FINAL REPORT OF

MINOR RESEARCH PROJECT

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Title

SURVEY AND CONTROL OF PLANT NEMATODE PARASITES FOR SUSTAINABLE AGRICULTURE

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.PROJECT REPORT FOR MINOR RESEARCH PROJECT

i)	Name of the researcher -	**Dr. Hansraj Kashinath Jadhav** (Principal investigator) Head, Dept of Zoology A. S. C. College, Naldurg. Dist. Osmanabad.
ii)	Title of the research project -	**"Study and Control of Plant Nematodes Parasites for Sustainable Agriculture"**
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Certified that the progress of research work done by Dr. H. K. Jadhav is satisfactory.

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PROJECT – REPORT

"SURVEY AND CONTROL OF PLANT NEMOTODE PARASITES FOR SUSTAINABLE AGRICULTURE".

Nematodes practices of animals were mentioned in early Egyptian records of 4500 B.C. but the existence of plant parasitic nematodes was unknown until 70$^{th\ century}$. This was not so unusual, because these nematodes vary from one third millimeter to 3 or 4 mm long and are only diameter of human hair. It was not until almost 100 years after discovery of microscope the discovery of wheat gall nematode, Agnince London on December 22, 1743 He published in Philosophical Transaction the following year that when small, black wheat galls were placed in water, many apparently lifeless filers in galls begin to move and migrated inter waters. We now know that when such wheat galls are kept in dry condition he nematode larvae may remain viable for more than the nematode larvae may remain viable for more than 25 years from a single gall up to 90000 nematode have been counted.

Though worldwide reorganization of nematodes as important casual agents of plant disease did not occur until middle of this century, nematodes were studied both in Europe and British Isles more than 100 years earlier. The discovery in 1855 that root – knot nematode (melodogyne sp.) caused galls on cucumber root reorganization that sugar beet nematode (Heterodrcescaphlii) damaged sugar beet, During this period published 1st comprehensive paper on free living nematodes were milestone of progress.

In united states, during early 1900's limited attention was given to study of plant nematode. During 1945-1955 several significant discoveries accelraced development of nematology as a separate development of plant nematology as a separate discipline. These were introduction of practical nematicides (Radopholussimilis) was cause spreading dedine disease of citry in florida, major producing region of potato (U.S) golden nematode (Heteroderarostochiensis) discovered, reorganization of serious damage caused by nematode feeding at root surfaces, recomtion of many interaction between nematode and other soil inhabiting organisms in plant disease complex including breakdown of disease resistance; By nematodes transmission of virus discovered. Because of these of transmission of virus discovered, research in nematology received increased attention and financial support.

During the past two decade despite important control measures for plant disease caused by nematode, and knowledge in most areas of plant nematology is limited, are

relatively primitive. Basic research in most areas of nematology has gained impetus only recently. In this important area of biology progress is Limited primarily by relatively few full time research activities have been limited. In recent years research activities have been limited by no of job opportunities rather than by no of nematologist. Both well trained scientist and suitable pre for these scientist are needed for rapid advancement of knowledge. Information is needed in such important areas as morphology and taxonomy ecology, physiology host parasite relationship ethology culturing symptomatology, like cydes, virus transmission and plant mltilion.

By lack of information from basic research progress in development of better control measure is limited. For small growers in developing countries with little or no equipment and with essentially only technical knowledge from their ancestors and for large producers with great deals of sophisticated equipment is more efficient and economical control are needed. These control measures must minimized losses from nematode attacks without adversely affecting environment and injuring user or other.

In general, nematode problems are most sever in warm areas of the world in soils that have been cropped intensively for long periods of time. Food production is virtually important to feed rapidly increasing population there is developing countries, such area located where food production is important to feed. There is one of most promising ways of increasing product on of food and fiber is protection of root from attack of nematode and other organism.

Support of programs to achieve their goals by Government and universities in all countries of world is urgently needed. Throughout world to assume maximum use of information to free exchange of information among hematologist Maris ability to control nematode information on important food crops would be inmeasurable and in combating impending famines.

ORIGIN OF RESEARCH PROBLEM.

In the present investigation we plan the assessment of plant nematode diversity from the economically important plants of Marathwada region Maharashtra, India. This project work also introduce new comers to the field need to learn the fundamentals of procedures & how to use a microscope to identify parasites by using newly designed protocols. The descriptions of common parasites can be used as reference for under standing a diversity anociated with plant & soil to monitoring report or as a quick reference for the experienced plant pathologist & agriculturist.

INTERDISCIPLINARY RELEVANCE.

Parasitism reflects alifestyle unearly one or more individual (the parasite) lives in close obligate association derives benefit such as nutrition as the hosts expense, parasites belongs to many different Phylogenetically district taxa and as such, display on variety of life – cycles and body forms. Virtually every plants species has parasites. Their parasites contribute significantly to biodiversity simply interns of the number and variety of species in existence.

Plant parasites infects plants resulting the appearance of symptoms on roots as well as on the above ground parts of plants, past symptoms may appear hypertrophy, necrosis etc.

Interns of habitat, pathogenic nematode are either ectoparasites enter root tissue but feed only on cells. Neoatology constitutes only a plant & soil nematology nematode are only plant parasites belonging to animal kingdom, appears like worms.

Experience has employ shown thod the majority of Nematlogist known nothing what so ever about Nematodes our result of that they almost always accept to resolve the case in questions according to the regulation & method of their own species. Better quality products will hello changes in rural economy for better. This may give rise to differences of option and even to legal proceedings & enormous decision may evenly come a considerable economic loss.

INTRODUCTION:

Nematodes are known as thread worms because the word nematode is derived from the Greek work Nemata, meaning thread. They are also known as "eel worms" due to their resemblance to eel fish and as round worms because of their round transverse section.

A typical nematode may be defined as a microscopic, ventrally arcuate animal which is pseudocoelomate, triploblastic, matamericallyunsegmented, bilaterally symmetrical, protostomiate and which does not possess respiratory and circulatory systems and cilia. Further, they are soft-bodied, hererotrophic and dioeceous in nature. They exhibit a serpentine motion through the lateral side (creeping side)

Plant Nematology, the study of plant parasitic nematodes is a young discipline. Plant nematology runs into other disciplines outside, parasitology and zoology. It is an integral part of crop protection besides environmentology. The varied effects of nematodes on plants at physiological, cellular and molecular levels hae not been precisely identified, though severe epidemics caused by plant parasitic nematodes have shattered the world-economy in past. During 1950's, over 90% of 22 million plants of black pepper were destroyed by yellows disease incited by Radopholussimilis in Indonesia, massive citrus plantation destruction due to R. similis occurred in florida; the carnage to sugarbeet due to Heteroderaschachtii in northern Europe and western U.S.A. and the extensive losses to potatoes due to golden nematode, Globoderarostochiensis nematodes. While these eye-opening epidemics led to some advancements in plant nematology, the techniques to determine the mechanism of and assess indirect damage cuased by various plant and soil nematodes have not been developed as yet. Consequently, the silent damage caused by various plant and soil nematodes continue to go undetected and even misconceived.

IMPORTANCE OF PLANT PARASITIC NEMATODES IN AGRICULTURE

Harmful effects of nematodes on crop plants were clearly demonstrated some 50 years ago when soil nematicides first became available commercially.

Nematodes can cause economic losses in a variety of ways. The economic importance of plant parasitic nematodes is usually associated with (i) types of the nematode, (ii) distribution of the nematode, (iii) nature of parasitism, (iv) host range, (v) virulence of the nematodes and (vi) inoculum level. Some estimates of crop losses due to plant parasitic nematodes are tabulated. The important nematode diseases of crop plants / trees are:

Ear - cockle disease of wheat, Molya disease of barley, Ufra disease of rice, White tip diseases of rice, Rice root nematode disease, Root - knot disease of rice, Tulip root and disease. of segging oats and rye, Cereal root - knot disease, Grass root - gall nematode disease of barley and grasses, Maize cyst - nematode diseases, Root - knot nematode of vegetables, Reniform nematode disease of vegetables, Golden nematode disease of potatoes,

Tuber dry rot disease of potato, Root - lesion nematode disease of potato, Sugarbeet - sickness or weariness, hunger roots disease or sugarbeet cyst - nematode disease, Cobb's root - gall or false root - knot disease of sugarbeet, Ruben faule or rugose brown lesion disease of sugarbeets and fodder beets, Stunt nematode disease of cabbage and cauliflower, Red root disease of celery, Onion bloat - disease, Cajanus cyst nematode disease, Yellow - dwarf - disease of soybea n, Stem nematode disease of alfalfa and red clover, Chrysanthemum foliar nematode disease, Reniform nematode disease of cotton, Root - knot - disease of cotton, Brown root - rot disease to tobacco, Black streak disease of bulbous iris, Brown - ring disease of narcissus, hyacinth and sweet potato, Bunchy rosette or witches broom disease of phlox, Slow decline of citrus, Spreading decline of citrus, Red ring disease of coconut palm, Root - rot of coconut and arecanut, Decline of bananas, Red plant, Spring crimp of dwarf, and Cauliflower diseases of strawberry, Yellows disease of black pepper, Root - lesion nematode disease of tea, Root - lesion disease of coffee, Bursaphelenchus pine wilt disease and nematode diseases of mushrooms. nematode In India, Barber (1901) first recorded a nematode disease of tea plants from South India and subsequently a number of diseases have been reported / investigated from the country. The root - knot nematode is considered to be the biggest threat to crop plants because of its polyphagous nature and wide distribution. Meloidogyne incognita has caused significant losses to vegetables crops which are on increase as the vegetables are being grown in the infested field year after year. Other species viz. , M. javanica on vegetables, M. arenaria on groundnut, M. graminicola on rice and M. hapla on various crops grown in the hilly region of India also cause severe losses to the crop plants. The cyst - forming nematodes are of great economic importance in India. Various species of Heterodera and Globodera cause tremendous yield losses in various crops. The Golden Cyst Nematode of Potato, Globoderarostochiensis is a severe problem in the Nilgiri hills of Ooty in Tamil Nadu where devastates potato crop. Strict quarantine and control measures enforced by the Government and the Co-operative Societies have restricted this nematode to an area of 2800 ha. The seed gall nematode Anguinatritici causes extensive damage to wheat in developing countries including India. In the U.S.A. and developed countries, it has been eradicated and is now a matter of curosity. The nematode is also involved in yellow slime or Tundu disease of wheat along with Corynebacteriumtritici. Radopholussimilis or the burrowing nematode is a serious pest of banana in South India. It is also known to be the causal organism of slow wilt disease of pepper in Karnataka. Two races of this nematode are known. Of these, the citrus race is not reported in India, while the banana race is prevalent in parts of our country. The stunt

nematode, Tylenchorhynchus spp. is a destructive pest of maize, wheat, ornamentals and certain fruit crops. The Stem and Bulb Nematode, Ditylenchusdipsaci and the Potato Rot Nematode, D. destructor are economically important on bulb crops (onions, garlic, narcissus etc.) and potato, respectively The two major economically important pests on rice are: Aphelenchoidesbesseyi causing the white tip disease of rice and Ditylenchusangustus causing ufra disease of rice.

Certain Dorylaimid nematodes like spp. of Xiphinema, Longidorus, Paralongidorus and Trichodorus are potential threats to ornamentals and fruit crops. They also act as vectors for NEPO (Nematode Polyhedral) and TOBRA (Tobacco Rattle) viruses. Besides these, Ditylenchusmyceliophagus and Aphelenchoidescomposticola can cause qualitative and quantitative losses to mushrooms. Other economically significant nematodes are Hoplolaimus sp. ,Helicotylenchus sp. , and certain Criconematids. etc. The reniform nematode, Rotylenchulusreniformis is a polyphagous and widely distributed nematode. It feeds on pulses, oilseed crops, vegetables and certain fruit crops. In India, two races of this nematode are known to be present. Race - A infests castor, cowpea and cotton, whereas, Race - B is host specific to cowpea only. The lesion nematode, Pratylenchus spp. causes damage to crops like wheat, maize, ornamentals and fruit crops. The main damage to the plant is due to the necrotic areas or lesions on the roots which are formed due to benzaldehyde and HCN produced as a result of enzymatic activity initiated by the nematode. In, India, the sub-tropical climate characterized by longer summers than winters, provides a favorable temperature range of 25-35 C to the nematodes. Several economically important genera of plant parasitic nematodes are able to complete all stages of their life cycle and multiply at a faster rate. This is leading to the build - up of a large nemic fauna in the cultivated land of the country year after year. The agricultural education, research and extension in India should, therefore, consider plant nematology as an important component of crop protection.

MORPHOLOGY OF PLANT PARASITIC NEMATODES

SHAPE AND SIZE OF NEMATODES

The nematode body, in majority of cases, is cylindrical in shape which may attain different body postures when killed with gentle heat. e.g. spiral or coiled as in case of *Helicotylenchus,* slightly ventrally arcuate in case of *Tylenchus sp.* and *Pratylenchus sp.* , open 'C' shaped in case of *Tylenchorhynchus sp.* , or straight as in the case of *Hoplolaimus sp.* In addition to the above, sexual dimorphism in certain nematodes also plays an important role in giving characteristic shapes the females of these nematodes. e.g. *Meloidogyne sp.* female attains a pear-shaped structure, while lemon shaped females are seen in the case of *Heterodera sp.* , The *Globodera sp.* female is round in shape and kidney or bean - shaped females are found in case of *Rotylenchulus reniformis.* The *Anguina* female is swollen with increase in the body width, and the females of *Tylenchulus semipenetrans* become saccate with a long neck region.

Size:

The size of the plant parasitic nematodes ranges between 200-800 µm and seldom goes beyond the length of 1 mm. The smallest plant parasitic nematode reported till now is *Paratylenchus* (150-180 µm) whereas the largest plant parasitic nematode is *Paralongidorus maximus* (8-10 mm). In non-plant parasitic nematodes, *Graffiala* is the smallest nematode with a size of 100-150 µm and the largest nematode ever reported is *Placentonema gigantissima* measuring 8 m in length. It is a parasite of the sperm whale.

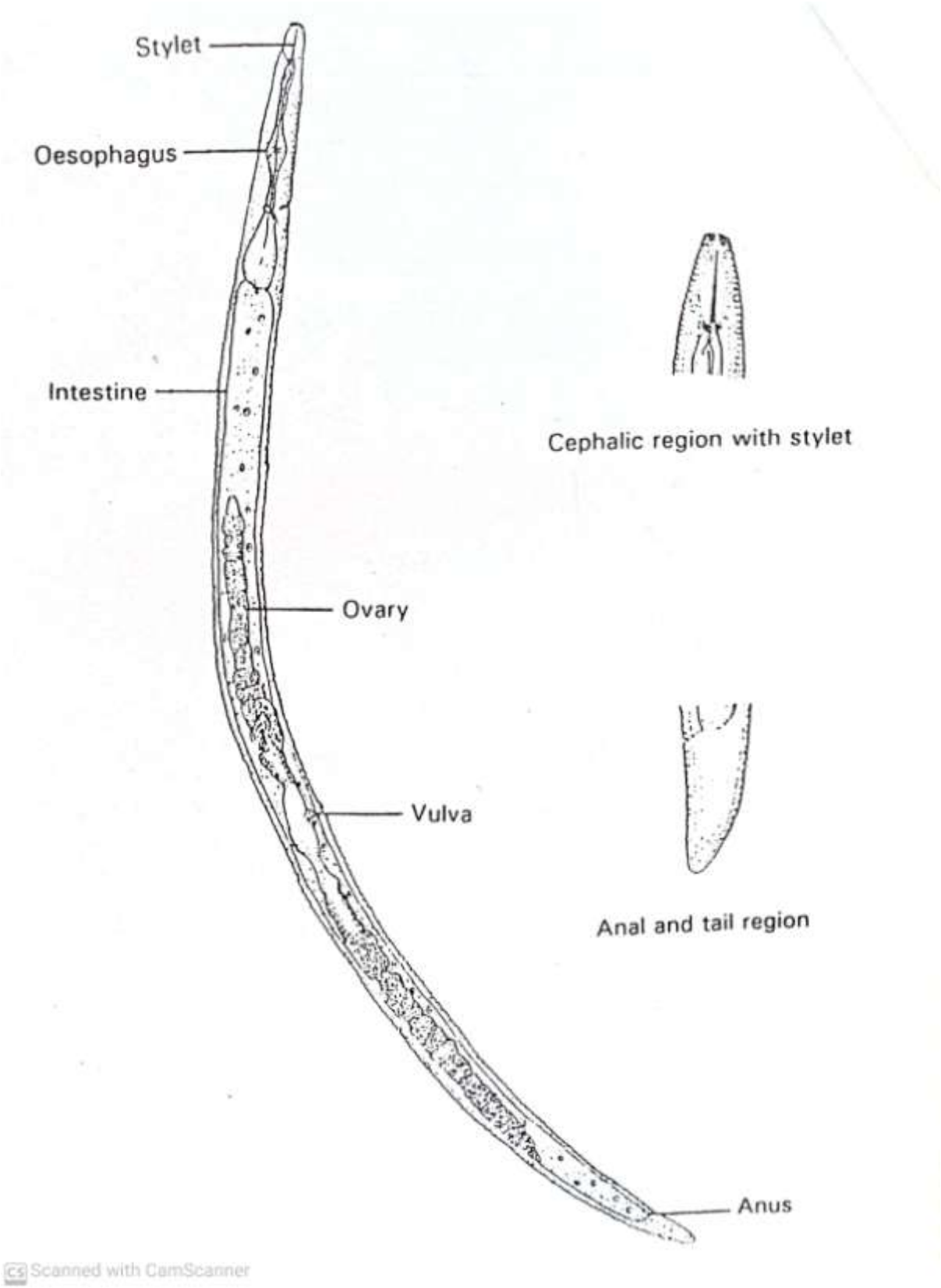

Fig.1. Structure of a Typical Nematode (*Tylenchorhynchus sp.*)

SURVEY AND SURVEILLANCE

The word survey holds its origin to the latin language meaning over all view. It can be defined as a general inspection to know the disease density, prevalence of nematode and collection of data for mapping. Surveillance is nearly synonymous to monitoring meaning close and usually constant observation of a pest. Objectives 1. To know the kinds of nematodes occurring in a particular locality, crop or season. 2. To find the association of nematode with symptoms in case of poorly growing crops. 3. To know the population dynamics of a particular nematode in relation to space and time. 4. To estimate the disease occurrence / crop - losses in case of pathogenic nematodes. 5. To know prevalence of various genera / species of plant parasitic or free living nematodes. Depending upon the purpose, surveys can be at macro or micro level.

(i) Macro - survey or Extensive survey: this type of survey is done for larger areas e.g. States, Districts, etc. It gives a general idea of the prevalence of a nematode. (ii) Micro - survey or Intensive survey: This type of survey is meant for a specific disease or nematode under consideration. Surveys can also be classified into the following three categories. (i) Farm survey- for this kind of survey, a field or farm is selected and the focus of the survey remains confined to the specific crop being grown in that farm. 53 (ii) Chance survey- this is carried out by Plant Protection and Quarantine Department at various sea ports and air ports to detect and prevent entry of any foreign pathogen. The survey is based on the factor of probability. (iii) Specially designed survey- it deals with a specific crop and the nematode which may parasitize it. e.g. , G. rostochiensis is a serious problem on potato in the Nilgiridistrict of Tamil Nadu. To prevent the spread of this serious disease to other areas, a specially designed survey methodology is followed. Such surveys require a great deal of finance and facilities for proper and constant vigilance. Field Sampling Methods The method depends upon presence / absence of susceptible crop, nature of crop (whether small annual plants, large annual plants, perennial plants, trees or vine crops), the kind of nematode expected and the type of injury it may cause. There are certain general principles applied to different sampling procedures. Except in ploughed fields where no crop is growing, samples are to be taken from the root zone to include as many feeder roots and as much surrounding soil as possible. In such spots, ecto- and endo-parasitic nematodes are most abundant. Before sampling, visual symptoms of the host crop should be taken into account. Spots where the sickly growing plants exist should be selected and the spots where healthy plants are growing should be considered as a check. No surface soil to be taken and sampling patterns be devised

to cover all the sections of the field in a systematic manner (from specific number of plants, at a given number of steps along a row or in a diagonal line across the field). Samples should not be taken from the boundaries of the field. The best containers for transportation and storage of samples are plastic bags with a capacity of 500 ml to 4 liters. The bags should be kept in an insulated box to prevent over - heating of the soil during transportation in hot weather. In general, a large number of small samples should be taken rather than a few large samples. If no susceptible crop, plants or weeds are growing, the field can be divided into sections i.e. in strips, grids or blocks and a pre-determined number of samples should be taken from each section. Usually, the soil is taken from the depth of 6-9 cm. The total weight of soil samples from about 1 ha should not be less than 10-20 kg. Where small annual plants are concerned, the best sample is the complete root system with adhering soil. In case of large plants, a part of the root system should be collected. Tree root samples should be taken from several sides of a tree at points where the feeder roots are most abundant. Roots are most important in sampling for endoparasitic nematodes and soil most important in sampling for ectoparasitic nematodes, but samples taken for either type of nematodes should include both roots and soil because certain stages of endoparasites are in the soil and ectoparasites might be partially embedded in roots . If the nematode injury is clearly visible and its identity appears to be correct, then depending on the objective of the collection of samples, they may be examined right in the field and discarded if required. Each polythene bag which may contain about 100-200 g of soil should be tightly closed with the help of a rubber band and labelled giving the following information:

1. Name of the locality, 2. Name of the crop, 3. Date of the collection, 4. Name of the collector, 5. Other relevant information like the previous crop grown in the field.

Extraction of Nematodes from Soil Samples

Cobb's Sifting and Decantation Method (Cobb, 1918)

This method is based on the principle of gravity. Heavier particles settle down more easily compared to the lighter ones. Nematodes being lighter in weight can be separated out from other matter using the principle of gravity. A set of sieves with specific mesh numbers is used for this purpose. The mesh numbers and the corresponding pore - size of these sieves are as follows.

A. B Fig. 5.3. Cobb's Sifting and Decantation Method (A) Soil in water is stirred thoroughly, (B) Suspension sifted through 16/20 mesh sieve, (C) Nematodes caught on 60-400 mesh sieves, (D) Nematodes poured into the beaker.

Mesh number 16 60 100 200 350 400

Pore size 1 mm 175 µ 140µ 65 µ 55 µ 30-45 µ 57

The 16 mesh number sieve is used to discard large stones or pebbles and the subsequent mesh numbers i.e. 60-400 to catch the nematodes on the basis of their body diameter and length. This method is combined with another method called Baermann Funnel Assembly Method described below.

Baermann Funnel Assembly Method (Baermann, 1917)

In this method, the final filtrate of the Cobb's sifting and decantation method is poured over a tissue paper, placed over a wire gauge fixed at the top of the funnel. Due to motality, the nematodes get caught into the water present in the belly of the funnel which can be drawn out after 24-36 h by opening the end of the tube attached to the funnel. The upper catch of either 100, 200, 350, or 400 mesh number can be placed on the tissue paper.

The smallest nematode which can be caught by 400 mesh no. sieve is Paratylenchus. The large nematodes like Paralongidorus, Xiphinema or Longidorus can be caught by 16 or 60 mesh number sieve.

Precautions:

Care should be taken to avoid splashing or other actions that may result in loss of nematodes from suspension. In general, this method works better with sandy soils. The suspension can be poured more successfully if the sieves are agitated slightly while pouring.

Baermann Funnel Technique modified by Christie and Parry (1951)

A hemi - spherical cloth bag made from fine muslin cloth having a ring of wire in the rim is used. The diameter of the ring is smaller than the diameter of the funnel. The funnel fitted with this bag can be used directly by keeping upper catch of 350 mesh number sieve or macerated plant tissues.

Staniland (1954) used small round sieves made from short length of copper tubing with 2½ - 3 cm diameter and having a piece of fine wire gauge at the bottom. The stem of the funnel is fitted with a sealed capillary tube. After 48 h the tube is broken and catch is drawn into a small quantity of water and the capillary tube is resealed for subsequent charge.

Fenwick Can Apparatus (Fenwick, 1940)

It is an apparatus for extraction of Heterodera cysts from soil. It comprises a slopy tank having false sloping bottom and a collar around its neck. The procedure involves the following steps.

1. Mix the soil thoroughly.
2. Fill the can with water and place the soil sample on the top sieve of 1 mm pore size.
3. Wash the sample into the apparatus via funnel. The coarse material is retained on the top sieve and the heavy soil particles sink to the bottom of the apparatus.
4. The floating cysts are carried off over the overflow of collar. Cysts, root-debris are collected on the sieve with 60 mm pore size, while the particles less than 60 mm pass through the sieve. The catch is poured into a wide bowl having sloping edge. The cysts float along the edge of the bowl and can be separated with camel hairbrush number 0 or 1.

The cysts are identified under the dissecting microscope using the top light. When required, the cysts are crushed either on glass slide or a cyst homoziniser is used.

Seinhorst Mistifier

The mistifier is used for extraction of endoparasitic nematodes from roots. It comprises large number of Baermann funnel assembly combined under a plastic cover. The top of the funnel supports a sieve having 3 legs and the base of the sieve just touches the brim of the funnel. The funnels are filled with water. The chopped roots of 1 cm length are kept at the top of the sieve. Water is sprayed upwards with a specially chosen nozzle giving a quantity of 100-500 ml / h at 1.5-2 atmospheric pressure of water supply. The nematode is caught in the belly of the funnel, which can be taken out by opening the funnel tube.

Staining of Plant Tissues for Location of Nematodes

Following stains can be used:

1. acid fuchsin and lactophenol (Lactic acid- 20 ml, Liquid phenol 20 ml, Glycerine- 40 ml and Distilled water- 20 ml) for roots
2. Cotton blue + lactophenol for leaves

Wash the roots to remove adhering soil, plunge the roots into warm (80 ° C) acid fuchsinlactophenol and leave them for about 1-2 minutes. Wash excess stain of roots by water and transfer roots to plain lactophenol to clear the plant tissue. Sometimes the material remains largely unstained while nematodes get stained.

FIXATIVES

These are chemicals or combinations of chemicals which fix the organs and their normal structural set up without disturbing the systems. They are of two kinds 1. Coagulum fixatives

2. Non - coagulum fixatives

Coagulum fixatives

The coagulum fixative produces a coagulum when mixed with an albumin solution. Absolute ethanol (C_2H5OH) has a low oxidation potential and acts as a denaturing coagulant of many proteins but it

does not coagulate nucleoproteins. The rate of penetration is moderate and it has excessive hardening and shrinking capacity.

Non - coagulum fixatives

The non - coagulative fixatives are formalin and acetic acid. Formaldehyde (HCHO) is a colorless gas and is highly soluble in water as HO (H_2CO) H to an extent of 40%. Formalin has a fast penetration capacity. It preserves proteins and glycogen by denaturing them. It fixes lipids but not fats. It has a shrinking action and strong hardening capacity. It can be washed out in water and in ethanol.

Acetic acid (CH, COOH) is a colorless liquid with a pungent smell and is miscible in water and ethanol. It precipitates nucleoproteins and by its well known swelling capacity, it is used in compound fixative with formalin which has a shrinking action. It can be washed in water and ethanol. Some of the common fixatives and their ingredients are as follows

F.A.A. (Formalin, Acetic Acid, Alcohol) Ethanol 96% Formalin Glacial Acetic Acid Distilled water F.A. 4:10 (Formalin, Acetic Acid) Formalin Glacial Acetic Acid Distilled water F.A. 4: 1 (Formalin, Acetic Acid) Formalin Glacial Acetic Acid Distilled water 100 ml 30 ml 5 ml 200 ml 10 ml 10 ml. 80 ml. 4 ml 1 ml. 95 ml.

T.A.F. (Triethanol amine, Formalin) Triethanol amine Formalin Distilled water Carnoy Fixative Glacial Acetic Acid Absolute ethanol Chloroform 2 ml. 7 ml. 91 ml. 1 part 6 parts 3 parts. After fixation in T.A.F. the nematodes remain remarkably lifelike. The solution remains stable over a long period. The constituents of Triethanol amine being hygroscopic, prevent specimens from drying up.

In F.A.A. , the ethanol shrinks the nematodes a little bit, which is sometimes useful for the study of structures such as incisures or lateral lines and annulations.

In F.A. 4:10 or F.A. 4: 1 one of the important drawback is that if Tylenchids are kept for a long period their stylet gets transparent and the cuticle of Trichodorus becomes swollen.

Formalin alone which is commonly used causes the nematode to appear granular. Baker (1945) suggested the addition of $CaCO_3$ to remove granulation as it neutralizes the free forming acids.

Hirschmann (1962) formulated carnoy fixative for the study of reproductive system.

KILLING OF NEMATODES

Heat and chemicals are commonly used for killing the nematodes. Killing should be done in a manner which stops the activity of protoplasm without distortion. For killing small samples, the nematodes are transferred onto a glass slide in a drop of water and slide is moved over the flame of a spirit lamp. The temperature of the slide should be such that it is just tolerable to the skin of the back of the hand. One can also use 0.5% acetic acid heated to 100 ° C in a closed tube. To kill and fix simultaneously, a drop of heated F.A 4:10 or 4: 1 can be placed on the nematode accommodated on the slide (Seinhorst, 1962).

Barbosa (1948) used ice water for relaxation and killing. Malek (1951) applied drop of a solution containing 24 g menthol dissolved in 10 ml. of 95% alcohol and 100 ml distilled water while Fanwick and Franklin (1942) used high concentration of chloroform vapor for 1½ hour. For larger samples, the beakers of samples should be kept for 5 minutes into an oven tuned to 60 ° C (Juckermann, 1960). Besides this, 5% formalin alone can kill and fix nematodes.

SLIDE PREPARATION

Preparation of semi permanent mounts (Franklin and Goodey, 1949)

Killed and fixed nematodes are transferred into a fixative disc, covered with lid and left overnight. The nematodes are then transferred into a small drop of lactophenol, the solution is evaporated by providing gentle heat, and then a drop of glycerol is added to it. The nematodes are transferred to another glass slide and mounted in glycerol and sealed with glyceal.

Permanent mount by slow glycerol method (Seinhorst, 1959):

The killed and fixed nematodes are transferred into a cavity block containing Seinhorst solution I (96% ethanol - 20 parts, Glycerol - 1 part, distilled water - 79 parts). This cavity block is kept into an airtight desiccator having alcohol for atleast 24 hours at room temperature. The nematodes are later transferred into Seinhorst solution II (96% ethanol- 95 parts, Glycerol- 5 parts) in another cavity block and kept in another desiccator containing calcium chloride at least for 24 hours. The nematodes are then mounted in anhydrous glycerol.

deMan's formula (deMan, 1880):

For taxonomic studies, the measurement of the nematodes are taken with the help of camera lucida and the body dimensions are tabulated by using deMan's formula. For tabulation of different body dimensions, following values are used:

n = Number of specimens

L = Total body length in mm.

a = Body length ÷ Greatest body width

b = Body length ÷Distance from anterior end to the junction of oesophagus and intestine

C = Body length ÷Tail length (anus to tail terminus)

c '= Body length / body width at the anal region

V = Distance of vulva from anterior end + Body length x 100

S.L. = Stylet length

Besides taking body dimensions, emphasis is also given on other morphological features like head - shape and sclerotisation, type of stylet and stylet knobs, type of oesophagus number and type of ovaries, position of amphids and phasmids, number of incisures in lateral field, tail length and shape etc.

On the basis of body dimensions and other morphological characters, the population of nematodes encountered is critically compared with already known species and accordingly the announcement of new or already known species is made.

Nematode Control in Crop Plants

Plant parasitic nematodes can be controlled by a variety of methods . These methods may not necessarily aim at complete killing of nemic fauna in the field - soil but are considered satisfactory if they ensure that nematode population remains below a certain level so that they are not economically detrimental . These methods employ physical , cultural , chemical , biological and regulatory approaches including use of resistant varieties and disease - free seed . PHYSICAL CONTROL Following are the methods of physical control of plant parasitic nematodes 1. Solar heat 2 .Steam 3.Hot water treatment 4.Irradiation 5.Osmotic pressure 6. Electricity

Solar Heat

Ploughing of field during hot summer months of May - June at 15 days interval exposes nematodes to the intense heat of the Sun due to turning of soil. In nursery beds , mulching of soil followed by light irrigation and covering of beds by polythene sheets leads to production of latent heat with high temperature which kills the nematodes . It also kills bacteria , fungi and weeds and some non target organisms .

Steam

It is generally injected into the soil through holes in the walls of steel pipes. There are two main types- horizontal perforated pipes which must be buried in trenches, or vertical spiked pipes, with pairs of outlets just above their pointed lower ends , connected by a horizontal supply pipe which lies on the soil surface . Partial sterilization of soil by steam is a long - established practice in glasshouses and for the preparation of seed and potting composts. Its cost limits its use to small seed or propagating beds .Insects , earthworms , nematodes , weed seeds , and many bacteria (including nitrifying bacteria) are killed by steam sterilization . Many of the ammonifyingbacteria , which form spores can survive at 100 ° C for several hours . These bacteria multiply rapidly after steaming , leading to liberation of quantities of ammonia that would otherwise be converted by nitrifying bacteria into nitrates (Anon . , 1959) . Injury to plants from excess ammonia can be avoided by balancing it with base fertilizers and , if necessary , allowing time for dispersal of free ammonia . Sometimes many mineral nutrients become more available as a result of steaming , which may be a drawback , e.g. , a small increase of micro - nutrients such as manganese may lead to phytotoxicity .

Hot Water Treatment

Many nematodes are less tolerant of heat than their host plants , especially when the plants are at a dormant stage , as with bulbs , tubers , perennial root - stocks , etc. Hot water proves a suitable medium for heat treatment , as it is cheap and readily available and partly because dry heat damages plants by causing water - loss . The first published record of hot - water treatment of living plant material for nematode control appears to be that of Marcinowski (1909) who treated ferns and begonias for 5 min at 50 ° C to kill Aphelenchoidesfragariae (then known as Aphelenchusormerodis) . In 1914 , Cobb reported that all stages of citrus nematode (Tylenchulussemipenetrans) were killed quickly in water at 54 or 60 ° C and that citrus roots survived temperatures sufficient to kill the nematode . He suggested hot - water treatment as a possible control method for citrus planting stock .

Irradiation

Different radiation treatments have been tried on various crops for the purpose of nematode control . X - ray exposures of embryonated eggs in females at 20,000 r (r = roentgen) , and y- rays from Cobalt source (Co) have been found effective . However , soil treatment to sufficient depths at such doses is impracticable and uneconomical . The radiation of plants is also undesirable because their roots are much more sensitive to radiation than the nematodes .

Osmotic Pressure

Addition of salts , charcoal and other compounds which create an osmotic stress on the nematodes has been tried for nematode control . However , this may increase salinity and alter soil mineral levels which may affect the crop plants adversely .

Electricity

Attempts have been made to control nematode pests by electric currents , usually applied to soil . The very high energy applications needed to render useful effect in soil treatments make this method unapplicable .

CULTURAL CONTROL

Cultural methods are attempts to adapt husbandary practices so as to minimise the effects of nematodes. Many of these practices are being used by farmers since times immemorial. Important methods of cultural control are briefly described below.

Manuring

Application of green manure in the fields ensures better plant establishment, enrichment of soil and development of nematophagous micro - organisms. These factors are beneficial in controlling the plant parasitic nematodes.

Fallowing

Leaving the field without cultivation, preferrably after ploughing or harrowing , exposes nematodes who starve and die . The method is uneconomical. Further wind in the empty field may cause soil erosion and weeds may grow in the field which may act as an alternate host for the nematode.

Flooding

The empty field is filled with water to kill the nematodes by asphyxiation (absence of oxygen for respiration) for a long period. The denaturation of organic matter in the soil produces toxic gases like H_2S, ammonia etc. which are also lethal to the nematodes. Flooding is not possible in arid zone areas and the method is uneconomical even in irrigated areas .Further, soil structure and chemical composition in the flooded field may change leading to the leaching of the nutrients. In certain cases, the longevity of nematodes may increase. Furthermore, this method is not effective against nematodes which parasitize rice crop.

Deep Summer Ploughing

The field is deep ploughed in summers and left as such for the high temperature and solar radiations to cause co - agulation of proteins and desiccation of exposed nematodes . In

nursery beds, light soil turning followed by light irrigation and subsequent covering by polythene sheets increases the temperature which kills nematodes .

Mixed Cropping

This method includes the cultivation of more than one crop in a field at a time. The losses on one crop can be supplemented by the production of another crop to a certain extent. While following this method, care should be taken in selection of crops keeping in mind the nature of nematode prevalent in the field. This method is suitable for field infested with a non - polyphagous nematode population which is moderate.

Time of Planting

Early or late sowing or planting discourages nematodes with their strict climatically regulated life cycles. They are not able to cause significant damage because the early sown crop matures enough so as not to allow or tolerate the damage caused by nematodes and on the other hand the nematodes die due to lack of food if the crop is sown late.

Trap Cropping

Two crops are grown in the field, out of which one is highly susceptible to the nematode present in the field. As a result, the nematodes attack this crop profusely. With careful timing, this crop is uprooted and the plants are burnt outside the field area. Thus the main crop faces little danger due to the nematodes.

Antagonistic Crops

Certain crops like mustard (Morgan , 1925 ; Triffitt , 1930) , marigold (Oostenbrink , 1958) , neem , etc. have chemicals or alkaloids as root exudates which repell or suppress the plant parasitic nematodes . e.g. , in case of marigold , a - terthinyl present in the plants is a strong repellent against many nematodes . Similarly mustard has Allylisothiocyanate which is also a strong repellent against many plant parasitic nematodes.

Sanitation and Removal of Diseased Plants

Removal of weeds , uprooting of diseased plants / plant - parts and their destruction is a significant step in hampering the perpetuation of disease in the next crop season . Early detection of infested crop plants and their removal from the fields as early as possible may check further spread of the nematode . Proper sanitation practices including removal of dead plant matter and debris of previous crop is also effective . It involves low cost , has minimum effect on non target microorganisms , and is non - toxic . However , in practice , it is very difficult to remove all infected plants .

Crop Rotation

Growing host crops at such intervals as will allow the increased nematode population to return to a safe level each time can reduce the crop losses . The success of this method would depend upon the knowledge of host range of the nematode , efficiency and susceptibility of the various hosts , population dynamics , and the relation between population and yield loss .

Use of Resistant Varieties and Certified Seeds

It is beneficial as the control on the neamtodes can be achieved through the cost of the seed and no additional and cumbersome processes are to be followed. Many resistant varieties have been reported from time to time by our plant breeders for different crops against different nematodes e.g. Raj Kiran (barley), Karnataka hybrid, SL-120, VNF- 8 (tomato) etc.

CHEMICAL CONTROL

Plant parasitic nematodes may be controlled by applying nematicidal chemicals to the soil or to the potential host plant. Protecting the host plant is the more attractive method because it requires much less active ingredient / unit area and avoids the need to treat the great bulk of soil in which the crop will be grown or is already growing.

Classification of Pesticides: Pesticides can be classified on the basis of their mode of entry, the mode of action by which they kill the target organism, and their chemical nature .

Classification on the Basis of Mode of Entry

Stomach poisons: These pesticides are taken orally by the target organism and as such these are to be made tasty and palatable to the target by mixing them with some food material. The common examples are lead arsanate, B.H.C. and Phosphomidon.

Contact poison: They enter into the body by contact and are directly absorbed by body wall. They get into the systems of the organism and cause death e.g. Methyl parathion, B.H.C.

Fumigants: These chemicals when applied get transformed into fumes which can be inhaled through respiration by the target organism e.g. CS_2, DD, EDB, etc.

Classification on the Basis of the Mode of Action

Physical poisons: They are usually heavy oils like tar oil which lead to asphyxiation and arrestment of respiration. Chemicals like aluminium oxide abrade the cuticle and promote loss of body moisture to kill nematodes.

Protoplasmic poisons: They destroy cellular protoplasm e.g. lead arsanate, copper acetoarsanate, etc.

Respiratory poisons: They lead to the blockage of cellular respiration or inactivation of cellular respiratory enzymes e.g. H.S. DD, EDB, etc.

Nerve poisons: These chemicals have anti acetylcholinesterase activity which leads to constant excitation of the nerves in the target organism. As a result, the organism faces convulsions, tremors, muscle paralysis and finally death e.g. Diazinon, Aldicarb, etc.

Classification on the Basis of Chemical Nature

The chemicals can be classified into following categories

Systemic inorganic compounds: These are systemic inorganic salts which act as stomach poisons and kill the target organism e.g. copper acetoarsanate, calcium arsanate, etc.

Synthetic organic compounds:

These compounds can be further divided into

1. Halogenated hydrocarbons e.g. Chloropicrin, DD, DBCP etc.

2. Organo phosphorus Compounds: Organic Phosphorus is the basic constituent in these compounds e.g. Dematon, Diazinon, etc.

3. Carbamates: Carbamate is the basic group in these compounds e.g. Carbaryl , Carbofuran , Aldicarb, etc.

4. Substituted phenols: In these compounds the phenol is substituted by any other group e.g. Binapacryl.

5. Thiocyanates: e.g. Lethone , Thanite , etc.

6. Flourinecompounds: e.g. Flourine sodium floroacetate

7. Sulphurcompounds: these include CS_2 , H_2S , Endosulphan , etc.

8. Synthetic pyrethroids: e.g. Cypermathrin , Decamethrin , etc.

Natural products

They include compounds like Nicotine, Pyrethrin, Urea, Oil Cakes, Sawdust, etc.

BIOLOGICAL CONTROL

Biological control is the exploitation of a living organism for reducing the pest population. It aims at increasing the number of parasites, predators and pathogens of nematodes in the soil. Biological control should receive more attention due to various reasons like high cost of nematicides and their toxicity to human beings and animals, time - consuming process of development of resistant variety and temporary nature of host resistance, negative aspects of cultural control , non - interference of biological agent with the environment , and a long term benefit. The success of biological control depends upon the

effectiveness of the biocontrolagent. A successful biological agent should have the following attributes.

1. **Ecological compatibility:** The agent should thrive well in the same environment as that of the target.

2. **Temporal compatibility:** The time required for maturation by the agent should synchronise with that of the target organism.

3. **Density:** The available agent population and the multiplication rate of the agent should be high.

4. **Reproductive potential:** The agent should have high reproductive potential to ensure high multiplication rate.

5. **Searching capacity:** The agent should search out the target quickly . 6. Dispersal capacity: The agent should spread in the soil very quickly.

7. **Host specificity:** The agent should be specific to the nematode which is to be controlled.

8. **Food requirement and habit:** The food requirement and habit of the agent should be such that it thrives well in the environment same as that of the nematode.

9. **Hyperparasitism:** The agent should not have hyperparasites of its own.

10. **Cultural ability:** The agent should be self culturing in field soil.

Predatory Nematodes

Predatory nematodes belonging to the order Mononchida are known to act as successful biological agents against plant parasitic nematodes. Thorne (1927) reported the feeding of H. schachtii larvae and males by Mononchuspapillatus. Other genera like Mononchoides ,Butlerius, Anatonchus and Diplogester are also known to act as biological agents against nematodes. Among the stylet bearing nematodes, Seinurafenuicaudata, certain Aphelenchoidessp, Discolaimus ,Actinolaimus and certain species of Dorylaimid nematodes are known to feed upon certain plant parasitic nematodes .

Nematophagous Fungi

Nematophagous fungi act as biological agents against plant parasitic nematodes . The presence of lectin on the fungal surface binds itself with carbohydrates on the nematode surface to facilitate penetration of cuticle by fungal hyphae . Certain endozoic fungi like Harposporiumanguillulae have sticky pointed spores which attach to the female body of the nematode , germinate by forming a germ tube and spread throughout the female body . Nematophthoragynophila is a potential biological agent against Heteroderasp .

Certain other nematophagous fungal genera are- Dectylaria ,Dectylella , Arthrobotrys , Verticillium , Ropalomyces , etc.

The beneficial effects of VasicularArbuscularMycorrhizae(VAM) fungi on plants can be through their adverse influence on plant parasitic nematodes . Certain VAM like Glomussp . , Gigosporasp . , Acaulosporasp .andSclerocystissp . are known to cause better plant establishment by increased phosphate uptake which improves resistance in plants . The increased soil particle aggregation leads to compactness of the soil which hampers nematode movement .Further, increased decomposition of soil organic matter produces toxic compounds and gases which are lethal to the plant parasitic nematodes.

Bacteria:

Adams and Eichenmuller (1963) first reported bacterial infection of Xiphinema americanum by Pseudomonas denitrificans. Bacteria parasitise the nematodes by formation of spores which adhere to the nematode body, enter into it and cause disruption of metabolic activities. Bacillus thuringensis , and Pasteuria . penetrans are the known successful biocontrol agents of plant parasitic nematodes .

Viruses

Loewenberg, Sullivan and Schuster (1959) reported a viral disease of Meloidogyne incognita in which the nematode larvae were found to be sluggish and no galls were formed on the plant roots. Not many cases of virus diseases of nematodes have been reported.

Predacious and Parasitic Animals

A number of tiny animals like amoebae, collembola, tardigrades, turbellarians, enchytraeids, mites etc. have been found feeding on nematodes. Thorne (1940) reported parasitism of a protozoan, Dubosquia penetrans on Pratylenchus brachyurus.

LEGAL (REGULATORY) CONTROL

Regulatory control of plant pests and diseases is the legal enforcement of measures planned to prevent them from spreading or having spread, from multiplying sufficiently to become intolerably troublesome. A nematode species can be localized in a particular area only. Such species are called as restricted species. When a nematode species is not allowed to enter a particular area, it is known as a prohibited species. On the other hand, some nematode species are omnipresent and they are termed as unrestricted species

Nematodes spread mainly through Air, Soil, Seed and Water.

Air: *Heterodera* cysts have been found to spread with wind currents. The problem is more severe in sandy soil areas where dust storms facilitate this type of spread.

Soil: Kradal (1959) reported the spread of 21,700 cysts by soil adhering on one quintal of potato seeds. Besides, spread from one area which is infested to a non - infested area can also take place by means of labour shoes, farm implements etc.

Seeds: *A. tritici* causing ear cockle disease of wheat is known to spread along with wheat seeds. Onion and leucerne bulbs spread the stem and bulb nematode D. dipsaci from one place to another.

Water: The field to field movement of irrigation water and rain water acts as a major mode of spread for nematodes. The knowledge of spread of nematodes can be utilized in devising measures to minimise / stop the spread of the nematode within a locality and from one locality to another. Plant Quarantine The Food and Agriculture Organization (FAO) of the United Nations has contributed significantly towards crop protection. The international plant protection organization, under the auspices of FAO, keeps watch on plant diseases occurring in any part of the world. With the help of its six regional branches, it provides information and suggests control measures through its official plant protection bulletin.

The six regional branches are

1. The European Plant Protection Organization (EPPO)
2. The Inter - African Phytosanitary Commission
3. The Plant Protection Committee for the South East Asia and Pacific region
4. Organisma International Regional de Sanidad Agroperciana
5. Convenio Interamericano de Protection Agricola
6. Near East Plant Protection Commission.

In addition, every country has its own domestic quarantine and it also assists the international programme. Modus Operandi of quarantines for healthy plant materials is through inspection and certification at the source of export and at the receiving end. Plant materials are detained to test for latent infections. For large consignments the diseases are checked by sampling method.

Any restriction to trade, which of course quarantine procedures cause, should be done when it is a must; and for that it is necessary to confirm that-

1. The pest will be introduced if not regulated
2. If introduced, the pest will be harmful.
3. That there are no natural methods of introduction of the pathogen (like wind dissemination).

For a pathogen which cannot be carried through plant materials or will not be harmful in the climatic conditions of the receiving country or can come through wind, there is no justification for restricting trade by quarantine regulations. For scientific purposes, special permits are issued to import cultures of pathogens or diseased materials . Plant quarantines are not very effective because they, usually, are not established soon enough and also because of biological or physical limitations in implementation.

Plant Quarantine Organization in India

In 1914, the Destructive Insects and Pests Act (DIPA) were passed by the Government of India, which forbids introduction of exotic pests and diseases into the country from abroad. The Agricultural Pests and Disease Acts of the various states prevent interstate spread of pests within the country.

The plant quarantine stations work at the major sea and airports, which check the entry of forbidden diseased materials. The plant protection adviser issues certificates and import permits.

Integrated Nematode Management

Since no individual method of nematode control is free from limitations, combination of two or more methods in a complementary manner should be preferred depending upon the feasibility. The effective components of integrated management system should be identified and then decision should be taken about components to be applied and the time of their application.

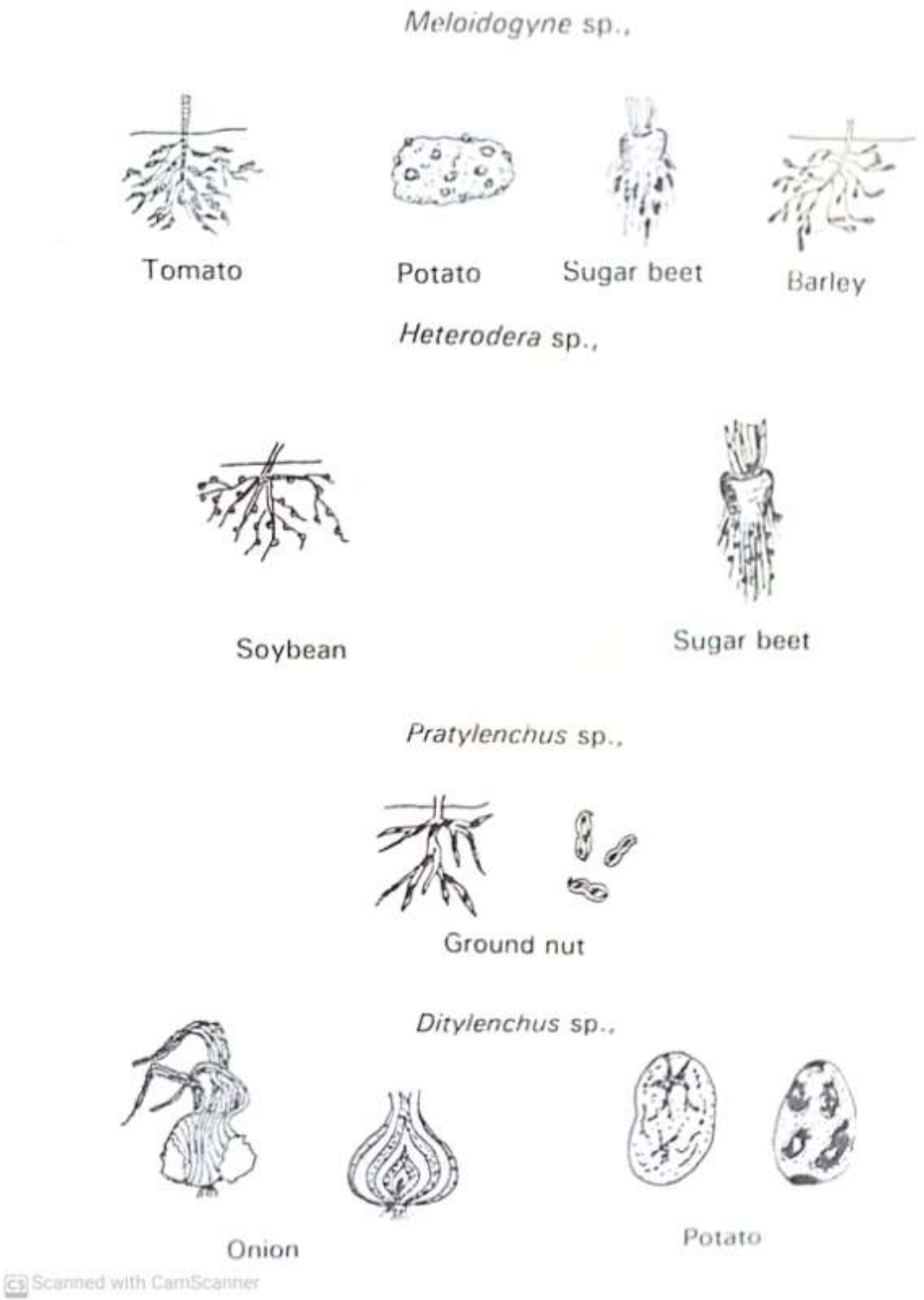

Fig. 2. Types of Symptoms caused by important plant parasitic nematodes.

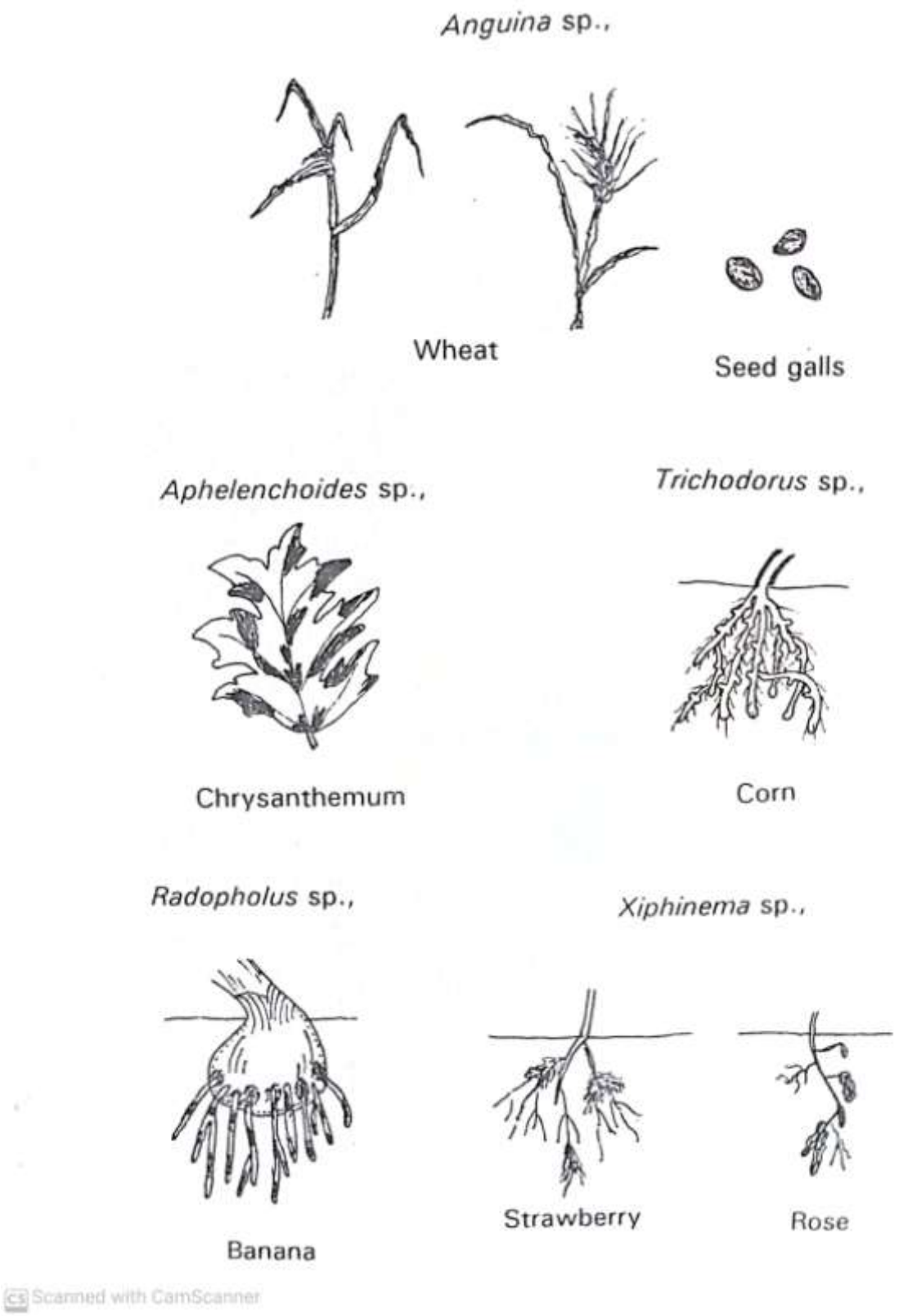

Fig. 3. Types of Symptoms caused by important plant parasitic nematodes.

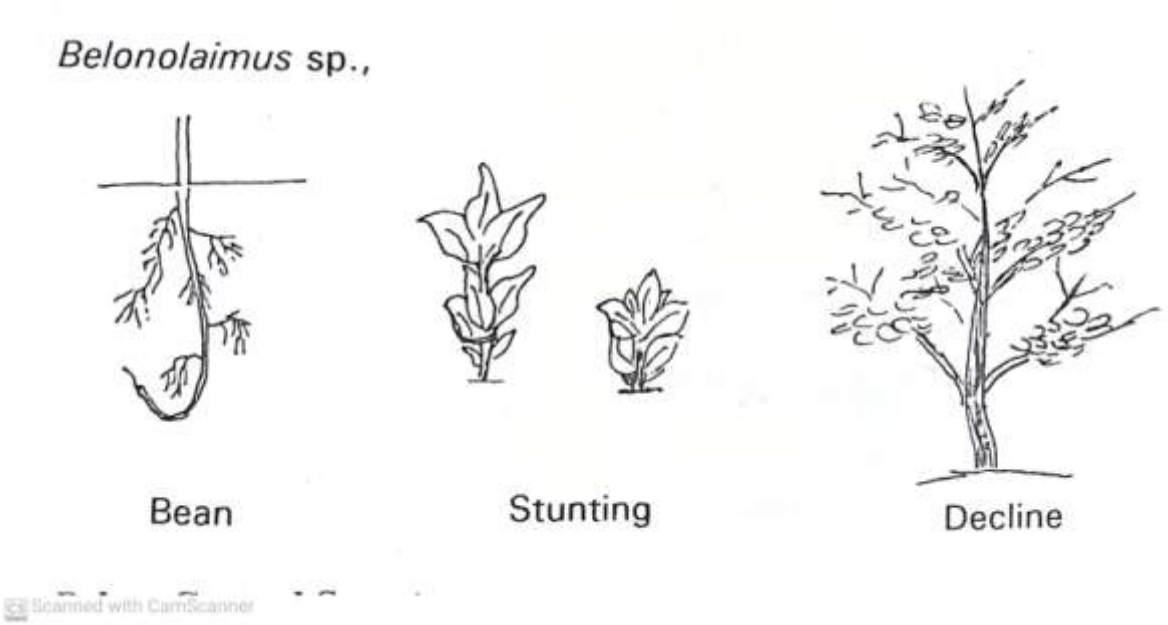

Fig. 4. Types of Symptoms caused by important plant parasitic nematodes.

1. ROOT - KNOT DISEASE

History and Importance

The root–knot nematode was first discovered by Berkley in 1855 . The nematode was found on cucumber in glasshouses of U.K. In India , it was first reported by Barber (1901) from tea plants in South India . Root - knot nematode is today the greatest threat to certain crops in tropics and sub - tropics because of its wide distribution , polyphagous nature and ability to complete several generations in a crop season .

Geographical Distribution

Root - knot nematode is widely distributed throughout the world . They are most prevalent in tropical and sub - tropical regions of the world , where summers are longer than winters . It is a cosmopolitan pest .

Symptoms

Specific symptoms include knots on roots which look like beaded roots . By observing the root one cannot see the adult female since it is found completely inside the roots . The egg mass can be seen within the knot which contains 200-500 eggs . The above ground general symptoms comprise chlorosis , wilting , stunting and smalling of leaf and fruit .

Causal Organism

Root - knot Nematode (*Meloidogyne spp* .)

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Sub - order	Tylenchina
Superfamily	Heteroderoidea
Family	Meloidogynidae
Sub - family	Meloidogyninae
Genus	*Meloidogyne*

Life Cycle

It is a sedentary endoparasitic nematode . The second stage larvae is the infective stage which infects the roots behind the root cap . It enters into the root , moves both inter- as well as intra- cellularly and feeds continuously till it reaches the vascular tissues . After second moult , it gets converted into third stage larvae which is a spike tailed stage . The sex differentiation starts from this stage if the conditions are favourable . The larvae get converted into fourth stage female and subsequently adult female which becomes pyriform . The neck region of the adult female remains embedded within the vascular tissues whereas the posterior body is found lying in the cortical zone . After attaining maturity , the female starts laying eggs into the gelatinous matrix secreted by the six rectal glands . The gelatinous matrix protects and nourishes the eggs in adverse environmental conditions . Each egg has one first stage larva which undergoes first moult within the egg . It then comes out from the egg as the second stage larva . This is the infective stage which can infect the root .

Several generations are completed in one crop season . No hatching factor is required . The duration of life cycle is dependent upon the prevailing temperature . At lower temperature during winters , it takes about 30-35 days but at 25-35 ° C it takes about 20-25 days to complete the life cycle . In adverse environmental conditions and with the non - availability of sufficient food and the feeding space , the production of male is more than that of females . Such males have two testes instead of one .

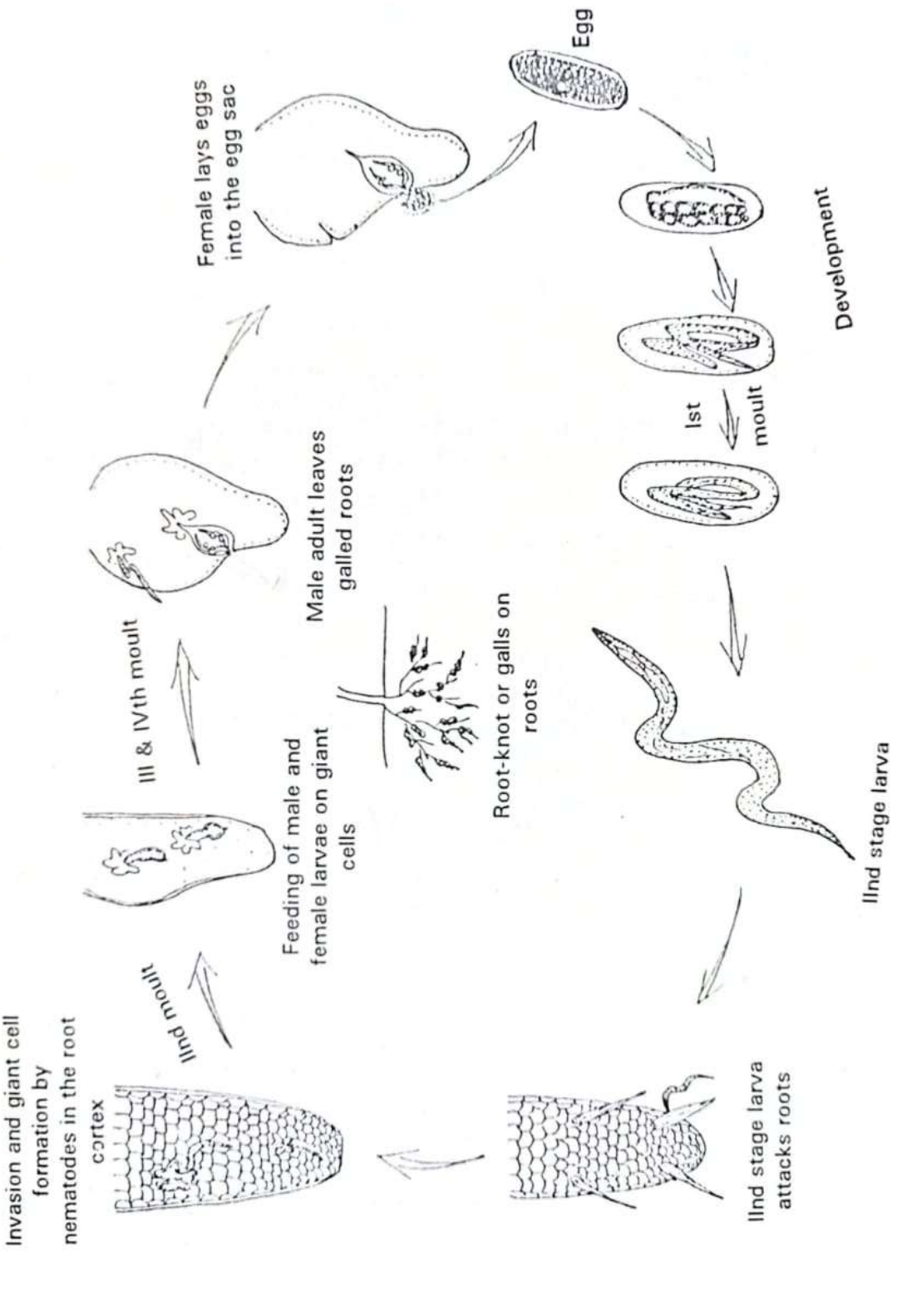

Fig. 5. Disease Cycle of Root-Knot nematode *Meloidogyne sp.*

Host Range

Root - knot nematode is polyphagous . It can infect more than 3,000 host plants belonging to all the plant families .

Host - Parasite Relationship

During feeding in the vascular tissues , the nematode enzyme acts upon tryptophan which is converted to I.A.A. (Indol Acetic Acid) . This hormone is responsible for the hypertrophy (cell enlargement) and hyperplasia (multiplication of cell) . These processes take place simultaneously resulting into formation of giant cell or syncytia . The syncytia are multi - nucleated , thick walled structures having dense protoplasm . They provide nourishment to the females . With the increase in size of nematode body and due to formation of syncytia , the surrounding tissues enlarge . This leads to the formation of knot on the roots .

Interactions with Other Organisms

The root - knot nematode may interact with other soil micro organisms like fungi and bacteria causing the complex diseases . The first report of a nematode- fungus complex was by Atkinson (1892) in Fusarium wilt of cotton . Meloidogyne incognita interacts with Phytophthora parasitica nicotianae causing black shank of tobacco ; Meloidogyne incognita interacts with Fusarium oxysporum causing cotton wilt ; and Meloidogyne incognita combines with Pseudomonas solanacearum causing tobacco wilt . These nematodes also have relationship with concomitant nematodes , which may be neutral , stimulatory or antagonistic.

Control

1. Deep summer ploughing : two to three deep summer ploughings in May and June at the interval of 15 days have been found effective in minimising the nematode population . Polythene mulching is effective .

2. Chemical control : use of Carbofuran 3G @ 1-2 kg a.i./ha is effective against the nematode .

3. Antagonistic crops : cultivation of antagonistic crops like mustard , is effective . Further , it also helps in reduction of the population of this nematode in the soil . Varma et al . (1978) found that growing of Tagetes patula or Sesamu orintale with egg plant increased yield of egg plant and reduced root - knot index as well as soil population of M. incognita , M. javanica and M. incognita var . acrita .

4 . Hot water treatment of infested plant material at 50 ° C for 10 min . is effective .

5 . Cultural practices like crop rotation , fallowing and use of organic manures are helpful . Amendement of soil with non edible oilseed cake of neem , karanj , mahua , etc. is useful .

Table No. 1.

Sr. No.	**Crop**	*Meloidogyne sp.*
1	Tomato	*M. incognita*
2	Maize	*M. incognita*
3	Soybean	*M. incognita*
4	Cotton	*M. incognita*

2. MOLYA DISEASE OF WHEAT AND BARLEY

History and Importance

It was first observed by Kühn (1874) in cereals in Germany . Prasad et al . (1959) reported the disease for the first time in India from Sikar district of Rajasthan . The nematode may cause 6-95 % loss in wheat in India . Geographical Distribution It has been reported in Germany , Sweden , Denmark , Netherlands , Japan , Norway , Australia , Canada , Israel , Italy , Poland , France , Greece , Yogoslavia , U.S.A. , Pakistan , Morocco , S. Africa , N. Africa and India . In India , the nematode is reported from Rajasthan , Maharashtra, Haryana , Punjab , Himachal Pradesh and Jammu & Kashmir .

Symptoms

Affected fields give a patchy appearance . After 2-3 years the disease may cover the whole field . The plants are characterised by stunted growth , general chlorosis , and stiffer , thinner and narrower leaf - blades . Tillering is reduced with culms getting thinner and weaker . Diseased plants flower prematurely and the ears , if formed , have very few grains . Infected roots are short , giving a bunchy appearance , and often bearing small gall - like formations . Fibrous root system is almost negligible .

Causal Organism

Cereal Cyst Nematode (*Heterodera avenae*)

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Sub - order	Tylenchina
Superfamily	Heteroderoidea
Family	Heteroderidae
Sub - family	Heteroderinae
Genus.	*Heterodera*

Life Cycle

The brown lemon - shaped cysts of H. avenae can be recovered from infested fields of wheat and barley . Each cyst may contain an average of 200 eggs and larvae . These eggs and larvae remain dormant until next host crop season . With the advent of cold temperature (about 20 ° C) , the infective second stage larvae start emerging from cyst . A hatching factor is not required for emergence . Penetration into roots takes place soon after emergence and larvae become sedentary . Further development within roots extends over a period of 3-4 weeks and involves three moults with successive enlargement of body size . A white , lemon - shaped body of females emerges out of the roots after 5-6 weeks of penetration . The female soon dies and the body wall hardens in the form of brown cyst within next few weeks . There is only one generation of nematode in a crop season . Male is elongated in shape . The development of male and female larvae is similar upto the second moult , after which , the males elongate and wriggle out at the final moult as adult male .

Spread and Survival

The nematode can spread through cultural operations , implements , labour feet and also through irrigation channels . Cyst can remain in soil for several years without host .

Host Range

H. avenae attacks **wheat** , barley , oats , rye , maize and other graminaceous plants . The only cruciferous plant reported as its host is *Senebiera pinnatifida* .

Host - Parasite Relationship

Host damage is governed to a large extent by soil moisture High soil moisture content of the soil can modify host parasite interaction in such a manner that better plant growth is obtained in spite of higher eelworm multiplication . The relationship between yield and nematode population density varies with the host plants . Wheat is more efficient host than barley.

Control

1. Crop rotation can reduce the crop losses . Cultivation of crops like carrot , fenugreek , onion , mustard , gram etc can cause even 55 % reduction in nematode population at the end of one year .

2. Deep summer ploughing is another effective strategy . 2-3 deep summer ploughings in May and June at an interval of 15 days minimise the nematode population .

3. Use of resistant varieties is indeed the most economical measure of nematode control . Rajkiran , RD - 2052 , C - 164 , DL350 , DL 375 and other varieties of barley are resistant . An Australian variety of wheat known as Katyl is known to be resistant against the nematode.

4. Chemical control is most effective method of nematode management . Use of Carbofuran

3G @ 1-1.5 Kg a.i./ha in wheat and 1 kg / ha in barley minimises the nematode infestation satisfactorily . Fensulfolthion , aldicarb sulfone and aldicarb at 1 per cent a.i./ha as seed dresser reduce penetration of wheat roots .

5. Integrated nematode management is most useful . Combination of nitrogen and DD or DBCP have given encouraging results . Integrating sowing time with nematicide application (aldicarb 2 kg a.i./ha with November sowing) has rendered better yields .

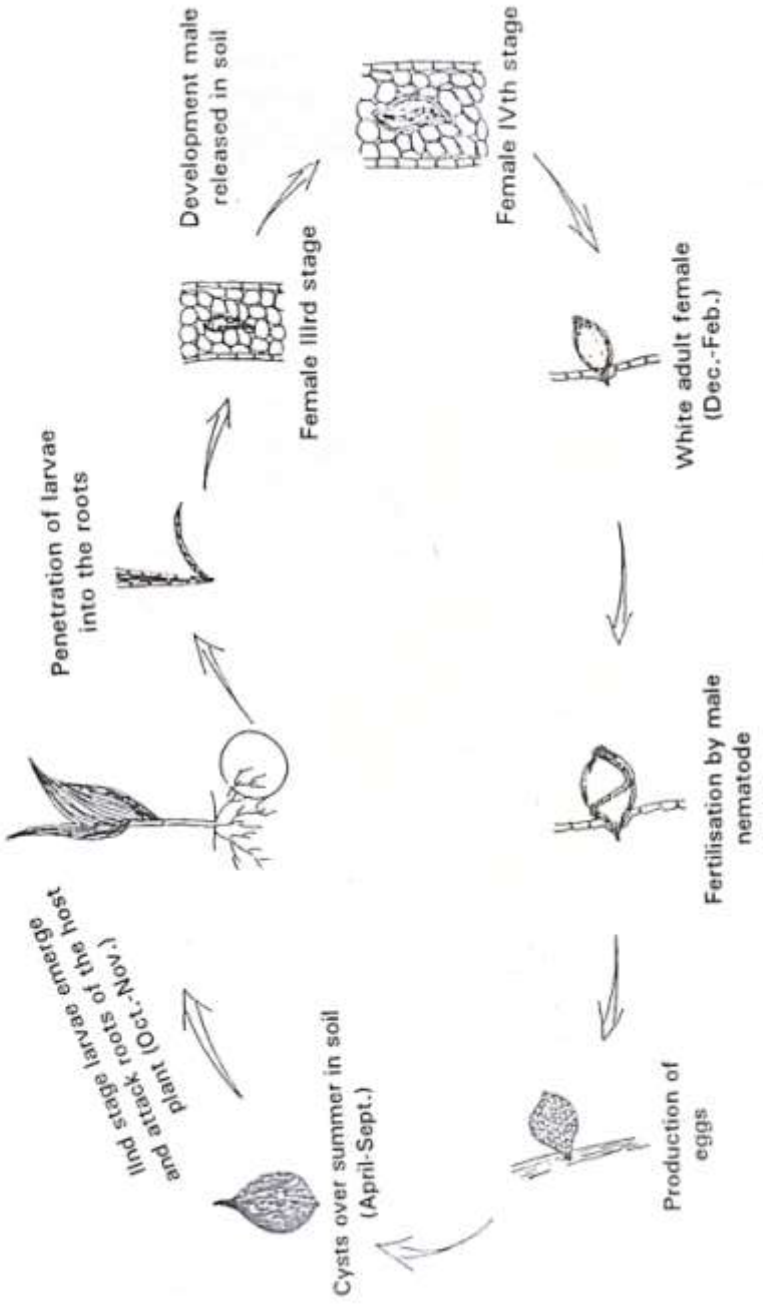

Fig. 6. Disease Cycle of cyst nematode *Heterodera sp.*

3. EAR - COCKLE DISEASE OF WHEAT

History and Importance

The ear - cockle disease was first discovered by Needham (1743) . It was the first plant parasitic nematode ever discovered (by Turbeville Needham who named it as Vibrio) . It is often associated with a bacterium Corynebacterium tritici and causes yellow ear rot which is commonly referred to as Tundu disease (Gupta and Swarup , 1972). It has been estimated that 2.5 to 8.5 % seed contamination may cause 30 to 70 % losses in yield. Effect of seed galls on flour quality could be adverse. If over 5 % by weight, it will deteriorate the flour colour, texture , odour and even taste of the bread .

Geographical Distribution In 1920, it was reported from all the five continents of the world. It has been eradicated from western Europe, USA, UK and Australia by using eradicating sieves. In India, its presence was first detected by Hutchinson in 1917. Chaudhary (1935) and Koshy and Swarup (1971) reported its occurrence from all over India .

Symptoms

Infested seedlings show slight swelling or enlargement of the basal part of stem. Leaves, emerging from these seedlings are twisted or crinkled, often folded with their tips held near the growing point. If severe infestation occurs, the seedlings may even die . The infested plants may show profuse till ering and may produce ear even 30-40 days earlier . The affected ears are usually shorter. In glumes , seed is replaced by the gall which is smaller , darker - coloured and proportionately shorter .

A. tritici when associated with Corynebacterium michiganensis var tritici becomes responsible for causing yellow ear rot or Tundu . The bacterium alone cannot cause Tundu . The Tundu disease is characterized by the production of a light yellow slime or gum on the leaf surface of young plants as well as on ears . This yellow slime , which can be seen trickling down the tissue in humid weather , becomes hard , brittle and brown on drying . Culms which are infested either die at the young stages or grow until heading .

Causal Organism

Wheat Seed Gall Nematode (*Anguina tritici*)

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Sub – order	Tylenchina
Super family	Anguinoidea
Family	Anguinidae

Sub - family Anguininae

Genus *Anguina*

Life Cycle

The seed galls fall on the ground during harvest and break down in soil to release second stage larvae which may be 3000-12,000 in number . Under Indian conditions , falling of galls in soil during harvest does not serve as source of inoculum as during rainy season , juveniles emerge and perish in absence of host . The larvae invade seedlings of the host plant and feed ectoparasitically around the growing - point and its leaf - sheath . When embryonic flower tissues are formed , the nematodes invade them and change themselves from ectoparasite to endoparasite . They develop into adults in the developing seed , which becomes a gall . Mating occurs and the females lay thousands of eggs . The adults die and the eggs hatch . The larvae moult once in the gall , so that when they leave it to infect new plants , they are in second stage . The second stage larvae in seed galls can live for many years .

Spread and Survival

The nematode is likely to be carried to long distances through galls mixed with seed . Year to year perpetuation is through seed contamination . The second stage larvae can live for many years in dried seed galls . Needham (1743) claimed the nematode survival for 25 years while Fielding (1951) revived larvae after 28 years .

Host Range Wheat (T. aestivum) , rye (Secale cereale) , emmer (Triticum dicoccum) , T. monococcum , T. ventricosum , spelt (T. spelta) , Avena sativa and Hordeum vulgare are susceptible hosts .

Host - Parasite Relationship

Histopathological studies have shown that a large number of second stage larvae of the nematode feed ectoparasitically on the growing point . At the time of flowering , the floral initials , instead of differentiating into staminate and carpellate tissues , grow to form cavities enclosing the larvae (Gupta and Swarup , 1968) . Various sugars and amino acids have been detected in galls (Midha and Swarup , 1974 ; Pathak et al . , 1983) .

Control

1. Drycleaning of seeds or brine floatation : use of nematode gall free seed is advisable if seed lot is mixed with galls . The seeds are dipped in 20 % brine solution , the nematode galls floating on the surface are removed , and the healthy seeds are washed 2-3 times with clean water and dried . Although salt solution removes all galls , the method is slow and combursome and it also affects seed germination . Seed cleaning with specific gravity table is quickest , safest and cheapest (Jat et al . , 1986) .

2. Hot water treatment : seed is presoaked for 4-6 hours in cold water , and then treated for 10 minutes in hot water at 54 ° C .

3. Seed certification : use of certified disease - free seeds can prevent the losses significantly .

4. Crop rotation : growing of non - host crops for 1-2 years is practically sufficient to manage the disease .

5. Resistant varieties : use of resistant varieties against wheat seed gall nematode can minimise the losses . In Iraq , cultivar Saber beg is resistant .

6. Rogueing : if infested plants are detected at an early stage they may be uprooted and burnt .

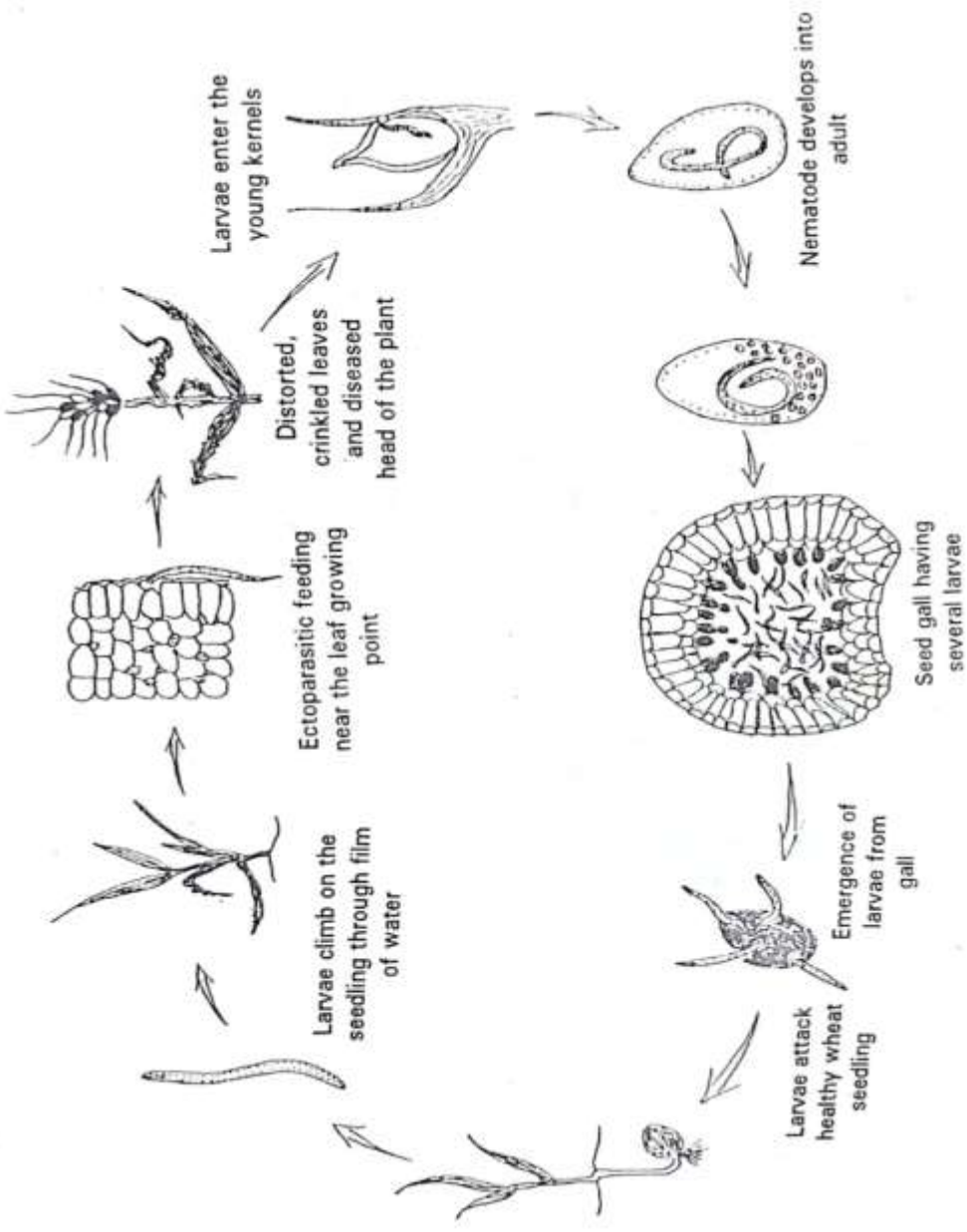

Fig. 7. Disease Cycle of wheat seed gall nematode *Anguina sp.*

4. RENIFORM NEMATODE DISEASE

History and Importance

The nematode was first described by Linford and Oliveira (1940) on roots of cowpea (Vigna sinensis) growing in pineapple field in Hawaii . Reniform nematode has been recognised as a major problem in India .

Geographical Distribution

Rotylenchulus reniformis is one of the important pests of vegetables grown in the tropical and semi - tropical areas of the world . It is known to occur in more than 30 countries .

Symptoms

In castorbeans, drying of leaf margins spread towards lamina which is followed by leaf - shedding . The axillary buds in the main stem develop into small leaves which shed - off . The roots become mildly discoloured while root - lets become dark brown and necrotic . The seeds become malformed and discoloured .

In tomato and pigeonpea , tender roots show traces of necrosis around the female heads . If infection is heavy then the roots show severe curling . The infected cotton plants become stunted and lose vigour . Their leaves lose dark green colour . The severe infection causes pruning of roots . Varied kinds of symptoms are induced in different crops which include hypertrophy , hyperplasia , thickening of cell walls , dense granular cytoplasm , enlargement of nucleus , formation of giant cells , syncytia , feeding peg , feeding tube and extensive damage to cortical tissues .

Causal Organism

Rotylenchulus reniformis

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Sub order	Criconematina
Super family	Tylenchuloidea
Family	Tylenchulidae
Sub family	Tylenchulinae
Genus	*Rotylenchulus*

Life Cycle

According to Linford and Oliviera (1940) it takes about 25 days from egg to egg . Further , it has been seen that 24-29 days are required for the completion of life cycle on ladies finger , 17-23 days on cotton , 19 days on soybean and 14 days on tomato .

Second stage larvae hatch in about 7-8 days in solanaceous crops but in 3 days in case of cantaloup , a cucurbit vegetable . Third and fourth stage larvae are non feeding and without stylet . After fourth stage or fourth moult , young females emerge which are infective and cannot develop further without feeding . Young females penetrate their body half way into the root and start swelling within three days of feeding . Egg laying is initiated from ninth day in case of solanaceous vegetables . Six unicellular pear shaped glands that open into vulva secrete the gelatinous matrix . Copulation occurs only when female begins to enlarge .

The nematode penetrates all along the root except the root tip . The extent of penetration may vary depending on the host variety and age of seedling .

R. reniformis is sexually dimorphic having kidney - shaped females and vermiform larvae and males . It is reported to be amphimictic and rarely parthenogenetic . Parthenogenitically produced eggs either do not hatch or young females developed prove to be non infective .

Immature females live freely in soil . The nematodes can spread from field to field through irrigation water , nursery stock , planting mateial and implements .

Host Range

The parasite is polyphagous . In India , it was first identified by Siddiqi and Basir (1959) on coffee and later recorded on several important hosts like tomato , brinjal , bhindi , sweet potato , cucumber , potato , ginger , onion , pigeon pea , chickpea , blackgram , beans , oilseeds , millets , certain fruits and ornamentals , etc.

Host - Parasite Relationship

R. reniformis is mainly a phloem feeder in case of castor , papaya and tomato whereas it remains confined to the pericycle in cowpea , tobacco and cauliflower. Hypertrophy , hyperplasia , thickening of cell walls , dense granular cytoplasm , enlargement of nucleus , giant cells / syncytia formation , feeding peg and feeding tube formation within the syncytium are the cellular modifications that occur due to feeding on host roots . Hypertrophied cells may also form a ring around the conducting tissues in different plants .

Ultrastructural changes induced by reniform nematode exhibit (i) initial phase : which includes partial cell wall lysis and separation and (ii) anabolic phase , which includes organelle proliferation and development accompanied by secondary wall deposition which provides nutrition for sessile female development .

Control

1. Non chemical methods like crop rotation , use of resistant varieties , application of organic amendments and host nutrition management can minimise the crop losses . Potential of biocontrol agents , fungal cultural filtrate and plant extracts remains to be exploited commercially .

2. Nematicides like DD , MBr , Tripate , Chloropic , Nemacur , Counter , Mocap , Fensulfothion , A 526321 , Aldicarb , Carbofuran and Vydate are promising for reniform nematode management in various crops .

5. THE CITRUS NEMATODE DISEASE (SLOW DECLINE OF CITRUS)

History and Importance

The citrus nematode was discovered in 1912 in California on the roots exhibiting a mottled appearance . Later , Cobb (1914) published detailed account of its distribution , morphology and life history . Siddiqi (1961) reported T. semipenetrans for the first time in India . This nematode causes a slow decline and is considered to be one of the factors responsible for the die - back disease of citrus trees in India . Per cent annual reduction of citrus trees due to this disease in India is 8.7 to 12.2 per cent .

Geographical Distribution

The nematode occurs in all citrus - growing countries . In India , it has been reported from U.P. (Siddiqi , 1961) Delhi , Rajasthan , Punjab , Maharashtra , W. Bengal , Assam , Himachal Pradesh , Sikkim , Orissa , Haryana , Bihar , Karnataka , Tamil Nadu and Andhra Pradesh .

Symptoms

The decline of the tree is gradual . Reduced tree vigour , chlorosis , falling of leaves , twig die - back and reduced fruit - production are the main symptoms of the disease . The nematode feeding is restricted to the cortical region of roots where a permanent feeding site is established . In case of heavy infestation , there may be more than 100 mature females per centimetre root . The role of secondary organisms like bacteria and pathogenic fungi in causing the disease syndrome is very important . In heavily infested roots , the cortex may separate from the vascular stele . As a result of continuous feeding and reproduction of the nematode , the feeder roots get destroyed particularly in the upper soil layers .

Causal Organism

Tylenchulus semipenetrans (Cobb, 1913).

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Sub order	Criconematina
Super family	Tylenchuloidea
Family	Tylenchulidae
Sub family	Tylenchulinae
Genus	Tylenchulus

Life Cycle

Van Gundy (1958) studied the life cycle in detail and found that eggs hatch in 12-14 days at 24 ° C . Sex differentiation was possible at second stage . The second stage female required 14 days to locate the root , fed on epidermal cells and mouled . Life cycle from egg to egg required 6-8 weeks .

Optimum temperature for nematode reproduction is 28-31 ° C . Nematode reproduction is high in soils with a clay content of 10 15 % along with a favourable pH of 5.6-7.6 .

Control

1. Preventive measures: Citrus nematode can easily be transported to new orchards through infested seedlings and budded plants . Therefore , nurseries should never be established near old citrus orchards and nursery soil should be sterilized before planting . Care should also be taken not to spread infection through tools , machinery and irrigation water used in infested groves .

2 . Chemical control: Bare root dip treatment is effective . It can be managed by bare root dip in water at 45 ° C for 25 min or in suitable concentrates of DBCP , Fensulfothion etc. Successful results have been achieved with pre - plant soil treatment with DD , MBr , DBCP used in irrigation water . Other chemicals viz . Ethoprophos , Carbofuran , Phorate , Dimethoate and Fensulfothion are also effective . Application of Carbofuran, Aldoxycarb or Oxamyl at 1.1 kg a.i./ha by low pressure drip irrigation system is beneficial .

3. Biological control: Incorporation of organic soil amendments into soil has reduced citrus nematode population. A Mononchus sp. was observed feeding on T. semipenetrans by Cobb (1914) . Several species of nematode trapping fungi like Arthrobotrys, Dactylella and Dactylaria have been found in association with citrus nematode.

4. Selections of Poncirus trifoliata vary in tolerance and can be utilized in developing resistant root stocks . Troyer and Carrizo citranges and trifoliate orange are highly promising. The hybrid, savage citrange (C. sinensis X Poncirus trifoliata) is moderately resistant .

6. THE BURROWING NEMATODE DISEASES

History and Importance

History and Importance Cobb (1893) first described burrowing nematode from necrotic root lesion of banana in Fizi . The disease of banana caused by Radopholus similis is known as root rot , blackhead , black head toppling or decline . In India, Nair et al. (1966) first reported the nematode on banana from Kerala . The losses in banana may range from 30 to 60 per cent. Geographical Distribution It is widely distributed all over the world and is a major economic problem in Fizi, Australia, New Zealand, Pacific Islands, central and south America and parts of Africa and Southeast Asia. In India, it is known to occur in Tamil Nadu, Kerala, Karnataka, Maharashtra and Gujarat.

Symptoms

On banana roots, reddish - brown cortical lesions appear. Root and rhizome necrosis is manifested to varying degrees. The affected plants show retarded growth, leaf yellowing and they bear smaller fruits. Feeding roots are invaded and destroyed as fast as they are formed. The lesioning of primary roots together with girdling and death of those roots which anchor the plant to the ground, destroy the plant totally.

Causal Organism

Radopholus similis (Cobb, 1893; Thorne, 1949).

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Sub order	Tylenchinea
Super family	Hoplolaimoidea
Family	Pratylenchidae
Sub family	Pratylenchinae
Genus	Radopholus

Life Cycle

R. similis is a migratory endoparasite. Its stages remain vermiform. Sexual dimorphism is apparent with adult males being somewhat degenerate and probably non - parasitic. The life cycle duration is 20-25 days.

Survival and Spread

Free - living stages and eggs do not survive in fallow fields or in soil stored in plastic bags for more than 12 weeks. After the plantation is destroyed, corn, if available may help survive the nematode. The spread is mainly through infested planting material. Other means of spread are: trimming sets , irrigation water , soil adhered to implements, tyres of motor vehicles and shoes of workers .

Host Range

R. similis is known to infect Musa sppl, Ipomoea batatas, pepper, arecanut, coconut , citrus , sugarcane , coffee , maize , avocado , tea . vegetables, ornamentals trees , grasses and weeds . Poucher et al . (1967) have reported 244 hosts of R. similis.

Host Parasite Relationship

Histopathology was first studied by Blake (1966). On entering the root, nematodes occupy the intercellular position in cortical parenchyma, where they feed on the cytoplasm of nearby cells and destroy them. Cavities begin develop. The continuous feeding and cavity formation leads to formation of tunnel. The tunnel increases laterally towards endodermis and produces a characteristic reddish - brown lesion around the cortex. The stele is not invaded but the root system is greatly reduced.

Control

1. Only certified planting material be used.

2. Disinfecting of banana sets is important. It may be done by paring, heat therapy and chemical treatment. For paring and prolinage, the infected portion of a set should be removed since the pared sets are prone to fungal attack , they should be treated with Bordeaux mixture or Carbofuran. For heat therapy, pared sets are dipped in hot water at 55 ° C for 25 min followed by fungicidal treatment.

3. Cultural Methods: Since the Banana Biotype apparently has a narrow host range and limited survival in soil in absence of a host , destruction of an infested plantation followed by planting of cover crop like Panicum maximum var. trichoglume. Phaseolus atroperpeureus may proved useful . According to Loof (1961), plantation of sugarcane in soil reduces *R. similis*. Flooding the soil for 5 months destroys not only Fusarium but also burrowing nematode (Loof, 1961). The practicability of this method is questionable under certain circumstances .

4. Use of resistant varieties is highly desirable. In citrus, Ridge Pineapple, Sweet orange and Estes rough lemon have resistance. In banana, tetraploids resulting from wild diploids, Gross Michel or Highgate triploids have exhibited resistance.

5. Chemical soil treatment: use of DD at 300 l / ha, E.D.B. at 150 kg / ha or DBCP at 40 1 / ha can increase yield by 30-50 per cent.

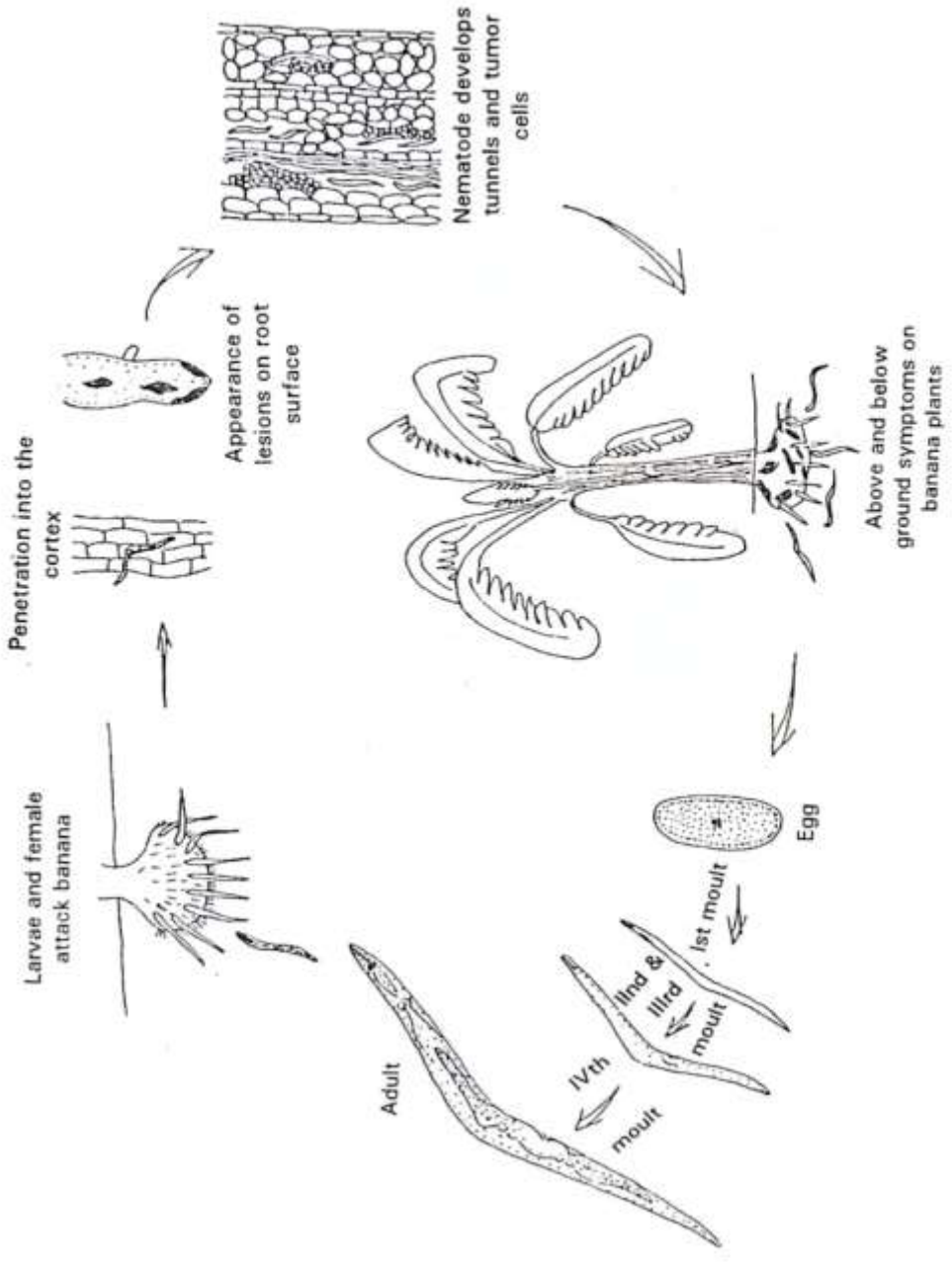

Fig. 8. Disease Cycle of burrowing nematode *Rodopholus sp.*

CONCLUSION

Biocontrol strategies for plant-parasitic-nematodes constitute a valid alternative to toxic chemical nematicides. Thereby, a wide diversity of effective strategies based on the use of filamentous fungi used as BCAs are described. They work through two main kinds of mechanisms of action, i.e., those that include the production of secondary metabolites (antibiosis), lytic enzymes, and space competition by *Trichoderma*. AMF directly acts providing higher nutrient and water uptake to the plant, modifying root morphology and altering the rhizosphere interactions, or competing for photosynthates , or colonization/infection sites. Endophytic fungi reduce the attack of the plant-parasitic nematodes by parasitism, by paralyzing the nematodes, through antibiosis, by lytic enzymes production and also by space competition. The second group of action mechanisms are the induction of plant defenses, such as the activation of SAR and ISR by *Trichoderma*, which seems also heritable. As well as, the modification of roots exudates, strigolactones production, plant secondary metabolites and enzymes production by AMF. Finally, the induction of SAR and ISR, the transport of chemical defense components through the plant and the strigolactones production by endophytic fungi.

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