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Short communication

Revisiting the modified Baermann extraction method: extraction efficiency of *Radopholus similis* using different extraction materials

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In order to determine the threat that plant-parasitic nematodes pose to crop production, it is essential to establish an accurate estimation of their presence. The extraction efficiency from soil, therefore, is a critical step in establishing disease pressures presented by nematodes or the conditions of soil health. To achieve high nematode extraction efficiency, various methods and modifications have been developed over time (Oostenbrink, 1954; Seinhorst, 1956; Harrison & Green, 1976; Viaene *et al*., 2021) with variations being introduced locally, depending on the availability or access to certain materials.

The simple extraction method was conceived and introduced by Baermann (1917), with a series of modifications made since to improve nematode recovery rate or adapt it to local conditions (Oostenbrink, 1954; Whitehead & Hemming, 1965; Rodríguez-Kábana & Pope, 1981; Coyne *et al*., 2007). Currently, it is among the most common extraction methods used. However, the nature of locally available or custom-made equipment for this method allows for great variations in extraction efficiency (Cesarz *et al*., 2019). The objective of the current study was to evaluate the plate method in our *NemAfrica* laboratory in Kenya. This is in part to determine a replicable and comparable protocol that we can use confidently and consistently across our experiments, and in part to enable the determination of a protocol that can be recommended across similar laboratory conditions. Considering size and motility variability between nematode genera, extraction efficiency also depends on the nematode genus (Verschoor & de Goede, 2000). For the purpose of the study we used *Radopholus similis* as a species with both male and female adults, both of which are motile, and to which we had access from *in vitro* cultures.

The experiment evaluated four key variable factors that may potentially affect extraction efficiency of *R. similis*, in a randomised complete block design with four replications and conducted three times. The four factors include: soil type (red ferralitic soil and sand), tap water volume (200 and 400 ml), soil quantity (50 and 100 ml) and filter types (19-cm-diam. milk filter (GD Textile Manufacturing), two-ply Kleenex facial tissue (Kim-Fay) and two-ply Velvex kitchen paper towel (Chandaria Industries)). The experimental unit was an individual modified Baermann plate receiving either one of the 24 treatment combinations of the four factors. Each experiment consisted of 96 samples in total.

The red ferralitic soil is a common soil type in Kenya, consisting of 83.3% clay, 7.7% silt and 9.0% sand; local river sand comprised 3.2% clay, 1.9% silt and 95% sand.

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Both soils were autoclaved, dried and sieved through 2 mm sieves. Nematode inoculum was collected from 2-month-old carrot discs and 400 mixed-stage *R. similis* were introduced across the soil surface in 200 μl of water and gently mixed for homogeneity. Soil was then spread evenly on either of the filter types, placed on top of a 15-cm-diam. plastic sieve (*ca* 2 mm aperture), placed on top of a plastic plate (Coyne *et al*., 2007) after adding water to each plate. During the nematode applications, 200 μl of nematode inoculum was sampled to individual 0.5 ml centrifuge tubes (n = 8) and the number of viable *R. similis* counted and classified to juvenile, female or male under a stereo microscope. Viability of nematodes was determined by nematode mobility as well as its reaction to a poke in the median bulb area with a handling needle. The Baermann plates were incubated at room temperature for 48 h before transferring the extraction suspension into 500 ml plastic beakers and allowing them to settle overnight before excess water was removed. The number of nematodes in the remaining 50 ml suspension was counted and classified as juvenile, female or male under a stereo microscope.

A mixed model three-way analysis of variance (ANOVA) was performed on data for each soil type separately using PROC MIXED in SAS (SAS Institute). Nematode parameter (*i.e.*, total number of nematodes recovered, relative number of females to total nematodes, males to total nematodes or juveniles to total nematodes) was included as a response variable. Explanatory variables included water volume, soil volume and filter type and all possible two-way and three-way interactions. When the effect of filter type was significant at $\alpha = 0.05$ level in a model, a multiple comparison procedure by least significant difference was conducted. Random effects of experiment and replication nested in the experiment were included in the models. Asymptotic standard errors and Wald tests for these covariance parameter estimates were produced to determine their contribution to the model. The model satisfied the statistical assumptions, thus no transformation was necessary.

Means (± standard deviation) of females, males and juveniles in the inoculum preparation were 324.5 (±17.9), 43.0 (±3.2) and 10.5 (±3.3) for the first experiment, 358.4 (±14.9), 12.9 (±2.6) and 3.9 (±2.5) for the second experiment, and 346.1 (±7.6), 31.8 (±7.6) and 37.6 (±5.5) for the third experiment, respectively. On average 17.3 nematodes for the first, 8.7 for the second and 18.6 for the third experiments were found immotile in the inoculum preparation.

Grand means of the number of recovered nematodes across experiments were 230.8 (range = 115-343) for red soil and 195.0 (range = 69-404) for sand. The mean recovery rate, calculated as (number of recovered nematodes)/(number of nematodes in the inoculum) × 100, for red soil was 53% for first, 62% for second and 63% for third experiments, and 41% for first, 45% for second and 63% for third experiments for sand. The mean recovery rate ranged from 18% for the treatment combination of 100 ml sand, 400 ml water, with kitchen paper towel from the second experiment to 97% for the treatment combination of 50 ml sand, 400 ml water, with facial tissue from the third experiment.

The extraction efficiency for total nematodes was lower ($P < 0.0001$) for kitchen paper towels relative to milk filters and facial tissues but no difference in the extraction efficiency was found between milk filters and facial tissues for both soil types ($P > 0.05$) (Fig. 1). Kitchen paper towels tend to be more absorbent than the other two filter types, which likely impedes nematode movement through the material. Milk filters are the most expensive option and are not locally available in some countries, such as Kenya, whereas facial tissues are more readily available locally and the same brand more reliably accessed. Although milk filters have been recommended as a better option than kitchen paper towels (Cesarz *et al*., 2019), our study demonstrates that facial tissue provides a more cost-effective alternative. Milk filters also led to relatively dirtier suspensions and allowed more soil particles to pass through. Passing the suspensions through a 25 μm sieve is recommended to clean such samples in a practical setting. Although milk filters are more expensive and lead to dirtier extractions, they are more durable than facial tissue and less easily damaged.

More ($P < 0.0001$) nematodes were recovered from red soil for 200 ml compared with 400 ml water volumes (Fig. 1), while no effect was observed for sand. The 200 ml water treatment just sufficiently submerged both soil types, whereas 400 ml water flooded samples to approx. 1 cm above the soil surface, which reduced nematode recovery in the higher clay content red soil but interestingly not in sand. This study evaluated these two water levels particularly to imitate conditions where soil was sufficiently submerged or flooded, thereby the water volume must be adjusted based on the size of sieve and plate.

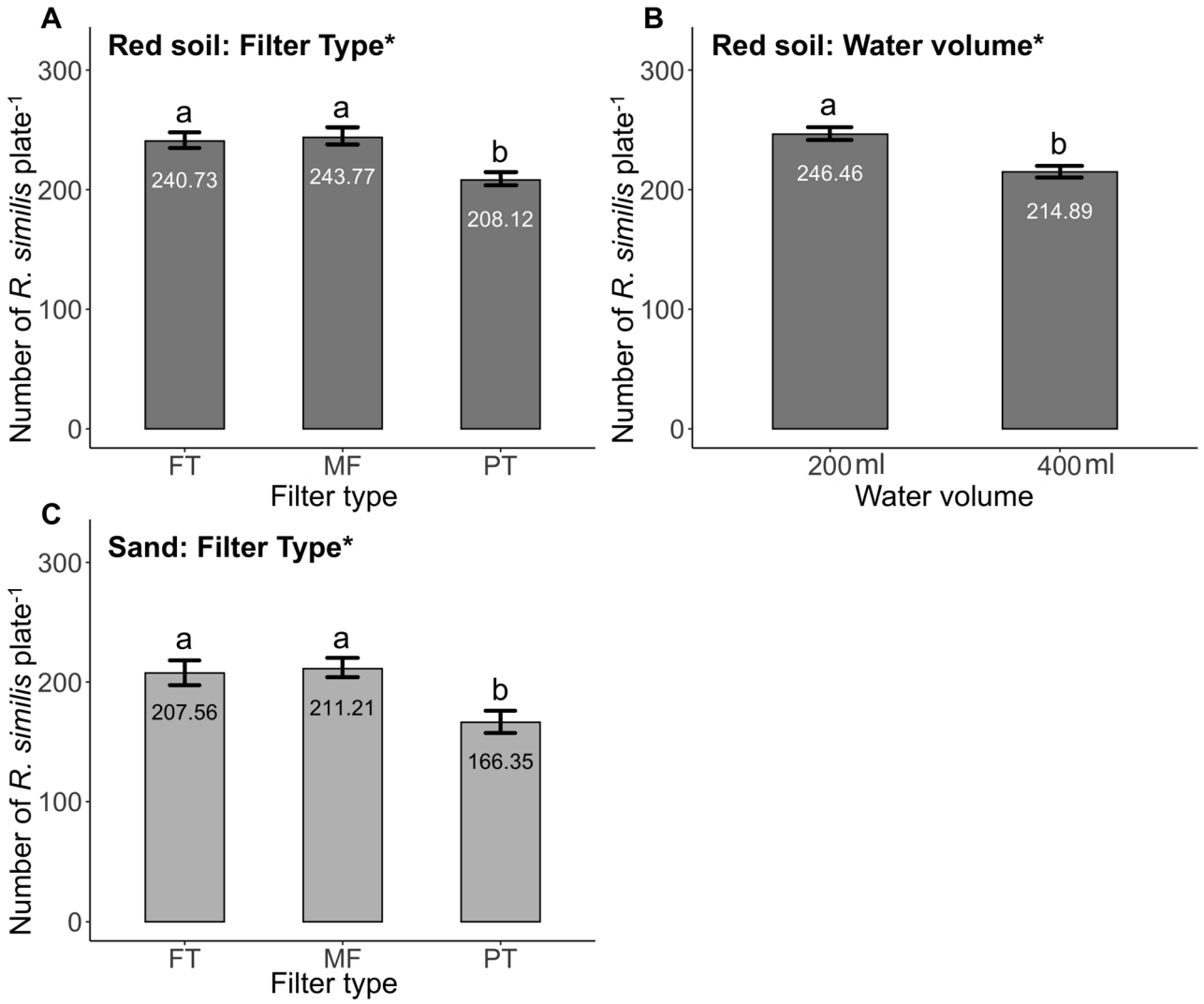

Fig. 1. Mean *Radopholus similis* recovery on modified Baermann plates for filter type (A) and water volume (B) with a red ferralitic soil and filter type for sand (C). FT = facial tissue, MF = milk filter and PT = kitchen paper towel. Data bars represent the mean ± standard error. An asterisk (*) denotes the significant factor effect ($P < 0.0001$). Letters represent significant differences among treatments in each graph. Data = means of three experiments (n = 144 for each soil type).

No difference in extraction efficiency was encountered from different soil volumes for either soil type. Generally, nematode assessment tends to be from 100 ml soil in most studies/laboratories and occasionally from 50 ml as viewed in various published studies or manuals (*e.g.*, Coyne *et al*. 2007). For both soil volumes, the depth of soil on the tray was less than 1 cm, above which Cesarz *et al*. (2019) reported smaller extraction efficiency. It appears therefore, that extraction efficiency does not significantly differ with soil layers below 1 cm depth.

None of the two-way or three-way interactions was significant ($P > 0.05$) for the total number of recovered nematodes for both soil types. The two-way interaction between filter type and water volume was nearly significant for sand ($P = 0.08$), with an extraction efficiency relatively greater for 400 ml volume of water for facial tissue, less for milk filters, and no difference for kitchen paper towels. This contradiction of water volume effect by filter types explains the insignificant effect of water volume alone to the extraction efficiency for sand.

For red soil, the relative number of females to total nematodes (F/T), males to total nematodes (M/T) and juveniles to total nematodes (J/T) were influenced ($P < 0.05$) by filter type (Facial tissue: F/T = 0.929, M/T = 0.049, J/T = 0.023, Milk filter: F/T = 0.907, M/T = 0.059, J/T = 0.034, Kitchen paper towel: F/T = 0.931, M/T = 0.046, J/T = 0.023). Higher numbers of males and juveniles passed through milk filters relative to other filter types, resulting in a smaller ratio of F/T and larger ratios of M/T and J/T. Water volume also significantly ($P < 0.05$) affected F/T and M/T (400 ml: F/T = 0.928, M/T = 0.047, 200 ml: F/T = 0.917, M/T = 0.056), with 400 ml leading to a greater F/T and lower M/T. No interaction was significant. Such effects of water volume and filter type were not observed for sand, with no significant effect on the gender ratios for any treatment (F/T = 0.913, M/T = 0.061, J/T = 0.026).

For both soil types, random effects of experiment and replications had no significant contributions to the model error, attesting less influence of time or indoor conditions, such as air circulation and temperature variations within an extraction room, to the extraction efficiency, although there may be variations of unknown source.

Our study demonstrates that different water volumes and filter types induced a relatively large variation in the extraction efficiency of nematodes, using *R. similis* as a test species, for two soil types. For good laboratory practice and to enable comparative assessment between studies, it is important that the techniques we use are standardised for consistency of results. Based on our findings, it is recommended to use either facial tissue or milk filter with a water volume that is sufficient to cover the soil layer, which should be less than 1 cm in order to achieve a higher and more consistent extraction efficiency. Facial tissue might be a more economical option as opposed to milk filter for laboratories with limited resources as in Kenya. Although this study was conducted on only one test species, the combination of 100 ml soil, 200 ml of water and facial tissues improved the total number of extracted nematodes from field soil samples by 133% at average compared to extraction with kitchen paper towels.

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References

Baermann, G. (1917). Eine einfache Methode zur Auffindung von *Ankylostomum* (Nematoden) larven in Erdproben. *Geneeskundig Tijdschrift voor Nederlandsch Indië* 57, 131-137.

Cesarz, S., Schulz, A.E., Beugnon, R. & Eisenhauer, N. (2019). Testing soil nematode extraction efficiency using different variations of the Baermann-funnel method. *Soil Organisms* 91, 61-72. DOI: 10.25674/so91201

Coyne, D., Nicol, J. & Claudius-Cole, A. (2007). *Practical plant nematology: a field and laboratory guide*. Ibadan, Nigeria, IITA, pp. 34-37.

Harrison, J.M. & Green, C.D. (1976). Comparison of centrifugal and other methods for standardization of extraction of nematodes from soil. *Annals of Applied Biology* 82, 299-308. DOI: 10.1111/j.1744-7348.1976.tb00565.x

Oostenbrink, M. (1954). Een doelmatige methode voor het toetsen van aaltjesbestrijdingsmiddelen in grond met *Hoplolaimus uniformis* als proefdier. *Mededelingen van de Landbouwhogeschool Gent* 19, 377-408.

Rodríguez-Kábana, R. & Pope, M.H. (1981). A simple incubation method for the extraction of nematodes from soil. *Nematropica* 11, 175-185.

Seinhorst, J.W. (1956). The quantitative extraction of nematodes from soil. *Nematologica* 1, 249-267. DOI: 10.1163/156854100509204

Verschoor, B.C. & de Goede, R.G.M. (2000). The nematode extraction efficiency of the Oostenbrink elutriator-cottonwool filter method with special reference to nematode body size and life strategy. *Nematology* 2, 325-342. DOI: 10.1163/156854100509204

Viaene, N., Hallmann, J. & Molendijk, L.P.G. (2021). Methods for nematode extraction. In: Perry, R.N., Hunt, D.J. & Subbotin, S.A. (Eds). *Techniques for work with plant and soil nematodes*. Wallingford, UK, CAB International, pp. 12-41.

Whitehead, A.G. & Hemming, J.R. (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* 55, 25-38. DOI: 10.1111/j.1744-7348.1965.tb07864.x