Original Article *Journal of Multidisciplinary Research Advancements (JOMRA), Vol. 3, No. 2 (2025), 112 – 118*
e-ISSN 2990-7721 Available online at https://www.nepjol.info/index.php/jomra

Diversity of Free Living Nematodes Under Agriculture Farming System in Kathmandu Valley

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Abstract: This study investigated the diversity and community composition of soil nematodes in tomato-growing farms across the Kathmandu Valley, Nepal. Fifty-four soil samples were collected from rhizospheres in three districts (Kathmandu, Bhaktapur, Lalitpur), revealing 14 families across five orders. Herbivorous nematodes dominated the community, followed by bacterivores, fungivores, omnivores, and predators. Ecological indices such as the Maturity Index (MI), Plant Parasitic Index (PPI), and Enrichment Index (EI) indicated varying levels of soil disturbance and fertility. The results underscore the role of nematodes as indicators of soil health and highlight the need for sustainable farming practices to maintain soil biodiversity.

Keywords: *Soil nematodes, Trophic groups, Ecological indices, Agroecosystem, Bioindicators, Nepal*

Conflicts of interest: None
Supporting agencies: None

Received 05.05.2025 Revised 28.10.2025 Accepted 09.11.2025

Cite This Article: Bagale, N., Dhakal, A. (2025). Diversity of Free Living Nematodes Under Agriculture Farming System in Kathmandu Valley. *Journal of Multidisciplinary Research Advancements*, 3(2), 112-118.

1. Introduction

Soil health and biodiversity are critical components of sustainable agricultural systems. Among the various soil organisms, nematodes occupy a unique ecological niche due to their abundance, trophic diversity, and role in soil processes, such as nutrient cycling, decomposition, and regulation of the soil food web (Van Der Heijden et al., 2008; Van Megen et al., 2009). The phylum Nematoda is one of the most species-rich groups of metazoans, found in diverse habitats ranging from marine and freshwater systems to terrestrial ecosystems (Persmark et al., 1992). In agroecosystems, nematodes contribute significantly to essential ecosystem services, including nutrient mineralization, organic matter decomposition, and suppression of soil-borne pathogens (Mulder et al., 2011).

Nematodes can be broadly categorised based on their feeding habits into herbivores (plant-parasitic), fungivores, bacterivores, predators, and omnivores (Yeates et al., 1993). Plant-parasitic nematodes (PPNs), including species like Meloidogyne, Pratylenchus, and Hoplolaimus, are notorious for reducing crop yields and biomass by affecting root structure and overall plant health (Bais et al., 2006; Adam et al., 2014). Conversely, free-living nematodes, particularly those in the bacterivore and fungivore groups, play beneficial roles in nutrient cycling and can serve as sensitive bioindicators of soil health and environmental conditions (Hu et al., 2015). Their rapid responses to changes in soil conditions, such as tillage, fertilisation, and organic amendments, make them ideal indicators of soil quality (Bileva et al., 2014).

Nematode indices, such as the Maturity Index (MI), Plant Parasitic Index (PPI), Channel Index (CI), Enrichment Index (EI), and Structure Index (SI), are increasingly employed in ecological studies to assess soil ecosystem status (Bongers, 1990; Ferris et al., 2001). These indices reflect nematode communities' functional and structural composition and help distinguish between disturbed, enriched, and mature soil ecosystems.

Tomato (Solanum lycopersicum), a member of the Solanaceae family, is one of the most widely cultivated vegetables globally and forms an integral part of the human diet (Missanga & Rubanza, 2018). In Nepal, tomato cultivation is economically significant and practised extensively across the Kathmandu Valley. However, soil-borne pests, particularly root-knot nematodes, are increasingly affecting tomato farms, which threaten productivity and crop quality (Keshari, 2004; Singh et al., 2011). The loss attributed to nematode infestations worldwide is estimated at around US$80 billion annually, with substantial underreporting in developing countries (Nicol et al., 2011; Jones et al., 2013).

This research is unique because it is one of the few studies in Nepal that specifically examines free-living nematode communities in tomato-growing agroecosystems. Kathmandu Valley is intensively farmed and agriculturally important, making it a key region for assessing how current farming practices influence nematode diversity and soil health. Unlike previous studies that focused mainly on identifying plant-parasitic nematodes, this study integrates trophic group composition and ecological indices to evaluate soil health and food web structure across multiple sites in the Kathmandu Valley. This study provides foundational data for designing integrated nematode management strategies and promoting sustainable agricultural practices in Nepal.

2. Materials and methods

2.1. Study Area

This study was conducted in the Kathmandu Valley of Nepal, which includes three districts: Kathmandu, Bhaktapur, and Lalitpur. The valley is located in the mid-hills region of Nepal, with an elevation ranging from 1,280 to 1,380 meters above sea level. Geographically, it lies between 27°27′N and 27°49′N latitude and 85°10′E and 85°32′E longitude. The region experiences a temperate climate, with average annual temperatures ranging from 11°C to 27°C and varying rainfall patterns across the seasons.

The six study sites selected for soil sampling included:

- Kathmandu District: Dharmasthali and Kavresthali
- Lalitpur District: Bhaisepati and Nakhu
- Bhaktapur District: Saraswati Khel and Bode

These sites represent agricultural lands where tomato (Solanum lycopersicum) is cultivated traditionally and commercially.

2.2. Experimental Design and Soil Sampling

A total of 54 soil samples were collected from the tomato rhizosphere. At each of the six sites, three individual tomato plants were selected. For each plant, three replicate soil samples were collected (3 plants × 3 replicates × 6 sites = 54 samples).

Soil was collected from the rhizosphere using a 5 cm diameter auger to a depth of 20 cm and stored in labelled polythene bags. Samples were transported to the laboratory within 24 hours, maintaining their moisture levels.

2.3. Nematode Extraction and Preservation

Nematodes were extracted from 100 grams of soil using the modified Cobb's sieving and decanting method described by S'Jacob and Van Bezooijen (1984). The extraction involved sequential sieving through meshes of decreasing sizes (125 µm to 45 µm), followed by a Baermann funnel technique using an 18 µm filter paper for 36 hours.

The nematodes were heat-killed and preserved in 4% triethanolamine formalin (TAF). Approximately 50 individuals were randomly selected from each sample for microscopic observation.

2.4. Identification of Nematodes

Nematodes were identified to the generic level under a compound microscope (Nikon Ci-L with DS-Fi-L2 camera) based on morphological features using identification keys provided by Bongers (1994). Identification criteria included:

- Structure of the esophagus and stoma
- Shape of tail and stylet
- Presence or absence of specific anatomical features such as basal bulbs, guiding rings, and amphids

Functional guilds and trophic groups (herbivores, bacterivores, fungivores, predators, and omnivores) were assigned following Yeates et al. (1993). Life-history strategies (coloniser-persister scale, c–p values) were designated according to Bongers (1990).

2.5. Soil Chemical Analysis

Soil organic carbon was measured by the Walkley and Black method (1934), and total nitrogen was determined using the Kjeldahl method (Jones, 1991). The C/N ratio was then calculated. Soil pH, temperature, and moisture content were also recorded during the sampling process.

2.6. Nematode Community Analysis and Indices

The following ecological indices were calculated:

- Maturity Index (MI): Indicator of environmental disturbance (Bongers, 1990)
- Plant-Parasitic Index (PPI): Reflects plant-feeding nematode pressure (Bongers et al., 1997)
- Channel Index (CI): Distinguishes between fungal and bacterial decomposition pathways (Ferris et al., 2001)
- Enrichment Index (EI) and Structure Index (SI): Indicate nutrient availability and food web complexity, respectively (Ferris et al., 2001)

Nematode metabolic footprints, representing carbon flow through different trophic groups, were also analyzed to assess soil food web function.

2.7. Statistical Analysis

Data was analyzed using General Linear Models (GLM) and Analysis of Variance (ANOVA) to compare nematode indices and diversity among the six study sites. The significance level was set at $p < 0.05$. Diversity indices such as the Shannon-Wiener Index were also computed for nematode community comparison.

3. Results and findings

3.1. Diversity and Abundance of Soil Nematodes

A total of 14 nematode families belonging to 5 different orders were identified from 54 soil samples collected from tomato-growing farms across Kathmandu Valley. The families observed included free-living and plant-parasitic groups, with considerable variation in their abundance across the study districts: Kathmandu, Bhaktapur, and Lalitpur.

The highest nematode population was recorded in Lalitpur, with 237 individuals per 100 cc of soil, indicating a rich and active soil ecosystem. Bhaktapur followed closely with 215 individuals, while Kathmandu had the lowest count at 57 individuals per 100 cc of soil, suggesting a relatively lower nematode abundance in its tomato-growing soils.

The dominant nematode families differed among the districts. In Kathmandu, the most prevalent family was Rhabditidae (58 individuals), followed by Tylenchidae (57) and Aphelenchidae (50), indicating a relatively even distribution of free-living and plant-associated groups. In Lalitpur, Hoplolaimidae was overwhelmingly dominant with 237 individuals, indicating a significant presence of plant-parasitic nematodes, followed by Cephalobidae (61) and Aphelenchidae (56). Similarly, Bhaktapur also had a high abundance of Hoplolaimidae (215), with Cephalobidae (106) and Aphelenchidae (55) being the next most common families. These differences highlight the site-specific variations in nematode community structure, likely influenced by local soil conditions and agricultural practices.

3.2. Functional Group Composition

Nematodes were classified into five trophic groups: herbivores, bacterivores, fungivores, predators, and omnivores. Herbivores were dominant in all study sites.

The nematode communities identified in the study were categorized into five primary trophic groups based on their feeding behavior: herbivores, bacterivores, fungivores, predators, and omnivores, as shown in Table 1. Among these groups, herbivores were the most dominant across all study sites, suggesting a significant impact of plant-parasitic nematodes on tomato cultivation in the Kathmandu Valley.

In Kathmandu, herbivores constituted approximately 56.35% of the total nematode population, followed by bacterivores (27.91%), fungivores (9.84%), and omnivores (5.90%). This distribution reflects a moderately balanced soil food web, though still dominated by plant feeders. In Lalitpur, herbivores were even more prominent, accounting for 64.70% of the nematode population. This was accompanied by fungivores (10.04%), omnivores (10.22%), and a notably lower percentage of bacterivores (15.05%), indicating potential stress or nutrient imbalance in the soil that favors plant-parasitic groups. In Bhaktapur, the trophic distribution was slightly more balanced, with herbivores (54.87%), bacterivores (23.19%), fungivores (10.35%), and omnivores (11.59%). Bhaktapur's relatively higher proportion of omnivores suggests a more functionally diverse nematode community and possibly better soil structure or resilience.

Predatory nematodes, which typically contribute to regulating soil biota and supporting a stable food web, were not observed significantly on any of the sites. Their absence might reflect environmental disturbances, limited prey availability, or soil conditions unfavorable for higher trophic-level organisms.

Table 1: Feeding type composition of nematode assemblage

District	Herbivores (%)	Bacterivores (%)	Fungivores (%)	Omnivores (%)
Kathmandu	56.35	27.91	9.84	5.90
Lalitpur	64.70	15.05	10.04	10.22

Bhaktapur	54.87	23.19	10.35	11.59

3.3. Ecological Indices of Nematode Communities

Ecological indices varied across the six sub-sites within the three districts, as shown in Table 2. The ecological indices used in this study provided valuable insights into the condition and functioning of soil ecosystems across six tomato-growing sub-sites in the Kathmandu Valley. These indices—Maturity Index (MI), Plant Parasitic Index (PPI), and various food web indicators—help assess levels of disturbance, nutrient enrichment, and biological complexity in soil nematode communities.

The Maturity Index (MI), which measures environmental disturbance, ranged from 2.15 in Bode to 2.74 in Bhaisepati. A lower MI value, as seen in Bode, suggests a more disturbed or enriched environment dominated by opportunistic nematodes. In contrast, a higher MI in Bhaisepati reflects a more mature and stable ecosystem with less anthropogenic disturbance.

The Plant Parasitic Index (PPI), which focuses on plant-feeding nematodes and reflects the vigor of host plants, varied from 2.85 to 3.16, with the highest value observed in Nakhu. This elevated PPI suggests a greater abundance or activity of plant-parasitic nematodes in Nakhu, possibly due to favorable conditions for their hosts or higher soil fertility supporting vigorous plant growth.

The PPI/MI ratio, a composite index used to assess soil enrichment and fertility, ranged from 0.68 in Bode to 0.87 in Bhaisepati. Higher ratios, such as in Bhaisepati, indicate nutrient-rich and less degraded soils, while lower values, like those in Bode, signal nutrient-poor conditions and potentially higher ecological stress.

In terms of food web indicators:

- The Channel Index (CI) was lowest in Dharmasthali (10.41), suggesting dominance of bacterial decomposition, and highest in Bhaisepati (54.59), indicating a greater role of fungal decomposition pathways.
- The Enrichment Index (EI), which reflects the availability of resources for opportunistic nematodes, peaked in Dharmasthali (82.70), implying high soil enrichment. It was lowest in Nakhu (47.31), suggesting limited organic input or lower microbial activity.
- The Structure Index (SI), a measure of soil food web complexity, was highest in Dharmasthali (82.34), followed closely by Kavresthali and Bhaisepati. Bode had the lowest SI (42.56), indicating a simpler food web and reduced ecological stability.

These indices reveal significant spatial variability in soil ecosystem health within the Kathmandu Valley. Some sites, like Bhaisepati and Dharmasthali, show indicators of more stable and enriched systems, while Bode exhibited signs of stress and reduced complexity.

Table 2: Index analysis of the soil nematodes

Site	MI	PPI	PPI/MI	Channel Index (CI)	Enrichment Index (EI)	Structure Index (SI)
Dharmasthali	2.42	2.85	0.85	10.41	82.70	82.34
Kavresthali	2.64	3.10	0.85	26.44	70.03	79.68
Bhaisepati	2.74	3.14	0.87	54.59	51.61	75.70
Nakhu	2.54	3.16	0.80	44.32	47.31	68.60
Saraswati Khel	2.39	2.97	0.80	28.45	69.72	72.03
Bode	2.15	3.15	0.68	29.70	53.05	42.56

3.4. Metabolic Footprint and Food Web Diagnostics

The metabolic footprint was highest in Dharmasthali (27.01) and lowest in Bode (11.99), indicating a greater carbon flow and food web activity in Dharmasthali. The enrichment footprint mirrored this trend, highlighting nutrient-responsive nematode activity in areas with high organic matter input.

- The Channel Index (CI) was lowest in Dharmasthali (10.41) and highest in Bhaisepati (54.59), suggesting a greater dominance of the fungal decomposition pathway in Bhaisepati.
- Sites with high Enrichment Indexes (EI), such as Dharmasthali and Kavresthali, indicated better nutrient availability and soil fertility.

3.5. Soil Chemical Analysis

Compost maturity index (Compost MI) ranged from 2.00 (Bode) to 2.97 (Saraswati Khel). The ratio of fungivores to bacterivores (F/(F+B)) was highest in Kavresthali (0.63), indicating high fungal activity.

A positive correlation was observed between nematode diversity and soil C/N ratio, suggesting that carbon and nitrogen availability significantly influenced nematode community structure.

4. Discussion

This study explored soil nematode communities' diversity and ecological dynamics in tomato-growing farms across the Kathmandu Valley. The results revealed significant variations in nematode abundance, functional composition, and ecological indices across different sites, reflecting the complex interactions between soil properties, land management practices, and nematode ecology.

The total abundance of nematodes was highest in Lalitpur, followed by Bhaktapur, and lowest in Kathmandu. This spatial variation may be attributed to differences in soil fertility, organic matter content, and agricultural intensification. The dominance of plant-parasitic nematodes, particularly Hoplolaimidae, in Lalitpur and Bhaktapur suggests a high degree of biotic stress on tomato crops. In contrast, Kathmandu's nematode community showed higher proportions of free-living families like Rhabditidae, possibly due to reduced chemical input or differing cultivation intensity.

Functional group composition further highlighted the predominance of herbivores across all sites, which aligns with findings from other tomato-cultivating regions where root-knot and other plant-parasitic nematodes are major threats (Singh et al., 2011; Nicol et al., 2011). The relatively higher presence of bacterivores and omnivores in Bhaktapur and fungivores in Lalitpur may indicate better organic matter decomposition and nutrient cycling, supporting more complex soil food webs (Yeates et al., 1993; Ferris et al., 2001). The findings, particularly the dominance of herbivorous/plant-parasitic nematodes and the low representation of predators and omnivores in intensively managed tomato plots, are consistent with studies from other tomato-growing regions, where continuous or high-input cultivation favours opportunistic plant feeders and simplifies soil food webs. Similar patterns were reported in intensive tomato systems in Kenya and in continuous tomato greenhouse cropping in China, which documented increased plant-parasitic nematodes and altered nematode-based indices under intensive management. The use of ecological indices (MI, PPI, CI, EI, SI) in our study follows the same diagnostic framework shown to detect enrichment and structural degradation in global studies (Ferris et al. 2001), and recent work also shows that host genotype and management practices can strongly shape tomato soil nematode communities, explaining site-level differences that were observed.

Ecological indices provided further insights into the health and maturity of soil ecosystems. The Maturity Index (MI), an indicator of environmental disturbance, was highest in Bhaisepati and lowest in Bode, suggesting that the former supports a more stable soil environment while the latter may be under greater anthropogenic pressure or environmental stress. Similarly, Structure Index (SI) and Enrichment Index (EI) values were highest in Dharmasthali, indicating active nutrient cycling and complex trophic interactions—a sign of healthy soil functioning (Ferris et al., 2001). In contrast, low SI in Bode indicates a simplified, possibly degraded ecosystem with less functional redundancy.

The Plant Parasitic Index (PPI) and PPI/MI ratio provided a nuanced understanding of enrichment and plant-nematode interactions. Sites with higher PPI and PPI/MI ratios, such as Nakhu and Bhaisepati, likely had more vigorous plant hosts and possibly higher soil fertility. However, the abundance of plant-parasitic nematodes also underscores the need for integrated nematode management strategies to reduce crop damage and maintain productivity.

Furthermore, the Channel Index (CI) revealed contrasting decomposition pathways among the sites. Low CI in Dharmasthali suggested bacterial-dominated decomposition, often associated with more active and faster nutrient turnover. At the same time, the high CI in Bhaisepati pointed to a stronger fungal pathway, which is common in soils with higher organic matter inputs or less disturbance. The metabolic footprint analysis supported these patterns. Dharmasthali exhibited the most significant footprint, which is indicative of a high level of soil biological activity and carbon turnover. On the other hand, Bode had the smallest footprint, pointing to a less active soil food web.

These findings are consistent with previous studies that emphasize the utility of nematode community indices in assessing soil quality, disturbance, and ecological resilience (Bongers, 1990; Neher, 2001; Ferris et al., 2001). The variation across sites also highlights the influence of localized farming practices on soil biota, such as pesticide use, organic amendments, and crop rotation. The nematode community structure and ecological indicators reveal that tomato farms in Kathmandu Valley vary widely in their soil health status. Sites like Bhaisepati and Dharmasthali show characteristics of mature, well-functioning ecosystems, whereas areas such as Bode may benefit from improved soil management practices. These results can guide farmers and agricultural planners in adopting sustainable practices to enhance soil biodiversity, productivity, and long-term ecological balance.

5. Conclusion

This study examined the diversity and functional composition of free-living and plant-parasitic nematodes in tomato-growing farms across Kathmandu Valley, Nepal. A total of 14 nematode families from five orders were identified, with significant variation in community structure across the study sites, Kathmandu, Bhaktapur, and Lalitpur. Hoplolaimidae, Cephalobidae, and Aphelenchidae emerged as dominant families in most locations.

Herbivorous nematodes were the most abundant functional group in all study areas, indicating a substantial presence of plant-parasitic nematodes that could threaten tomato crop productivity. The analysis of ecological indices such as the Maturity Index (MI), Plant Parasitic Index (PPI), and Structure and Enrichment Indices revealed varying levels of soil disturbance, fertility, and food web complexity. Bhaisepati and Dharmasthali demonstrated higher MI, EI, and SI values, pointing to more stable and enriched soil ecosystems. At the same time, Bode showed signs of ecological stress and reduced biological complexity.

The study also highlighted the role of nematodes as sensitive indicators of soil health. Their community structure and associated indices provide valuable insights into the ecological impacts of agricultural practices and can inform strategies for sustainable soil management.

Based on the findings of the study, the following recommendations can be made:

- Reducing chemical inputs and increasing organic matter through compost or green manure can enhance the diversity and resilience of soil biota, especially beneficial nematodes such as bacterivores and omnivores.
- Strategies such as crop rotation, resistant tomato varieties, and biological control agents should be adopted to manage plant-parasitic nematodes and reduce yield losses.
- Soil health monitoring programs should incorporate regular assessments of nematode community structure and ecological indices (e.g., MI, PPI, EI) to detect early signs of degradation or enrichment.
- Since this study was limited to a single season, future research should include multi-seasonal and longitudinal studies better to understand temporal dynamics and long-term trends in nematode ecology.
- Training and capacity-building programs for farmers on soil biology, the role of nematodes, and sustainable management practices can contribute to more informed decision-making and improved agricultural sustainability.

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