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By

Seenivasan Nagachandrabose,
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PREFACE

Nematodes are unique animals with wide diversity in their structural organization, feeding, and habitat. Their size ranges from microscopic to a few meters. Nematodes are widespread on the earth and live in various habitats, from the soil and water ecosystems of the deep ocean to hilltops and even in deserts, the Arctic, and Antarctica. Their mouth parts are variable among species and genera according to their feeding habits. They may be feeding on fungi, bacteria, yeast, algae, and microbes that exist in the soil. Even a few groups of predatory nematodes feed on smaller nematodes. However, many nematodes live as parasites on plants and animals, including humans, domestic animals, insects, etc. Likewise, a unique group of nematodes in the soil that feed on plants is called phytonematodes or plant-parasitic nematodes. The extent of damage caused by phytonematodes may vary from a slight injury to the total loss of plants or trees. Phytonematodes possess a syringe-like mouthpart called a, "stylet" with which they feed on plant parts. Most phytonematodes live in soil and feed on underground parts such as roots, tubers, or bulbs. Due to this subterranean activity, diagnosing nematode-damaged or infected plants is complex. Very few can infect foliar parts under favorable humid conditions and feed on leaves, stalks, inflorescences, and seeds.

The phytonematodes are reported to cause an annual yield loss of 125 billion US dollars globally. In India alone, phytonematodes damage 24 crop plants and cause severe yield loss in agriculture and horticulture crops worth 21068 million rupees. Farmers are now practicing chemical control methods for nematode management. Particularly they repeated use carbofuran to achieve desirable nematode control. Farmers can avoid a 50% loss by nematodes by applying chemical nematicides. But the toxic chemical treatment in the soil leads to many problems, such as health issues for applicators, lethal effects on non-target soil organisms, and groundwater contamination. Also, the application of chemical nematicides is so expensive. Hence, it is time to explore alternative, safe, and inexpensive natural control methods to manage nematodes in crop plants.

In general, organic acids formed during the decomposition of organic materials are reported to have nematocidal activities. Humic acid, a naturally formed organic acid that can be extracted by various procedures, has now been widely used by farmers to enhance the growth and yield of many crop plants. Primarily, it is obtained from lignite or coal mines.

Humic acid typically contains heterocyclic compounds with carboxylic, phenolic, and carbonyl functional groups. Because of the nature of their functional groups, it is considered an alternative to nematicides. Hence, the use of humic acid for phytonematode control is emerging as an essential tool. Several books are available on phytonematode control using chemicals, biocontrol agents, and bioproducts. However, there is no book to explain the importance of humic acid in phytonematode control. This book's driving force is to fulfill farmers' requirements for using humic acid in crop plants for nematode management. This simple, easily understandable basic book appeals to extension personnel and researchers.

In this textbook, we have explained the following: what a nematode is, the role of organic methods and organic acids in nematode control, the role of humic acid in agriculture, plant disease control, phytonematode control, and how humic acid controls nematodes. Research on phytonematode management using humic acid on bananas and citrus nematodes, its compatibility with available biocontrol agents, and its prospects are also included in this book. It is my hope that this book will satisfy the needs of farmers, agricultural extension workers, and life science researchers.

Seenivasan Nagachandrabose
Sankaranarayanan Chellappa
Subramanian K.S.

August 2021

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INTRODUCTION TO PHYTONEMATODES

Nematodes are a crucial invertebrate animal group of organisms that exist in all the environments on earth, from the deep ocean to hilltops and hot desert habitats to Arctic and Antarctic habitats. Nematodes are classified or grouped based on their feeding habitat as bacterial feeders, fungal feeders, algal feeders, predators (feed on smaller-sized nematodes), plant feeders (plant-parasitic nematodes), omnivores (feed on microbes as well as plants), and animal feeders (parasites that feed on animals, including humans and vertebrate insects). The majority of nematodes live as parasites on other living hosts. Plant-parasitic nematodes, or phytonematodes, are a special group that feeds on plants with some unique characteristics to all other groups of nematodes.

Even though nematodes inhabit virtually every territory on the planet, they are strikingly similar in morphology and life stages. Notwithstanding their primary intricacy, certain essential standards are common to all nematodes. Nematodes are triploblastic (formation of three germ layers during embryogenesis), bilaterally symmetrical (when cutting a nematode longitudinally in a dorsoventral plane, it gives two halves), unsegmented (however, nematodes look like a segmented animal due to the presence of annulations or markings on the cuticle), pseudocoelomate (false body cavity), vermiform (tapering at both ends), and colorless. Many phytonematodes are slender with elongated bodies, fusiform-shaped, or spindle-shaped. The nematode body is circularly-shaped in cross-section view, and its anterior and posterior ends are tapered in nature. Nematodes' body lengths range from 0.2 mm to 11.0 mm. The pin nematode (*Paratylenchus*) is the smallest, whereas the needle nematode (*Paralongidorus maximus*) is the longest. The body width of plant-parasitic nematodes ranges from 0.01 to 0.05 mm. Though most plant-parasitic nematodes are vermiform, a few species or genera have distinct shapes, especially adult females of root-knot nematodes (pear or saccate shape), cyst nematodes (globular – *Globodera*; lemon shape – *Heterodera*), reniform nematodes (reniform, bean, or kidney-shaped), and citrus nematodes (irregularly saccate shaped). These specially shaped nematodes have higher reproductive potential (produce eggs in mass) than others (lay an egg in a single). Though nematodes are bilaterally symmetric organisms, they have triradiate symmetry in the esophagus region, tetraradiate symmetry in the hypodermal chord region, and hexaradiate symmetry in the lip region. The asymmetry occurs in nematode body regions where parts of the reproductive system, intestinal system, and excretory system exist. In nematodes, the male and female are separated by whether they have one or two gonads. The gonads open externally through a separate opening called the vulva in females. In contrast, males' reproductive and digestive systems combine at the distal end of the rectum and open exteriorly through a common opening called the cloaca.

The nematodes do not have distinct body parts. Hence, nematologists divide the nematodes into four body parts called head, neck, body, and tail. The region of the mouth (from the anterior part (lips) up to the base of the stylet) is called the head region; the portion or regions of the esophagus (from the stylet base to the cardia) is the neck region; the intestinal region (from the cardia to the anal opening) is the body region and the distal end from the anus to the tip of the nematode is called the tail region.

From a longitudinal view, the nematode body has four different regions: the dorsal side, the ventral side, the right lateral side and the left lateral side. The natural openings of nematodes, like the excretory pore, vulva, and anus, are located on the ventral side, but the mouth or oral opening is present in the anterior terminus. The nematode body comprises different organ systems, including the digestive, excretory, nervous, and reproductive systems. There is no definite body system for respiration and circulatory purposes. Respiration in nematodes happens through the cuticle and other membranes by simple diffusion. Similarly, fluids present in the false body cavity, or pseudocoelum, do the duties of the circulatory system.

Characteristics of members of the phylum Nemata.

1. Lives in different ecosystems or habitats such as soil, rivers, ponds, freshwater, and the ocean either as free-living organisms or parasites.
2. They are triploblastic animals with unsegmented and bilaterally symmetrical bodies having false body cavities, or pseudocoelum.
3. They are vermiform or fusiform with a circular shape in cross-section.
4. Their body is covered with a triple-layered cuticle.

5. Six life stages exist in their life cycle: eggs, juveniles (J1, J2, J3, and J4), and adults.
6. Four moltings occur during their growth periods.
7. The mouth or oral opening has six lips and 16 papillae (sense organs) on the lips.
8. Nematodes have a pair of sense organs in the head region called amphids.
9. Muscles are arranged longitudinally in the body wall region and are corrected with nerves in four hypodermal chord regions.
10. The excretory system in nematodes is either gland cell (renette) or tubular/canal structured.
11. The nervous system has a circum oesophageal commissure, which encircles the esophagus in a region called the nerve ring or brain of nematodes.
12. The hypodermis has four thickenings: one dorsal, one ventral, and two lateral regions, which house the longitudinal nerves originating from the brain or circum-oesophageal commissure.

Characteristics of plant-parasitic nematodes

The following three characteristics are prevalent among plant-parasitic nematodes.

1. Their size is almost microscopic.
2. They live as obligate parasites on plants, which means they need live host plants to feed, grow, and multiply, meaning that they must have living plant tissue to feed, grow, and reproduce.
3. All phytonematodes have a syringe-like mouthpart called a stylet and feed on plant parts.

Types of phytonematodes

Very few nematodes can survive in above-ground parts of plants like seeds, stems, leaves, and inflorescences. But most phytonematodes are underground feeders and prefer mainly roots (feeder roots) and feed on underground stems, pseudostems, tubers, and bulbs. The subterranean feeding habits of phytonematodes, make it challenging to diagnose nematode problems in crop plants.

The major nematodes associated with many crops are as follows;

1. Root-knot nematodes (*Meloidogyne* spp.)

2. Seed gall nematodes (*Anguina tritici*)
3. Cyst nematodes (*Heterodera* spp.)
4. Potato cyst nematodes (*Globodera* spp.)
5. Lesion nematodes (*Pratylenchus* spp.)
6. Burrowing nematodes (*Radopholus similis*)
7. Stem and bulb nematodes (*Ditylenchus* spp.)
8. Citrus nematodes (*Tylenchulus semipenetrans*)
9. Reniform nematodes (*Rotylenchulus reniformis*)
10. Spiral nematodes (*Helicotylenchus* spp.)
11. Dagger nematodes (*Xiphinema* spp.)

Based on the feeding pattern, phytonematodes are categorized into ectoparasites, semi-endoparasites, and endoparasites. Ectoparasitic nematodes remain outside the plant and use their style to feed on the cells without entering the roots. Ectoparasites are the most commonly observed phytonematode species under field conditions but are considered less damaging. Stunt nematodes (*Tylenchorhynchus* spp.), lance nematodes (*Hoplolaimus* spp.), and spiral nematodes (*Helicotylenchus* spp.) are some ectoparasitic nematodes. Semi-endoparasitic nematodes can partially penetrate the plant roots and feed on cell contents by developing permanent feeding cells, or syncytia, on plant roots. Reniform nematodes (*Rotylenchulus reniformis*); and citrus nematodes (*Tylenchulus semipenetrans*) are semi-endoparasites. Endoparasitic nematodes enter the roots completely. They are classified into two types: i). Migratory endoparasites migrate through root tissues by destructively feeding on plant cells. Examples are lesion nematodes (*Pratylenchus* spp.) and burrowing nematodes (*Radopholus similis*); ii). Sedentary endoparasitic nematodes are the most damaging plant-parasitic nematode genera worldwide. They establish within the roots by developing specialized feeding sites, for example, cyst nematodes (*Heterodera* and *Globodera*) and root-knot nematodes (*Meloidogyne* spp.). Foliar nematodes feed on above-ground plant parts such as stems, leaves, and inflorescence. For example, white-tip nematodes (*Aphelenchoides besseyi*) and ufra nematodes (*Ditylenchus angustus*) are foliar feeders of rice plants.

1. Nematodes feed on foliage

a. Leaf, flower, and bulb feeders

This subgroup consists of four major nematode genera: *Anguina tritici* (wheat seed gall nematode), *Orina phyllobia* (leaf-feeding nematode on silver nightshade weed plants), *Aphelenchoides* spp. (leaf and bud nematode), and *Ditylenchus* spp. (stem and bulb nematode).

b. Tree trunk feeder

This group consists of two nematodes, *viz.*, *Bursaphelenchus cocophilus* (coconut red ring nematode) and *Bursaphelenchus xylophilus* (pine wilt nematode).

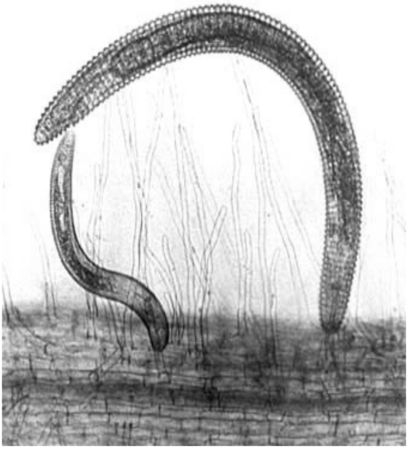
2. Nematodes feed on roots and below-ground parts.

This group of nematodes is further classified primarily into three; i. ectoparasites, ii. semi-endoparasites, and iii. endoparasites.

Ectoparasites	Semi-endoparasites	Endoparasites
• Sedentory (Sheath nematode) • Migratory (Dagger nematode)	• Reniform nematode • Citrus nematode	• Sedentory (*Meloidogyne*, *Globodera* and *Heterodera*) • Migratory (*Radohpholus*, *Pratylenchus* and *Hirschmanniella*)

Ectoparasites: They live in soil and freely move around the roots. They feed by inserting a stylet and sucking plant sap through the stylet. They feed on root epidermis layers.

Ectoparasite - Migratory: This group of ectoparasites spends their whole life in the soil and whenever they need food, insert stylet and suck the food. After feeding, they detach the stylet and move to another part of the root. Here, the nematodes' stylet attachment to the roots is temporary only. E.g., ring nematode (*Criconemoides*), stubby root nematode (*Trichodorus christie*), and pin nematode (*Paratylenchus* spp.).



Criconemoides (Courtesy: S.W.Westcott)

Ectoparasite – Sedentary:

This group of ectoparasites also inserts their stylet inside the roots but keeps it for an extended period once they are attached to the roots. The attachment of the stylet is somehow permanent, and they feed in the same location for an extended period. E.g., sheath nematodes (*Hecyliophora arenaria*) and sessile nematodes (*Cacopaurus pestis*).

Semi-endoparasites:

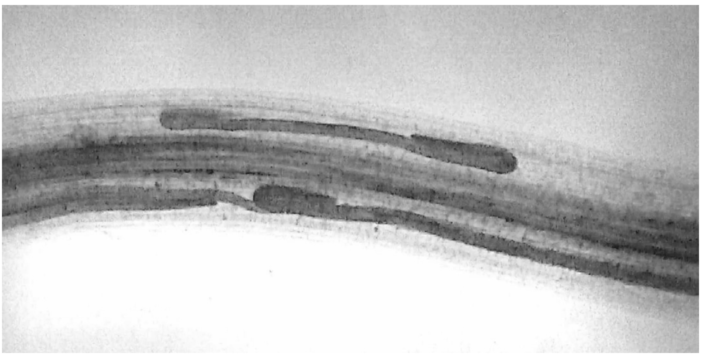
This category of nematodes inserts their stylet along with the head and neck region inside the root and the remaining body is exposed to soil. They prefer the root cortex or endodermis region (the outer layer of endodermis only) to establish a feeding site. E.g. reniform nematode (*Rotylenchulus reniformis*) and citrus nematode (*Tylenchulus semipenetrans*).

Rotylenchulus reniformis

Endoparasites:

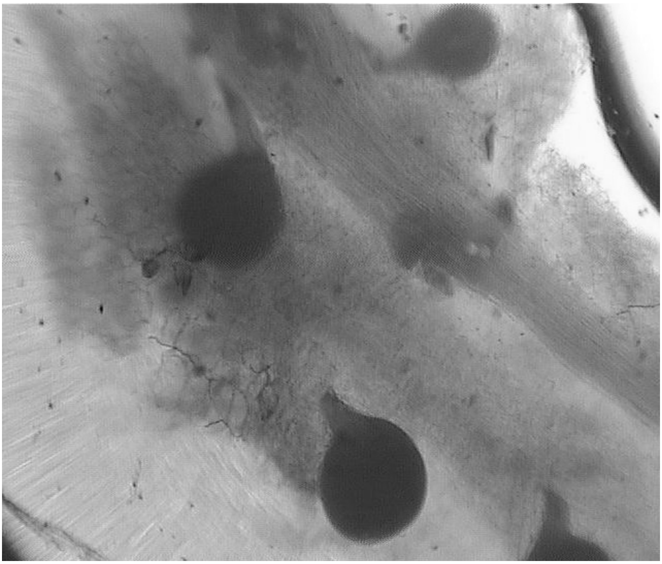
Endoparasitic nematodes ultimately enter the root. The major part of their life cycle is within the roots.

a. **Endoparasites-Migratory**: This kind of nematode, once it enters inside the root, freely moves inside the roots. They are primarily root-cortical feeders, and they continuously feed on cortex cells. If they finish all the portions of roots, they will exit from (e.g.) *Hirschmanniella* spp., *Pratylenchus* spp., *Radopholus similis,* etc., These nematodes move in the cortical parenchyma of the host root. While migrating, they feed on cells, multiply, and cause necrotic lesions.



Hirschmanniella oryzae on rice roots

b. Endoparasites-Sedentary: Sedentary endo-parasitic nematodes enter the roots completely and fix a feeding site. *Meloidogyne, Globodera,* and *Heterodera* prefer the pericycle cells on the root endodermis. Once they establish a feeding site with typical nurse cells for nourishment, they stop moving and become sedentary.



Meloidogyne

Types of injury

Since most of the nematodes are damaging the roots, the nematode-infected plants look like water- and nutrient-deficient plants.

- Leaf chlorosis (yellowing) or discoloration of foliage
- Stunted plant growth
- The plant does not respond to routine fertilizer applications.
- A tendency for rapid wilting, and plants recover slowly from wilting.
- Dieback and the gradual decline of woody plants
- Nematode-infected plants are more prone to weed problems. The infected plants are weak enough to compete with weeds.

The most distinctive nematode symptoms on roots are as follows;

- Root galls or knots
- Root lesions or root necrosis
- Excessive root hairs

Injury caused by foliar-feeding nematodes

1. Leaf discoloration:

- The rice white tip nematode, *Aphelenchoides besseyi,* feeds on young leaf tips, causing typical ‘white tip’ damage in rice.
- In Chrysanthemum, *Aphelenchoides besseyi* feed on leaves, making them yellow.

Whitetip symptom in rice due to *Aphelenchoides besseyi* (Source: TNAU)

2. Dead or devitalized buds: The strawberry foliar nematode, *Aphelenchoides fragariae,* infests the growing point of plants, killing them and resulting in ‘blind plants.’

Dead buds in strawberry by *Aphelenchoide fragariae*
(Source: MV McKenry and PA Roberts)

3. Seed galls: It is caused by *Anguina tritici* in wheat seeds. The *A. tritici* feeding on leaves and stems moved to seeds during the inflorescence maturation stage. A single-galled seed may have thousands of nematodes. *Anguina tritici* can survive up to 28 years in galled seeds.

Wheat seed gall
(Source: R.S. Hussey, Nemapix picture set, Bugwood.org**)**

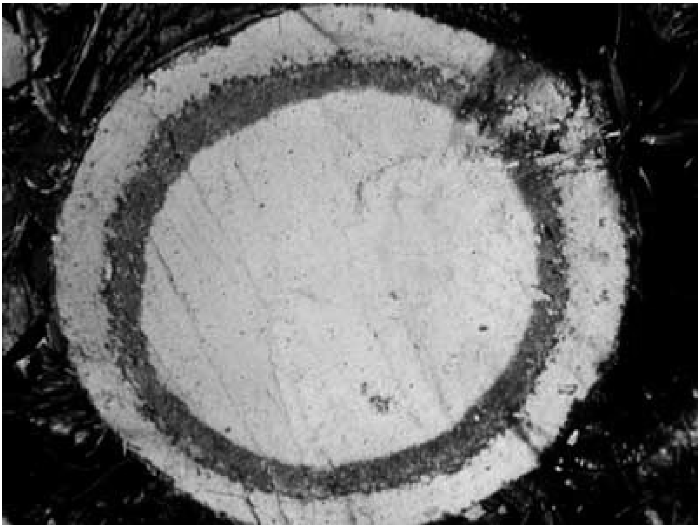
4. Twisting of leaves and stem: The stem and bulb nematode *Ditylenchus dipsaci* causes twisting and curling of the basal leaves in infested onion plants.

Onion leaf infected with *Ditylenchus dipsaci*
(Source: University of Minnesota)

5. Crinkled stem and distorted foliage: The *A. tritici* in young wheat plants survive in growing tips and lead to the formation of crinkled stems or distorted foliage in wheat.

Wheat plants infested with *Anguinta tritici*
(Source: Mohamedova, M & Piperkoka, N)

6. Necrosis and discoloration: The red ring nematode, *Rhadinaphelenchus cocophilus,* is the causal agent of coconut red ring disease in central and southern America and the Caribbean Islands. The nematode, along with the diseased tissues, is transmitted by a palm weevil, *Rhynchophorus palmarum,* and deposited in the leaf axils of healthy plants. Palms of bearing age are more susceptible to infection. Symptoms appear as yellowing of older leaves from tip to base, gradually extending to inner leaves, leading to drooping and death of leaves. About 3 cm beneath the stem surface, a ring of reddish necrotic tissue, about 3 cm wide, extends the entire length of the stem, roots, and petioles.



Red ring disease on coconut caused by *Rhadinaphelenchus cocophilus* (Source: Sasser, 1971)

7. Lesions on leaves and stem: The foliar nematode, *Aphelechoides ritzemabosi,* feeds ectoparasitically on buds and endoparasitically on leaves. The nematodes ascend the stem in a thin film of water covering the plant, enter the leaves through stomatal openings, and feed on the mesophyll tissues. The symptoms appear as tiny brown spots, which enlarge to become interveinal angular spots. Nematode feeding on buds results in a 'blind' plant (*A. fragariae* on strawberry) or undersized and distorted flowers (*A. ritzemabosi* in chrysanthemum).

Leaf lesions in chrysanthemum caused by *A. ritzemabosi*
(Source: Sasser, 1971)

Damage caused by root-sucking nematodes

Root-feeding or sucking nematodes damage the the roots and express symptoms on roots. The nematode-infected plants also express symptoms on their leaves or foliage. Hence, we can classify the damages into root and foliar symptoms due to root feeders.

a. Foliar damage
b. Root damage

a. Foliar damages

1. Stunted growth: Nematode-infected plants appear stunted. Infected plants' growth becomes stunted or not normal compared to uninfected plants. Commonly, stunted symptoms appear in patches due to the patchy distribution of nematodes. E.g., potato plants infected with *Globodera rostochiensis* and wheat plants with *Heterodera avenae* express stunted growth.

Stunted tomato plant due to *Meloidogyne incognita*
(Source: TNAU)

2. **Foliage discoloration**: The nematode infected plants always exhibit yellowing or discoloration of the foliage. E.g., yellowing of foliage in coffee plants by lesion nematode *P. coffeae,* pale green leaves of potato due to *G. rostochiensis* infection, and yellowing and drying of orange or acid lime leaves due to the citrus nematode, *Tylenchulus semipenetrans*.

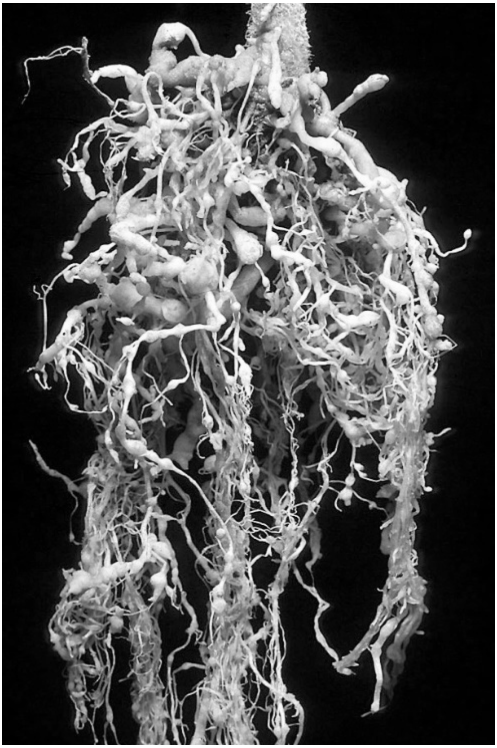
Rice leaf discolouration due to *Meloidogyne graminicola*
(Source: TNAU)

3. **Wilt symptom:** Nematode-infected plants also express wilt symptoms. Mainly, root-knot nematode-infected tomato and tobacco plants express day-wilt damage. The plants look like wilted plants during the day but recover during the evening. The wilt symptoms appear due to nematodes, even when plants are given sufficient water and fertilizers.

Wilted guava plant due to root-knot nematode, *Meloidogyne enterolobii*
(Source: TNAU)

B. Root damages

1. **Gall or knot formation:** Root-knot nematodes (*Meloidogyne* spp.), false root-knot nematodes (*Nacobbus batatiformis*), sheath nematodes (*Hemicycliophora arenaria*), stem and bulb nematodes (*Hemicycliophora arenaria*), dagger nematodes (*Xiphinema index*), and needle nematodes (*Longidorus* spp.) are gall-forming nematodes. *Meloidogyne* spp. produces typical root galls. However, the gall size may vary according to the host plant and *Meloidogyne* spp. involved. The galls produced by *M. hapla* are smaller than those of *M. incognita*. *M. enterolobii* caused simple galls as well as compound galls. *M. incognita* on cucumber causes larger galls. *Xiphinema* (on grapes, onion, rose, and turmeric) and *Longidorus* (on turmeric) are causing smaller-sized root tip galls.



Root-knot galls on tomato
(Source: TNAU)

2. **Root Lesion:** Migratory endoparasitic nematodes like *R. similis* on banana, *P. coffeae* on coffee, and *Hirschmanniella oryzae* on rice cause lesion damage. The lesions appear at the root due to feeding on cortical tissues. Lesion color may vary, i.e., a reddish-brown lesion by *R. similis* on banana, brown lesions by *P. coffeae,* and *H. oryzae*.

Root lesions in banana caused by *Radopholus similis*

3. **Reduction of root:** As nematodes prefer to feed at the root tip, further root growth is arrested. As a balanced reaction, the root forms many branches near the arrested root tip. However, reduction of the root system by nematodes may lead to three different root reductions, such as coarse root, stubby root, and curly tip.

Coarse root: Nematode infection arrests the proliferation or growth of the lateral root system. It leads to having the main root alone without any lateral feeder roots. *Paratrichodorus* spp. causes these kinds of symptoms.