COMMUNITY ANALYSIS OF PLANT PARASITIC AND FREE LIVING NEMATODES ASSOCIATED WITH RICE AND SOYBEAN PLANTATION FROM PAKISTAN

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Abstract. Variety of nematode community indices have been proposed for purposes of environmental monitoring. During three phases of surveys (2005-08), thirty nine nematode genera other than root-knot nematodes were found associated with rice crop plants while 33 from soybean in the surveyed areas of rice and soybean. Nematodes were placed into five different groups (Yeates et al., 1993) viz., herbivores, fungivores, bacteriovores, omnivores and predators. In the present study highly abundant group is herbivores, followed by bacteriovores, fungivores, predators, and omnivores were encountered from rice and soybean fields. The occurrence percentage was observed in per 500 g soil for each nematode genus. All species of nematodes associated with soybean are first records of nematodes associated with soybean from Pakistan. Nematode communities were analyzed by mean abundance, prominence value, importance value and cluster analysis based on nematode numbers and presence or absence of nematode, respectively. Community relationship revealed the overall dominance of herbivores (*Hirschmanniella* and *Helicotylenchus*) and fungivores (*Aphelenchoides*) in rice and *Tylenchorhynchus* (herbivores), *Aphelenchoides* (fungivores), *Panagrolaimus* and *Acrobelus* (becteriovores) from soybean in all terms of community analysis. While the UPGMA cluster analysis showed that Multan and Dir localities exhibit close similarity coefficient in soybean and in rice Thatta and Sawat showed more similar coefficient or the nematode incidence
Keywords: *cluster analysis, glycine max, nematodes, survey, nematode community*

Introduction

Rice **(***Oriza sativa* L.**)** is the staple food of more than two billion people, predominantly in Asia where more than 90% of the word' s rice is grown and consumed (Bridge etal., 1990). Worldwide, rice yield losses due to plant parasitic nematodes are estimated at 10% (Sasser and Freckman, 1987). The association of nematodes with rice in Punjab was reported by Anwar and Khan (1973) along with thirteen nematode genera including *Hirschmanniella oryza* and *Radopholus oryza*. Maqbool and Hashmi (1982) found plant parasitic nematodes in high frequency from rice growing areas of Pakistan. Maqbool (1983-84) reported *Tylenchorhynchus rassicae, T. clarus, T. mashoodi* and *Basiria graminicola,* respectively from soil around the roots of rice from Khyber Pakhtunkhawa (KP). Khan and Bilqees (1994) described a new species *Basiria bajorensis* from Bajore agency of paddy crops. Maqbool and Shahina (2001), was given biodiversity of nematode fauna of different Pakistani crops including rice. Musarrat et al. (2006) reported *M. incognita* from rice growing area of Sindh and KP, Pakistan. A review article on root knot nematode, Meloidogyne of Pakistan was given by Shahina et al. (2009).

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Soybean (*Glycine max* (L.) Merrill) is one of the most important oilseed crop in the world. The soybean cyst (*Heterodera glycines*) and root-knot (*Meloidogyne* spp.) nematodes have received the greatest emphasis in breeding program, contributing up to 4% crop losses (Good, 1973). Maqbool (1981) in a general survey of crops reported the occurrence of root-knot nematode (*M. hapla*) on soybean. Severity of *M. incognita* on soybean was reported from Sindh and KP by Musarrat et al. (2006).More than 100 species of plant parasitic nematodes have been associated with soybean (Schmitt and Noel, 1984), the major pest species being *Heterodera glycines, Meloidogyne* spp., (*M.incognita, M. arenaria* and *M. javanica*) and *Rotylenchulus reniformis*. These nematodes are estimated to cause annual yield losses of over 10% to soybean on a worldwide basis (Sasser and Freckman, 1987).The aim of this study was to determine the percentage occurrence and community analysis of plant parasitic and free living soil nematodes.

Material and methods

Survey and sample collection

Extensive surveys were carried out from primary and secondary growing areas of rice and soybean from different agro ecological zones of Pakistan (except Balochistan) since 2005-2008. A total of 277 randomly chosen fields from 41 locations were visited and composite soil samples were taken from the rhizosphare of crop plants on each field (*Table 1, Fig.1A & B*). in KPK area, zone 1 includes northern mountainous areas of the country and irrigated rice is grown either in flat valleys.the climate is sub-humid monsoon with 750-1000 mm average rainfall,mostly concentrated in summer. While in Punjab area, zone 2 lies in the broad strip of land between rivers Ravi and Chenab where both canal and sub soil water are used for irrigation.the climate is sub humid, sub-tropical type with 400 to 700 mm of rainfall mostly in July-August. In Sindh area, Zone 4 includes Indus delta which consists of vast spill flats and basins. The climate is arid tropical marine with no marked season.

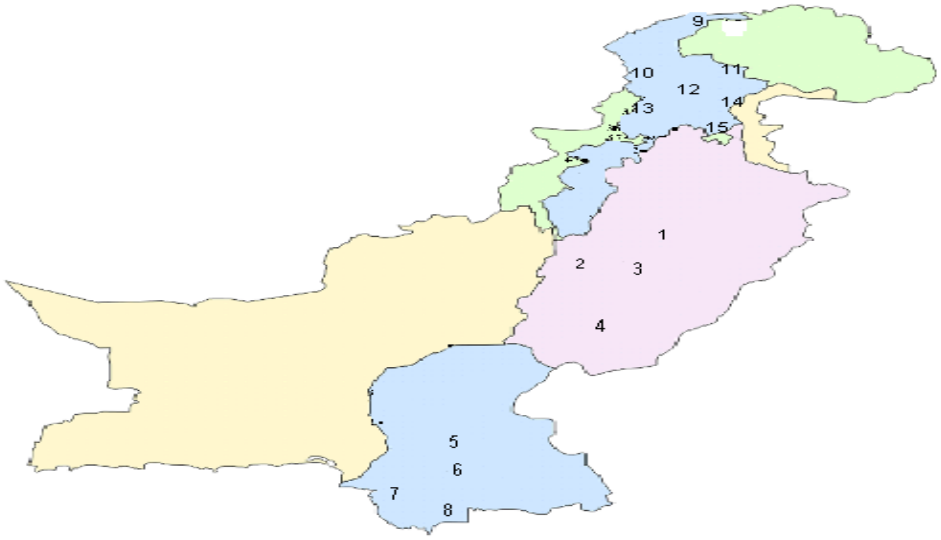

Figure 1A. *Geographic localities of soybean growing areas of Pakistan.*
Localities: 1-Faisalabad, 2-Multan, 3-Vehari, 4-Rahimyar Khan, 5-Sangharh, 6-Hyderabad, 7- Thatta, 8-Badin, 9-Kurram Agency, 10-Dir, 11-Swat, 12-Mansehra, 13-Malakand, 14- Abbottabad, 15-Hazara.

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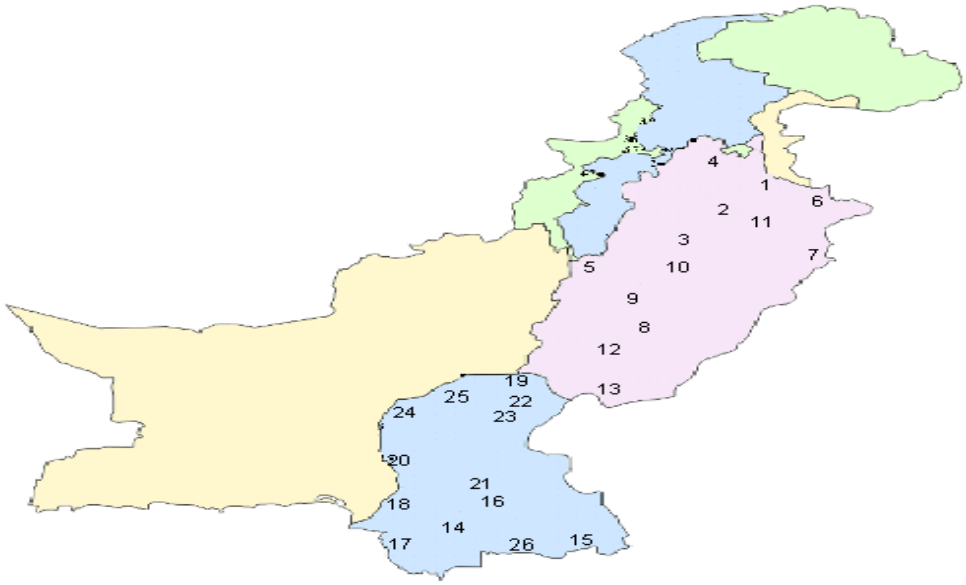

Figure 1B. *Geographic localities of rice growing areas of Pakistan.*
Localities: 1-Sialkot, 2-Gujranwala, 3-Sheikhupura, 4-Okara, 5-Jhang, 6-Narowal, 7-Kasur, 8-Pakpattan, 9-Sahiwal, 10-Faisalabad, 11-Hafizabad, 12-Vehari, 13-Rahimyar Khan, 14-Hyderabad, 15-Badin, 16-Sangharh, 17-Thatta, 18-Dadu, 19-Jaccobabad, 20-Larkana, 21-Nawabshah, 22-Sukkur, 23-Khairpur, 24-Shikarpur, 25-Nasirabad, 26- Gharo.

Extraction of nematodes

Extraction of plant parasitic and free living nematodes from soil was made by Cobb's sieving and Baermann funnel techniques (Baermann, 1917 and Cobb, 1918, respectively).

Quantitative analysis

For the community analysis nematodes were counted in an open counting chamber with only 5 ml extracted nematode suspension by a counter under binocular microscope. Process was repeated 3 times and average of three readings gave the number of nematodes per unit of soil.

Data analysis

Community analysis of phytoparasitic and free living nematodes in rice and soybean fields of Pakistan was done by the using of Norton techniques (1978).

The similarity matrix based on the quantitative analysis (presence (1)/absence (0) of nematodes, which used to establish the similarity between localities on the basis of Jacord's coefficient of similarity (Rohlf, 2005). Dandrogram constructed on the basis of data was related to the localities. All computations were carried out using the NTsys packages, version 2.2 (Rohlf, 2005).

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Table 1. *Surveyed localities of rice and soybean of Pakistan.*

S.#	Localities of rice	Latitude	Longitude	S.#	Localities of soybean	Latitude	Longitude
1	Rahimyar Khan	28°30`N	70°25`E	1	Multan	30°15`N	71°36`E
2	Sialkot	32°30`N	74°31`E	2	Vehari	29°15`N	71°30`E
3	Hafizabad	32°05`N	73°40`E	3	Faisalabad	31°25`N	73°09`E
4	Sialkot	32°15`N	74°52`E	4	Rahimyar Khan	28°30`N	70°25`E
5	Gujranwala	32°10`N	74°12`E	5	Hyderabad	25°23`N	68°24`E
6	Narowal	32°06`N	74°52`E	6	Badin	24°38`N	68°54`E
7	Jhang	31°15`N	74°22`E	7	Sangharh	26°20`N	68°57`E
8	Sheikhupura	30°32`N	71°80`E	8	Thatta	33°35`N	74°14`E
9	Sahiwal	30°45`N	73°80`E	9	Hazara	33°59`N	72°56`E
10	Okara	30°50`N	73°31`E	10	Swat	34°40`N	72°52`E
11	Vehari	29°15`N	71°30`E	11	Dir	35°12`N	71°53`E
12	Kasur	31°07`N	74°27`E	12	Kurram Agency	34°40`N	71°55`E
13	Pakpattan	31°21`N	73°24`E	13	Mansehra	34°25`N	71°50`E
14	Faisalabad	31°25`N	73°09`E	14	Malakand Agency	34°40`N	71°55`E
15	Nawabshah	26°15`N	68°25`E	15	Abbottabad	34°26`N	71°52`E
16	Sukkur	28°55`N	68°55`E				
17	Khairpur	27°06`N	87°44`E				
18	Sangharh	26°20`N	68°57`E				
19	Hyderabad	25°23`N	68°24`E				
20	Badin	24°38`N	68°54`E				
21	Thatta	33°35`N	74°14`E				
22	Dadu	26°06`N	67°45`E				
23	Jaccobabad	28°17`N	68°26`E				
24	Larkana	27°32`N	68°18`E				
25	Nasirabad	27°32`N	69°18`E				
26	Gharo	24°44`N	67°35`E				

Results

Percentage occurrence of nematode genera in rice plantations

The nematode genera encountered from rice plantations were identified and characterized into different groups as follows: Out of 39 nematode genera 15 belongs to harbivores viz., (*Basiria*, *Boleodorus*, *Criconemoides*, *Ditylenchus*, *Helicotylenchus*, *Heterodera*, *Hirschmanniella*, *Hoplolaimus*, *Longidorus*, *Merlinius*, *Paratylenchus*, *Pratylenchus*, *Psilenchus*, *Tylenchorhynchus* and *Xiphinema*); while four genera (*Aphelenchoides*, *Aphelenchus*, *Filenchus* and *Dorylaimus*) belong to fungivores. Six genera (*Clarkus*, *Ironus*, *Laimydorus*, *Mononchus*, *Mylonchulus* and *Oinchus*); and two genera (*Eudorylaimus* and *Mesodorylaimus*) of predators and omnivores were encountered respectively. Among these nematodes the second highest genera were bacteriovores (*Acrobelus*, *Cephalobus*, *Mesorhabditis*, *Diplogaster*, *Diploscapter*, *Discolaimium*, *Eucephalobus*, *Geomonhystera*, *Panagrolaimus*, *Plectus*, *Rhabditis* and *Zeldia*).

The most frequently occurring nematode genera were *Hirschmanniella*, *Aphelenchoides*, *Helicotylenchus* and *Tylenchorhynchus* from rice cultivated areas

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which were surveyed. These nematodes were found in relatively high densities as compare to other plant parasitic nematodes from surveyed areas of both provinces (*Table 2*).

Table 2. *Occurrence (percentage) of nematode genera regarding their diverse groups from rice fields of Pakistan during 2005-08.*

Trophic groups of nematode	Punjab			Sindh		
	2005-06	2006-07	2007-08	2005-06	2006-07	2007-08
Herbivores nematodes						
Basiria	3	4	1	2	3	0.99
Boleodorus	1	-	-	2.5	2	-
Criconemoides	1	0.9	-	-	-	1
Ditylenchus	10	12	9.8	5	3.9	6.3
Helicotylenchus	60	62	55	72	66	58
Heterodera	1	-	-	-	-	-
Hirschmanniella	66	58	62	90	95	88
Hoplolaimus	5	4	3	1	2.5	1.5
Longidorus	1	-	-	0.9	0.4	-
Merlinius	1	-	1.5	2	1.5	0.9
Paratylenchus	3.8	6	3	2	1	6.3
Pratylenchus	1	0.9	1	0.8	1.5	0.44
Psilenchus	1	-	-	1	0.9	1
Tylenchorhynchus	20	15	11	25	45	35
Xiphinema	2	1	3	1	-	-
Fungivores						
Aphelenchoides	60	45	73	70	71	62
Aphelenchus	32	30	18	25	40	20
Dorylaimus	2	1	3	3.3	4	2
Filenchus	1	0.9	0.8	-	-	1
Bacteriovores						
Acrobelus	25	20	18	40	39	33.2
Cephalobus	15	25	33	60	65	70
Mesorhabditis	20	15	23	35	38	33
Diplogaster	13	15	20	60	55	51
Diploscapter	8	10	7	15	13	11
Discolaimium	6	4	3	10	12	18
Eucephalobus	2	2.5	1	3	5	6.5
Geomonhystera	15	20	22	10	11	13
Panagrolaimus	18	11	19	23	42	40
Plectus	8	6	4	25	33	26
Rhabditis	15	13	10	8	6.5	10
Zeldia	15	22	23	11	13	20
Omnivores						
Eudorylaimus	1	-	-	3.5	4	2.5
Mesodorylaimus	3	1	1	1	2	1
Predators						
Clarkus	55	50	45	65	66	62
Ironus	1	1	-	0.8	0.6	1
Laimydorus	8	6	10	30	10	28
Mononchus	-	-	1	0.9	2	1
Mylonchulus	10	8	7	22	25	33
Oinchus	1	-	-	2	1	-

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The samples contained genera of diverse group with a range of 5 to 39 %. Studies on % occurrence of these groups revealed that herbivores dominated the entire nematode community in % occurrence (39%) followed by bacteriovores (31%), predators (15%), fungivores (10%) and omnivores (5%) (*Fig. 2A*).

In terms of overall % occurrence of nematode genera among five different groups, *Hirschmanniella* (herbivores) was most frequently occurred genus with the highest % occurrence (21%), followed by *Aphelenchoides* (fungivores) and *Helicotylenchus* (herbivores) sharing the same % occurrence (17%). The second highly abundance nematode genus was predator *Clarkus* (16%) followed by bacteriovores group with *Cephalobus* (16%), *Diplogaster* (10%) and *Panagrolaimus* (7%) in the surveyed areas of rice during three years (2005-2008) (*Fig. 2B*).

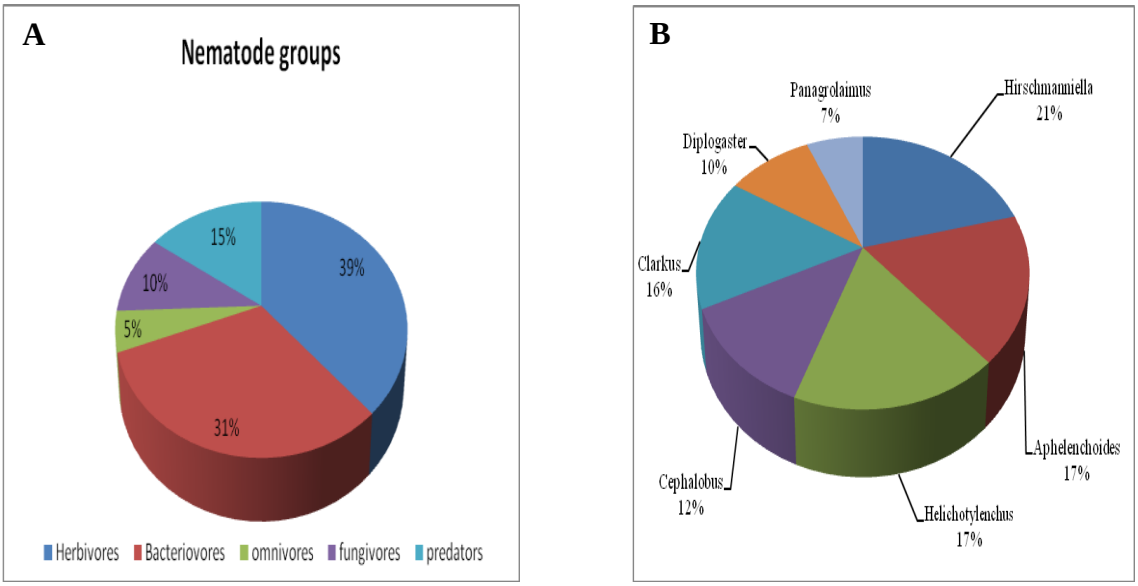

Figure 2. *A. Different groups of nematodes in rice; B. Occurrence (%) of nematode genera in rice surveyed areas.*

Percentage occurrence of nematode genera in soybean plantations

Soil analysis revealed the presence of 33 nematode genera encountered from soybean crop plantations during 2005 to 2008 surveys from Sindh, Punjab and KP (*Table. 3A-B; Fig. 3A-B*). These genera belonged to five diverse groups in which herbivores contain 15 nematode genera, fungivores with 6 genera; bacteriovores have 10 genera; predators contain 3 genera and only one genus representing the omnivores. Among these five groups herbivores dominated with the highest percentage occurrence (40%), followed by bacteriovores (30%), fungivores (18%), predators (9%) and omnivores (3%) in the nematode community structure (*Fig. 3A*).

In terms of overall % occurrence of nematode genera, *Helicotylenchus* (29%) was the most occurred (herbivores) genus whereas *Tylenchorhynchus* (herbivores, 17%) followed by *Aphelenchoides* (fungivores, 12%) were encountered in abundance from all fields of soybean crop .However other nematode groups such as bacteriovores: *Panagrolaimus* (18%), *Plectus* (14%) and *Acrobelus* (10%) frequently encountered from soybean soil samples (*Fig. 3B*).

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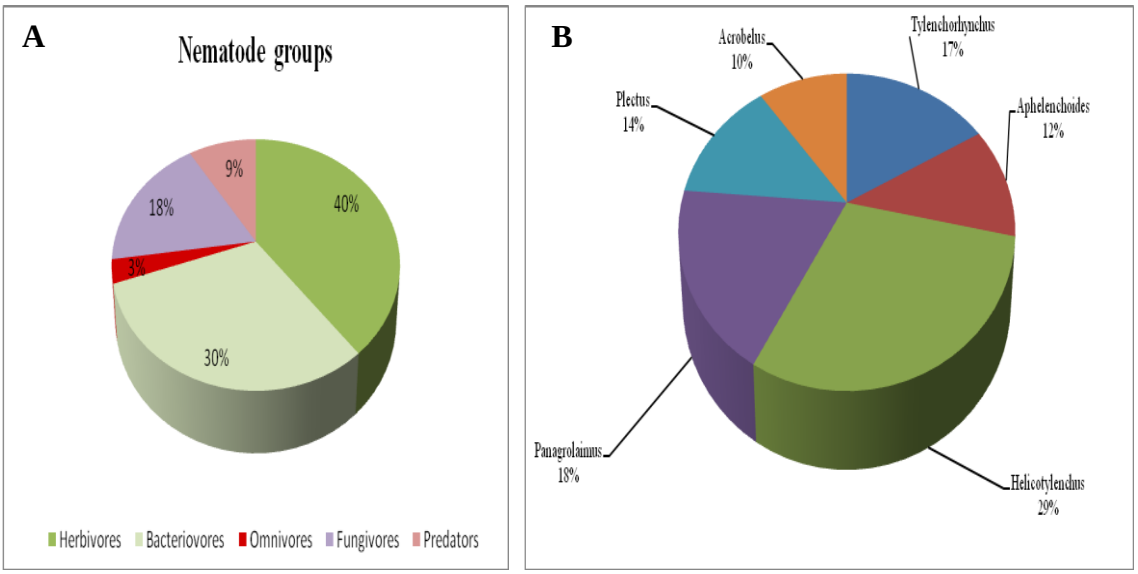

Figure 3. *A. Different groups of nematodes from soybean; B. % occurrence of nematode genera in soybean surveyed areas.*

Table 3A. *Percentage occurrence of nematode genera regarding their diverse groups from soybean fields of Pakistan during 2005-08.*

Nematode genera	**Punjab**			**Sindh**			**Khyber Pakhtunkhwa**		
Herbivores	2005-06	2006-07	2007-08	2005-06	2006-07	2007-08	2005-06	2006-07	2007-08
Boleodorus spp.	2	1	1	1	0.4	-	1	-	-
Criconemoides spp.	1	1	4	3.5	1	2	1	2.5	2
Ditylenchus spp.	7	10	10	8	5	3	-	2	-
Gracilacus spp.	-	-	-	1	0	2	-	-	-
Helicotylenchus spp.	36	60	53	42	55	63	41	33	52
Heterodera spp.	0.5	1	-	-	-	-	-	-	-
Hoplolaimus spp.	0.6	1	-	2	1	5	4	-	-
Longidorus spp.	0.2	1	-	-	-	1	0.4	0.2	-
Neodolichorhynchus spp.	-	-	-	3	5	-	2	5	3
Paratylenchus spp.	1	6	8	4	7	8	3	6	4
Psilenchus spp.	0.2	1	0.6	-	-	1	1	-	-
Tylenchorhynchus spp.	25	35	30	29	28	26	22	24	30
Xiphinema spp.	5	9	10	3	4	1	3	2	-
Fungivores									
Aphelenchoides spp.	25	30	15	20	22	28	15	16	-

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Aphelenchus spp.	15	20	30	23	34	37	20	10	10
Dorylaimus spp.	15	13	10	9	7	3	2	-	1
Discolaimus spp.	1	2	1	2	3	1	1	1.5	-
Filenchus spp.	4	3.6	5	3	2	1	1	3	1
Nygolaimus spp.	1	1.5	-	1	1	1	-	-	-

Table 3B. *Percentage occurrence of diverse groups of nematodes from soybean fields of Pakistan during 2005-08.*

Nematode genera	**Punjab**			**Sindh**			**Khyber Pakhtunkhwa**		
	2005-06	2006-07	2007-08	2005-06	2006-07	2007-08	2005-06	2006-07	2007-08
Bacteriovores									
Acrobelus spp.	12	10	19	25	36	30	8	6	5
Cephalobus spp.	30	25	20	15	19	20	15	13	12
Diplogaster spp.	5	8	20	15	14	18	6	8	3
Diploscapter spp.	1	0	1	0.4	0.5	-	1	-	-
Mesodorylaimus spp.	8	6	7	4	3	5	1	-	-
Panagrolaimus spp.	40	35	55	35	30	29	12	18	19
Plectus spp.	18	22	15	35	25	40	20	15	10
Rhabditids spp.	7	5	6.8	3	5	2	1	1.5	-
Rhabdolaimus spp.	3	2	1	1	3	4	1	-	-
Seleborca	1	2	1	0	0	1	2	1	1
Predators									
Laimydorus spp.	1	1	-	-	-	1	-	-	-
Mononchus spp.	5	6	8	2	4	6	1	3	5
Mylonchulus spp.	6	3	1	5	6	4	2	1	2
Omnivores									
Eudorylaimus spp.	-	5	2	8	10	15	4	2	-

Community analysis of nematode genera in rice fields

To exhibit the relationship of nematodes associated with host plants the community analysis was performed which provides information concerning frequency (absolute and relative frequency) and density (absolute and relative density) of nematodes on studied plant hosts.

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Community relationship revealed the overall dominance of herbivores (*Hirschmanniella* and *Helicotylenchus*) and fungivores (*Aphelenchoides*) in all terms of community analysis (*Table 4*).

Table 4. *Community analysis of plant parasitic and free-living soil nematodes in rice growing areas of Pakistan.*

Nematode genera	A.F	R.F	A.D	R.D	P.V
Plant parasitic nematodes					
Aphelenchoides spp.	27.9	6.2	233	5.9	16.7
Aphelenchus spp.	17.3	4.6	107	4.9	10.5
Basiria spp.	4.2	1.4	22	1.2	1.6
Boleodorus spp.	3.4	1.0	25	1.5	1.2
Criconemoides spp.	3.2	1.0	24	1.6	1.8
Ditylenchus spp.	3.8	1.8	66	3.3	3.2
Filenchus spp.	1.5	0.41	66	3.5	2.9
Helicotylenchus spp.	23	6.1	121	5.5	13.9
Heterodera spp.	3.6	2.0	8.0	2.1	2.0
Hirschmanniella spp.	66.2	12.8	253	9.8	33
Hoplolaimus spp.	15.3	5.1	110	4.9	1.2
Longidorous spp.	4.3	1.5	20	1.2	1.5
Merlinius spp.	1.6	0.51	39	1.6	1.14
Paratylenchus spp.	2.1	0.21	22	0.6	1.0
Pratylenchus	1.5	0.5	32	1.5	1.12
Psilenchus spp.	1.6	0.3	55	2.3	1.8
Tylenchorhynchus spp.	41.3	12.8	223	9.4	31
Xiphinema spp.	3.7	1.0	28	1.4	1.42
Free- living soil nematodes					
Acrobelus spp.	23.5	5.9	326	5.8	4.2
Cephalobus spp.	16.4	6.2	255	4.7	3.2
Cervidellus spp.	11.2	2.3	99	0.36	3.2
Clarkus	1.3	0.5	66	3.4	2.6
Diplogaster spp.	9.6	6.4	110	5.3	3.5
Diploscapter spp.	8.5	3.2	88	2.1	1.9
Discolaimium spp.	9.5	2.4	49	2.1	3.1
Dorylaimus spp.	1.9	0.34	26	1.3	2.8
Eucephalobus spp.	5.6	0.6	44	1.3	1.5
Eudorylaimus spp.	1.3	0.54	20	1.6	2.1
Geomonhystera spp.	9.8	1.2	33	2.3	2.3
Ironus spp.	8.5	2.4	22	1.6	1.3
Laimydorus spp.	1.1	0.5	15	1.32	2.4
Mesodorylaimus	8.3	3.1	79	2.0	1.8
Mononchus spp.	5.4	1.5	110	5.0	6.3
Mylonchulus	4.6	1.4	98.5	4.8	5.9
Oinchus spp.	3.2	0.4	9	1.0	1.1
Panagrolaimus spp.	14.2	5.6	99	2.3	2.1
Plectus spp.	25.3	5.8	206	5.6	7.3
Rhabditis spp.	11.3	2.5	76	3.3	2.6
Zeldia spp.	5.6	0.5	45	2.6	1.3

Absolute frequency (A.F) = (No of samples containing a species / Total samples collected) 100; Relative frequency (R.F) = (Frequency of a species in a sample/ Sum of frequencies of all species present) 100, Absolute density (A.D) = (No of individuals of a species in a sample/ volume of the sample) 100; Relative density (R.D) = No of individuals of a species in a sample / Total no. of individuals in the sample) 100; Prominence value (P.V) = (Absolute density/ Absolute frequency).

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Frequency

Hirschmanniella (AF= 66.2%) was the most prevalent genus in the entire plant parasitic nematode community, followed by *Tylenchorhynchus* (AF= 41.3%); *Aphelenchoides* (AF= 27.9%) and *Helicotylenchus* (AF= 23.0%), while the least prevalent genus was *Filenchus* (AF= 1.5%).

Among the free-living soil nematodes, *Plectus* (AF= 25.3%) and *Acrobelus* (AF= 23.5%) were the most prevalent genera. *Laimydorus* (AF= 1.1%) was the least prevalent genus in rice fields (*Table 4*).

Absolute density

Among all the nematode genera recorded, *Hirschmanniella* had maximum absolute density (AD= 253/ sample), followed by *Aphelenchoides* (AD= 233/ sample) and *Tylenchorhynchus* (AD= 223/ sample), while the genus *Longidorus* had lowest absolute density (AD= 20.0/ sample). In free-living soil nematode group studies, *Acrobelus* had the highest absolute density (AD= 326 / sample) followed by *Cephalobus* (AD= 255/ sample) and *Plectus* (AD= 206/ sample), while *Oinchus* had the lowest mean density 9 per sample.

Prominence value

The rice nematode genus *Hirschmanniella* had the highest prominence value (PV= 33.0), followed by *Tylenchorhynchus* (PV= 31.0), while the least PV was found in genus *Paratylenchus* (PV= 1.0). Among the free-living soil nematodes *Plectus* had maximum PV (7.3), followed by *Mononchus* (PV= 6.3), while the least prominence value was recorded in *Oinchus* (PV=1.1).

Community analysis of nematode genera in soybean plantations

Detailed analysis of different parameters for nematode community analysis has been provided in the *Table 5* for the soybean crop.

Frequency

Among the plant parasites, the most frequently encountered genus was *Tylenchorhynchus* (AF= 45.8%); followed by *Aphelenchoides* (AF= 45.3%), whereas *Boleodorus* was least frequent (AF= 1.3%) genus. Genus *Panagrolaimus* and *Acrobelus* were the most prevalent genera among the free-living soil nematodes with absolute frequencies (AF) 40% and 36%, respectively whereas the least frequent genus was *Diploscapter* (AF= 1.5%).

Absolute density

Genus *Tylenchorhynchus* had the highest absolute density (AD= 222), followed by *Aphelenchoides* (AD= 205) while the least density was of the genus *Gracilacus* (AD= 12). Among the free-living soil nematodes *Panagrolaimus* and *Acrobelus* were the most dominant genera in the entire nematode community in soybean fields with AD= 253 and AD=221, respectively. Whereas *Diploscapter* was the least dominant genus with AD= 22.

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Prominence value

Community analysis of plant parasitic nematodes associated with soybean crop revealed that highest prominence value of *Tylenchorhynchus* (PV= 32) and *Aphelenchoides* was noted as second most prevalent genus with PV= 31, while the least prominence values (PV= 1.1 and PV= 1.2) were recorded for *Filenchus* and *Longidorus* genera respectively.

In case of free-living soil nematodes maximum prominence value was recorded in genus *Acrobelus* (PV=33), while the least prominence value (PV=1.2) was recorded in the genus *Diploscapter* (*Table 5*).

Table 5. *Community analysis of plant parasitic and free-living soil nematodes in soybean growing areas of Pakistan.*

Nematode genera	A.F	R.F	A.D	R.D	P.V
Plant parasitic nematodes					
Aphelenchoides	45.3	12.4	205	9.4	31
Aphelenchus	15.3	5.5	106	4.8	10.5
Boleodorus	1.3	0.51	55	2.5	1.7
Criconemoides	3.8	1.3	38	1.6	1.6
Ditylenchus	3.7	1.0	33	1.4	1.4
Filenchus	1.9	0.5	35	1.5	1.1
Gracilacus	1.6	0.21	12	2.1	1.6
Helicotylenchus	20	7.3	109	5.5	9.8
Heterodera	5.5	1.5	44	5.0	6.2
Hoplolaimus	3.8	1.0	31	1.4	1.3
Longidorous	1.9	0.5	23	1.3	1.2
Neodolichorhynchus	2.9	1.0	30	4.3	1.4
Paratylenchus	3.7	1.5	36	1.5	1.5
Psilenchus	1.8	0.51	45	2.6	1.3
Tylenchorhynchus	45.8	11.8	222	9.5	32
Xiphinema	5.7	1.5	96	4.0	5.2
Free-living soil nematodes					
Acrobelus	36	2.5	221	8.9	33
Cephalobus	22	5.6	110	3.6	7.5
Diplogaster	1.9	2.5	33	2.4	2.1
Diploscapter	1.5	0.5	22	1.3	1.2
Discolaimus	1.8	0.4	39	2.6	1.7
Dorylaimus	5.7	1.5	110	5.0	6.2
Eudorylaimus	4.9	1.3	102	4.8	5.5
Laimydorus	1.9	2.6	59	2.6	2.1
Mesodorylaimus	7.6	2.0	93	4.2	6.1
Mononchus	3.8	1.9	35	1.5	1.5
Mylonchulus	2.2	1.5	40	2.0	1.6
Nygolaimus	3.6	1.2	69	2.8	2.9
Panagrolaimus	40	11.3	253	5.6	12
Plectus	18.3	4.3	196	4.6	10.7
Rhabditis	5.3	2.3	59	2.6	2.1
Rhabdolaimus	5.8	1.6	39	1.2	1.5
Seleborca	1.6	0.5	45	2.4	1.5

Absolute frequency (A.F) = (No of samples containing a species/ Total samples collected) 100; Relative frequency (R.F) = (Frequency of a species in a sample/ Sum of frequencies of all species present) 100; Absolute density (A.D) = (No of individuals of a species in a sample/ volume of the sample) 100; Relative density (R.D) = No of individuals of a species in a sample/ Total no. of individuals in the sample) 100; Prominence value (P.V) = (Absolute density/ Absolute frequency).

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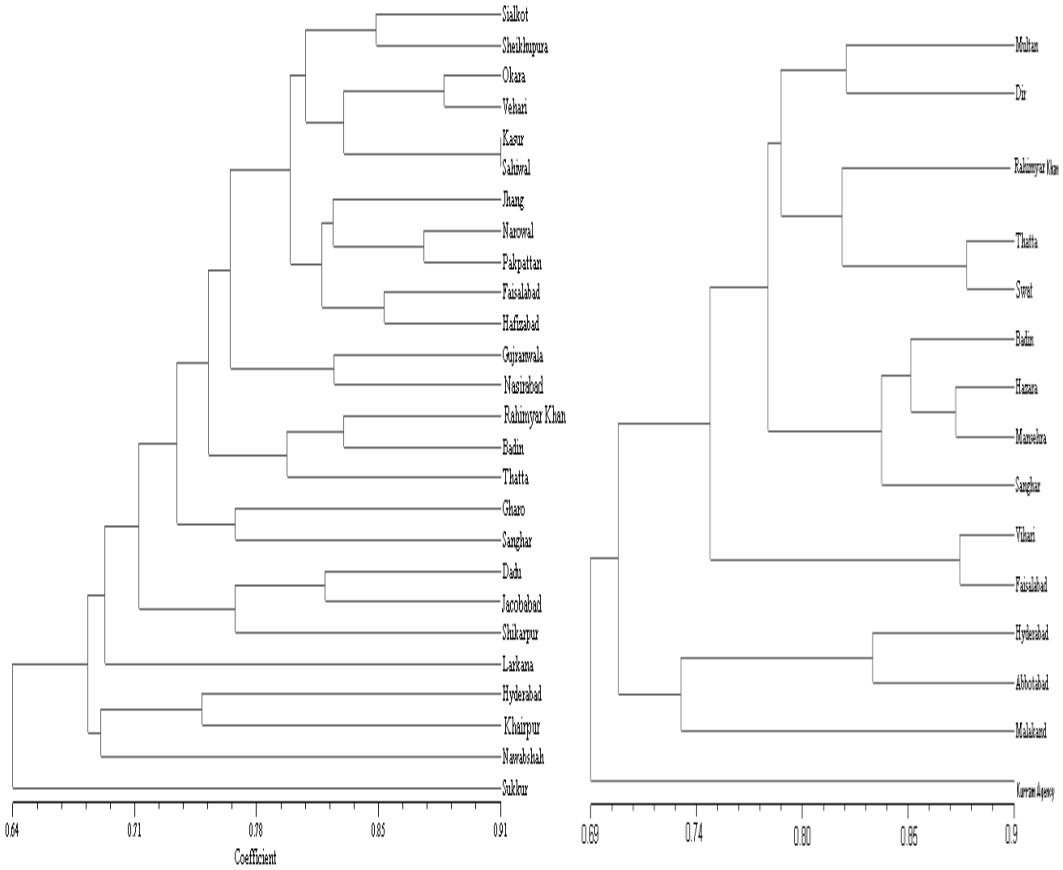
Cluster analysis of rice and soybean localities

A similarity matrix based on the proportion of shared nematode community was used to establish the level of relatedness between the different rice and soybean localities of Pakistan. Similarity of closest areas was estimated ranged from 0.64 to 0.91. Two localities of rice Kasoor and Sahiwal (Punjab) were the closest localities for the presence of plant parasitic nematodes with the other tested localities; whereas, Nasirabad and Sukkur (Sindh) showed very low similarities (64 %) for the all nematode genera, but both localities have same nematode genus i.e., *Hirschmanniella*.

Similarities of nematode density obtained from quantitative data were used to create a cluster diagram. Cluster analysis, based on Nei and Li's similarity coefficients using UPGMA dendrogram, showed that all 26 rice growing localities of Pakistan could be easily distinguished based on the information generated by the community analysis of nematodes.

As expected, localities were distinguished in 9 cluster groups corresponding to the Sindh and Punjab localities, with additional sub clusters and a few independent localities (*Fig. 4*). In cluster analysis those areas which soil samples having abundant density of nematodes genera showing a high frequency of nematode relatedness. While observing the clustering pattern, it is evident that in most of the cases the diversity level among the localities of the rice areas in close proximity is very narrow, forming small sub-clusters with very little linkage distances. Low difference within certain locations may be due to shorter history of cultivation.



Figures 4 and 5. *Dendrogram resulting from average linkage of 26 localities of rice and 15 localities of soybean growing areas of Pakistan based on nematode communities*

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A similarity matrix based on the presence/absence of nematodes data was used to construct the ratio of similarity between the different soybean growing localities of three provinces of Pakistan. Similarity coefficients among various soybean growing areas of Pakistan ranged from 0.69 to 0.90 were presented.

The UPGMA cluster analysis showed that all 15 soybean growing areas could be easily distinguished based on the information generated by the presence or absence of nematodes. As expected, areas were separated into 5 distinct groups. Group A lines comprised with the similarity coefficient of 0.82 (82 %). The localities of Multan and Dir showed close similarity coefficient. Thatta and Swat 0.87 (87 %) show more similar coefficient for the nematode incidence. Kurram Agency did not fall in any group, might be this area having dissimilar nematode fauna /or climate conditions and would have some difference of nematode density (*Fig. 5*).

Discussion

Nematodes co-exist together in different environment (Boag and Yeates, 1998), however, their frequency, density and diversity varies depending upon ecological and edaphic factors (Sohlenuis, 1979; Khatoon et al., 2001). Nematodes may form the most significant group for community indicator analysis because more information exists on their taxonomy and feeding groups (Gupta and Yeates, 1997) than for other macrofauna. Free-living nematodes are very important and beneficial in the decomposition of organic material and the recycling of nutrients in soil. Nematode bacterivores and fungivores do not feed directly on soil organic matter, but on the bacteria and fungi which decompose organic matter. The presence and feeding of these nematodes speed up the decomposition process. Their feeding recycles minerals and other nutrients from bacteria, fungi, and other substrates and returns them to the soil where they are accessible to plant roots.

Detailed study has revealed that host associated nematodes were more in rice than soybean cultivated areas of Pakistan. A total of 39 genera were identified from rice while 33 genera were recorded from soybean from 41 localities throughout Pakistan. Among these nematodes, *Hirschmanniella* spp., was predominant and reduced yield production Tiwari and Kumar (1996) reported *Hirschmanniella* spp., infection in irrigated rice. Many researchers described the *Hirschmanniella* spp., on rice fields from various parts of the country (Maqbool and Shahina, 2001). Rose et al. (1967) reported that *Tylenchorhynchus claytoni* suppressed yield of soybean by 21 % in microplots and Ahmad et al. (2001) studied the occurrence of plant parasitic nematodes in soybean fields of Bundel khand region. Also adding to the parasitic load were *Aphelenchoide*s spp., *Helicotylenchus* spp., and *Tylenchorhynchus* spp., which were found in large number of samples of rice and soybean examined. However, present findings are in confirmation of the results of previous surveys carried out by many researchers that worked on similar objectives viz., Khan and Shaukat (1999), Ahmad and Nadeem (1983), Sher (1963), Robbins et al. (1982).

On the basis of trophic composition nematodes were grouped in five categories: herbivores, bacteriovores, omnivores, fungivores and predators. Herbivores representing the highest number of genera and also the highest abundance in both rice and soybean crops. While within the taxonomic groups, *Hirschmanniella* was the most frequently encountered genus (21%) dominating the entire nematode community in rice and it could be related to its cosmopolitan nature of occurrence and specific host (rice). Similarly, *Helicotylenchus* was the most frequently occurring genus in soybean which may be due to its cosmopolitan and polyphagous nature. *Aphelenchoides*, *Clarkus* and *Cephalobus*

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showed comparatively high frequency and density than for other trophic group of nematodes due to mono-cropping. In rice and soybean herbivores showed highest abundance which was related to the abundance of other trophic groups. It may be concluded that a highly significant positive correlation exists between herbivores species of both rice and soybean samples. These relationships are broadly in line with findings of the Debabrata et al. (2007) and Yeates et al. (1993) who reported that free-living bacteriovorous, fungivorous and omnivorous species comprise 52 % of the total nematode genera and 26 % of the terrestrial genera. Higher population of herbivores may also be attributed to the fact that, the excessive use of fertilizers and other manures, add excessive nutrient to the soil. These findings are similar to the study of Tomar et al. (2006).

Cluster analysis was also performed based on nematode communities. The resulted dendogram showed that diversity level among the 26 localities of the rice areas in close proximity is very narrow, while the UPGMA cluster analysis showed that all 15 soybean growing areas could be easily distinguished. The localities of Multan and Dir showed close similarity coefficient. Thatta and Sawat showed more similar coefficient or the nematode incidence. Kurram Agency did not fall in any group and observed to be more distantly related to all groups.

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