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A high throughput investigation on transfer tools for nematodes in various suspension

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Abstract

This study evaluates the efficiency of three different tools-forceps, needle and wire pick for transferring Root-Knot Nematode (RKN) females in various suspension media (water, formalin and lactophenol). The performance of each tool was assessed based on picking time, number of females transferred per minute, damage rates and overall picking efficiency. The wire picks consistently demonstrated the highest picking efficiency, achieving 99% in water suspension, 98% in formalin suspension and 98% in lactophenol suspension. It outperformed both the forceps and needle in terms of speed, precision and minimal damage. The forceps showed lower efficiency and higher damage, particularly in water and lactophenol suspensions, while the needle, though more efficient than the forceps, was less effective compared to the wire pick in all scenarios. These findings highlight the wire pick as the most effective tool for RKN female and vermiform nematode transfer across different media and emphasize the need for selecting appropriate tools to optimize nematode handling in research and practical applications.

Keywords

forceps; needle; nematode handling tools; picking efficiency; root-knot nematodes; suspension media; wire pick

Introduction

Nematodes are one of the most abundant groups of organisms on Earth with an estimated one million species of which only a fraction has been described (1–3). They inhabit a wide range of environments, including soil, water and as parasites of plants and animals (4-6). In agricultural systems, nematodes can be both beneficial and harmful. Beneficial nematodes, such as those from the families Steinernematidae and Heterorhabditidae, are used in biological control of insect pests (7). However, plant-parasitic nematodes (PPNs) are significant agricultural pests, causing annual crop losses estimated at $100 billion worldwide (8).

Understanding nematode biology and behaviour is essential for developing effective control strategies and mitigating their impact on crops and human health. Nematode picking, the process of manually isolating individual nematodes from a sample, is a fundamental technique used in various areas of nematode research. It is crucial for several applications. Picking allows researchers to isolate individual nematodes for morphological characterization and identification, which is essential for taxonomic studies and understanding nematode diversity (9). Additionally, it enables the isolation of specific nematodes for DNA extraction and genetic analysis, providing insights into population structure, genetic diversity and the evolution of nematode

populations (10). This technique is also crucial in bioassays, where specific nematodes are required for testing the effects of different stimuli, such as chemical compounds, essential oils or microbial antagonists (4). Furthermore, picking facilitates the isolation of nematodes for detailed microscopic examination, revealing internal structures, developmental stages and other features relevant to nematode biology (9). The accurate handling and isolation of nematodes are essential for research and management. Various techniques have been developed to extract and handle nematodes from soil and plant tissues. These methods include flotation, sieving and the use of centrifugal flotation techniques (11, 12). However, after extraction, the challenge remains to accurately isolate specific life stages, particularly swollen females and vermiform nematodes, which are critical for further investigation such as molecular analysis. Traditional tools for nematode handling include fine forceps and needles. Forceps are commonly used for their ability to grasp nematodes, but their effectiveness is limited by the risk of exerting too much pressure, leading to physical damage (13). Needles, on the other hand, offer precision but can be cumbersome and time-consuming to use, especially when dealing with delicate or large nematodes (14). Both tools require considerable skill and practice to use effectively.

The limitations of traditional tools in handling swollen females and larger vermiform nematodes are well-documented. These nematodes are often more fragile and susceptible to damage during handling. For instance, root-knot nematodes (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* spp.) have swollen female stages that are critical for identification and study but are easily damaged by forceps or needles (15). Similarly, migratory endoparasitic nematodes like *Pratylenchus* spp. require delicate handling to avoid damage during isolation. In response to these limitations, various innovative tools and techniques have been developed. Some researchers have explored the use of micro-manipulation devices and automated systems for nematode handling (16). These technologies, while promising, are often expensive and require specialized training, limiting their widespread adoption.

The concept of a wire pick tool for nematode handling is inspired by the need for a simple, cost-effective and efficient tool that minimizes damage to delicate nematodes. Similar tools have been used in other areas of micro-manipulation, such as in entomology and cell biology, where precision and minimal physical stress are paramount (17). Preliminary studies on the use of wire tools for nematode handling have shown promising results. For example, the use of fine wire loops has been reported to improve the accuracy of nematode isolation and reduce damage rates compared to traditional tools (18). However, systematic studies comparing the efficacy of wire picks to forceps and needles specifically for swollen females and vermiform nematodes are lacking.

A pick is a basic tool required in nematology to isolate a desired nematode from a suspension containing different species of nematodes. The nematode pick is generally made from feathers, eyelashes, plastic bristles and thin metal points and is mainly used for handling the vermiform nematodes. However, for handling swollen females of root-knot nematodes (RKN), *Meloidogyne* spp. specialized picks are not available. The simple forceps are still used in laboratories to pick the swollen females. Moreover, extreme care is required with the forceps to pick the swollen female nematodes without crushing or damaging them. As the uncrushed or undamaged swollen females of RKN are prerequisites for classical taxonomy and molecular diagnosis, we have developed an electric wire-based simple pick for handling swollen females and vermiform nematodes.

This study aims to evaluate the performance of the wire pick tool in comparison to traditional forceps, needles and picks. We hypothesize that the wire pick tool will demonstrate superior performance in terms of handling time, precision and reduced damage rates, thus proving to be a valuable addition to the nematologist's toolkit with the following objectives:

- To design and fabricate a simple wire pick tool for handling swollen females and vermiform nematodes. To compare the effectiveness of the wire pick tool with traditional picks, forceps and needles through experimental trials.
- To assess the handling time, precision and damage rates associated with each tool.
- To provide recommendations for the use of the wire pick tool in nematological research and applications.

By addressing these objectives, we aim to contribute to the development of more efficient and precise methods for nematode handling, ultimately enhancing the capabilities of researchers and practitioners in the field of nematology.

Materials and Methods

Electric wire-based nematode pick preparation

The wire pick tool was designed to provide a simple, efficient and cost-effective method for handling swollen females and larger vermiform nematodes. A piece of 10-15 cm long ordinary wire (No. 10) used for electrical conductivity for households is taken for making a pick (Fig. 1). First 10 cm of wire served as a handle for the pick. The remaining 2.5 cm of plastic insulation is removed carefully using a sharp knife or scalpel blade without damaging the copper threads. Only 4-6 copper wire threads were retained and others were removed using scalpel blades under a stereo zoom microscope. The flexible copper threads are made in to bowel shaped and the distance between the copper threads was adjusted to 0.3-0.6 mm to retain the root-knot nematode females (The dimension of the root-knot nematode females: Pear-shaped and about 0.4-1.3 mm long by 0.27-0.75 mm wide). It is better to select the copper threads thickness as near as the dimension of the swollen females.

Nematode culture

Nematodes were obtained from mixed soil samples collected from agricultural fields, containing a variety of nematode species, including swollen females and larger vermiform stages of root-knot (*Meloidogyne* spp.), cyst (*Heterodera* spp.) and root-lesion (*Pratylenchus* spp.) nematodes. The nematodes were extracted using a modified Baermann funnel technique, which involved placing soil samples on a mesh

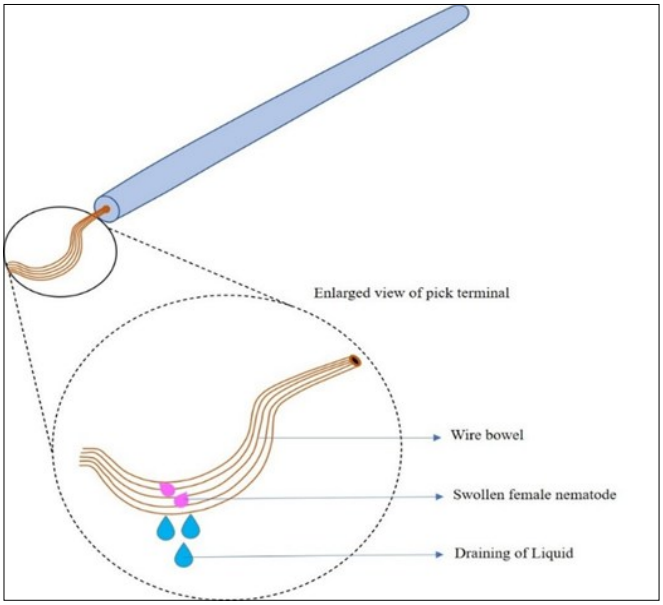

Fig. 1. A simple tool for picking swollen females and vermiform nematodes. A custom-designed tool for efficiently picking swollen female and vermiform nematodes. The tool features a fine-tipped wire or needle mounted on a handle, designed to allow precise and gentle transfer of nematodes from various suspension media. The tip's shape and material provide stability and reduce the risk of damaging delicate nematode structures, particularly useful for handling swollen females in sensitive molecular or morphological studies. This tool offers an ergonomic grip, enhancing control and reducing hand fatigue during prolonged use. The design enables easy sterilization and re-use, making it a practical option for nematode handling across research applications.

screen over water in a funnel. This setup allowed nematodes to migrate into the water over a 48-hour period, after which they were collected from the bottom of the funnel and stored in distilled water at 4 °C until use (19).

Nematode picking efficiency

The root-knot nematode, *Meloidogyne incognita*, was cultured in a susceptible plant, *Plectranthus forskohlii* (Syn *Coleus Forskohlii*). The RKN-infested roots were taken and the adhering sands were gently washed using tap water. From that, approximately 150 swollen root-knot nematode females were teased out and collected in a Petri dish. Forceps, needles, conventional picks and wire picks were used to pick 100 nematodes each, from the beaker containing female RKNs into another beaker. A stopwatch was used to measure the time taken for transferring 100 female RKN and the number of nematodes transferred per minute. Due care was taken to shift only a single nematode at a time. The damage in the transferred female RKN was observed and quantified under a stereo microscope (Lawrence Mayo, Model; LM-52-3621 elegant). The Picking Efficiency (PE) was computed based on the formula described below.

$$\text{Picking Efficiency (PE)} = \frac{\text{(TFT)-(TFD)}}{\text{(TFT) x 100}} \quad \text{Eqn. 1}$$

TFT - Total number of females transferred; TFD - Number of females damaged after transfer

Statistical Analysis

A completely randomized design (CRD) was employed to evaluate the performance of the wire pick tool compared to traditional forceps and needles. Three treatments were established: forceps, needle and wire pick tool, with each treatment replicated five times. Each replicate consisted of 30 nematodes. The handling procedure involved selecting 30 swollen females and larger vermiform nematodes from the extracted suspension using a stereomicroscope. Each tool was then used to pick and transfer the selected nematodes from a Petri dish to a microscope slide with a drop of water. The handling time for each nematode was recorded in seconds using a stopwatch and any physical damage to the nematodes was noted under the microscope. Damage was defined as any visible physical harm or deformation. An Analysis of Variance (ANOVA) was conducted to test for significant differences between the tools in terms of handling time and damage rate. The model used for the ANOVA included the overall mean, the effect of the treatment and the random error associated with the observation. The Standard Error of Difference (SEd) between treatment means was calculated using the mean square error (MSE) from the ANOVA and the number of replicates. The Critical Difference (CD) at a 0.005 significance level was calculated using the critical value of the t-distribution for a two-tailed test at the desired significance level and degrees of freedom. Pairwise comparisons of treatment means were made using the CD value, with treatments grouped based on significant differences. Statistical analyses were performed using R software, specifically employing the 'agricolae' package for the ANOVA and post-hoc tests. This detailed methodological approach ensured a robust evaluation of the wire pick tool's performance compared to traditional handling tools.

Results

The efficiency of different tools for transferring Root-Knot Nematode (RKN) females in various suspensions was assessed across three different solutions: water, formalin and lactophenol. The wire pick's efficiency (99%) in water suspension underscores its potential for applications requiring speed and precision, significantly outperforming both the forceps (46%) and needle (92%). The wire pick required the least amount of time (2.5 min to pick 100 females) and had the highest number of females transferred per minute (52.44), coupled with minimal damage (0.24%). Conversely, the forceps took the longest (4.94 min), had a lower transfer rate (18.22 females per min) and caused higher damage (9.80%). Conventional pick required an average of 11.34 min (range: 9.8-13.6) to transfer 100 RKN females, with a transfer rate of 10.92 females per minute (range: 6-15). Damage to nematodes was minimal, with only 0.89 females (range: 0-2) damaged on average, resulting in 99.1 undamaged females (range: 98-100) and a picking efficiency of 91%. The needle was more efficient than the forceps but less than the wire pick, taking 10.32 min to pick 100 females and transferring 14.87 females per min with 1.01% damage (Table 1). In formalin suspension, the wire pick again showed the highest efficiency (98%), with a quick picking time of 2.8 minutes and a high transfer rate of 36.6 females per min. It also resulted in minimal damage (0.6%). The forceps, while faster than the needle (3.34 min), still had a lower efficiency (68%) compared to the wire pick, transferring 23.8 females per min but causing more damage (7.6%). Using the conventional pick took an average of 11.98 min (range:

Table 1. Picking efficiency of different tools to transfer RKN females in water suspension

Picking tools	Time taken to pick 100 females	Number of females transferred per minute	Number of females damaged	Number of females without damage	Picking efficiency (PE=TFT-TFD/TFT × 100) (%)
Forceps	4.94^{a*} (4.2 - 5.3)	18.22^{b} (13 - 21)	9.80^{b*} (8 - 12)	90.2^{a*} (88 - 93)	46
Needle	10.32^{b*} (8.7 - 11.4)	14.87^{b*} (9 - 21)	1.01^{a*} (0 - 2)	99.0^{b} (98 - 100)	93
Conventional pick	11.34^{b*} (9.8 - 13.6)	10.92^{b*} (6 - 15)	0.89^{a*} (0 - 2)	99.1^{b} (98 - 100)	91
Wire pick	2.5^{a*} (1.9 - 3.1)	52.44^{a*} (36 - 68)	0.24^{a} (0 - 1)	99.8^{b} (99 - 100)	99
SEd	0.56	5.28	0.92	0.92	
CD (p=0.05)	1.93	18.11	3.16	2.01	

* pairwise comparisons are significant @ 5% (p = 0.05) by Tukey's HSD test. The table presents the results of a study comparing the efficiency and outcomes of three different tools (forceps, needle and wire pick) used for picking females in Water suspension, with statistical significance tested using Tukey's HSD test at a 5% significance level.

10.1-13.6) to transfer 100 females, with a rate of 12.92 females per min (range: 9-15). The tool damaged an average of 2.47 females (range: 0-2), leaving 97.5 females undamaged (range: 98-100) and yielding a picking efficiency of 80%. The needle, taking 9.54 min, had the lowest transfer rate (11.2 females per min) and also the highest damage rate (3.02%), resulting in a lower efficiency (73%) (Table 2). In lactophenol suspension, the wire pick continued to exhibit superior performance with an efficiency of 98%, picking 100 females in just 1.32 min, transferring 71.2 females per min and causing minimal damage (1.4%). Using the conventional pick in lactophenol suspension took an average of 5.12 min (range: 4.6-5.9) to transfer 100 RKN females, with a transfer rate of 15.06 females per min (range: 11-18). The average number of damaged females was 1.6 (range: 0-3), leaving 98.4 females (range: 97-100) without damage. This resulted in a picking efficiency of 80%. The needle also performed well with an efficiency of 86%, taking 6.02 min to pick 100 females and causing only 1.6% damage. The forceps, although quicker than the needle (4.06 min), had the lowest efficiency (55%), transferring 23.6 females per min but resulting in higher damage (10.4%) (Table 3).

Overall, the wire pick consistently demonstrated the highest picking efficiency across all suspension types, highlighting its effectiveness in transferring RKN females with minimal damage and maximum speed. The forceps and needle, while functional, showed lower efficiencies and higher damage rates, particularly in water and formalin suspensions.

Discussion

The comparative analysis of various picking tools for transferring Root-Knot Nematode (RKN) females demonstrates the wire pick's consistent superiority across different suspension media. In water suspension, the wire pick achieved a picking efficiency of 99%, transferring 100 females in just 2.5 min, resulting in a transfer rate of 52.44 females per min. This finding aligns with existing literature, which highlights its ability to minimize damage and improve handling speed (20, 21). In contrast, the forceps exhibited a much lower efficiency of 46% and a transfer rate of only 18.22 females per min, making it less suitable for handling delicate nematodes due to its gripping nature, which caused 9.8% damage. Although the needle performed better than the forceps, with a 92% efficiency, it still

Table 2. Picking efficiency of different tools to transfer RKN females in formalin suspension

Picking tools	Time taken to pick 100 females	Number of females transferred per minute	Number of females damaged	Number of females without damage	Picking efficiency (PE=TFT-TFD/TFT × 100) (%)
Forceps	3.34^{a*} (2.8 - 4.1)	23.8^{b} (19 - 30)	7.6^{a} (4 - 12)	92.4^{a*} (88 - 96)	68
Needle	9.54^{b} (8.8 - 11.1)	11.2^{b} (8 - 17)	3.02^{a*} (2 - 5)	96.9^{b*} (95 - 98)	73
Conventional pick	11.98^{c*} (10.1 - 13.6)	12.92^{b*} (9 - 15)	2.47^{a*} (0 - 2)	97.5^{b} (98 - 100)	80
Wire pick	2.8^{a*} (2.4 - 3.5)	36.6^{a*} (32 - 49)	0.6^{a} (0 - 2)	99.4^{c*} (98 - 100)	98
SEd	0.43	3.67	1.25	1.71	
CD (p=0.05)	1.64	13.85	4.73	6.45	

* pairwise comparisons are significant @ 5 % (p = 0.05) by Tukey's HSD test.

Table 3. Picking efficiency of different tools to transfer RKN females in lactophenol suspension

Picking tools	Time taken to pick 100 females	Number of females transferred per minute	Number of females damaged	Number of females without damage	Picking efficiency (PE=TFT-TFD/TFT × 100) (%)
Forceps	4.06^{c*} (3.4 - 4.7)	23.6^{b*} (17 - 31)	10.4^{b} (4 - 17)	89.6^{b} (83 - 96)	55
Needle	6.02^{b*} (5.4 - 6.5)	12.0^{b*} (8 - 15)	1.6^{a*} (0 - 4)	98.4^{a} (96 - 100)	86
Conventional pick	5.12^{c*} (4.6 - 5.9)	15.06^{b*} (11- 18)	1.6^{a*} (0 - 3)	98.4^{a} (97 - 100)	80
Wire pick	1.32^{a*} (0.8 - 1.8)	71.2^{a*} (57 - 100)	1.4^{a} (0 - 3)	98.6^{a} (98 - 100)	98
SEd	0.27	7.87	2.37	2.39	
CD (p=0.05)	1.05	29.69	8.97	9.01	

* pairwise comparisons are significant @ 5 % (p = 0.05) by Tukey's HSD test.

inflicted 1.01% damage and required significantly more time (10.32 min). These results reinforced prior studies that outline the needle's limitations compared to the wire pick (22, 23). In formalin suspension, the wire pick again excelled, achieving 98% efficiency with a picking time of 2.8 min and a transfer rate of 36.6 females per min. While the forceps demonstrated improved efficiency (68%) and a quicker picking time (3.34 min) compared to their performance in water suspension, they caused more damage (7.6%), consistent with reports of reduced control in viscous media (24, 25). The needle's performance in this medium was again limited by higher damage rates and slower handling times. The higher viscosity of lactophenol suspension further highlighted the advantages of the wire pick. It achieved 98 % efficiency with a rapid picking time of 1.32 min and a transfer rate of 71.2 females per min, confirming its effectiveness in thicker media (27). Conversely, the forceps had the lowest efficiency at 55% and inflicted the highest damage at 10.4%, while the needle, with an efficiency of 86%, offered better precision than the forceps but still fell short of the wire pick's performance (28, 29). Although the conventional pick was not the most efficient, it demonstrated balanced performance across all media. In water, it achieved a 91% efficiency with a moderate transfer rate, making it a reliable choice for tasks requiring gentle handling. It maintained an efficiency of 80 % in both formalin and lactophenol, demonstrating adaptability to denser media while ensuring low damage rates. Nematode picking is a meticulous process involving several steps to ensure the isolation and preservation of nematodes for research purposes. The initial step is sample collection and processing, which varies according to the nematode species and research goals. Soil, plant roots and water are common sources; each requiring different collection tools like soil corers for soil samples, careful excavation for root samples and plankton nets for water. To process these samples, methods such as the Baermann funnel, sugar flotation and sieving are used to isolate nematodes by separating them from debris and concentrating them for further examination. Sample preservation is also critical to maintaining the viability and morphological integrity of nematodes, using refrigeration at 4 °C for short-term storage, freezing at -80 °C for long-term preservation, or chemical fixation for morphological studies.

Nematode picking requires specialized equipment, particularly microscopes and picking tools. Dissecting microscopes (10x - 40x magnification) are used for initial sample inspection, while compound microscopes (40x - 1000x magnification) allow detailed morphological study. Picking tools vary based on the nematode size and the sample medium: fine forceps for solid surfaces, Pasteur pipettes and micropipettes for liquid samples and micro-needles for high-precision applications like genetic analysis. Techniques such as direct picking with forceps, transferring with a loop and washing with buffer solutions are used depending on the sample type and precision needed. After picking, nematodes are often mounted on microscope slides for observation. Temporary mounts with water or glycerin are used for quick morphological checks, whereas permanent mounts with Canada balsam or Hoyer's solution allow for long-term study. During observation, researchers assess characteristics such as size, cuticle patterns, head structures, tail features and internal anatomy, which aid in species identification.

Several factors influence the choice of tools and techniques, including nematode size and morphology, sample type, research objectives and the skill level of the researcher. Smaller nematodes require finer tools and different sample types may necessitate specific handling approaches. Emerging technologies, such as automated picking systems, microfluidic devices and laser capture microdissection (LCM), are enhancing the efficiency and precision of nematode picking. Automated systems use robotic arms and image analysis, microfluidic devices offer controlled manipulation and LCM provides precision for genetic studies, representing the future of nematode picking in research.

The consistent superiority of the wire pick across all suspension types highlights its optimal design for handling nematodes with minimal damage and maximum efficiency. The findings are in line with studies that advocate for the use of wire picks in nematode research due to their precision and speed (20, 24). The forceps and needle, while functional, showed lower efficiencies and higher damage rates in various suspensions. This suggests that while these tools can be used, they may not be ideal for all scenarios. Specifically, forceps may require more careful handling to avoid damaging delicate nematodes and needles may be better suited for specific applications where high precision is less critical (25, 26). Advancements in nematode handling techniques are poised to revolutionize research by overcoming the limitations of traditional manual methods. While tools like wire picks, forceps and needles are effective, they are labour-intensive and prone to variability. Emerging technologies, particularly microfluidic platforms, offer significant potential for precision and automation. Systems like the COPAS sorter and the "WormFarm" chip demonstrate how microfluidics can enable high-throughput assays and real-time tracking of nematode behaviour and morphology (30, 31). However, adapting microfluidics for larger nematodes, such as plant- and animal-parasitic species, remains a challenge due to their size and fragility. Optimizing chip designs and flow dynamics for gentle yet efficient handling is essential. Integrating machine learning and automated imaging into these platforms could further enhance data acquisition and analysis (32). By combining the precision of traditional methods with the efficiency of automation, future innovations will enable researchers to address complex biological questions with greater accuracy, opening new avenues in nematode research across agriculture, medicine and environmental sciences.

Conclusion

The results underscore the importance of selecting the appropriate tool for nematode handling, depending on the suspension medium and the required precision. The wire pick's consistent performance indicates that it is the optimal choice for researchers and practitioners who need an efficient and gentle method for transferring nematodes. Additionally, the pick is easy to prepare and causes little to no damage to swollen nematode females during handling. It is particularly useful for molecular studies that require quick picking without harming the swollen females. Nematode picking is a fundamental technique in various areas of nematode research. Selecting the right tools and techniques necessitates careful consideration of the nematode's size and morphology, the sample type, the

research objectives and the experience and skill level of the researcher. Advancements in emerging technologies continue to enhance the efficiency and accuracy of nematode picking, creating new opportunities in research. Future research should focus on refining tool designs and evaluating additional tools to improve nematode handling practices. Overall, the findings of this study provide valuable insights into the effectiveness of nematode handling tools, establishing a foundation for improved methodologies in nematode research and management.

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Authors' contributions

KP carried out the experiment and wrote the original draft. BJ supervised, validated, reviewed and edited the manuscript. AA supplied the necessary resources and interpreted the results. BJ and SP involved in data validation. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

References

1. Yang J, Wu X, Chen Y, Yang Z, Liu J, Wu D, Wang D. Combined attributes of soil nematode communities as indicators of grassland degradation. Ecological Indicators. 2021;131:108215. https://doi.org/10.1016/j.ecolind.2021.108215
2. Rae R, Riebesell M, Dinkelacker I, Wang Q, Herrmann M, Weller AM, et al. Isolation of naturally associated bacteria of necromenic *Pristionchus* nematodes and fitness consequences. J Exper Biol. 2008;211(12):1927-36. https://doi.org/10.1242/jeb.014944
3. Blaxter LM, De Ley P, Garey RJ, Liu XL, Scheldeman P, Vierstraete A, et al. A molecular evolutionary framework for the phylum Nematoda. Nature. 1998;392:71-75. https://doi.org/10.1038/32160
4. Jorge F, Perera A, Poulin R, Roca V, Carretero MA. Getting there and around: Host range oscillations during colonization of the Canary Islands by the parasitic nematode *Spauligodon*. Molecular Ecology. 2018;27(2):533–49. https://doi.org/10.1111/mec.14458
5. Soliman GM, Ameen HH, Abdel-Aziz SM, El-Sayed GM. *In vitro* evaluation of some isolated bacteria against the plant parasite nematode *Meloidogyne incognita*. Bulletin of the National Research Centre. 2019;43:1-7. https://doi.org/10.1186/s42269-019-0200-0
6. Gugino BK, Ludwig JW, Abawi GS. An on-farm bioassay for assessing *Meloidogyne hapla* infestations as a decision management tool. Crop Protection. 2008;27(3-5):785-91. https://doi.org/10.1016/j.cropro.2007.11.004
7. Grewal PS, Ehlers RU, Shapiro-Ilan DI. Nematodes as biocontrol agents. CABI Publishing; 2005. https://doi.org/10.1079/9780851990170.0000
8. Nicol JM, Turner SJ, Coyne DL, den Nijs L, Hockland S, Maafi ZT. Current nematode threats to world agriculture. In: Jones J, Gheysen G, Fenoll C, editors. Genomics and molecular genetics of plant-nematode interactions. Springer. 2011.p.21-44. https://doi.org/10.1007/978-94-007-0434-3_2
9. Xu M, Liu Q, Zhang Z, Liu X. Response of free-living marine nematodes to the southern Yellow Sea cold water mass. Marine Pollution Bulletin. 2016;105(1):58-64. https://doi.org/10.1016/j.marpolbul.2016.02.067
10. Campos-Herrera R, El-Borai FE, Stuart RJ, Graham JH, Duncan LW. Entomopathogenic nematodes, phoretic *Paenibacillus* spp. and the use of real-time quantitative PCR to explore soil food webs in Florida citrus groves. Journal of Invertebrate Pathology. 2011;108(1):30-39. https://doi.org/10.1016/j.jip.2011.06.005
11. Jenkins WR. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter. 1964;48:692.
12. Oostenbrink M. Estimating nematode populations by some selected methods. Nematology. 1960;6:85-102.
13. Hooper DJ. Handling, fixing, staining and mounting nematodes. In: Southey JF, editor. Laboratory methods for work with plant and soil nematodes. Her Majesty's Stationery Office; 1986. p. 59-80 https://doi.org/10.1163/187529286x00408
14. Eisenback JD, Hunt DJ. Handling, fixing, staining and mounting nematodes. In: Perry RN, Moens M, Hunt DJ, editors. Techniques for work with plant and soil nematodes. CABI; 2021.p.71-87 https://doi.org/10.1079/9781786391759.0071
15. Perry RN, Moens M. Plant nematology. CAB International; 2006. https://doi.org/10.1079/9781845930561.0000
16. Bongers T, Ferris H. Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology and Evolution. 1999;14(6):224-28. https://doi.org/10.1016/s0169-5347(98)01583-3
17. Gaugler R, Kaya HK. Entomopathogenic nematodes in biological control. CRC Press; 2018. https://doi.org/10.1201/9781351071741
18. Flegg JJM. Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb's decanting and sieving technique. Annals of Applied Biology. 1967;60(3):429-37. https://doi.org/10.1111/j.1744-7348.1967.tb04497.x
19. Barker KR, Carter CC, Sasser JN, editors. Nematode extraction and bioassays. In: An Advanced Treatise on Meloidogyne, Vol. II: Methodology. Raleigh, NC: North Carolina State University Graphics; 1985. p. 19-35
20. Walia RK, Bajaj HK. General techniques for nematode identification with light microscopy. In: Identification of Important Plant Parasitic Nematodes. Department of Nematology, CCS HAU Hisar; 2010. p. 1-11
21. Montarry J, Mimee B, Danchin EG, Koutsovoulos GD, Ste-Croix DT, Grenier E. Recent advances in population genomics of plant-parasitic nematodes. Phytopathology. 2021;111(1):40-48. https://doi.org/10.1094/phyto-09-20-0418-rvw
22. Perry RN, Hunt DJ, Subbotin SA, editors. Techniques for work with plant and soil nematodes. CABI; 2020. https://doi.org/10.1079/9781786391759.0000
23. Atkinson HJ. Root-knot nematodes (*Meloidogyne* species) systematics, biology and control. Physiological Plant Pathology. 1980;16(2):301-02. https://doi.org/10.1016/0048-4059(80)90044-2
24. Stevenson JA. Plant pathology: An advanced treatise. JG Horsfall, AE Dimond, editors. In: The Diseased Plant. Academic Press; 1959. p. 674 https://doi.org/10.1126/science.131.3410.1368
25. Whitehead AG, Hemming JR. A comparison of methods for extracting nematodes from soil. Annual Applied Biology. 1965;55(2):25-38. https://doi.org/10.1111/j.1744-7348.1965.tb07864
26. Senthilkumar M, Amaresan N, Sankaranarayanan A. Extraction of nematodes from plant materials. In: Plant-Microbe Interactions. Springer Protocols Handbooks. Humana, New York, NY; 2021. p. 77 https://doi.org/10.1007/978-1-0716-1080-0_77
27. Viaene N, Hallmann J, Molendijk LPG. Methods for nematode extraction. In: Perry RN, Hunt DJ, Subbotin SA, editors. Techniques

for work with plant and soil nematodes. CABI; 2021. p. 12-41. https://doi.org/10.1079/9781786391759.0012

28. Cobb NA. A nematode formula. Bulletin of the U.S. Department of Agriculture; 1890. https://doi.org/10.5962/bhl.title.46872

29. Stirling GR. Biological control of plant-parasitic nematodes: An ecological perspective, a review of progress and opportunities for further research. In: Davies K, Spiegel Y, editors. Biological Control of Plant-Parasitic Nematodes. Progress in Biological Control, vol 11. Springer, Dordrecht; 2011. p. 1-38 https://doi.org/10.1007/978-1-4020-9648-8_1

30. Boyd WA, McBride SJ, Rice RJ, Snyder DW, Freedman JH. A high-throughput method for assessing chemical toxicity using a *Caenorhabditis elegans* reproduction assay. Toxicology and Applied Pharmacology. 2010;245(2):153-59. https://doi.org/10.1016/j.taap.2010.02.014

31. Xian B, Shen J, Chen W, Sun N, Qiao N, Jiang D, et al. Worm Farm: a quantitative control and measurement device towards automated *Caenorhabditis elegans* aging analysis. Aging Cell. 2013;12(3):398-409. https://doi.org/10.1111/acel.12063

32. Buckingham SD, Partridge FA, Sattelle DB. Automated, high-throughput, motility analysis in *Caenorhabditis elegans* and parasitic nematodes: Applications in the search for new anthelmintics. Int J Parasitol: Drugs and Drug Res. 2014;4(3):226-32. https://doi.org/10.1016/j.ijpddr.2014.10.004