Classical and Molecular Methods of Identification as well as Estimating Nematodes - A Comparative Review

Abstract

Changing climatic scenarios accompanied with harsh ecological conditions are forcing various groups of organisms to evolve and adapt in different dimensions. Predominantly in the field of agriculture and crop science, several categories of new pests along with diseases are emerging voraciously causing havoc and causing panic-stricken situations for the farmers. Among the pests, nematode is one of the unnoticed categories contributing significantly to economic loss to the farmers. Due to monotonous crop protection practices in some of the areas as well as drastic changes in the environmental factors, new species of nematode are evolving and attacking economically important crop species. For that, we have to understand the pattern of feeding, oviposition site, life cycle, host searching behaviour, parasitic stages and susceptible host stage for attacking in order to form proper management practices. For understanding its mechanisms and behaviour, our prime concern will be its identification and taxonomic classification by various conventional as well as molecular approaches. Molecular approaches are turning out to be a useful and vigorous tool for the identification of the phylum *Nematoda* replacing conventional ones.

Keywords: Climate change, Conventional and molecular techniques, Estimation, Identification, Nematodes

INTRODUCTION

Thread worms or Pinworms are a group of mysterious fauna within the kingdom of animalia to be studied. It belongs to the phylum *Nematoda*. The generalised scientific classification starts from Kingdom: *Animalia*, Phylum: *Nematoda* followed by different classes and ending in species [24]. Nematodes can be long back around 6000-4000 B.C., as mentioned in *Vedas.* Vedic people termed these organisms as *"Krmis"* which signifies worm [25]. Its rich diversity encourages us to be a keen observer as well as motivates us to further study what is going on with this group of organisms in terms of its behaviour, biology, physico-chemical properties *etc.* that draws our attention [25]. They take shelter in a wide variety of areas of different habitats such as soil, plants, animals, water and human beings. It won't be surprising if we can trace them from hot water springs to shivering polar regions [37]; [47]. Their structural and physiological phenomena enable them to survive in such diversified conditions which are no match to each other. They exhibit such variants of character although belonging to the same category which can be well understood from the fact that if on one side a nematode can be a plant or an animal pathogen, on the other hand, some nematodes are being widely utilised for genetic studies such as *Pratylenchus penetrans* or lesion nematode is a well-established plant pathogen causing linear necrotic lesions in most of the plants [39] while *Caenorhabditis elegans* is widely used for genetic studies as well as it has grabbed its attention from the view point of cytologists, toxicologists and breeder all around the globe [30]. In terms of its size also, it exhibits a tremendous distinctiveness. This can be explained with some authentic examples such as the size of the plant parasitic nematodes ranging from 300-1000 micrometres while the longest or so-called largest nematode is *Placentonema gigantissima*,

which shocks everyone with its length being 8.4 m or 28 feet and a diameter of 2.5 cm. *Placentonema gigantissima* feeds on the endometrium of the female sperm whale and was first and foremost found in the islands of Kuril, 1950 [25].

Most of the studies of biological science exclude this group of organisms in terms of its importance as they cause lesser damage to the crop plants. But lesser we know that due to the changing climate scenario as well as rising temperature unusual changes as well as interesting phenomena are found among this group regarding its habitat searching, host finding, host acceptance and many more are expected from various groups of scientists [25]. So, once we notice a different species resembling a coiled worm, it becomes our prime duty to trace its lineage and go for its identification. It is very important to construct the phylogenetic tree of the newly found species and compare its characteristics with the other members of the family. Taxonomic studies involve various parameters for the identification of an organism [20]. For profiling the taxonomical framework, one has to undergo basic as well as advanced scientific techniques for initial identification of the organism.

This article reviews various conventional as well as molecular techniques for the identification of nematodes including its variants of population estimation methods to finally establish the phylogenetic tree for species identification and characterization, so that further studies can be conducted. It also includes a comparative study of works done by different scientists all across the globe to finally draw a conclusion as to how much of the species they have been able to identify through various techniques and which of these methods is more efficient.

EXTRACTION METHODS

Before moving towards its phylogenetic analysis, we have to undergo a series of procedures such as sampling, isolation of the nematodes from the source material of our

interests, processing, *in-vitro* culture and microscopic evaluation [7]. Here, a brief outline of the methods used for the extraction of nematodes from soil and its related substrates, plant material as well as cysts is mentioned below in table 1:

Table 1: Different procedures of isolation

Isolation from soil and other related substrates		
Name of the method	**Principle followed**	**Suitable for**
Cobb's Method	Variation in size, shape along with precipitation rate between nematodes and soil particles	Isolation of active nematodes
Flegg modified Cobb method [17]	Variation in size, shape along with precipitation rate between nematodes and soil particles	Isolation of active nematodes
Sole decanting method	Variation in specific gravity between nematodes and soil as well as nematode mobility	Isolation of active nematodes
Erlenmeyer method	Variation in size, shape along with precipitation rate between nematodes and soil particles	Isolation of active nematodes
Centrifugal floatation method [12]	Variation in specific gravity between nematodes and other substrates	Isolation of active and non-active nematodes
Oostenbrink elutriator [35]	Variation in size, shape along with precipitation rate between nematodes and soil particles	Isolation of active and non-active nematodes
Isolation of cysts		
Name of the method	**Procedure followed**	**Suitable for**
Fenwick can method [16]	Floating properties of cysts, the difference between size, shape and colour between cysts and other substrates	Separation of cysts from dry soil

Kort's cyst extraction elutriator [29]	Variation in precipitation rate along with size between cysts and soil particles	Extraction of cysts from soil
Baunacke method [6]	Floating properties of cysts, the difference between size, shape and colour between cysts and other substrates	Separation of cysts from dry soil
Seinhorst cyst extraction elutriator [44]	Variation in precipitation rate along with size between cysts and soil particles	Extraction of cysts from soil
Modified Seinhorst method [43]	Variation in precipitation rate along with size between cysts and soil particles	Extraction of cysts from soil
Wye washer [51]	Variation in structure along with size between debris and cysts	Extraction of cysts from dried debris and soil particles
Isolation from plant material		
Name of the method	**Procedure followed**	**Suitable for**
Baermann funnel method [5]	Makes use of nemtaode mobility	Isolation of active nemtaodes
Funnel spray method [42]	Makes use of nematode mobility and precipitation rate	Isolation of active nematodes
Root incubation [32]	Makes use of nematode mobility	Isolation of motile nematode stages
Blender nematode filter method	Makes used of nematode mobility	Isolation of active nematodes
Dissection	Physical presence	Diagnosis purpose
Blender centrifugal flotation method [12]	Variation in specific gravity	Isolation of active and in-active nematodes

CONVENTIONAL COMBINED WITH MOLECULAR METHODS OF IDENTIFICATION AND ESTIMATION

Conventional methods of identification and characterisation include Polymerase Chain Reaction (PCR), Shape of the head, number of annules, body length, length of the stylet, shape

of the stylet knob, structure of the lateral fields, presence/absence and frame of the spermatheca, sculpt of the female tail terminus, shape and span of the spicule and gubernaculum are all crucial morphological recognition characteristics in round worms [22] and other ratio measurements which were proposed by De Man [13] are as follows:

L = Total body length in microns or millimetres

a = Body length ÷ greatest width

b = Body length ÷ oesophagus length

b' = Body length ÷ oesophagus length from the lips to the end of the oesophageal glands

c = Body length ÷ tail length

c' = Body length ÷ body width at the anus

G1 = Overall length of the anterior ovary from vulva x 100 ÷ body length

G2 = Overall length of the posterior ovary from vulva x 100 ÷ body length

V = Distance of the vulva from the lips x 100 ÷ body length [50].

Advantages of conventional methods:

1. It is comparatively easy and handy.
2. It requires less time.
3. It does not involve sophisticated types of equipment.
4. It is less expensive and does not burn a hole in our pockets.
5. It does not require highly trained personnals rather than basic skilled trainees.

Disadvantages of conventional methods:

1. The effect of several factors such as geographic location, host plant, nutrition and other environmental phenomena that guide the evolution among the species is unavoidable.

2. It can be challenging if it consists of variegated species.
3. It is not the ultimatum to finally conclude that so-called organism belongs to this particular genus or any other taxa of classification.

A summarized figure comprising of possible numbers of conventional as well as specialised techniques with examples of nematode that were identified are enlisted below in figure 1:

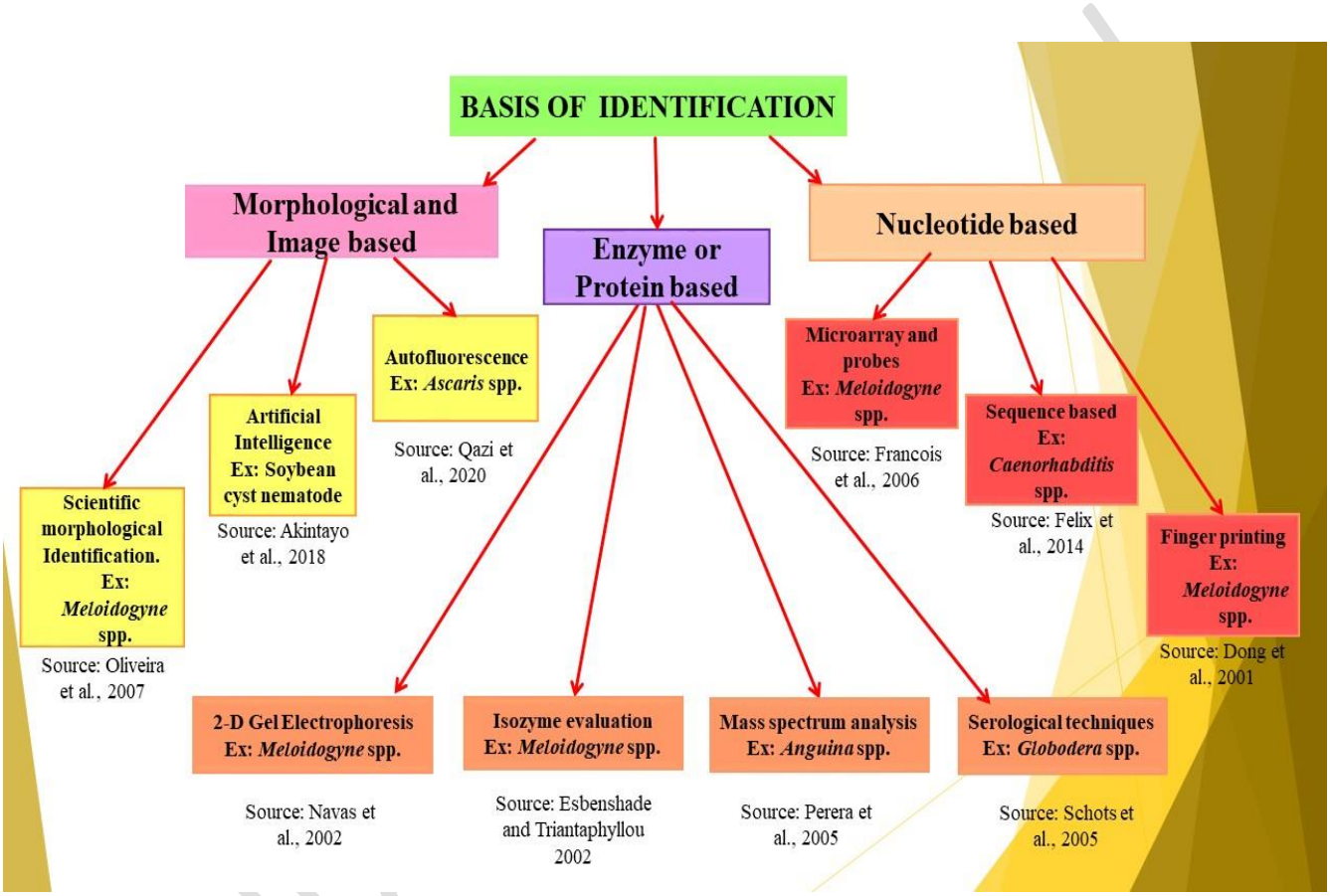

Figure 1: Conventional and specialised techniques for identification of nematodes

Table 2 lists the different techniques or tools for arranging nematodes in different taxa

Table 2: A comparative study of nucleotide, enzyme or biochemical, morphological, barcoding techniques or tools used for placing nematodes into different taxa

Nucleotide based				
Name of the techniques	**Pros**	**Cons**	**Nematodes identified so far and compared**	**References**

A. Micro array and probes- 1. SCARs (Sequence characterised amplified regions) 2. DNA microarray 3. Satellite DNA based like pMfd satellite DNA 4. TaqMan qPCR	They are very specific and accurate in binding to the destined sites and highly reproducible	High costs, spotted microarrays are not highly specific, issues of dye effectiveness and low signal analysis	1. *Meloidogyne chitwoodi.* 2. *Meloidogyne fallax, Meloidogyne hapla.* 3. *Meloidogyne fallax, Bursaphalenchoides xylophilus* 4. *Meloidogyne minor, Paratrichodorus allius*	[18]; [19]; [26]; [40]
B. Sequence based 1. Nuclear DNA 2. Mitochondrial DNA 3. Whole genome	Sequence information is easily accessible, comparisons can be made easily and accurate identification	Lack of authenticity of some data stored in GenBank, and lack of technical facilities	1. Two specific primers for *Heterodera avenae* and *Heterodera filipjevi* 2. *Meloidogyne* spp.	[48]; [53]
C. Fingerprinting 1. PCR-RFLP 2. gDNA-RFLP 3. RAPD and SRAP 4. RT-PCR	Accurate and precise identification along with information	Primers should be designed accurately, DGGE is labour-intensive	1. *Meloidogyne floridensis, Heterodera medicaginis*	[1]; [3]; [23]; [31]; [34]; [40]; [45]; [46]

5. DGGE (denaturing gradient gel electrophoresis)	about the relatedness of different organisms		2. *Meloidogyne incognita*, *Globodera tabacum* complex 3. *Meloidogyne* spp. complex, 7 species of *Stenernematidae* 4. 14 *Meloidogyne* spp. and *Cooperia curticei*	
D. SrRNA markers	Ease of application and takes less time	It is less variable than ITS (internal transcribed spacer) thereby less suitable	*Caenorhabditis elegans*, Chromadorian nematodes, Ascaridoids, Strongylida Rhabditida, Spirudida and Tylenchida (by 5.8 SrRNA analysis)	[10]; [14]
E. SSU (small sub-unit) rDNA analysis	Ease of application and takes less time, target molecule identification	Offers medium resolution in some cases	Aschelminthes, 3 Secernentea clades, Chromadorida and 2	[52]

	and determination of evolutionary rates		Adenophorean clades	
Enzyme or Biochemical based				
Name of the techniques	**Pros**	**Cons**	**Nematodes identified so far and compared**	**References**
A. 2-D gel analysis	The resolution of complex protein markers and allows evolutionary inferences	The degree of polymorphism depends upon the number of samples evaluated,	4 species of root-knot nematodes (*Meloidogyne* spp.)	[33]
B. Mass spectrum analysis or Matrix-assisted laser desorption/ionization (MALDI)	It is very selective; it is not susceptible to changes in microorganism growth protocols	It is costly to operate, and the accuracy of detection might be impacted by the culture period of the nematode to be analysed	*Anguina tritici*, *Anguina funesta*, *Meloidogyne javanica*, *Paragodius tricuspidatus*, *Spinochordodes tellinii*	[9]; [36]
C. Isozyme evaluation or Multi Locus Enzyme Electrophoresis (MEE)	Distinguishes major species	Takes more time and labour-intensive	*Meloidogyne* spp.	[15]
D. Serological evaluation (mono and polyclonal antibodies)	Precise and accurate	Specificity is more in a small number of	*Heterodera glycines*, *Meloidogyne*	[4]; [8]; [27]; [41]

		samples to be analysed	*incognita*, *Meloidogyne javanica*, *Globodera rostochiensis*, *Globodera pallida*	
Morphological and Image-based				
Name of the techniques	**Pros**	**Cons**	**Nematodes identified so far and compared**	**References**
A. Artificial intelligence 1. Convolutional Selective Autoencoder (CSAE) 2. WorMachine	Detection of minute objects, can handle a large number of samples, fast and accurate distinction	Depends on the input of software data and its handling	1. Soybean cyst nematode 2. *Caenorhabditis elegans*	[2]; [21]
B. Autofluorescence	Exclusion of added artificial dyes, advances in terms of the light microscope	Each and every species does not liberate fluorescence spectra	*Ascaris lumbricoides* and *Ascaris suum*	[38]
C. Microscopic image analysis	Detection of external features invisible to the naked eye	Unable to differentiate the masked environmental effects on	1. *Meloidogyne incognita*, *Meloidogyne hapla*, *Meloidogyne*	[11]; [49]

	such as the number of striations, shape of the caudal alae, shape of the body, shape and size of the head *etc*.	heredity at the genetic and molecular level	*javanica*, *Meloidogyne arenaria* 2. *Globodera* spp. and *Heterodera* spp.	
Barcoding				
Name of the techniques	**Pros**	**Cons**	**Nematodes identified so far and compared**	**References**
Meta barcoding	Capacity to instantaneously determine each single species inside complicated multi-ingredient and processed mixtures	Skilled personnals are required for the identification purpose	*Anisakis simplex*, *Panagrellus redivivus*, *Turbatrix aceti* and *Caenorhabditis elegans*	[28]

CONCLUSION

The goal of taxonomy is to assist individuals in comprehending biodiversity, categorising creatures and communicating biological knowledge. Scientific naming is required for taxonomic discourse and correct name is only achievable with type specimens and associated visual information. This, however, is not always practicable, especially when working with environmental materials (eDNA). Nevertheless, it is now widely acknowledged that there is a lack of phenotypic traits to adequately characterise biological variety and the

utilization of genetic data to enhance and/or overcome this constraint is routine. Conversely, a clade is more relevant if its members have distinct biological characteristics, as opposed to the taxon just representing a collection of people with identical physical or molecular characteristics. Because of the relative simplicity of molecular approaches, numerous new taxa have been identified; some based just on genomic sequences. Those taxa might have been hard to characterise visually not just due to a lack of experts and significant morphological distinctions, but also because the individuals of these are difficult to cultivate. Taxa found using distinct molecular techniques, on the other hand, are not necessarily consistent; for example, when sequenced data from different parts of the DNA is utilised in separate research, or when sequence data obtained from the same DNA region is interpreted differently across investigations. Similarly, taxa based on morphometric characteristics may not necessarily correlate to those derived from genetic data and likewise.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, *etc*) and text-to-image generators have been used during the writing or editing of manuscripts.

REFERENCES

1. Abd ElAzim AM, Khashaba EH, Ibrahim SA. Genetic polymorphism among seven entomopathogenic nematode species (Steinernematidae) revealed by RAPD and SRAP analyses. Egyptian Journal of Biological Pest Control. 2019; 29:1-7.

2. Akintayo A, Tylka GL, Singh AK, Ganapathysubramanian B, Singh A, Sarkar S. A deep learning framework to discern and count microscopic nematode eggs. Scientific Reports. 2018; 8:1-11.

3. Amarante MRV, Bassetto CC, Neves JH, Amarante AFT. Species-specific PCR for the identification of *Cooperia curticei* (Nematoda: Trichostrongylidae) in sheep. Journal of Helminthology. 2014; 88:447-452.

4. Atkinson HJ, Harris PD, Halk EJ, Novitski C, Leighton-Sands J, Nolan P, Fox PC. Monoclonal antibodies to the soya bean cyst nematode, *Heterodera glycines.* Annals of Applied Biology. 1988; 112(3):459-469.

5. Baermann, G. A simple method for the detection of Ankylostomum (nematode) larvae in soil tests. A simple method for the detection of Ankylostomum (nematode) larvae in soil tests; 1917; 41-47.

6. Baunacke W. Investigations on the biology and control of the beet nematodes Heterodera schachtii Schmidt. P. Parey. 1922;11: 185-288.

7. Berhanu M, Gebeyaw DT, Kefale D, Kang Y. Overview of nematophagous fungi, isolation techniques, and their role in biological control of helminthic parasites: A literature review. Acta Entomology and Zoology. 2024; 5(1):133-143.

8. Bird AF. Serological studies on the plant parasitic nematode, *Meloidogyne javanica.* Experimental Parasitology. 1964; 15:350-360.

9. Biron DG, Joly C, Marche L, Galeotti N, Calcagno V, Schmidt-Rhaesa A, Renault L, Thomas F. First analysis of the proteome in two nematomorph species, *Paragordius tricuspidatus* (*Chordodidae*) and *Spinochordodes tellinii* (*Spinochordodidae*). Infection, Genetics and Evolution. 2005; 5:167-175.

10. Chilton NB, Hoste H, Hung GC, Beveridge I, Gasser, RB. The 5.8 S rDNA sequences of 18 species of bursate nematodes (order Strongylida): Comparison with rhabditid and tylenchid nematodes. International Journal for Parasitology. 1997; 27(1):119-124.

11. Chitwood BG. 'Root-knot nematodes'. Part 1. A revision of the genus *Meloidogyne Goeldi*, 1887. Proceedings of the Helminthological society of Washington. 1949; 16(2):90-114.

12. Coolen WA, D'Herde CJ. A method for the quantitative extraction of nematodes from plant tissue. State Agriculture Research Centre, Ghent, Belgium, 1972; p. 77.

13. De Man JG. The native nematodes living freely in the pure earth and in fresh water (Vol. 1). Brill. 1880.

14. Ellis RE, Sulston JE, Coulson AR. The rDNA of C. elegans: sequence and structure. Nucleic acids research. 1986; 14(5):2345-64.

15. Esbenshade PR, Triantaphyllou AC. Isozyme phenotypes for the identification of Meloidogyne species. Journal of Nematology. 1990; 22(1):10.

16. Fenwick DW. Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. Journal of helminthology. 1940; 18(4):155-72.

17. Flegg JJ. 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.

18. François C, Castagnone C, Boonham N, Tomlinson J, Lawson R, Hockland SU, Quill J, Vieira P, Mota M, Castagnone-Sereno PH. Satellite DNA as a target for TaqMan real-time PCR detection of the pinewood nematode, *Bursaphelenchus xylophilus*. Molecular Plant Pathology. 2007; 8(6):803-9.

19. François C, Kebdani N, Barker I, Tomlinson J, Boonham N, Castagnone-Sereno P. Towards specific diagnosis of plant-parasitic nematodes using DNA oligonucleotide

microarray technology: a case study with the quarantine species Meloidogyne chitwoodi. Molecular and Cellular Probes. 2006; 20(1):64-9.

20. Hägerbäumer A, Höss S, Heininger P, Traunspurger W. Experimental studies with nematodes in ecotoxicology: an overview. Journal of nematology. 2015; 47(1):11.

21. Hakim A, Mor Y, Toker IA, Levine A, Neuhof M, Markovitz Y, Rechavi O. WorMachine: machine learning-based phenotypic analysis tool for worms. BMC biology. 2018; 16:1-1.

22. Handoo ZA, Carta LK, Skantar AM. Taxonomy, morphology and phylogenetics of coffee-associated root-lesion nematodes, *Pratylenchus* spp. Plant-parasitic nematodes of coffee. 2008; 29-50.

23. Handoo ZA, Skantar AM, Hafez SL, Kantor MR, Hult MN, Rogers SA. Molecular and morphological characterization of the alfalfa cyst nematode, from Utah. Journal of Nematology. 2020; 52(1):1-4.

24. Hanelt B, Thomas F, Schmidt-Rhaesa A. Biology of the phylum Nematomorpha. Advances in parasitology. 2005; 59:243-305.

25. Hoeppli R. The knowledge of parasites and parasitic infections from ancient times to the 17th century. Experimental Parasitology. 1956; 5(4):398-419.

26. Huang D, Yan G, Gudmestad N, Skantar A. Quantification of *Paratrichodorus allius* in DNA extracted from soil using TaqMan Probe and SYBR Green real-time PCR assays. Nematology. 2017; 19(8):987-1001.

27. Hussey RS. Monoclonal antibodies to secretory granules in esophageal glands of Meloidogyne species. Journal of Nematology. 1989; 21(3):392.

28. Knot IE, Zouganelis GD, Weedall GD, Wich SA, Rae R. DNA barcoding of nematodes using the MinION. Frontiers in Ecology and Evolution. 2020; 8:100.

29. Kort, J. A technique for the extraction of *Heterodera* cysts from wet soil and for the estimation of their egg and larval content. Verslagen en Mededelingen, Plantenziektenkundige Dienst, Wageningen Netherlands. 1960; 233:6.

30. Long NP, Kang JS, Kim HM. Caenorhabditis elegans: a model organism in the toxicity assessment of environmental pollutants. Environmental Science and Pollution Research. 2023; 30(14):39273-87.

31. Marché L, Valette S, Grenier E, Mugniéry D. Intra-species DNA polymorphism in the tobacco cyst nematode complex (*Globodera tabacum*) using AFLP. Genome. 2001; 44(6):941-6.

32. Mountain WB, Patrick ZA. The peach replant problem in Ontario: VII. The pathogenicity of Pratylenchus penetrans (Cobb, 1917) Filip. & Stek. 1941. Canadian Journal of Botany. 1959; 37(3):459-70.

33. Navas A, López JA, Espárrago G, Camafeita E, Albar JP. Protein variability in Meloidogyne spp.(Nematoda: Meloidogynidae) revealed by two-dimensional gel electrophoresis and mass spectrometry. Journal of Proteome Research. 2002; 1(5):421-7.

34. Naz IS, Rius JE, Blok V, Ahmad MU, Ali SA. Species identification of root knot nematodes in Pakistan by random amplified polymorphic DNA (RAPD-PCR). Sarhad Journal of Agriculture. 2013; 29(1):71-8.

35. Oostenbrink M. Estimating nematode populations by some selected methods. Nematology. 1960; 85-102.

36. Perera MR, Vanstone VA, Jones MG. A novel approach to identify plant parasitic nematodes using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Rapid Communications in Mass Spectrometry: An International Journal

Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry. 2005; 19(11):1454-60.

37. Perry RN, Moens M. Introduction to plant-parasitic nematodes; modes of parasitism. Genomics and molecular genetics of plant-nematode interactions. 2011; 3-20.
38. Qazi F, Khalid A, Poddar A, Tetienne JP, Nadarajah A, Aburto-Medina A, Shahsavari E, Shukla R, Prawer S, Ball AS, Tomljenovic-Hanic S. Real-time detection and identification of nematode eggs genus and species through optical imaging. Scientific Reports. 2020; 10(1):7219.
39. Saikai K, MacGuidwin AE. Difference in lesion formation by male and female. Journal of Nematology. 2020; 52(1):1-9.
40. Sapkota R, Skantar AM, Nicolaisen M. A TaqMan real-time PCR assay for detection of Meloidogyne hapla in root galls and in soil. Nematology. 2016; 18(2):147-54.
41. Schots A, Hermsen T, Schouten S, Gommers FJ, Egberts E. Serological differentiation of the potato-cyst nematodes Globodera pallida and G. rostochiensis: II. Preparation and characterization of species specific monoclonal antibodies. Hybridoma. 1989; 8(4):401-13.
42. Seinhorst JW. The significance of the soil condition for the occurrence of damage by the stem nematode *Ditylenchus dipsaci* (Kuehn) Filipjev. *Tijdschrift over Plantenziekten*. 1950; 56:289-348.
43. Seinhorst JW. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica. 1959; 4:67-69.
44. Seinhorst JW. Methods for the extraction of *Heterodera* cysts from not previously dried soil samples. Nematologica. 1964; 10:87-94.
45. Semblat JP, Wajnberg E, Dalmasso A, Abad P, Castagnone-Sereno P. High-resolution DNA fingerprinting of parthenogenetic root-knot nematodes using AFLP analysis. Molecular Ecology. 1998; 7(1):119-25.

46. Smith T, Brito JA, Han H, Kaur R, Cetintas R, Dickson DW. Identification of the peach root-knot nematode, Meloidogyne floridensis, using mtDNA PCR-RFLP. Nematropica. 2015; 45(1):138-43.

47. Tahseen Q. Nematodes in aquatic environments: adaptations and survival strategies. Biodiversity Journal. 2012; 3(1):13-40.

48. Toumi F, Waeyenberge L, Viaene N, Dababat A, Nicol JM, Ogbonnaya F, Moens M. Development of two species-specific primer sets to detect the cereal cyst nematodes Heterodera avenae and Heterodera filipjevi. European Journal of Plant Pathology. 2013; 136:613-24.

49. Turner SJ, Subbotin SA. Cyst nematodes. Plant nematology. 2006; 91-122.

50. Van Bezooijen J. Methods and techniques for nematology. Wageningen, The Netherlands: Wageningen University; 2006.

51. Winfield AL, Enfield MA, Foreman JH. A column elutriator for extracting cyst nematodes and other small invertebrates from soil samples. Annals of Applied Biology. 1987; 111(1):223-31.

52. Winnepenninckx B, Backeljau T, Mackey LY, Brooks JM, De Wachter R, Kumar S, Garey JR. 18S rRNA data indicate that Aschelminthes are polyphyletic in origin and consist of at least three distinct clades. Molecular Biology and Evolution. 1995; 12(6):1132-7.

53. Ye W, Zeng Y, Kerns J. Molecular characterisation and diagnosis of root-knot nematodes (*Meloidogyne* spp.) from turfgrasses in North Carolina, USA. PLoS One. 2015; 10(11):e0143556.