Efficiency of Extraction of Nematodes by Flotation-Sieving Using Molasses and Sugar and by Elutriation

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Abstract: Blackstrap molasses was studied as an economical substitute for sucrose in the preparation of an extracting solution for removal of nematodes from soil by the flotation-sieving technique. The maximal number of nematodes extracted from soil was obtained with molasses solutions with specific gravity values in the range 1.000-1.073. Results of studies on the relation between size of soil sample and the amount of extracting solution are presented. In paired comparisons, a molasses solution with sp. gr. = 1.100 at 27 C extracted greater numbers of plant parasitic, dorylaimoid, mononchoid, and other soil nematodes than did the standard 1.0 M sucrose solution (sp. gr. = 1.100); the superiority of the molasses solution is attributed to its higher viscosity. The molasses method also was superior or equal in efficiency to the elutriation technique. *Key Words:* Extraction method, nematode analyses, diagnostic technique.

A rapid and inexpensive method for the extraction of nematodes from soil is of particular importance in diagnostic and research laboratories where large numbers of samples are processed daily (1). Development and subsequent modifications of the elutriation technique (6, 8) simplified the extraction of nematodes from soil, compared to the sieving method of Cobb (9) or the Baermann funnel (9). The centrifugal-flotation method (4) reduced the time of operation required by other methods, but did not significantly affect the efficiency of extraction for most nematode species. Further improvement was attained by Byrd et al. (3) with the introduction of flocculating agents, which made possible development of the flotation-sieving procedure; this simple and efficient method permitted very rapid extraction (2.0-2.5 min/sample). One disadvantage of this method is that it requires a 1.0 M sucrose solution as the flotation medium, a feature which may become economically limiting in laboratories where large numbers of samples are processed daily. Recently, (5) we suggested the use of blackstrap molasses as a less expensive (US $0.03/Kg) substitute for sugar in the preparation of the extracting solution.

The purpose of this paper is to report results of studies made to determine conditions and variables important in the flotation extraction of nematodes with molasses, to present comparative data on the efficiencies of sucrose and molasses extracting solutions in the technique, and to evaluate the use of molasses in the flotation-sieving technique compared with the elutriation technique (6).

MATERIALS AND METHODS

Extraction procedures: The extraction procedure used was essentially as described by Byrd et al. (3) with some modifications (5). A 426-μm (40-mesh) sieve was used over a 38-μm (400-mesh) only when soils contained large amounts of debris.

Preparation of extracting solutions: Enough blackstrap molasses (sp. gr. = 1.300 at 37 C) to produce 4 liters of extracting fluid of the required specific gravity was measured and transferred to a 4-liter bottle containing 250 ml of tap water and 4 ml of a 1.25% (w/v) aqueous solution of flocculating agent (Separan® NP 10, Dow Chemical Co., Midland, Michigan). The final volume in the bottle was adjusted to the 4-liter mark with tap water. Our standard molasses solution (sp. gr. = 1.100 at 27 C) was a 25% (v/v) solution of blackstrap molasses in water. The standard sucrose (1.0 M) contained 12.5 μg/ml of Separan® NP 10 (3).

Physical variables: Determinations of specific gravity were made at 27 C with standard hydrometers. Kinematic viscosities of solutions were determined at 27 C using Cannon-Fenske viscosimeters (size 300) immersed in a water bath coupled to a Sargent Thermonitor Model ST. Each determination was performed 10 times and values obtained were expressed in centistokes.

Types of experiments.—1) Tests of specific gravity of molasses solution in relation to number of nematodes collected.—A series of seven molasses extracting solutions varying in specific gravity from 1.000 to 1.152 were prepared. Fractions of each solution were used for checking viscosity and specific

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gravity. To study the relation between specific gravity of the extracting solution and number of nematodes recovered, soils from three different fields known to contain a wide variety of plant parasitic nematode genera were used. Each soil was well mixed and eight samples taken for testing at each specific gravity for a total of 56 determinations per soil. Soils were processed on the day they were collected.

—2) Sample size and final volume of extracting solution.—This study consisted of two experiments. One involved a fixed volume (350 ml) in the mixing beaker with varying amounts of soil. In this experiment sample size was 50, 100, 150, and 200 ml. Each sample size was replicated four times. The soil used was a Dothan loamy sand containing several genera of plant parasitic nematodes.

The second experiment involved varying the final volume in the mixing beaker with a fixed soil sample size of either 50 or 100 cc. The final volumes were 350, 500, and 600 ml for each sample size. Each of these conditions was replicated four times. Soil for this study was a Norfolk sandy loam from a field maintained under continuous cotton culture.

—3) Comparative tests.—The efficiency of the standard molasses solution for extracting nematodes from soil was compared with the extraction efficiency of a 1.0 M sucrose solution, and with the elutriation procedure of Seinhorst (6). For the first comparison, a variety of soil samples differing in texture and crop history were collected from different fields in Alabama and each processed with the two flotation solutions on the same day using the standard procedure. The soil samples chosen contained a very wide range of both numbers and kinds of nematodes.

A smaller number of soil samples was used in comparisons with the elutriation method. Soil samples of 450 cc were used for elutriation. All comparisons were made on the same day for each soil sample.

Statistical analyses: All data were analyzed using procedures for analysis of variance. Unless otherwise stated, all differences referred to in the text were significant, $P = 0.05$. Correlation coefficients and equations for curve fitting were determined by following standard procedures (7).

RESULTS

Comparison of viscosities of molasses and sucrose solutions: Determinations of kinematic viscosity (centistokes, ct) indicated that the molasses solutions were more viscous than the sucrose solutions (Fig. 1-A). When viscosity data were expressed logarithmically, the relationship between kinematic viscosity and specific gravity was linear. Equations obtained from these data indicate that the slope for the molasses solution was 1.218 times greater than that for the sucrose solutions.

Effect of specific gravity of molasses solution on the number of nematodes collected: For each species, the data represent the percent of the total number of nematodes extracted at each specific gravity (Fig. 1-B). For most nematode species, the number extracted was directly related to increasing specific gravity within the range of 1.000 to 1.050 or 1.073, depending on the nematode species to be extracted (Fig. 1-B). The increase in the number of *Xiphinema americanum* (Cobb) and larvae of *Meloidogyne* extracted was very sharp within the range of specific gravity 1.000 to 1.050 or 1.073; the corresponding increments for *Pratylenchus scribneri* (Steiner), *Helicotylenchus dihystera* (Cobb) Sher for dorylaimoid and for other nematodes in the genera *Ditylenchus, Tylenchus,* or mononchoid types were not so pronounced and followed the pattern depicted for saprophagous types in Fig. 1-B. The small increment observed for *Trichodorus christiei* (Allen), was not significant. The use of solutions with specific gravities between 1.050 and 1.100 resulted in either small significant increase in number of nematodes extracted (*X. americanum*), no further statistically significant change (saprophagous nematodes), or a significant ($P < 0.01$) decline in the number extracted (*T. christiei*). The use of solutions with specific gravities greater than 1.100 resulted in a lower recovery of nematodes compared to that with specific gravities equal to 1.050 or 1.073.

Relation between size of soil sample and final volume in the mixing beaker: The data are expressed as percentage of the value obtained using a 50-cc soil sample (Fig. 1-C, D). Increasing the sample size from 50 cc to 100 cc resulted in some increase in numbers extracted of *Meloidogyne* larvae, *P. scribneri, H. dihystera,* dorylaimoids, saprophagous nematodes, and species of *Tylenchus* and *Ditylenchus;* the pattern obtained is

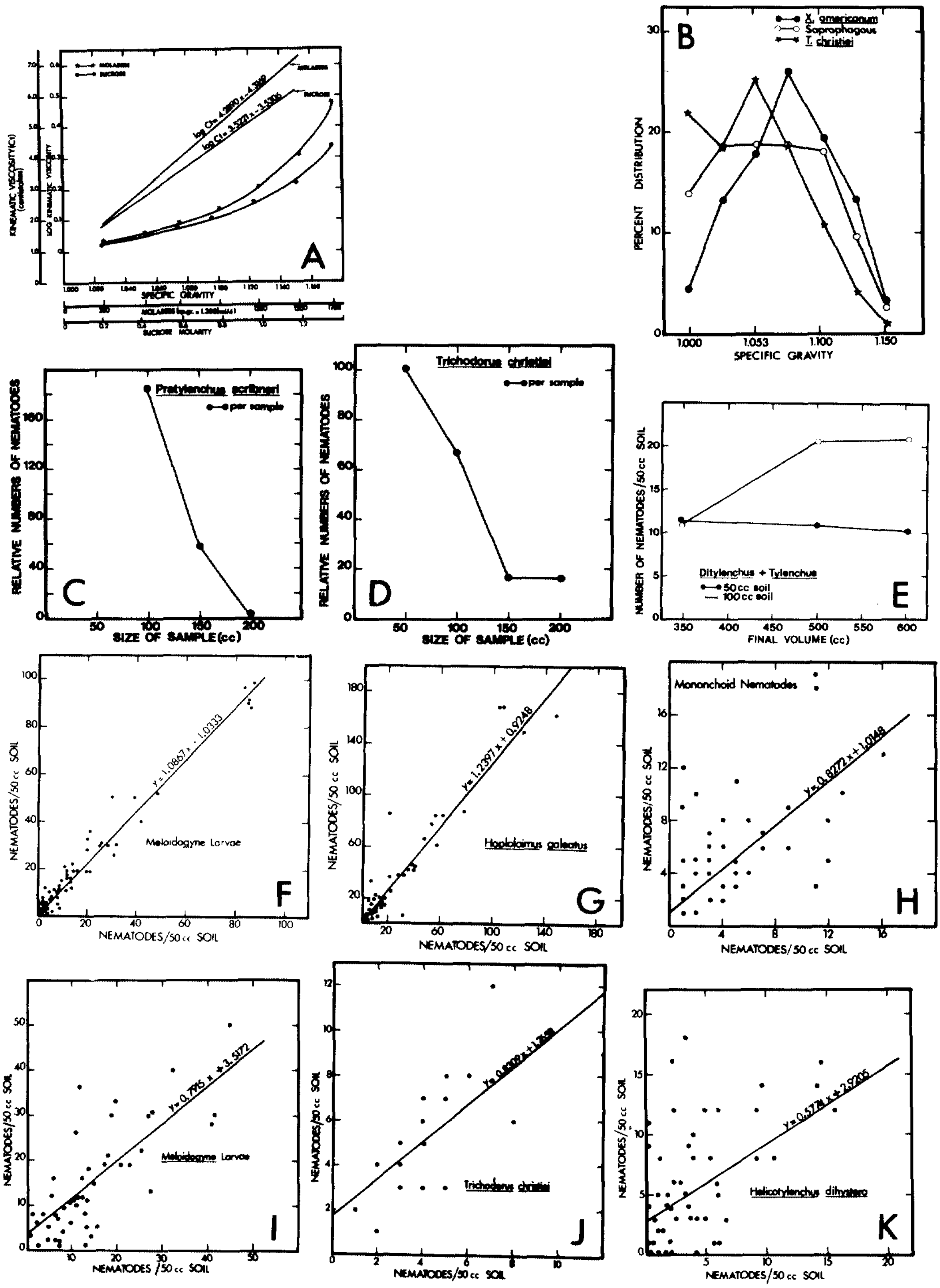

FIG. 1-(A to K). Extraction of nematodes from soil with aqueous molasses and sucrose solutions. **A.** Relation between viscosity and specific gravity of the molasses and sucrose solutions. **B.** Relation of specific gravity of molasses solution to the relative number of nematodes extracted from soil. Effect on number of nematodes extracted by changes in soil sample size using a constant (350-ml) final volume in the mixing beaker (**C**, **D**), and by changes in final volume in the beaker using soil samples of fixed size (**E**). Relation between the number of nematodes extracted with molasses solution (Y-axis) and the corresponding numbers obtained with sucrose (**F**, **G**, **H**), or by elutriation (**I**, **J**, **K**).

TABLE 1. Comparison of molasses (sp. gr. = 1.100) and (1.0 M) sucrose solutions for the extracting of nematodes from soil with the flotation-sieving technique.

Nematode species and types	No. recovered per 50 ml		No. samples	Linear correlation coefficient (r)	Avg. no./ 50 cc[a]	Range[a]	Standard deviation[a]	Increased efficiency with molasses (%)
	Molasses	Sucrose						
Meloidogyne larvae	2,508	2,151	165	+ 0.99**	13	0-296	44	17
Xiphinema americanum	251	203	94	+ 0.81**	2	0-25	3	24
Helicotylenchus dihystera	1,475	1,047	189	+ 0.80**	6	0-184	15	41
Hoplolaimus galeatus	1,924	1,446	142	+ 0.96**	10	0-148	22	33
Trichodorus christiei	118	91	24	+ 0.75**	4	0-9	3	30
Tylenchus + *Ditylenchus*	794	603	142	+ 0.73**	4	0-20	4	32
Dorylaimoid	1,669	1,329	166	+ 0.88**	8	0-53	8	26
Saprophagous	5,251	3,990	190	+ 0.90**	21	0-187	21	32
Mononchoid	330	275	101	+ 0.73**	3	0-16	3	20

[a]Calculated from results obtained with sucrose solution.

illustrated for *P. scribneri* (Fig. 1-C). With the exception of *P. scribneri*, the increments observed were not proportional to the increase in sample size; this was particularly evident when results were considered on the basis of nematodes per 50 ml soil. Numbers of *T. christiei* (Fig. 1-D), *X. americanum*, and mononchoid nematodes declined sharply when sample size was doubled from 50 cc to 100 cc. Further increments in sample size above 100 cc gave a smaller number of nematodes per 50 cc for all species and groups studied.

Data for nematodes in the genera *Ditylenchus* and *Tylenchus* (Fig. 1-E) illustrate typical results obtained when sample size was maintained constant and the final volume was varied from 350 to 600 ml. These changes in volume produced no significant variation in the number of nematodes extracted from 50 cc of soil. With a 100-cc soil sample, significant increments in the number of nematodes extracted were obtained when volume was increased from 350 to 500 ml; this increment was equivalent to double the number of nematodes extracted from the 50-cc sample. No further increase in the number of nematodes extracted occurred for the 100-cc sample when the volume was increased from 500 to 600 ml.

Efficiency: Comparisons of the molasses and sucrose solutions for extraction of plant-parasitic and other nematodes are presented in Table 1. Data comprised the percentage of increased efficiency in extraction using molasses solution and the average number of nematodes extracted with sucrose solution from samples for these comparisons. The standard deviation and range are given to indicate the degree of variation in concentration of nematodes in these samples. The linear correlation coefficient (r) is also included to indicate the closeness of association between data obtained with the two extracting solutions. In addition, Fig. 1-(F to H) presents graphs of typical data from these comparisons. Graphs were constructed by plotting the number of nematodes extracted from each sample with the use of molasses (Y-axis) against the corresponding number extracted with the sucrose solution (X-axis). Each graph includes the linear equation describing the relation between the two sets of data and a sufficient number of points to visualize the amount of "scatter" obtained; no attempt was made to represent all points. Comparisons resulting in coincident points were represented by a single dot on the graph.

The data (Table 1) show that results obtained with molasses solution maintained a very close relation with those obtained with the sucrose solution as indicated by the highly significant correlation coefficient between the sets of results. Although the degree of association for root-knot nematode larvae (Fig. 1-F), *Hoplolaimus galeatus* (Cobb) Sher (Fig. 1-G), *X. americanum*, and for

TABLE 2. Comparison of the flotation-sieving technique using molasses solution (sp. gr. = 1.100) with the elutriation method of Seinhorst for recovery of nematode larvae from soil.

Nematode species and types	No. recovered per 50 ml: Molasses	No. recovered per 50 ml: Elutriation	No. samples	Linear correlation coefficient (r)	Avg. no./50 cc[a]	Range[a]	Standard deviation[a]	Increased efficiency with molasses (%)
Meloidogyne larvae	746	729	48	+ 0.78**	15	0-45	13	2
Helicotylenchus dihystera	351	244	72	+ 0.66**	3	0-16	6	44
Hoplolaimus galeatus	303	167	24	+ 0.61**	7	0-43	9	81
Trichodorus christiei	118	98	24	+ 0.79**	4	0-12	2	20
Saprophagous	1,317	1,007	72	+ 0.46**	14	0-37	10	31

[a]Calculated from results obtained with the elutriation method.

dorylaimoid and saprophagous nematodes demonstrated almost perfect linear correlations (Table 1), those for *H. dihystera* and for mononchoid (Fig. 1-H) and other nematodes indicated a looser association between results with the two solutions. Table 1 shows that the molasses solution was consistently superior to the sucrose solution in the extraction of nematodes from the samples.

Table 2 presents data from comparisons between the flotation sieving method with molasses solution and the elutriation method. Fig. 1-(I to K) are graphs showing representative plots of results obtained with molasses solution (Y-axis) and the elutriation method (X-axis). Since the soil sample size for the elutriation method was 450 cc, results with this method were converted to a 50-cc basis before calculation of their relation to results with the flotation-sieving method.

Results obtained with the molasses solution maintained a close relation with results obtained using the elutriation technique; the closeness of this association was supported by the highly significant correlation coefficients calculated from the two sets of results. In all cases, the relation significantly shows a degree of scatter depending on the kind of nematode. The correlation was stronger for root-knot nematode larvae (Fig. 1-I) and *T. christiei* (Fig. 1-J), than for saprophagous nematodes. The degree of association for data on *H. dihystera* (Fig. 1-K) and *H. galeatus* was intermediate. The molasses-flotation-sieving technique was superior to the elutriation technique for extracting all nematodes in these samples (Table 2).

DISCUSSION

Extraction of nematodes by the flotation-sieving technique depends on differences in the sedimentation velocity of nematodes and other suspended particles. Physical factors operative in this partitioning can be interpreted by Stoke's law:

$$v = \frac{2gr^2\,(d^1\text{-}d)}{9\eta} \quad \text{(I)}$$

where v represents the sedimentation velocity, g the acceleration due to gravity, r and d^1 the radius and density of the particle, respectively, d the density, and η the viscosity of the suspending fluid. In applying this formula, d can be considered equal to the specific gravity of the fluid and nematodes or other small particles as spheres with a radius = 1. The term $2gr^2/9$ then becomes a constant (K) and

$$v = \frac{K(d^1\text{-}d)}{\eta} \quad \text{(II)}$$

In flotation-sieving techniques, the value of v for nematodes is minimized by increasing d, so that d^1-d = 0 or becomes negative. This results in suspension (v = 0) or flotation ($v < 0$) of the animals and a good sedimentation velocity for denser soil particles. Minimization of d^1-d was attained for most nematodes by increasing the specific gravity of the molasses extracting solution from 1.000 to 1.050 or 1.073. Further increments in the value of d beyond 1.100 progressively slowed sedimentation of denser soil particles, so that some of these particles were transferred over into the sieve, and the amount of clear liquid available for decanting was reduced. This resulted in cloudy samples and lower numbers of nematodes extracted.

Studies of specific gravity indicate that the standard molasses solution (sp. gr. = 1.100) chosen for routine extraction of nematodes is likely to extract the greatest number of nematodes from any given soil sample. Occasionally, solutions having lower specific gravity may be valuable for extraction of stubby-root nematodes or other species which behave similarly.

Results from studies on the relationship between size of soil sample and final volume in the mixing beaker indicate that for each soil sample size there exists an optimal volume of extracting solution. Once the optimal ratio between the volumes of extracting solution and amounts of soil is attained for any shape of container, additional solution causes no increase in the number of nematodes extracted.

Comparisons between the molasses solution and the sucrose solution confirmed our earlier report (5) from a smaller number of samples, in that results obtained with either solution are linearly related to those obtained with the other solution on the same samples. Statistical analyses of these results showed a consistent superiority of the molasses solution for nematode extraction. An explanation of this phenomenon probably lies in the slightly higher viscosity of the molasses solution. At constant specific gravity, changes in sedimentation velocity dv in relation to changes in viscosity $d\eta$ can be derived from equation II as:

$$\frac{dv}{d\eta} = \frac{-A}{\eta^2} \qquad \text{(III)}$$

where A is a constant. Equation III indicates that small differences in viscosity can greatly influence extraction of nematodes since dv is inversely related to the square of η. The higher viscosity of the molasses solution could then result in increased numbers of nematodes extracted.

Comparisons between the flotation-sieving technique using molasses solution and the elutriation technique of Seinhorst indicate good correlations between the two methods. These results agree with those obtained by Barker et al. (2), who found in a seasonal study that results with the flotation-sieving technique paralleled those obtained with other methods. The slight degree of superiority shown by the molasses technique over the elutriation method in the extraction of root-knot nematode larvae is not significant. The superiority shown by the molasses technique for extracting lance, spiral, stubby root, and saprophagous nematodes is statistically significant and probably relates to the size, shape, and weight of these nematodes. In comparing the molasses flotation-sieving method and the elutriation technique, lower counts should be expected from the elutriation technique since it extracts living nematodes only.

In conclusion, these studies show that blackstrap molasses, where available, provides an efficient and economical substitute for sucrose in the extracting solution for the flotation-sieving method. The mathematical interpretation of results advanced here may be useful in the development of new or more efficient extracting solutions from soil.

LITERATURE CITED

1. BARKER, K. R., and C. J. NUSBAUM. 1971. Pages 281-301 *in* B. M. Zuckerman, W. F. Mai, and R. A. Rohde, eds. Plant parasitic nematodes, Vol. I. Academic Press, New York.
2. BARKER, K. R., C. J. NUSBAUM, and L. A. NELSON. 1969. Seasonal population dynamics of selected plant-parasitic nematodes as measured by three extraction procedures. J. Nematol. 1:232-239.
3. BYRD, D. W., JR., C. J. NUSBAUM, and K. R. BARKER. 1966. A rapid flotation-sieving technique for extracting nematodes from soil. Plant Dis. Rep. 50:954-957.
4. JENKINS, W. R. 1964. A rapid centrifugal-flotation technique for extracting nematodes from soil. Plant Dis. Rep. 48:692.
5. RODRÍGUEZ-KÁBANA, R., and P. S. KING. 1972. The use of sugarcane molasses as an economical substitute in the extraction of nematodes from soil by the flotation-sieving technique. Plant Dis. Rep. 56:1093-1096.
6. SEINHORST, J. W. 1956. The quantitative extraction of nematodes from soil. Nematologica 1:249-267.
7. SNEDECOR, G. W. 1956. Statistical methods. The Iowa State College Press, Ames, Iowa. 534 p.
8. SOUTHEY, J. F. (ed.). 1970. Laboratory methods for work with plant and soil nematodes. Tech. Bull. No. 2, Min. Agric., Fish. Food., Her Majesty's Stationery Office, London. 72 p.
9. THORNE, G. 1961. Principles of nematology. McGraw-Hill, New York, 533 p.