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**Protocol status:** Working
We use this protocol and it's working

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# ROOT-KNOT NEMATODE EGG EXTRACTION

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## ABSTRACT

Nematodes of the genus *Meloidogyne* (the root-knot nematodes) are among the most economically damaging plant-parasitic nematodes affecting horticultural and field crops worldwide. *Meloidogyne* spp. infest a wide range of plant species, including many major crop plants such as tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*), cotton (*Gossypium* spp.), coffee (*Coffea* spp.), maize (*Zea mays*), and rice (*Oryza sativa*).

There are several techniques for extracting plant-parasitic nematodes from soil samples, roots, or aerial parts of plants. To select the most appropriate method, it is important to contemplate the time available for processing the samples, the effectiveness of each procedure in relation to the purpose of the extraction, and the equipment a method requires. In addition, it is essential to consider some characteristics of the nematode genus or species to be extracted, such as:

a) the parasitic habit (whether a nematode is a sedentary or migratory endoparasite, an ecto-endoparasite, an ectoparasite, etc.),
b) the life cycle (what stages are found in soil or plant tissues), and
c) the size and displacement capacity.

For the extraction of root-knot nematode eggs, it is necessary to consider the entire root system as well as the number of roots to process. This protocol for egg extraction from root galls was developed for the facilities and materials available in UC Riverside's Department of Nematology. However, it can be adapted to different situations in other laboratories.

## IMAGE ATTRIBUTION

Tomato roots infected with the root-knot nematode *Meloidogyne incognita*. Godinez-Vidal D. 2023.

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## GUIDELINES

This root-knot nematode egg extraction protocol was adjusted using Howard (1990) and Hussey & Barker (1973) as references. Furthermore, this protocol was developed for the facilities and materials available in UC Riverside's Department of Nematology. However, it can be adapted to different situations in other laboratories.

## MATERIALS

Plastic apron (the protocol requires working with bleach)
Goggles (the protocol requires working with bleach)
Scissors
Blender or shaker
1,000-mL graduated cylinder
Plastic buckets
170-, 500-, and 500-Mesh sieves
Plastic beaker
Bleach (concentrated solution)
Brown paper towels
Mallet
Wash bottle

## SAFETY WARNINGS

Remember to take measures before working with bleach, such as using closed-toe shoes, appropriate clothing, protective goggles, and gloves.

## BEFORE START INSTRUCTIONS

Before beginning, be aware that the materials and shaker equipment to start are available.

Consider modifying the bleach concentration for the extraction based on the level of active ingredient of the bleach used. The 10% bleach solution that many protocols show is based on 5.25%-6% a.i. bleach. However, if using more concentrated bleach from popular brands, the concentration needs to be adjusted from 10% down to 8.25%, which means that 67 mL rather than 100 mL of bleach need to be dissolved in water for a total volume of 1 L to obtain a final concentration of 5.25-6% a.i.

# PREPARATION OF THE LABORATORY SPACE

1 Wash around the sink with hot tap water and lay brown paper towels on both sides of the sink, covering the work area.

## PREPARATION OF MATERIALS

2 Using hot tap water, wash the plastic buckets, the three sieves, the graduated cylinder, and the pair of scissors.

## PROCEDURE

3 Cut the tops (leaves, branches, and main stem) off the infected plants. Wash the roots by dipping the soil in a plastic bucket full of water. Rinse the roots by dipping them in a second plastic bucket full of water until all the soil particles have been removed from the roots.

4 Cut the clean roots with scissors and remove the remaining main plant stem.

5 Prepare a 10% bleach solution by pouring 100 mL of bleach into the graduated cylinder. Add water to a final volume of 1,000 mL (before preparing the bleach solution, check the “Before Starting” section for more information).

6 Place the roots of about one plant into the clean blender or shaker. Cover the roots with 10% bleach, just enough to cover them entirely, which should take c. 350 mL to 1 L.

7 Blend or shake the roots for 3 mins.

8 Meanwhile, place the 170-, 500-, and 500-mesh sieves on the plastic bucket in the following order: the 500-mesh sieves at the bottom and the 170-mesh sieve at the top (eggs will be collected from the two 500-mesh sieves).

9 Pour the blended or shaken roots into the top sieve of the stack of sieves and rinse with tap water. Rinse until the white bleach has disappeared. Use the mallet to hit the sieves gently on their sides to prevent the sieve pores from clogging. Discard the plant material collected in the top sieve.

10 Rinse the debris in the second sieve (500-mesh sieve) until all bleach has disappeared, and gently gather the root-knot nematode eggs on one side of the sieve. Collect the eggs with tap water into a clean plastic beaker using a wash bottle.

11 Gently rinse the fine debris in the last 500-mesh sieve until all bleach has disappeared. Carefully gather the root-knot nematode eggs again on one side of this last sieve, so no eggs will be lost through the screen. Use the wash bottle to collect the eggs into the plastic beaker.

12 Pass the water that was poured into the plastic bucket through the bottom 500-mesh sieve to collect any eggs that may have escaped. Gather the nematode eggs and collect them in a different plastic beaker. If this egg suspension is clean enough (no soil particles visible), then both egg suspensions can be poured together into the same beaker for further quantification.

13 Repeat until all the root material has been processed.