Community analysis of nematodes associated with forage crops in Bhubaneswar, Odisha

Mukesh Kumar Patra, Pranaya Pradhan and Mahantheshwara B

Abstract
A survey was carried out to study the distribution of plant parasitic nematodes associated with forage crops in different places of Bhubaneswar of state Odisha, India. Around 140 soil samples were collected from the rhizosphere of forage crops (Hybrid Napier, Maize, Cowpea, Lemongrass, Bajra, Rice bean, Guinea grass) across the different 5 places of Bhubaneswar. Nematodes were isolated by Cobb’s sieving and decanting method followed by Modified Baermann’s funnel technique. Estimation of mean nematode population per 200 cc soil samples revealed the presence of Rotylenchulus reniformis, Tylenchorynchus maschooi, Pratylenchus zeae, Xiphinema insigne, Calosoma heterocephala, Helicotylenchus dihystera, Hoplolaimus indicus, Aphelenchus avenae, Criconemella ornata, Rhabditis, Mononchids, Mylonchulus, Dorylaimid exhibiting varying population densities. Among the different plant parasitic nematodes Xiphinema insigne was highest frequency of occurrence 110.00 with Absolute frequency (78.57%) followed by Hoplolaimus indicus (75.71%). More over Rotylenchulus reniformis was found the most prominent species with highest prominence value (80.60) followed by Xiphinema insigne (60.59), with the lowest prominence value (2.47) recorded in Criconemella ornata. Among free living nematodes Rhabditis were the highest frequency of occurrence (136.00) with absolute frequency (97.14%) followed by Dorylaimid (94.29%). Rhabditis also having the highest prominence value (86.78) followed by Mononchid, Dorylaimid and lowest Mylonchulus (19.61).

Keywords: Community analysis, prominence value, forage crops, nematode, Odisha

1. Introduction
Bhubaneswar is the capital of the Indian state of Odisha located in the Eastern part of Odisha, lying between 85.84° east longitude and 20.27° north latitude. Bhubaneswar has a tropical savanna climate. The annual mean temperature is $27.4^\circ$C ($81.3^\circ$F); monthly mean temperatures are $22$–$32^\circ$C ($72$–$90^\circ$F). Summers (March to June) are hot and humid; during dry spells, maximum temperatures often exceed $40^\circ$C ($104^\circ$F) in May and June. Winter lasts for only about ten weeks, with seasonal lows dipping to 15–18 °C ($59$–$64^\circ$F) in December and January. The highest recorded temperature is $46.5^\circ$C ($115.7^\circ$F), and the lowest is 8 °C ($46^\circ$F).

Forage crops are grown in rangelands and pastures $^{[1]}$. Rangelands and pastures are the most important natural resource in the area and support the local livestock industry, which is the main income source of the region. These crops are grass and legume plant species that are grown for livestock feed as well as land conservation and reclamation. Forage crops can be grazed directly by animals in pastures, or conserved for winter feeding as hay or silage. Forage crops are prone to attack by plant parasitic nematodes $^{[2,3,4]}$. Since in Odisha, the study of nematodes in forage crops is few and far between $^{[5]}$, it triggered to study the association of nematodes in forage crops grown in Bhubaneswar.

2. Materials and Methods
A survey of the different location of Bhubaneswar of state Odisha was conducted during the year kharif, 2016 to determine the occurrence of various plant parasitic nematodes associated with forage crops. Composite soil samples collected and processed within a week after the collection. Altogether 140 soil samples were collected from various plants belongs to forage such as Hybrid Napier, Maize, Cowpea, Lemongrass, Bajra, Rice bean, Guinea grass in different places under Bhubaneswar, Odisha. Extraction of the nematodes was done by Cobb’s sieving and decanting method followed by Baermann’s funnel technique and nematode suspension was obtained $^{[6]}$. 

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Killing of nematodes and fixing in formalin glycerol fixative was used followed by storing the fixed nematode population/200 cc soil sample was done in a multi chambered counting dish under a stereoscopic binocular microscope. Infected roots were stained in cotton blue-lacto phenol solution and observed for the presence of nematodes. Plant parasitic nematode communities were analyzed for computing absolute frequency, relative frequency, absolute density, relative density and prominence value as per the following formulae given by [17].

\[
\text{Absolute frequency} = \frac{\text{Number of samples containing a species}}{\text{Number of samples collected}} \times 100
\]

\[
\text{Relative frequency} = \frac{\text{Frequency of species}}{\text{Sum of frequency of all species}} \times 100
\]

\[
\text{Absolute density} = \text{Number of individuals per unit of soil.}
\]

3. Results and Discussion

During the year 2016 one hundred forty soil samples were scooped from the rhizosphere of various forage plants like Hybrid Napier, Maize, Cowpea, Lemongrass, Bajra, Rice bean, Guinea grass in different places under Bhubaneswar city, the capital of Odisha state. After processing the soil samples through Cobb’s sieving and decanting technique followed by Modified Baermann’s technique, clean nematode suspension was obtained. The nematode suspension includes various plant parasitic and free-living nematodes. Community structure of plant parasitic and free-living nematodes associated with these seven forage plants in different places from Bhubaneswar has been presented in the Table-1 and Table-2.

Table 1: Occurrence and distribution of nematode species associated with different forage crops in localities of Bhubaneswar, Odisha, India

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Name of the Forage crops</th>
<th>No. of sample collected</th>
<th>Soil sample containing nematode species (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hybrid Napier</td>
<td>20</td>
<td>Aa  12  4  20  8  4  0  16  20  16  12  16</td>
</tr>
<tr>
<td>2</td>
<td>Maize</td>
<td>20</td>
<td>Co  12  4  14  12  0  12  0  8  16  12  0  20</td>
</tr>
<tr>
<td>3</td>
<td>Cowpea</td>
<td>20</td>
<td>Hi  12  10  20  0  16  20  16  12  20  20  16  0</td>
</tr>
<tr>
<td>4</td>
<td>Lemon grass</td>
<td>20</td>
<td>Pz  10  20  8  0  20  0  16  12  20  20  10  0</td>
</tr>
<tr>
<td>5</td>
<td>Bajra</td>
<td>20</td>
<td>Rr  12  20  0  20  10  10  0  20  20  10  0  20</td>
</tr>
<tr>
<td>6</td>
<td>Rice bean</td>
<td>20</td>
<td>Tm  12  20  0  10  10  20  0  10  20  20  10  0</td>
</tr>
<tr>
<td>7</td>
<td>Guinea grass</td>
<td>20</td>
<td>Xi  10  20  0  0  10  10  0  20  20  0  10  0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>140</td>
<td>D  86  48  106  20  110  32  94  42  136</td>
</tr>
</tbody>
</table>

Aphelechnus avenue (Aa), Caloosia heterocephala (Ch), Criconemella ornata (Co), Helicotylenchus dihystrera (Hd) Hoplolaaimus indicus (Hi), Pratylenchus zea (Pz), Rotylenchulus reniformis (Rr), Tylenchorhynchus mashhoodi (Tm), Xiphinema insigne (Xi), Dorylaimid (D), Monchonch (M), Mylonchus (My), Rhabditid (R)

The analysis of nematodes communities revealed the association of 13 nematode species with these forage crops. Among the different plant parasitic nematodes Xiphinema insigne was highest frequency of occurrence (110), followed by Hoplolaaimus indicus (106), Caloosia heterocephala (104) and Helicotylenchus dihystrera (86) with Absolute frequency higher Xiphinema insigne (78.57%), followed by Helicotylenchus dihystrera (75.71%), Caloosia (74.29%), Helicotylenchus dihystrera (61.43%), Rotylenchulus reniformis (34.29%), Pratylenchus zea (32.86%), Criconemella ornata (25.71%) with same absolute frequency Aphelechnus avenue and Tylenchorhynchus mashhoodi (14.29%). Relative frequency of Xiphinema insigne was recorded maximum (11.22%) followed by Hoplolaaimus indicus (10.82%), Caloosia heterocephala (10.61%), Helicotylenchus dihystrera (8.78%), Rotylenchulus reniformis (4.90%), Pratylenchus zea (4.69%), Criconemella ornata (3.67%) with same frequency (2.04%) found in Aphelechnus avenue and Tylenchorhynchus mashhoodi. Rotylenchulus reniformis occurred in high densities, which was 137.75 nematodes per 200 cc soil followed by Tylenchorhynchus mashhoodi (191.47), Xiphinema insigne (68.36), Caloosia heterocephala (64.23), Helicotylenchus dihystrera (63.95), Hoplolaaimus indicus (38.15), Aphelechnus avenue (18), Criconemella ornata (4.88). Among these plant parasitic nematodes Rotylenchulus reniformis was found above pathogenic level which cause disease incidence in forage crops in different places of Bhubaneswar. Other nematodes species reflected a low density which was below pathogenic level. Highest Relative density was recorded in Rotylenchulus reniformis (16.84%) followed by Tylenchorhynchus mashhoodi (16.26%), Pratylenchus zea (11.29%), Xiphinema insigne (8.36%), Caloosia heterocephala (7.85%), Helicotylenchus dihystrera (7.82), Hoplolaaimus indicus (4.67), Aphelechnus avenue (2.20%), Criconemella ornata (0.60%).

Table 2: Prominence, frequency of occurrence and population density of nematodes associated with forage crops in Bhubaneswar, Odisha

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Nematode species</th>
<th>Total no. of samples collected</th>
<th>No. of samples containing the species</th>
<th>Absolute Frequency (%)</th>
<th>Relative Frequency (%)</th>
<th>Absolute density</th>
<th>Relative density (%)</th>
<th>Prominence value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aphelechnus avenue</td>
<td>140</td>
<td>20</td>
<td>14.29</td>
<td>2.04</td>
<td>18</td>
<td>2.20</td>
<td>6.80</td>
</tr>
<tr>
<td>2</td>
<td>Caloosia heterocephala</td>
<td>140</td>
<td>104</td>
<td>74.29</td>
<td>10.61</td>
<td>64.23</td>
<td>7.85</td>
<td>55.36</td>
</tr>
<tr>
<td>3</td>
<td>Criconemella ornata</td>
<td>140</td>
<td>36</td>
<td>25.71</td>
<td>3.67</td>
<td>0.848</td>
<td>0.60</td>
<td>2.47</td>
</tr>
<tr>
<td>4</td>
<td>Helicotylenchus dihystrera</td>
<td>140</td>
<td>86</td>
<td>61.43</td>
<td>8.78</td>
<td>63.95</td>
<td>7.82</td>
<td>50.12</td>
</tr>
<tr>
<td>5</td>
<td>Hoplolaaimus indicus</td>
<td>140</td>
<td>106</td>
<td>75.71</td>
<td>10.82</td>
<td>38.15</td>
<td>4.67</td>
<td>33.20</td>
</tr>
<tr>
<td>6</td>
<td>Pratylenchus zea</td>
<td>140</td>
<td>46</td>
<td>32.86</td>
<td>4.69</td>
<td>91.47</td>
<td>11.19</td>
<td>52.43</td>
</tr>
</tbody>
</table>

\[
\text{Relative density} = \frac{\text{Number of individuals of a species in a sample}}{\text{Total of all individuals in a sample}} \times 100
\]

\[
\text{Prominence value (PV)} = \text{Absolute Density} \times \text{Absolute Frequency}
\]
In consideration to prominence value of plant parasitic nematodes *Rotylenchulus reniformis* exhibited highest prominence value (80.60) acting as the most prominent species next to *Xiphinema insigne* (60.59), *Caloosia heterocephala* (55.36), *Pratylenchus zeae* (52.43), *Tylenchorynchus mashhoodi* (50.27), *Helicotylenchus dihystera* (50.12), *Hoplolaimus indicus* (33.20), *Aphelechus avenue* (6.80), *Criconemella ornata* (2.47). So far as the free living nematodes are concerned, *Rhabditis* were having higher frequency of occurrence (136) with absolute frequency (97.14%) followed by *Dorylaimid* (94.29%), *Mononchid* (67.14%) and *Mylenchulus* (30%). *Rhabditis* was also having highest density (88.05%) followed by *Mononchid* (37.55%), *Dorylaimid* (27.48%) and *Mylenchulus* (19.61%). Among nematodes encountered from 140 samples, overall *Rhabditis* and *Dorylaimid* were adjudged as the most frequent nematodes but *Rotylenchulus reniformis* was found the most predominant species in context to density of prominence value. This is in agreement with results undertaken by [8], who recorded the highest density and prominence value of *Rotylenchulus reniformis* in Cowpea as forage crop from Udaipur, Rajasthan and Chittorgarh district of Rajasthan. [9] Stated that the problems in maize due to *Pratylenchus zeae*, *Meloidogyne spp.*, *Heterodera spp.*, *Tylenchorhynchus spp.*, *Hoplolaimus spp* is more. It correlated to the presence of *Pratylenchus zeae* in Bhubaneswar and also *Caloosia heterocephala* as highest number in Maize. [2] Reviewed *Meloidogyne spp.*, *Rotylenchulus reniformis*, *Heterodera spp.*, *Pratylenchus zeae*, *Tylenchorhynchus spp.*, *Helicotylenchus dihystera* are the major nematode spp. parasites infecting forage crops. In Bhubaneswar *Meloidogyne spp* is not found in forage crops as due to some edaphic factors, [10,11] suggested that *Meloidogyne spp.* are not frequently encountered nematodes in natural and dry land agricultural areas in Turkey, but they are widespread in coastal greenhouses and in the main vegetable growing areas close to rivers.

4. Conclusion

It is concluded that both plant parasitic nematode (*Rotylenchulus reniformis*) and the bacterial feeding nematode (*Rhabditis*) are the prominent nematode in forage crops in Bhubaneswar. Since *Rhabditis* exhibited highest frequency of occurrence and these are the indicator of soil health, acting as soil organic matter decomposer, this reflected the antagonistic effect on plant parasitic nematodes reducing their population below the economic threshold level in most of the samples.

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6. References