# A comparison of mustard, household detergent and formalin as vermifuges for earthworm sampling

Gaute Bø Grønstøl, Torstein Solhøy & Mass Kåre Løyning

Published on paper: 2000.
Published online: 2024-10-11.
ISSN 1502-4873 (paper).
ISSN 1891-5396 (electronic).
doi: https://doi.org/10.5324/fn.v20i0.6000.

Grønstøl, G.B., Solhøy, T., & Løyning, M.K. 2000. A comparison of mustard, household detergent and formalin as vermifuges for earthworm sampling. - Fauna norv. 20: 27-30.

The efficiencies of three vermifuges (household detergent, mustard and formalin) in extracting earthworms were compared in a pasture in western Norway. Relative efficiency of detergent and mustard compared to formalin with respect to number of earthworms extracted, was 79% and 66%, respectively. No significant differences were found among the three treatments in proportion of juveniles, earthworm size, number of earthworms or biovolume of emerging earthworms. Two genera of earthworms were represented in the samples, *Allolobophora* spp. and *Lumbricus* spp. Genus composition differed among the three treatments, formalin extracting the highest percentage of *Allolobophora* spp. and mustard the lowest. The relative efficiency of the three vermifuges differed from what has been found in an earlier comparable study. Both mustard and household detergent are less detrimental to health of vegetation, earthworms and field workers than formalin, and given their relatively high efficiency as vermifuges, they might in many cases be preferable to formalin.

*Gaute Bø Grønstøl (corresponding author, E-mail: gaute.gronstol@zoo.uib.no), Torstein Solhøy & Mass Kåre Løyning. Address: Department of Zoology, University of Bergen, Allégt. 41, N-5007, Norway.*

## INTRODUCTION

Sampling of earthworms has proven useful in several contexts aside of studies of earthworm biology. It has been used to estimate habitat quality of waders and mammals, e.g. golden plovers (*Pluvialis apricaria*), lapwings (*Vanellus vanellus*), curlews (*Numenius arquata*) and badgers (*Meles meles*) (Bengtson 1976, Barnard & Thompson 1985, Baines 1990, Berg 1993, Dasilva et al. 1993, Grønstøl 1996). The degree of soil pollution can be monitored by analysing earthworm abundance and the quantity of pollutants that are assimilated by earthworms (e.g. Pizl & Josens 1995). The diversity and density of earthworm communities may provide estimates of the decomposition rate and productivity of an area (e.g. Curry & Byrne 1992). Earthworm sampling may also provide a useful pedagogic tool in undergraduate ecology courses, where it can be used to demonstrate in the field concepts like sampling design, population density, spatial aggregation, ecological niches, biodiversity and habitat segregation.

Extraction of earthworms using a vermifuge is in many cases preferable to hand sorting soil samples. It is less time consuming, and soil and vegetation cover need not be removed. Formalin is the most commonly used vermifuge (e.g. Raw 1959, Lee 1985) but has the drawback that it is toxic to vegetation, harmful or even lethal to the earthworms (Gunn 1992), and carcinogenic and allergenic to humans. In this study we compare the effectiveness of mustard and household detergent as vermifuges with that of formalin. The results are further compared with those of Gunn (1992) who did a similar study.

## METHODS AND MATERIAL

Sampling was conducted at Rosendal in western Norway (59°59'N, 6°02'E) on a pasture with an approximately 1-2 cm high vegetation grazed by sheep. The soil was fertile brown soil with a low sand content and pH at around 5.6-5.8. Six sampling squares of 0.5m x 0.5m were laid out along each of three parallel transect lines at 10m intervals. The distance between the transect lines was three metres. Each square of a sampling triplet was treated with different test substances, and the types of vermifuges were systematically alternated along the transects so that in each transect two squares were treated with each type of vermifuge. Treatment substances were mixtures of water and a) Dijon mustard of the brand «Maille» (30 ml $l^{-1}$), traditional flavour, imported from France (content: water, mustard seeds, vinegar, salt, and citric acid), b) household detergent used for dishwashing of the brand «Zalo» (40 ml $l^{-1}$) (without phosphate, containing 15-30% anionic tensids, 5-15% non-ionic amphoteric tensids, <5% preservatives, <0.03% 1.2-benzisotiazolin-3-on) manufactured by Lilleborg A/S and c) formalin (6 ml of 36% $l^{-1}$ or 0.2%). On each square three litres of solution was applied, and earthworms were collected for 10 min. This was done twice in succession on each square. Towards the end of the second period very few earthworms emerged. Biovolume, which correlates tightly with dry weight (Nordström & Rundgren 1972a), was measured using a graduated cylinder. Earthworms were counted, and genus composition and proportion of juveniles (individuals with no clitellum) were determined. Sampling was done from 5-7 May 1997, and the weather was cloudy with some light rain showers with temperatures around 3 to 6°C.

## RESULTS

Differences between vermifuges in number of earthworms, biovolume, and mean size (biovolume divided by number of earthworms extracted) were analysed using three-way ANOVA (Leven's test for homogeneity of variance of groups of different treatments: $p > 0.05$ for all three tests). No significant differences were found in any of the above measurements (Table 1), however, the power was relatively low and judging by the mean values, the trend was that formalin was the most effective, and mustard the least effective vermifuge.

Two genera of earthworms (*Lumbricus* ssp. and *Allolobophora* ssp.) were represented in the samples. More than 95% of the *Lumbricus* species were *L. rubellus*, the rest was *L. terrestris*. The *Allolobophora* representatives were mainly *Allolobophora caliginosa*. A chi-square test revealed that the ratio *Allolobophora* ssp. to *Lumbricus* ssp. was significantly different among the three treatments, with formalin producing the highest and mustard the lowest ratio ($X^2$=7.68, df=2, $p<0.025$; Table 2). There were no significant differences in the proportion of juveniles extracted by the three different vermifuges neither when considering separate genera nor pooled earthworms ($X^2$<1, df=2, $p>0.95$, for all three comparisons; Table 2).

## DISCUSSION

The general density of earthworms was high (Table 1, Figure 1), as is often found in pastures (Nordström & Rundgren 1973). When considering earthworm numbers, size, biovolume, and age distribution, both mustard and detergent seemed to be nearly as, if not equally, efficient vermifuges as formalin (no statistically significant differences). But the efficiencies of mustard and detergent were lower than formalin in extracting *Allolobophora* spp, which is known to reside deeper in the substrate than *L. rubellus* and overlapping with the distribution of *L. terrestris* (Rundgren 1975). This might be explained by a shallower impact range of mustard- and detergent solution due to their higher viscosity. In this context it should also be noted that Nordström & Rundgren (1972b) found that formalin extraction tended to underestimate density of juvenile *Allolobophora* spp. while predicting well *Lumbricus* spp. densities in general. This effect should not confound conclusions of this study, since our main aim was not so much to report precise earthworm density estimates as to compare efficacy of different extraction solutions.

**Table 1.** Efficiencies of formalin, detergent and mustard as vermifuges in extracting earthworms. n = 6 for each treatment. Statistical data describes the treatment effect, in a three-factor ANOVA (effects explained by the other factors, i.e. triplets and transect lines, are not included in the table).

| | Formalin | | Detergent | | Mustard | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE | F-ratio | P | Power |
| No of worms $m^{-2}$ | 378 | 46 | 298 | 51 | 249 | 72 | 1.6 | 0.26 | 0.25 |
| Biovolume $m^{-2}$ in ml | 107 | 13 | 84 | 14 | 63 | 20 | 3.01 | 0.11 | 0.43 |
| Mean size in ml | 0.29 | 0.02 | 0.28 | 0.01 | 0.24 | 0.02 | 2.17 | 0.18 | 0.32 |

**Table 2.** Genus composition and distribution of juvenile vs. adult earthworms in samples extracted by formalin, detergent and mustard, respectively. Actual numbers of earthworms extracted are given in brackets.

| | Formalin | | Detergent | | Mustard | |
|---|---|---|---|---|---|---|
| | % total | % juv. | % total | % juv. | % total | % juv. |
| *Allolobophora* spp. | 91 | 81 | 87 | 78 | 85 | 81 |
| | (515) | (415) | (391) | (306) | (317) | (256) |
| *Lumbricus* spp. | 9 | 83 | 13 | 88 | 15 | 86 |
| | (52) | (43) | (56) | (49) | (56) | (48) |

*L. terrestris* is a deep burrower compared to *L. rubellus*, hence one might suspect that the efficacy of vermifuge extraction should be relatively lower for this species due to dilution or soil absorption of the extraction fluid. However, in pastures in southern Sweden, Nordström & Rundgren (1972b) found that formalin extraction was as efficient as hand sorting on estimating populations of both *L. terrestris* and *L. rubellus* in April and August. *Allolobophora* species are deep burrowers and have as a group a more homogenous biology and distribution than what is found when comparing *L. terrestris* and *L. rubellus* (Rundgren 1975). Based on the above considerations we deemed it acceptable to pool earthworms to genera when analysing relative efficacy of the extraction solutions.

Gunn (1992) showed that the effectiveness of mustard increased with increasing concentration from 50-250 ml $l^{-1}$, so by using higher concentrations of mustard and detergent than we used, one might possibly increase the efficiency of these vermifuges to the level of formalin. If the extraction efficiencies of mustard and detergent do not increase with increased concentrations, the scale of the differences might be evaluated using larger sample sizes, which would reduce the standard errors and increase the testing power (Table 1). Then one might pin down differences at a more fine grained level than this study could. If, as indicated by mean densities found in this study, mustard and detergent are slightly less efficient than formalin, one could perhaps identify the genus- or species specific factors by which the numbers should be multiplied in order to be comparable to results of studies using formalin. This might be useful since formalin traditionally has been used to estimate earthworm abundance (Lee 1985).

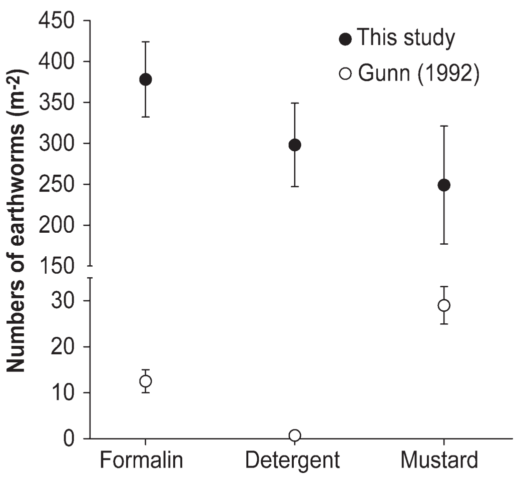


Figure 1.
Earthworm densities estimated using formalin, houshold detergent and mustard. Results from this study are compared with results from Gunn (1992) who did a similar study. Error bars signify one standard error, n=6 in all groups.

Our results contrasted somewhat with Gunn's (1992) findings from a similar study comparing the efficiency of formalin, household detergent and mustard (Fig 1). Using the same number of sampling squares, Gunn (1992) found mustard (15 ml $l^{-1}$) to be the best vermifuge yielding significantly more earthworms than formalin (0.2%). Formalin extracted a significantly higher number of earthworms than household detergent (15 ml $l^{-1}$) which had virtually no effect.

Discrepancies between ours and Gunn's results may have several causes. 1) Differences in soil characteristics (density, water saturation and temperature) might perhaps affect the penetration depth of different substances, and it is conceivable that differences in soil composition or acidity might lead to chemical compounds in detergent or mustard being neutralized or chemically tied up to a different extent. 2) Chemical compositions of and concentration of detergent or mustard differed. In this study we used about double the concentrations of detergent and mustard than Gunn (1992). 3) Efficiency of vermifuge extraction may vary between earthworm species, so the different results might be explained by differences in species or genus composition between sites.

Different kinds of detergents and mustard might differ in their earthworm extraction efficiency, which emphasize the need to calibrate the relative efficiency of alternative vermifuges to formalin in pre-experimental tests before replacing formalin. Reports of such comparisons should provide a description of the substance used (brand, manufacturer, chemical composition and concentration), a listing of the species/genera extracted and a description of the habitat/soil that is sampled.

To conclude, our results partly support Gunn's conclusion that mustard might be a good substitute for formalin, which is toxic to plants, study animals and field workers. Our results, however, suggested that the household detergent we used, «Zalo», is an even better alternative, being at least as effective as mustard, much cheaper, easy to obtain, biodegradable and not harmful to skin.


## ACKNOWLEDGMENTS

Thanks to enthusiastic ecology students at the University of Bergen for field work.



## SAMMENDRAG

### En sammenligning av effektiviteten til sennep, oppvaskmiddel og formalin som utdrivningsagenser ved estimering av meitemarktetthet

Det har lenge vært vanlig å bruke formalin som utdrivningsagens i studier som involverer tetthetsestimater av meitemark.

Formalin er toksisk for vegetasjon, sterkt skadelig for meitemark og kreft- og allergifremkallende hos mennesker. Dette gir et insitament til å finne alternative ufarlige utdrivningsagenser som er like effektive som formalin. I dette studiet testet vi hvor effektivt oppvaskmiddel (Zalo) og sennep (Dijon) var i forhold til formalin som utdrivningsagens. Med hensyn til antall meitemark var effektiviteten til løsninger med oppvaskmiddel og sennep henholdsvis 79% og 66% relativt til formalin. To slekter meitemark, *Allolobophora* ssp. og *Lumbricus* ssp., var representerte i prøvene. Andelen av *Allolobophora* ssp. i forhold til *Lumbricus* ssp. var signifikant forskjellig mellom løsningene; formalin hadde den høyeste andelen av *Allolobophora* ssp. og sennep den laveste. Vi fant ingen signifikante forskjeller mellom utdrivningsagensene med hensyn til størrelse, antall, biovolum og andel juvenile meitemark. Den relative effektiviteten av formalin, oppvaskmiddel og sennep skilte seg fra resultater rapportert av Gunn (1992). Dette kan skyldes forskjeller mellom studieområdene med hensyn til jordtyper, konsentrasjoner, typer av vaskemiddel og sennep brukt, og/eller ulik artssammensetning av meitemark.

Tatt i betraktning at både oppvaskmiddel og sennep viste seg å være rimelig effektive, og at de er betydelig mindre helseskadelige enn formalin, vil disse løsningene i mange sammenhenger være gode alternativer til bruk av formalin ved utdrivning av meitemark.

## REFERENCES

Baines, D. 1990. The roles of predation, food and agricultural practice in determining the breeding success of the lapwing *Vanellus vanellus*. - Journal of Animal Ecology 59: 915-929.

Barnard, C.J. & Thompson, D.B.A. 1985. Gulls and plovers. - Croom Helm, Kent.

Bengtson, S.A. 1976. Effect of bird predation on lumbricid populations. - Oikos 27: 9-12.

Berg, Å. 1993. Food resources and foraging success of Curlews *Numenius arquata* in different farmland habitats. - Ornis Fennica 70: 22-31.

Curry, J.P. & Byrne, D. 1992. The role of earthworms in straw decomposition and nitrogen turnover in arable land in Ireland. - Soil Biology and Biochemistry 24: 1409-1412.

Dasilva, J., Woodroffe, R. & Macdonald, D.W. 1993. Habitat, food availability and group territoriality in the european badger, *Meles meles*. - Oecologia 95: 558-564.

Gunn, A. 1992. The use of mustard to estimate earthworm populations. - Pedobiologia 36: 65-67.

Grønstøl, G.B. 1996. Aerobatic components in the songflight display of male lapwings as cues in female choice. - Ardea 84: 45-55.

Lee, K.E. 1985. Earthworms their ecology and relationships with soils and land use. - Academic Press, Australia.

Nordström, S. & Rundgren, S. 1972a. Volumetric estimation of body size in lumbricids. - Oikos 23: 278-280.

Nordström, S. & Rundgren, S. 1972b. Methods of sampling lumbricids. - Oikos 23: 344-352.

Nordström, S. & Rundgren, S. 1973. Associations of lumbricids in Southern Sweden. - Pedobiologia 13: 301-326.

Rundgren, S. 1975. Vertical distribution of lumbricids in southern Sweden. - Oikos 26: 299-306.

Pizl, V. & Josens, G. 1995. Earthworm communities along a gradient of urbanization. - Environmental pollution 90: 7-14.

Raw, F. 1959. Estimating earthworm populations by using formalin. - Nature 184: 1661-1662.