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PLANT PARASITIC NEMATODE (PPNS) INVESTIGATION IN SELECTED SITE OF LOWER HILLS OF UTTARAKHAND, INDIA

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Abstract

These findings can be valuable tools for long-term monitoring and for comprehending regional soil. Nematodes are essential components of soil the soil community capable of surviving extreme and diverse climatic conditions. The diversity and distribution of soil nematodes were studied from the agricultural crop from the selected localities of Pauri Garhwal (1,814 mASL, 29.18ºC to 14.26ºC), A total of 110 soil samples were collected attitudinally from subarea of Pauri Gharwal. The present study revealed that the nematode diversity consist of 54 genera. The nematode diversity structure also demonstrated sensitivity to various other climatic parameters. Elevation significantly influenced biodiversity indices, diversity composition, and trophic levels. Nematode genera richness and all regular indexes indicated lower biodiversity of soil nematodes at higher altitudes. These findings can be valuable tools for long-term monitoring and for comprehending regional soil health in the face of changing environmental conditions.

Keywords : biodiversity indices, climatic parameters, Pauri Garhwal

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Introduction

Soil is a large reservoir of biodiversity. Soil diversity are among the most species rich components of terrestrial ecosystems (Anderson, 1975; Usher *et al*., 1979; Giller, 1996). India is one of the twelve mega biodiversity regions with 7.7% genetic resources of the world. Nematodes, as one component of the soil ecosystem interact with biotic and abiotic factors and adapt themselves to their environment even if the environment threatens to change. If they are not able to do so, they become extinct in due course. So, they are considered as important biological indicators because of their tremendous diversity. The plant parasitic nematodes are distributed worldwide and possess a broad host range of economically important crops. They are recognized as causing more economic damage to food crops. Damage due to plant parasitic nematode causes poor growth, decline in the quality and yield of the crop and reduction in the resistance to stressors, such as, drought, diseases, etc. Damaged roots fail to absorb water and fertilizers effectively, leading to additional losses. The nematode diversity structure of hilly region show sensitivity to various other climatic parameters. Elevation significantly influenced biodiversity indices, diversity composition, and trophic levels.

Material and Method

2.1 Study Site

The present investigation was carried out in district of Uttrakhand viz., Pauri Garhwal (1,814 m above sea level)..

2.2 Extraction of Nematodes

Baermann-funnel method was used for nematode extraction from the soil as described by Viglierchino and Schmitt, (1983). The residue on the sieve was collected in a beaker and placed cover a tissue-lined small course sieve. The sieve was then gently put on a Baermann's funnel apparatus, which consisted of a regular funnel with a short length of rubber tubing attached to the stem and closed by a clamp or pinchcock as stoppered.

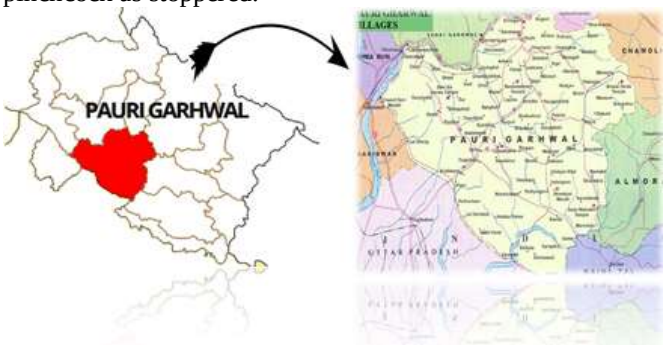

Figure 1. Geographic position of study site (Pauri Gharwal) villages

The funnel was supported in an upright position and filled with sufficient water to touch the bottom of the sieve. The funnel was fitted with a sieve. Retained soil was placed on top layered of tissue paper on top of the wire screen. The sides of the tissue paper towards the corner to cover the wire screen. The sample was then placed in the rack on top of a Baermann funnel. Water was poured all the way to the top of the funnel. The nematodes passed through the tissue and the sieve into the clean water of the funnel (motility of nematode separated them from the inert debris of the course sieve). Gravity caused them to settle at the bottom of the funnel, where they were collected after 24-48 hrs. The nematodes were now ready for counting and identification under a dissecting microscope.

2.3 Nematode Counting

Nematodes were counted in Counting dish with 3 ml extracted homogeneous nematode suspension by a counter under a stereoscopic binocular microscope (Nikon SMZ645). This process was repeated 3 times and the average number of nematodes was noted for per unit of soil. Nematodes were identified to the genus level as per the taxonomic key and classified into their trophic groups is described by Yeates *et al*. (1993). From the samples, one nematode of each taxon was preceded the permanent slides. Each sample had a slide consisting of the identified genera.

Table 1. Prevalence of plant parasitic nematodes species at District Pauri Gharwal and Blockwise

Sampling Region	Subarea (Block/ Village)	No. of samples	Isolated Nematode Genera	Ph range
Pauri Gharwal 78° 24'N to 79° 23'E 1,814 mASL	Kotdwar	10	*Hoplolaimus, Longidorus, Paratylenchus, Psilenchus, Tylenchus, Aporcelaimellus, Actinolaimus, Aporcelinus, Aquatides, Discolaimodes, Discolaimus, Ironus, Iotonchus, Laimydorus, Mylonchulus, Aporcella, Mononchoides longicauda, Parahadronchus, Tripyla, Nygellus, Nygolaimus, Amphidorylaimus, Ischiyodorylaimus, Labronema, Allodorylaimus, Mesodorylaimus, Microdorylaimu,, Moshajia, Oxydirus, Porodorylaimus, Pungentus, Acrobeles, Acrobeloides, Achromadora Acrobelophis, Alaimus, Cephalobus, Chiloplectus, Mesorhabditis, Oscheius, Monhystera, Panagrellus, Plectus, Teratolobus, , Tryplina Ditylenchus, Dorylaimellus, Dorylaimoides, Leptonchus, Tylencholaimellus , Aglenchus, Aphelenhoides, Axonchium, Basiriotyleptus, Belondira, Tyleptus*	6.7-7.5
	Bariyon	5		
	Jitpur	10		
	Haldua Parao	8		
	Sinala	7		
	Ghendwara	5		
	Indargaon	10		
	Devaprayag	5		
	Simkhet	5		
	Paukhal	7		
	Marwara	8		
	Banjkot	5		
	Dugadda	5		
	Satpali	8		
	Amotha	7		

1.1 Soil Physiochemical Properties

The pH of collected soil samples was measured by pH meter (Systronics pH system 362). For measuring the pH of each sample, 20g of soil sample was dissolved in 100ml of distilled water. The pH meter was calibrated before taking the readings. For proper dissolution, the solution was stirred with a magnetic stirrer (Spinet), and the reading was measured with a pH meter. For the estimation of electrical conductivity (EC), the conductivity cell was first calibrated with 0.01 M KCl. After calibrations, reading was taken from the digital TDS/ Conductivity meter (model: MK-509). For the available phosphorus (AP), 0.5 M NaHCO3 used as extractant (Olsen *et al.,* 1954) and reading was obtained from the Spectrophotometer. Likewise, for available nitrogen (AN), alkaline permanganate method was adopted given by Subbiah and Asija (1956). The reading was obtained from the automatic N- analyzer. Organic carbon estimation was done by wet oxidation method (Hausenbuiller, 1976). The soil texture classification was done by using the hydrometer and USDA triangle methods. The sand, silt and clay percent were calculated by pipette or hydrometer method (Gee and Or, 2002). The fraction of sand, clay, and silt was calculated using the following equations (Bouyoucos, 1962).

i. Clay % = Corrected hydrometer reading at 6hrs; 52 min ×100/wt of sample
ii. Silt % = Corrected hydrometer readings at 40sec ×100/wt of sample - % of clay
iii. Sand % = 100% - %Silt - %Clay

The values have been obtained from the mean of soil samples (± standard error) from each region.

1.2 Diversity Indices

1.2.1 Trophic and Food Web Indices

The trophic (feeding) categories were identified using classification's system Yeates *et al.* (1993) of as plant parasites known as phytophagous/herbivores, bacteriophagous/bacterivores (bacterial feeders), mycophagous/fungivores (fungal feeders), predators/predaceous (carnivore), and omnivores while allocation of colonisation-persistence (c-p) values was made after Bongers (1990). Plant Parasitic Index (PPI) was calculated to estimate the relative state of the substrata of the three zones. Trophic diversity was calculated by the trophic diversity index (TDI) as given by Heip *et al.* (1988).

2.5.2 Frequency (N): The recoverences of taxon in the collected samples. The frequency of a nematode genus represents the number of samples in which it is found.

Absolute Frequency = $\frac{\text{No. of samples containing a species}}{\text{No. of samples collected}}$ x 100

Relative Frequency = $\frac{\text{Frequency of a species}}{\text{Sum of frequencies of all spp.}}$ x 100

Absolute Density = $\frac{\text{No. of individuals of a species in a sample}}{\text{Volume or mass or units of the sample}}$ x 100

Relative Density = $\frac{\text{No. of individuals of a species in a sample}}{\text{Total of all individuals in a sample}}$ x 100

Prominence Value = $\frac{\text{Absolute density} \times \sqrt{\text{Absolute frequency}}}{100}$

1.2.2 Diversity Index

Diversity of trophic groups, genus richness and maturity indices were computed. These community indices were calculated as follows:

The Shannon Wiener, sometimes called the Shannon Weaver Index, is a measurement of diversity that takes into account both the genus richness and the proportion of each genus within the community (Begon *et al.,* 1996). The Shannon entropy quantifies the uncertainty (entropy or degree of surprise) associated with this prediction. It was calculated as mentioned below:

Simpson Index (D) = $1-\Sigma n\ (n-1)/N\ (N-1)$ or $=\Sigma\ (Pi)^2$

Simpson Index of diversity = (1-D)

Simpson Reciprocal index = 1/D

(Simpson Index (D), Simpson Index of diversity and Simpson Reciprocal index based on dominant species present)

Shannon-Wiener Index (H') = -Σ Pi ln Pi (Shannon and Wiener, 1949)

Plant Parasitic Index (PPI) = 1/NΣ (CP) ni (Bongers *et al.*, 1997)

Where,

Pi = proportion of individual of taxon i in the total population ($Pi= n/N)^2$

n = total number of individuals of each species

N = total no of organism of all species

ni = the total number individuals of plant parasitic nematodes

vi = c-p value of the family

N = total number of individuals of all species

1.2.3 Statistical Analysis

The morphometric data obtained through measurements was analyzed statistically where descriptive analysis was performed and all measurements were presented in micrometres (except ratio and percentage) and in the form of mean ± standard deviation (range). The statistical analyses were done by SPSS using software version 20.0. Graphs were prepared by using Graph Pad Prism Software. Nematode abundance was based on trophic groups (Yeates *et al.*, 1993), and assigned to functional guilds then classified along the colonisation-persistence gradient (c-p values) according to Bongers (1990) (Bongers and Bongers, 1998; Ferris *et al.*, 2001). The values were further arranged to functional guilds which were portions of trophic groups that share the same c-p value determining the effect of the treatments on abundance of Plant Parasitic Nematodes (PPN) and Free Living Nematodes (FLN). Analysis of variance was represented in different sets for the edaphic factor of the study, and generally, the data were expressed as the mean ± SD using SPSS 20.0 software. The trophic groups and taxonomic groups were analyzed by using non-parametric one way ANOVA. Analysis of edaphic factor was done by using two-way ANOVA where the abundance of nematode group as response and edaphic (pH) traits as factors.

Result

The nematode survey from hilly region encountered from the 110 soil samples were identified based on trophic levels characterized into different groups (Table 3). The pH of the soil was 5.4–8.8, and the texture of the soil was found to be loam, clay loam, and sandy loam. In total, more than 8,204 specimens/individuals were isolated from 54 genera from Pauri gharwal district.

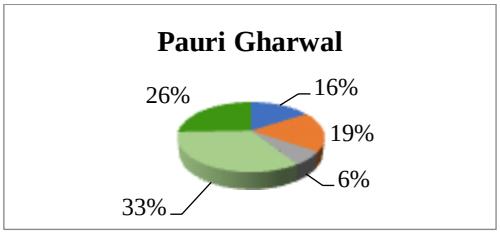

Figure 2. Categorization of the total nematode population based on feeding habits (of the collected soil samples from the District Pauri Gharwal India. Data represented in the form of mean ± SEM.

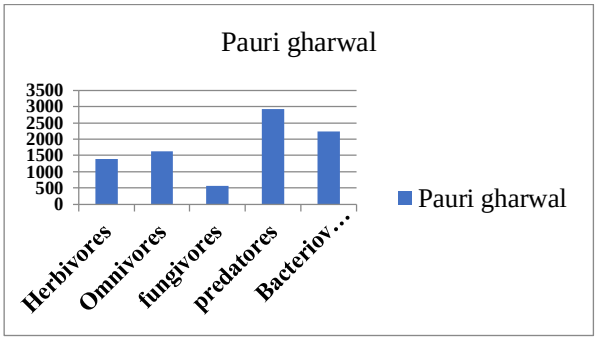

Figure 3. Diversity of nematodes from the Pauri Gharwal of Uttrakhand of India. Different bar patterns represent different feeding groups (herbivores, omnivores, bacterivores, predators, and fungivores).

Table 3. Categorization of the total nematode population (54 identified genera) obtained from the food crop soil samples from Pauri Gharwal of Uttrakhand of India. on the basis of trophic level (feeding habits).

Trophic Level	Name of genera	Pauri Gharwal Prevalence of nematode
Herbivores	*Hoplolaimus, Longidorus, Paratylenchus, Psilenchus mixus, Tylenchus*	**1398.00**
Omnivores	*Amphidorylaimus, Crassolaibium Dorylaimus, Eumenicus, Ischiyodorylaimus, Labronema, Allodorylaimus, Mesodorylaimus, Microdorylaimu,, Moshajia, Oxydirus, Amphidorylaimus, Labronema, Opisthodorylaimus, Porodorylaimus, Pungentus*	**2164.00**
Bacteriovores	*Tylencholaimus, Aglenchus, Diptherophora, Aphelenchus, Ditylenchus, Dorylaimellus, Dorylaimoides, Leptonchus, Tylencholaimellus , Aglenchus, Aphelenhoides, Axonchium, Basiriotyleptus, Belondira, Tyleptus*	**665.00**
Predators	*Aporcelaimellus, Actinolaimus, Aporcelinus, Aquatides, Discolaimodes, Discolaimus, Ironus, Iotonchus, Laimydorus, Mylonchulus, Aporcella, Mononchoides longicauda, Parahadronchus, Tripyla, Nygellus, Nygolaimus*	**1471**
Fungivores	*Acrobeles, Acrobeloides, Achromadora Acrobelophis, Alaimus, Cephalobus, Chiloplectus, Mesorhabditis, Oscheius, Monhystera, Panagrellus, Plectus, Teratolobus, , Tryplina*	**2506**

2.3.1 Altitudinal diversity of Plant Parasitic Nematodes of Pauri Gharwal region

An analysis of nematode diversity showed total 54 genera found associated with food crop of sandy clay soils (Table: 4) In Dehradun region (1,814 km mASL) genera rich group was predators followed by omnivores. In frequency analysis highest absolute frequency genera was Hoplolaimus seinhrosti (26.67 %) followed by Iotonchus indicus (26.67 %), Mesorhabditis vernalis (23.33 %), Aporcelinus abeokutaensis (21.67 %) and Labronema deoriaensis (20.00 %) and least frequent genera found in total diversity analysis was Diptherophora alami (3.33 %), Longidorus elongates, Actinolaimus armatus, Dorylaimellus indicus, Discolaimus texanus, Aporcelaimellus obtusicaudatus (5.00 %), Iotonchus monhystera, Aquatides minutus, Nygellus subclavatus, Nygolaimus timmi, Acrobelophis minimus (6.67 %), Ditylenchus dipsaci, Dorylaimoides constrictus (8.33 %). Absolute densities analysis had highest value Mesorhabditis vernalis (1053.33 %), Paratylenchus similis (771.66 %), Porodorylaimus sturhani (503.33 %), Iotonchus indicus (385.00 %), Mylonchulus contractus (510 %) and least absolute densities Discolaimodes bulbiferus (105.00 %), Ironus dentifurcatus (76.66%) and Discolaimus texanus (86.66 %). Likewise Relative frequency (RF) having highest value Hoplolaimus seinhrosti (39.02 %), Paratylenchus similis, Psilenchus mixus (19.51 %) and least Relative

frequency (RF) Actinolaimus armatus, Aporcelaimellus obtusicaudatus (1.9 %), Nygellus subclavatus, Nygolaimus timmi (2.6 %) and Mesorhabditis minuta (5.17 %). The Prminence Value of nematode diversity having highest value Hoplolaimus seinhrosti (39.84), Paratylenchus similis (28.17) Iotonchus indicus (19.88) and Mylonchulus contractus (19.75) and least prominence Value Dorylaimellus indicus (1.2), Dorylaimoides constrictus (1.7), Aporcelaimellus obtusicaudatus (2.3) and Discolaimodes bulbiferus (2.7). The Relative Density (RD) having highest value Hoplolaimus seinhrosti, Paratylenchus similis (33.11), Mesorhabditis vernalis (28.20), Belondira apitica (25.26 %), %) and least frequent genera Ironus dentifurcatus (1.5 %), Discolaimus texanus (1.77) and Discolaimus similis (2.2 %).

Table 4. Pauri Gharwal region: In total, 54 genera were encountered from 110 soil samples. The bold values represent the prominence value of plant parasitic nematodes genera.

S. No.	Name of genera	Absolute frequency (AF)	Relative frequency (RF)	Absolute Density (AD)	Relative Density (RD)	Prminence Value (PV)
	Herbivores					
1.	*Hoplolaimus seinhrosti*	26.67	39.02	771.66	33.11	**39.84**
2.	*Longidorus elongatus*	5.00	7.31	105.00	4.50	**2.34**
3.	*Paratylenchus similis*	13.33	19.51	771.66	33.11	**28.17**
4.	*Psilenchus mixus*	13.33	19.51	431.66	18.52	**15.76**
5.	*Tylenchus arcuatus*	10.00	14.63	250.00	10.73	**7.9**
	omnivores					
6.	*Dorylaimus stagnalis*	15.00	12.32	300.00	11.07	**11.61**
7.	*Eumenicus monhystera*	10.00	8.21	143.33	5.29	**4.53**
8.	*Labronema deoriaensis*	20.00	16.43	183.33	6.76	**8.1**
9.	*Labronema baqrii*	13.33	10.95	383.33	14.15	**13.99**
10.	*Mesodorylaimus bainsi*	13.33	10.95	136.66	5.04	**4.99**
11.	*Mesodorylaimus paralitoralis*	13.33	10.95	271.66	10.031	**9.9**
12.	*Mesodorylaimus arvensis*	6.67	5.47	106.66	3.9	**2.75**
13.	*Allodorylaimus irritans*	8.33	6.8	426.66	15.75	**12.31**
14.	*Porodorylaimus sturhani*	10.00	8.21	503.33	18.58	**15.91**
15.	*Pungentus angulatus*	11.67	9.5	253.33	9.35	**8.65**
	Fungivores					
16.	*Aglenchus agricola*	15.00	18.36	126.66	13.52	**4.906**
17.	*Aphelenchus avenae*	10.00	12.24	170.00	18.14	**5.3**
18.	*Belondira apitica*	20.00	24.48	236.66	25.26	**10.58**
19.	*Diptherophora alami*	3.33	4.08	41.66	4.44	**0.76**
20.	*Ditylenchus dipsaci*	8.33	10.20	111.66	11.92	**3.2**
21.	*Dorylaimellus indicus*	5.00	6.12	56.66	6.05	**1.2**
22.	*Dorylaimoides constrictus*	8.33	10.2	60.00	6.4	**1.7**
23.	*Tylencholaimellus acutus*	11.67	14.28	133.33	14.23	**4.5**
	Predators					
24.	*Actinolaimus armatus*	5.00	1.9	56.66	1.16	**1.26**
25.	*Aporcelinus falcicaudatus*	13.33	5.2	186.66	3.82	**6.8**
26.	*Aporcelinus abeokutaensis*	21.67	8.49	343.33	7.03	**15.98**
27.	*Aporcelaimellus obtusicaudatus*	5.00	1.9	105.00	2.15	**2.3**
28.	*Aporcelinus granuliferus*	13.33	5.2	296.66	6.08	**10.83**
29.	*Aquatides minutus*	6.67	2.6	106.66	2.18	**2.7**
30.	*Aporcelaimellus malagasi*	10.00	3.9	200.00	4.1	**6.3**
31.	*Discolaimodes bulbiferus*	6.67	2.6	105.00	2.15	**2.7**
32.	*Discolaimus major*	21.67	8.49	238.33	4.88	**11.09**
33.	*Iotonchus monhystera*	6.67	2.6	120.00	2.4	**3.09**
34.	*Iotonchus indicus*	26.67	10.45	385.00	7.8	**19.88**
35.	*Ironus dentifurcatus*	10.00	3.9	76.66	1.5	**2.42**
36.	*Laimydorus papillatus*	10.00	3.9	160.00	3.2	**5.06**
37.	*Laimydorus siddiqi*	5.00	1.96	103.33	2.11	**2.3**
38.	*Aporcella*	20.00	7.8	141.66	2.9	**6.33**
39.	*Parahadronchus shakili*	10.00	3.92	420.00	8.609	**13.28**
40.	*Mylonchulus contractus*	15.00	5.8	510.00	10.45	**19.75**
41.	*Discolaimus similis*	8.33	3.26	108.33	2.2	**3.12**
42.	*Discolaimus texanus*	5.00	1.96	86.66	1.77	**1.93**
43.	*Mylonchulus viasis*	10.00	3.9	385.00	7.8	**12.17**
44.	*Nygellus subclavatus*	6.67	2.6	213.00	4.3	**5.5**
45.	*Nygolaimus timmi*	6.67	2.6	270.00	5.53	**6.9**
46.	*Tripyla arenicola*	11.67	4.5	260.00	5.3	**8.88**
	Bacterivores					
47.	*Achromadora indica*	10.00	10.34	535.00	14.32	**16.91**
48.	*Acrobeloides conoidis*	18.33	18.96	670.00	17.93	**28.68**
49.	*Acrobeloides nanus*	8.33	8.6	426.66	11.42	**12.31**
50.	*Acrobelophis minimus*	6.67	6.8	126.66	3.39	**3.2**
51.	*Mesorhabditis vernalis*	23.33	24.13	1053.33	28.20	**50.88**
52.	*Mesorhabditis minuta*	5.00	5.17	120.00	3.2	**2.6**
53.	*Oscheius vulvastriatus*	13.33	13.79	543.33	14.54	**19.84**
54.	*Panagrellus dubius*	11.66	12.06	260.00	6.96	**8.88**

Table 5. various ecological and diversity indices of Haridwar, Dehradun and Pauri Gharwal districts of the Uttarakhand. Different Indices show value in Std ± Average by SPSS using software version 20.0.

Indices	Pauri Gharwal soil (Loam sand, Loam)
Shannon Weiner Index (H')	0.0183039 ± 0.021874
Simpson Index (D)	0.024362 ± 0.98561
Index of Diversity (1-D)	737.803 ± 1303.388
Reciprocal Index 1/D	0.150187 ± 0.126841
Plant Parasitic Index (PPI)	39.73 ± 75.43

Conclusion

The Altitudinal diversity analysis, frequency, indices and density of nematode population were found to be depending on various factors. These factors are PH, temperature, humidity, nature of soil playing important role in the diversity dynamics. Present survey in district of Uttrakhand show high predators generic diversity having 31 genera were categorized on the basis of feeding habit habits i.e., herbivores, fungivores, omnivores, bacteriovores and predators. In Pauri Gharwal region predators group are dominant due soil nature (sandy). Food crop of Pauri Gharwal region have low diversity of plant parasitic nematode, bacteriovores but rich in predators diversity indices which can be inferred as the diversity of nematodes has been affected due to the presence of high disturbances like various agricultural practices in the particular region. Edaphic factors play a major role in deciding the diversity of the nematodes in a particular ecosystem. The diversity analysis of two districts found to be highly infested with the PPN infection and one district infected with predators because of soil nature. Furthermore, the fertility of the agricultural fields reduces which is a sign to wake early to take comprehensive measures for the protection.

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