

PRACTICAL MANUAL
on
**Nematodes of Horticultural crops
and their Management**

Course No. HPP 229 Credit Hrs. 2(1+1)

For B.Sc. (Horticulture) II Year students



Dr. Usha

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Department of Entomology
College of Agriculture
Rani Lakshmi Bai Central Agricultural University, Jhansi-284003

Syllabus:

Practical: Methods of sampling and extraction of nematodes from soil and plant parts, killing, fixing and preparation of temporary and permanent nematode mounts. Nematicides and their use. Collection and preservation of 20 plant species/parts damaged by plant parasitic nematodes.

Name of Student

Roll No.

Batch

Session

Semester

Course Name :

Course No. :

Credit

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CERTIFICATE

This is to certify that Shri./Km.ID No..... has completed the practical of course.....course No. as per the syllabus of B.Sc. (Hons.) Agriculture/ Horticulture/ Forestry semester in the year.....in the respective lab/field of College.

Date:

Course Teacher

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Exercise No. 2

Object: To learn extraction of Nematode from soil by Baermann's funnel technique

Requirements:

Procedure:

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Advantages:

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Disadvantages:

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Exercise No. 4

Object: To separate the nematode cysts by Modified Fenwick Can method

Requirements:

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Procedure:

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Exercise No. 7

Object: To study the mounting of the nematode for identification

Requirements:

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Procedure:

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Practical No. 9

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

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

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Problem: 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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Object: To study the diseased material of 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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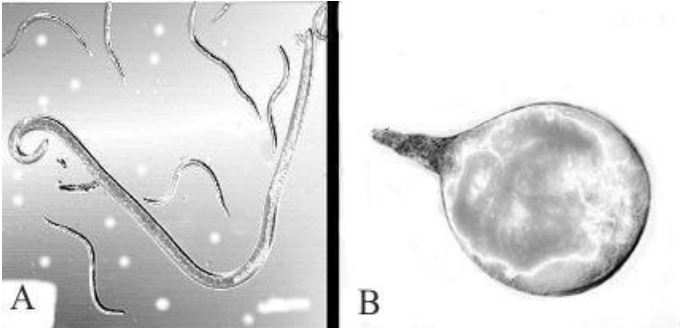
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Exercise No. 11

Object: To study the diseased material of citrus nematode

Scientific Name:

Common Name:

Classification:

Phylum:

Class:

Order:

Family:

Genus:

Species:

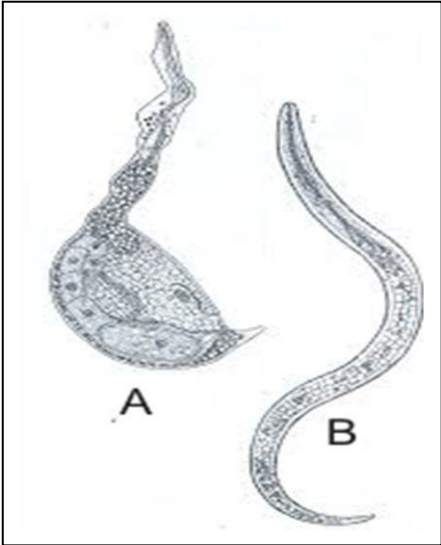
Symptoms:

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General

features:

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Exercise No.12

Object: To study the diseased material of 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. 13

Object: To study the diseased material of 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. 14

Object: To study the diseased material of Burrowing Nematode

Scientific Name:

Common Name:

Classification:

Phylum:

Class:

Order:

Family:

Genus:

Species:

Symptoms:

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General features:

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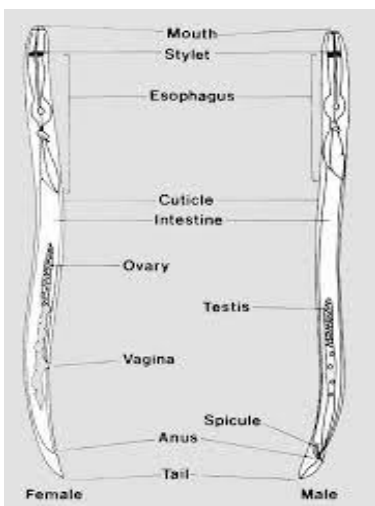
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APPENDICES

SAMPLING OF NEMATODES FROM FIELD

Procedure:

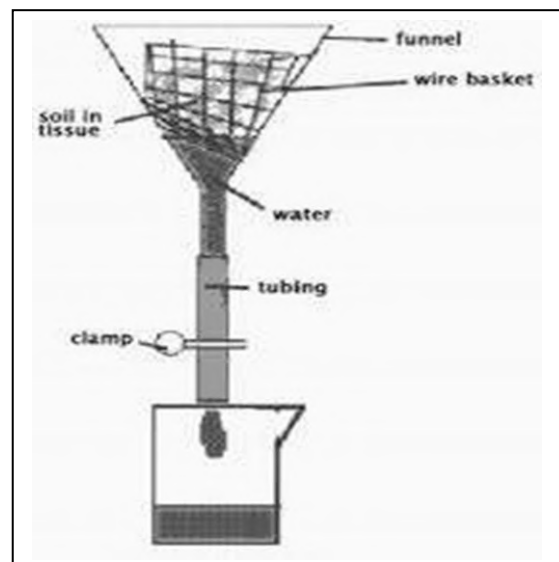
1. Sampling device depends on the type of soil and depth of sampling. In light soil a tube auger is an ideal tool. In case of heavy textured soil a hand shovel or spade is most suitable.
2. Nematodes are not homogeneously distributed in a field. Random or stratified survey becomes essential to estimate the extent of areas infested. The best site to sample for nematode is where feeder root or feeding site for nematodes are abundant. Usually 50-100 cm away from base and 5-30 cm deep site are preferred for sample collection.
3. Sampling of soil roots or both has to be deciding depend on the mode of parasitism of the nematode.
4. Prepare a composite sample by combining the core and sub samples of 200 cm³ soil drawn after mixing of sample. 5-10g root at early stage of crop is enough for nematode extraction and their study.
5. The soil samples should be collected in polythene bags and secured with rubber band with a proper label inside the bag with all the details like date, place, crop, soil type and history of previous crop etc.
6. Usually store the soil samples at low temperature about 10-15° C.

EXTRACTION OF NEMATODE FROM SOIL BY BAERMANN'S FUNNEL TECHNIQUE

Requirements: Glass funnel, Funnel stand, Steel wire net, Beaker, Tissue paper, Pinch clamp and Rubber tubing.

Procedure

1. Fix the funnel on a funnel stand and having rubber tubing (3") attached to the end of funnel stem with pinch clamp on rubber tubing.
2. Place a molded steel wire net in the funnel and fill the funnel nearly to top with fresh tap water.
3. Eliminate air trapped in the funnel stem by pinching the clamp and allowing water to drain off until level is about ½ above bottom of wire basket.
4. Cover the inside of basket with double tissue paper and carefully place the material for extraction into the tissue lined basket. This material may be soil, small root cut into half inch pieces to fit into funnel, bulb, tuber or corm cut into small pieces and label each funnel with sample number and date.
5. Add fresh water daily to funnel to maintain the original water level.
6. Leave funnel undistributed for 2-5 days at room temperature and at the end of waiting period hold below the end of rubber tubing and open the pinch clamp fully to drain off about 10 ml of water containing the nematodes which have settled down to the bottom of apparatus.



Advantages: The technique is simple and the equipment is inexpensive. Recovery of active nematodes from very small samples is fairly good.

Disadvantages: Lack of aeration in the water reduces the movement of nematodes, thus hindering their recovery. Recovery of active nematodes from large samples is poor. The funnel capacity is less, hence may be too small to be a representative.

EXTRACTION OF THE NEMATODE FROM SOIL BY COBB'S DECANTING AND SIEVING METHOD

Materials: 20-mesh sieve (833 µm aperture); 200-mesh sieve (74 µm aperture); 325-mesh sieve (43 µm aperture); Coarse

sieve (1 cm aperture); Two stainless steel bowls or plastic buckets; 250 ml beaker; 600 ml beaker; Coarse spray water bottle

Procedure

1. Review Potential extraction errors
2. Mix the soil sample and pass through a coarse sieve to remove rocks, roots etc
3. Take a 600 cc sub-sample of soil and pack lightly into a beaker for uniformity
4. Place soil in one of the buckets or pans half-filled with water.
5. Sieving and decanting process (various combinations of the following):
6. Mix soil and water by stirring with hand or paddle. Allow to stand until water almost stops swirling
7. Pour all but the heavy sediment through a 20-mesh sieve into a second bucket and discard the residue in first bucket. Discard the material retained on the sieve
8. Stir the material present in second bucket; allow to stand until water almost stops swirling
9. Pour all but the heavy sediment through a 200-mesh sieve into the first bucket, discard the residue in the second bucket.
10. Backwash the material retained on a 200-mesh sieve, which includes large nematodes, into a 250 ml beaker.
11. Stir the material in the first bucket. Allow to stand until water almost stops swirling.
12. Pour all but the heavy sediment through a 325-mesh sieve into a second bucket; discard the residue present in the first bucket.
13. Backwash the material retained on a 325-mesh sieve, which includes small to mid-sized nematodes and silty material, into a 250 ml beaker.
14. Sample present in the 250 ml beaker will probably be too dirty for direct viewing. So, it may be placed on Baermann Funnel or subjected to sucrose-centrifugation. This combined procedure allows the extraction of nematodes from larger volumes of soil.

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.

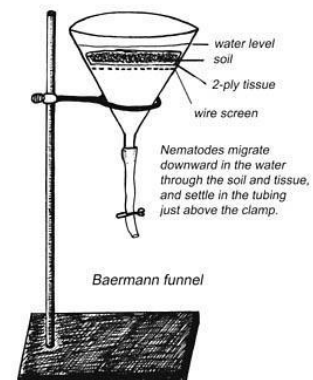
Disadvantages: The method requires expensive sieves and an experienced worker. Nematodes are difficult to see because of fine particles.

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

1. Mix the dried soil thoroughly and take a soil sample of 50-100 ml.
2. 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.
3. Wash the residue from sieve into white bowl and floating cysts along the edge of bowl are picked up with camel's hair brush.
4. Collect the cysts in a drop of water on glass slide and examine.



KILLING AND FIXING NEMATODES

Step1: Collect live nematode specimens in distilled or deionized water in a small beaker or watch glass. Concentrate the nematodes in a minimal volume of water and add equal volume of hot (90C) fixative solution and buffered formalin (Humason, 1972) to it. Nematodes may be killed with heat before adding fixative, though adding hot fixative directly is also effective. Buffered formalin provides very good fixation. Leave the specimens in the fixative for 1-2 days. Nematodes may be stored in buffered formalin indefinitely; it does not clear characters. Buffered formalin solution is prepared as follows:

Requirement: Formalin (ca 40% formaldehyde)-10.0 ml; Water-90 ml; Sodium acid phosphate-0.4 g; Anhydrous disodium phosphate-0.65 g

Step II. Processing Specimens to glycerin

1. Prepare the following two solutions and keep them at room temperature

Seinhorst I solution: 20 parts 95% ethanol; 1 parts glycerin; 79 parts water

Seinhorst II solution: 95 parts 95% ethanol; 5 parts glycerin

Place fixed nematodes in a BPI dish. Draw-off excessive fixative and concentrate the nematodes in a small volume. Add 6-8 ml of Seinhorst solution 1 to the nematode suspension. (A very small quantity of rose Bengal, acid fuchsin, or aqueous picric acid may be added to the solution to stain the nematodes. This is optional. Place the open BPI dish in a larger closed glass container with 95% ethanol at the bottom, and place in oven at 35-40C for at least 12 hours. This removes most of the water in the BPI dish. (Do not close or allow ethanol from the glass container to over-flow into the BPI dish.). Remove the dishes from oven and draw-off the excess Seinhorst solution1 from the BPI dish using a pipette under a dissecting microscope to avoid loss of specimens. Add Seinhorst solution 2 to the BPI dish, place it in a partially covered Petri-dish and keep it in oven at 40C.

Several hours (at least 3 hours) later, draw-off excess solution from the BPI dish and repeat step 5. Keep the dishes in oven until all the alcohol has evaporated (at least 3 hours) and nematodes are in pure glycerin.

MOUNTING NEMATODES

Temporary Mounts

1. Place a small drop of the fixative in the center of a clean glass slide.
2. Using a nematode pick under a dissecting microscope, pick up the desired specimens and place them in the fixative on the center of the slide.
3. Place the slide under the dissecting microscope, and arrange the nematodes in the centre of the slide and bottom of the drop.
4. Place glass wool (about 5mm in length) or glass microbeads in a triangular position near the edge of the drop.
5. Place a cover glass (18mm wide) gently over the drop using a forcep or supporting it with a needle. Draw off excess fixative carefully using filter paper.

Permanent Mounts

1. Fix a clean cover glass (25mm wide) in the center of a Cobb aluminium slide by supporting with appropriate size white cardboard pieces.
2. Place a small drop of anhydrous glycerin in the centre of the cover glass on the aluminium slide.
3. Pick up nematodes from the fixative, as in step 2 of (A), and place them in the glycerin drop.
4. Arrange the nematodes in the center of the slide and place glass wool as in steps 3-4 of (A).
5. Carefully place a cover glass (18mm wide) over the drop, and seal the edges of the cover glass as in steps of 5-6 of (A).
6. After the sealant has dried, a second coat of sealant may be added. Allow to dry, label the slides on the white cardboard, and examine under a compound microscope. Excess of glycerin on the slide is difficult to remove and can cause smudges, which interferes with the sealing process.
7. Store the slides in a flat position to avoid settling of nematodes towards the edge of the cover glass.
8. Use of aluminum slides enables viewing of the nematodes from both sides of the slides.

STAINING OF THE NEMATODES IN PLANT TISSUE (Acid Fuchsin Lactophenol Method)

Requirements: Acid Fuchsin, Lactophenol, hot plate or spirit lamp, beaker and Petri plate.

Procedure:

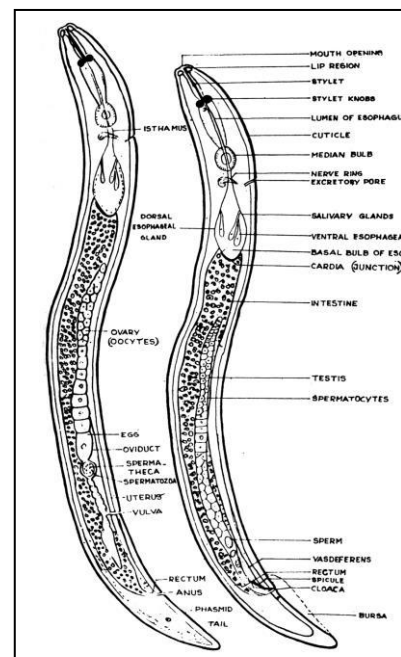
1. Wash the plant material free from soil.
2. Heat the staining solution (1% Acid Fuchsin in 100 cc Lactophenol) to boiling and immerse the plant tissue for 1-3 minutes.
3. Remove the material from staining solution and wash off the excess stain with cold water.
4. Store in de-staining solution (clear lactophenol solution without acid fuchsin) until properly de-stained.
5. The plant tissue will lose its colour and becomes translucent and stained nematode is clearly visible.

PLANT PARASITIC NEMATODES

Morphology: Adult plant parasitic nematodes are elongated worms ranging in length from about 0.30mm to over 5.0mm. The anterior end tapers to a rounded or truncated lip region, the body proper is more or less cylindrical, and the posterior end tapers to a terminus which may be pointed or hemispherical. Proportions of the elongated body vary greatly. Females have greatly expanded bodies, sometimes nearly spherical, but always with a distinct neck. The adult males are always slender worms. Plant parasitic nematodes have no appendages. The mouth of a nematode is at the ANTERIOR end, and the terminus is at the POSTERIOR end. The excretory pore, vulva, and anus are on the VENTRAL side; and the opposite side is called DORSAL. The right and left sides are called LATERAL. The cuticle is attached to several other layers of tissue, which are separated laterally, dorsally and ventrally by chords. These contain nerves, excretory organs, etc., and separate four bands of muscles, which move the body.

A. Alimentary canal – The alimentary canal starts at the mouth and ends at the anus. It includes the oesophagus, intestine, intestine, and rectum

B. Stylet – In plant parasitic nematodes of the “Tylenchida” group, the mouth contains a stylet or mouth spear, a hardened, hollow, cuticular 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.

- C. **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. Posterior to the median bulb, the oesophagus contains three glands, one dorsal and two subventral, each with a nucleus. Three glands may form a terminal bulb to which the intestine is 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.
- D. **Dorsal Gland Orifice** – This in most species of plant parasitic nematodes 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.
- E. **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.
- F. **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.

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.

ROOT KNOT NEMATODE

Scientific name: *Meloidogyne spp.*

Common name: Root knot nematode

Classification:

Phylum	Nematoda
Class	Secernentia
Order	Tylenchida
Super family	Heteroderoidea
Family	Meloidogynidae
Sub family	Meloidogyninae
Genus	<i>Meloidogyne</i>
Species	<i>incognita</i>

1. Infested roots show number of galls of various size different from bacterial nodules.
2. Female as well as third and fourth stage larvae are sedentary endoparasitic in roots.
3. Females are saccate, spheroid with distinct neck.
4. Male and larvae are elongate.
5. Stylet strong with rounded knobs in male, in female slender with strong basal knobs.
6. Oesophagus with large medium bulb followed by a short isthmus.
7. Vulva and anus of females are opposite to neck and surrounded by a pattern of fine lines resembling human fingerprints.

CITRUS NEMATODE

Scientific name: *Tylenchulus semipenetrans*

Common name: Citrus Nematode

Classification:

Phylum	Nematoda
Class	Secernentia
Order	Tylenchida
Super family	Criconematoidea
Family	Tylenchulidae
Genus	<i>Tylenchulus</i>

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.

CYST NEMATODE

Scientific name: *Heterodera spp.*

Common name: Cyst Nematode

Classification:

Phylum	Nematoda
Class	Secernentia
Order	Tylenchida
Super family	Tylenchoidea
Family	<i>Heteroderoidea</i>
Sub family	<i>Heteroderidae</i>
Genus	<i>Heterodera</i>

1. Parasitic on many plants. Adult female with neck embedded in plant roots and body exposed. Larvae, males and cyst are found in soil.
2. Females are typically swollen, lemon shaped, white or yellow in colour and cysts are hard brown in colour.
3. Stylet is short in male with rounded basal knobs.

LESION NEMATODE**Scientific name:** *Pratylenchus spp.***Common name:** Lesion nematode**Classification:**

Phylum	Nematoda
Class	Secernentia
Order	Tylenchida
Sub order	Tylenchina
Super family	Tylenchoidea
Family	Pratylenchidae
Genus	<i>Pratylenchus</i>

1. The roots of diseased plants show discrete elliptical lesion in the initial stage which are yellow, brown or black in colour.
2. The nematodes are small, vermiform body gradually tapering posterior and migratory endoparasitic.
3. The head is relatively broad and continuous with body.
4. Vulva in posterior region, female genital tract monodelphic, prodelphic.
5. In male bursa enclosing train terminus.

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

Phylum	Nematoda
Class	Secernentia
Order	Tylenchida
Super family	Tylenchoidea
Family	Pratylenchidae
Genus	<i>Radopholus</i>

1. It is migratory endoparasite and burrows in the host plant tissue, which appears on root surface in the form of deep lesions.
2. In female stylet is short and stout and in male very slender and rudimentary.
3. In female tail is taper with plain end and in male long with bursa.

RENIFORM NEMATODE**Scientific name:** *Rotylenchulus spp.***Common name:** Reniform Nematode**Classification:**

Phylum	Nematoda
Class	Secernentia
Order	Tylenchida
Super family	Tylenchoidea
Family	Haplolaimidae
Sub family	Rotylenchinae
Genus	<i>Rotylenchulus</i>

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.

NEMATODE MANAGEMENT

Nematicide:

- A nematicide is a type of chemical pesticide used to kill plant-parasitic nematodes.
- Nematicides have tended to be broad-spectrum toxicants possessing high volatility or other properties promoting migration through the soil.

Nematicide Properties and Efficacy:

1. For all nematicides, consider properties relative to movement in soil:
 - water solubility
 - vapor pressure, volatility, fumigant action
 - Henry's constant (k_H) - affinity for water
 - method of incorporation or movement in soil
2. Nematicides in soil are in dynamic equilibrium among the three soil phases: 1) solids (adsorbed to clay), 2) soil solution, and 3) soil air.
 - Non-fumigants are mainly distributed in phases 1 and 2, movement is by mass flow.
 - Fumigant movement determined by vapor pressure and k_H , move 1000x faster in air than in water.
 - Fumigant movement is affected by low and high soil temperatures, low and high soil moisture, clay or silt content, organic matter content; e.g., problems with 1,3-D efficacy in the Tulelake region were attributed to the narrow temperature and moisture window prior to potato planting, and high adsorption in highly organic soils.
 - Consider the principle of attempting to deliver a standard dosage to the target (standard concentration*time product; e.g, 50 ppm for 4 days and 100 ppm for 2 days both give a dosage of 200 ppm-day)

Types of Nematicides:

- Natural Nematicides
- Artificial Nematicides: Fumigants; Carbamates; Organophosphates

Natural Nematicides:

- Garlic developed polysulfide product
- Neem Cakes
- Root exudates of MARIGOLD
- Nematophagous fungi-*Paecilomyces*

Artificial Nematicides:

- These are artificially synthesized chemical compounds that are highly toxic and in some extinct even carcinogenic used for nematode control in a field.
- They might be fumigants, carbamates or organophosphates.

- **Carbamate nematicides** (benomyl, carbofuran, carbosulfan, cloethocarb)
 - ✓ **oxime carbamate nematicides** (alanycarb, aldicarb, aldoxycarb, oxamyl, tirpate)
- **Fumigant nematicides** (carbon disulfide, cyanogen, 1,2-dichloropropane, 1,3-dichloropropene, dimethyl disulfide, methyl bromide, methyl iodide, sodium tetrathiocarbonate)
- **Organophosphorus nematicides**
 - ✓ **organophosphate nematicides** (diamidafos, fenamiphos, fosthietan, phosphamidon)
 - ✓ **organothiophosphate nematicides** (cadusafos, chlorpyrifos, dichlofenthion, dimethoate, ethoprophos, fensulfothion, fosthiazate, heterophos, isamidofos, isazofos, phorate, phosphocarb, terbufos, thionazin, triazophos)
 - ✓ **phosphonothioate nematicides** (imicyafos, mecarphon)
- **unclassified nematicides** (acetoprole, benclonthiaz, chloropicrin, cyclobutrifluram, dazomet, DBCP, DCIP, fluazaindolizine, fluensulfone, furfural, metam, methyl isothiocyanate, tiozazafen, xylenols)
- **Avermectin nematicides** (abamectin)
- **Botanical nematicides** (carvacrol)

Organic cakes: Neem cake, Mustard cake, Linseed cake, castor cake and Jetropha cake

Trap crops: Mustard, *Tagetes*, *Asparagus*, *Crotolaria* and *Margosa*

Biopesticides:

- a) **Plant products:** Neem formulations (Nimbicidine, Achook, Neem oil and NSK)
- b) **Bacteria:** *pasteuria penetrans*, *Pseudomonas* and *Bacillus subtilis*

c) **Fungi:** *Paecilomyces lilacinus*, *Trichoderma* spp. and VAM fungi

d) **Predatory Nematodes:** Mononchids, Aphelenchids, Dorylaimids and Diplogasterids

NEMATICIDES CURRENTLY AVAILABLE IN MARKETS

Chemical name	Trade name	Formulation
Fumigants		
Methyl bromide	Dowfume	Gas
1,3 dichloropropene	Telone/DD-95	Liquid
Ethylene dibromide ¹	Dowfume W-85	Liquid
Metam-sodium	Vapam	Liquid
Dazomet	Basamid	Dust (prill)
Methyl isothiocyanate	Di-Trapex	Liquid
Chloropicrin ¹	Larvacide	Liquid
Organophosphates		
Thionazin	Nemafos	Granular or emulsifiable liquid
Ethoprophos	Mocap	Granular or emulsifiable liquid
Fenamiphos	Nemacur	Granular or emulsifiable liquid
Fensulfothion	Dasanit	Granular
Terbufos	Counter	Granular
Isazofos	Miral	Granular or emulsifiable liquid
Ebufos	Rugby	Granular or emulsifiable liquid
Carbamates		
Aldicarb	Temik	Granular
Aldoxycarb	Standak	Flowable
Oxamyl	Vydate	Granular or emulsifiable liquid
Carbofuran	Furadan/Curaterr	Granular or flowable
Cleothocarb	Lance	Granular

RECOMMENDED NEMATICIDAL DOSAGES AND TREATMENTS FOR SOME IMPORTANT CROPS

Crop	Nematode pest	Nematicide	Application rate	Application techniques
Potato	Globodera spp.	Aldicarb	2.24-3.36	Incorporated in row
		Oxamyl	4.0-5.5	
		Carbofuran	4.0-5.5	
Tomato, cucurbits	Meloidogyne spp.	Aldicarb	3.36	Incorporated in 30-cm bands
		Ethoprophos	0.9-2.9	Incorporated in bands
		Oxamyl	0.6-1.2	Incorporated in bands
		Fenamiphos	1.6-3.3	Incorporated in bands
		Dazomet	30-50 g/m ²	Incorporated in bands and irrigated Time interval before planting
Citrus	Tylenchulus semipenetrans	Fenamiphos	10.8-21.6	Annual treatment applied along drip-line
		Aldicarb	5.5-11.0	Annual treatment applied along drip-line
Grape	Meloidogyne spp.	Fenamiphos	10.0	In bands for nursery use
	Xiphinema index	Aldicarb	5-10	In bands for nursery use
Banana	Radopholus similis	Carbofuran	2-4 ga.i. per plant	Applied around plant 2-3 times per year
	Helicotylenchus multicinctus	Ethoprophos	2-4 ga.i. per plant	Applied around plant 2-3 times per year
	Pratylenchus spp.	Fenamiphos	2-4 ga.i. per plant	Applied around plant 2-3 times per year

	Meloidogyne spp.	Isazofos	2-4 ga.i. per plant	Applied around plant 2-3 times per year
		Ebufos	2-4 ga.i. per plant	Applied around plant 2-3 times per year