T.R.I. ADVISORY CIRCULAR No.PM 5

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SAMPLING FOR NEMATODE ANALYSIS

(This Advisory Circular supersedes the Advisory Circular PM 5 Serial No. 03/10 issued in December 2010 and related previous Advisory Circulars)

1. Introduction

All species of pathogenic nematodes of tea inhabit roots and soil. However, their number and activity will depend on soil factors such as soil temperature, moisture, depth, use of agro-chemicals and organic matter etc. and plant factors such as age, cultivar and pruning cycle.

Tea fields or nurseries may show symptoms caused by nematodes such as slow decline in growth, yellowing of leaves, premature flowering and fruiting and stunted growth etc. due to other stress factors such as drought, water deficit, clayeyness, hard pan and water logging conditions, nutrient imbalances etc. Before taking samples for nematode estimation, such factors should be ruled out.

In order to assure a realistic nematode estimation, the sampling procedure should be different in a nursery and a field. Necessary sampling should be done to represent different cultivars, ages, conditions and symptoms of nematodes and weaknesses in plants of nursery or field.

Nematode diagnosis is also proposed as an internal quarantine measure in assuring use of nematode free and healthy planting materials restricting dissemination of parasitic nematode species through planting materials in the tea growing areas.

2. Sampling for Diagnostic Purposes

Early detection helps to check on the spread of nematode infestation. It could also curtail the extent of damage by enabling the adoption of appropriate and timely corrective measures. Sampling should therefore, be done at critical points where nematode introduction, establishment and or development could possibly occur. Such vigilance is most essential amongst nursery, newly planted young tea fields and mature tea. Infestation can only be confirmed by microscopic examination of soil and root samples.

Soil and root sampling in the nursery/field should be assigned to a responsible person and be carried out under close supervision. An analytical report would only be as good as the sample submitted.

3. Sampling in the Nursery

Sampling should commence about six months after the putting out of cuttings, or seed, in the nursery, when adequate roots have been developed. It is appropriate to sample the nursery prior to restacking plants. A minimum of 10 g of root material is needed per sample and the method adopted for sampling is as follows.

a. Take at least 5 plants at random from each bed of 1,000 plants.
b. Cut off the feeder roots, after removing the plants from the bags, and transfer the feeder roots to a clean polythene bag.
c. Cover the root sample with some moist soil to avoid drying.

d. Label each bag with Estate name, Division, Cultivar, Bed No., Age, Date of sampling and time.

In order to facilitate interpretation of the results of analysis and to give appropriate recommendations, it is necessary to send the duly filled Nursery History Fact Sheet issued from TRI.

4. Requisites of Field Sampling

Soil or root samples in the field should be drawn when the soil is adequately moist for at least 2 weeks.

Soil and root sampling should be avoided after any soil disturbances such as fertilizer, soil agrochemical applications and / or organic matter additions. A minimum of one-month period should be given for sampling.

It is also necessary to submit the duly perfected Crop and Field History Fact Sheet issued from TRI along with the samples to receive a proper diagnostic report.

4.1. Sampling in New Clearings and Mature Fields

Sampling of tea fields suspected with nematode infestation is important for diagnostic purpose and necessary recommendations. Both soil and roots are required for detecting the presence of nematodes in tea fields. The following method should be adopted to draw representative soil samples.

Additional samples may also be taken separately from fields and or patches and weak areas showing possible nematode symptoms. In fields due for pruning, sampling is recommended to be drawn 6 months prior to intended month of prune.

Divide the field into blocks of 2 ha each using natural boundaries such as footpaths etc. wherever possible (Figure 1).

a. Collect 25-30 samples at random points from each such 2 ha block.

b. Collect about 50 g soil from each sampling point. The samples should be taken 15 cm (6") away from the base of the bush and at a depth of 15-25 cm (6-10") with an Auger or ordinary crow bar ('Alavangoe").

c. Also collect feeder roots from each sampling point.

d. Pool the soil and root samples obtained from respective blocks and make about 500 g of the composite soil sample along with roots in a clean polythene bag and seal the bag properly.

e. Label each bag using a permanent marker with Estate name, Division, Field No., Cultivar, Block No., Age, Date of sampling and time. Also, a tag with above details may be inserted.

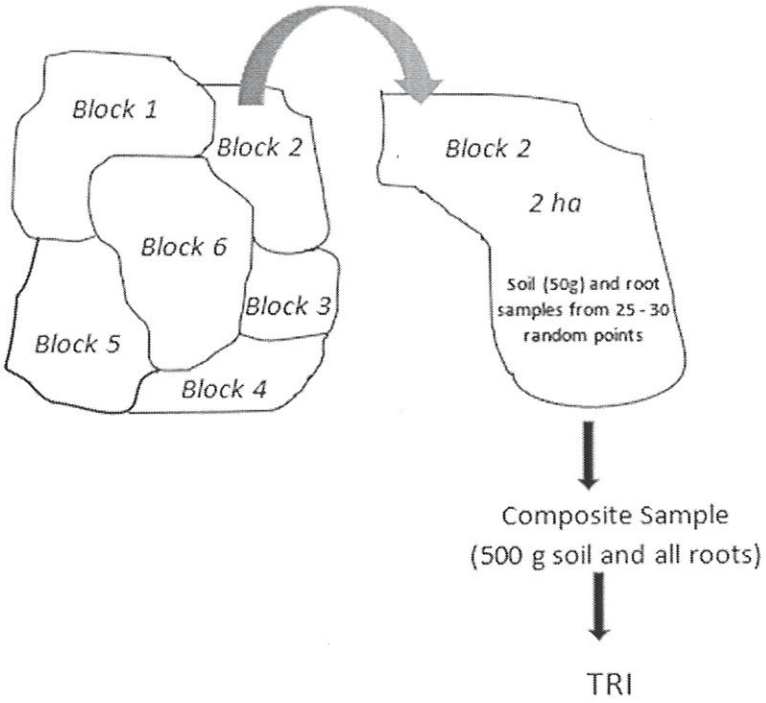

Figure 1: Method of collecting representative root and soil samples from young and mature tea fields for nematode estimation

4.2. Sampling in Mature Fields Prior to Uprooting

Soil and root populations of the parasitic nematode species in tea fields are expected to be aggravated with time. Hence, the residual nematode populations in mature tea fields due for uprooting would possibly serve as a source of inoculum in new clearings.

Hence, sampling is recommended to be drawn 6 months prior to intended month of uprooting to diagnose the nematode infestation level for adopting appropriate nematode management practices.

In fields due for pruning, sampling is recommended to be drawn 6 months prior to intended month of prune.

In fields identified for uprooting, sampling is recommended to be drawn 6 months prior to intended month of uprooting to diagnose the nematode infestation level for adopting appropriate nematode management practices.

5. Dispatch of Samples to TRI

The samples should be kept away from direct sunlight or any other condition that may promote heat during sampling and transport to avoid overheating in order to ascertain the life of the resident nematode populations.

The samples should be dispatched to the nearest Nematology Laboratories at Talawakelle, Hantane, and Ratnapura. Samples need to reach the laboratories compulsorily within 48 hours of sampling along with duly perfected Crop and Field / Nursery History Data sheet. Undue delay could lead to dehydration and death of nematodes, thereby resulting in erroneous estimates of nematode counts.

For a pre-arranged appointment to dispatch of samples to the TRI, please contact the respective Nematology laboratories.

6. Important

Please note that the Institute does not accept the following samples for nematode estimation.

a. **Water Samples:** Testing of water for estimating nematodes is a laborious exercise which requires advanced techniques for physical recovery of nematodes from a large volume of water.

   If an estate is in a nematode prone area and known to have nematode infested fields, one should assume that the water courses running through the fields are contaminated. For further details on preventing contamination, please refer to the Advisory Circular PM 8.

b. **Soil for Filling Nursery Bags:** It is mandatory that all soils intended to use for tea nursery work be sterilized. For selection of soils for nurseries and soil sterilizing techniques, please refer to the Advisory Circular PN 2.

Tea Research Institute of Sri Lanka
Talawakelle