RECOVERY OF *PRATYLENCHUS LOOSI* FROM SOIL SAMPLES

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The current method of extracting eelworms from soil (The Baermann funnel technique) gives wide variation among replicates and recovers only 4-5% of the eelworms present. By omitting certain steps in the extraction process, the variation was reduced, more than four times the number of eelworms were extracted and the time of processing was decreased by six to seven hours.

Introduction

The most accurate method of assessing numbers of eelworms in soil samples will undoubtedly be to sort out a requisite sample and count the worms directly. Laborious as this may sound, this was the method used by some earlier workers (Stockli 1943 ; Mindermann 1956). This method is extremely slow and is not at all satisfactory when large numbers of soil samples have to be analysed within a short period of time. Subsequently, more rapid methods came to be devised at the expense of accuracy. The object of the present investigation was to compare the accuracy of several methods of extracting eelworms from soil and to determine their suitability for the extraction of *Pratylenchus loosi* Loof from tea soils.

Materials and methods

A thoroughly mixed sample of fumigated soil was divided into several 100 g subsamples and each was inoculated with 200 *P. loosi*. Following inoculation, the soil was well mixed and for each technique of extraction, six inoculated subsamples were used as replicates.

1 — *Baermann funnel technique*

The method was first introduced by Baermann in 1917 and since then several modifications of the basic technique have been made. Details of the method currently used at the TRI are given below.

A glass funnel 15 cm in diameter, with a rubber tubing attached to the stem is filled with water after clamping the tube. The soil (100 g) is then transferred to a 60-mesh sieve, nine cm in diameter and four cm in depth with a disposable nylon-cotton-wool filter at the bottom. The sieve containing the soil sample is then gently lowered into the funnel and the water level is adjusted until the soil sample is just submerged (Figure 1). It is left overnight (16-18 hr) and on the following morning 10-15 ml of water is run off the funnel into a watch glass (Step a). The eelworms are allowed to settle at the bottom of the watch glass over a period of three hours when the surface water is gently sucked out with a pipette until only about half the previous volume remains (Step b). The volume of suspension now remaining is sucked into a pipette (Figure 2), which is then clamped vertically (Step c). The eelworms are allowed to settle and concentrate at the lower end of the pipette over a period of three to four hours, when three to five drops are collected from the pipette onto a slide. The eelworms are then counted under a compound microscope.

The technique depends on the ability of the eelworms to move through the soil, through the filter, into the surrounding water where they sink and collect at

the base of the funnel. Step b and c, which take about six to seven hours, are used to concentrate the eelworms into a few drops of water. The time taken for the entire process of extraction is about 22 to 25 hours.

2 — *'Modified' Baermann funnel technique*

This method is basically the same as the above, except that the steps for concentrating the eelworms (b and c) are omitted. After setting up the funnel overnight, the following morning a measured volume of water (5-20 ml) is run from the funnel into a small beaker (Figure 3). Without further concentration, one ml aliquots are transferred to a counting slide (Figure 3) and the eelworms counted under a stereoscopic microscope. An average count of three such aliquots is determined and then multiplied by the total volume (5-20 ml) ; this gives the counts per 100 g soil. The total time taken up to the point of counting is about 16 to 18 hours.

3 — *'Oostenbrink-Baerman' method*

In this method, developed by Oostenbrink (1960), a shallow pan containing water is used instead of the normal glass funnel. A weighed quantity of soil (say 100 g) is transferred into a broad sieve, 16 cm in diameter and three cm deep with a nylon-cotton-wool filter at the bottom. The sieve containing the soil is gently lowered into the pan containing sufficient water to just submerge the soil. The method is in effect another modification of the Baermann funnel technique, but the soil is more thinly spread, providing a better opportunity for eelworms to move through the filter. The following morning, the water contained in the pan is collected and made up to volume (100 ml). Eelworm estimation is made by taking one ml aliquots and the total number is calculated as given in Method 2.

4 — *Rapid flotation-centrifugation technique*

The method was first adapted to phyto-nematodes by Mindermann (1956), and later modified by Caveness & Jensen (1955) and Jenkins (1964). The soil (100 g) is transferred to a one litre beaker and stirred thoroughly with about 500 ml of water and allowed to stand for about 30 sec. The mixture is then decanted through a 30-mesh coarse sieve into another beaker, to remove all stones and big roots. The process is repeated two or three times and the filtrates are all pooled together into a pail. After about 45 sec, when most of the heavier soil particles have settled to the bottom, the filtrate containing the suspension of eelworms and fine soil particles is decanted through a fine 325-mesh sieve and the residue remaining on the sieve (eelworms + fine soil particles) is washed down into a beaker with about 200 ml of water.

The suspension is then centrifuged for about five min at 3000 rpm. At the end of this process, the eelworms settle to the bottom along with the soil residue. The supernatant is carefully poured out and a sugar solution (one lb sugar/litre water) added to each tube and the solution well mixed with the sediment and centrifuged for 30-45 sec. The specific gravity of the sugar solution being high, the eelworms float in the supernatant while the soil particles settle to the bottom. The supernatant is poured onto a 325-mesh screen and the sugar is rinsed off the eelworms with fresh water. The eelworms are then washed down from the sieve into a beaker with fresh water and made up to volume (100 ml). With sufficient skill, the whole process of extracting eelworms from soil takes less than 15 min. The method, being a mechanical one, recovers both live and dead worms.

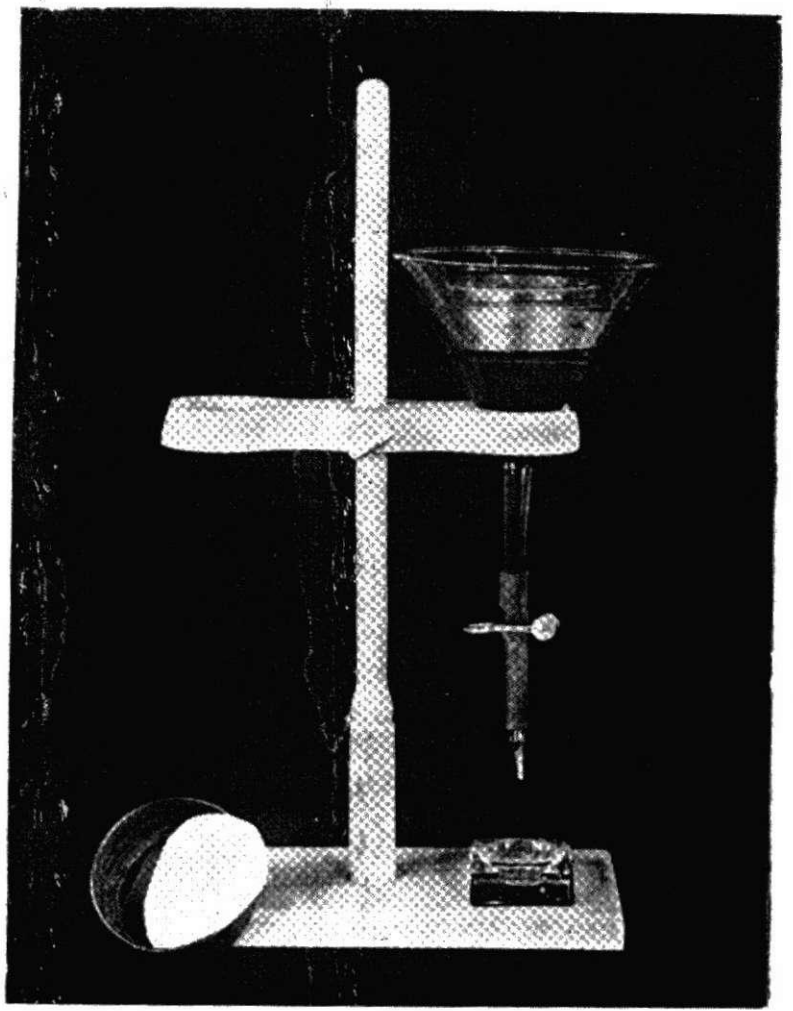

FIGURE 1—*The Baermann funnel technique for the extraction of nematodes from soil*

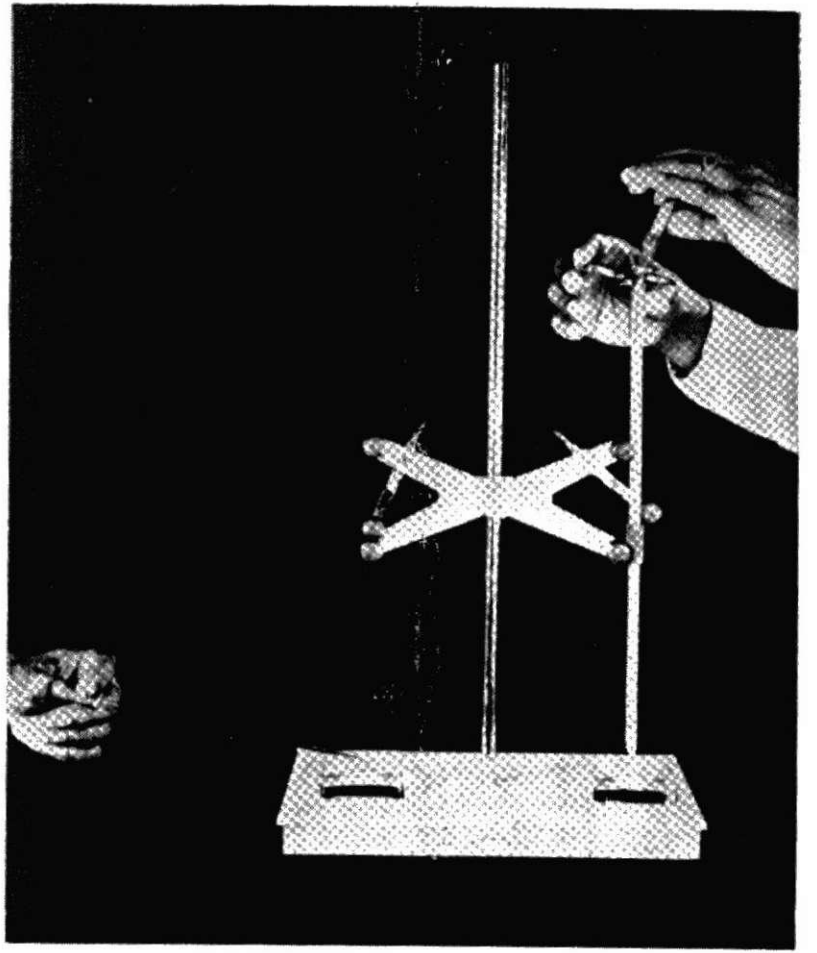

FIGURE 2—*Concentration of a nematode suspension after extraction from the soil by the Baermann funnel technique*

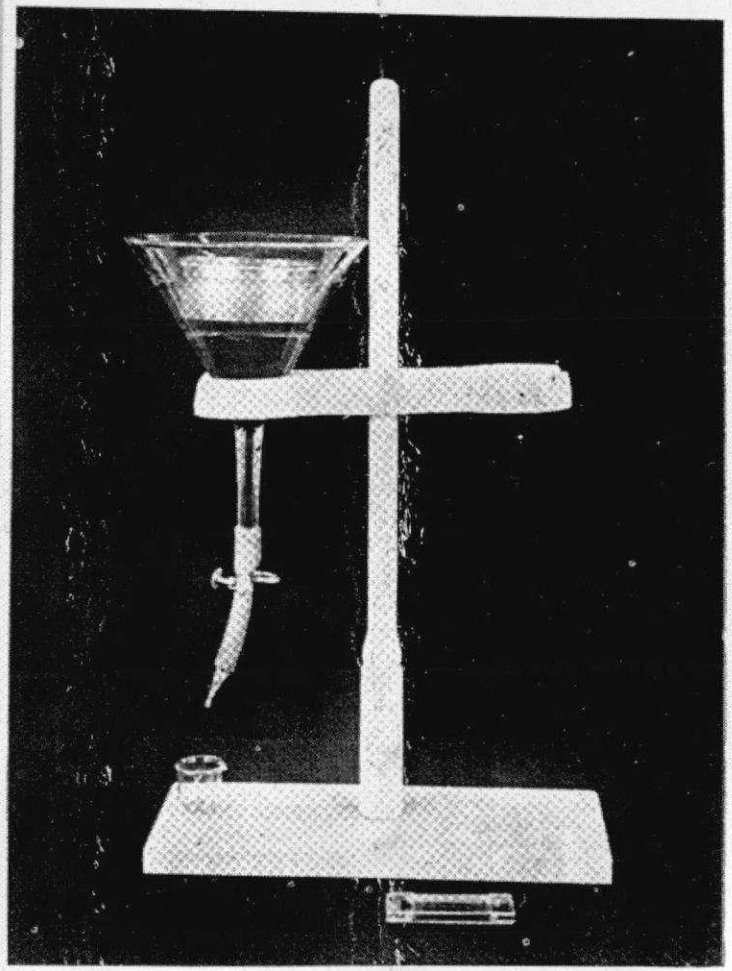

FIGURE 3—*The modified Baermann funnel technique for the extraction of nematodes from soil*

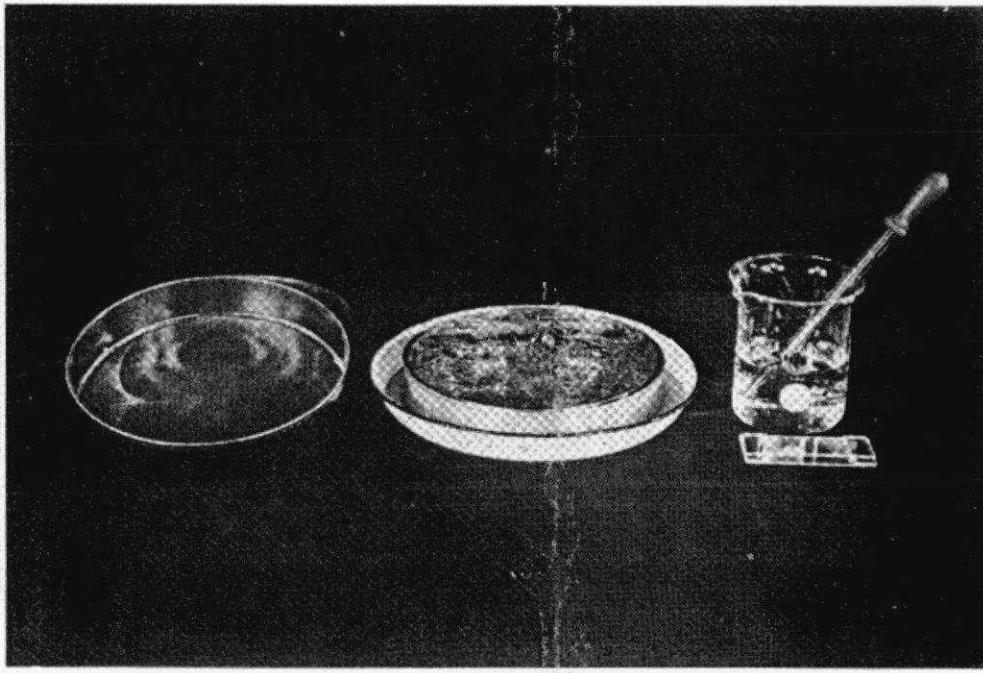

FIGURE 4—*The Oostenbrink-Baermann method of extracting nematodes from soil*

Results and discussion

The results obtained by using the four extraction techniques investigated are presented in Table 1.

TABLE 1—*Numbers of* P. loosi *recovered from 100 g soil by four different techniques*

Technique	Number of *P.loosi* extracted						Mean	Variation co-efficient %	Recovery %
1-Baermann funnel	8	9	2	8	19	9	9.2	60	4.6
2-'Modified' Baermann funnel	27	73	27	47	20	33	37.8	51	18.9
3-'Oostenbrink-Baerman'	118	170	63	122	180	63	119.3	42	59.7
4-Flotation-centrifugation	51	0	0	45	53	0	25.0	109	12.4

Transformed Data (Square root transformation)

Technique	1	4	2	3
Mean recovery	3.088	4.057	6.088	10.749

$P < 0.05 = 2.675$

It is clear that the four methods of extraction differ considerably, both in efficiency and in variation among samples. The least efficient method is the normal Baermann funnel technique, where recovery was only 4.6%, less than a quarter of the recovery by the modified Baerman funnel technique. As the initial step in the two processes is identical, the difference must be due to steps b and c when the extracted eelworms are concentrated. In step b, the removal of surface water from the watch glass probably causes turbulance and eelworms are removed with the water. In step c, when eelworms are transferred from the watch glass to a pipette, some may be left behind (Figure 2).

Although it would appear that the Oostenbrink-Baermann method is the best because it gave the highest recovery and the lowest vatiation, this is almost certainly due to the soil being spread out much more thinly than in the previous two methods. In the latter the soil is three to four cm deep compared with a few mm in the Oostenbrink-Baermann method. The deeper the soil, the less chance the eelworms have of moving through the filter into the surrounding water. Aeration will also be poorer in the Baermann funnels and it is likely that the eelworms are less active and some may die. One considerable disadvantage of the Oostenbrink-Baermann method is that it cannot detect low levels of infestation. The final volume of suspension containing eelworms is at least 100 ml and only three aliquots, each of one ml are taken for counting. If only a few eelworms are present, it is likely that none will be counted. It can be calculated that 33 eelworms is the minimum that can be detected in 100 ml of suspension.

This disadvantage also applies to the modified Baermann funnel technique. The smaller the volume of water, the lower the minimum detectable level in the suspension. As only 5-20 ml are drawn off for counting in the modified Baerman funnel technique, even with a few eelworms present in the suspension, it is likely that most of them would be detected.

In order to see if there is any difference in the number of eelworms recovered with different volumes of water drawn off the funnel, aliquots of 5, 10 and 20 ml were taken and numbers estimated (Table 2). The different volumes had no significant effect on the numbers of eelworms extracted, but the coefficient of variation among replicates was least (38%) with the five ml aliquot. Five ml seems, therefore, to be the appropriate volume for analysis.

TABLE 2—*Numbers of* P.loosi *recovered from 100 g soil samples drawing out different volumes of water using the 'modified' Baermann funnel technique*

Volume drawn (ml)	Number of *P.loosi* recovered 1	2	3	4	5	6	Mean	Std error	Coefficient of Variation %
5	90	62	53	43	52	28	54.7	± 8.48	38.0
10	40	43	43	40	97	27	48.3	±10.03	50.8
20	27	87	73	73	33	--	58.6	±11.99	45.8

Transformed data (Square Root transformation)

Mean recoveries between treatments were not significantly different.

The minimum number of *P.loosi* that can be detected in the five ml suspension is $= 1/3 \times 5 = 1.6$.

The flotation-centrifugation technique gives poor recovery and very high variation among samples and does not appear to be a suitable method for the extraction of *P.loosi* from tea soils. Its only virtue is speed, and it may have use in the rapid estimation of eelworm numbers when accuracy is not important.

Soil populations and extraction technique

All extraction methods tested have some disadvantages and it is of interest to compare their performance on eelworm infested soils. This is presented in Table 3.

TABLE 3—*Least detectable number of P.loosi in suspension and the corresponding least detectable field population per acre by the different extraction techniques*

Method of extraction	Recovery %	Lowest detectable level in suspension	Lowest detectable level/100 g soil	Corresponding* population in field/ acre
1	4.6	1.0	22	198 $\times 10^6$
2	18.9	1.6	9	81 $\times 10^6$
3	59.7	33.3	56	504 $\times 10^6$
4	12.4	33.3	268	241.2×10^7

* The appropriate volume of soil in one acre in the 1st 6" zone is 6×18^8 ml. Assuming 1 ml of soil = 1.5g this volume of soil weighs 9×10^8 kg.

There is still considerable room for improvement, but method 2 is evidently the best, being able to detect up to as few as nine eelworms per 100 g soil (81×10^6/ acre) and with a relatively low coefficient of variation (38%). In addition, the time of processing the samples is six to seven hours less than for the currently used Baermann funnel method.

If a negative result (count of 0) is obtained by processing the soil even by method 2, the corresponding field population need not be zero, on the other hand, as the limit of recovery is up to 81×10^6 eelworms/acre, the field population could be anywhere on the scale between zero and 81×10^6/acre.

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