NEMATODES EXTRACTION

Extraction of nematodes from roots:

1. Wash the roots and place in a petridish filled with clean water.

2. Dissect the tissues using fine needle under a dissecting microscope(15-50X). Pick up the emerging nematodes, egg masses etc. from the suspension using a pointed brush.

Extraction of nematodes through soil:

1. Take about 100g soil and put in a beaker and add about 1 litre of water and stir about 15 seconds and the supernatant is pass through a 106µm sieve. Repeat the procedure.

2. The supernatant is again passes through 38µm sieve. The particles in the sieve can be collected and added to the centrifuge tube.

3. Distribute the nematode suspension equally in the centrifuge tubes. Balance the centrifuge tubes with water and centrifuge for 4 minutes at 1800g.Decant the supernatant over a 10µm sieve to collect the nematodes which do not precipitate.

4. Add $MgSO_4$ and centrifuge 3 minutes at 1800g. Pour the supernatant on 10µm sieve. Rinse the sieve with water using a wash bottle and collect the nematodes in beaker.

Counting of nematodes in the suspension

1. Nematode suspensions are made up to a fixed volume (100 ml) by adding water with a wash-bottle.

2. Carefully mix the suspension in the beaker, using air from an aquarium

pump or sucking in and out using a pipette.

3. Draw a fixed volume (1 or 2 ml) using a micropipette and place it in a counting dish. Similarly take another sub-sample in another dish.

4. Allow the nematodes to settle down and count them at 25-50x magnification. Take at least two counts for a sample

5. Calculate the number of nematodes present in the sample drawn on volume or weight basis, as the case may be using the following formula.

N = (n1 x v2) nematodes per ml sample
v1x v3
with n_1 = number of nematodes in v_1
v_1 = volume (ml) of the counted suspension obtained from v_2
v_2 = volume (ml) of the extracted sample (total suspension)
v_3 = volume (ml) of the sample
If nematode count has to be given on weight basis, replace v_3 with W, the weight of the sample.