nigrescens* from grasshopper locusts and other insect. The insect become infested with *M. nigrescens* by feeding on vegetation where the dark brown nematode eggs are deposited. The host is vulnerable throughout life cycle. Developing female nematode remains longer in the host. It affects morphology and physiology of insect. The gut and oviduct become compress and distorted. Cuticle becomes discolored and softened. Inhibition of molting reduced excretion, sterilization of female host. It is always fatal to host.

Entomophagic nematodes:

A symbiotic bacteria *Xenorhabdus* spp. is associated with *Steinernema* spp., *Photorhabdus* spp. with *Heterorhabditis.* The bacteria are responsible for death of host. It occurs primary/secondary from the bacterium *P. luminescens* is bioluminescent.

The free living J_3 larval of nematodes are resistant to desiccation and can survival for several months. They enter through mouth /anus. On entering in to host, they penetrate the wall of alimentary canal and move to body cavity. The host haemolymph taken by the nematodes and accumulates in anterior part of their intestine. The bacteria in pouch multiply and release through the anus of nematode body of the host insect. In the host, the bacteria multiply and cause **septicemia.** The nematodes develop on bacteria and in decomposed tissues of host insect. The host dies in about 48 hrs. after infection. High effective against lepidopterans *Spodoptera litura, Helicoverpa armigera, Papilio demoleus* and *Agrotis segtum.*

Entomophilic Nematode:

1. *Neotylenchus* -*Scirpophaga novella*
2. *Panagrolaimus* -*Chlo zonellus*
3. *Rhabditis* -*Chilo zonellus*
4. *Mermithids* -*Cirphis*
5. *Mermis* -*Amsacta moorei*
6. *Agamermis* -*Trypozyza incertulas*
7. *Hexamermis* - *Spodoptera litura, Scirpophaga novella, Chilo infuscatellus.*

ENTOMOPATHOGENIC NEMATODES (EPN)

Entomopathogenic nematodes (EPN) are beneficial nematodes parasitizing crop insects, particularly lepidopterans and coleopterans and are effectively used as biopesticidesagainst a vide variety of insect pests. The impressive attributes of EPN have stimulated strong commercial interest in nematodes as biological insecticides and are percived as viable alternative to chemicals in IPM programme.

Difference between EPN & PPN:

Entomopathogenic nematode and plant paracitic nematodes differ both in structure and behaviour from each other. A comparative table gives the most significant differences between the two groups.

Sr. No	**Entomopathogenic Nematode (EPN)**	**Plant Parasitic Namatode (PPN)**
1	Parasitic on insect pests and pass one of the stage of their life cycle in insect pests. They never parasitise or damage to plant	Parasitise mainly root system of plants and pass one of the stage of their life cycle in/ on the root tissues of plant as endoparasitic/ ectoparasitic nematode.
2	Beneficial to Agricultural crops by attacking insects pests by killing them quickely.	Harmful to Agricultural crops damage and alter physiology of crop plants, produce abnormalities, knots, lesion on root system, introduced fungal pathogen inside roots and aggravate wilt and root-rot diseases.
3	Increase crop yield by killing crop pests as a biological control agent	Inflicting 15% yield loss on an average in Agricultural crops globally as a parasite.
4	Size 0.3-1.5 mm (very small)	Size upto 4 mm (small to medium)
5	Absence of stylet	Presence of stylet
6	EPN feed on bacteria and decomposing host insect.	PPN feed on the plant part mainly root to get their nourishment.
7	Life cycle completes within a week.	Life cycle completes in 20-30 days.

EPN have many attributes which make them a good and promising biocontrol agent. They often behave like insecticide or other plant protection chemicals. They can be easily incorporated as a component of IPM programme.

Some typical characters of EPN:

- Ablity to search (chemoreceptors) the target insect in soil, plant surface.
- Quick kill of the target insect through release of bacteria.
- Broad host range: *Coleoptera, Lepidoptera, Diptera, Orthoptera, Homoptera etc.* Can be recovered from soil and mass multiplied with in a short period.
- Easily cultured on artificial diet or living hosts. Can be stored for longer perriod in soil, cadaver, in partially desiccated state and recieved when needed.
- They are compatible with many pesticide can be formulated as dust, sprays, capsules, grannules *etc.*and applied spraying EPN suspention or through irrigation system.
- These are safe to vertebrates, plants and non-target organisms.
- They are environmentaly safe and self perpetuating in nature.
- No registration required.

EPN falling three genera namely *Steinernema, Neosteirnema* and *Heterorhabditis.*

Dignostic characters between *Steinernema* and *Heterorhabditis.*

Steinernema	***Heterorhabditis.***
Symbiotic bacteria	
Species associated-*Xenorhabdus*. Bacteria location- within specialized intestinal vesicle.	Species associated-*Photorhabdus.* Bacterial
Adults	
Excretory pore located anterior to nerve ring. Spicule ventraly arcute. Bursa absent. Genital papillar – 10-11 pairs.	Excretory pore located posterior to nerve ring. Spicule nearly straight. Bursa present. Genital papillae-9 pairs.
Infective juveniles	
Excretory pore anterior to nerve ring	Excretory pore posterior to nerve ring
Luminescence – No	Luminescence – Yes
Colour of dead larva- Black	Colour of dead larva- Red, pink.

Identification of Entomopathogenic Nematode:

Adult: Obligate parasite, adult stage founding the haemolymph of infected insect, adults are amphidelphic, stylet absent, amphid aperture located on lateral lips.

Female: Amphidelphic, didelphic with reflexed ovaries, vulva media, functional only during mating, mature females ovoviviparous, developing larvae consume the entire body content and eventually filling the females, females cuticle degenrates and larvae are released.

Male: Testes single, reflexed, spicule paired, separate with or without vallum, gubernaculum present, genital papillae nipple shaped.

Infective juvenile: Third stage is dauer stage or infective in nature and found in soil. Stoma and anus closed, Excretory pore on nerve ring mouthregin armed. Tail elongate. Capable of killing wide range of insects due to septicemia caused by symbiotic bacteria.

Nematode biology:

Juvenile of EPN pass through four stages. The first two stage may be developed on food material.

The parasitic cycle of nematodes is initiated by the third state IJS (infective juveniles). These non-feeding juveniles locate and invade suitable host insects through natural body openings (i.e. anus, mouth and spiracles).

Once inside the host, nematodes invade the haemocoel and release the symbiotic bacteria that are held in nematode intestine. The bacteria cause a septicemia, killing the host with in 24-72 hrs. The IJS feed on the rapidly multiplying bacteria and disintegrated host tissues. About 2-3 generations of the nematodes are completed within the host cadaver. When food reserves are deplected the reproduction ceases and the offspring develop into resistant IJS that disperse from the dead host and are able to survive in the environment and to seek out new hosts. Soon after death, the cadevers turn flaccid and starts changing coloure. In case of wax moth larvae and depending upone nematodes species, *Steinernematid* killed insects turn to various shades of brown, orchre, whereas *Heterorhabditid* killd insect turn red, brick red, purple orange, yellow and some times green. The colour of the cadever is attributed to the associated bacterium, especially photorhabdus that have pigmens. If the cuticle is transparent, nematodes are visible incide the cadever.

LECTURE NO. : - 15
MODE OF ACTION

- Ovicidal
- Inhibition of egg hatching
- Lack of penetrability of larvae
- Mortality in larvae
- Toxic to Nematodes/ Nematicidal
- Nematostatic
- Inability to complete life cycle
- Inhibition of egg laying capacity of females
- Directly toxic
- Disturbing pH
- Creation of unfavorable condition for nematode development & multiplication

LECTURE NO. : - 16
MASS PRODUCTION TECHNIQUES FOR EPN

The EPN are multiplied either on a suitable host (*in vivo*) on a semi-synthetic diet (*in vitro*). Both the techniques of mass production of EPN have their own advantages and limitations

In *vivo* production

EPN are baited out and multiplied on host insects. Three host insects viz';

i) *Galleria mellonella*
ii) *Corcyra cephalonica*
iii) *Helicoverpa armigera.*

The method of preparation of diet and mass multiplication of host insect.

i. *Galleria mellonella:*

Culture of *G. mellonella* can be done and easily maintained in laboratory. The ingredients for the artificial diet of *G. mellonella* are as follows.

Part-A		**Part –B**	
Corn flour	200 g	Glycerin	150 ml
Wheat bran	100 g	Honey	150 ml
Skimmed milk power	100 g		
Yeast tablets	50 g		

Yeast tablets are grinded into a fine powder and mixed with corn flour, wheat bran and milk powder. Glycerin and honey are mixed separately. Finally, part A and B are mixed thoroughly and homogenous mixture is prepared. The content of artificial diet is distributed in two plastic containers (5 lit. capacity). About 1000 first or second instar *G. mellonella* larvae are released in each container and incubated at 350C. The larvae will be ready for use within three weeks. If the temperature is < 350C development of larvae will be slow.

ii. Corcyra cephalonica:

1. Broken sorghum or wheat grain	1 kg
2. Maize meal	1.0 kg
3. Rice broken	500 gm.
4. Streptomycin	0.5 gm.
5. Yeast powder	1 gm.

About 1.00 CC Corcyra eggs aremixed with this and perforated lid is secured and kept for about a month. The fully grown larvae are utilized for multiplication of EPN.

iii. *Helicoverpa armigera:*

The ingredients for artificial diet of *H. armigera are*

Chickpea flour	84 g	Casein protein rich purified	10 g
Agar agar	11 g	Cholesterol/vegetable oil	0.1 ml
Yeast extract powder	11 g	Methyl P'-4 hydroxybenzoate	2 g
Sorbic acid	1 g	Streptomycin sulphate	0.01 g
Ascorbic acid	5 g	Distilled water	600 ml

Yeast extract, sorbic acid casein, cholesterol methyl P-4 hydroxybenzoate and Streptomycin sulphate are mixed well in a grinder with 400 ml water. In another container, agar agar is added in200 ml distilled water heated. All the ingredients are thoroughly mixed in grinder. Thereafter, the ingredient is poured in petri plates/vial and kept at room temp. to cool down. Now, diet is ready for use *H. armigera* larvae are collected from field of chickpea or pigeon pea on maintained on artificial diet under laboratory condition.

Production of EPN *in vivo*

Production of EPN on insect is generally done by using while trap method.

Insect are inoculated with EPN on a petridish lined with filter paper. After 2-5 days, infected insects are transferred to the white trap. This method consists of a dish on which the cadavers rest on inverted watch glass, surrounded by water the central dish containing the cadavers provides a moist surroundings for the EPN emergence from cadaver. New progeny of infective juveniles that emerge from cadaver migrate to the surrounding water where they are trapped and subsequently harvested. This method has the advantage that the IJS migrate away from host cadaver on emergence and continue to do so until the body contents of the host are consumed.

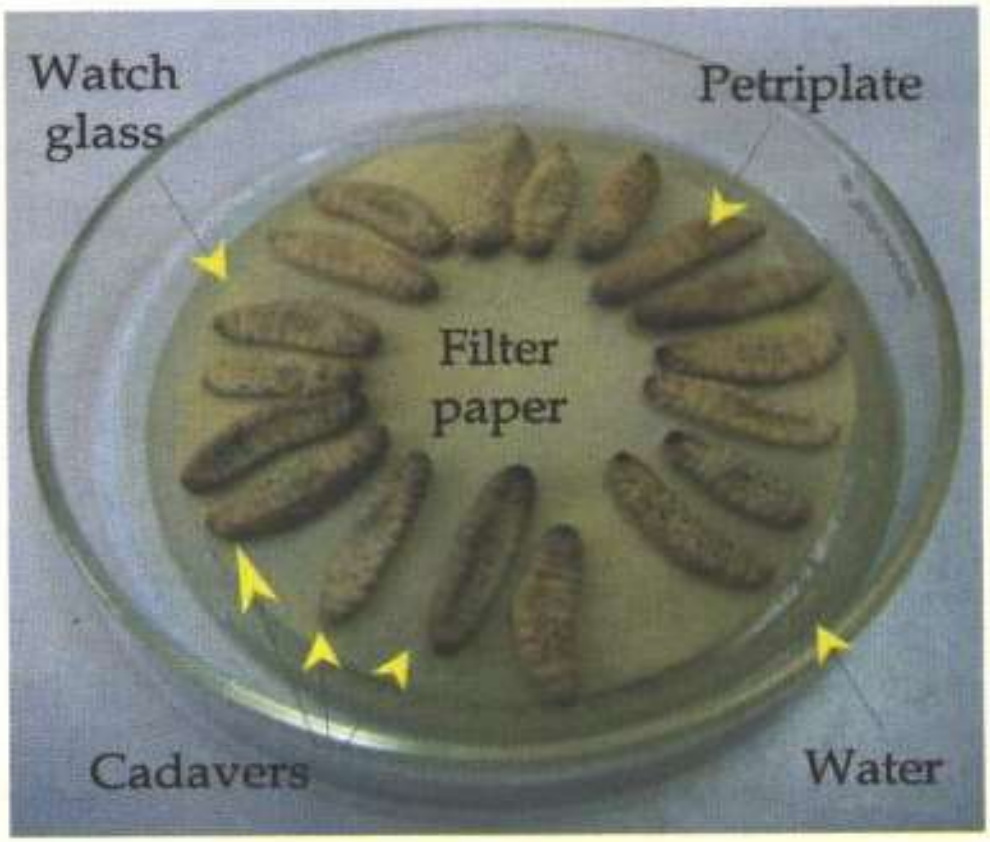

Fig. 26. White trap method for harvesting EPN emerged from *G. mellonella* cadavers

Production of EPN *in vitro*

For in vitro production of EPN, various media have been reported. The following media have been proposed and their ingredients are as follows:

- **Wout's medium:** Nutrient broth (0.88 g), soybean flour (14.40 g), groundnut oil (10.40 g) and distilled water (60 ml).
- **Modified Wout's medium:** Nutrient broth (0.50 g), yeast extract (0.20 g), soybean flour (16.00 g), groundnut oil (12.00 g) and distilled water (30 ml).
- **Wheat flour medium:** Wheat flour (15 g), Kabuli gram flour (5 g), beef extract (5 g), yeast extract (6 g), agar 1% (1 g), coconut oil (6 g) and distilled water (60 ml).
- **Modified wheat flour medium:** Wheat flour (15 g), Kabuli gram flour (5 g), beef extract (5 g), yeast extract (1 g), agar 1% (1 g), groundnut oil (10 g) and distilled water (60 ml).
- **Egg yolk media I:** SDEY (7 g), yeast extract (2 g), NaCl (0.8 g), oil (15 g) and distilled water (60 ml).
- **Egg yolk media II:** SDEY (10 g), yeast extract (5 g), NaCl (0.8 g), oil (12 g) and distilled water (60 ml).
- **Egg yolk media modified:** SDEY (7 g), soybean flour (20 g), yeast extract (2 g), NaCl (0.8 g), oil (15 g) and distilled water (60 ml).
- **Dog biscuit medium:** Dog biscuit (15 g), yeast extract (1 g), peptone (3 g), agar (2 g), oil (10 g) and distilled water (60 ml).
- **Modified dog biscuit medium:** Dog biscuit (20 g), yeast extract (1 g), peptone (3 g), beef extract (5 g), oil (7 g) and distilled water (100 ml).

Mix the ingredients and coat on to polyether polyurethane from cut into small cubic pieces till the latter soaked in the media (1.5 g of foam chips, 8-9 g medium, w/w). The flask filled with foam-media impregnated water and autoclaved for 20 min at 121^0C and allowed to cool. Fresh infective juveniles extracted from insect will be used each time to avoid the prior inoculation of bacteria in the flasks. The nematodes should be inoculated aseptically in to the flask @ 1000 Ijs/flask. Incubate the sealed flask at 280C for 25 days. The colonies of EPN will start appearing on the wall of flask after two weeks post inocolation.

After 25 days of inoculation infective juveniles will be harvested. Piled up the foam chips on 100 mesh sieve. Place the sieve in a pan of distilled water overnight. The nematodes seddiments pass through a 250 mesh sieve in a pan of water. About 95% infective juveniles migrate into the water within 2 hour and sterilized with 0.1% Hymine solution, wash thrice with sterile distilled water and collect and store at room temperature. After concentrating nematode suspension with the help of micro filtration assembly. EPN will be kept till further use.

(Techniques for mass production of Entomopathogenic Nematodes See in practical mannual)