INDEX

CERTIFICATE

This is to certify that Shri/Ku . _____ Enrol. No._____ has completed the practical work of Course No . H/ENTO-365(1+1) (Nematode Pests of Horticulture Crops and Their Management) as per the Syllabus of B.Sc.(Hons)Horti Third Year, Sixth Semester in the laboratory/field of College as prescribed by M.C.A.E.R. Pune.

Date : **Course Teacher**

EXERCISE NO. 1
COLLECTION OF SOIL AND ROOT SMAPLES

Objective : Qualitative and quantitative analysis of nematode populations.

Materials required : Soil auger or hand hoe, polythene gag, aluminium foil label, rubber band.

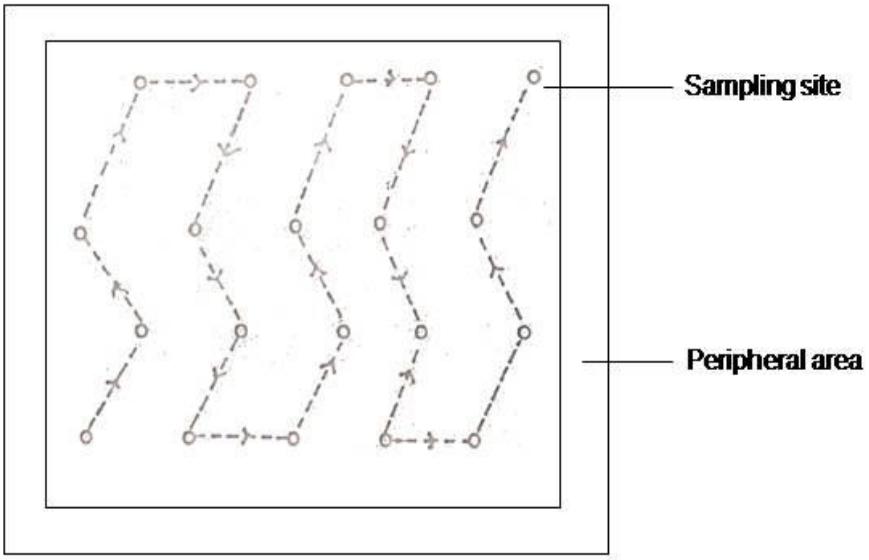
Procedure

1. Sampling from field crops

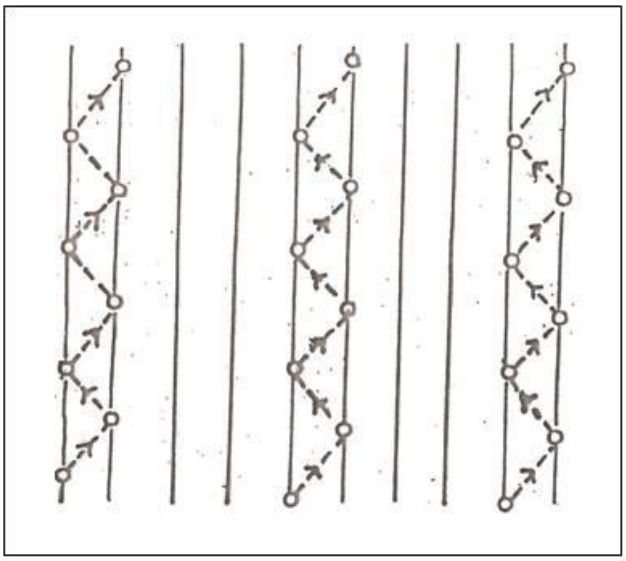
- Leave about 1m peripheral area of the field.
- Remove 2-3 cm upper layer of the soil with the help of a hand hoe.
- Collect 50-100 cc soil along with feeder roots up to a depth of 15-20 cm (subsample). Draw 10-20 such subsamples from one hectare area in a zig-zag manner (Fig. 1.1A)
- Put all the subsamples in the same polythene bag (composite sample)
- Put an aluminium foil label bearing the sample number in the polythene bag and tie it with a rubber band.
- Write the details (see labeling) separately in a note-book.

2. Sampling from vegetable crops

- Select 6 rows of a field (2 from the beginning, 2 from the middle and 2 from the far end of the field).
- Collect 8-10 subsamples up to a depth of 20-30 cm from each pair of rows in a zig zag manner (Fig. 1.1B).
- Put all the subsamples in the same ploythene bag and label it.



A. Field Crops



B. Vegetable field

Fig. 1.1 Pattern of sample collection

3. Sampling from an orchard

- Take 2 subsamples from one tree up to a depth of 30 to 60 cm (feeder root zone) depending upon the age of the tree.
- Collect subsamples from 10 trees randomly form one hectare area (Gig. 1.2A).
- Pool all the subsamples in the same polythene bag and label it.

4. Sampling from a tree

- Collect 5 subsamples each from around the main stem and drip line of the tree (Fit. 1.2B) by the method described above.
- The depth of the sampling will very with the kind and age of the tree.
- Put all the subsamples in the same polythene bag and label it.

Labelling : Write sample number on an aluminium foil label, fold it and put in the polythene bag. Tie the mouth of the polythene bag with a rubber band. Note down following information in a note-book.

- Sample number
- Host plant
- Date of sampling
- Farmer's name and address
- Previous cropping sequence.
- Condition of the crop. Symptoms, if any.

Storage of the samples : Samples should be stored in a refrigerator (about 10^0C) for a few days if immediate processing is not possible.

EXERCISE NO. 2
EXTRACTIN OF NEMATODES FROM SOIL SAMPLES

Objective : To extract various types of nematodes from a soil sample for qualitative and quantitative estimation.

Materials required : Beakers, enameled metallic pas, Petri-plates, sieves, soil sample, facial tissue papers, wire nets, glass funnels, funnel stand, rubber tube, glass vials.

Methods

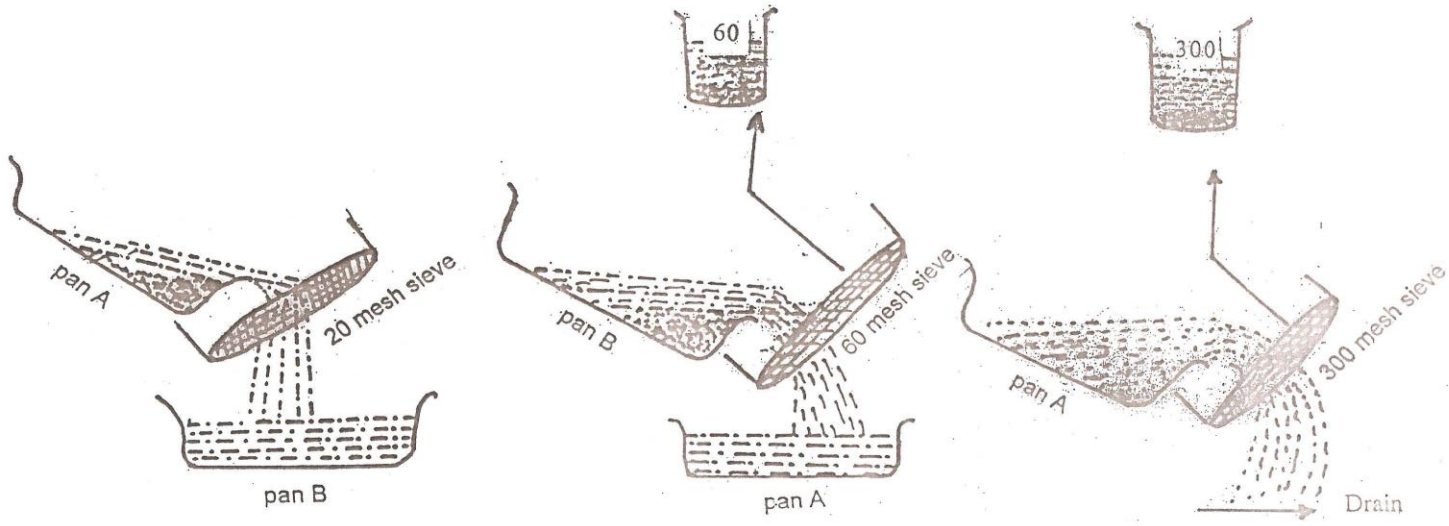
1. Cobb's decanting and sieving technique

Principle

- The soil particles and nematodes settle at different rates due to difference in their specific gravity.
- Different sized nematodes are retained on sieves of different pore sizes.

Procedure (Fig. 2.1)

- Take out the composite sample in an enameled metallic pan, mix it thoroughly and take 250 cc for processing. Store the remaining sample in a refrigerator.
- Transfer 250 cc soil to Pan A and add about one litre water, mix well breaking clods and clumps.
- Wait for 10-20 seconds and pass this soil suspension through a 20 mesh sieve (pore size 840 μm), collecting the filtrate in pan B. Wash the pan A.
- Collect roots present on the 20-mesh sieve in a beaker and discard the remaining material.
- Stir the suspension of pan B gently, wait for a few seconds and pour it through a 60-mesh sieve (Pore size 250 μm) (pore size 250μm) in pan A. collect the residue left over 60-mesh sieve in a beaker and label it as 60.
- Pass the contents of pan A through a 300-mesh sieve (pore size 53 μm). Discard the suspension passed through the sieve.



Cobb's Decanting and Sieving Technique

Fig. 2.1

- Collect the residue left over 300 mesh sieve in a beaker and label as 300.
- Examine the contents of beakers labeled as 60 for cyst nematodes directly under a stereomicroscope.
- Further process 300-mesh residue by the following techniques.

2. Baermann's Funnel Technique

Principle

The active and motile nematodes pass through the tissue paper and get collected at the base of rubber tube/specimen vial due to movement and gravitational force whereas inert soil particles/debris remain on the tissue paper.

Procedure (Fig. 2.2)

- Process the soil sample by Cobb's decanting and sieving technique as above.
- Take a glas funnel with a piece of rubber tube (10-12"long) bearing a glass vial (5ml capacity) attached to its distal end.
- Fill the funnel assembly with water and press the rubber tube gently to remove the air bubbles.
- Transfer the sieved nematode suspension (labeled as 300) to a moulded piece of wire net covered with a double layered tissue paper and place it over the funnel.
- Add water till it touches the lower surface of the wire net.
- After 24-48 hours remove the glass vial and observe the nematodes under a stereoscopic binocular microscope.

Advantages

- Clear nematode suspension, free of debris etc. is obtained.
- The nematodes are collected in a small amount of water.

Disadvantages

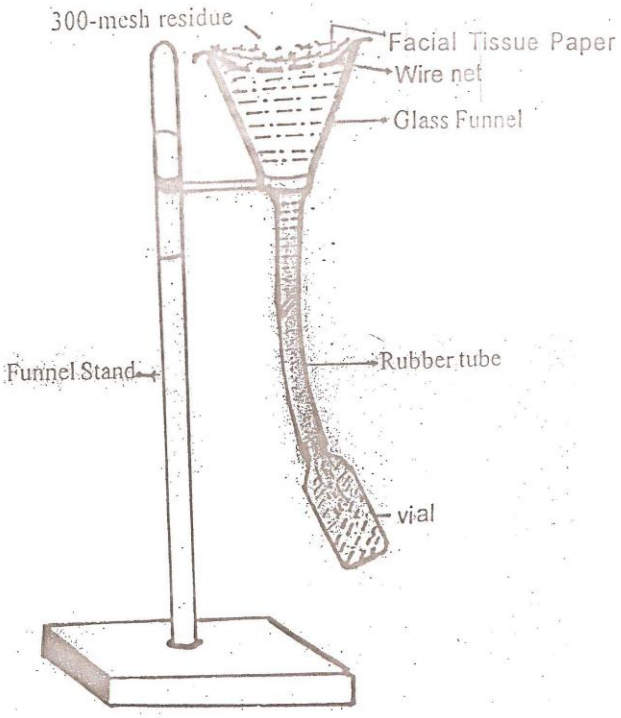
- It is a time consuming.
- Nematodes may lose their activity/viability due to lack of oxygen.
- Sedentary and slow moving nematodes can not be extracted.

3. Modified Baermann's funnel Technique (Fig. 2.2)

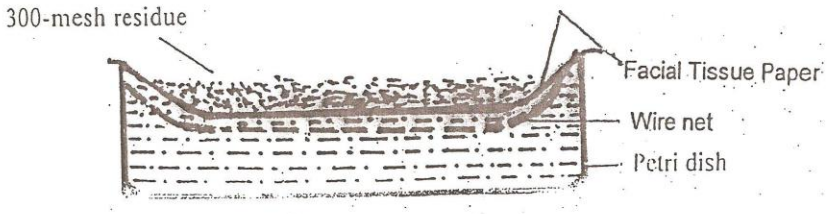
In this technique Petri-plate is used in place of funnel. Other steps are same as described above for Baermann's funnel method and the nematodes get collected in Petri-plate.

Advantage

Maximum recovery of active nematodes is achieved by this method because the nematodes do not lose their activity/viability since the oxygen is always available.



Baemann's Funnel Technique



Modified Baermann's Funnel Technique

Fig. 2.2

EXERCISE NO. 3
COLLECTION OF NEMATODES FROM PLANT MATERIAL

Objective : To extract nematodes form plant parts for qualitative and quantitative estimation.

Materials required : Infested plant material, scissors, Petri-plates, wire guage, facial tissue paper, waring blender, acid fuchsin, lactophenol, etc.

1. Direct examination technique

Procedure

- Wash the infested plant material thoroughly and chop it into small pieces.
- Put this material in a Petri-plate containing water.
- The migratory semi endo/endoparasitic nematodes come out of the chopped material into water by 24 hours and can be seen directly under stereomicriscope.
- Alternatively the hopped material can be processed by modified Baermann's technique.

2. Waring blender technique

Procedure

- Wash the roots under tap water to remove adhering soil particles.
- Chop the roots to 0.5-1.0 cm pieces, and transfer them to a blender containing about 100 ml water.
- Operate the blender for 15 seconds.
- Take out this material from the blender and put it on the modified Baermann's funnel assembly as described earlier.

3. Acid fuchshin staining technique

This technique is mainly used for detecting sedentary semi-endo and endoparasitic nematodes. The stained but dead nematodes can be dissected out of the roots under a stereomicroscope.

Procedure

- Wash the root gently under tap water to remove the soil particles.
- Remove the excess water with a blotting paper.
- Cut/chop the roots to small pieces (about 1 cm).
- Take a known quantity of chopped roots (0.5 to 1 g) and wrap them in a piece of muslin.
- Boil them in 0.1% acid fuchisin lactophenol solution for 1-3 min depending upon the hardness of the root.
- Remove the excess stain under running tap water.
- Leave the stained root bits in plain lactophenol (lactic acid 1 part + glycerine 2 parts + phenol 1 part + water 1 part) overnight to destain the roots.
- Examine the roots under a stereomicroscope. The nematodes appear red in more or less transparent root tissues.

EXERCISE NO. 4
COUNTING AND PICKING OF NEMATODES

Objective : i. To estimate the number of the nematodes in a given suspension.
ii. To transfer nematodes form one solution to another.

Materials required : Counting dish, tally counter, nematode suspension, pipette, stereozoom microscope, pick, etc.

1. Nematode counting

Direct counting method : This method is used when the nematode population in the suspension is very low.

Procedure

- Transfer the nematode suspension provided to a counting dish (Fig. 5).
- Keep the counting dish under the stereo microscope and wait for a few seconds so that nematodes get settled at its base.
- Adjust one small square of the counting dish from one side under stereo microscope and record the nematode number using tally counter.
- Proceed to the next square and likewise count from all the squares occupied by the nematode suspension.

Dilution method : This method is used when the nematode population in the suspension is very high.

Procedure

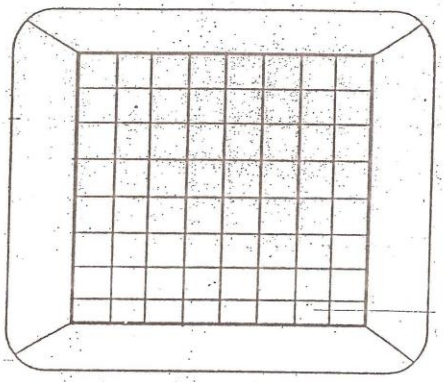
- Take the nematode suspension in a beaker and make a volume of 100 ml by adding water.
- Bubble the diluted suspension vigorously with a pipette.
- Transfer 5 ml of nematode suspension to a counting dish and count the nematodes as described above.
- Repeat this process 3 times.

- Calculate the average number of nematodes per ml.
- Multiply with 20 to compute the total number of nematodes present in the sample.

Nematode picking

Procedure

- Take the nematode suspension in cavity block or petriplate and focus the nematode under a low magnification of stereozoom microscope.
- Touch the desired nematode with a pick (fig. 5) gently by giving a slight jerk, the nematode will start floating in the water column. Refocus the nematode under microscope.
- Bring the pick underneath the nematode and lift it with a jerk.
- Transfer it to a drop of water placed over a glass slide.



Counting dish

Nematode pick

Fig. 5

EXERCISE NO. 5

KILLING, FIXING, CLEARING AND MOUNTING OF NEMATODES

Objective : To prepare semipermanent and permanent whole mounts of nematodes.

Materials required : Calcium chloride (anhydrous), cavity blocks, desiccator, ethanol, formalin, lactophenol, nematode suspension, oven, glass slides, cover slips, glass wool, glycering, nail polish.

1. Killing and fixing

- Take live nematode suspension in a test tube.
- Add 8% boiling formaldehyde solution in equal volume.
- Leave this fixed suspension in specimen vial and cork it. Let is stand for minimum 24 hours.

2. Clearing

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

Lactophenol (Rapid) method

Procedure

- Take a drop of lactophenol on a slide.
- Warm it to 55-60^{0}C for 1-2 min.
- Transfer the desired nematode to a drop of warm lactophenol.
- The nematode will become transparent and is ready for mounting in lactophenol (semipermanent mount)

Glycerol ethanol (Seinhorst's) method

Procedure

- Take Seinhorst's solution-I (96% ethanol – 20 ml, glycerine – 1ml, distilled water – 79 ml) in a cavity block and transfer the desired nematodes into it.
- Cover the cavity block partly and put it in a glass vessel containing 96% alcohol.
- Place the glass vessel in an oven at 40^0 C for 12 hours.
- Refill the cavity block Seinhorst's Solution-II (Glycerine – 5 parts + 96% alcohol – 95 parts).
- Cover the cavity block partly and leave it in oven at 40^0C for 4 hours.
- The nematodes are left in the pure glycerine and are ready for mounting in glycerine (permanent mount).

3. Mounting

Procedure

- Take a drop of lactophenol / anhydrous glycerine on a glass slide.
- Pick 2-3 cleared nematode and transfer them on to the slide.
- Arrange them parallel)under stereomicroscope) ensuring the nematodes are resting on the surface of the slide.
- Pick three glass wool pieces (diameter equal to nematode) and arrange in the drop on the slide radially.
- Take a coverslip and put it over the drop of lactophenol / glycerine and tap it gently.
- Remove the excess of lactophenol / glycerine with the help of a blotting paper.
- Seal it with nail polish first at three places and after a few minutes ring it with the nail polish. After 5 minutes each, give two coatings of nail polish again the label the slide (Fig. 6).

Precautions

- The thickness of the glass wool should be almost same or slightly more than the thickness of the nematode.

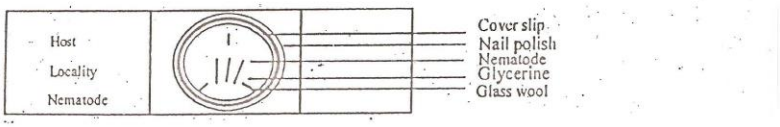

Host
Locality
Nematode
Cover slip
Nail polish
Nematode
Glycerine
Glass wool

Fig. 6.

EXERCISE NO. 6
TEMPORARY MOUNTS

- Take live nematode suspension in a test tube.
- Add 8% boiling formaldehyde solution in equal volume.
- Leave this fixed suspension in specimen vial and cork it. Let it stand for minimum 24 hours.
- Take a drop of mounting fluid (Water or 3% formaldehyde) on glass slide.
- Pick as many as 10 nematodes from above suspension (specimen vial) and transfer them on to the slide.
- Examine the slide under stereomicroscope to make sure that all the nematodes are at the bottom of the mounting field and towards the middle of the drop.
- Pick cover glass supports by using the wet pick and place them at the edges of the drop.
- Holding a cover glass in the forceps, gently lower it over the drop. Ideally, the drop should be just large enough so that the cover glass rest on the supports with no excess mounting fluid. If there is excess mounting fluid, absorb it with a small piece of filter paper (blotter, paper towel, or other absorbent papers) watching through the microscope to insure your nematodes and not drawn from under the cover glass.
- Seal the slide by painting the sealing material around the edge of the cover glass. This is best done under the lowest power of the dissecting microscope to be sure hat the edge of the cover glass is sealed.
- It is good practice to label slides as son as they are made.

EXERCISE NO. 7
METHODS OF NEMATODES CONTROL

Objective : Acquaintance with nematode control methods feasible in laboratory / nematode infested field plots.

Chemical methods

The following methods can be used to economise nematicidal use.

- Seed coating (for bold seeded crops) : Treat 100 g okra seed with carbosulfan (Marshal 25 ST) @ 3% (w/w). Use treated seed for sowing in root-knot nematode infested field plots.
- Bare rot dip treatment (for transplanted crops) : Prepare 1000 ppm concentration of phosphomidan. Dip the roots of root-knot nematode infected tomato seedlings in this solution for 1 hour. Transplant the seedlings in nematode infested field plots.
- Nursery bed treatment (for transplanted crops) : Broadcast carbofuran (Furadan 3G) @ 2 Kg a.i./ha (7g/sq.m.) to root-knot nematode nursery beds. Mix in soil before sowing tomato seed.
- Basin area treatment (for perennial crops) : Apply carbofuran (Furadan 3G) @ 4 Kg a.i./ha (13g/sq.m.) in about 9 sq.m. area around citrus tree infected with citrus nematode. Mix the chemical in soil and give light irrigation.

Physical methods

- Water floatation / Salt sedimentation : Put one part of wheat seed contaminated with cockles of seedgall nematode in a container filled with plain water and the second part in another container filled with 20% brine solution. Stir the seed with a stick. Separate the galls floating on the surface with a sieve. Assess the efficacy of both the methods.
- Hot water treatment : Take one volume of paddy seed infested with white tip nematode in a container. Add two volumes of plain water and two volumes of boiling water to it and stir the seed with a stick. After 10 min. drain off the water and dry the seeds. Compare the treated and untreated seeds for nematode recovery.

Cultural methods

- **Soil solarization :** Cover one field plot with LDPE clear plastic sheet (400 gauge) for 15 days. Compare for nematode population with uncovered field plot.
- **Rabbing :** Spread rice husk @ 20 Kg/sq.m. on root-knot nematode infested nursery bed and burn it. Compare for nematode population with untreated field plot.

Host resistance

Uproot five tomato plants each from root-knot nematode infested field plots planted with a resistant variety (Hisar Lalit) and a susceptible variety (HS-101). Count the number of galls on each plant and compare.

Biological control

Observe the following biocontrol agents from slides (Fig. 18).

- Nematode captured by a predacious fungus.
- Nematode egg infected with a parasitic fungus, *Paecilomyces lilacinus*.
- Root-knot nematode's second stage juvenile encumbered with spores, nad female infected with a bacterial parasite, *Pasteuria penetrans*.

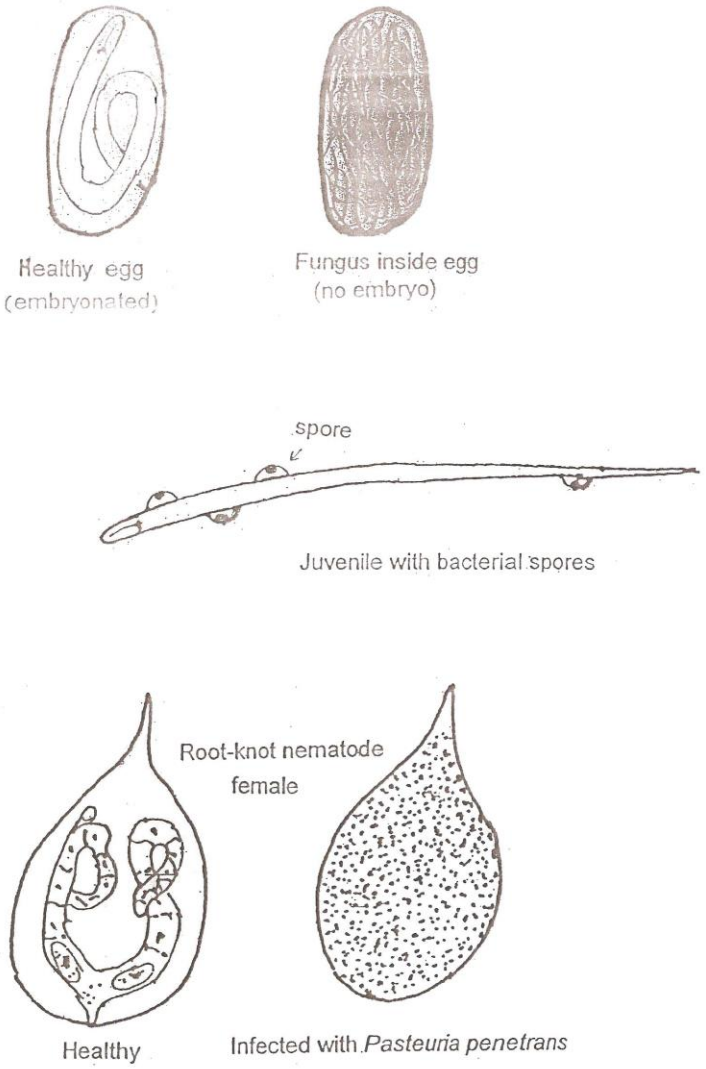

Healthy egg
(embryonated)
Fungus inside egg
(no embryo)
spore
Juvenile with bacterial spores
Root-knot nematode
female
Healthy
Infected with *Pasteuria penetrans*

Fig. 18.

EXERCISE NO. 8

NEMATICIDES AND THEIR USE

Plant parasitic nematodes may be controlled by applying nematicidal chemicals to the soil or to the potential host plant. The main advantage of chemical control over that of other methods is that, the nematodes may be reduced within the short period after the application of chemical. This method is generally practiced when cultural and biological control fails to check the nematode populations. The chemicals are also used when there is very heavy infestation and proper resistant varieties are not available for growing. The main objectives of chemical soil treatment are to protect the crop form nematode damage, prevent nematode multiplication, improve the growth of plants and finally improve the quality and quantity of the produce.

Types of nematicides

The nematicides which are in commercial use may be grouped into two, fumigants and non fumigants. The fumigants include compounds belonging to halogenated hydrocarbon and isothiocynate groups, while the non-fumigants consist of organo phosphates and carbamates.

A] Halogenated hydrocarbons group

1) **DD mixture –** DD is a mixture of 1-3 dicholoropropene and 1-2, dichloropropane, in 2:1 ratio. It is a dark brown volatile liquid. It kills nematodes, soil insects and wire worms and even some fungi at high dosages. DD is generally used @ 400-500 liters per hactare as a preplant soil fumigant.

2) **EDB (1,2-dibromoethane) –** It is heavy, colourless volatile liquid with choloroform like odour, poorly soluble in water and well soluble in organic solvents. It kills nematodes as well as insects. EDB can not be used for certain bromine sensitive plants like onion, garlic, lilly etc. It is generally use @ 60 lit./ha.

3) **Methylbromide (CH_3Br) –** It is bromomethane and in the pure form, is a colourless liquid. It is odourless and toxic to humans; it kills nematodes, fungi,

and insect which are present in soil. It is usually used for fumigating potting soil or the soil in the green house, seed beds and plant beds.

4) **DBCP (CH_3Br CHBr. CH_2Cl) –** It is heavy straw coloured volatile liquid. It can be injected into the soil like other soil fumigants or an emulsifiable concentrate can be used with irrigation water. This is generally used @ 20 to 50 lit/ha depending upon the crop and soil type. It is a potential nematicide capable of giving a high degree of nematode control.

B] Isothiocynate Group

1) **Dazomet (3, 5 dimethyl-tetrahydro 1, 3, 5, 2, H thiadiazinethion) –** It is crystalline substance, poorly soluble in water and well in chloroform. It has a complex effect with nematicidal, insecticidal, fungicidal and herbicidal action. It is applied as a pre-plant soil fumigant @ 20-35 g/sq.m.

2) **Trapex (methyl isothiocynate CHNCS) –** This chemical is a 20 percent product of an organic solvent, generally xylol, with or without emulsifier. It is used to control the nematodes, soil insects and weeds. It been found effective against cyst forming nematodes.

C] Organo phosphates group

The introduction of contact and systemic granular menaticides in the seventies has provided very effective chemicals for nematode control. These are as follows

1) **Parathion –** It is contact poison and was used first of all by Dimock and Ford (1950) to control chrysanthemum foliar nematode. Chemically it is O, O-diethyl o (P-intro-phenyl) phosphorothioate

2) **Dichlofenthion –** The compound is not volatile enough to function effectively as a soil fumigant, hence generally it is emulsified with water and applied as a drench. It is less phytotoxic and kills many kinds of nematodes at normal dose. It is usually used @ 150 lit./ha.

3) **Thionazin –** It is most effective against sting nematode of maize. Thionazin is also effective against the root-knot nematode attacking vegetables. This can also be used as seedling bare root dip treatment @600 to 800 ppm for nematodes control.

4) **Fensulfothion –** It is promising nematicide and soil insecticide. The active ingredient disperses in soil via the water system so that the chemical easily comes in contact with nematodes. It is generally used @ 40 to 80 Kg/ha.

5) **Ethoprop –** It is a broad spectrum pesticides acting as nematicide and soil insecticide. The chemical is non fumigant, non systemic and has efficient contact action with good soil movement and residual properties. It can be used @ 40 to 80 kg/ha.

6) **Phorate –** It is systemic pesticide and is absorbed by roots and subsequently translocated to aerial parts of the plant. It persists for several weeks protecting the crop not only from nematodes but also from sucking insects. It is generally used @ 20 to 30 kg/ha.

D] Dithiocarbamate groups

These are comparatively of recent origin, which do not have phosphorus and chlorine compounds. These are the derivatives of carbamid acid.

1) **Carbofuran –** It is systemic nematicide and effective against a variety of nematodes. It is also used for seed treatment to control the nematodes during the early stage of plant growth. It is generally applied @ 30 to 50 kg/ha.

2) **Oxamyl –** It is systemic nematicide which can be sprayed to the foliar to control the nematodes on roots. It also controls foliage nematodes. It is used @ 10-20 lit/ha.

3) **Methomyl –** It is a systemic nematicide formulated as 90% wettable powder and used at the rate of 40-80 kg/ha.

Types of Treatment

The nematicides can be used in following ways.

1) Pre-plant treatment – The fumigants which belong to halogenated hydrocarbons are usually phytotoxic which must be applied in the soil before planting. About 3-4 weeks waiting period should be provided.

2) Treatment at the planting time – Broadcasting the granules or spraying the emulsifiable concentrates on the crop or at the time of sowing/planting is the normal method of application in case of organophosphates and carbamates. These chemicals are less phytotoxic, hence safely used at the time of planting.

3) Post plant treatment – Organophosphates and carbonates being less phytotoxic can also be used in the standing crop. In perennial crops, such type of treatment is generally practiced.

4) Bare root dip treatment – Roots of young seedlings or nursery planting material infested with endoparasitic nematodes can be disinfested. Nursery stock of perennials are generally exposed to nematicide solution of about 6hrs. while roots of tender seedlings require 10-30 minute exposure.

5) Seed treatment – Some systemic nematicides have been tested as seed dressing material to minimize the cost of treatment. Carbosulfan can be used as seed treatment for the control of nematodes.

6) Foliar treatment – Some systemic chemicals, e.g. oxamyl can be sprayed on foliage and translocated throughout the plant to effect the nematodes on roots. Foliar application of promising nematicides could also be used in combination with soil application of granular nematicides.