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Popular Article

Extraction of Nematodes from Soil

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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.

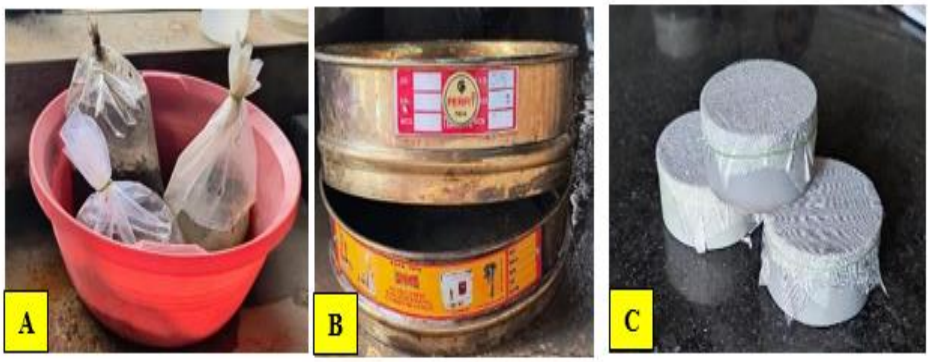

Fig. 1. A) Soil sample, B) 325 & 25 mesh sieves and C) Filter rings.

Materials Required

1. Soil sample from farmer's field
2. Wash bottles
3. Distilled Water/ sterile water
4. Trays
5. 2 Washing tubs (plastic)
6. Stirring rod
7. Sieves of 325 mesh and 25 mesh size
8. Glass bowls
9. Sponge/ Foam
10. Kinfe

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11. Nematode filter ring (made of PVC ring, bandage cloth and tissue paper)
12. Stereomicroscope
13. Scissor
14. Rubber bands

Procedure:

- Collect the soil sample of about 250-500 grams and place in a washing tub containing known volume (500-1000ml) of water and stir well. Make sure to break all the clods.
- Allow the soil to get wet and settle for more than 4 hours.
- In the working space, place the 25-mesh sized sieve over the 325-mesh sieve over another washing tub for soil wash. There should not be any gap between the sieves.
- Now pour the supernatant into this washing tub
- As the supernatant passes through the 25-mesh sieve followed by 325 mesh sieves, most ofthe sediments are filtered out.
- The nematodes are trapped in the 325-mesh sieve.

Fig. 2. Sample wash with water using 25 and 325 mesh sieves.

- Rinse the contents of 325 mesh sieve carefully and transfer them to a glass bowl.
- Avoid pouring the sediments to the glass bowl.
- To the remaining soil sample, addwater and repeat the above process of soil wash for up to 5 times.
- Repeat the above process for at least 3 times, as we will obtain a clear solution devoid of most of the soil impurities.
- Meanwhile, we have to prepare the nematode filter ring

Fig. 3. Transferring water with nematodes in to the bowls

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Ring preparations:

- For that, take a PVC ring, cover it with a tissue paper and make it wet.
- This helps to bind the tissue paper well tothe ring and above it, place a piece of bandage cloth.
- Tie them properly with a rubber band and make sure that it's airtight.
- Place the ring over a sponge, and to which transfer the clear solution containing soil nematodes.
- As the water percolates, transfer the ring to another glass bowl containing water for nematode extension.
- Leave the set up for 24 hours for the nematode extension period.
- After 24 hours, we can observe and identify thenematode using stereomicroscope

Fig. 4. Filter ring for nematodes extraction.

Observations:

- Under the stereomicroscope, migratory plant parasitic nematodes can be seen along with other saprophytes of the rhizosphere.
- The fast moving and random ones are the saprophytes whereas the sluggish, coiled ones are the nematodes.

Fig. 5. Nematodes observation under stereomicroscope.

Precautions:

- Not suitable for very large commercial soil samples
- Prefer light soils over heavy soils as the latter would clog the sieve.
- Highly inactive nematodes can be lost.
- Deal the glass wares with utmost care.
- The nematode filter ring should be made properly

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