LECTURE NO. 1

DEFINITION AND HISTORY OF DEVELOPMENT OF NEMATOLOGY

Nematode :

The word Nematode was derived from greek word “Nema” meaning thread and “oides” meaning resembling.

Nematodes are defined as triploblastic, bilaterally symmetrical, multicellular, un-segmented generally microscopic worms with single cavity (pseudocoelomic).

Nematology:

Nematology is an important branch of biological science, which deals with a complex, diverse group of round worms known as nematodes that occur worldwide in essentially all environments. OR

Study of nematodes is known as Nematology.

History of Nematology:

Nematology research, like most fields of science, has its foundations in observations and the recording of these observations. The earliest written account of a nematode "sighting," as it were, may be found in the Pentateuch of the Old Testament in the Bible, in the Fourth Book of Moses called Numbers: "And the Lord sent fiery serpents among the people, and they bit the people; and much people of Israel died".

Although no empirical data exists to test the hypothesis, many nematologists assume and circumstantial evidence suggests the "fiery serpents" to be the Guinea worm, *Dracunculus medinensis*, as this nematode is known to inhabit the region near the Red Sea.

Before 1750, a large number of nematode observations were recorded, many by the notable great minds of ancient civilization. Hippocrates (ca. 420 B.C.), Aristotle (ca. 350 B.C.), Celsus (ca 10 B.C.), Galen (ca. 180 A.D.) and Redi (1684) all described nematodes parasitizing humans or other large animals and birds.

Borellu (1653) was the first to observe and describe a free-living nematode, which he dubbed the "vinegar eel;" and Tyson (1683) used a crude microscope to describe the rough anatomy of the human intestinal roundworm, *Ascaris lumbricoides*.

Other well-known microscopists spent time observing and describing free-living and animal-parasitic nematodes: Hooke (1683), Leeuwenhoek (1722), Needham (1743), and Spallanzani (1769) are among these.

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

From 1750 to the early 1900s, nematology research continued to be descriptive and taxonomic, focusing primarily on free-living nematodes and plant and animal parasites.

Berkely (1855) - Determination of root knot nematode, *Meloidogyne* spp. to cause root galls on cucumber plants in green house in England.

Kuhn (1857) – Reported the stem and bulb nematode, *Ditylenchus dipsaci* infesting the heads of teassel.

Kuhn in (1874) is thought to be the first to use soil fumigation to control nematodes, applying carbon disulfide treatments in sugar beet fields in Germany.

Atkinson (1892) – First report of root knot nematode and *Fusarium* complex in vascular wilt of cotton.

In Europe from 1870 to 1910, nematological research focused heavily on controlling the sugar beet nematode as sugar beet production became an important economy during this time in the Old World.

In 1918, the first permanent nematology field station was constructed in the U.S. Post Office in Salt Lake City, Utah under the direction of Harry B. Shaw, after scientists observed the sugar beet nematode in a field south of the city.

Nathan Cobb (1918) also called the “Father of American Nematology” published his Contributions to a Science of Nematology and his lab manual "Estimating the Nema Population of Soil." These two publications provide definitive resources for many methods and apparatus used in nematology even to this day.

T. Goodey (1933) - Published book on “Plant parasitic nematodes and the diseases they cause”.

Christie (1945) – Description of the nematicidal value of EDB. In 1955 the European Society of Nematologists was founded. In 1961 The Society of Nematologists was founded in the USA. In 1969 the Journal of Nematology was first published by the Society of Nematologists in USA.

History of Nematology in India:

Nematology as a separate branch of Agriculture Science in India has been recognized only about 37 years back. The history and development of Nemtology in India have been listed below in chronological order.

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

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

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

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

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

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

1961 – Nematology laboratory established at Agricultural College and Research Institute, Coimbatore, with the assistance of Rockfeller Foundation and Indian Council of Agricultural Research.

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

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

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

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

1966 – Division of Nematology established at IARI, New Delhi

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

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

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

1971 – Indian Journal of Nematology published.

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

1972 – First All India Nematology Workshop held at IARI, New Delhi
1973 – Fifth South – East Asian Nematology Course at New Delhi.
1975 – Sixth South – East Asian Nematology Course at New Delhi.
1976 – Summer Institute in Phytonematology held at Allahabad.
1977 – Department of Nematology established at Haryana Agriculture Unviersity, Hisar.
1977 – All India Co-ordinated Research Project (AICRP) on nematode pests of crops and their control started functioning in 14 centres in India with its Project Co-ordinator at IARI, New Delhi.
1979 – M.Sc. (Ag.) Plant Nematology course started at Tamil Nadu Agricultural University, Coimbatore.
1979- All India Nematology Workshop and Symposium held at Orissa University of Agricultural University, Coimbatore
1979 – All India Nematology Workshop and Symposium held at Orissa University of Agriculture and Technology, Bhubaneswar
1979 – Seventh South – East Asian Nematology course at New Delhi.
1981 – Department of Nematology established at Tamil Nadu Agricultural University, Coimbatore.
1981 – All India Nematology Workshop and Symposium held at Tamil Nadu Agricultural University, Coimbatore.
1982 – Department of Nematology established at Rajendra Agriculture University, PUSA, Bihar
1983 – All India Nematology Workshop and Symposium held at Solan, Himachal Pradesh.
1985 – All India Nematology Workshop and Symposium held at Udaipur, Rajasthan.
1986 – National Conference on Nematology held at IARI, New Delhi
1987 – All India Nematology Workshop at Govt. Agriculture College, Pune.
1987 – Group Discussion on Nematological problems of Plantation crops held at Sugarcane Breeding Institute, Coimbatore.
1992 – Silver Jubilee Celebration of Division of Nematology, IARI, New Delhi.
1992 – Summer Institute on “Management of Plant Parasitic nematodes in different crops” organized by ICAR at Haryana Agricultural University, Hisar.
1995 – All India Nematology Workshop and National Symposium on Nematode problems of India held at IARI, New Delhi. 1997 – Summer School on “Problems and Progress in Nematology during the past one decade”
was organized by ICAR at IARI, New Delhi.
1998 – Afro – Asian Nematology Conference held during April 1998 at Coimbatore.
1999 – National seminar on “Nematological Research in India: Challenges and preparedness for the new millennium” at C.S. Azad University of Agriculture and Technology, Kanpur.
2000 – National Nematology Symposium on “Integrated Nematode Management” held at OUAT, Bhubaneshwar, Orissa.
2001 – National Congress on “Centenary of Nematology in India: Appraisal and Future plans” at IARI, New Delhi.
2003 – Winter school on “Biological Control of Plant Parasitic nematodes” at Department of Entomology, TNAU, Coimbatore.
2005 – National Symposium on Recent Advances and Research Priorities in Indian Nematology at IARI, New Delhi – 9-10 December.
2007 – Workshop on Nematology in India - Achievements and Oppurtunities was held at Division of Nematology, IARI, New Delhi – 5-7 March.

LECTURE NO. 2

ECONOMIC IMPORTANCE AND GENERAL CHARACTERISTICS OF PLANT PARASITIC NEMATODES

Economic Importance of Nematodes in Agriculture:

On global basis, about 34 per cent of crop is lost annually due to diseases, insects and weeds. The plant parasitic nematodes play a vital role in the production of crops, rarely there is any crop free from their attack whether in the field, orchards, kitchen garden or green house. Pioneer 1983 stated that about 2000 species of Phytonematode belonging to about 200 genera have been described so far, while it is estimated that about 42000 species of phytonematodes are present. Many species of nematodes are important parasites of plants and animals, whereas others are beneficial to agriculture and the environment. Annual crop losses due to these obligate parasites have been estimated to be about $ 78 billion worldwide and $8 billion for U.S. growers. The estimated annual crop loss in Tamil Nadu is around Rs. 200 crores.

In the United States, the nematodes are known to cause six per cent loss in field crops, ($ 100 million / year), 12 per cent loss in fruits and nuts ($ 225 million / year), 11 per cent loss in vegetables ($ 267 million / year) and 10 per cent loss in ornamental ($ 60 million / year).

Information of crop losses caused by nematode in India is limited to a few estimates. In India, the cereal cyst nematode, *Heterodera avenae* causes the "molya" disease of wheat and barley in Rajasthan, Punjab, Haryana, Himachal Pradesh and Jammu and Kashmir. The loss due to this nematode is about 32 million rupees in wheat and 25 million rupees for barley in Rajasthan State alone.

The seed gall nematode, *Anguina tritici* causes the "ear-cockle" disease of wheat in North India. This nematode along with the bacterium, *Clavibacter tritici* causes "tundu" or "yellow slime" disease. The overall damage is about one per cent, but in severe infestation, the loss may even go up to 80 per cent. The annual loss due to this nematode in North India is about 10,000 tonnes of wheat costing 70 million rupees.

The potato cyst nematode, *Globodera rostochinensis* and *G. pallida* are serious problem in the nilgiris and Kodaikanal hills in potato. About 3000 hectares are infested by this nematode. Total failure of the crop has been recorded under severe infestation.

The root lesion nematode, *Pratylenchus coffeae* is a serious pest of coffee in South India. About 1000 hectares are infested by this nematode. Annual loss is about 20 million rupees.

The burrowing nematode, *Radopholus citrophilus* causes "spreading decline" of citrus in Florida. *R. similis* causes "pepper yellows" in Indonesia and "banana rhizome rot" in various parts of the world. This nematode is a serious pest in banana, coconut, arecanut and ginger. It is also responsible for "slow wilt" of pepper in Karnataka.

The citrus nematode, *Tylenchulus semipenetrans* is responsible for "slow decline" disease of citrus. It is suggested that the citrus nematode is also one among the factors responsible for "die-back disease" of citrus trees in India. The total annual reduction in the citrus crop due to the nematode infestation is estimated at 15 per cent. The life span of the citrus trees is also reduced. The severe infestation in acid lime gardens are observed in Perambalur district of Tamil Nadu.

The reniform nematode, *Rotylenchulus reniformis* has been reported to cause 14.9, 8.1, 6.0, 13.2 and 8.7 per cent loss in yield of cotton, maize, fingermillet, cowpea and black gram, respectively.

The root-knot nematodes, *Meloidogyne* spp. produces galls on roots of many vegetable crops, pulses, some fruit crops, tobacco and ornamental crops and lead to severe yield loss. This nematode is mostly polyphagous and attack more that 3000 species of plants.

The percent yield loss due to root-knot nematodes in vegetable crops has been studied under All India Coordinated Research Project (Nematodes) and estimated to the tune of 28-47 per cent in tomato, 26.2-50.0 in brinjal, 19.7-33.0 in chillies, 6.0 % in okra, 38.0-47.2 in bitter gourd, and 18.0-33.0 in melon. Avoidable yield losses due to *Meloidogyne incognita* were estimated to be 43.48 and 28.60 per cent in French bean and cowpea, respectively.

Estimated annual losses due to nematodes for selected world crops

Crops	Number of estimates per crop	Food and Agriculture Organization production estimates (1000 MT)	Estimated yield losses due to Nematodes (%)
Banana	78	2097	19.7
Barley	49	171635	6.3
Cassava	25	129020	8.4
Citrus	102	56100	14.2
Cocoa	13	1660	10.5
Coffee	36	5210	15.0
Corn	125	449255	10.2
Cotton (Lint)	85	17794	10.7
Field bean	70	19508	10.9
Oat	37	43355	4.2
Peanut	69	20611	12.0
Potato	141	312209	12.2
Rice	64	469959	10.0
Sorghum	53	70698	6.9
Soybean	91	89893	10.6
Sugarbeet	51	293478	10.9
Sugarcane	65	935769	15.3
Sweet potato	67	117337	10.2
Tea	16	2218	8.2
Tobacco	92	6205	14.7
Wheat	89	521682	7.0

The examples mentioned above include only a small portion of the important nematode problems found in India. Besides acting alone, they are also known to be associated with bacteria, fungi and viruses in causing complex plant disease which further increases the losses in yield of various crops. The nematode problem is much more important in developing countries, in tropical or subtropical regions which are most suitable place for the reproduction and activity of the nematodes.

General characteristics of plant parasitic nematodes:

1. They inhibit marine, fresh water and terrestrial environments as a free living and parasites.
2. They are bilaterally symmetrical, triploblastic, unsegmented and pseudo-coelomates, body fluid under high pressure.
3. Nematodes are elongated, cylindrical, but sometimes fusiform, pear shaped, spheroid, kidney shaped.
4. Vermiform, round in cross-section, covered with a three layered cuticle secreted by epidermal cells.
5. Growth accompanied by moulting of juvenile stages, usually four juvenile stages.
6. Oral opening surrounded by six lips and sixteen sensory structure.
7. The mouth is followed by stoma or mouth cavity which bears stylet or spear in plant parasitic nematodes by which they pierce the cell walls of the host and ingest the food.
8. The stoma is followed by pharynx or oesophagous then intestine and a rectum ending into a ventral terminal and sub terminal anus in females and or cloaca in males.
9. Possess unique cephalic sense organs called amphids.
10. Body wall contains only longitudinal muscles connected to longitudinal nerve chords by processes extending from each muscle.
11. Unique excretory system containing gland cells or a set of collecting tubes.
12. Longitudinal nerve chords housed within the thickening of the hypodermis.
13. Has no circulatory system (no blood system).
14. Reproduction normally sexual and gonochoristic (sexes are separate).
15. Males are always smaller than females.
16. Feed on just about everything. Live just about everywhere, many species are endoparasites.

LECTURE NO. 3, 4 AND 5

GENERAL MORPHOLOGY, ANOTOMY AND BIOLOGY OF NEMATODES

Morphology and Anatomy of plant Parasitic Nematodes:

Body shape and size: Nematode show great variation in their external and internal organs. The plant parasitic nematodes are slender, elongated; spindle shaped or fusiform organisms. In majority of species the two sexes look alike, although the males are generally slightly smaller than females. Sexual dimorphism occurs in some members of Tylenchida, where females become swollen and males are vermiform within same species, *e.g. Anguina, Meloidogyne, Heterodera, Rotylenchulus, Tylenchulus etc*. Their size may vary from 0.2 mm *(Paratylenchus) to* about 12mm (*Paralongidorus spimikis).* Their width ranges from 0.01 to 0.5 mm. In some cases body width may be less than 1% of its length, *e.g., Ecphyadophora* or as much as 15%, *e.g., Criconema.* The free living, non parasitic nematodes are generally larger while the largest animal parasitic nematodes may have a length of some centimeter to a meter or more. The nematode parasitizing whale fish is about 27 feet long.

Body posture : The nematodes, when relaxed by gental heat, either lie straight *(Pratylenchus)* or slightly ventrally curved *(Hoplolaimus)* or curved in 'C' shape *(Tylenchorhynchus)* or form a spiral *(Helicotylenchus).* The curvature of the body is always towards ventral side except in case of *Dorsalla* which curved in a 'C' shape towards dorsal side.

Segmentation, colouration and symmetry: The body is not metamerically segmented internally. Appearance of segmentation in some nematodes is given by the cuticle, *e.g., Criconema.* Generally nematodes are colourless or whitish with slight yellowish tint depending upon the chemical composition of the cuticle. Basically, the nematode body is bilaterally symmetrical, *i.e.,* if cut into two through the sagital plane, the two halves are mirror images of each other. But radial symmetry is found in the anterior region, whereas intestine, excretory and reproductive systems exhibit asymmetry.

Body regions : The nematode body is not divisible into definite regions, but there are certain subdivisions which have been used without much morphological basis . The anterior parts of the body having the mouth, lips and stoma is called as head and it is

continuous with main body. Sometimes it is set off by a constriction. Beginning at the anus or cloaca and extending to the posterior extremity is the tail. Longitudinally, the body can be divided into four zones; the ventral which has the natural openings *viz*., excretory pore, anus or cloaca and vulva, the side opposite to ventral is dorsal. The other two sides are right and left laterals.

Lip region: The lip region also called as head exhibits great variations. In some nematodes it may be completely merged with the body *(Ecphyadophora),* in others it may be truncate as in *Duotylenchus.* In many nematodes the head is slightly to prominently demarcated from the body or may distinctly set off e.g. *Dolichondus.*

Tail It is the post-anal elongation of the body present in all stages of nematodes (male, female and larva). Différent structures; phasmids, papillae, pores, alae, bursa, setae, caudal glands, spinnerets etc. are found in the tail. In some nematodes, the intestine extends into the tail to form blind sac. The tail is locomotory organ which helps in swimming to aquatic nematodes. The other structures present on tail are used for different purposes, *e.g.,* caudal alae, bursa and genital papillae help in reproductive activity.

General structure of nematode:

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

(a) Outer body tube or Body wall

(b) Inner body tube or Alimentary canal

(c) Body cavity or Pseudo-coelom in which excretory, nervous and reproductive systems are present.

(A) Outer body tube : The outer body tube comprises three layers, cuticle, hypodermis (epidermis) and somatic muscle layer.

(I) Cuticle : Cuticle is the outer most covering of the body wall which is non-cellular, semipermeable and tough layer secreted by the epidermal cells derived from the ectoderm. It serves as an exoskeleton and protect the inner soft body tissues. It is either smooth or ornamental with annulations, punctuations etc.. The cuticle supports musculature regulates permeability and maintains the turgor pressure of the body. It invades all the natural openings of body including, the mouth, rectum, cloaca, vagina, excretory pore, amphids and phasmids. The cuticle lining of these organs are shed

off at each moult. The cuticle is composed three primary layers a) Cortical layer b) Medium layer and c) Basal layer.

The following are the important external structures of the cuticle.

Punctuations: These are minute, round dots arranged in a definite pattern. They act as structures for strengthening the cuticle rather than as pore canals through which cuticular proteins may be transported.

Transverse striations: The outer cuticle of the Tylenchids is marked with transverse striations or annulations which gave them a segmented appearance. The markings or ridges which are arranged transversely are called as striae and the space between the striae is known as interstrial region. When the striae are deep, it is called annulation and deep interstrial region is known as annula.

Longitudinal striations : Just like transverse lines, the longitudinal cuticular markings are also present which generally take the form of longitudinal ridges and alae.

(A) **Ridges :** These are raised area which extend through the length of body and found on sub median as well as on the lateral surface.

B) **Alae :** These are thickenings or projections of the cuticle found in the lateral or sub lateral region. The following three types of alae are found in nematodes.

(a) **Longitudinal alae :** They are sub lateral in position and generally 4 in number. The area between two alae is known as lateral field, which lie above the lateral chord.

(b) **Cervical alae :** These are confined to the anterior or cervical region of the nematode body, *i.e.,* on head or labial area. These are found in animal parasitic nematodes.

(c) **Caudal alae :** The wing like processes of cuticle found in caudal region (tail) in male for clasping the female during copulation.

Special modifications of cuticle

1. Suckers : A special oval or rounded modification of the cuticle only on some males and functions in copulation.

2. Medio-ventral pre-anal : In certain group of nematodes (Rhabditida) in the middle of ventral just anterior to anal, there is a thickening of cuticle.

3. Rosette : These are punctuation patterns of the cuticle surrounding genital papillae.

4. Plectanes : These are cross striated cuticular plates which function to support genital papillae of some males.

Cuticular layering *:* The nematode cuticle is 3 layered structure with many sub layers. It consists of an outer layer (cortical), a middle layer (matrix or median) and an inner layer (fibre or basal). In some nematodes there are only 2 layers, *e.g.,* adult females of Heteroderidae.

(i) **Cortical layer :** It is the outer most hyaline layer with regularly spaced annulations. In the larger nematodes it is often divided into an external cortical layer and an internal cortical layer. The external cortical layer has been considered to be a keratin chemically. The presence of disulphide group in this layer is responsible for its resistant properties.

(ii) **Matrix or median layer***:* The average thickness of this layer is variable and ranges from 0.1 to 0.5 µ chemically; the median layer consists of protein which resemble collagen.

(iii) **Fibre layer or basal layer :** The basal layer consists of regularly arranged vertical rods or striations. It is composed of a protein with very close linkage between the molecules, resulting in a resistant layer.

(II) Hypodermis: The hypodermis lies between the cuticle and somatic layer r and responsible for the formation of cuticle. It is also called as epidermis or sub cuticle which is a thin layer and characteristically thickened in the dorsal, ventral and lateral positions to form four longitudinal hypodermal (epidermal) chords. The two lateral chords are more prominent than the dorsal and ventral ones. The chords protrude the pseudocoelomic cavity between the somatic muscles and divide them into 4 quadrants. In higher nematodes this is rich in lipids and glycogen which serve as a source of reserve energy. It is one of the most metabolically active regions of the nematode body.

Hypodermal glands : Various types of hypodermal glands are found in different species of nematodes which act as either osmotic or ionic regulators. The caudal glands are usually 3 to 5 in number situated in the tail, produce an adhesive secretions which serve to anchor the nematodes. The hypodermal glands are also associated with some sensory organs viz., amphids, deirids and phasmids.

(III) Somatic muscle layer : Somatic muscle cells are arranged longitudinally beneath the hypodermis in the interchordal zones. The somatic musculature is composed of a single layer or more or less spindle-shaped cells attached to the hypodermis in the four inter chordal zone. On the basis of arrangements the following types of cells are found.

(a) Holomyarian : having 2 muscle cells in each zone.

(b) Meromyarian : 2 or 5 muscle cells in each interchordal zone.

(c) Polymyarian : More than 5 muscle cells per zone.

On the basis of shape of cells, they may be grouped into three types

(i) Platymyarian : A flat type of cell with contractile elements limited only to the base lying closest to the epidermis.

(ii) Coelomyarian : A 'U' shaped type of cell in which the muscle fibre are adjacent and perpendicular to the hypodermis and extend along the sides of the muscle cell for varying distances.

(iii) Circomyarian : A type of muscle cell in which the muscle fibres completely surround the cytoplasm.

In addition to somatic muscle there are specialized muscles associated with different organs, *e.g.,* cephalic muscle, somata-oesophageal muscle, somata-intestinal muscle,

copulatory muscle, spicular muscle, gubernacular muscle, vulval muscle etc.

(B) Inner body tube or digestive system:

The inner body tube of the nematode body forms the gut or alimentary canal into which some glands open. It is distinguishable into three distinct regions namely, stomodeum (foregut), mesenteron (mid gut) and the proctodeum (hind gut). The fore gut and hind gut are invaginations which are lined with cuticle. On moulting, the linings of the stomodeum and proctodeum are shed with cuticle. The stomodeum incorporates the stoma, oesophagus and the oesophago-intestinal valve leading to mesenteron. The proctodeum is comparatively small and includes the rectum in females and the cloaca in males. The middle portion of the alimentary canal is the mid gut or intestine, which is endodermal in origin. The ducts of oesophageal and rectal glands enter into stomodeum and proctodeum, respectively.

Stomodeum (Foregut): 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 nematodes. Generally, there are 6 lips 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 oesophagus. In order Tylenchida the stoma is armed with protrusible hollow stylet which is formed by the fusion of stomal wall hence called as stomatostylet, which is used for piercing into plant cells. In order Dorylaimida the stylet arises in two pieces, a buccal portion embedded in the oesophageal wall and a replaceable stylet odontostyle or onchiostyle. The replaceable stylet is formed within the oesophagus.

Pharynx or oesophagus : It is the largest part of the stomodeum and found between stoma and intestine. The pharynx is mainly a food transporter pumping food from the low pressure stoma to the high pressure intestine. Internally, the pharynx is lined with cuticle and externally by a membrane (basal lamella). It contains radial muscle, oesophageal glands and valves, which prevent the regurgitation of food. The oesophagus shows great diversity in form. The three part cylindrical

oesophagus has three well defined regions, corpus, isthmus and basal bulb.

Mesenteron (Midgut) : The intestine is hollow generally straight tube, formed from a single layer of epithelial cells, backed by a well developed basal lamina. The plasma membrane which lines the lumen of the intestine is thrown into the fine finger like projection known as microvilli. They increase the surface area of the intestine and are both secretary and absorptive function. The food moves in the intestine by the ingestion of more food and also by the locomotory activity of the nematode.

Proctodeum (Hindgut) : The rectum is a short narrow tube lined by cuticle, connecting the intestine in the slit like anus in the female and the cloaca in the male. The pylorus, an intestine rectal valve formed by the intestinal cells is generally present which separates the intestine from rectum. In some nematodes rectal glands, 3 in females and 6 in males, are present. They are responsible for production of gelatinous matrix in which eggs are laid.

(C) Body cavity (Pseudocoelom) : The body cavity of nematodes differs from the true coelomic cavity of the animals that it is not completely lined with the tissue of mesodermal origin. Since, it is devoid of mesodermal lining hence called as pseudocoelom. Externally it is lined by somatic muscle cells, which are mesodermal in origin, and internally by the cells of alimentary canal, which are ectodermal in origin. The pseudocoelom is filled with fluid which is rich in protein and other dissolved substances, which bathes all the internal organs and functions as part of the turgor pressure system on the exo-skeleton. The nervous, excretory and reproductive systems are present in pseudocoelom. Besides, this variety of cells (pesudocoelomocytes) and fibrous tissue (mesentries) etc. are also present within the body cavity.

THE NERVOUS SYSTEM : The nervous system in plant parasitic nematodes is not well studied, particularly in Tylenchida due to their small body size. Nervous system of animal parasitic nematode eg. *Ascaris* was studied and described in details. In nematodes, a central and a peripheral nervous system can be identified.

Central nervous system : Also known as brain consist of nerve ring associated with ganglia and nerve. The nerve ring or circum-oesophageal commissure is a 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 obliquely 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 and 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, amphideal nerve, amphids, phasmid, deirids, hemizonid, hemizonian and other associated structures.

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
(b) Latero dorsal nerve
(c) Latero-ventral nerve
(d) Ventral nerve
(e) Ventro lateral nerve
(f) Dorso lateral nerve

REPRODUCTIVE SYSTEM :

The nematodes are dioecious or amphioxus having as separate males and females within a species. Generally the males are lesser in number than females or may be completely absent, which indicates a tendency towards hermaphroditism and parthenogenesis. Hermaphroditism means individuals which function both as male and females.

Female reproductive system:

The nematodes may have a single ovary, the female is called monodelphic and when there are two ovaries it is called as didelphic. When a single gonad is present, it may be either directed towards anterior to vulva monodelphic, prodelphic or posterior to vulva monodelphic, opisthodelphic. When two gonads are present, these are usually on the opposite sides of vulva (out stretched), *i.e.,* one towards the anterior and the other towards posterior end of body, called as didelphic-amphidelphic. In some nematodes

(Meloidogyne, Heterodera etc.) both the gonads are directed towards the anterior end of the body with reflexed ovaries, it is didelphic- prodelphic.

The female gonad typically consists of an ovary, oviduct, uterus, vagina and vulva. The ovary is a hollow, elongate tube lie with flattened epithelial cells and has few to a large number of oocytes. 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 the growth zone which constitutes the greater part of the ovary. The oocytes or germ cells in this zone become big and ripe which are generally arranged in a single row. After maturity they are called oogonia.

The oviduct is next to growth zone of ovary. The oocytes when ripe they pass into oviduct through a tiny canal formed by the oviduct cells. The oviduct is made up of high columnar epithelial cells. In some nematodes where sperms are stored, a part of oviduct may serve as spermatheca. However in others, the spermatheca is in the proximal parts of the uterus or in the post vulvar sac at the distal end of the gonad.

Uterus is the largest and most complex part of the gonad, serves and functions for fertilization, egg shell formations and laying of eggs. Vagina opens through female gonopore, the vulva. In Tylenchida, the vulva is generally a transverse slit and position varies from species to species. The eggs are expelled through vulva which is normally situated in the middle of the body. Size and shape of egg is variable, however, it is usually oval, elliptical or rounded. The eggs are covered by three distinct membrane viz; an outer protein layer secreted by the uterine wall, a chitinous and vitelline layers are secreted by the egg it self. These three layers are not present in all nematodes. The gelatinous matrix (egg sac) is secreted in *Meloiqogyne* by rectal glands in which eggs are deposited and it protects the eggs from adverse environments. Generally, the eggs are laid outside the body, where the embryonic development takes place (exotoky). However, sometimes the eggs develop within the body of female (endotoxy) without being laid such nematodes may either be viviparous of ovoviviparous. In cyst forming nematodes, the female produce eggs but these hatches inside the cysts.

Male reproduction system:

The nematodes may have either one testis are called monarchic or two testis are called diorchic. In diorchic forms the testes are outstretheed i.e., directed opposite to

each other exception being *Meloidogyne* where they are in parallel position. The male reproductive system generally consists of three primordial parts; the testis, seminal vesicle and vas deference. In the testis the germinal and growth zones easily can be distinguished. In germinal zone spermatogonial division takes place, while in growth zones spermatocytes increase in size. The spermatocytes are arranged in single or double rows, but may sometimes in multiple rows. Sperms are formed at the posterior end of testis and are stored in the seminal vesicle.

Then follows the **vas deference** which consists of an anterior glandular region and a posterior muscular region and containing the ejaculatory duct at the posterior end. The ejaculatory duct helps in the ejection of sperms during fertilization. It tapers gradually and opens ventrally into the cloaca. The cloaca is provided with male copulatory structures, such as spicules, gubemaculums etc. Male nematodes have a pair of copulatory picules which are lodged in the spicular pouches. The spicular pouches are the outgrowths of the dorsal wall of the rectum. Spicules are tubular sensory structures covered by sclerotized cuticle. Its function to open the vulva for the transfer of sperms during copulation.

Gubernaculum : It is a sclerotized plate like structure located dorsal to the spicules and formed by the dorsal wall of the specular pouch.

Excretory system:

Different types of excretory system are found in nematodes which assigned an excretory function. Morphologically the excretory system is of two type (i) Renette or glandular type {ii) Canalicular or Tubular type.

Glandular type : It consists of a large cell in the pseudocoelomic cavity known as ventral gland (renette) which is connected to the excretory pore by a short or long duct that usually terminate in an ampule and open through a pore on ventral side. The whole system lies in the pseudocoelom.

Tubular type : Generally following 4 main types of systems are present and found in Class Secernentea.

(a) Simple H shaped

(b) Rhabditoid type

(c) Ascaridid type

(d) Asymmetric type

The excretory system also plays important role in osmoregulation, in addition to excretion so it is also called excretory secretary system.

Respiratory and c i r c u l a t o r y systems:

Organs for these two systems are lacking in nematodes, but the systems do work. Pseudocoelomic fluid in the body cavity serve the function of circulatory system. Oxygen for respiration is taken by body cuticle through diffusion.

General Biology of nematodes:

The life history of plant parasitic nematode is direct or simple.

- Four moults and five stages
- Eggs : 200 to 250 (Oval)

(Singly in soil or root or in gelatinous matrix.)

- Hatching : 2 to 3 days
- Juvenile stage (Vermiform) : 22 to 25 days
- Adult female or male : 2 to 3 days

(females vermiform or different shapes, male vermiform)

- Life cycle : 25 to 30 days
- Reproduction : Sexual or parthenogenetic
- Sex ratio : 1:1

The six stages are there, egg, first stage larva or juvenile (L1), second stage larva or juvenile (L2), third stage larva or juvenile (L3), fourth stage larva or juvenile (L4) and adult.

LECTURE NO. 6

CLASSIFICATION OF NEMATODES

- Phylum - Nematoda (nematodes)

Class : Adenophorea

Subclass : Chromadoria

Order - Araeolaimida
Order - Desmodorida
Order - Desmoscolecida
Order - Monhysterida

Subclass : Enoplia

Order - Dorylaimida
Order - Enoplida
Order - Mermithida
Order - Muspiceida
Order - Trichocephalida

Class : Secernentea

Order - Aphelenchida
Order - Ascaridida
Order - Camallanida
Order - Diplogasterida
Order - Rhabdiasida
Order - Rhabditida
Order - Spirurida
Order - Strongylida
Order - Tylenchida

Diagnostic characters of class Secrenentea and Adenophorea (Aphasmida)

Secernentea (Phasmida)	Adenophorea (Aphasmida)
Amphidial opening is one the head i.e. near the lip region.	Amphids open behind the head post labial.
Lateral canals open into the end excretory duct.	Lateral canals and excretory duct in a cell.
Oesophagus is divided into procorpus, Median bulb, isthumus and basal bulb.	Oesophagus is cylindrical with an enlarged glandular base.
Male tail with bursa (Caudal alae)	Male tail lacks bursa but possess genital papillae.
Caudal glands are absent.	Caudal glands are present
Phasmids are present.	Phasmids are absent.
The mesenterial tissues are less developed.	The mesenterial tissues are well developed.

Taxonomic classification of nematodes:

Phylum - Nematoda

Class (2)

Adenophorea : i. Lateral canal absent
ii. Caudal glands present

Secernentea
i. Lateral canal present
ii. Caudal glands absent

Order (5)

Dorylaimida **i. Stylet without knobs** **ii. Two part oesophagus**
III. Annulation not visible

Sub-order (1)

Dorylaimina

Superfamily (2)

Dorylaimoidea : I. Long body **Trichodoroidea I. Short body**

Family (1) **Family (1)**

Longidoridae : i. Attenuated spear (stylet knob absent) **Trichodoridae : i. Onchiostyle is long**

Genus (3) ***Longidorus, Xiphinema,*** **Genus (2)** ***Trichodorus Paratrichodorus***

Class - Secernentea

Order(5)

Tylechida

i. Stylet with knob **ii. Three part oesophagus** **iii. Annulation**

Sub-order (2)

Tylenchina
i. DEG situated in procorpus

Aphelenchina
i. DEG situated in median bulb

Super family(5)

Super family (1)

Aphelenchoidea
i. Head with bowl shaped frontal disc.

Tylenchoidea
i. Vermiform
ii. Bursa present
iii. Eggs outside body

Heteroderoidea
i. Female swollen
ii. Eggs retained in body

Criconematoidea
i. Procorpus fused with median bulb

Family (6) **Tylenchidae, Tylenchorhynchidae, Dolichodoridae, Belonolaimidae, Pratylenchidae, Hoplolaimidae**

Family

Tylenchidae
i. Ovary one
ii. Tail attenuated (Rounded)

Sub-family (6)

Tylenchinae
i. Pyriform to clavate

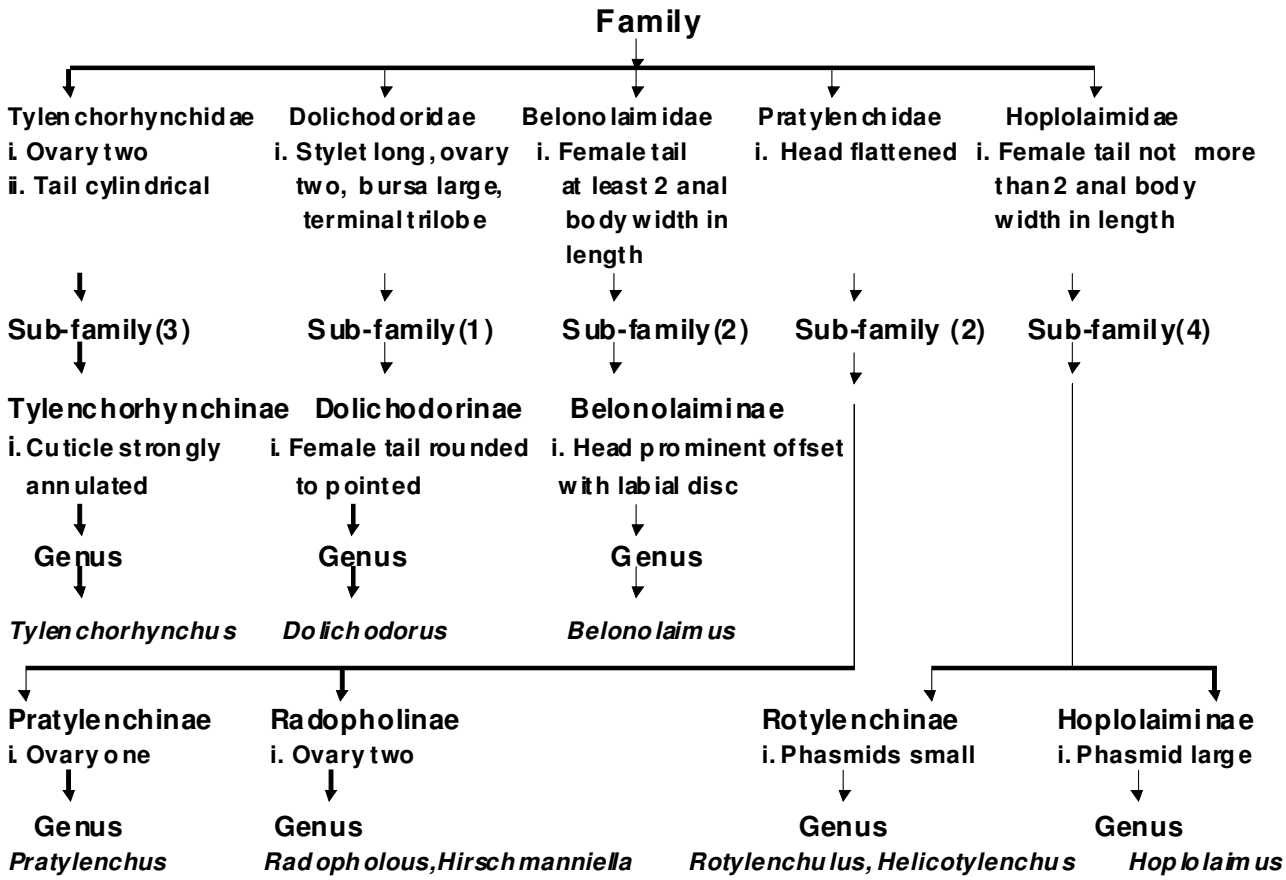
Ditylenchinae
i. Body cuticle exceptionally thick

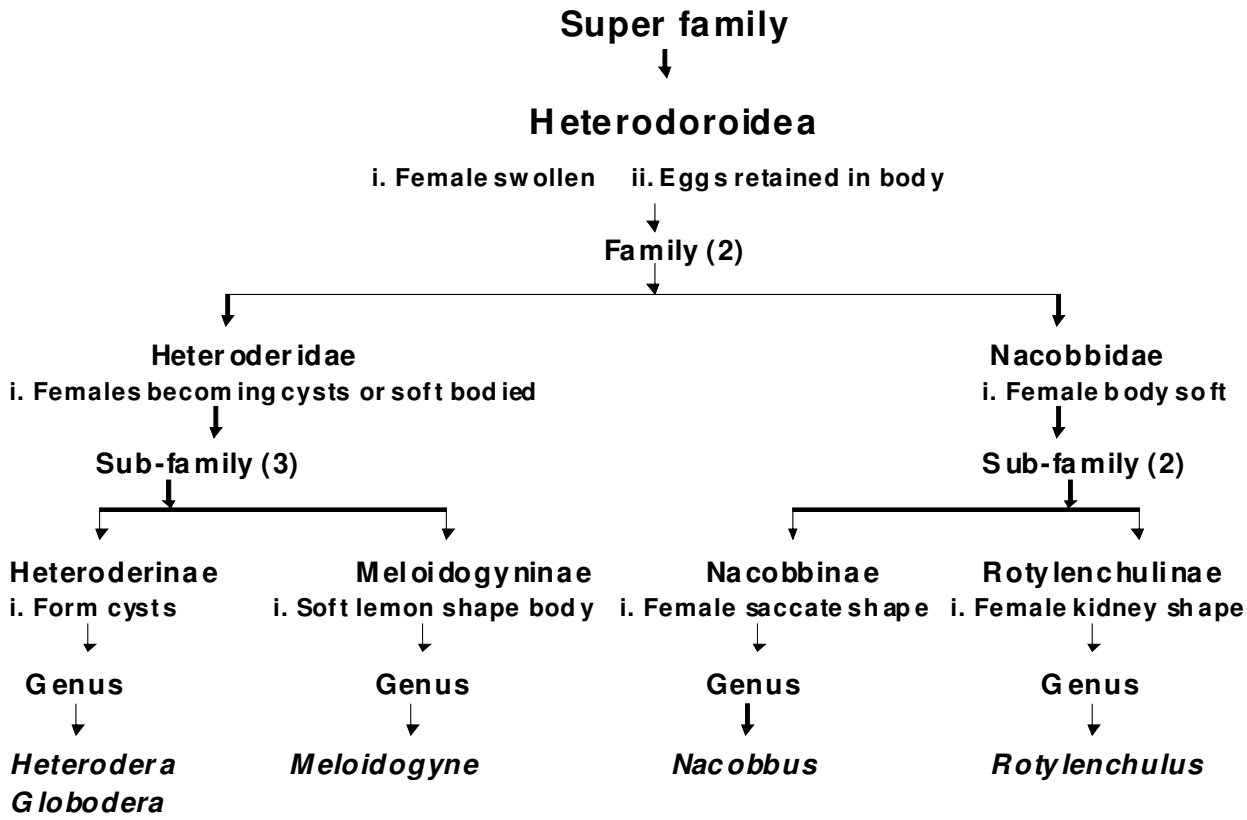
Anguininae
i. Stout, ovary upto basal bulb

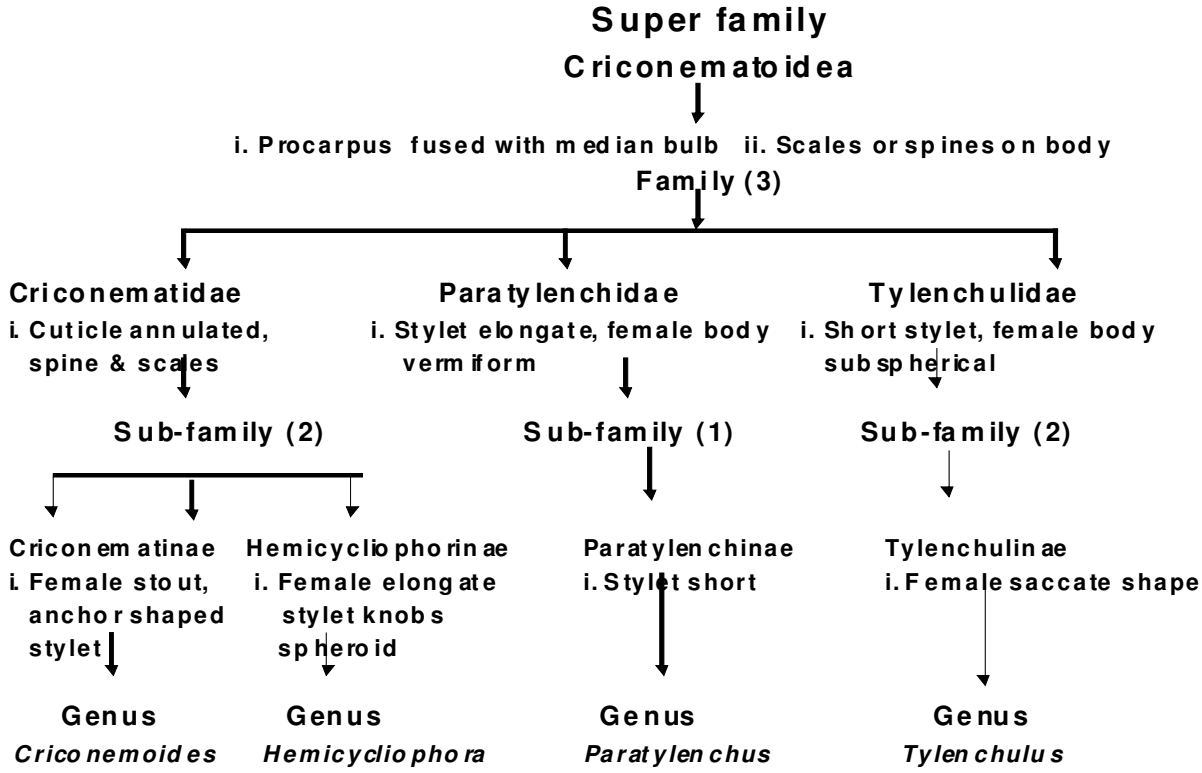
Genus
Tylenchulus

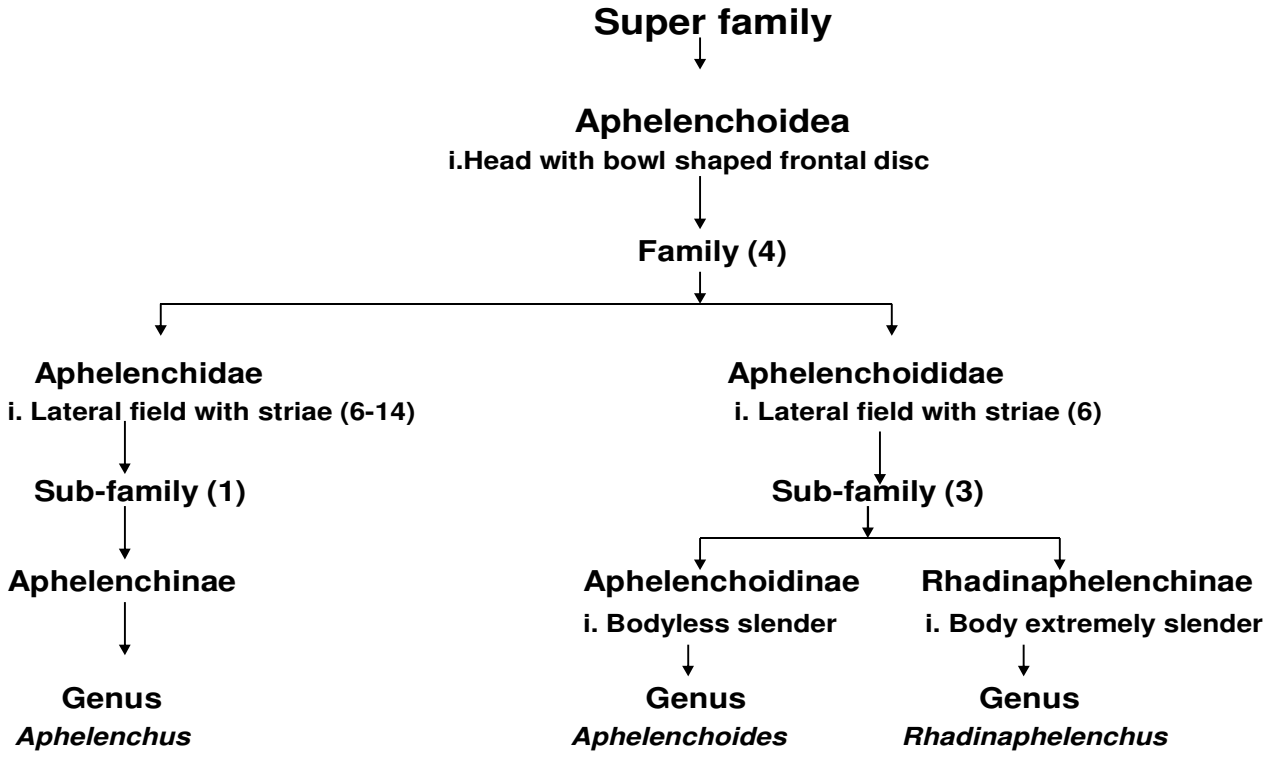
Genus
Ditylenchus

Genus
Anguina




Super family
Heterodoroidea
i. Female swollen ii. Eggs retained in body
Family (2)
Heteroderidae
i. Females becoming cysts or soft bodied
Sub-family (3)
Heteroderinae
i. Form cysts
Genus
Heterodera
Globodera
Meloidogyninae
i. Soft lemon shape body
Genus
Meloidogyne
Nacobbidae
i. Female body soft
Sub-family (2)
Nacobbinae
i. Female saccate shape
Genus
Nacobbus
Rotylenchulinae
i. Female kidney shape
Genus
Rotylenchulus


Super family
Criconematoidea
i. Procarpus fused with median bulb ii. Scales or spines on body
Family (3)
Criconematidae
i. Cuticle annulated, spine & scales
Sub-family (2)
Criconematinae
i. Female stout, anchor shaped stylet
Genus
Criconemoides
Hemicycliophorinae
i. Female elongate stylet knobs spheroid
Genus
Hemicycliophora
Paratylenchidae
i. Stylet elongate, female body vermiform
Sub-family (1)
Paratylenchinae
i. Stylet short
Genus
Paratylenchus
Tylenchulidae
i. Short stylet, female body subspherical
Sub-family (2)
Tylenchulinae
i. Female saccate shape
Genus
Tylenchulus



Taxonomical status and description of important plant parasitic nematodes of Tylenchoidea:

Family : Anguinidae

Genus : *Ditylenchus*: Thin body cuticle, stylet small with basal knobs, oesophageal gland with distinct basal bulb with a small lobe projecting part of intestine, female tail elongate and conoid; ovary one.

Genus : *Anguina*: Body cuticle thin, stylet small with basal knobs, ovary with one or two flextures, oocytes in multiple rows arranged in rachis. Occuring in wheat seed gall.

Family : Pratylenchidae

Subfamily : Pratylenchinae

Genus : *Pratylenchus*: Oesophageal gland overlaps the intestine ventrally. Single ovary.

Genus : *Radopholus*: Oesphageal gland overlaps the intestine dorsally, tail terminus rounded. Two ovaries.

Family : Hoplolaimidae

Subfamily : Hoploaiminae

Genus : *Hoplolamus*: Well developed cephalic frame work phasmids large not opposite to each other, stylet well developed, massive basal knobs with anterior projections, two ovaries.

Genus : *Scutellonema*: Large phasmids opposite to each other on tail.

Genus : *Helicotylenchus*: Dorsal oesophageal gland open away from the base of stylet, ventral overlapping.

Subfamily : Rotylenchulinae
Genus : *Rotylenchulus*: Dorsal oesophageal gland open near the base of stylet, dorsal overlapping of oesophageal gland, two ovaries, males with weak stylet, adanal bursa.

Family : Heteroderidae
Subfamily : Heteroderinae
Genus : *Heterodera*: Pyriform or lemon shape; female body becoming cyst.
Genus : *Globodera*: Round or globular cysts.
Subfamily : Meloidogyninae
Genus : *Meloidogyne*: Body cuticle thin, without spines, vulva and anus close together, terminal, eggs laid in gelatinous matrix. Adult female pyriform with tapering neck.

IMPORTANT PLANT PARASATIC NEMATODES:

1. **Root-knot nematode, *Meloidogyne* Species**

Diagnosis: Female : Swollen, subspherical, sedentary; cuticle annulated. Stylet with well developed basal knobs. Gonads didelphic (genital tract normal), prodelphic (uterus projected anteriorly), elongated and convulated (Folded or coiled). Eggs laid in gelatinous matrix outside the nematode body. Anus and vulva terminal surrounded by particular striae on the cuticle known as perineal pattern.

Male: Long and vermiform, spear well developed, tail short without caudal alae.

Second stage juvenile: Small vermiform**,** stylet small with slight knobs. Tail elongated, conoid without hyaline portion.

Third and fourth instar juveniles: Swollen with spike tail, without stylet, found inside the roots.

Important species:

(i) *Meloidogyne incognita*
(ii) *M. javanica*
(iii) *M. arenaria*
(iv) *M. hapla*

Distribution: Throughout the country

Hosts: Vegetables, pulses, fruit, plantation and fibre crops, ornamental plants.

Biology:

Root know nematodes are sedentary parasites. The second stage juveniles are infective and established a feeding site involving vascular tissues, endodermis and pericycle cells. The feeding site comprises of 8-12 multinecleate ‘giant cells’ (Fig. 21). The second stage juveniles start swelling and develop to third and fourth stages. After final moult, male aquires vermiform

shape and come out into the soil. Female attain sac-like shape and lay eggs on the root generally by parthenogenesis, and nematodes complete 7-8 generations in a year.

Disease caused: Root knot disease.

Symptoms: Diseased plant shows yellowing of leaves, stunted growth, reduced vigour of the plants, reduced size and number of fruits.

Formation of root knots or galls on the roots is the typical symptom caused by this nematode on various crops. The galls may vary in size and shape depending upon initial nematode population, species of the nematode, type of the plant etc. (Fig. 21). Initially the galls are small (primary galls) but at later stage of plant growth big (compound) galls are formed due to reinfection.

Under severe infestation, these nematodes also predispose the attack of certain weak fungi and bacteria and kill the plants.

In leguminous plants, gall produced by nematode differ from bacterial nodules in the following respects.

Nodule	Gall
• Attached on the side of the root • Can be detached easily • Soft in consistency	Swelling of root itself Can not be detached They are hard

Control:

- Two to three deep ploughings at 10-15 days interval during May/June.
- Rotations with cereal crops.
- Apply Carbofuran (Furadan 3G) @ 7 gm/m^3 in the nursery beds or transplanted vegetable crops.
- Grow resistant tomato varieties – Hisar Lalit, PNR 7 or SL- 20.
- In grapevines, apply carbofuran @ 13 g/ m^3 around vine trunk (9 m^2) 3 weeks after pruning.

2. Burrowing nematode, *Radopholus similis*

Diagnosis : Marked sexual dimorphism

Female : Vermiform; head low, cephalic sclerotization prominent, stylet with well developed knobs; esophagus overlapping intestine dorsally; Amphidelphic; tail elongate, conoid with

rounded terminus.

Male : Vermiform, stylet and esophagus degenerate, caudal alae complete.

Disease caused : Black head or toppling disease of banana

Distribution : Andhra Pradesh, Karnataka, Kerala, Maharashtra, Tamil Nadu, Gujarat, Goa.

Hosts : Banana, pepper, coffee, coconut and Arecanut etc.

Biology : These are migratory endoparasites. Larvae and adults of both the sexes are infective and enter the young roots at some distance from the root tip and move through the air channels between the radial lamillae of the cortical cells forming cavities in the root. Eggs are deposited scattered within the roots.

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.

Control:

- Paring and Hot- water treatment of sets at 55 ^{0}C for 25 minutes.
- Dip pared sets in mud slurry, sprinkle carbofuran @ 1.2 g a.i., dry in shade before planting.

3. Citrus nematode, *Tylenchulus semipenetrans*

Diagnosis:

Female : Small sized, neck long, inside the root tissues, posterior portion swollen. Stylet small, esophagus criconematid type, excretory pore near middle of the body, post uterine sac present.

Male : Stylet and oesophagus degenerated, caudal alae absent.

2nd stage juveniles: Small vermiform, stylet short, esophagus criconematid type, excretory pore near mid body, tail conical.

Disease caused : Slow decline of citrus, citrus dieback.

Distribution : Throughout the country.

Hosts : Citrus species.

Biology : It is sedentary semiendoparasite. Second stage juveniles infect the roots by inserting the head region in the cortical layers of the roots. A feeding site involving 6-10 cells (nurse cells) is formed. Further moults occur and adult female continues feeding on nurse cells with 1/4th of

the body remaining inside the root. Infection near the parent feeding sites results in the formation of colonies. At maturity the females excrete gelatinous matrix into which eggs are deposited. Male development takes place in soil.

Symptoms : The diseased trees show reduction in growth and vigour 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 gelatinous matrix excreted by the adult females. Cortex of highly infested feeder roots decays and gets sloughed off easily.

Control:

- Apply carbofuran @ 13 g/m^3 around tree trunk (9 m^2 area) just before flowering, followed by light irrigation.
- Apply carbofuran @ 7 g/m^3 (as above) + neem cake @ 1 kg/tree just before flowering.

4. Reniform nematode, *Rotylenchulus reniformis*

Diagnosis (Fig. 22):

Mature females : Kidney-shaped, swollen, anterior portion inside the root tissue. Stylet strong, Amphidelphic, tail short, peg like, eggs laid in gelatinous matrix.

Immature females: Vermiform, 'C' shaped, cephalic sclerotization and stylet well developed. Opening of dorsal oesophageal gland much posterior in corpus region, oesophageal glands overlapping, vulva in the middle of the body, Amphidelphic. Tail elongate, conoid.

Male : Stylet and oesophagus reduced, caudal alae rudimentary.

2nd stage juvenile: Similar to immature females but lack sex organs.

3rd and 4th stage juveniles: With superimposed cuticle.

Distribution : Throughout the country.

Hosts : Vegetable, pulse and oilseed crops, sugarcane, tobacco, banana, grapevine, cotton, citrus etc.

Biology : Sedentary semi-endoparasite of roots, this nematode is unique in that adult female is infective stage. Vermiform female insert its anterior most region of the body into the roots and starts swelling, becoming kidney-shaped. Gelatinous matrix is secreted by the female around the body in which eggs are deposited. Second stage juveniles undergo three moults in the soil. Third and fourth stage juveniles do not shed the old cuticle. The nematode can feed upon

cortical, pericycle, endodermis as well as phloem cells and results in the formation of syncytia due to hypertrophy and hyperplasia of the cells.

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

Control:

- Crop rotation with resistant or immune plant species is recommended. These include mustard (*Brassica nigra*), oats, onion, sugarcane, and sun hemp (*Crotalaria juncea*) (Robinson et al. 1997, Caswell *et al*. 1991). Pineapple is rotated with sugarcane in Puerto Rico (Roman 1964). Sorghum, maize and reniform nematode resistant soybeans are recommended as rotation crops for cotton (Starr and Page 1990).

5. Root lesion nematode, *Pratylenchus spp.*

Female – Vermiform, lip region flat, stylet long with rounded basal knobs. The esophagus overlaps intestine ventrally. Ovary single, vulva posterior, tail cylindrical with smooth or crenate terminus.

Male : With smooth or crenate terminus. Stylet and esophagus fully formed, tail elongate conoid enveloped with caudal alae.

Important species : *P. coffeae,* P. zeae and *P. thornei*

Distribution : Widely distributed in most of the tropical regions of Asia (India, Thailand and Japan), Africa, Australia and America.

Hosts : *P. coffeae :* Citrus, banana, coffee; *P. zeae :* Maize, *P. thornei :* Pulses.

Biology : These are migratory endoparasites. Larvae and adults of both the sexes are infective and enter the young roots at some distance from the root tip and move through the air channels between the radial lamillae of the cortical cells forming cavities in the root. Eggs are deposited scattered within the roots. The egg to egg cycle is completed in about 27 days at 26-32 ^{0}C.

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:

- Fallowing for a period of six months or longer.
- Crop rotation with non-host crops.
- Use of disease free planting material.
- Storage of large corms in the sun for two weeks prior to planting.
- Use of cover crop calapogonium.
- Removal of infested portions of corm before planting.
- Hot water treatment at 55 ^{0}C for 15-25min.
- Paring and Pralinage.
- Carbofuran 3 g @ 40g/ plant at 90 days after planting.
- Application of neem cake.

6. Spiral nematode, *Helicotylenchus spp.*

Female : Body 'C' shaped when relaxed, lip region, hemi-spherical, slightly offset, stylet moderately long, dorsal esophageal gland orifice typically located more than one half stylet length, posterior to stylet knob, ovaries two, vulva posterior to middle of body, Tail rounded to nearly pointed, tail of males short and with bursa.

Male : Similar to female except sexual dimorphism

Distribution : Throughout the country.

Host : Banana, cocoa, sweet potato, citrus, sugarcane, mango, fig, cassava, coffee, maize, rice, grapevine, orange etc.

Biology : Endo and ecto-parasitic on many plants, all stages found in soil and roots. Newly laid eggs hatched within 48-51 hours and first moult is believed to take place outside the egg shortly after hatching. Second stage larvae undergo 2nd, 3rd and 4th moults. During 4th moult the male and female differentiate due to gonad development.

Symptoms : The nematodes attack root cortex and produce necrotic lesions. **Banana spiral nematode:** *Helicotylenchus multicinctus* - Infested plants remain stunted, take longer to mature, yield smaller bunches and degenerate faster than uninfested plants. Extensive, discrete, superficial necrotic brown lesions on root surface which may even coleasce. The thin secondary and tertiary roots are also attacked and necrosis is more pronounced which

eventually leads to debility of entire plant. The nematode also penetrates the corms, causing red lesions Infested plants remain stunted, take longer to mature, yield smaller bunches and degenerate faster than uninfested plants.

Control:

- Heavy mulching of organic matter minimizes the population of *H. multicinctus*.
- Rice or green gram grown after the crop reduces the population of *H. multicinctus*.
- **Physical** methods include removal of infested tissues by paring and disinfecting them in hot water at 55^0 C for 20 minutes before planting. In Kerala, the practice of 'sun drying' the rhizome before planting has been found effective in control of nematodes.
- Carbofuran and phorate at 2 g a.i./plant at planting are the most effective and popular granular nematicides used against banana nematodes. Ethoprop at 2-3g a.i./plant or carbofuran at 1.2 g a.i./ plant reduce the nematodes population with high cost benefit ratio.
- A few germplasms like **Kadali, Kunnan, Pisang seribu** and **Ayiranka Poovan** are known to support low nematode populations.
- Integrations of paring of suckers and hot water dipping at 55^o C for 20 min., neem cake 400 g per plant and carbofuran 20 g per plant before planting significantly reduce the nematode population and improve the plant growth.

7. Cyst nematode, *Heterodera and Globodera spp.*

Heterodera: **Diagnosis:**

Female : Body ovoid to globose with protruding neck and vulval cone, white in colour, cuticle with lac-like or zig zag pattern. Stylet strong with well developed knobs. Genital tract didelphic, prodelphic, eggs retain inside body and some may be laid in gelatinous matrix.

Male : Body long vermiform with posterior twist. Stylet fairly stout with knobs. Tail short, caudal alae absent.

Second stage juveniles: Vermiform, stylet fairly stout with well formed knobs. Oesophagous overlapping intestine. Tail elongated, conoid and with hyaline portion.

Cyst : Lemon shaped with a vulval cone. Vulva surrounded by transparent thin walled area which breaks down on maturity to form birth pore/fenestra. Eggs/juveniles retained inside body.

Sr. No.	Important species	Distribution	Hosts
(i)	Cereal cyst nematode, *H. arenae*	North India	Wheat and Barley
(ii)	Pigeon pea cyst nematode, *H. cajani*	Widespread	All pulses, sesame, cluster bean etc.
(iii)	Maize cyst nematode, *H. zeae*	Maize growing areas	Maize
(iv)	Rice cyst nematode *H. oryzicola*	Kerela, M.P., Orissa, West Bengal	Rice and banana

Biology: Sedentary endoparasites of root. Second stage juveniles infect the roots and establish a feeding site (syncytium) within the vascular bundles, in which 8-10 adjacent cells enlarge and coalesce resembling a multinucleate cytoplasmic mass. Third and fourth stage juveniles gradually swell and posterior end starts protruding out from the roots; lemon shaped white females appear on the root surface, only their neck portion is inside the root. Eggs are laid inside the female body. Male vermiform, leave the roots. Females gradually turn brown, detach from the roots and remain in the soil during off-season as cyst stage enclosing eggs within. *H. aveane* is active during rabi season and completes only one generation. *H. cajani* and *H. zeae* multiply during kharif season and complete several generations.

Symptoms: The diseased plants show yellowing of leaves, stunted growth, reduced tillering inspite of sufficient moisture and fertilizer in the field. Ear heads if formed, are very small known as **'Molya'** disease' in Rajasthan and Haryana.

Control: For *H. avenae* on wheat and barley.

- Two or three summer ploughings at 10-15 days interval during May/June.
- Rotation with mustard, chickpea for one to two years.
- Grow resistant barley varieties C-164, Rajkiran.
- Apply carbofuran @ 1-2 kg a.i./ha.

Globodera: **Diagnosis:**

Female: Body globose or ovoid, terminal proturbance absent.

Cyst: Circumfenestrate, vulval slit less than 15 µm, usually lost on cysts from soil.

Important species :

Globodera rostochiensis – Potato cyst nematode or Golden nematode

G. pallida - Potato cyst nematode or Golden nematode

Distribution : Nilgiri and Kodai Kanal hills in Tamil Nadu.

Hosts : Potato, tomato, brinjal.

Biology : Same as in *Heterodera*

Symptoms : 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:

- Rotation with pea, cabbage, carrot, cauliflower, French bean etc. during autumn season.
- Grow resistant potato varieties like Kufri Swarna, Kufri Thenmalai.

8. Dagger nematode, *Xiphinema spp.*

Diagnosis:

Female: Body elongate-cylindrical, forming open spiral with a greater curvature in posterior half, without annulations. Stylet typically long, very slender with fanged knobs and 'guiding ring'. Oesophagus with slender anterior tube and wide posterior part. Ovaries one or two. Vulva near the middle of body or near posterior end of oesophagus when only one ovary is present. Tail bluntly rounded or with projections on ventral side in both males and females.

Male : Extremely rare, not essential for reproduction. Body shape, lip region, oesophagus, tail etc. similar to female.

Distribution : Throughout the country

Hosts : Grapevine, fig, rose, mulberry, citrus etc.

Biology : Adults and larvae are migratory root ectoparasites. The nematode does well in heavy soils, with quicker population build-up and shorter duration of life, as the temperature increases. The females feed only on the root tips, which causes root galling, while young larvae feed in the peripheral region of the roots and no galling symptoms. Reproduction by parthenogenesis. The eggs hatch in 6 to 8 days and the first moult takes place outside the egg. 2^{nd} , 3^{rd} and 4^{th} moults occurs at 6 days interval. The nematode complete its life cycle with in 22 to 27 days at 24 ^{0}C.

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

9. RICE STEM NEMATODE, *Ditylenchus angustus*

Diagnosis :

Female : Swollen 'C' shaped, stylet small with delicate knobs, basal oesoplageal bulb not overlapping the intestine, cardia absent, vulva in posterior region of the body, ovary single, prodelphic, tail elongate.

Male: Similar to female but more slender, caudal alae sub-terminal.

Diseased caused: Ufra disease of rice.

Distribution: Assam, West Bengal.

Host: Rice.

Biology: Fourth stage juvenile and adult female form nematode 'wool' in the absence of the host on the stubbles. On getting water, the nematodes become active and enter the whorl of the leaf sheath of rice seedlings. Four to five generations can be completed there, after which nematodes enter the stem and ear primordial. They undergo quiescence when the plant ripens.

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

At the reproductive phase, the nematodes collect around the floral primordial and feed upon the developing earhead. As a result, each ear either emerges as crinckled or twisted with many empty spikelets (ripe ufra) or does not emerge at all (swollen ufra). Infected plants remain stunted and their leaves often wilt.

Control:

- Burning of stubbles, straw after crop is over.
- Rotation with jute, sesamum.

Host – Parasitoid relationship

The plant parasitic nematodes are obligate parasites and most of them feed on subterranean plant parts. Plant parasitic nematodes confined to three orders; Dorylamida, Tylenchida and Aphlenchida. Nematode feeding on plant tissues may cause either mechanical or biochemical injury. Most common enzymes released by nematodes during injury to plant parts are cellulose, protease and amylase. These enzymes brings about the alteration in plant parts like cell division in meristimatic tissues may be increased or decreased resulting into hypertrophy or hyperplasia or may die due to formation of necrotic tissues. A successful host-

parasitoid relationship results in appearance of symptoms on host plants. The symptoms of injuries caused by plant parasitic nematodes can be divided into two categories.

Symptoms of nematode diseases can be classified as

I. Symptoms produced by above ground feeding nematodes

II. Symptoms produced by below ground feeding nematodes

I. Symptoms produced by above ground feeding nematodes:

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

2. Dead or devitalized bud: Straw berry plant infected *A. fragariae*, affects the growing points and kill the plant.

3. Seed galls: In wheat, *Anguina tritici* larvae enter into the flower primordium and developed into galls. Nematode can survive upto (28 year) large time inside the cockeled grain.

4. Twisting of leaves and stem: In onion, basal leaves become twisted when infected by *Ditylenchus dipsaci*. Top leaves in rice crop become twisted with *D. angustus.*

5. Crinkled or distorted stem and foliage: Seed gall nematode, *A. tritici* infest the growing point which cause crackle on distortion stem and leaves.

6. Necrosis and discolouration: Red ring in coconut caused by *Rhadinaphelenchus cocophilus*. Due to infestation coloured circular area appeared in the trunk.

7. Lesions on leaves and stem: Small yellowish spots are appeared on onion stem and leaves due to *D. dipsaci*, lesion caused by *A. ritzemabosi* on Chrysanthemum.

II. Symptoms produced by below ground feeding nematodes:

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

a) Above ground symptoms

b) Below ground symptoms

A) Above ground symptoms:

1. Stunting: Reduced plant growth, and the plants can not able to withsatand adverse conditions. Patches of stunted plants appears in the field e.g. potato due to *Globodera rostochiensis*, in generally due to *Heterodera cajani* and in wheat by *Heterodera avenae*.

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

3. Decline and die back: In banana, decline and die-back are caused by *Radopholus similis,* spreading decline in citrus due to *R. citophilus* and slow decline of citrus due to *Tylenchulus semipenetrans*. In grapevine slow decline is caused by *Meloidogyne* spp.

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

B) Below ground symptoms:

1) Root galls or knots: Characteristic root galls produced by root-knot nematode, *M. incognita,* falls root knots produced by *Nacobus* on sugarbeeet and tomato. Small galls are produced by *Hemicycliphora* on lemon. *Ditylencus* on wheat/oat, *Xiphenema* on rose.

2) Root Lesions: Penetration and movement of nematodes in the root causes typical root lesions. *e.g. Partylenchus* sp, *Radopholus* in Banana, *Partylenchus* in Coffee, Rice root nematode in Rice.

3) Reduced root system: Due to feeding on the tip of root / root lets growth is arrested and root produced branches which produced the stubby root, curly roots, coarse root.

a) Coarse roots: *Paratrichodorus* arrest the growth of lateral roots. Least to open root system with only main root system without lateral system.

b) Stubby roots: The lateral roots produced excessive root lets.

c) Curly tip: In the injury caused by *Xiphinema* spp. nematodes retards the elongation of roots causes curling of roots known as fish hook sysmptoms.

4) Root proliferation: Increase root growth or excessive branching due to nematode infestation. The infested plant produced excessive root hairs at the point of infestation. e.g. Trichoderoot ojstei, Nacobbus, *Heterodera, Meloidogyne, Partylenchus*

5) Root rot: Nematode feeds on fleshy structure resulting in rooting. *e.g. Ditylenchus destructor* on potato.

6) Root surface necrosis: Sever injury caused by *T. Semipenetrans* on citrus caused complete decortication of roots and results root necrosis.

7) Cluster of sprouts on tubers: Cluster of shot and swollen sprouts are formed due to *D. dipsaci o*n tuber.

Classification of plant parasitic nematodes on the basis of parasitism/habitat:

Basically they are divided into two groups:

1. Below ground feeder
2. Above ground feeder

Nematodes parasitizing the below ground parts can be further divided in the following categories on the basis of their feeding habits

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

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

A) Migratory ectoparasite:

e.g. *Criconemoides, Paratylenchus, Trichodorus*. Entire life cycle spend free in soil feeding externally on leaves, plants, deposit egg in soil when host disturbed the detached.

B) Sendentary ectoparasite:

E.g. *Hemicycliophora arenaria, Cocopaurus pestis.* Attachment of nematodes to roots system is permanent.

2. Semi-endoparasitic nematodes: E.g.*Rotylenchulus reniformis, Tylenchulus semipentrans.* The anterior part of the nematode head and neck being permanently fixed in the cortex and posterior part extends free into soil.

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

a) Migratory endoparasite: E.g. *Hirschmanniella, Partylenchus and Radopholus similis.* This nematode are found in cortical parenchyma of roots while migrating they feed on cell, multiply and cause necrotic region.

b) Sedentary endoparasite: E.g. *Heterodera, Meloidogyne.* The second stage larvae penetrate the root lets and become sedentary throughout the life cycle inside the root cortex.

Interaction of nematodes with micro-organisms:

Plant parasitic nematodes favour the establishment of secondary pathogen viz., fungi, bacteria, virus. The nematodes cause mechanical wounded which favours the entry of micro organisms. In association of nematode and pathogen break the resistance in resistant genotype. The nematode also alter the host that colonization by the secondary pathogen.

Various kinds of associations and host parasite relationship has been reviewed by many nematologists (Pitcher, 1962 and 1965 , Powell 1979).

- **Nematode bacterium associations** - There are various interactions on nematode bacterium associations. Studies wilt in potato, banana etc. Generally the nematodes predispose the host to infection by this bacterium.
- **Nematode plant virus association** - Intraction between nematode and virus are better known through the role of nematodes as vectors of soil-borne plant virus. There are reports of association in which the Virus increases or decreases nematode population.
- **Nematode fungus association** – There are various types of nematodes involved in intraction between nematode and fungi. Research on disease complex involving nematodes and fungi, especially those involving Meloidogyne spp. And wilt-inducing fungi of recognized.

Nematode – Fungi interaction:

Crop	Name of the disease	Nematode	Fungus	Role of nematode
Cotton	Damping off	*Meloidogyne incognita,* *M. acrita*	*Rhizoctonia solani* *Pythium spp.*	Assists Assists
	Vascular wilt	*M. incognita* *Rotylenchulus* *B. longicaudatus*	*Fusarium oxysporum* *F. vasinfectum* *F.oxysporum* *F.oxysporum*	Assists Assists Assists
Tobacco	Damping off	*M. incognita* *M. acrita,* *M. incognita,*	*P. debryamum* *Alterneria tenusis*	Assists Assists
	Vascular wilt	*M. incognita* *M. incognita acrita*	*F.oxysporum* *P. parasitica*	Assists Assists
Banana	Vascular wilt	*R. similis*	*F.oxysporum cubense*	Assists
Tomato	Cortical rot	*Globodera rostochiensis*	*R. solani*	Assists
	Vascular wilt	*Meloidogyne* spp.	*F. oxysporum*	Assists
Potato	Damping off	*D.destructor*	*P. infestans*	Assists

	Cortical rot	*G.rostochiensis*	*R. solani*	Assists
Onion	Damping off	*D. dispasaci*	*Botrytis allii*	Assists
Brinjal	Vascular wilt	*P. penetrans*	*V. albo-atrum*	Assists
Pea	Vascular wilt	*Pratylenchus* spp. *P. penetrans* *Hoplolaimus* spp.	*F. oxysporum* *F. pisi* *F. oxysporum*	Assists Assists Assists
Soybean	Damping of	*M. javanica*	*R. solani*	Assists
	Vascular wilt	*Heterodera*	*Fusarium* sp.	Assists
Cowpea	Vascular wilt	*M.Javanica*	*F. oxysporum* sp.	Assists
Lucerne	Vascular wilt	*M. hapla*	*F. oxysporum* sp.	Assists
Wheat	Stem rot	*Anguina tritici*	*Dilophospora*	Essential
	Wheat rot	*H. avenae*	*R. solani*	Assists

Nematode – Bacterium interaction:

Crop	Name of the disease	Nematode	Bacterium	Role of nematode
Wheat	Tundu	*A. tritici*	*C. tritici*	Essential
Tomato	Vascular wilt	*M. hapla*	*P. solanacearum*	Assists
		M. incognita		
	Vascular wilt	*Helicotylenchus*	*P. solanacearum*	Assists
	Canker	*M. incognita*	*C. michiganens*	Assists
Potato	Vascular wilt	*Meloidogyne*	*P. solanacerum*	Assists
Lucerne	Vascular wilt (crown buds)	*D. dipsaci*	*C. insidiosum*	Assists

Nematode – Virus interaction:

Nematode serve as vector of virus. Raski and Goheen (1958) 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	*X. diversicaudatum*
Grapevine fan	*X. index*
Yellow mosaic	*X. index*
Tobacco ring spot	*X. americanum*
Cowpea mosaic	*X. basiri*
Tomato black ring, beet ring spot	*L. elongatus*
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. similis
Pea early browning	*P. anemones, P. pachydermus,*
	P. teres, T. viruliferus

The nematode 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*.

Transmission:

1. Retention to close biological association between virus and vector as in *X.index*.
2. Retention of virus mechanically as in *Longidorus* in the inner surface of cuticle lining of lumen as in *Longidorus and Paralongidorus,* cuticle lining of style extension and oesophagus in *Xiphinema.*

The virus particles are released into plant cell with the help of oesophagus.

NEMATODE MANAGEMENT:

Nematodes are thread-like roundworms invisible to the naked eye. Species parasitic on plants attack roots and other plant parts, causing stunting and yield reduction. Nematode-infected plants are not only weakened, but their root systems are more susceptible to secondary infections by fungi or bacteria. For example, nematode-infested okra plantings are often attacked by *Fusarium* wilt. Not all nematodes are plant pathogens, however. The majority of nematode species live in the soil on decaying plant material where they are active in decomposition and nutrient recycling. Some species parasitize other nematodes or insects.

Correct identification is the first step when a nematode problem is suspected. The second step is to determine whether populations are high enough to threaten the crop. Most state soil testing services can analyze for nematodes. Soil samples taken during warm weather and when plants are growing are more useful in revealing nematode problems than samples taken in winter or from fallow land. Their reproductive rate is slower at cooler temperatures, so

populations build up more slowly. Thus, cool season crops are less likely to be damaged. Early spring potatoes, for example, are rarely damaged by nematodes.

Plant parasitic nematode 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.

Different Methods of nematode management:

Plant parasitic nematode 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.

1. Regulatory method
2. Physical method
3. Cultural method
4. Biological method
5. Chemical method

(I) Regulatory method:

It is method of pest and disease control to impose legal enforcement to prevent them from spreading. The principle involved in enacting quarantine is exclusion of nematode from entering into an area which is not infested, in order to avoid the spread.

Quarantine principle are traditionally employed to restrict the movement of infected plant materials and contaminated soil into a state or country. Shipment of planting material may be treated to kill the population of nematodes. Quarantine also prevent the movement of infected plant and soil to move out to other nematode free area. E.g. Soybean cyst nematode, *Heterodera glycines* state quarantine of *Globodera* on potato during 1941. 1953 *Radopholus similis* on citrus was brought under state quarantine Act.

Plant quarantine in India:

The destructive insects and pest Act, 1914 was pass by Govt. of India which restrict introduction of exotic pest and disease into country from abroad. The Agricultural Pest and Disease Act of various states to prevent the inter state spread of pest within country. The Plant Protection Advisor to the Govt. of India to under the inspection and treatment e.g. Regulation

have been made against 1. G.*rostochensis* 2. *Radinaphelenchus cocophilus* red ring of coconut. Domestic quarantine regulation have also been imposed for the purpose of restriction of movement from Tamil Nadu to other part.

Prevention of nematode spread:

Nematode spread through air, soil, seed, plant material or water. Cysts of *Heterodera* can be easily dispersed by wind current. This type of spread is common in sand, loan soil where the dust storm facilitate the spread.

Soil adhering potato tubers saved as main source when used or seed material. Farm implements also carry some amount of soil, animal, laour also carry the some amount of soil. *Anguina tritici* responsible for ear cockle which known to spread alongwith seeds, onion bulb, *Ditylenchus dipsaci* from one place to other. Movement of irrigation and flood which may also passive dispersal of nematodes.

The following stem to be taken:

1. Only nematode free seed / planting material should be used for raising of crop.
2. The seed should be sown where the nematode population is not known to exist.
3. Regularly estimate the population of nematode under cropped area.
4. Seed born nematodes can be controlled by fumigation, hot water treatment.

Prevention of nematode multiplication:

Multiplication of nematode can be prevented withdrawing or temporarily stopping cultivation of susceptible host plant. Seed certification can be adopted e.g. Banana sucker *R. similis,* Potato seed free from cyst nematode, wheat seed from *A. tritici*.

(II) Physical method:

It is very easy to kill nematode in laboratory by exposing heat, irradiation, osmotic pressure.

Heat: Sterilization of soil by allowing steam is a practice in soil used in green house, seed beds and small area cultivation. Insects, weeds, seeds, nematodes, bacteria or fungi are killed by steam sterilization. The soil surface needs to cover during steaming operation. The plastic sheets are used to sterilize the soil, lab / pot culture experiment autoclaves are used to sterilize the soil.

Hot water treatment: Hot water treatment is commonly used for controlling nematodes. Prior to planting the seed material like banana suckers, onion bulb, tubers, seed, roots, can be dipped in hot water at 50 – 55 ^{O}C for 10 minutes.

Irradiation: It also kill the nematodes. Cysts *G.rostochiensis* exposed to 20,000 γ contained only dead eggs, 40, 000 γ exposure loss their egges contents *D.myceliophagus* on mushroom compost to γ rays between 48,000 to 96,000 γ inactivated nematode. UV light also kill the nematodes not practically.

Osmotic pressure: 100 % nematode mortality when sucrose or dextrose were added to nematode infested soil @ 1 to 5% by weight not practical.

Washing process: The nematode containing soil adhering to potato, tubers, bulbs and other planting material should be washed carefully to avoid spreading of nematode in new area.

Seed cleaning: Remove the seed galls from the normally healthy material.

Ultrasonic: Untasonics have little effect on *Heterodera* not feasible practically.

(III) Cultural methods:

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

Selection of healthy seed material: Propagation of vegetative part from healthy plant. Golden nematode of potato, *R.similis*, *Helicotylenchus, Partylenchus* of banana can be eliminated by selecting nematode free plant material. Like wise wheat gal, rice white tip.

Adjusting the time of planting: Life cycle depend on the climatic factors. Adjust the planting helps to avoid nematode damage. When the crop planted in winter whome the temperature of soil is low where the activity of nematode can not be active at low temperature early potato and sugar beat grow in soil during cold and escape the cyst nematode.

Fallowing: Leaving the field without cultivation, preferably after ploughing helps to expose the nematodes to sunlight and nematodes die due to starvation without host plants.

Deep summer ploughing: During onset of summer the infested filed is ploughed with disc, reversible plough which exposed to hot sun which increase the soil temperature and kill the nematodes. Raising of nursery beds for the vegetable crop can be prepared during summer, covered with polythene sheet which enhanced the temperature by 5 – 10 ^{O}C resulting the killing of nematode. Soil solarisation using polythene sheet.

Manuring: Raising green manure crops and enrichment of FYM, oil cake, neem cake pressmud cake, poultry manures etc in soil encourages the development of predacious nematodes, *Mononchus* sp and other antagonistic microbes in the soil which check the nematodes in the field.

Flooding: Flooding can be done when ample irrigation water is available. Under submerged conditions, anaerobic the nematodes by asphyxiation. Chemical lethal to nematodes such as hydrogen sulphide (H_2S), NH_4 are released in flooded conditions which kill the nematodes.

Antagonistic crops: Crop like mustard, marigold and neem have a chemical alkaloids as root exudes which repel / suppress the plant parasitic nematodes.

Marigold (*Tagetes* spp) plant the α terthinyl and bithinyl compounds are present throughout the plants root tip. Mustard allyl isothiocyante pangola grase pyrocatechol are present.

Removal and destruction of infected plants: Early detection of infested plants and removal to helps to nematode stored. After harvest, stubbles / trey plant should be removed. Ploughed and exposed to soil.

Use of resistant varieties: Nematode resistant *M. incognita* – Nemared, Nematex, Hisar lalit, Atkison – tomato, potato – Kufri suvarna, *G.rostochiensis*.

(IV) Biological method:

Biological control aims to manipulate the parasites, predators and pathogens of nematodes in rhizosphere in order to control the plant parasitic nematode. Addition of organic amendments like FYM, oil cake, green manures, pressmud cake, etc encourage the multiplication of nematode antagonistic microbes which intern checks the plant parasitic nematodes.

By addition of several amendments in soil act in several ways releasing formic, acitic, propionic and butric acids are released during microbial decomposition. NH_4 and H_2S gases are also released during decomposition which are toxic to plant parasitic nematodes. The nematode antagonistic microbes are multiplied fast due to addition of organic matter.

Predacious nematodes: It is having specialized stoma with teeth to catch and swallow the plant parasitic nematodes. The organic amendments helps in encourages in multiplication of predacious nematodes (*Mononchus*) and other genera *Diplogaster* and *Tripyla* species.

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

Nematode trapping fungi (NTF):

The nematode trapping fungi have adhesive network and sticky knob produced by mycelium to catch the plant parasitic nematodes.

1. **Sticky branches:** Fungal mycelia have short branches and anastamose to form loops. The nematode traps in loop.
2. **Sticky networm:** The mycelium curl around anastamoses with similar branches. Adhesive networm of mycelium to hold the nematode e.g. *Arthrobotrys* sp.
3. **Sticky knobs:** Small spherical lobes are present in one / two celled lateral hyphae. Terminal lope is sticky to hold nematode e.g. *Monacrosporium ellipsospora*.

Constricting rings: Short hyphal branch curls back on it self and anastamoses and forming a ring. When the nematode enters the ring and contact inner wall, the ring cell bulge inwards filling the lumen of the ring and kill nematode *M. bembicoides*.

Non-constricting rings: The ring become non-adhesive trap and ineffective structure and kill the nematode.

Endozoic fungi: Usually enter in the nematode by germ tube and penetrate into cuticle form sticky pore. The fungal hyphae ramify throughout the nematode body, absorb contents and multiply. The hyphae emerged out from dead body. E.g. *Catenaria vermicola* on sugarcane nematode.

Parasitic fungi: *Paecilomyces lilacinus* is an effective e.g. parasite on many nematodes. The parasitic fungi is particularly effective against *Meloidogyne, Heterodera, Rotylenchulus, Tylenchulus*. The fungus attacks the egg as they are deposited in a group as mass effective against cyst, root-knot, tomato, brinjal betelvine, banana and citrus.

Bacteria:

The recent studies have shown in influence of introduced microbial antagonist in controlling parasitic nematode. Seed treatment with *Pseudomonas fluorescens* have been found to reduced the cyst nematode; *H.cajani* in cowpea.

The rhizobacteria viz., *Bacillus cereus, P.fluorescens* were found effective agent *M.incognita* in tomato, banana. *Pasteuria penetrans* was found to be very effective against the

root-knot nematodes in many crops. It is most effective against J_2 of root knot can be seen attached to cuticle of nematode.

(V) Chemical method:

(See under Exercise No. 8 in the Practical Manual)

Entomophilic Nematodes:

Nematode associated with insects are referred as entomophilic, entomogenus and entomophagus nematodes. They belongs to the superfamilies Tylenchoidea, Rhabditoidea, Oxyuroidea and Mermithoidea of the phylum nematode.

ENTOMOPATHOGENIC NEMATODES (EPN)

Enomopathogenic nematodes (EPN) are beneficial nematodes parasitizing crop insects, particularly lepidopterans and coleopterans and are effectively used as biopesticide against a vide variey of insect pests. The impressive attributes of EPN have stimulated strong commercial interest in nematodes as biological insecticides and are perceived as viable alternative to chemicals in IPM programme. A symbiotic bacteria *Xenorhabdus* spp. is associated with *Steinernema* spp, and *Photorhabdus* sp. With *Heterorhabditis*. The bacteria is responsible for death of host. It occurs primary / secondary from the bacterium *P. luminescens* are bioluminescent.

Mode of action

The free living J_3 larval of nematodes are resistant to desiccation and can survival for several months. They enter thought mouth / Anus. On entering into host, they penetrate the wall of alimentary canal and move to body cavity. The host haemolymph taken by the nematodes and accumulates in the 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 segetum.*

Difference between EPN and PPN:

Entomopathogenic nematode and plant parasitic nematode differ both in structure and behaviour from each other:

Entomopathogenic Nematode (EPN)	Plan Parasitic Nematode (PPN)
Parasitic insect pests and pass one of the stage of their life cycle in insect pests. They never parasitise or damage plants.	Parasitise mainly root system of plants and pass one of the stage of their life cycle in / or the root tissues of plant as endoparasitic / ectoparasitic nematode.
Beneficial to agricultural crops by attacking insects pests by killing them quickly.	Harmful to agricultural crops damage and alter phyhsiology of crop plants, produce abnormalities, knots, lesion on root system, introduce fungal pathogen inside roots and aggravate wilt and root-rot diseases.
Increases 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.
Size 0.3-1.5 mm (very small)	Size upto 4 mm (small to medium)
Absence of stylet	Presence of stylet
EPN feed on bacteria and decomposing host insect.	PPN feed on the plant parts mainly roots to get their nourishment.
Life cycle - Generally completes within a week.	**Life cycle –** Generally complete 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.

Identification of EPN

Adult: Obligate parasite, adult stages founding the haemolymph of infected insect, adults are amphimictic, stylet absent, amphid aperature 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 contents and eventually filling the females, females cuticle degenerates and larvae are relased.

Male: Testes single, relfexed, spicule paired, separate with or without vallum, gbernaculum 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 or not armed. Tail elongate. Capable of killing wide range of insects due to speticaemia caused by symbiotic bacteria.

Nematode biology: Juveniles of EPN pass through 4 stages. The first two stage may be developed on food material.

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

Techniques for mass production: The EPN are multiplied either on a suitable host (*in vivo*) on a semisynthetic 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) *Corycera cephalonica* iii) *Helicoverpa armigera. G. mellonella* larva has been found to be more susceptible for EPN. Yield per larva ranges from 1-3.5 x 10^5 IJS and roughly, 5000 *G. mellonella* larvae are needed to treat an area o one hectare @ 2.5 x 10^9 IJS.

In case of *H. armigera* and *Corcycera cephalonica* larvae, the yield of IJS is 1-3 x 10^5 and 0.5-2 x 10^5, respectively.

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 35 ^{O}C. The larvae will be ready for use with in three weeks. If the temperature is < 35 ^{O}C development of larvae will be slow.

After 2-3 weeks, the larvae are drawn for multiplication of EPN.

ii. *Corcycera cephalonica:*

1. Broken sorghum or wheat grain	-	1 kg
2. Maize meal	-	1 kg
3. Rice broken	-	500 gm
4. Streptomycin	-	0.5 gm

5. Yeast powder - 1 gm

About 1.00 CC Corcyra eggs are mixed 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	Cholestrol / 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 in 200 ml distilled water heated. All the ingredients are thoroughly mixed in grinder. Thereafter, the ingredient is poured in Petri plates / vival and kept at room temperature to cool down. Now, diet is ready for use. *H. armigera* larvae are collected from field of chickpea or pigeonpea 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.

Insects 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 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 IJS migrate away from host cadaver on emergence and continue to do so until the body contents of the host are consumed.

Formulations: EPN can be formulated either with active nematodes in various substrates like Sponge, Vermiculture, soil gel, powder and granules.