

EXERCISE NO. 9**ISOLATION OF NEMATODES FROM SOIL AND PLANT****MATERIAL****1. WHITE HEAD AND HEMMING TRAY METHOD** (White-head and Hemming, 1965)**MATERIAL**

Soil sample, Trays perforated and none perforated, tissue paper, beakers

PROCEDURE:

- Wash the infested roots with egg masses thoroughly under tap water.
- Place the soil or roots in the perforated tray lined with tissue paper.
- Place it on non-perforated tray having sufficient water.
- The water should just touch the surface and material should not float.
- After 24 hours, collect the water in beaker and allow settling for one hour.
- When the juveniles settle, the excess of water should siphoned out until about 100ml remain.
- Then bubble nematode suspensions a pipette and count three replications of 2 ml aliquots in a counting dish with the help of low power stereomicroscope.

2. BAERMANN FUNNEL TECHNIQUE**MATERIAL**

Soil sample, sieves set, sauce pan, shovel, Baermann funnel, muslin cloth, tissue paper, plastic bucket.

PROCEDURE

- Wrap a quantity of soil in tissue paper or muslin cloth and suspend it partly in contact with water on a glass funnel.
- Close the ring of the tube with a spring clip.
- Nematodes move in wet soil, fall into the water, and collect behind the clip.
- After 12-24 hrs of incubation collect some quantity of water and locate nematodes under stereoscope.

ISOLATION FROM ROOTS:

- Cut infected roots into 1 cm pieces.
- Wrap the pieces in a muslin cloth and suspend over Baermann funnel so that it partly touches the surface of water.
- After few hours of incubation, collect nematode suspension in a Petri dish.

3. RECOVERY OF NEMATODES FROM SOIL BY COBB'S SIFTING AND GRAVITY METHOD

- Dip about 500 cm³ of soil to 3-4 liter of water in a bucket and stir vigorously to release the nematodes.
- After 1 minute without stirring soil particles settle to bottom leaving the nematodes in suspension.
- Decant the suspension into a second bucket through a nest of sieves with opening of 200, 250, 325 mesh/inch.
- Return the fluid to the first bucket.
- Repeat the entire process, stirring as before the soil settle, and decanting through sieves. Finally pass the water from behind the sieve into a beaker.
- If the soil has much inorganic matter the nematodes should be permitted to migrate through a fine cloth fastened over a beaker and then inverted over a funnel of water.
- Whitehead and Hemming tray method can also be used for the isolation of nematodes from both soil and roots.

4. INCUBATION METHOD OF EXTRACTION

This method is also called as root incubation in Plastic bags or jars

MATERIAL

Plastic bags, jars, conical flask or similar vessel

PROCEDURE

- Cut or chop the tissues finely and mix together.
- Weigh out the sample/sub sample.
- Place the sample in a closed plastic jar or vessel with a small quantity of water usually 10- 20 mL and label it.
- Remove the water from jar daily and collect in a beaker.
- Replace the water for each decanting and repeat the same process for 2-7 days.
- Count the number of nematodes/mL of water or store for future use.

QUESTIONS

1. Why sieves of different openings are used?
2. What are the advantages and disadvantages of the Whitehead and Hemming tray method?
3. What is the disadvantage of incubation method?