Protocols for killing, fixing & visualizing Plant Parasitic nematodes for Laboratory studies

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Introduction

Nematodes are microscopic organisms that significantly affect plant health and agricultural productivity. Studying their morphology and behavior is essential for effective management. Nematode killing is a crucial step in most studies because live nematodes can become distorted and spoiled when treated with cold fixatives. There are several methods for killing and fixing nematodes to ensure optimal preservation.

Killing and Fixatives

Several fixatives have been recommended for killing and fixing nematodes. The primary advantage of fixatives is to preserve the nematode's morphology and arrest post-mortem changes in cells and tissues. The best results are obtained when nematodes are killed quickly and fixed immediately.

Killing by heat

Collect nematode specimens in a small drop of water placed in a cavity block. Heat formalin-acetic acid fixative (4:1 or 4:10) to 100°C or slightly above, or place nematodes in a drop of water on a plain or cavity slide. Hold the slide over a small flame, moving it about for 5–6 seconds. Observe the nematodes carefully with the naked eye or under a stereoscopic microscope; they will twist briefly and suddenly straighten, indicating death.

Fixatives

Composition of F. A. 4:1 or 4:10

Formalin (40%formaldehyde)	= 10 ml
Glacial Acetic Acid	= 1or 10 ml
Distilled water	= up to 100 ml

Other fixatives commonly used are:

1) TAF

Formalin (40% formaldehyde)	= 7 ml
Triethanolamine	= 2 ml
Distilled water	= 91 ml

2) FAA

95% ethanol	= 20 ml
Formalin (40% formaldehyde)	= 6 ml
Glacial Acetic Acid	= 1 ml
Distilled water	= 40 ml

Processing of nematodes

Fixed nematodes often have unclear internal structures due to a granular appearance in the intestine. This can be resolved by clearing specimens using lactophenol or glycerol-based solutions.

Rapid Lactophenol Method

Composition

Phenol (liquid)	20 g
Lactic acid	20 g
Glycerol	40 g
Distilled water	20 ml

Warm the phenol with water until dissolved and then add the lactic acid and glycerol.

Glycerol-Ethanol

Transfer nematodes from the fixative to a small cavity block or other deep dish containing 0.5 ml of the following solution (I)

96% ethanol	20 parts
Glycerol	1 part
Distilled water	79 parts

Place the cavity block/dish in a desiccator containing about 1/10 of its volume of 96% ethanol for at least 12 h in an oven at 35-40 C. By doing so, almost all the water is evaporated and nematodes are left in a mixture of glycerol and ethanol.

Top up the cavity block/dish with a solution (II) of 5 parts glycerol and 95 parts 96% ethanol and place it in a partly closed Petri dish, to allow slow evaporation of the ethanol and maintained for at least 3 h at 40 C. The nematodes are then left in pure glycerol and are ready for mounting.

Slow method to glycerol

Transfer nematodes, after fixation, to the following dilute glycerol solution in a cavity block or small syracuse watch glass.

Glycerol	= 1.5 ml
Distilled water	= 98.5 ml

A trace of copper sulphate or a little thymol must be added to prevent growth of moulds. Place the dish containing the nematodes in a partially closed Petri dish and transfer the latter to a desiccator for atleast 4 weeks. The dilute glycerol evaporates very slowly and leaves the nematode in pure glycerol.

Staining of roots

Nematodes in the roots can be stained without duly staining the plant tissues, by any of the following methods

Lactophenol-acid fuchsin method

1. Wash root material to remove soil and debris.

2. Immerse roots in boiling 0.1% acid-fuchsin lactophenol for 1–3 minutes (adjust based on root toughness).

3. Rinse the roots in running water to remove excess stain.

4. Place roots in liquid phenol for differentiation for few hours

5. Examine the material under a stereoscopic microscope. Stained nematodes will appear red, contrasting with root tissues.

Byrd's Technique

Wash the infected roots free of soil or other adhering debris .Dip the roots in sodium hypochlorite (2.5%) for 1 min. Wash the roots again 3 to 4 times in running water .Keep the roots in plain water for atleast 4 hours and preferably for overnight to make roots free of bleach.

Preparation of stock solution

Acid fuchsin	2.5 g
Glacial Acetic Acid	250 ml
Distilled water	750 ml

Dilute the stock solution in the ratio of 1: 8 with water. Keep roots in the boiling diluted stock solution for 30 seconds. Wash the stained roots with running water to remove excessive stain. Dry the roots in two folds of blotting paper. Transfer the roots in glycerine. Examine the roots under stereoscopic binocular microscope.

Conclusion

Efficient killing, fixation, and staining techniques are essential for studying nematode structures. Fixed specimens can be stored for long durations, while root staining methods help identify nematodes within plant tissues. These techniques provide valuable tools for nematological research, aiding in plant pathology and agricultural pest management.

References

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