PRACTICAL MANUAL

Plant Nematology

Course No.: PPA 504 Credit Hrs. 3(2+1)

M.Sc. (Agri.) Plant Pathology

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2024

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Kanpur - 208001

Syllabus: Plant Nematology (PPA 504)

Practical: Studies on kinds of nematodes- free-living, animal, insect and plant parasites, nematode extraction from soil, extraction of migratory endoparasites, staining for sedentary endoparasites, examination of different life stages of important plant parasitic nematodes, their symptoms and histopathology.

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Course Teacher

CONTENTS

Exercise No. 1

Object: Studies on kinds of nematode's: free-living, animal, insect and plant parasites

Free-living: .....

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Animal parasitic: .....

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Insect Prasitic: .....

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Plant Parasitic: .....

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Exercise No. 2

Object: Symptoms of nematode damage

Above-ground feeders symptoms: .....

Below-ground feeders symptoms: ……..

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Exercise No. 3

Object: Collection of infested soil and plant samples

Requirements: .....

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Where & When to sample? .....

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Procedure: (How to sample?) .....

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Exercise No. 4

Object: Nematode extraction from soil and plant material

Requirements: ……

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Procedure: ……

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Baermann's Funnel Technique

Requirements: ……

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Procedure: ……

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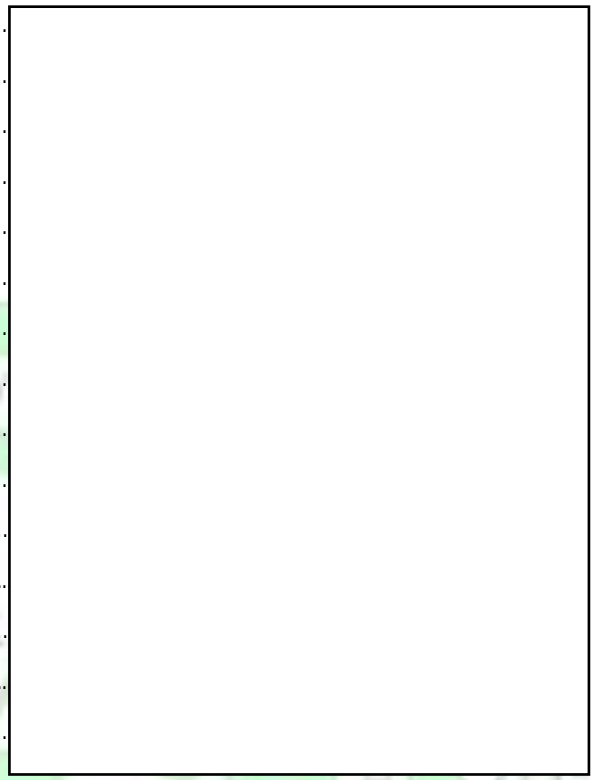


Cobb's Decanting and Sieving Method

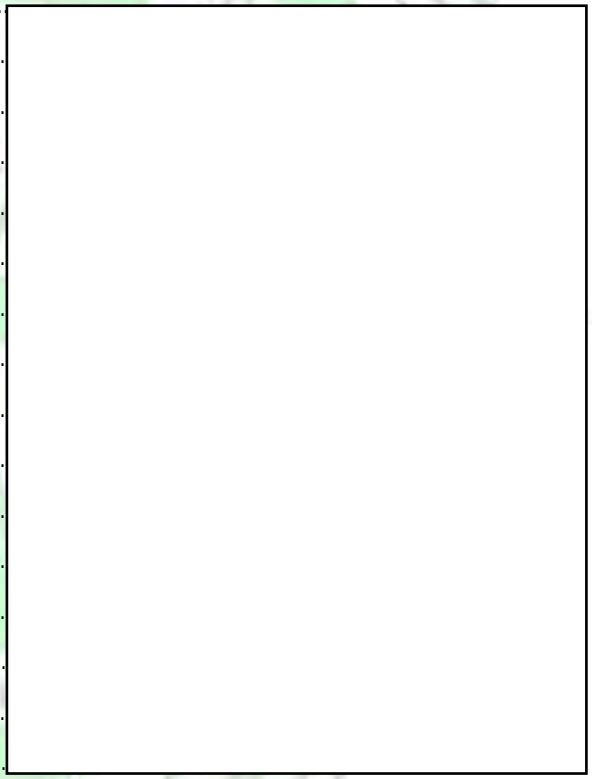
Requirements:

Procedure:

Modified Fenwick Can Method

Requirements:

Procedure:



Exercise No. 5

Object: Killing, fixing and preservation of nematode for identification

Requirements: .....

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Procedure: .....

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Clearing: .....

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Lactophenol Method: .....

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Procedure: .....

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Mounting of nematode: .....

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Precaution:

Exercise No. 6

Object: Study about migratory and sedentary endoparasites

Migratory endoparasites: ……

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Sedentary endoparasites: ……

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Exercise No. 7

Object: To stain nematodes in plant tissue (Acid Fuchsin Lactophenol Method)

Requirements: .....

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Procedure: .....

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Practical No. 8

Objectives: To get familiar with different morphological features of plant parasitic nematodes

Draw a neat well-labeled diagram indicating different morphological features of typical male and female plant parasitic nematode.

Introduction: .....

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Elaborate different morphological features plant parasitic nematode.

Alimentary canal: .....

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Stylet: .....

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Oesophagus: .....

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Dorsal Gland Orifice: .....

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Median bulb: .....

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Intestine: ……

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Excretory system:……

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Reproductive system:……

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Nervous system: ……

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Exercise No. 9

Object: Study on Root Knot nematode

Scientific Name: ..... **Common Name:** .....

Classification

Phylum: ..... **Class:** .....

Order: ..... **Family:** .....

Genus: ..... **Species:** .....

Symptoms: .....

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General features: .....

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Exercise No. 10

Object: Study on Cyst Nematode

Scientific Name: ..... Common Name: .....

Classification

Phylum: ..... **Class:** .....

Order: ..... **Family:** .....

Genus: ..... **Species:** .....

Symptoms: .....

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General features: .....

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Exercise No.11

Object: Study on Burrowing Nematode

Scientific Name: .... **Common Name:** ....

Classification

Phylum: .... **Class:** ....

Order: .... **Family:** ....

Genus: .... **Species:** ....

Symptoms: ....

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General features: ....

Exercise No. 12

Object: Study on Citrus Nematode

Scientific Name: ..... **Common Name:** .....

Classification

Phylum: ..... **Class:** .....

Order: ..... **Family:** .....

Genus: ..... **Species:** .....

Symptoms: .....

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General features: .....

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Exercise No. 13

Object: Study on Reniform Nematode

Scientific Name: .... **Common Name:** ....

Classification

Phylum: .... **Class:** ....

Order: .... **Family:** ....

Genus: .... **Species:** ....

Symptoms: ....

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General features: ....

Exercise No. 14

Object: Study on Wheat Seed Gall Nematode

Scientific Name: ..... **Common Name:** .....

Classification

Phylum: ..... **Class:** .....

Order: ..... **Family:** .....

Genus: ..... **Species:** .....

Symptoms: .....

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General features: .....

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Exercise No. 15

Object: Study on Lance and Stunt Nematode

Scientific Name: ..... **Common Name:** .....

Classification

Phylum: ..... **Class:** .....

Order: ..... **Family:** .....

Genus: ..... **Species:** .....

Symptoms: .....

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General features: .....

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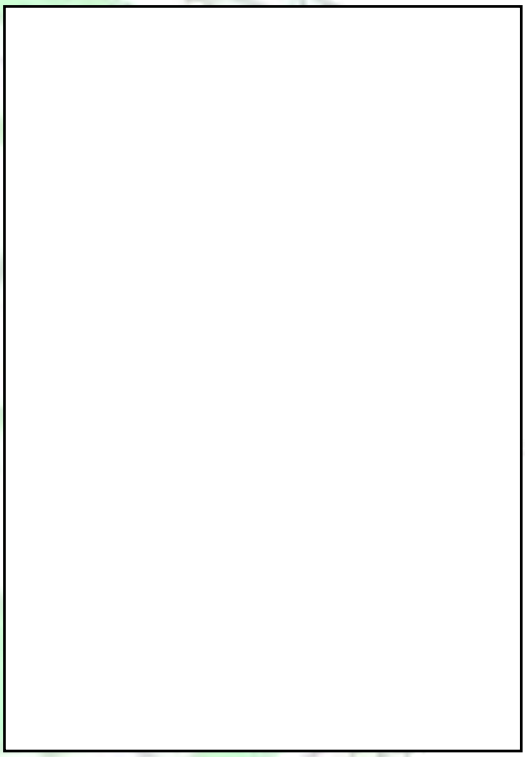

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Scientific Name: .... Common Name: ....

Classification

Phylum: .... Class: ....

Order: .... Family: ....

Genus: .... Species: ....

Symptoms: ....

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General features: ....

Exercise No. 16

Object: Study on Lesion Nematode

Scientific Name: ..... **Common Name:** .....

Classification

Phylum: ..... **Class:** .....

Order: ..... **Family:** .....

Genus: ..... **Species:** .....

Symptoms: .....

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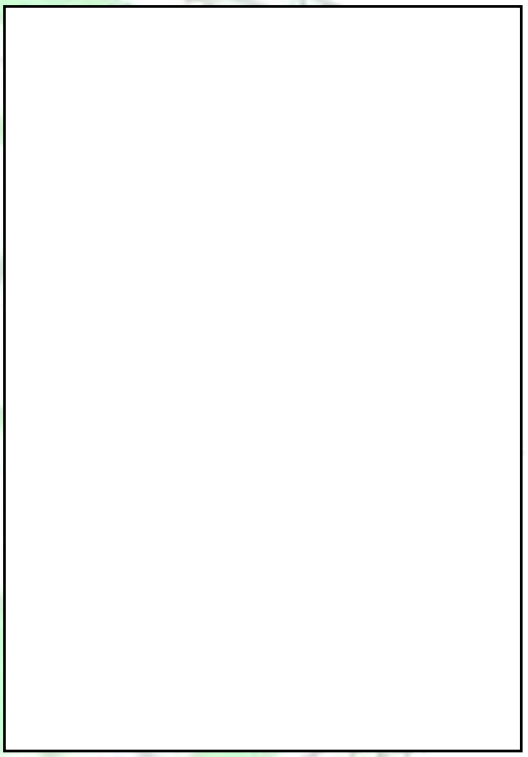
General features: .....



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Exercise No. 17

Object: Study on Dagger and Stubby Nematode

Scientific Name: ………………………… **Common Name:** …………………………

Classification

Phylum: ………………………… **Class:** …………………………

Order: ………………………… **Family:** …………………………

Genus: ………………………… **Species:** …………………………

Symptoms: …………………………………………………………

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General features: …………………………………………………………

Scientific Name: .... Common Name: ....

Classification

Phylum: .... Class: ....

Order: .... Family: ....

Genus: .... Species: ....

Symptoms: ....

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General features: ....

Exercise No. 18

Object: Study on Stem and Bulb Nematode

Scientific Name: ..... **Common Name:** .....

Classification

Phylum: ..... **Class:** .....

Order: ..... **Family:** .....

Genus: ..... **Species:** .....

Symptoms: .....

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General features: .....

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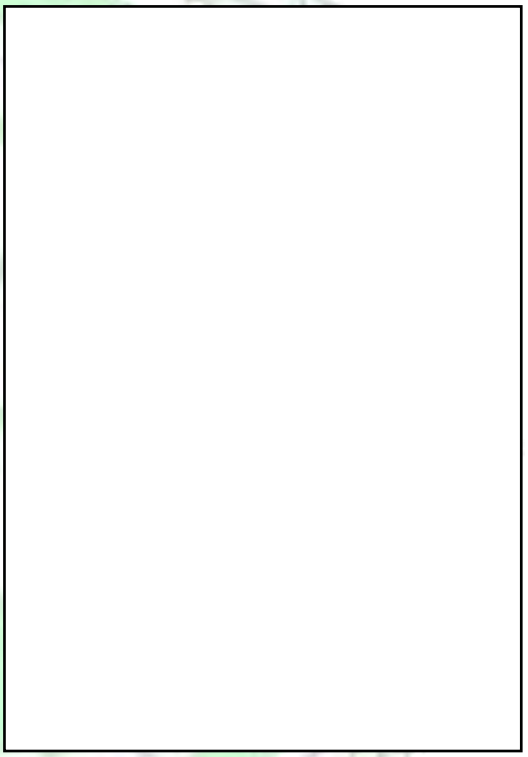

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APPENDIX

GLOSSARY

Basal knobs: Protuberance (usually three) at the base of the stylet, functioning as posterior points of attachment of the stylet muscles; also called stylet knobs.

Bilateral symmetry: Body devisable into two equal counterparts or mirror image halves through dorso-ventral plane.

Buccal/mouth cavity: The cylindrical or sub-globular structure connecting the oral opening with the interior portion of oesophagus and armed with a protrusible spear or stylet; also called buccal capsule.

Bursa: Lateral cuticular extension present at the posterior end of the male nematode, clasps the female during copulation.

Chlorosis: The plant physiological disorder in which the chlorophyll is deficient and photosynthesis is limited.

Cloaca: Common part of male digestive and reproductive systems that opens outside through a common cloacal aperture, emptying through the anus. Cuticle Non cellular, external covering of body wall, secreted by hyphodermis.

Cyst: The dark, hardened body or oxidized cuticle of dead adult female of *Globodera* and *Heterodera* species that provide protection to eggs contained within it.

Disease: Harmful physiological processes due to continuous irritation by a primary causal agent.

Ectoparasite: A parasite living and feeding on a host from outside.

Egg mass: Gelatinous matrix secreted by female nematode in which eggs are laid.

Embryogenesis: Sequential development of embryo from single celled egg.

Endoparasite: A parasite that enters the host tissue and feeds from within.

Egg shell: Outermost covering of egg containing chitin inside where the development of embryo takes place.

Gall: An abnormal swelling or localized outgrowth (tumor) on roots, often more or less spherical or fusiform, composed of unorganized cells produce by the plant as a result of attack by nematode (e.g., *Meloidogyne* sp.)

Genital primordium: The group of initial cells in juveniles which give rise to reproductive system of adults.

Giant cells: A multinucleate mass of protoplasm formed by the division of nucleus without cell division or cell wall formation (e.g., in *Meloidogyne* infested tissue).

Gonads: A sex organ; part of reproductive system responsible for production of germ cells or gametes (ova/sperm). Ovary in female and testis in male.

Gonoduct: Part of reproductive system responsible for carrying germ cells from gonads to the exterior.

Gubernaculum: The grooved, sclerotized plate like hard structure located to the posterior to the spicules of male nematode; guide the movement of spicules.

Hatching: The process involving the emergence of juvenile from egg shell, eclosion.

Hyperplasia: An abnormal increase in the number of cells of a tissue.

Hypertrophy: An abnormal increase in the size of cells of a tissue.

Hypodermal chords: Dorsal, ventral and lateral protrusions of hypodermis into the pseudocoelom.

Hypodermis: A thin, cellular or syncytial layer of body wall lying beneath the cuticle, responsible for the production and maintenance of cuticle.

Infective stage: The stage in the life cycle of a parasite that invades the host tissue and establishes the infection.

Isthmus: The narrow portion of the oesophagus between the metacarpus and the basal region or posterior bulb.

Juvenile or larva: Any of four life stages of nematode between embryo and adult; an immature nematode which does not yet have functional gonads.

Knots: A localized abnormal swelling of plant tissue due to infestation of nematode. .

Median bulb: The enlarged, usually ovate, posterior portion of carpus (in the middle portion of oesophagus), frequently containing a valve.

Migratory: Capable of moving about freely, within or outside the host; vagrant.

Morphology: Study of forms, structure and development of organisms.

Moulting: To shed or cast off cuticle or body encasement in the transition from one developmental stage to the next to permit growth; ecdysis.

Nematode: Worm-like organism defined as triploblastic, bilaterally symmetrical, un-segmented, pseudocoelomate, invertebrates, with 4 hypodermal chords, a tri-radiate oesophagus, circum-oesophageal nerve ring and lacking specialized organs for respiration and circulation.

Nerve ring: The centre of nervous system or brain in nematode, in the form of a ring encircle the oesophagus, composed of nerve fibers, cell bodies of neurons and associated ganglia; also called the circum-oesophageal commissure.

Oesophagus: Anterior portion of alimentary canal between stoma and intestine, lined internally with cuticle.

Parthenogenesis: Reproduction of egg without fertilization.

Perineal pattern: A fine, wavy, fingerprint-like characteristic arrangements of cuticular striae near the perennial (area around anus, vulva, phasmid and tail) region in *Meloidogyne* females. This pattern is more or less an identifying characteristics of the species.

Pseudocoelom: The space between wider outer tube (body wall) and shorter inner body tube (alimentary canal) of nematode body; also called as body cavity. It is devoid of mesodermal epithelial lining.

Pseudocoelomic fluid: The watery lymph like nutritive fluid in the pseudocoelom in which internal organs are suspended.

Rhizosphere: The area in soil immediately surrounding plant roots and influenced by them.

Sedentary: Staying at a place, stationary, sessile.

Spicule: A pair of heavily cuticularized male copulatory organ.

Stylet or spear: A scletorized, slender, axially located, hollow feeding structure located in the buccal cavity or mouth cavity.

Syncytium: A multinucleate mass of protoplasm form by the dissolution of walls of adjacent cells.

Triploblastic: Possessing three germ layers *i.e.* ecto, meso and endoderm.

Triradiate: Having three radiating sides (used for the lumen of oesophagus).

SYMPTOMS OF NEMATODE DAMAGE

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

- **Dead or devitalized buds** - Nematode infection kills growing buds e.g. *Aphelenchoides fragariae* on strawberry.
- **Crinkled stems and foliage -** e.g. Wheat gall nematode, *Anguina tritici* Ufra disease of rice, *Ditylenchus angustus.*
- **Seed galls –** e.g. Wheat gall nematode, *Anguina tritici* larva enter into the flower primordium and develops in to a gall.
- **Necrosis & discolouration** – e.g., Red ring disease of coconut, *Rhadinaphelenchus cocophilus*. Due to the infestation, red coloured circular area appear in the trunk of infested palm.
- **Leaf lesions** - Symptom on broad-leafed foliage plants e.g., Chrysanthemum foliar nematode, *Aphelenchoides ritzemabosi.*
- **Twisting of leaves and stem-** e.g., in onion basal leaves become twisted when infested with *Ditylenchus dipsaci*.
- **Leaf discolouration-** The leaf tip become white in rice due to rice white tip nematode *Aphelenchoides besseyi.*

Below-ground feeder symptoms: The nematode infest and feed on root portion and exhibit symptoms on below ground plant part as well as on the above ground plants parts and they are classified as: Above ground symptoms & Below ground symptoms

Above ground symptoms:-

- **Stunting:** Reduced plant growth and the plant cannot able to withstand in adverse conditions. Patches of stunted plants appears in the field e.g., *Heterodera avenae* – Molya disease in wheat & barley. *Globodera rostochinensis* – Golden nematode in potato
- **Discolouration of foliage:** Also due to nutritional deficiency e.g. Root lesion nematode- *Pratylenchus coffeae*, White tip nematode- *Aphelenchoides besseyi,* Citrus nematode- *Tylenchulus semipenetrans.*
- **Wilting:** e.g. Root-knot nematodes, *Meloidogyne* spp.
- **Decline and die back:** e.g., In banana decline and die back are caused by *Radopholus similis*.

Below ground symptoms:-

- **Root galling:** e.g. *Meloidogyne* spp. – Characteristic galls on host roots; *Nacobbus* spp – Larger galls on beet & tomato; *Ditylenchus radicicola* – Small galls on cereals; *Hemicycliophora arenaria* – Galling on lemon roots; *Xiphinema diversicaudatum* - Galling on roses
- **Reduced root system:** Due to nematode feeding the root tip growth is arrested and the root produced branches. This may be of various kinds such as coarse root, stubby root and curly root.
  a. **Stubby roots –** Stubby branches or rootlets arranged in cluster e.g., *Trichodorus christiei* on corn
  b. **Coarse root –** Lateral roots stopped growth with no branches e.g., *Belonolaimus longicaudatus* on corn.
  c. **Curly root –** The nematode retard the elongation of roots and cause curling of roots known as 'Fish hook' symptom e.g., Injury caused by *Xiphinema* spp.
- **Root lesions:** Necrotic lesions e.g., *Pratylenchus* spp. (soybean), *Radopholus similis* (citrus & banana), *Helicotylenchus multicinctus* (banana).
- **Rotting:** Nematode + Micro-organisms e.g., *Ditylenchus destructor* – potato rot.
- **Excessive root branching:** e.g. *Meloidogyne hapla* in tomato when many are present. As the females mature, the cysts, which can contain hundreds of eggs, harden and turn brown or black.

COLLECTION OF INFESTED SOIL AND PLANT SAMPLES

Equipment's required: Equipment needed for nematode estimation are soil probe, bucket, polythene bags, soil auger, hand hoe, spade, rubber band, label's etc.

Where & When to sample? Select the plant or crop to be sampled as per the objective of the study. Sample in locations in which plants do not grow properly, could be an area of nematode damage. Such areas are often circular to oval in outline, and occasionally in row crops poor growth may follow the rows. Soil samples therefore, should be taken from around the roots of plants that are not growing properly.

Soil samples can be collected at any time of year, as long as the sampling tool can be inserted into the ground to the proper depth. Avoid collecting excessively wet or dry soils, as it is difficult to prepare samples for analysis under these conditions.

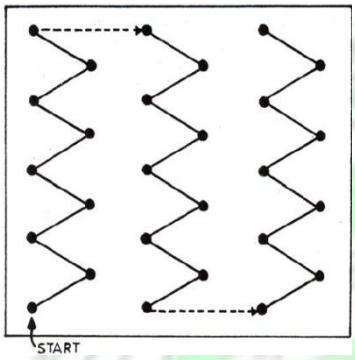
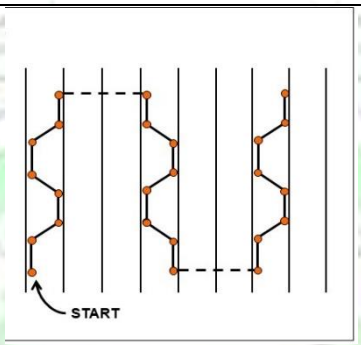
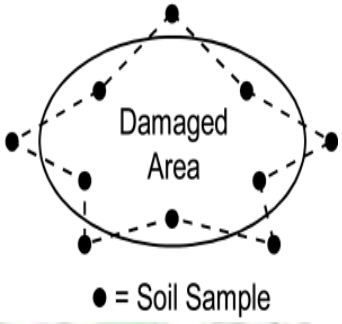
How to sample?

1. Scrap off upper 5-6 cm of soil surface with a hand hoe. And dig to 15-20 cm (up to 45 cm in case of perennials) to collect

fine roots and adjacent soil. If the plants are small then dig up the whole plant.

2. Collect 15-20 samples from one hectare in a *zig-zag* manner for field crops. For vegetable crops, select six rows in that two from beginning, two from middle and two from end and draw 6-10 samples from each row. In case of fruit trees, collect 20 samples (two samples from randomly collected 10 plants)
3. Mix the samples thoroughly to make a composite sample. Take about 500gm of soil sample at last.
4. Keep the soil sample in the polythene bag and label it with tags having specification such as locality, date, collectors name, crop name etc.
5. The samples should be protected from drying or excessive heat. They should be stored in refrigerator if possible at 5^0C.
6. Bring to laboratory and extract nematode as soon as possible.

Recommended pattern for soil sampling (Baker, 1985)

		
Collecting soil sample from a fallow field	Collecting soil sample from root zone of annual crop or widely spaced crops	Collecting soil sample from ornamental or perennial plant

EXTRACTION OF NEMATODE

Extraction from plant material by maceration method

Materials required: infected plant root with root-knot nematodes, Baermann funnel, scalpel, tap water, blender, glass slides, 60,325 mesh sieve, watch glass, stereoscope/ binocular microscope.

Procedure:

- Wash the roots showing galls thoroughly with tap water.
- Observe carefully primary and compound galls.
- Cut out the galls from root material. Avoid empty galls which have been completely decayed by soil parasitic fungi.
- Cut the galls into small pieces using a scalpel.
- Grind the galls with small amount of water by using a blender about 20 seconds.
- Pour the grinded material over a 60 mesh sieve, discard the residues and collect the filtrate.
- Pour the filtrate in to a 325 mesh sieve and collect the residue in a beaker by pouring water gently on the beaker of the sieve.
- Pour the filtrate into a Baermann funnel, leave for overnight. Then the collect the nematode in the watch glass and examine under stereoscope or binocular microscope

Extraction from soil

Baermann funnel: This method was introduced by Baermann in 1917 for the extraction of motile nematodes by using a funnel.

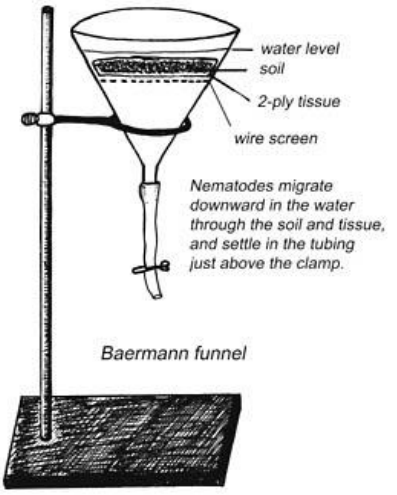
Materials required: Soil sample, Funnel with a piece of soft silicone tube attached to the stem, closed with a squeezer clip, Piece of cheesecloth, Petri dish and beaker, Tissue paper, counting slide (Cavity slide), Stereo microscope, Tap water etc.

Procedure:

1. Place soil on the cotton-wool milk filter (cheese cloth)/ facial tissue paper which is held by a support; maximum thickness of soil layer is 2–3 mm.
2. Place support with sample into the funnel.
3. Add water from the side until bottom of the sieve just touches the water;
4. Nematodes start to leave the soil and pass through the cotton-wool milk filter / facial tissue paper, sink to the bottom of the funnel stem or dish, respectively;
5. Collect the nematodes after 24–72 h in a glass beaker by opening the spring or screw clip of the funnel stem.
6. Let the nematodes settle in the glass beaker and remove the supernatant or pass suspension over a 20 or 25 lm sieve

to reduce the volume of water.

7. Examine nematodes within a counting slide (Cavity slide) at 25–40 magnification using a dissecting or inverse microscope (stereo microscope) or transfer of nematodes on a microscope slide with the help of handling needle for inspection at higher magnification in a compound microscope.



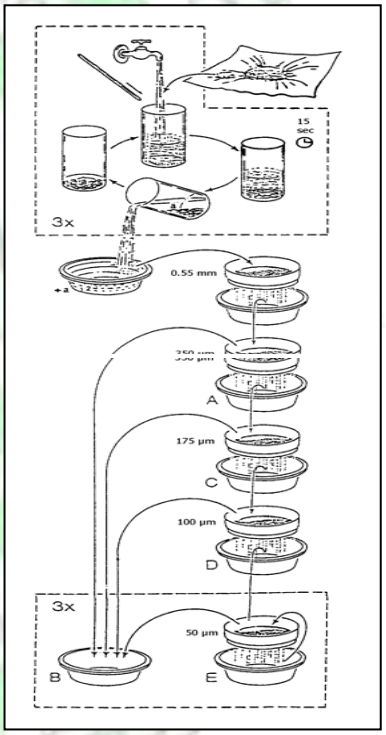
Advantages:

1. It is a simple and cost effective method.
2. This method use less amount of water in the extraction of nematode.
3. In this method final suspension is much clean then other method.
4. This method has good recovery of **motile nematodes** from small samples.

Disadvantages:

1. Nematodes extraction from a large sample up to 250 ml is much difficult.
2. Lack of aeration in the soil reduces nematode movement.
3. Poor recovery of relatively **immotile nematodes** (e.g., *Xiphinema*, *Hemicycliophora*, *Criconemoides*)

Cobb's Sieving Method:



Materials required: Soil sample, Different size sieves (20, 60,100, 200,300 & 350 mesh), Beaker (250 ml), Tap water, Wash bottle, Tissue paper, Stereo-microscope.

Procedure:

- Take soil sample and water into a beaker in the 1:3 ratio.
- Make the suspension of above sample and left it for 15 seconds for settled down and decant the supernatant in a bowl.
- Stir the remaining sediment again with water after 15 seconds; decant the supernatant in the same plastic bowl.
- The remaining sediment in the beaker can be discarded.
- Pass the suspension through a 20 or 60 mesh sieve into second bowl.
- Shake the sieve for the passing of nematodes which are submerged in the suspension, from the sieve and remaining debris in the 20 or 60 mesh sieves can be discarded.
- This suspension again pass through 100 to 150 mesh sieve into another or third bowl and discard residues left in the second bowl.
- And this suspension again passes through a 200 mesh sieve into another bowl and discarded residues left in the third bowl.
- Collect the catch remaining material on sieve by rinsing with water in a 250 ml beaker and separate it.
- Pass the all third bowl suspension through 350 mesh sieve into another bowl and discarded residues of the third bowl. Collect the catch on sieve by rinsing with water in a 250 ml beaker.
- Sample present in 250 ml beaker will be probably dirty due to presence of fine soil particle for direct viewing, thus it may be placed on modified Baermann funnel technique in which place a facial tissue paper on a moulded coarse aluminium mesh. Wet the tissue paper and transfer the beaker collected dirty sample on to it. Put the aluminium mesh over the Petri plate containing water (water should always touch the content of mesh). Keep the filtrate undisturbed for 24 hours.
- Examine the petri plates contents filter sample under stereo-microscope for active nematode.

Advantages: The method is not dependent on nematode movement; sluggish nematodes are recovered. It allows the recovery of most nematodes from large soil samples. Nematodes are available for direct examination in less than half an hour.

Disadvantages: The method requires expensive sieves and an experienced worker. Nematodes are difficult to see because of fine particles.

Precaution:

1. Aperture of fine sieve commonly blocked with soil particles, so blocking of sieve should be avoided by shaking.
2. Tear sieve should not be used for the extraction.
3. The soil mix properly with water and break all clumps for preparation of suspension.

SEPARATION OF THE NEMATODE CYSTS BY MODIFIED FENWICK CAN METHOD

Requirements: Modified Fenwick Can with top sieve, funnel and drain plug, one sieve of 20 and 60 mesh each, white bowl and camel's brush No. 1.

Procedure

A. Mix the dried soil thoroughly and take a soil sample of 50-100 ml.
B. Place the dried sample on top sieve and wash into apparatus. Coarse material is retained on top sieve, heavy soil particle would settle in bottom of apparatus and the dried floating cysts are carried off over the overflow collar and collected on the 60-mesh sieve.
C. Wash the residue from sieve into white bowl and floating cysts along the edge of bowl are picked up with camel's hair brush.
D. Collect the cysts in a drop of water on glass slide and examine.

*Setup for a basic Fenwick extraction method. A= Sieve; B= Water can; C=Over flow collar: D=Outlet; E: Sieve; F: Cyst collector.

KILLING, FIXING AND PRESERVING EXTRACTED NEMATODE

Materials required: Small glass vial, Distilled water, Cavity block, pure glycerine, Formalin, Cavity block, Lactophenol, Nematode suspension, Cover slip, Glass wool and Nail polish

Procedure:

1. Pure nematode sample collect from plant and soil after extraction.
2. Concentrate nematode suspension as collected in the test tube by allowing the nematode to settle down in a test tube for about half an hour.
3. And carefully removing the top half of water with the help of dropper
4. Boil 8% formalin (8 ml formaldehyde solution + 92 ml of water) and pour it over nematode suspension.
5. Amount of formalin solution should be equal to the water containing nematode so that the worms are ultimately in 4% formalin solution.
6. Levelled on sample and it can be used for further study.
7. Nematodes thus killed and fixed can be preserved or further processed for slide preparation after 24 hour.

Clearing: Internal structure of nematodes specially the reproductive organs are obscured due to the presence of food material in the intestine. To make the contents of transparent, clearing is done by either of following method.

Lactophenol method

Procedure

1. Take a drop of lactophenol on a slide.
2. Warm it to 55-600 c for 1-2 min.
3. Transfer the nematode to drop of warm lactophenol.
4. The nematode will become transparent and ready for mounting in lactophenol for semi- permanent mounts.

Mounting of nematode

1. Place a small drop of lactophenol /glycerine in the centre of a clean glass slide.
2. Pick up the nematode and place them in the fixative on the centre of slide.
3. Place the slide under stereomicroscope and arrange the nematode the centre of slide and bottom of drop.
4. Place three glass wool (about 5mm in length) in a triangular position near edge of the drop.
5. Place cover slip and put over the drop of lactophenol/glycerine and tap it gently.
6. Remove the excess of lactophenol/glycerine with the help of blotting paper.
7. Apply nail police or favicol with a small brush to edge of the cover slip to seal it.

Precaution:

1. The formalin solution should be 8% neither more nor less.
2. The formalin solution properly boiled.
3. Amount of formalin solution should be equal to the water containing nematode.

The most suitable fixatives are:

TAF: Triethanolamine: 2ml; Formalin (40% formaldehyde): 7ml; Distilled water: 91ml

The fixative remains stable for a long time and nematode appearance remains lifelike because the specimens do not dry out.

FA: (4:1): Formalin (40% formaldehyde): 10ml; Glacial acetic acid (propionic acid): 1ml; Distilled water: 89ml

In FA 4:1 nematodes maintain their structure though they may become discoloured after some time.

Formalin glycerol: Formalin (40% formaldehyde): 10ml; Glycerol: 1ml; Distilled water: 89ml

This has the advantage of keeping the nematode from drying out even if the vials are not properly sealed. Again take great care with all these fixatives as they are dangerous to health.

STAINING OF NEMATODE IN INFECTED PLANT TISSUE

Material required: Phenol - 20ml; Lactic acid - 20ml; Glycerine - 40ml; Distilled water - 20ml; Cotton Blue - 1g

Procedure:

1. Mix the above ingredients to prepare glycerine – lactophenol solution.
2. Dissolve 1g of cotton blue in 100ml of distilled water.
3. Take 5ml of cotton blue solution and mix in 100 ml of glycerine-lactophenol solution to prepare staining solution.
4. Wash the infected roots of plants through the tape water to free it from soil.
5. Heat the staining solution up to boiling and dip the infected roots for 3-4 minutes. Both plant tissue and nematode will be stained in this hot solution.
6. Bring out the infected plant material from the staining solution and wash it in tap water to remove excess stain.
7. Dip the material in destining solution (clear lactophenol solution without cotton blue) until it is properly de-stained.
8. In the solution plant tissue will lose its colour slowly and become translucent while the stained nematode will be clearly visible.
9. After teasing the material under binocular microscope the nematode can be observed.

Migratory Endoparasitic Nematodes: Migratory endoparasites can move into, though, and out from host tissues at any stage of development (except the egg). Migratory endoparasites generally live and feed in tender tissues such as the root cortex. They burrow through the tissue, breaking open many cells after feeding on them. Cells surrounding the feeding became necrotic by toxic materials liberated from the disrupted cells. The relatively large areas of dead cells usually turn brown to become small spots or lesions big enough to see, and are often easily colonized by fungi. Root rot diseases are often associated with infestations of migratory endoparasitic nematodes like *Radopholus* spp. The most important examples are species of burrowing and lesion nematodes. The nematode, *Radopholus similis*, causes the spreading decline disease of citrus. It is the subject of strict (and expensive) quarantine regulations for ornamentals, nursery stock, and other growing plants being exported and can severely limit growth of many ornamental plants. The foliar (*Aphelenchoides* spp.) nematodes feed on or inside the leaves and buds of ferns, strawberries, chrysanthemums, and many kinds of foliage ornamentals, and cause distortion or death of buds, leaf distortion, or yellow to dark brown lesions between major veins of leaves. Other nematodes which attack plants of above ground cause leaf or seed galls and still others cause deterioration of the bulbs and necks of onions and their relatives, but are not common here. Ectoparasitic and migratory endoparasitic nematodes generally deposit their eggs singly as they are produced, wherever the female happens to be in the soil or plant.

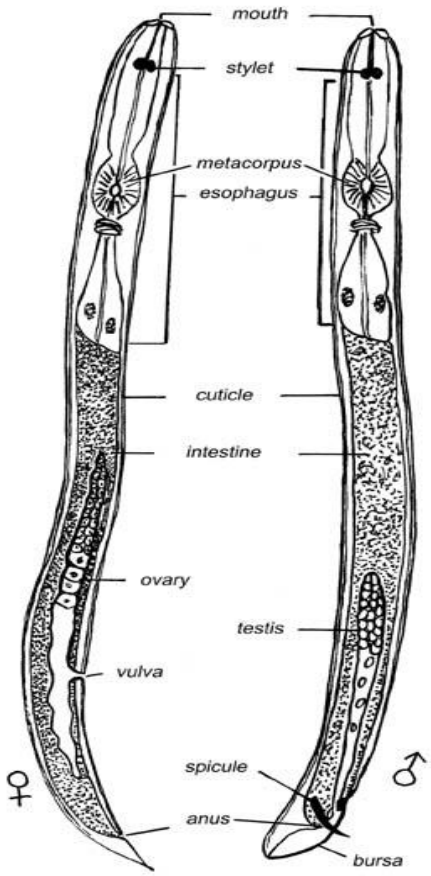
Sedentary Endoparasitic Nematodes: Sedentary endoparasitic nematodes include root knot (*Meloidogyne* spp.), cyst (*Heterodera* spp.), reniform (*Rotylenchulus* spp.), and citrus (*Tylenchulus semipenetrans*) nematodes. In most of these species, the second stage juvenile is the "infective" stage, which moves through the soil. Second stage juveniles locate host roots and enter them. They then establish a suitable feeding site within the root tissues. Once a feeding site is selected, the nematode injects growth regulating substances into the cells near its head, causing some of those cells to enlarge. These "giant" or "nurse" cells become specialized cells for feeding uninterruptedly by nematodes. At the same time, the nematode becomes immobile, and the body swells to a round, lemon, kidney, or ovoid form. Mature females of the sedentary endoparasitic nematodes generally produce large numbers of eggs which remain in their bodies or accumulate in masses attached to their bodies.

Morphology of Nematode

Introduction

1. "Nematode" is a greek word (nema = thread, oides = form) i.e. thread like organisms as they look like tiny threads moving under microscope.
2. Nematodes are triploblastic, bilaterally symmetrical, unsegmented, pseudocoelomate animals showing Sexual dimorphism.
3. They are more or less cylindrical, fusiform or pear shaped or otherwise modified particularly in adult females, example Filiform e.g. *Xiphinema, Longidorus, Paralongidorus* ;Sausage shaped or plump e.g. *Criconemoid* group; Pyriform or flask shaped - e.g. Females of *Meloidogyne spp.*and *Heterodera* cysts and Kidney shaped or Reniform- eg. *Rotylenchulus reniformis*.
4. Plant nematodes are generally microscopic with their body size ranging from 0.3 to 2.0 mm in which smallest - *Paratylenchus minutus* and longest - *Paralongidorus epimikis* (up to one cm in length).
5. The mouth opening is generally anterior and is usually surrounded by lips bearing sensory organs. The mouth is followed by mouth cavity or stoma.
6. An oesophagus, intestine and a rectum terminating in a ventral, terminal or sub terminal anus in females or a cloacal opening in males. The body is covered with cuticle.
7. The body wall is composed of a hypodermis or epithelium, situated beneath the cuticle, and a single layer of muscle.
8. The male reproductive system opens directly into the rectum, forming a cloaca, while the female has separate opening, the ventrally situated vulva.
9. Excretory and nervous system are present but there is no respiration and circulatory system.

Parts:

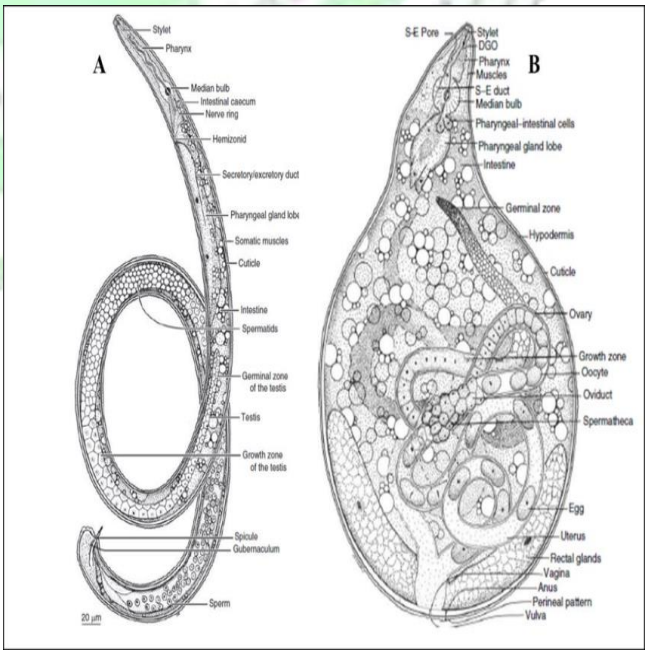


1. **Stylet:** In plant parasitic nematodes of the "Tylenchida" group, the mouth contains a stylet of mouth spear, a hardened, hollow, culticular structure similar to a hypodermic needle. Muscles are attached to three knobs at the posterior end of Stylet and extend forward. They are used to pull the stylet forward so that it projects from the mouth opening and can be used to pierce plant cells. The food of the nematode is taken through the stylet. The nematode ingested food through stylet.
2. **Oesophagus:** A slender tube is attached to the posterior end of the stylet .This is the oesophageal tube leading to the median bulb, which in turn is attached by means of another slender tube to the intestine. The three glands may form a terminal bulb to which the intestine 1s attached, or may form a lobe lying alongside the intestine. In either case, the dorsal gland has a duct leading anteriorly through the median bulb and connecting with the oesophageal tube. The connection is called the dorsal gland orifice.
3. **Dorsal gland orifice:** Most species of plant parasitic nematodes it is located behind the stylet at a distance seldom exceeding the stylet length and generally much closer. At this point there is an opening into the oesophageal tube and often an abrupt bend in it.
4. **Median bulb:** The median bulb contains a "valve" to which muscle fibres are attached. In cross section this structure is tri-radiate. When activated by muscles it functions as a pump, sucking food through the stylet and forcing it into intestine.
5. Intestine: It is a simple tube with walls one cell thick. It functions as a storage organ and is usually filled with globules of fatty substances. Posteriorly it narrows to a rectum, which terminates at the anus.
6. **Excretory system:** Nematodes have an excretory system, but in the plant parasites, the only part usually seen is a section of the excretory tube leading to the excretory pore.
7. **Reproductive system:** The female may has one or two ovaries in which eggs are formed. The male reproductive organs consist of one or two testis and associated structures, two spicules and a gubernaculum. In addition, some species have bursa.
8. **Nervous system:** Plant parasitic nematodes have a highly developed nervous system, though very little of it can be seen except for the nerve ring and *hemizonid*.

ROOT KNOT NEMATODE

Scientific name: *Meloidogyne* spp.
Common name: Root knot nematode
Classification:

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Super family	Heteroderoidea
Family	Meloidogynidae
Sub family	Meloidogyninae
Genus	*Meloidogyne*
Species	*Incognita*



Root-knot infested plants show stunted growth, curled and wilted leaves and several galls in roots. Yield of tomato is considerably reduced. Control of above nematode could be achieved by cross rotation and soil fumigation.

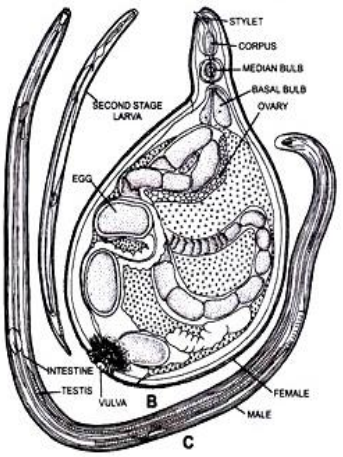
1. Females measure 0.45-0.76 mm in length and males 0.87-1.17 mm.

2. Body of female oval with a distinct neck. Cephalic framework highly sclerotisted. Oesophagus tylenchoid.
3. Spear or stylet measures 10-14 micron and contains rounded knobs.
4. Vulva and anus terminal.
5. Cuticular striations around vulva form characteristic perennial pattern. Dorsal arch high, irregular and marked by several zigzag and broken stare. Vulvular width 21-23 mm.
6. Male is vermiform with single testis, curved spicule and thickened gubernaculum.
7. Live cycle includes egg, first stage larva, infective second stage larva, male and female third and fourth stage larva and adults. Second stage larva penetrates epidermis of roots causing giant-cell formation.

CYST NEMATODE

Scientific name: *Heterodera* spp.
Common name: Cyst Nematode
Classification:

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Super family	Tylenchoidea
Family	Heteroderoidea
Sub family	Heteroderidae
Genus	*Heterodera*



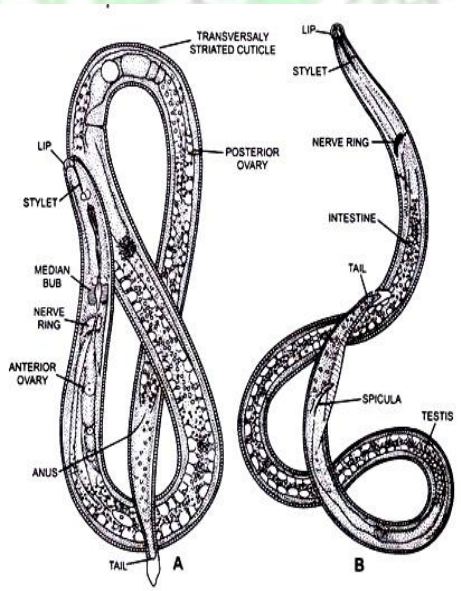
Infected potato plant reveals curling and wilting of leaves, stunted growth and finally plant dies. Yield of potato is reduced due to above nematode. Infestation can be controlled by nematicide treatment fumigation and crop rotation.

1. Female with sac like body and a neck measures 0.5-0.8 mm in length. Female contains large amount of eggs.
2. Body cuticle is marked with punctuations giving striated appearance.
3. Spear with rounded knobs is distinct. Vulva posterior.
4. Males cylindrically elongated measuring 1.0-1.1 mm is length. Lip region is marked by 6-8 annules.
5. Testis single, spicule pointed and hemizonids adjacent to excretory pore.
6. Life-cycle consists of egg, first stage large, second stage (infective larva), third stage larva, fourth stage larva and fifth or adult stage. Cyst contains second stage larvae and when next crop is sown they infect roots, cause giant cell formation in which they accumulate. Larvae after moulting change into adult male and female. Female attaches to roots and transforms itself into cyst.

BURROWING NEMATODE

Scientific name: *Radopholus similis*
Common name: Burrowing nematode
Classification:

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Super family	Tylenchoidea
Family	Pratylenchidae
Genus	*Radopholus*
Species	*Similis*



The burrowing nematode causes severe damage to banana plants. It causes necrosis in roots and rhizomes which is manifested by retarded growth, leaf yellowing and falling of mature plant. Hot water treatment of roots and rhizomes kills nematodes.

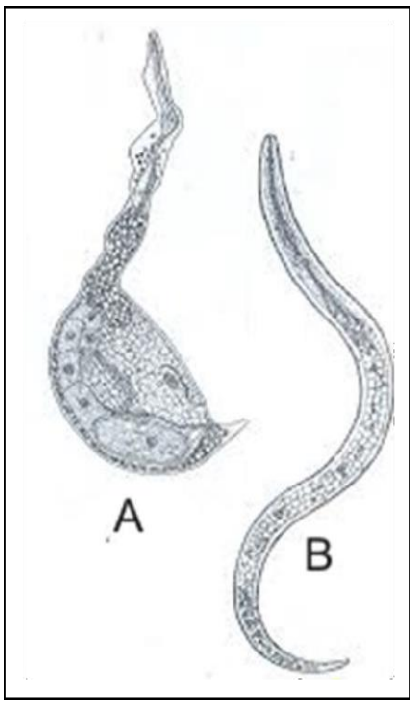
1. Females and males measure 0.5-0.7 mm is length.
2. Head slightly narrow and lip region is rounded marked by 3 striae.
3. Body is covered by transversally striated cuticle lateral field contains 3 incisors.
4. Spear or stylet is strong and well-developed.
5. Median bulb of oesophagus is spherical and its basal lobe much elongated containing 3 nuclei.
6. Anterior ovary extends up to median bulb and posterior ovary into the intestine.
7. Testis outstretched Gubernaculum and spicule present.

8. Life-cycle includes egg → first stage large → second stage infective larva, third stage larva, fourth stage larva and fifth or adult stage. Roots containing these larvae remain in soil waiting to insect a new crop.

CITRUS NEMATODE

Scientific name: *Tylenchulus semipenetrans*
Common name: Citrus Nematode
Classification:

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Super family	Criconematoidea
Family	Tylenchulidae
Sub family	Tylenchulinae
Genus	*Tylenchulus*
Species	*semipenetrans*



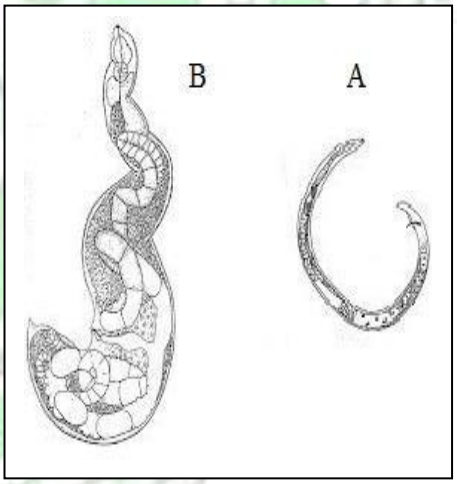
The diseased trees show reduction in growth and vigour with yellowing of leaves. Such trees show gradual dieback symptoms starting from the uppermost portion. Roots of infected trees appear larger in diameter and darker than the healthy trees mainly due to adherence of soil particles to the gelationous matrix excreted by the adult females. Cortex of highly infested feeder roots decays and gets sloughed off easily.

1. Semi-endoparasitic on citrus roots. Slender young females, males and larvae are found in soil while mature obese females protrude from roots often in cluster.
2. Stylet is small in larvae and male but well developed in female.
3. Vulva is prominent in the posterior end of young and adult female.

RENIFORM NEMATODE

Scientific name: *Rotylenchulus reniformis*
Common name: Reniform Nematode
Classification:

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Super family	Tylenchoidea
Family	Hoplolaimidae
Sub family	Rotylenchinae
Genus	*Rotylenchulus*
Species	*reniformis*

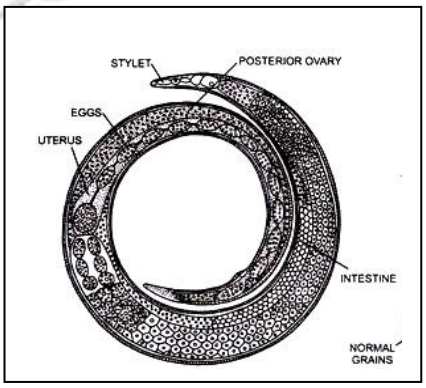


1. Infested roots show pin head like structure on roots where mature females with only their neck embedded in roots and body covered with egg masses, larvae, males and immature females found in soil.
2. Mature females typically reniform (kidney shaped), male body slender and small.

WHEAT SEED GALL NEMATODE

Scientific name: *Anguina tritici*
Common name: Gall nematode
Classification:

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Sub order	Tylenchina
Family	Anguinidae
Genus	*Anguina*
Species	*tritici*



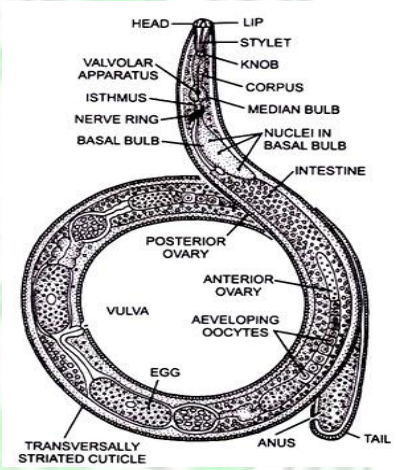
In the fields, *Anguina tritici* infected plants can be distinguished by wrinkled and twisted leaves, reduced and irregular inflorescence and as dying plant. Nematode causes characteristic cockle disease as shown in figure. Yield of wheat is reduced by *A. tritici* infection. Contamination of wheat beyond 5 percent affects colour, texture and taste of chapattis. Disease can be controlled by dry cleaning of seeds, hot water treatment and crop rotation.

1. It is first plant parasitic nematode to be discovered.
2. Males measure 2.4 mm and females 3.8 mm in length. Body of males is straight while that of females is spiral.
3. Lateral fields indistinct. Deirids and plasmids absent.
4. Anterior end contains stylet or spear measuring 8-10 micron in length.
5. Oesophageal gland of female abnormal. There is a secondary storage gland between nerve ring and basal bulb of oesophagus.
6. Ovary has 2 flexors. Male contains spermatheca gubernaculum and spicules.
7. Life cycle (Biology). Seeds containing galls when sown burst releasing 800-33000 second stage (L2-stage) larvae. These penetrate roots and come into shoots. As seeds develop inflorescence, L2 stage larvae reach seeds and cause gall formation.
8. In galls, male and female mature and copulate. Females produce eggs which hatch into first stage larvae, these larvae after 24 hours moult and change into second stage. They remain in seeds and ready to infect new host plants in subsequent sowing.

LANCE NEMATODE

Scientific name: *Hoplolaimus caronatus*
Common name: Lance Nematode
Classification:

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Super family	Tylenchoidea
Family	Hoplolaimidae
Genus	*Hoplolaimus*
Species	*caronatus*



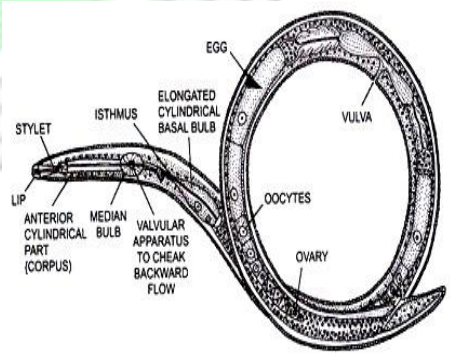
These tiny nematodes cause stunting of growth of plants. They destroy epidermis, vascular bundles, xylem and phloem. They also cause plugging of xylem elements and hence stunted growth of plants. Their infestation reduces the yield of cereals and vegetables.

1. Females and males measure 1.2-1.6 mm and 1.18-1.2 mm in length, respectively. Lip region of female is bluntly conoid and that of male spheroid Deirids and Hemizonids indistinct.
2. Stylets strongly developed with elongated, fureate and forward pointing knobs.
3. Ovaries are outstretched with developing oocytes.
4. Spermatheca is spheroid filled with granular sperms.
5. Male has bursa, Spicule straight, gubernaculum trough like and Telamon is curved.

STUNT NEMATODE

Scientific name: *Tylenchorhynchus* spp.
Common name: Stunt Nematode
Classification:

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Super family	Tylenchoidea
Family	Belonolaimidae
Genus	*Tylenchorhynchus*



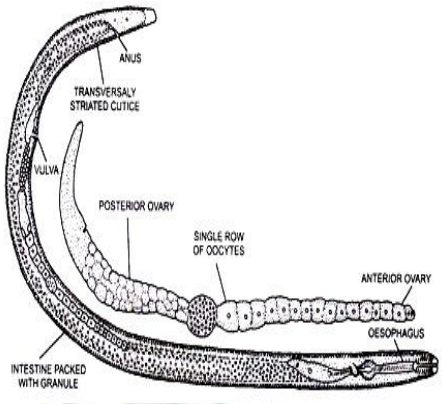
Younger seedlings are very susceptible to damage causing stunted growth. Yield of cabbage and cauliflower is very much reduced.

1. Females measure 0.7-1.0 mm in length and males 0.67-1.0 mm.
2. Cuticle is coarsely started. Lateral fields with 4 incisors.
3. Stylet 22-28 microns with prominent cupped knobs.
4. Oesophagus contains anterior cylindrical part, median bulb slightly ovoid and basal bulb elongated and pyriform.
5. Ovaries and Testis outstretched, spicule tylenchoid and gubernaculum trough like.

LESION NEMATODE

Scientific name: *Pratylenchus thornei*
Common name: Lesion Nematode
Classification:

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Super family	Tylenchoidea
Family	Pratylenchidae
Genus	*Pratylenchus*
Species	*thornei*



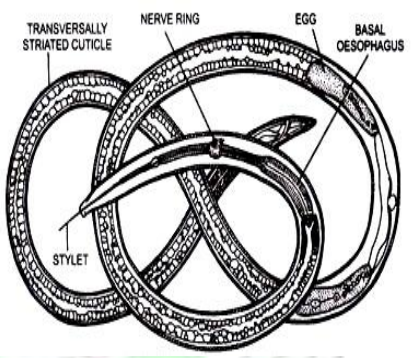
Above nematodes cause stunted growth. It destroys cortex region of the maize plant causing formation of large cavities and hypertrophy.

1. Females and males are cylindrical measuring 0.45-0.77 and 0.5-0.57 mm in length, respectively.
2. Body cuticle has fine transverse striations.
3. Lip region contains 3 annules.
4. Anterior region is truncated. Basal plate of cephalic framework is characteristic of above species.
5. Stylet measuring 17-19 microns in length with strong knobs. Stylet is divided into 3 parts namely proorhabdion, mesorhabalion and telorbabdion.
6. Median bulb of oesophagus, nerve ring, excretory pose and oesophageal glands are clearly seen.
7. Gonads are monodelpline and outstretched.
8. Life-cycle involves egg → first stage larva, second stage larva (infective stage), third stage larva, fourth stage larva and fifth stage or adult stage.

DAGGER NEMATODE

Scientific name: *Xiphinema americanum*
Common name: Dagger Nematode
Classification:

Phylum	Nematoda
Class	Enoplea
Order	Dorylaimida
Super family	Tylenchoidea
Family	Longidoridae
Genus	*Xiphinema*
Species	*americanum*



Dagger destroys, epiblema of infested plants and opens way for various viral and bacterial infections. Xiphinema index acts as a vector for fan leaf virus of grapevine.

1. Females and Males measure 1.5-2.0 mm and 1.6-2.0 mm respectively.
2. Lip region contains 2 circlets of 6 and 10 papillae lateral cords about one-fourth of body width.
3. Stylet in 120-140 micron in length. Pores arranged in 2 rows. Oesophagus consists of long slender anterior regions and basal bulb. Oesophagus leads into intestine which has course refractive granules in its cells.
4. Ovaries reflexed and testis 2 in number.
5. Life-cycle involves egg, first stage larva, second stage larva, third stage larva, fourth stage larva and adult male and female.

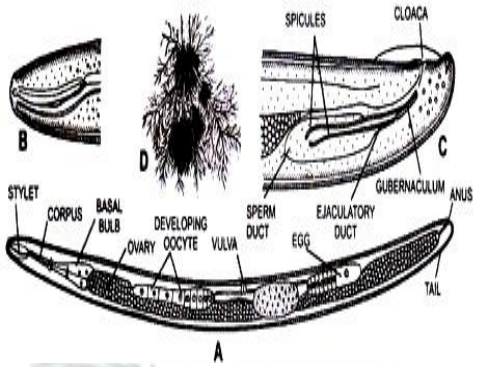
STUBBY NEMATODE

Scientific name: *Trichodorus christie*
Common name: Stubby Nematode
Classification:

Phylum	Nematoda
Class	Adenophorea
Order	Triplonchida
Super family	Tylenchoidea
Family	Trichodoridae
Genus	*Trichodorus*
Species	*christie*

Stubby root nematode is notorious causing stunted growth of the host plant. It feeds on root-tips causing necrosis, loss of meristematic tissues and shortening of mitotic regions and thickening of protoxylem. Infestation could be controlled by fumigation of soil and crop rotation.

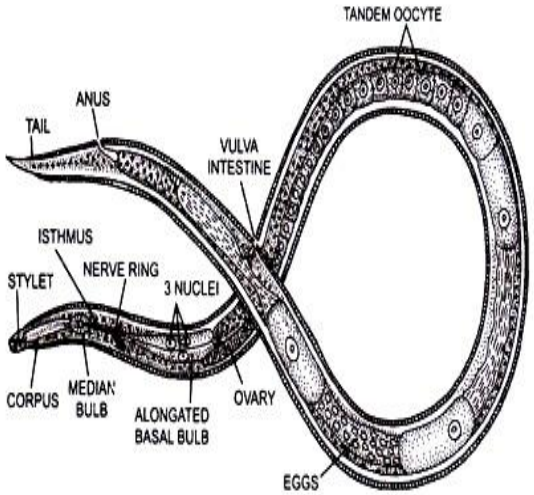
1. Females measure 0.46-0.7 mm and males 0.58-0.6 mm.
2. Body more or less cylindrical. Excretory pore near the base of oesophagus.
3. Oesophagus slightly overlaps the anterior end of intestine. Oesophageal papillae absent.
4. Spicule is straight measuring 63 micron. Gubernaculum present.
5. Life-cycle consists of egg, first stage, second stage, third stage, fourth stages larvae and adults.



STEM AND BULB NEMATODE

Scientific name: *Ditylenchus dipsaci*
Common name: Stem and Bulb Nematode
Classification:

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Family	Anguinidae
Genus	*Ditylenchus*
Species	*dipsaci*



Teasel plants (Gokhroo) produce threads for textile industry. Threads are by burs (spiny seed cases). Above nematode infect burs which assume Puffy appearance and affect the yield of threads. Yield of alfalfa, rye, oat, onion is also affected.

1. Both males and females measure 1.0-1.3 mm in length.
2. Body is covered by transversally striated cuticle. Lateral field is marked by incisors. Deirids usually visible near the base of neck.
3. Stylet with strongly developed knobs and less than 15 microns.
4. Basal oesophageal bulb with the usual 3 prominent and 2 indistinct nuclei.
5. Ovary single. Oocytes tandem. Testes outstretched.