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COMPARATIVE EFFICIENCY IN DIFFERENT METHODS OF EXTRACTING NEMATODE FROM SOIL AND PLANT MATERIAL

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ABSTRACT
Nematodes cause lot of damage to crops, in order to identify and control them the proper quantification of its population in the soil and/or plant should be known through the use of different extraction methods. These extraction methods were used and compared for suitability in terms of the life stages of nematodes extracted from soil and plant material. Seinhorst method is found to be the most appropriate technique for extraction of cyst nematode, while the Automatic Zonal Centrifugation was the most suitable in extracting mostly all stages of the nematodes from both soil and plant materials. Different techniques have its own advantages and disadvantages in which the choice of the desired technique depends mainly on the target stage of the nematode that an individual will be working with or to the purpose of extraction.

KEY WORDS: Baermann, Cobb's extraction, zonal centrifugation, Seinhorst, *Meloidogyne*.

INTRODUCTION

Nematodes are found in a wide variety of habitats, free-living nematodes live in the soil and in freshwater and marine sands and muds. In soil, they are important components of nutrient turnover. Other nematodes are parasites of almost every species of animal, including humans and plant and cause enormous social and economic damage. They are also known as 'eelworms' and 'roundworms'. Nematodes are typically worm-like, except they are unsegmented. Essentially, the body form is similar to a flexible cylinder with a rounded head and a pointed tail. They need at least a film of water for active movement, which is an undulating motion similar to that of an eel. The majority of nematodes are microscopic in size, although some species that parasitize animals are much larger and can grow to several centimeters in length. To have an idea about the quality and quantity of the nematode population in soil and plant, appropriate extraction technique should be followed. The choice of an extraction method depends on the aim of the extraction, time and equipment available, the required efficiency and preference of the person performing the extraction. The efficiency of a method varies with nematodes genera, life stage, material used, soil type, moisture content of the soil and performance of the person carrying out the extraction. Viglierchio and Schmitt (1983) compared nematode extraction techniques and have been studied for the needs of the individuals. The main objective of extraction is to get a good specimen at a desired quantity for further observation and study. Selection of different methods in extracting the soil nematodes depends on various parameters. Some methods are more effective for some group of nematodes than others and the efficiency of nematode extraction can vary.

Therefore, it is very important to learn about the different extraction methods of the nematodes appropriate for different species. A number of methods have been developed for the extraction of nematode from soil and plant materials. To know the efficacy of different methods, research has been carried out by following different method at ILVO, Merelbeke and at faculty of Biology, K.L. Ledeganckstraat, Ghent University. Gent, Belgium.

The objectives of the work include:

A. Extraction of Cyst Nematode:

1. To extract cyst nematodes from soil using stirring and Seinhorst method.
2. To compare the two methods in terms of the total number of cysts recovered.
3. To know the advantage(s) and disadvantage(s) of each method.

B. Extraction of Vermiform Nematodes from Soil:

1. To undergo extraction of vermiform nematodes from soil by Cobb's decanting and sieving + pie-pan method and Automatic Zonal Centrifugation (AZC) method.
2. To make comparison of the two methods based on the number of nematodes collected per genus.
3. To identify the advantage(s) and drawback(s) of each method.

C. Extraction of Nematodes from Roots:

1. To perform extraction of nematodes from plant roots using Automatic Zonal Centrifuge and Baermann funnel methods.
2. To distinguish the advantage(s) and disadvantage(s) of using each method.

METHODOLOGY

1. Nematode Extraction

A. Extraction of Cyst Nematode

The soil sample is taken from the field infected with *Heterodera* for stirring and Seinhorst methods. In stirring method, 300 grams of dried soil sample was used and processed (Persmark Lotta *et al.,* 1992), while in Seinhorst method, soil were not dried but was kept in a

container to conserve moisture and 500 grams of the soil sample was taken for the extraction. Cysts were extracted using the Seinhorst method. Collected cysts from the two methods were counted under a stereomicroscope in the next preceding day.

B. Extraction of Vermiform Nematodes from Soil

1. Automatic Zonal Centrifugation (AZC) method

For this method, 200 ml of soil sample was taken. Organic fraction of the soil samples were separated with the mineral fraction. This was done by placing the soil sample into a 350µm sieve placed on a shaker machine while slowly pouring water over it. A beaker was at the bottom of the sieve to collect the mineral fraction of the sample while the debris was collected from the sieve. The debris (containing the organic sample) was then blended at high speed before it was mixed to the mineral fraction in the beaker. The solution containing the mineral and organic fraction was then processed through the Automatic Zonal Centrifuge. MgSO4 and kaolin content of the machine were checked. The AZC machine can take only half of the sample, thus, from the 200 ml soil sample, only 100ml was actually processed using the machine. The clear nematode suspension was then collected in a small beaker and was set aside for counting and identification of nematodes.

2. Cobb's Decanting and Sieving Method + Pie Pan Method

Soil sample (300 ml) was taken and measured using a beaker for the extraction, then processed by using different size sieves. The suspension from the last sieve was collected for processing using the pie pan or cotton wool method to get clearer suspension of nematode. One day later the filter is removed and the suspension is collected in a beaker. Identification and counting was done in the following day.

C. Extraction of Nematodes from Roots

1. Baermann Funnel Method

Ten grams of root knot infected tomato roots were weighed for this extraction method. Baermann funnel were set-up with the root samples based on the procedures followed by Riggs et al., 1997. Nematode suspension was collected at every 2 days interval for three weeks. The Collected nematodes were counted and recorded. Water was added into the funnel everyday for the free movement of nematode and to avoid drying of roots.

2. Automatic Zonal Centrifugation (AZC) Method – Roots

For this method corn roots were washed gently with tap water and cut into small pieces (about 1/2 cm length). Ten grams of root samples were blended at high speed for 1 minute. It was then processed in the AZC machine for extraction. The resulting count of the nematode was only 5 grams since the machine processed only half of the sample.

RESULTS AND DISCUSSION

A. Extraction of Cyst Nematode: Stirring and Seinhorst methods

The average data revealed that Seinhorst method yields slightly more cysts than the stirring method (Figure 1). It is expected by theory that Seinhorst method can yield more number of cysts because both old and younger cysts can be extracted from it while in stirring method only the old cysts filled with air bubbles are usually extracted. As stated by Southwood and Henderson (2000), this method might well be adopted for single-species studies on other organisms; those of a comparatively uniform size and mass. Stirring method is simple and easy to follow, does not require sophisticated apparatus (needs only a beaker and spoon) and can also give better recovery of cyst if properly done although in this method there is a need to dry first the soil thus results cannot be given right away. On the other hand, Seinhorst method, aside that it needs the apparatus which is difficult to construct and expensive, it also needs some protocol to follow to give an efficient result.

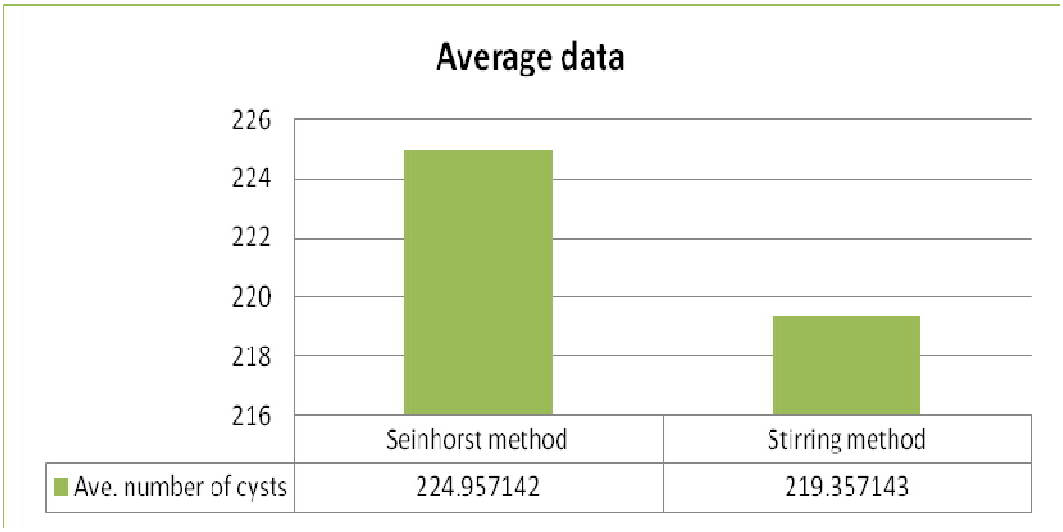

FIGURE 1. Average data on number of cysts collected under Seinhorst and stirring method.
(Seinhorst count was converted from 500-300 grams by ratio and proportion for equal basis of comparison)

However, the use of Seinhorst method can result to extraction of both light and heavy cysts which the stirring method cannot provide and since the apparatus can process even up to 1 kg of soil sample as compared to stirring which is only limited to 300 grams. Faster results can also be obtained because samples can be directly process without the need to dry them; hence it's called 'wet method' of extracting cyst nematodes from soil. In the Netherlands, elutriation with elutriators is also preferred as this will take time per soil sample and it avoids the messy use of large numbers of containers filled with water as up to 700 soil samples per day will be processed in commercial laboratories (Been *et. al*., 2007).

2. Extraction of Vermiform Nematodes from Soil

Number of nematodes per genus from average data was higher in the AZC than in Cobb's method particularly in Pratylenchus and Meloidogyne genera (Figure 2) indicating that AZC was more efficient in extracting vermiform nematodes from the sample. By theory, AZC method can yield more number of nematodes than the Cobb's method. The slight discrepancy in the Tylenchorhynchus count (which is higher in Cobb than in AZC), might be due to confusion sometimes with the genus Pratylenchus. In Cobb's method, the extraction requires more sieving, and in this process nematodes can be lost. While in the AZC method, minimal sieving is done and in addition the organic fraction of the soil sample was also included in the process whereas in Cobb's method, it was not included. Also in Cobb's + pie pan method, only mobile stages of nematodes are collected, unlike in the AZC in which immobile stages (including eggs) can also be extracted from the sample. Bellvert et al., (2008) also found out that Schuiling Centrifuge was more efficient than the Fenwick can method, this may be due to different sample sizes used for extraction.

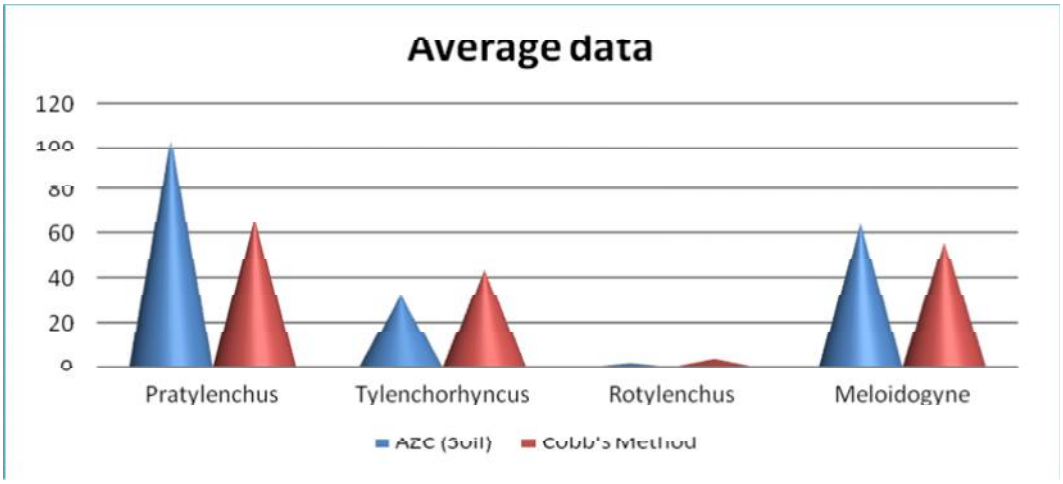

FIGURE 2. Average data of the four genera of nematodes collected in AZC and Cobb's method of extracting nematodes from the soil. (Cobb's count was converted from 300 to 200 ml by ratio and proportion to have an equal basis of comparison)

As to their advantages and disadvantages/drawbacks, Cobb's method is easy to perform and does not need sophisticated material unlike the AZC method but Cobb's method is labor intensive and less efficient (as manifested by low recovery of nematodes, and can extract only mobile stages of nematodes) and therefore not very accurate for quantitative sampling. On the other hand, AZC method gave a clean nematode suspension and a higher recovery of nematodes (due to inclusion of organic fraction of the sample and the ability to extract immobile stages of nematodes as well) and the process was faster compared to Cobb's method. Viaene et. al. (2007) further stated that the inferiority of the sieving method compared to centrifuging for retrieval of eggs be caused by two factors: the aperture of 20µm was too large or eggs were lost during handling such as splashing it with water when rinsing.

However, the AZC machine is quite expensive and needs expertise to operate the entire process. The extraction fluid may have negative effects on the nematodes' shape and its vitality thereby affecting identification (Van Bezooijen, 2006). Therefore, this method (AZC) is not quite good in extracting nematodes intended for taxonomic purposes.

3. Extraction of Nematodes from Roots

Automatic Zonal Centrifugation (AZC)

Average data shows a relatively great number of Pratylenchus compared to the other three (3) species of nematodes (Table 1). The higher number of Pratylenchus followed by Meloidogyne in the roots can be accounted to the fact that these nematodes are endoparasitic while Tylenchorhynchus, Rotylenchus juveniles are ectoparasitic ones. On the other hand, the presence of other nematodes suggests that AZC method can extract different kinds of nematodes and of different sizes. Moreover, all life stages including the eggs were found on the suspension. This can also be one of the advantages of this method aside from its higher efficiency and less laborious being fully automated. The disadvantages are the same as mentioned above (AZC soil extraction).

TABLE 1. Average number of nematode genera collected from maize roots under AZC.

Nematode species	Counts
Pratylenchus	830.50
Tylenchorhynchus	8.35
Rotylenchus	0
Meloidogyne	116.50

Baermann Funnel Method

Meloidogyne counts were observed within the duration of the experiment if certain pattern or evolution can be seen. The graph (Fig. 3) showed the highest count of nematodes (all juveniles) during the first counting then the counts decreases until the 7th day, then it slightly goes up during the 8th day while continued to decrease until the termination of the set-up. This particular set-up might have the mature galls at the time set-up was made thus most of the juveniles came out during the first counting then declined up to the 7th counting. The probable cause of the slight increase during the 8th counting might be attributed to the new batch of juveniles coming out but this time only in less quantity that might be attributed to the location (not in its natural environment) and duration of the set-up.

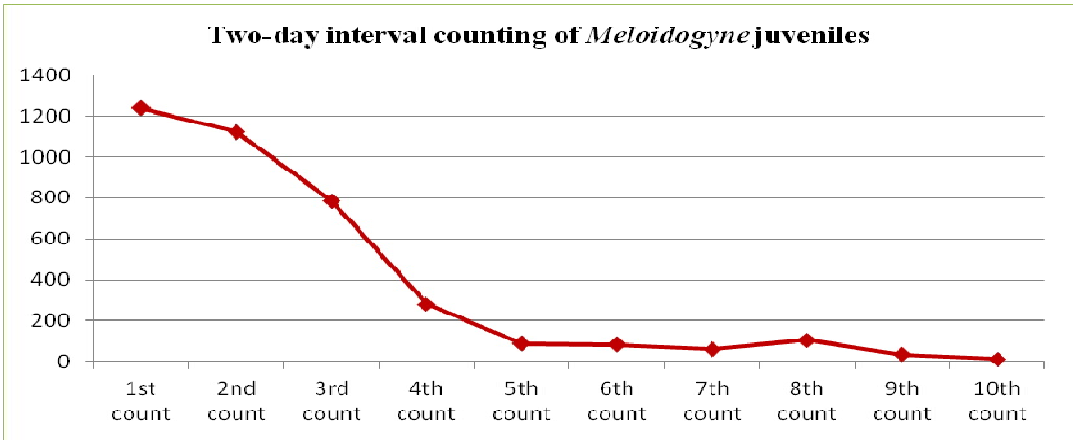

FIGURE 3. Meloidogyne juvenile counts at 2-day interval set-up under Baermann funnel

This observation primarily suggests that the counting of nematodes using this method can vary with different time therefore it is necessary to determine the number of days by which peak of nematode population count occur. Thus, Baermann funnel method is not good for quantitative research; it can only extract nematodes that are mobile in fact only juvenile stage was found during the counting. In addition, Ravichandra (2010) mentioned that in this method recovery of active nematodes from large samples is poor. However, Baermann funnel method is not laborious; it is simple and requires only cheap materials to establish the set-up.

In conclusion, the different techniques or methods of extracting nematodes (either from soil and roots or the host plant itself) pose each of the different pros and cons with its different features. The choice of the technique matters most to the target stage of the nematode that an individual (whether a researcher or student) will be investigating or to the purpose of the extraction to be dealt with whether for taxonomical or quantitative research.

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