

Original Research Article

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Nematode Extraction from Banana (*Musa* AAA) and Plantain (*Musa* AAB) Roots at Nematlab, Ecuador

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ABSTRACT

Keywords

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A description of the procedure used for nematode extraction from banana (*Musa* AAA) and plantain (*Musa* AAB) roots at the Nematology Laboratory of Nematlab, Ecuador is given. This procedure is an adaptation of the method of Taylor and Loegering (1953) with modifications based on experimental results. A literature review on nematodes that attack *Musa* roots, their root symptoms and the procedures used for their extraction in other banana and plantain producing countries is included with economic thresholds suggested. Finally, the advantages of the described methodology are indicated.

Introduction

Nematodes are the main pathogen of the banana root system (Stirling 2023, Sikora *et al.*, 2018, Nega, 2015, Guzmán-Piedrahita, 2011a, 2011b, Volcy, 2011, Dubois and Coyne, 2011, Quénéhervé, 2008, Gowen *et al.*, 2005). The most important nematodes in relation to the damage they cause and their occurrence in *Musa* are *Radopholus similis*, *Helicotylenchus multicinctus*, *H. dihystra*, *Meloidogyne incognita*, *M. javanica*, *Pratylenchus coffeae* and *P. goodeyi* (Baker *et al.*, 2014, Volcy, 2011, Quénéhervé, 2008, Gowen *et al.*, 2005, Gowen, 2000a, 2000b, 1995, De Waele, 2000, De Waele

and Davide, 1998, Bridge *et al.*, 1997, 1995, Afreh-Nuamah, 1995, Fogain, 1994, Gowen and Quénéhervé, 1990, Siddiqi, 1973).

In Latin American banana producing countries, usually only polyspecific communities occur, consisting of a mixture mainly of *Radopholus similis*, *Helicotylenchus* spp. and with low frequency and numbers of *Pratylenchus* spp. and *Meloidogyne* spp. Nematodes delay foliar emission, lengthen the crop's vegetative cycle, reduce bunch weight and yield (Medina *et al.*, 2022, Chávez *et al.*, 2020, 2016, Jaramillo *et al.*, 2023, 2019, Quénéhervé *et al.*, 1991a, 1991b).

Nematode management decisions are based on periodic monitoring of their populations in roots. To estimate the number of nematodes in roots, it is necessary to separate them from the roots and perform counts. There are several methods for nematode extraction from roots (Shokoohi, 2025, Hallmann and Subbotin, 2018, Marais *et al.*, 2017, OEPP/EPPO, 2013, Ravichandra, 2010, Coyne *et al.*, 2007, Bezooijen, 2006, Zuckerman *et al.*, 1990, Hooper *et al.*, 2005, Hooper, 1990, 1986, Southey, 1986, McKenry and Roberts, 1985, Barker, 1985, Ayoub, 1980). Comparisons between individual methods and between methods and combinations of them for the extraction of banana root nematodes have been carried out by Araya *et al.*, (1995), Paneque *et al.*, (1988), Alvarado-Soto and López-Chaves (1981), Escobar and Rodríguez-Kabana (1980), Quimí and Villacís (1977), and Gowen and Edmunds (1973).

An extraction method is of quantitative value only when it provides an accurate estimate of the true number of nematodes present in the samples with minimal error (Brown *et al.*, 1987). The decision to use a particular method is primarily related to the availability of the required equipment and the required extraction efficiency. However, the most important factor is that the method used is standardized for all samples and investigations in each laboratory since the goal is to relate expectations of crop loss to nematode population densities (Ferris, 1987). Several experiments (Chávez and Araya, 2016, Araya *et al.*, 1998, 1995, Araya and Carrillo, 1996, 1995, Araya and Centeno, 1997, Araya and Cheves, 1996, Araya and Obando, 1997, Araya and Vargas 1997, 1996) have been conducted to optimize the Taylor and Loegering (1953) method for banana (*Musa* AAA) and plantain (*Musa* AAB) root nematode extraction. The objective of this publication is to describe the procedure that is used in the nematology laboratory of NEMALAB, Ecuador.

Root symptoms in nematode attack on plants

When *Musa* (banana and plantain) roots are still white and creamy, it does not mean that they are free of nematodes. As indicated by Mai (1985), McKenry and Roberts (1985), Ayoub (1980), extensive loss of yield can occur when one or more nematode species may be feeding on a given root plant, without showing obvious or specific plant symptoms. It is known that in white-creamy roots infected with nematodes histological and physiological cell alterations occur (Jones *et al.*, 2016, Haegeman *et al.*, 2010, Grunewald *et al.*, 2009, Wyss,

2002, Blake, 1966) which restrict water and nutrients uptake (Lilley *et al.*, 2024, Sikora *et al.*, 2018, Haegeman *et al.*, 2010, Wilcox-Lee and Loria, 1987). Then most of the time the damage goes unnoticed due to the hidden nature of nematodes and the non-specific damage symptoms, which can be confused with nutrient deficiencies, drought or other soil pest or pathogen problems as indicated by Lahm *et al.*, (2017).

***Radopholus similis* Musa root symptoms**

Radopholus similis is an obligated biotrophic migratory endoparasite that enters roots either from soil or through the corm tissue (Blake, 1966yy) and penetrates roots primarily through the root tip or cap, but any part along the root can be invaded (Holdeman, 1986, Stover, 1972, Blake, 1966, Loos, 1962, Loos and Loos, 1960). In roots, the nematode actively moves through the cortical parenchyma, destroying cells as juveniles and adult stages feed on the cell cytoplasm (Oramas and Román, 1987). Cells adjacent to the nematode separate, and their nuclei and nucleoli enlarge. The nucleus disintegrates, the cell wall ruptures, and a cavity forms through which the nematode moves (Román, 1978). These cavities and galleries enlarge and lengthen as the nematode migrates, but without crossing the endodermis (Dropkin, 1989, Blake, 1966). In general, the vascular cylinder of the root is not invaded by *R. similis* (Elsen, 2002, Oramas and Román, 1987, Román, 1978), but Loos and Loos (1960) reported that it does invade when roots are infected by *Fusarium*. Araya and De Waele (2004) found that this nematode can be found in the vascular cylinder of banana roots in low numbers. In citrus roots, the most notable response to *R. similis* feeding is the formation of tumors in the pericycle, this reaction occurs when the nematode passes through the endodermis and migrates to the vascular cylinder of the root (Maggenti, 1981). *Radopholus similis* can remain within banana roots for three or four generations or until the root dies.

Radopholus similis causes dark brown and reddish-brown lesions along the entire root (Figure 1A), on the root epidermis, cortical parenchyma, and vascular cylinder (Sikora *et al.*, 2018, Guzmán- Piedrahita, 2011a, Volcy 2011, Jackson *et al.*, 2003, Orton and Siddiqi, 1973, Blake, 1966). These lesions penetrate and cross the cortical parenchyma and sometimes reach the vascular cylinder (Araya and De Waele, 2004, Valette *et al.*, 1997, Mateille, 1994). The lesions generally coalesce, causing atrophy of the root tissue. In severe infestations, *R. similis* migrates to the corm, as described

by Román (1978), where it causes reddish-brown to black lesions that often spread even to the corms of newly sprouted suckers. This nematode induces more root rot than *Meloidogyne* spp. The base of the stem often has corky areas, caused by high infections that have led to the loss of both anchoring and absorbing roots.

***Helicotylenchus* spp. Musa root symptoms**

Helicotylenchus spp. is an obligate biotrophic migratory ecto-endoparasite (Sikora *et al.*, 2018, Guzmán-Piedrahita, 2011b, Orion and Bar-Eyal, 1995, Blake, 1966) that enter roots either from soil or through the corm tissue (Blake, 1966) and induces necrotic lesions on the surface of the roots (Siddiqi, 1973). Small feeder roots may be destroyed, and those remaining may appear necrotic and fall off when the roots are handled. Larger roots show small black surface lesions and larger necrotic areas. When roots are cut longitudinally, most of the damage will be confined to the outer surface of the root, while little internal damage is evident unless severe necrosis and decay has begun.

Ecto-endoparasitic nematodes such as *Helicotylenchus* spp. feed along the surface of roots and, in some cases, for extended periods at specific sites, extracting nutrients from the deeper root tissues without causing apparent or noticeable damage. The histology of *H. multincinctus* isolated from banana roots was studied by Orion and Bar-Eyal (1995) in *Arabidopsis thaliana*. They observed that the nematode concentrates around the root apex or calyptra, near the root hairs, but any part along the root can be infected.

The entire nematode body penetrates the root to feed within 36 hours of inoculation (Blake, 1972, 1966). Females, and probably males, feed directly from the root parenchyma cells at a depth of 4 to 6 cells (Blake, 1972, 1966). *Helicotylenchus* spp. feeds on the outer cells of the root epidermis, it produces superficial lesions, circular or elongated in shape and brown, reddish-brown, or dark in color (Figure 1B-C) at the point of infection where the nematode establishes itself to feed (Orion and Bar-Eyal, 1995).

When populations are high, the lesions coalesce, forming elongated necrotic areas. In severe infections, when the roots split longitudinally in half, the progression of necrosis toward the vascular cylinder is observed (Gowen, 2000b, McSorley and Parrado, 1986).

Many of the fine roots may be destroyed by the nematode, and those that remain appear necrotic and detach easily when handled (McSorley and Parrado, 1986). Tertiary, secondary, and primary roots exhibit superficial black lesions and necrotic areas. Only when very severe damage occurs do fruiting plants that are not supported or that have been exposed to strong winds fall.

***Meloidogyne* spp. Musa root symptoms**

These nematodes are not thought to be as important as the more destructive lesion forming species which in mixed populations are generally dominant. They are obligated biotrophic sedentary endoparasites that cause considerable deformation and stunting of roots. *Meloidogyne* spp. are commonly found in sandy soils (Sarah, 1989, Ouénéhervé, 1988, Boncato and Davide, 1980a) and populations may be greater in the more distant parts of the root system (Pinochet, 1977). Root-knot nematodes are a problem in Taiwan where they are the major nematode pest and, in some localities, new to bananas where the introduction of *R. similis* and *Pratylenchus* spp. has been avoided (Vargas and Araya, 2004, Gowen and Ouénéhervé, 1990).

The infective juvenile stages penetrate roots intracellularly, and root invasion occurs within 6 hours and continues for up to 24 hours (Burton, 1987). Direct penetration into the roots causes cell death. When the nematodes move through the cortex, they can feed on cortical cells, but in most cases, feeding occurs on endodermal or pericyclic cells. *Meloidogyne* spp. induces specific feeding sites by forming giant cells, which are multinucleated cells resulting from multiple mitoses in the absence of cytokinin's.

Adiko (1989), inoculating *M. incognita* into *in vitro* propagated *Musa* AAB cv. Horse plants grown in 10 L pots, observed proliferation of fine roots at the infection points as it is observed in field conditions (Figure 1D). He also reported root deformation and irregular root thickening as it is found in commercial field samplings (Figure 1E) and reported the formation of galls.

In greenhouse experiments with banana plants inoculated with *M. incognita* and grown in 1.8 L pots, the formation of corky, suberous tissue was observed, resulting in irregular swellings and thickenings, and a corrugated epidermis of the primary and secondary roots. These symptoms are consistent with those reported by Stanton and Cobon (2000) in banana and

those indicated by Adiko (1989) in plantain. When the infection occurs at the root tips, growth ceases, and new roots proliferate at the infection point (Figure 1D). At the end of the thin and fine roots, some galls characteristic of infection by this nematode can be observed. Generally, *Meloidogyne* spp. does not invade the corm (Pinochet, 1977), but Quénéhervé and Cadet (1985) and De Waele and Davide (1998) found that it can colonize the outer layers of the corm up to 7 cm deep.

***Pratylenchus* spp. *Musa* root symptoms**

Pratylenchus coffeae is an obligatory biotrophic migratory endoparasite that enters roots either from soil or through the corm tissue (Blake, 1966). Infections caused by *P. coffeae* are very similar to those caused by *R. similis* (Van den Bergh, 2002, Blake, 1972, 1966), and in practice, they are very difficult to differentiate. It causes black necrotic lesions (Figure 1F) with surface cracks. When roots are cut longitudinally, typical brownish-red lesions (Figure 1F) are seen along the cortex. The damage it caused is probably underestimated because of the similarity of the necrosis of the root and rhizome to that caused by *R. similis*.

For technicians familiar with the symptoms of nematodes in *Musa* roots it is easy and common to distinguish the damage it caused from that induced by *Helicotylenchus* spp. and *Meloidogyne* spp. When the damage is advanced, dark, brown, and reddish-brown lesions are frequently observed on the outermost region of the roots. These lesions penetrate and cross the root tissue. The lesions generally coalesce, causing atrophy of the root tissue. In severe infections, the nematodes migrate to the corm, causing reddish-brown to black lesions that often spread even to the corms of newly sprouted suckers. It is common to observe extensive areas of purple, reddish-brown or necrotic color in the epidermis and cortical parenchyma of the root, resulting in cracking and detachment.

Dropkin (1989) states that it can damage the vascular cylinder of the root. Root colonization induces cavities in the cortical parenchyma (Pinochet 1978). Lesions and necrotic areas are also observed in the corm (Bridge *et al.*, 1997, Quénéhervé and Cadet, 1985, Siddiqi, 1972). Oramas and Román (1987) and Pinochet (1978) found destroyed cells, galleries, necrosis, and all developmental stages of the nematode in the cortical zone of roots damaged by *P. coffeae*.

Banana and plantain roots nematode extraction procedures

The liquefying-sieving method introduced by Taylor and Loegering (1953) is the basis for the recovery of nematodes from banana and plantain roots in the producing countries of Latin America.

This method is commonly used in Honduras (Tarté *et al.*, 1981, Stover and Fielding, 1958), Colombia (Gómez, 1980), Ecuador (Larrea-Bernal *et al.*, 2025, Arreaga *et al.*, 1989, Escobar and Rodríguez-Kabana, 1980), and Jamaica (Hutton, 1978).

Also, it is used on other banana producing countries like the Canary Islands (Rodríguez, 1990), West Africa (Bridge *et al.*, 1995, Vilardebo and Guerout, 1976), Oman (Mani and Al Hinai, 1996), Cameroon (Price, 1994), Vietnam (Inge and Nguyen, 2000) and Philippines (Boncato and Davide, 1980a, 1980b), with modifications by each author.

The incubation method is used in Florida, USA (McSorley and Parrado, 1981) and Egypt (Youssef and Aboul-Eid, 1996), while in Australia (Stanton and Cobon, 2000), Burkina Faso (Sawadogo *et al.*, 2001) and Ivory Coast (Adiko and Badou N'Guessan, 2001) they use the mist chamber. In Uganda (Barekye *et al.*, 2000, Speijer and De Waele, 2001), Tanzania (Speijer and Bosch, 1996), Nigeria (Speijer *et al.*, 2001), and Venezuela (Haddad *et al.*, 1975), a combination of methods is used, and after liquefying the roots, the nematodes are extracted using a modified Baermann funnel method (Hooper, 1990). In Dominica, Saint Lucia, St. Vincent and Grenada, after liquefying the roots, the suspension is incubated with hydrogen peroxide for 2 days (Ambrose, 1984).

In Kenya, Satyanarayana *et al.*, (2000) extract nematodes by placing 2-5 cm root pieces directly into Baermann funnels. Guzmán *et al.*, (1994) in Mexico and Daneel *et al.*, (1999) in South Africa liquefy the roots, sieve the suspension, and centrifuge it in a sugar solution. Some researchers also combine root maceration with centrifugation and flotation (Binks and Gowen, 1996) or with Baermann funnels using hydrogen peroxide (Whyte and Gowen, 1974). Hahn *et al.*, (1996) extract nematodes from roots by flotation and centrifugation. Depending on the researchers and with the passage of time, there are also differences in the methods used, even within the same country.

Economic thresholds for banana nematodes

In Ecuador, using the Taylor and Loegering (1953) method, ANEMAGRO-Análisis Nematológico para el Agro S.A. (2013) consider 10000 *R. similis* for 100 g of total roots in samples taken in recently flowered plants or 2000 *R. similis* by 100 g of living (functional) roots. In samples taken in front of the follower suckers the recommendation is 3000 *R. similis* by 100 g of total roots or 1000 *R. similis* by 100 g of living (functional) roots (ANEMAGRO, 2013). Also, in Ecuador, with the same method, INIAP - Instituto Nacional Autónomo de Investigaciones Agropecuarias (2018) suggest 10000 *R. similis* by 100 g of total roots in samples taken in the interspace between the mother plant and its respective follower sucker, and when the samples are taken in front of the follower suckers with a height between 1.5 and 2.0 m 2500 *R. similis* by 100 g of total roots.

In Dominica, Saint Lucia, Saint Vincent, Grenada, and parts of Jamaica, the established threshold is 4000 nematodes per 100 g of roots (Ambrose, 1984), where the nematodes are extracted by macerating the roots for 10 seconds in a kitchen blender and incubating the solution with hydrogen peroxide for 2 days. In Costa Rica and other Central American countries (Figuerola, 1994, Tarté and Pinochet, 1981), Colombia (López, 1976, Gómez, 1980), and the Philippines (Boncato and Davide, 1980b), 10000 *Radopholus similis* per 100 g of roots are considered the economic threshold using the Taylor and Loegering (1953) method. However, Pinochet (1987) considered 20000 *R. similis* per 100 g of roots as an acceptable amount for areas of Central America, and CORBANA in Costa Rica uses a threshold of 10000 *R. similis* per 100 g of roots for samples taken from the interspace between the parent plant and the follower, and 8000 individuals when the sample comes from the follower (Araya, 2002). Chávez *et al.*, (2020) in Ecuador with nematode populations lower than 5000 nematodes per 100 g of roots using the root maceration reported that those population levels were not enough to develop root symptoms but it reduced ratooning and yield. The same observation was made by Medina *et al.*, (2022) in Costa Rica, where no root symptoms were observed with population lower than 5000 nematodes per 100 g of roots, but it reduced ratooning and yield.

Guerout (1972) determined a tolerance limit of 1000 *R. similis* per 100 g of roots in Ivory Coast, also using the Taylor and Loegering (1953) method. In South Africa, Jones and Milne, 1982, cited by Sarah (1989),

established a threshold of 20 *R. similis* per 100 g of roots, but the method was not mentioned. In Tenerife, using the root maceration method, they recommend treatment when *Pratylenchus* populations exceed between 9000 and 12000 and when *Meloidogyne* populations exceed 1000 per 100 g of roots (Perera and Molina, n.d.).

Rajendran *et al.*, (1979) found that if the nematode population (*R. similis*, *Pratylenchus coffeae*; *Helicotylenchus multicinctus*) exceeds 2000 per 10 g of roots, there is a drastic reduction in yield, however, the method used to extract the nematodes was not described. Rodríguez and Rodríguez (1977) suggested for another nematode, also a migratory endoparasite of banana, *Pratylenchus goodeyi*, a population between 6000 and 8000 per 100 g of roots. Nematode extraction was performed by combining root blending with sieve washing and incubation.

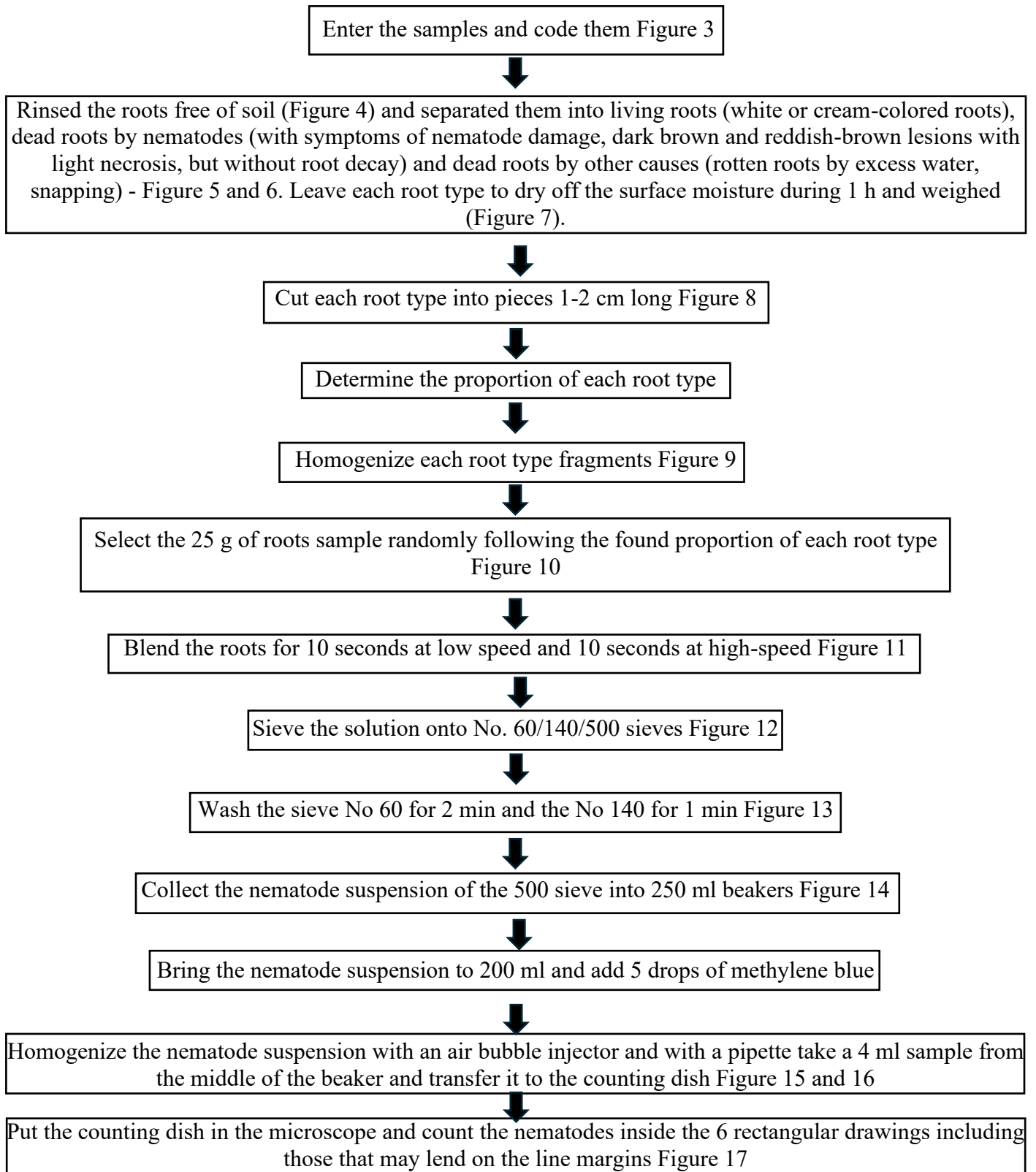
In Philippines Davide (1992) reported a significant yield reduction in terms of bunch weight when plants were inoculated with beyond 1000 *R. similis* by plant. Bunch weight was reduced by 60.5, 50.0, 40.3 and 14.3% with *R. similis* inoculum levels of 4000, 3000, 2000 and 1000 per plant, respectively. In Costa Rica (Moens and Araya, 2002) in individual plants inoculated with 1000 *R. similis* or 1000 *Pratylenchus coffeae* or 1000 *Meloidogyne incognita* by plant found a bunch weight reduction of 6.4 kg (27 %), 5.6 kg (24 %), and 7.5 kg (32 %), respectively. Given that the four nematode genera cause damage to the crop, for the implementation of their management options, the reference values given should be for the population of all the phytoparasitic nematodes present, as has been suggested by Jaramillo *et al.*, (2019), Aguirre *et al.*, (2016a, 2016b), Salguero *et al.*, (2016), Ramclam and Araya (2006).

Economic thresholds established with a particular method cannot correspond to and be applied with another method. Furthermore, for population values to be fully comparable and equitable, they must have been obtained using the same method and with the same steps. That is, if we consider the Taylor and Loegering (1953) method, the same blending times must be used, employing blenders of the same brand and speeds, the same root segment sizes, the same volumes of water for blending, the blender container must be of the same capacity and shape, the sieves used must have the same opening, placed in the same order and with the same washing times.

Figure.1 A-F **A)** dark brown and reddish-brown lesions on roots cut longitudinally, that penetrate and cross the cortical parenchyma and sometimes reach the vascular tissue caused by *Radopholus similis*, **B)** reddish-brown spots that can coalesce and form necrotic areas on the root epidermis caused by *Helicotylenchus* spp., **C)** Brownish stains on the cut root lengthwise induced by *Helicotylenchus* spp., **D)** root proliferation at the *Meloidogyne* spp. infection point, **E)** irregular root thickening induced by *Meloidogyne* ssp., **F)** dark brown and reddish-brown lesions on roots epidermis caused by *Pratylenchus coffeae*.



Figure.2 Schematic representation of the nematode extraction procedure used in the nematology laboratory NEMALAB, Ecuador.



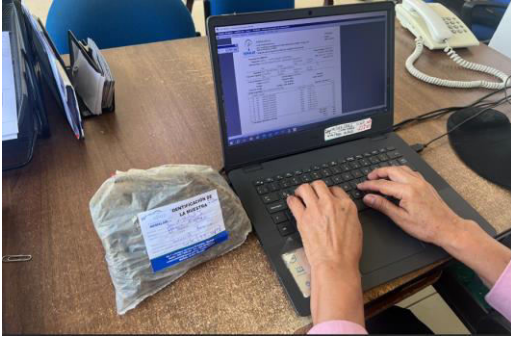


Figure 3. Receipt of root samples in the laboratory, registration and coding.



Figure 4. Washing the roots free of soil.



Figure 5. Root separation into living roots (left), dead roots by nematodes (center), and dead roots by other causes (right).



Figure 6. Left: dead roots by nematodes, center: living roots, right: dead roots by other causes.



Figure 7. Weigh each root type.



Figure 8. Cut each root type crosswise into pieces between 1 and 2 cm long.



Figure.9 Homogenize each root type.

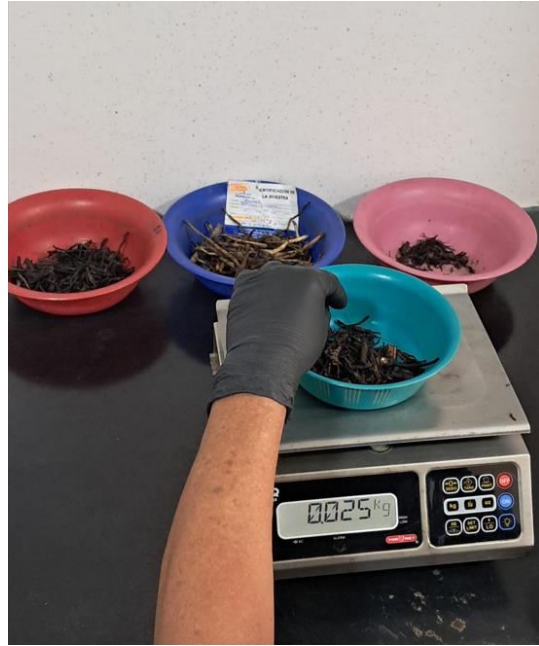


Figure.10 Recollection of the 25 g root sample.



Figure.11 Blend the roots in 150 ml of water for 10 seconds at low speed followed by 10 seconds at high speed.



Figure.12 Sieve the liquefied solution onto 0.25/0.106/0.025 mm mesh.



Figure.13 Wash the 0.25 mm sieve for 2 minutes and then the 0.106 mm sieve for 1 minute.



Figure.14 Collect and wash the nematodes of the 0.025 mm sieve into a 250 ml beaker.

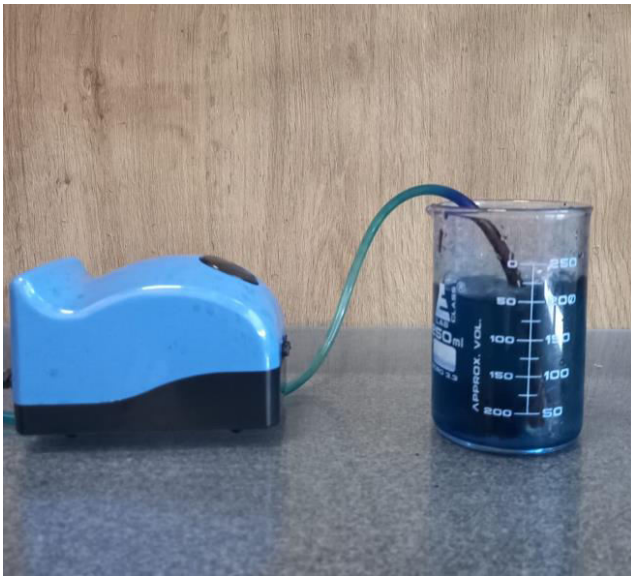


Figure.15 Homogenize the nematode suspension with an air bubble injector for 30 seconds.

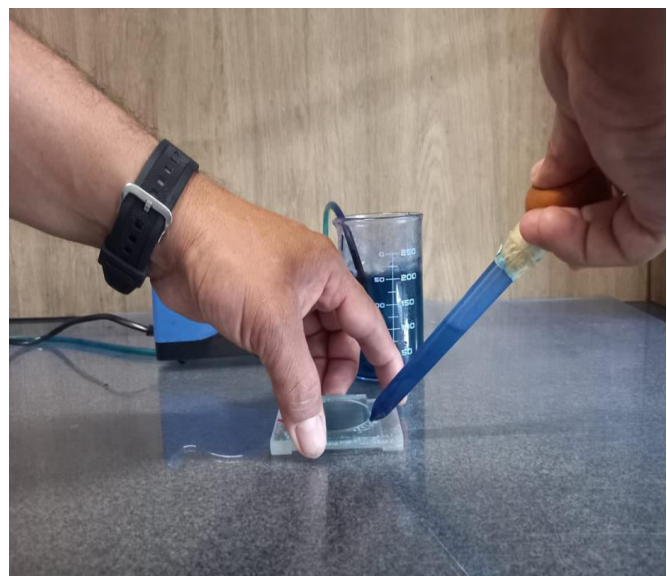


Figure.16 With a pipette take a 4 ml sample and put into the counting dish.



Figure.17 Identify and enumerate the nematodes present in the aliquot using an Olympus BH microscope at 4x magnification.

Even more, for those values to be comparable, the root samples must be taken from the same place and plant reference.

1. Root samples are received at the laboratory, registered, and individually coded for processing (Figure 3). When they cannot be processed fresh, they are stored in a refrigerator Indurama serie RS-10989-593 adjusted to 8-10 °C until processing, a period that normally does not exceed 48 hours.

2. Each sample is rinsed free of soil (Figure 4) with potable water and then roots are separated into living roots (white or cream-colored roots), dead roots by nematodes (with symptoms of nematode damage, reddish-brown lesions on the outer part of the roots penetrating throughout the cortex and then turns necrotic along the entire root; in the epidermis, cortical parenchyma, and vascular cylinder, but without root decay) and dead roots by other causes (rotten roots by excess water, snapping) - Figure 5 and 6.

During the root separation process, if in some roots, it is necessary to cut some damaged parts, they are classified accordingly. Leave each root type to dry off the surface moisture for 1 hour and weigh each individually (Figure 7).

3. Cut each root type crosswise into pieces between 1 and 2 cm long and place them in the corresponding colored labeled plastic container Figure 8.

4. Each root type (Figure 7) is weighed separately on a Fisher Scientific serie 10309201 scale of 710 g ± 1 g and the proportion of each type is determined. For example, in a root sample of 132 g of total roots, with 80.1 g of living roots, 48.9 g of dead roots by nematodes, and 3 g of dead roots by other causes, there would be 60.7% of living roots, 37% of dead roots by nematodes, and 2.3% of dead roots by other causes. Then the sample size used of 25 g, would have of living roots $25 * 60.7\% / 100 = 15.17$ g, of dead roots by nematodes $25 * 37\% / 100 = 9.25$ g and of dead roots by other causes $25 * 2.3\% / 100 = 0.57$ g. $15.17 + 9.25 + 0.57 = 24.99$ g.

5. Root pieces of each root type are homogenized (Figure 9) by manual mixing. Then, the 25 g root sample is taken and weighed. Extract in three times the required weight of each root type and put together into the plastic container. The remaining roots of each type are stored until the nematode count is performed on the 25 g sample, just in case to require repeating the analysis. Then pour it into the blender container (Figure 11) where 150 ml of potable water is added.

6. Blend (Figure 11) the roots at low speed for 10 seconds followed by 10 seconds at high speed in a kitchen blender (Osterizer; Sunbeam-Oster).

7. Sieve the liquefied solution through a set of overlapping sieves (Figure 12) from top to bottom of 0.25/0.106/0.025 mm (No. 60/140/500 mesh).

8. With water without pressure and in a circular wave wash the 0.25 mm sieve for 2 minutes and then the 0.106 mm sieve for 1 minute (Figure 13).

9. With a plastic wash bottle containing water, collect and wash the contents of the 0.025 mm sieve into a 250 ml beaker (Figure 14).

10. With water, bring the nematode suspension to a final volume of 200 ml and add 5 drops of methylene blue solution (5 g L⁻¹ of distilled water) to stain the root parts that remain. When the sample cannot be read immediately, the beaker containing the suspension is stored in a refrigerator chamber, maintaining the temperature between 8 and 10 °C.

11. Homogenize the nematode suspension with an air bubble injector (Figure 15) for 30 seconds.

12. Using a graduated pipette take a 4 ml aliquot from the middle of the beaker and transfer it to the counting dish with 6 rectangles (Figure 16) which is equivalent to 2 ml effective solution for nematode reading.

13. Identify and enumerate the nematodes present in the aliquot using an Olympus BH microscope at 4x magnification (Figure 17). The data are first converted to the number of nematodes in 200 ml of solution and then to 100 g of roots. Therefore, the number of nematodes in the sample would be: nematodes x (200 ml of suspension / 2 ml aliquot x 100 g of reported roots / 25 g of processed roots), which is equivalent to a correction factor of 400. Then, the value obtained from the 2 ml reading is multiplied by 400 and is equivalent to the number of nematodes in 100 g of roots.

Advantages of the root nematode extraction procedure

The method allows the recovery of both active and inactive nematodes. Although it involves several sequential steps, apart from reading, all of them can be performed by non-specialized personnel. The required

equipment is readily available, and except for the microscope and fine sieve, the price is affordable.

Author Contributions

César Chávez: Conceived the original idea, designed the diagram and help to write the manuscript. César Chávez-Santos: Planned and took the photos and help to write the manuscript. Orlando Aguirre: Planned the photos and help to write the manuscript. M. Araya: Organize and structure the information, write the draft, and review it.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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