

A MODIFIED EXTRACTION METHOD FOR ACCURATE ESTIMATION OF CITRUS NEMATODE POPULATION FROM FIELD SAMPLES

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ABSTRACT: The efficiency of extraction of the citrus nematode, *Tylenchulus semipenetrans* was dependent upon method, transport temperature, soil texture, interval of time recovery and sample size. More nematodes were recovered by direct extraction with Baermann incubation plates (modified Baermann) than sieving prior to Baermann incubation. Great nematode recovery was obtained when soil samples were transported in ice chest than open air. Nematode collected at the end of three days interval yielded 35.8%, 28.5% and 58.0% of the total number of nematode recovered daily (for 3 days) from sandy loam, loamy clay and clay soils, respectively. Number of nematode recovered per soil unit was decreased with the subsample size increasing.

Key words: citrus, nematode, extraction efficiency, *Tylenchulus semipenetrans*.

INTRODUCTION

The extraction of nematodes from their environment continues to be a vexing problem. More knowledge is needed for the parameters limiting the efficiency

of the various available methods. Quantitative nematode extraction methodology needs serious attention (Viglierchio and Schmitt, 1983 b). Storage time and temperature can greatly affect the relative efficiencies of nematode

extraction methods (Barker *et al.*, 1969 b). Temperature at the time of extraction should be reported when motility dependent methods are used (McSorley, 1987). Difficulty in using centrifugation technique in clay soil has been reported with large number of subsamples. Also, nematode recovery from smaller subsample is rarely inferior even on this soil (Ayala *et al.*, 1963). Extraction efficiency depends on several factors including the methods, soil type, nematode species and size of samples (Viglierchio and Schmitt, 1983 b and McSorley and Frederick, 1991).

The aim of these experiments was to compare sieving prior to Baermann incubation with direct Baermann incubation (without sieving) for extraction of *T. semipenetrans* and some factors affecting the extraction efficiency.

MATERIALS AND METHODS

Several experiments were conducted to evaluate various aspects of the sampling and sample processing. Soil samples were collected from different soil textures of citrus field (15 cores) and mixed carefully.

1. Efficiency of modified Baermann extraction method:

An aliquot of 250 cm³ soil was taken and soaked in tap water for nematode extraction by sieving through 60 and 400 mesh sieves. Active nematodes present in the suspension were obtained from the fine sieve and extracted by Baermann plates utilizing tissue paper. Also, an aliquot of 100 cm³ soil without sieving was put directly (modified Baermann) onto tissue paper on plates, each treatment replicated three times. The nematode stages were collected and counted after 48 hours for each of the two methods.

2. The effect of transport temperature:

Fifteen soil samples collected from citrus fields were divided to two parts. The first part was transported in ice chests containing ice bags (13–18 °C) and the second part was transported in open air (28–33 °C). Care was taken to keep samples of both treatments out of direct sunlight. The samples were extracted by Baermann plates and counted after 1, 2, 3, 4, 5, 7 and 10

days. The experiment was conducted twice.

3. Effect of soil type and time of extraction:

Nematodes were extracted directly from soil using Baermann plates (modified Baermann) as previously described. Nematodes were collected and counted after 1, 2, 3, 4, 5, 7 and 10 days. Three soil textures; sandy loam soil (consisted of 72% sand, 10% silt and 18% clay), loamy clay soil (22% sand, 44% silt and 34% clay) and clay soil (30% sand, 22% silt and 48% clay) according to the mechanical analysis were used. To determine whether there is seasonality in extraction efficiency, the experiment was conducted once in September and again in January.

4. Nematode recovery at the end of a given time interval:

Nematodes were extracted directly (modified Baermann) at the end of 3 days compared to that of nematodes recovered daily over the same time interval.

5. Sampling size:

The total number of citrus nematodes collected from sub-

samples 200, 150 and 100 cm³ soil were counted and calculated as juveniles and males/100 cm³. Three soil textures previously described were used in the experiment. Three replicates were conducted with all treatments in all experiments.

RESULTS

More *Tylenchulus semipenetrans* juveniles and males were extracted with direct action (modified Baermann) ($P < 0.01$) than sieving prior Baermann incubation (Table 1). Some differences were observed between the two extraction methods. The percentages of recovered nematodes by the sieving prior Baermann were 26.9, 23.3 and 56.1% compared with recovered nematodes by direct extraction (modified Baermann) from clay, loamy clay and sandy loam soils, respectively.

The use of ice chests to cool soil samples prior to extraction increased seasonally the juveniles and males of *T. semipenetrans* (Fig. 1). An average of 70% of the extracted nematodes were recovered during the first three days out of the ten days by modified Baermann method (Fig. 2 A & B).

Numbers of nematode recovered after three days were not affected by season when data were pooled across all soil textures.

Table (1): Comparison of Baermann plates alone or in combination with sieving for extraction of *Tylenchulus semipenetrans* from soil samples.

Soil type	Number of extracted nematodes		%
	Plates	Sieves/plates	
Clay	2534	681	26.9
Loamy clay	2044	477	23.3
Sandy loam	3981	2235	56.1

However, in two of the soil textures (sandy loam and clay), cumulative recovery numbers after 3 days out of the ten days counts were approximately 20% lower in winter than summer.

Recovered nematodes from Baermann incubation units daily were highly significant rather than at the end of the three day time

interval (Table 2). This trend was observed when the nematodes were recovered from the three soil textures. Collection of nematodes one time after 3rd day was lower than those of daily collection, since their percentage reductions were 35.8, 28.5 and 58.7 from sandy loam, loamy clay and clay soils, respectively.

Table (2): Comparison of the extraction efficiency and precision of *Tylenchulus semipenetrans* from Baermann plates when samples are recovered daily for three days once at the end of three days.

Day	Sandy loam		Loamy clay		Clay	
	Daily	3 rd day	Daily	3 rd day	Daily	3 rd day
1	1333*		2604		1408	
2	2187		1950		720	
3	680	1503	578	1462	433	1503
Total	4200	1503	5132	1462	2561	1503
Coefficient of variation	14	4	2	17	5	10

* Juveniles and males/100 cm³ soil.

Nematode recovery per soil unit was decreased with the increase of subsample size in all tested soil textures (Table 3). The

greatest yield of all total recovered nematodes from the three soil textures were obtained with the low sampling size (100 cm³), since

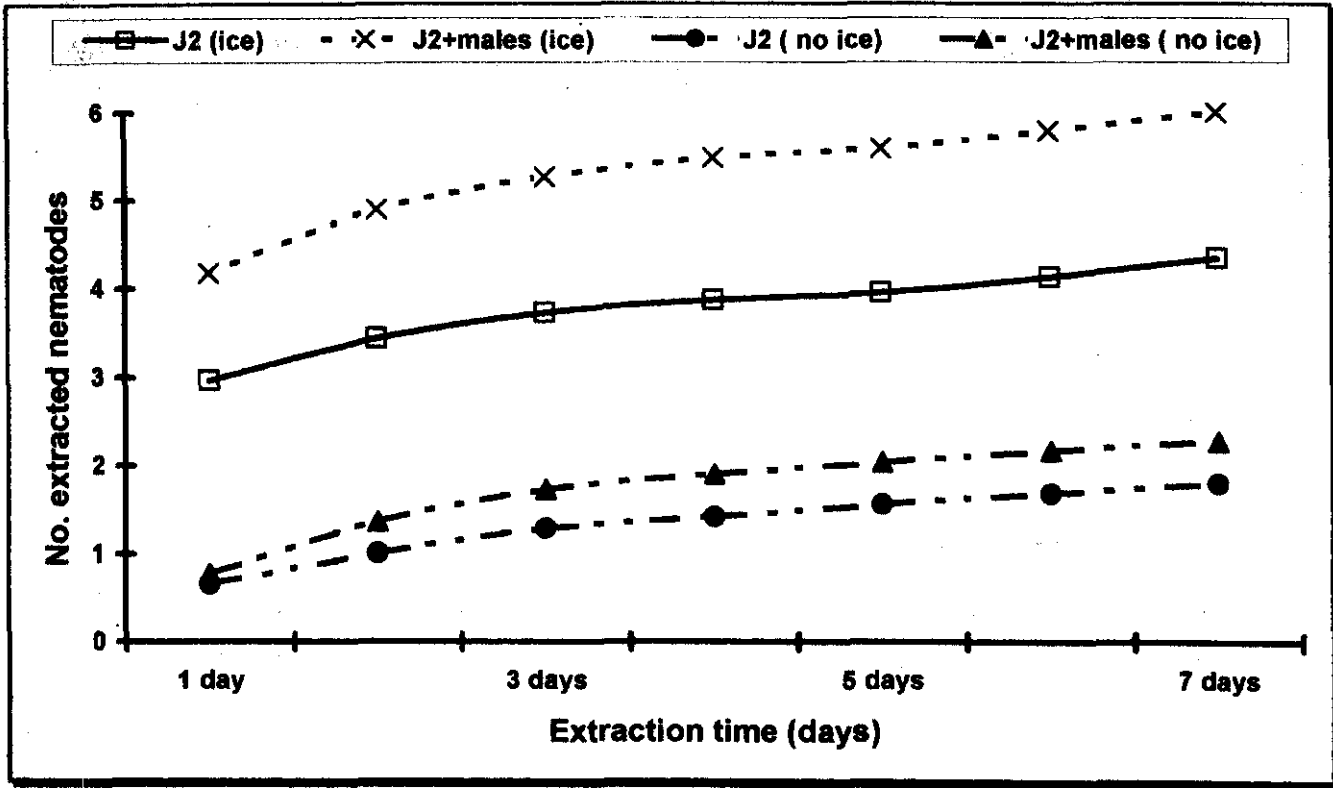


Fig. (1): Effect of transport temperature on extraction recovery of *Tylenchulus semipenetrans* in soil.

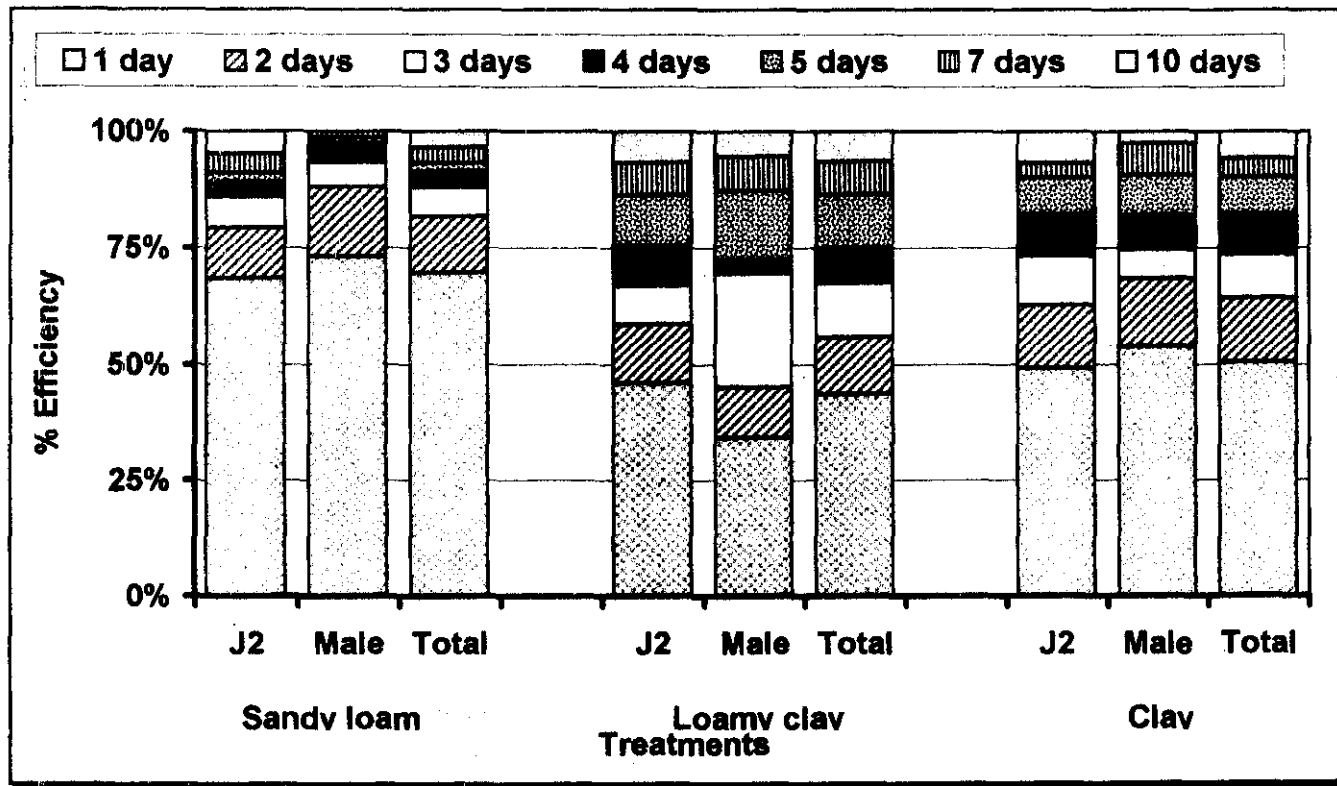


Fig. (2A): Effect of soil type on extraction efficiency of Baermann plates, samples collected in summer.

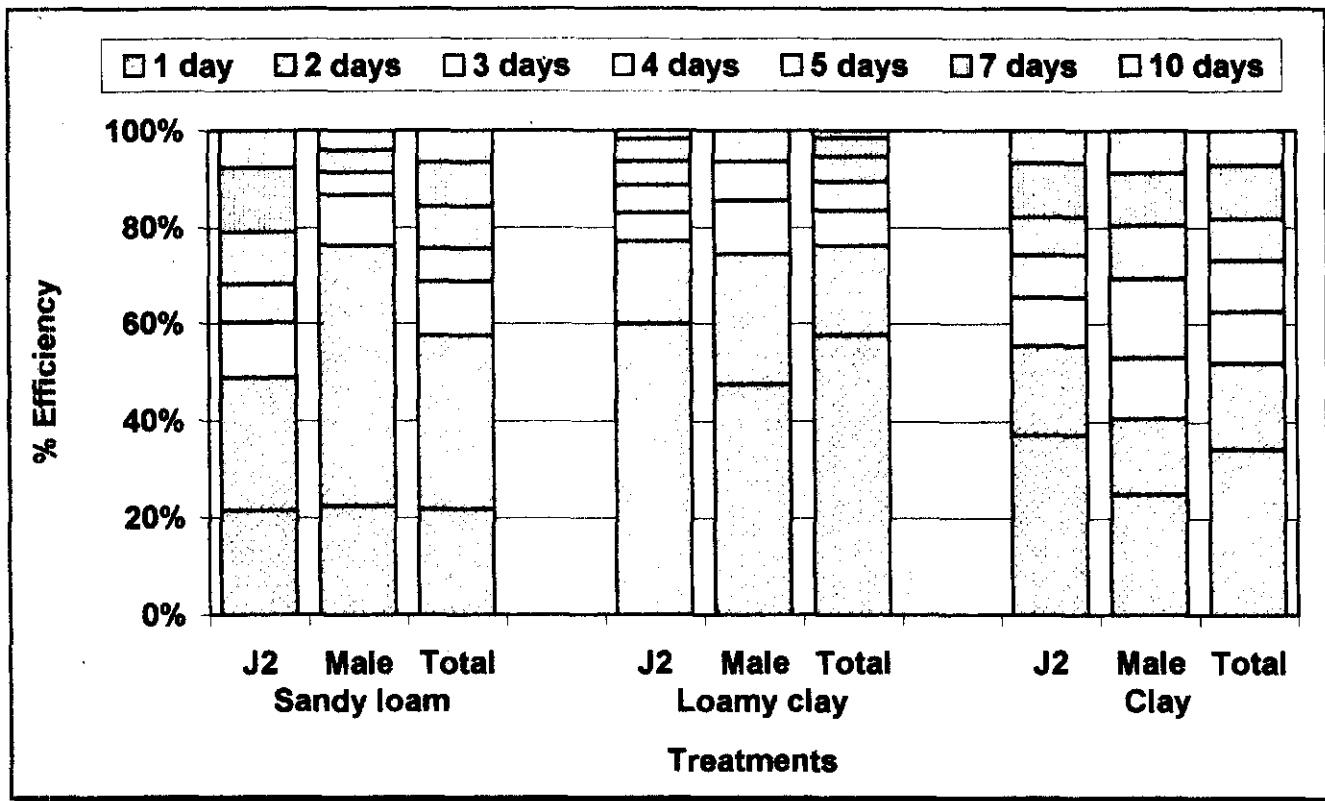


Fig. (2B): Effect of soil texture on extraction efficiency of Baermann plates, samples collected in winter.

75.9% were obtained with the medium size (150 cm³), while 62.3% were recovered with large subsample size (200 cm³). Loamy clay soil was gained more efficiency of nematode recovery,

since 62.6% of nematode recovered from large subsample size (200 cm³), while 74.1% were recovered with medium subsample size of these extracted with the low subsample size (100 cm³).

Table (3): Effect of subsample size on extraction efficiency and precision for recovery of *Tylenchulus semipenetrans* from soils of three different textures.

Subsampling size (cm ³)	Sandy loam		Loamy clay		Clay	
	Mean*	cv	Mean	cv	Mean	cv
200	3279	13	4389	5	2391	5
150	4331	4	5194	5	2718	5
100	5027	11	7006	2	4102	9

* Juveniles and males/100 cm³ soil.

DISCUSSION

The term extraction efficiency is used to indicate the proportion of the nematode population that is removed from soil or plant tissue. There are many reasons why knowledge of extraction efficiencies is necessary and appropriate (Ferris, 1987).

The relatively high recovery of *T. semipenetrans* from all soil textures on Baermann plates is encouraging and probably applicable in most field textures. Although for many nematode species, extraction by modified

Baermann method is considered to be more variable (Barker *et al.*, 1969b; McSorley, 1987). Sieving before incubation of Baermann tray was extracted *Belenolaimus logicaudatus* much more efficient (McSorley and Frederick, 1991). This work was done in sandy soil, while our studies in loamy sand, loamy clay and clay soil. Obtained results of transporting temperature demonstrate how temperature affects the efficiency of nematode extraction (Barker *et al.*, 1969a) and greatly influence the number of recovered nematodes during certain periods of year (Barker *et al.*, 1969b). Our results showed

that, cooling samples with ice was an effective method to recover higher nematode numbers.

Based on the results of these experiments, three days were established as an acceptable extraction time that will permit accurate estimate of *T. semipenetrans* densities from all soil textures. Viglierchio and Schmitt (1983a) reported that extraction efficiency can vary substantially with soil type. This finding was the same in summer and winter with approximately 20% lower in winter. The low recoveries during winter may reflect decreased nematodes motility (Barker *et al.*, 1969a). Numbers of nematodes were related positively to root mass density and root concentration of reducing sugars, starch and total nonstructural carbohydrates. Numbers of nematodes were related inversely to soil moisture and root lignin content (Duncan *et al.*, 1993).

High nematode yield was obtained with daily recovery of a citrus nematode from field samples. Same trend was obtained also from the three different soil textures. It seems that, we must use this technique to recover a large number of citrus nematode to estimate accurate population. The

relatively high recovery of *T. semipenetrans* from the low sampling soil size (100 cm³) on Baermann units is encouraging and probably applicable in most field situations and soil textures.

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طريقة معدلة لزيادة كفاءة الاستخلاص والتقدير الدقيق لأعداد نيماتودا الموالح من العينات الحقلية

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تم دراسة تأثير كفاءة استخلاص العينات المحتوية على نيماتودا الموالح بعمليات الغسيل باستخدام المناخل أحجام ٦٠ و ٤٠٠ ثقب فى البوصة الطولية مقارنة بوحدات برمان المعدلة بنقع العينات فى الوحدات دون المرور بعملية الغسيل ، كذلك دراسة تأثير درجة الحرارة أثناء نقل العينات من الحقل إلى المعمل باستخدام ثلاجة الرحلات المحتوية على أكياس من الثلج وتراوح حرارتها ما بين ١٣-١٨ م مقارنة بالنقل خارج الثلاجة والتي تراوحت بين ٢٨-٣٣ م ، كما درست كفاءة عمليات استخلاص ثلاث أنواع مختلفة من التربة (الطينية الرملية والطينية الطميية والطينية).

كذلك درست كفاءة استخلاص النيماتودا خلال ثلاث أيام إما بالتجميع اليومي أو التجميع بعد ثلاث أيام مباشرة وكذا درس حجم العينات التى يجرى عليها عمليات الاستخلاص ٢٠٠ ، ١٥٠ ، ١٠٠ سم تربة.

وكانت النتائج المتحصل عليها كما يلى:

١. زادت كفاءة عمليات الاستخلاص بوحدات برمان المعدلة عن استخدام المناخل مع وحدات البرمان فى أنواع التربة المختلفة بنسب ٢٦,٩% ، ٢٣,٣% و ٥٦,١% لأنواع التربة الطينية ، والطينية الطميية والطينية الرملية.
٢. أدى استخدام ثلاجة الرحلات لتبريد عينات التربة أثناء النقل إلى زيادة أعداد اليرقات والذكور موسمياً.
٣. تم استخلاص حوالى ٧٠% من إجمالى أعداد النيماتودا فى أول ثلاث أيام بوحدات برمان المعدلة.
٤. لم تتأثر أعداد النيماتودا عند تمريرها خلال أنواع التربة المختلفة باختلاف الموسم ولكن كانت أقل فى نوعى التربة الطميية الرملية والطينية بحوالى ٢٠% فى الشتاء عنه فى الصيف.
٥. كان التجميع اليومي للنيماتودا على مدار ثلاث أيام أكثر معنوية عنه عند التجميع بعد ثلاث أيام فى أنواع التربة المختلفة.
٦. أعطى نسبة استخلاص تم الحصول عليها عند استخدام حجم ١٠٠ سم^٣ تربة بالنقع يليها ١٥٠ سم^٣ ثم ٢٠٠ سم^٣ فى أنواع التربة المختلفة.