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Popular Article

Extraction of Plant Parasitic Nematodes Via Roots

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Nematodes are an integral part of the soil biome, ranging from one to ten million per square meter of soil. In order to understand and study the dynamics of the nematode population, their extraction from the soil is a crucial step. Moreover, the attack of plant parasitic nematodes may pave the way for other pathogens to enter. So, their detection is not only necessary for diagnosing nematode damage but also to prevent the formation of other disease complexes. Based on certain parameters like nematode size, sedimentation rate between them, and soil particles, it is possible to extract the nematode population by sieving and decantation methods. This is one of the simple and basic methodologies adopted for extracting nematodes. However, for isolating large, slow-moving sedentary nematodes, other methods are recommended. By this method, we can obtain most of the active, inactive, or dead nematodes.

Materials required:

- Scissors: Used to cut root samples into thin pieces.
- Glass bowls: are used to retain strained samples in PVC rings.
- Petri dish: A Petri dish is used to store chopped root bits.
- Bandage cloth: Bandage fabric covers one end of the PVC ring for straining.
- Tissue paper: It is used to cover one end of the PVC ring underneath the bandage.
- Rubber bands: It help hold tissue paper and bandages in place.
- Wear disposable gloves to prevent contamination and potential risks.
- Foam/Sponge: This is used to filter the root sample so that the filtering foam absorbs the water from the root sample.

- Stereo microscope: This instrument is used to examine an extended root sample for the presence or absence of plant parasitic nematodes.
- Nematode filter rings, which are made up of PVC rings, tissue paper, and bandage fabric, are used to strain the root sample.

Fig.1. Infected tomato plant

Fig.2. Infected root (galls)

Procedure:

- Collect a sample of the root depending on the crop, 25–100 g of roots per total sample is adequate.
- The diseased tomato plant bits or roots that have galls and lesions are put in a container and let to soak for an extended period of time.
- Gently wash the affected tomato roots to get rid of any sand and dirt
- Cut the roots into tiny pieces with a knife or scissors while wearing gloves and place them in a Petri dish filled with water.
- Use a little water to wash your gloved hands into the Petri dish to get rid of any leftover nematode or root remnants.
- Place the sliced or teased root sample for a duration of 4 to 12 hours.

Ring preparations:

- This aids in properly gluing the tissue paper to the ring, and a piece of bandage fabric is placed over it. Make sure the rubber band tie is airtight and tie them securely.
- After covering the sponge with the ring, pour the clear solution containing the soil
  nematodes onto it.
- Move the ring to a different glass bowl with water for nematode expansion as the water seeps through

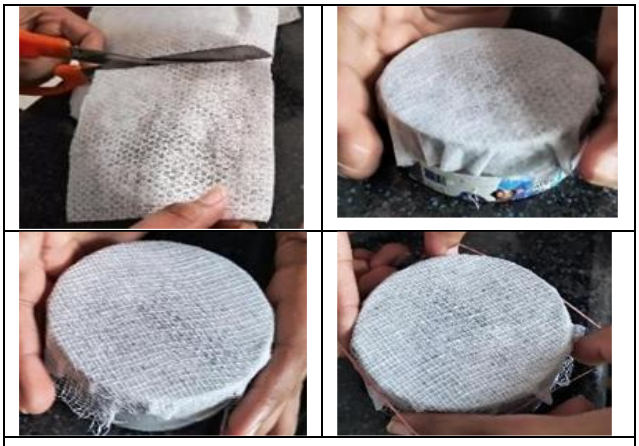

Fig.3. Making rings for nematode extraction

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- During the 24-hour nematode extraction period, leave the setup in place. Using a stereomicroscope, we can view and identify the nematode after 24 hours.

Fig.4. Left for extension period

Observation:

- Plant parasite nematodes and other rhizosphere saprophytes are visible under the stereomicroscope.
- Whereas nematodes are slow-moving, coiled organisms, saprophytes are quick-moving, random organisms.

Fig. 5. Nematodes observation under stereomicroscope

Precautions:

Thoroughly wash all bowls and sieves with warm water. Thoroughly wash countertop, sink and surrounding work area with warm water. Disinfect with 70% ethanol. Place bowls and other equipment's upside down to drain on countertop

- Extremely dormant nematodes may disappear.
- Handle the glass essentials with extreme caution.
- The nematode filter ring needs to be set up precisely.
- Ensure that the roots are not too dry or too fresh.
- Prior to extraction, sterilize every piece of equipment.
- When extracting, use pure, contaminant-free water.
- When extracting, take care not to harm the root structure.
- Take care when handling samples to avoid losing nematodes.
- To get rid of debris, filter the extraction solution.
- To make nematode detection easier, use appropriate lighting.
- If you won't be examining the removed nematodes right away, store them in a cold, dark area.
- Clearly label samples to prevent confusion.

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