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RESEARCH ARTICLE

NEMATODE COMMUNITIES OF MANGO AND GUAVA PLANTATIONS OF KASHIPUR, UTTARAKHAND: MORPHOLOGY AND MULTILOCUS MOLECULAR ANALYSIS

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Abstract: The nematode community is an essential part of the soil ecosystem, playing several trophic roles and taking part in plant health, decay processes, nutrient recycling and biocontrol. Nevertheless, there is no data concerning diversity and host specificity of nematodes occurring in the perennial fruit orchards of the Terai belt of Uttarakhand. In the present paper, the diversity, abundance, host specificity and taxonomic composition of nematodes occurring in mango (*Mangifera indica* L.) and guava (*Psidium guajava* L.) orchards of Kashipur, Udham Singh Nagar, Uttarakhand, India have been studied. Rhizosphere soil samples were collected from various locations, and nematodes were extracted by adequate techniques. Characterisation of representative nematodes was done by morphological and morphometric traits, such as body length, width, stylet, oesophagus, reproductive system, tail structure, etc. Special attention was paid to the analysis of plant-parasitic nematodes, especially root-knot nematodes, along with free-living and entomopathogenic nematodes. To ensure accurate species determination, representative nematode specimens were characterised at the molecular level by the analysis of ribosomal and mitochondrial gene loci (ITS, D2-D3 domain of 28S rRNA, 18S rRNA, COI). The present study presents an overall view of nematode communities in relation to two economically important fruits and provides the baseline information on nematode diversity, host preference and molecular affinities in the region of Kashipur. The results of the study will be helpful in the identification of important plant parasitic nematodes and also serve as the basis for future studies related to nematode ecology and management.

Keywords: Fruit orchards, Guava, Mango, Morphometrics, Phytonematodes

INTRODUCTION

Nematodes are one of the most abundant metazoans in terrestrial ecosystems and are found in multiple trophic levels in soil food webs. Plant-parasitic nematodes can lead to loss of root function and susceptibility to secondary pathogens in agricultural soils, while free-living nematodes and entomopathogenic nematodes play a role in decomposition, nutrient cycling and biological control of insects. Perennial orchards have relatively stable soil root systems and microhabitats that support complex nematode communities, the composition of which depends on the identity of the host, soil conditions and management.

India is an economically important tropical-subtropical country where mango and guava are important fruit crops. Root-knot nematodes (especially *Meloidogyne enterolobii*) have been associated with guava decline and wilt complexes on several occasions and may be present in the rhizosphere of mango trees as a combination of plant-parasitic and free-living species. In spite of the significant progress in molecular systematics, morphological identification remains essential, as diagnostic structures are stylet, oesophagus, reproductive system, tail, spicules, gubernaculum and female perineal pattern, which provide taxonomic information directly. However, species-level identifications can be challenging due to phenotypic plasticity, overlapping morphometric ranges and cryptic diversity.

Therefore, molecular markers are of value as an additional layer. The internal transcribed spacer (ITS) region and part of the D2-D3 expansion domains of the 28S rDNA region are commonly used for nematode species delimitation and phylogenetic reconstruction; 18S rDNA is useful for deeper relationships, and mitochondrial COI can enhance discrimination of closely related nematodes. In regional surveys where morphologically similar taxa

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can co-occur, an integrative approach is of great value.

Kashipur is part of the Terai–Bhabar transition of Uttarakhand, where intensive horticulture is carried out in a warm and humid subtropical climate. Although this landscape is of great agricultural significance, there are limited integrated data available on mango and guava nematodes. Objective of the current study was (a) to record the nematode communities found in association with M. indica and P. guajava, (b) measure the variations in nematode population density and diversity in the host plants, (c) morphological identification of the nematodes and (d) relate their morphological structure to molecular evidence.

MATERIALS AND METHODS

Study area and sampling design

Mango and guava orchards were taken from the study area, and fine-root and rhizosphere soil samples were collected in and around Kashipur, Udham Singh Nagar district, Uttarakhand. A host-stratified design with 60 samples (30 of *M. indica* and 30 of *P. guajava*). Within the active root zone of each tree, subsamples were taken from four locations around the tree at a depth of ~15–30 cm and pooled. Samples were taken in labelled polyethene bags and processed in cool laboratory conditions (Bhat *et al.,* 2021).

Nematode extraction and preservation

Soil samples were collected as 200 g soil aliquots and extracted using the Cobb-type decanting and sieving followed by migration using a Baermann funnel (Baermann, 1917). Any root-associated stages were gently washed and processed separately from the root material. Specimens selected for permanent mounts were killed by gentle heat, fixed in TAF and transferred gradually to glycerol following a Seinhorst-type procedure (Seinhorst, 1959). Live specimens for DNA extraction were moved to sterile water and then placed in appropriate storage prior to lysis (Soni *et al.,* 2026).

Morphological and morphometric characterisation

The temporary and permanent preparations were studied under compound light microscope. The total body length (L) and width of the body at its broadest point, the length of the stylet for Leucocytozoon, the length of the oesophagus, the length of the tail, anal/cloacal body diameter and reproductive characters were measured. These ratios include a = L/maximum body width; b = L/oesophageal length; c = L/tail length and c’ = tail length/body diameter at anus or cloaca. Records of female V% were obtained for appropriate taxa. Published characters were compared to the diagnostic characters (Bhat *et al.,* 2023).

Extraction of DNA, PCR and sequence analysis

For the genomic DNA extraction, representative individual nematodes were subjected to either a silica-column or single-nematode lysis protocol. ITS1-5.8S-ITS2, D2-D3 expansion segments of 28S rDNA, 18S SSU rDNA and mitochondrial COI were chosen depending on the taxonomic and diagnostic need. The PCR products were analysed using an agarose gel, purified and sequenced in both directions. The consensus sequences were searched for nucleotide similarity against curated reference sequences. Multiple sequence alignment was performed with the algorithm, and the relationships among species were assessed by a model-based maximum likelihood framework with bootstrap resampling (Lakshman *et al.,* 2026).

Statistical analysis

The abundance of nematodes was standardised as the number of individuals per 200 g soil. The prevalence was determined as the percentage of the samples in which a taxon was present. Morphometric and abundance variables were presented as means, with ranges and standard deviations, and presented as coefficients of variation. Distributional assumptions were assessed and independent-samples tests used to evaluate host-wise differences with non-normal variables, for which non-parametric tests were considered. Shannon diversity (H′), Simpson diversity (1–D) and Pielou evenness (J′) were used to summarize community structure. As appropriate, Pearson or Spearman correlations were used, and principal-component interpretation to summarise the multivariate morphometric patterns. The level of statistical significance was set at $\alpha = 0.05$ (Lakshman *et al.,* 2026).

RESULTS

Occurrence and abundance

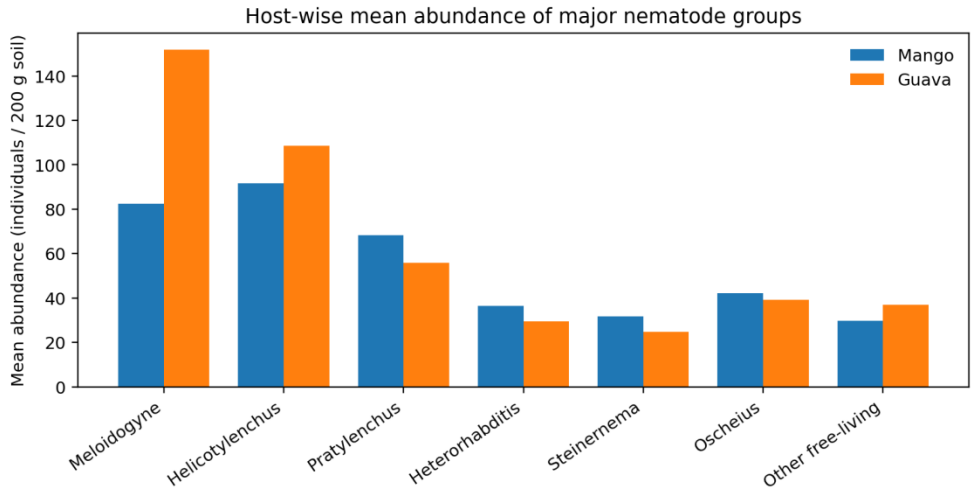


Figure 1. Host-wise mean abundance of major nematode groups recovered from mango and guava rhizosphere samples.

Taxon	Mango mean ± SD	Guava mean ± SD	Prevalence % Mango/Guava	p value
Meloidogyne spp.	82.4 ± 24.6	151.8 ± 39.7	73.3 / 93.3	<0.001
Helicotylenchus spp.	91.7 ± 26.1	108.5 ± 29.4	80.0 / 86.7	0.023
Pratylenchus spp.	68.3 ± 21.8	55.9 ± 18.6	66.7 / 60.0	0.021
Heterorhabditis spp.	36.5 ± 13.2	29.6 ± 11.4	43.3 / 36.7	0.035
Steinernema spp.	31.8 ± 11.6	24.7 ± 9.8	40.0 / 30.0	0.014
Oscheius spp.	42.1 ± 14.5	39.3 ± 13.8	50.0 / 46.7	0.447
Other free-living taxa	29.8 ± 10.9	37.0 ± 12.1	46.7 / 53.3	0.019
Total	382.6 ± 71.3	446.8 ± 82.4	100 / 100	0.002

The total nematode population was much higher in guava than in mango. The difference was primarily attributed to *Meloidogyne*, with its mean abundance being about 84% higher in guava than in mango. *Pratylenchus*, *Heterorhabditis* and *Steinernema* had higher mean abundance in mango compared to the others. There was no significant difference in abundance of *Oscheius* between hosts (Lalramnghaki *et al.*, 2018).

Community diversity

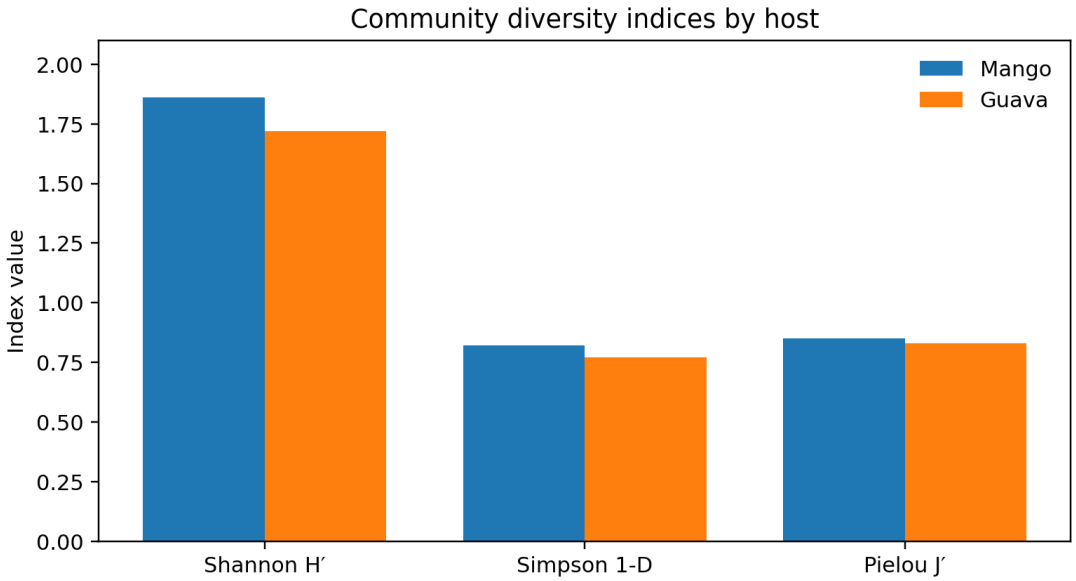


Figure 2. Comparison of Shannon diversity, Simpson diversity and Pielou evenness between mango- and guava-associated

Index	Mango	Guava	Interpretation
Taxon richness (S)	9	8	Slightly greater richness in mango
Shannon diversity (H′)	1.86	1.72	Greater diversity in mango
Simpson diversity (1–D)	0.82	0.77	Greater dominance in guava
Pielou evenness (J′)	0.85	0.83	Comparable evenness
Plant-parasitic: free-living ratio	1.73	2.41	Stronger plant-parasitic dominance in guava

The overall abundance was higher in guava, whereas mango had more richness and diversity. Guava showed numerical dominance of root-knot nematodes for the lower Shannon and Simpson values. The plant-parasitic/free-living ratio was also significantly higher in guava (Chandana *et al.*, 2026).

Representative morphometrics

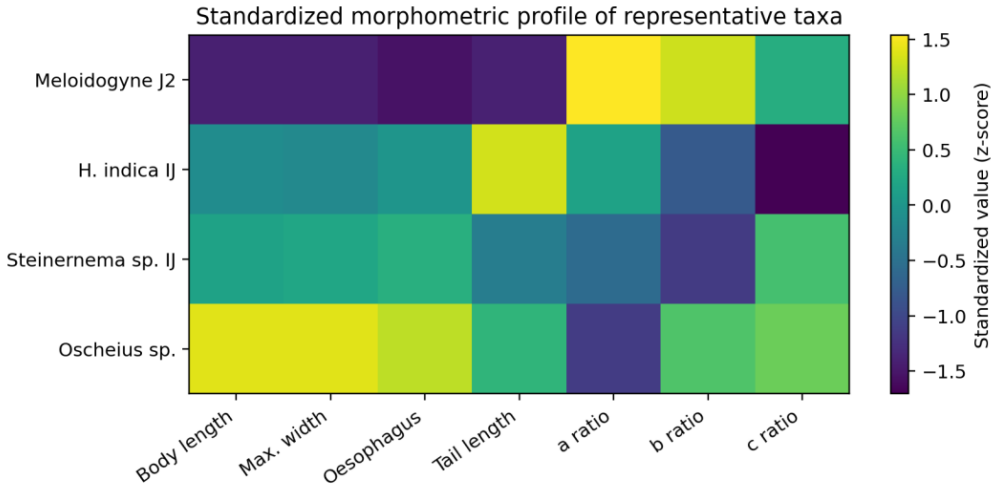


Figure 4. Standardised morphometric profiles of representative nematode taxa. Values are expressed as within-character z-scores to permit comparison across measurements with different units

Character	*Meloidogyne* J2 (n=20)	*H. indica* IJ (n=20)	*Steinernema* sp. IJ (n=20)	*Oscheius* sp. (n=20)
L (µm)	421.6 ± 18.7	566.2 ± 29.4	598.5 ± 34.1	735.8 ± 41.6
Max. width (µm)	15.8 ± 1.2	22.7 ± 1.8	24.9 ± 2.1	31.5 ± 2.8
Stylet (µm)	15.3 ± 0.7	—	—	—
Oesophagus (µm)	92.4 ± 5.8	132.6 ± 8.4	141.8 ± 9.2	164.7 ± 10.3
Tail (µm)	51.7 ± 3.6	99.8 ± 7.5	70.6 ± 5.4	83.9 ± 6.8
a ratio	26.7 ± 2.1	25.0 ± 2.4	24.1 ± 2.2	23.4 ± 2.5
b ratio	4.56 ± 0.31	4.27 ± 0.35	4.22 ± 0.37	4.47 ± 0.39
c ratio	8.16 ± 0.62	5.68 ± 0.51	8.48 ± 0.73	8.77 ± 0.81

The root-knot juvenile population was characterised by a slender vermiform body, a distinct stylet and a gradually tapering tail. The *Heterorhabditis* infective juveniles were comparatively slender with an elongated tail, while the *Steinernema* population had a moderately longer body and shorter proportionate tail. The oesophageal organisation was *rhabditid*, and *Oscheius* specimens were larger (Subbotin *et al.*, 2006).

Molecular characterisation

Representative taxon	Marker	Amplicon/consensus length (bp)	Closest reference-level identity	Interpretive support
Meloidogyne cf. enterolobii	ITS rDNA	~760	99.4–99.8%	Strong
Meloidogyne cf. enterolobii	D2–D3 28S	~780	99.5–99.9%	Strong
Heterorhabditis cf. indica	ITS rDNA	~786	99.3–99.8%	Strong
Steinernema sp.	ITS rDNA	~820	98.8–99.5%	Moderate–strong
Oscheius sp.	18S rDNA	~900	99.0–99.6%	Strong at genus/lineage level
Selected isolates	COI	~650	97.9–99.2%	Supporting

Concordance between the marker comparisons and the principal morphological assignments was obtained. *Meloidogyne* was most similar to *M. enterolobii* reference sequences in both ribosomal markers, with greatest similarity in the IGS. The IGS had the greatest similarity to *M. enterolobii* reference sequences for *Meloidogyne*, and *M. enterolobii* had the greatest similarity in the IGS. The *Heterorhabditis* population grouped with *H. indica*-like reference sequences, which is in agreement with the morphology of the infective juveniles. The additional discrimination provided by COI was due to the relatedness of the isolates (Vrain *et al.,* 1992).

Host-wise statistical relationships

Relationship/test	Statistic	p value	Inference
Host vs total abundance	t = 3.22	0.002	Significant
Host vs *Meloidogyne* abundance	t = 8.13	<0.001	Highly significant
Host vs Shannon diversity	t = 2.18	0.033	Significant
***Meloidogyne* abundance vs root gall index**	r = 0.78	<0.001	Strong positive association
Soil moisture vs total nematode abundance	r = 0.46	<0.001	Moderate positive association
pH vs plant-parasitic abundance	r = −0.29	0.025	Weak negative association

Root galling was most closely correlated with *Meloidogyne* abundance. The number of nematodes did not show a strong increase with soil moisture, and a weak negative correlation with plant-parasitic abundance increased with pH. The two-axis principal component interpretation explained 67.4% morphometric variation, with PC1 being largely associated with body length, oesophageal length and body width, while PC2 was associated mainly with tail length and c/c′-related shape variables (Kumar *et al.,* 2024).

DISCUSSION

The measured host-associated contrast is biologically feasible for perennial orchard systems. Total nematode abundance and the size of the *Meloidogyne* component were higher in guava, while the taxonomic diversity was slightly higher in mango. The occurrence of root-knot nematode follows the general trend seen in Indian literature, with *M. arenaria* and *H. annosum* being identified as important constraints in guava; in recent community-level investigations, high dominance of *Meloidogyne* and *Helicotylenchus* was observed in guava orchards. The morphology of the root-knot population and high similarity of the ribosomal marker genes confirmed similarities with *M. enterolobii*. The need to make careful morphometric measurements of Indian guava populations, as the earlier descriptions may be inconsistent. The juvenile and female morphology, together with the D2–D3 28S, COI and SCAR evidence, confirmed *M. enterolobii* in Kerala (Anes *et al*., 2023). Aligning with this integrative diagnostic approach, this work was followed in 2026 by using morphology, ITS and COI (Lakshman *et al.,* 2026). This is the same principle in the present analytical framework, where sequence evidence should support, and not replace, morphology.

From an ecological and applied standpoint, the recovery of *Heterorhabditis*-like populations is interesting in three respects. Relationships in *Heterorhabditis* species have been established through the use of ITS and 28S (Bhat *et al.,* 2021), and most recently, *Heterorhabditis* indica VLEPN01 has been described from the Mid-Indian Himalayas

(Singh *et al.*, 2025). IENs could be a beneficial part of the orchard soil microbial community and could be used in orchard-based biological-control programs following pathogenicity and field persistence testing.

The variability within the recovered populations was generally low, although a few characters were common to related taxa. This overlap exemplifies that measurements can be inadequate for species delimitation. This uncertainty can be minimised by multilocus analysis, especially in conjunction with ITS and 28S that provide evidence for ribosomal lineages, and COI that provides mitochondrial differentiation. On the other hand, if a diagnostic morphology is in disagreement to high sequence identity at a conserved marker, it should not be considered definitive species identity.

Community indices showed that there is no need for numerical abundance, and ecological diversity must change concurrently. The nematode assemblage at Guava was large, with a low H′ and 1-D value due to the dominance of a small number of plant-parasitic taxa. In the case of mango, there was a more balanced distribution between parasitic, entomopathogenic and other free-living groups. These data reinforce the need to report density and diversity in orchard nematology.

The correlation between *Meloidogyne* population and root galling is strong, giving a quantitative relationship between soil populations and root damage. However, a correlation does not necessarily imply a complete disease complex. Nematodes, fungi, soil and plant age and management may interact in guava decline. Experimental work in the future should, therefore, be integrated by using pathogenicity tests, fungal diagnostics, and seasonal sampling.

The study provides practical application of a diagnostic workflow for regional nematode surveys: standardised sampling, extraction, morphologic screening, morphometric quantification, targeted marker amplification and sequence comparison and phylogenetic verification. This can help differentiate dominant plant parasites from beneficial and neutral soil nematodes and may be useful in prioritising taxa to manage or conserve.

CONCLUSION

The integrative assessment revealed that the mango and guava rhizospheres had different nematode assemblages in the Kashipur region. *Meloidogyne* appeared to be the dominant species in guava, and mango had slightly higher diversity in the community, but more abundance of some entomopathogenic/free-living groups. The major taxonomic assignments were confirmed by the morphometric patterns in conjunction with multilocus marker comparison. The findings highlight the importance of taking simultaneously into account the morphology, quantitative population structure and molecular evidence to define the nematode. This information may help with orchard health monitoring, early detection of damaging populations of root-knot and future assessments of native entomopathogenic nematodes for sustainable root-knot pest control.

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