

Diversity of nematode fauna associated with cotton fields of high temperature areas of Sindh, Pakistan

A.N. Ashfaq¹, F. Shahina¹ and S. Dawar²

¹National Nematological Research Centre, University of Karachi, 75270- Karachi, Pakistan

²Department of Botany, University of Karachi-75270 Karachi, Pakistan

†Corresponding author: shahinafayyaz@gmail.com

Abstract

Diversity of nematode fauna in relation to their occurrence in high temperature cotton growing areas of Sindh by using Euclidean distance has been estimated. The fauna obtained in June 2017 and June 2018 surveys of cotton fields of five Districts Sanghar, Mirpurkhas, Umerkot, Mititari and Tando Allah Yar of Sindh where temperature in May to July raised up to 40°C -50°C. The data was subjected to construct the dendrogram for interpretation on the basis of their prevalence. In order to estimate nematode species diversity, richness and evenness, the data has been submitted for calculating the diversity parameters such as Dominance (D), Simpson's diversity index (1-D), Shannon diversity index (H'), Evenness (e), Brillouin Diversity Index (HB), Menhinik Diversity, Margalef's richness index, Equitability (J) and Fisher's alpha. All estimated parameters of diversity has interrelation among all nematode genera in particular but no correlation have been found establish between genera with reference to temperature fluctuation during studied years.

Keywords: Prevalence, Diversity index, Species richness, Cotton fields, Nematode fauna, Sindh.

Global warming is the present-day challenge and it raised concern for agriculture point of view. Fungi and nematodes are major microfauna and important constituents of soil food webs. The functional features of nematodes usually influenced by the abiotic characteristics and therefore any change in the conditions those are easily reflected in various forms, can affect the population dynamics of nematodes. Nematodes due to most numerous soil mesofauna are of significant interest that occupies all trophic consumer grades within the soil food web. In this context, community structure can provide vigorous perceptions about many aspects of ecosystem function (De Ruiter *et al.*, 2005; Ritz & Trudgill, 1999).

In complex food web nematode interaction with other soil organism ecosystem services which include maintenance of soil structure, biocontrol of pests and diseases, carbon sequestration, nutrient cycling and soil detoxification.

Nematodes interact with other soil organisms in complex food webs to provide essential functions and ecosystem services which include maintenance of soil structure, carbon sequestration, bio-control of pests and diseases, soil detoxification and nutrient cycling. For the last decade has been witnessed that loss of diversity is highly attributed due to agricultural intensification, pollution, modification of global carbon, greenhouse effect, increased warming and nitrogen cycles (Asner *et al.*, 1997). The

conclusively documented status of below-ground biodiversity is not yet achieved however, little is known about the effects of temperature on the diversity especially in the tropics (Bardgett & Putten, 2014).

Temperature has been kept count as influential factor that affects generation time (Evans & Fisher, 1970), rate of development (Bergeson, 1959), egg production and hatching (Bergeson 1959), (Greet, 1978), movement (Dusenbery *et al.*, 1978), size (Evans & Fisher, 1970) and respiration (Elliott *et al.*, 1980). The effects of temperature has shown varied effect on these regular functions, and thus the best way to measuring the population response is by evaluating optimum temperature for a species, as it depicts the outline of several processes affected by temperature. Generally, nematode communities are receptive to changes in food web (Yeates, 1987) environment and temperature (Samoiloff, 1987; Wasilewska, 1989) and can be quantified through Euclidean Distance and Diversity index.

The aim of present study was to investigate the effect of high temperature on the diversity of nematode communities of cotton fields of five districts of Sindh province, Pakistan. The areas under study are main cotton growing for the last fifteen years and considered to be high temperature areas of the country. Drip irrigation system is typically used there. Standard diversity indexes have been used for estimation.

Materials and Methods

During June, 2017 and June, 2018, comprehensive surveys were carried out in regular cotton growing areas of Sindh, Pakistan. Five Districts Sanghar, Mirpurkhas, Umerkot, Mititari and Tando Allah Yar were selected on the basis of the regular cotton growing fields for the last ten years. Details of studied areas are given in Table 1, recorded at the time of sampling. 100 samples from each locality were collected and processed as per Baermann (1917) and Cobb method (1918). Quantitative and qualitative analysis were performed and data was subjected to analysis.

Table 1. Details of cotton growing areas of Sindh, Pakistan in June, 2017 and June, 2018.

S. No.	Locality	Temperature*		Humidity %*		Longitude	Latitude
		2017	2018	2017	2018		
1.	Sanghar	43°C	48°C	90	85	26.0436 °N	68.9480 °E
2.	Mirpurkhas	41°C	46°C	58	64	25.5065 °N	69.0136 °E
3.	Umerkot	50°C	49°C	40	55	25.3549 °N	69.7376 °E
4.	Mititari	42°C	47°C	65	48	25.7519 °N	68.4770 °E
5.	TandoAllahyar	45°C	46°C	78	70	25.4351 °N	68.7332 °E

*Temperature and humidity were recorded at the time of sampling

Euclidean Distance in the basis of nematode occurrence and Diversity Index of species in a community of high temperature cotton growing fields were estimated. Different parameters such as Dominance (D), Simpson's diversity index (1-D), Shannon diversity index (H'), Evenness (e), Brillouin Diversity Index (HB), Menhinik Diversity, Margalef's richness index, Equitability (J) and Fisher's alpha were calculated and presented in tabulated form whereas graphical presentation was also furnished for few parameters.

Formula configuration of different parameters used to measure the diversity are:

- Dominance D: Dominance = 1-Simpson index.
 $D = \sum (ni - 1) / (n - 1)$.
- Simpson's Diversity Index: (SDI)
 $D = 1 - \sum n(n-1) / (N)(N-1)$
- Shannon Weiner Diversity Index:
 $H' = -\sum p_i \log p_i$
- Evenness (e): $J' = H' / H'_{\max}$
- Brillouin Diversity index: The Brillouin index, HB, is calculated using:

$$HB = \frac{\ln M - \sum_{i=1}^s \ln n_i!}{N}$$

- Menhinick Diversity Index: A diversity index, taking into account the number of individuals as well as number of taxa. Menhinick's richness index- the ratio of the number of taxa to the square root of sample size.
- Margalef's richness index: $(S-1)/\ln(n)$, where S is the number of taxa, and n is the number of individuals.
- Equitability (J') = $H'/H \max = H'/\log S$
- Simpson's Diversity Index: is a measure of diversity. In ecology, it is often used to quantify the biodiversity of a habitat.
- Fisher's alpha:

$$S = a * \ln(1 + n/a)$$

Results and Discussion

Euclidean Distance of nematode fauna associated with different cotton field of Sindh, Pakistan: The population based data obtained from the sequential surveys (June, 2017 and June, 2018) of five different cotton growing areas viz., Sanghar, Mirpurkhas, Umerkot, Mititari, and Tando Allah Yar of Sindh Province, Pakistan has been studied.

District Sanghar: The nematode fauna found from Sanghar in the year 2017 is divided into two major groups on the basis of their population dynamics contains thirteen nematode in Group I and nine nematode in Group II, Group I is further divided into 3 Subgroups that ranging the population (20-25%) 15% and 5-10% Group II is also divided onto 3 sub clade, comprised of nine nematode species and their population ranged from 30-35%, 40% and 45-50%.

In the next year 2018, the nematode population was found to be increased but with different grouping pattern. The whole data is comprised of 20 nematode genera. The population based cluster dendrogram is divided into 2 major groups .Group I is further divided into three sub-

groups containing 12 nematodes which ranged the population 5-30%. The Group II is divided into 2 subclade with population % ranged between 50-65% and 75-85%. The highest population 75-85% is found in for the subgroup containing *Hoplolaimus columbus*, *Aphelenchus avenae* and *Meloidogyne incognita* and *Pratylenchus penetrans* (Fig. 1a & b).

District Mirpurkhas: Nematode population dynamics of district Mirpurkhas in 2017-2018 the nematode fauna associated with cotton field of District Mirpurkhas is mainly divided into two Groups in 2017. Group I is largely diversified on the basis of prevalence of nematode genera. The Group II consists of three plant parasitic nematodes with high population percentages i.e., *Helicotylenchus* 60%, *Rotylenchulus reniformis* and *Aphelenchus avenae* both with 65%.

In the year 2018, the overall population was increased but different grouping patterns compared to the previous year data. The obtained data of occurrence is divided into 2 major Groups on the basis of occurrence percentage. The Group1 consist of 19 nematode genera and their percentages ranged between 5-65% that further splits into two subgroups. Subgroup 1 contains 13 nematodes in which four are plant parasitic, and their prevalence is not significantly diversified i.e., ranged between 5-30% whereas subgroup II has 6 nematode genera with 45-65% of their presence. The group-II is found to be less diversified but with high percentages range between 85-100% for 4 nematode genera i.e., *Malenchus*, *Pratylenchus*, *Tylenchorhynchus* and *Aphelenchus* (Fig. 2 a & b).

District Umerkot: During the survey 21 nematode genera were detected in 2017. Out of 21, 10 genera were of parasitic type. The obtained population is diversified but with less population differences i.e., mostly of the nematode genera are prevalent under 2 major groups that ranged between 5-30% and 35-65% of occurrence. The other group is comprised of only three genera i.e. *Filenchus*, *Rotylenchulus*

and *Aphelenchus* having occurrence percentages 80-85% and 95%, respectively.

The next survey conducted during 2018, the population studies and their presence of different nematode genera have shown non-significant difference. Two groups have clearly separate divisions. In the Group I, 19 nematode species are placed. Maximum number of genera have percentage occurrence between 5-35% while remaining genera of this group have 45-65% of occurrence. Three genera are clustered forming a separate group “Group-II” *Rotylenchulus*, *Aphelenchus* and *Filenchus* with high percentages i.e., 80-100% (Fig. 3 a & b).

District Mitiari: The obtained data of nematode population of district Mitiari subjected to cluster analysis on the basis of percentages occurrence of different nematode fauna has been grouped on prevalence diversity. The entire population comprises of 20 genera which further divided into two groups. Group I contains 11 nematodes and their occurrence % ranged between 5-35% minimum is for *Helicotylenchus*, *Pratylenchus*

i.e., 5% and maximum for *Dorylaimellus* (A) *parvulus* i.e., 35%.

Group-II accommodate 9 genera and has more diversified population in sense of occurrence % of different genera that ranged between 40-75%. Most prevalent nematode genera are *Aphelenchus*, *Longidorus* and *Tylenchorhynchus* i.e., 75% of occurrence. Root-knot nematode is found with 50% and other 4 parasitic nematode genera i.e., *Ditylenchus*, *Heterodera* that have 55% and 50% occurrence, respectively. *Filenchus* and *Rotylenchulus* are grouped under the same and both have same occurrence % i.e., 60%.

In the next year 2018, the whole nematode fauna of cotton fields of District Mitiari was found to be much variable and significantly varied with the previous year population. The obtained nematode genera are divided into two groups. Group1 contains 12 genera and ranged between 30-55% whereas Group 11 contains 7 genera and % of occurrence ranged within 75-100% (Fig. 4 a & b).

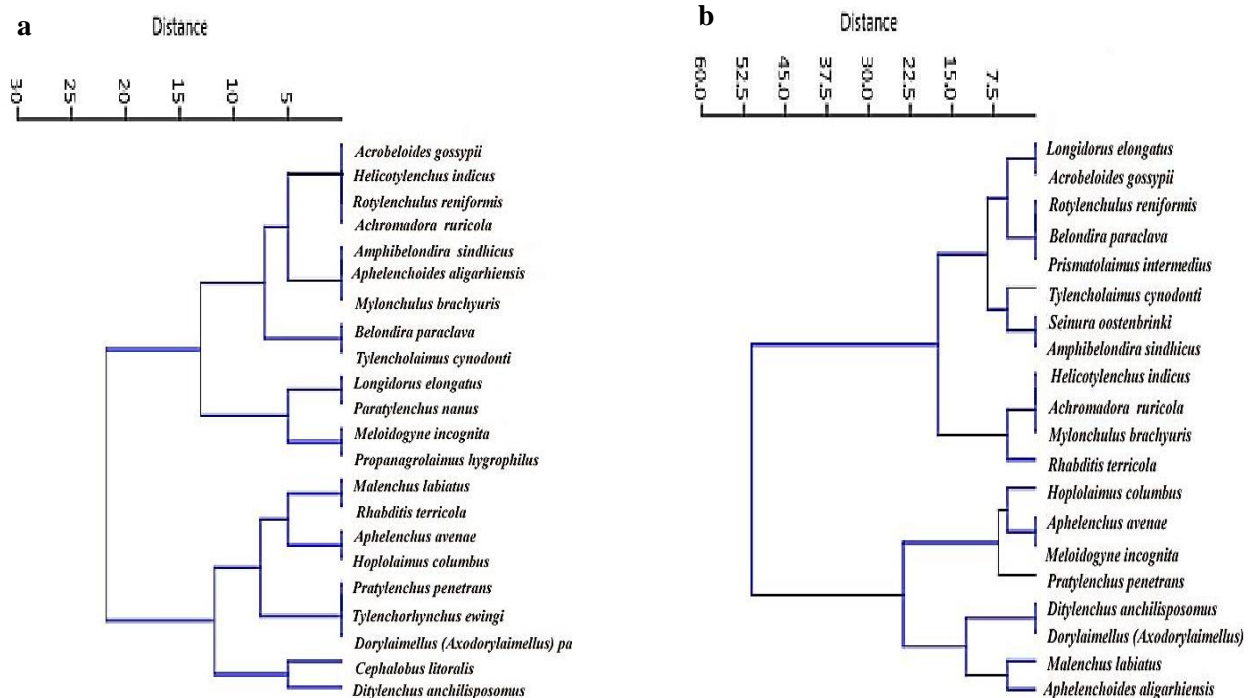


Fig. 1a & b. Cluster analysis of occurrence percentages of nematode fauna of Sanghar in 2017-18.

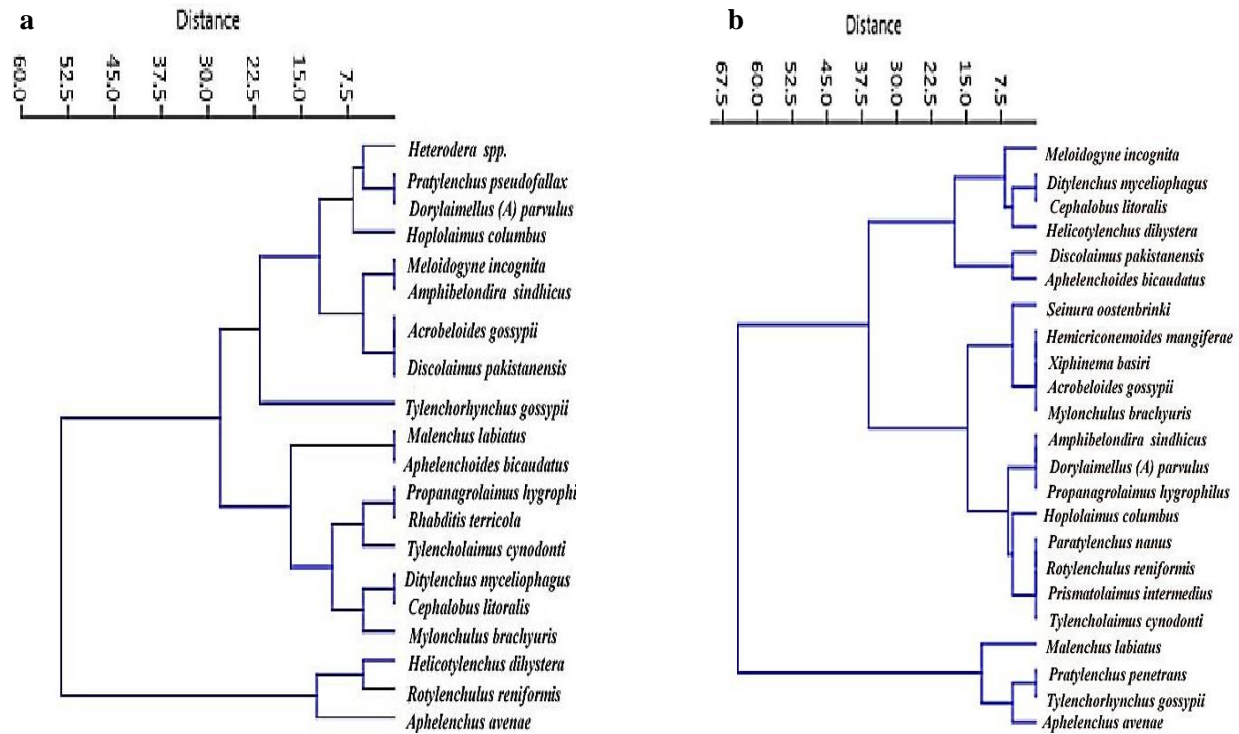


Fig. 2a & b. Cluster analysis of occurrence percentages of nematode fauna of Mirpurkhas in 2017-18.

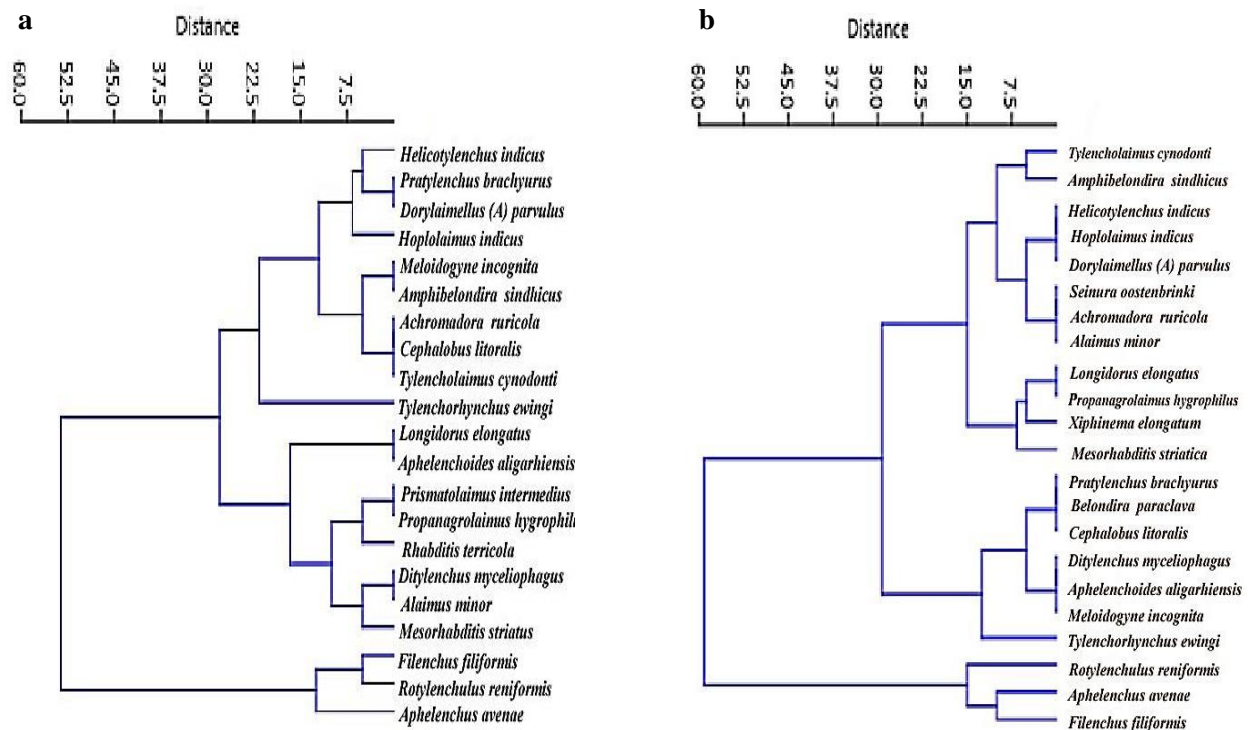


Fig. 3a & b. Cluster analysis of occurrence percentages of nematode fauna of Umerkot in 2017-18.

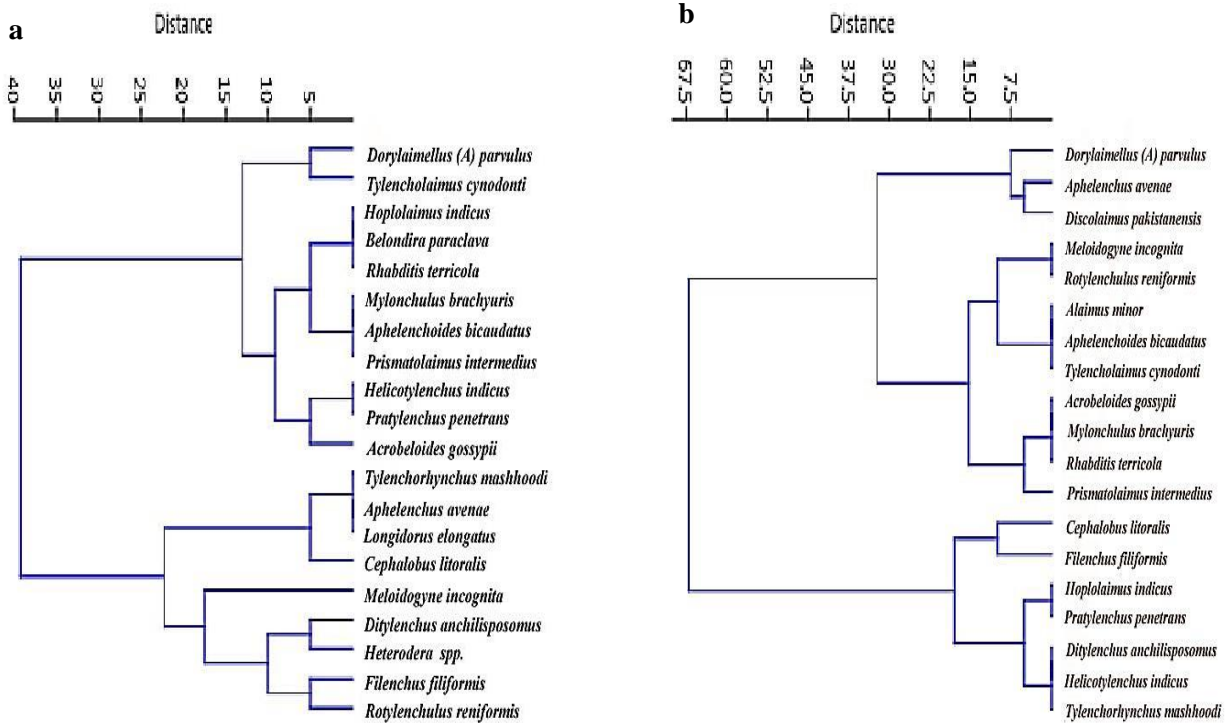


Fig. 4a & b. Cluster analysis of occurrence percentages of nematode fauna of Mitiari in 2017-18.

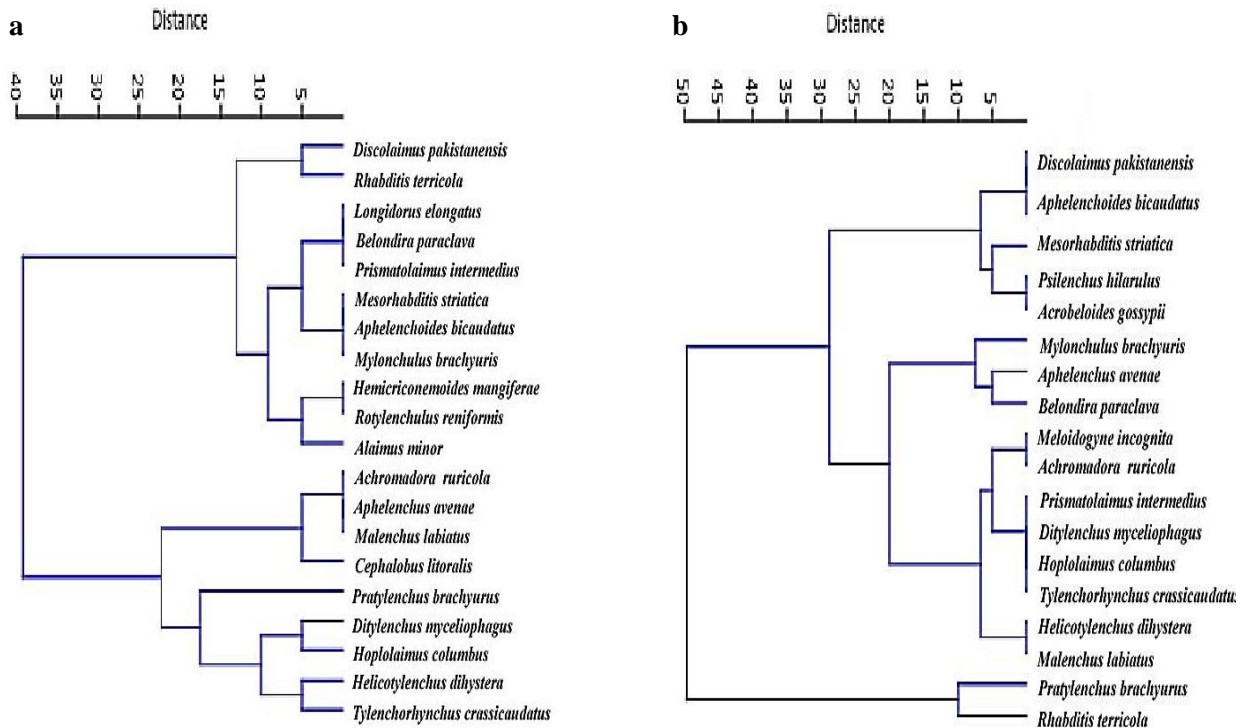


Fig. 5a & b. Cluster analysis of occurrence percentages of nematode fauna of Tando Allah Yar in 2018.

District Tando Allah Yar: The nematode population of the District Tando Allah Yar is not much diversified on the basis of their percentages of occurrence. Most of the genera are gathered under the same group “Group I” and their percentages not exceed more than 35%. In the Group II plant parasitic nematodes *Pratylenchus*, *Ditylenchus*, *Hoplolaimus*, *Helicotylenchus* and *Tylenchorhynchus* have high percentages ranged from 60-85%.

In the year 2018, only 2 genera *Pratylenchus* and *Rhabditis* is placed in a separate group having occurrence percentages 85 and 75 respectively whereas rest of 16 genera are accommodate in a group having occurrence percentages ranged from 5-60% (Fig. 5a & b).

Diversity index of nematode fauna of different Districts of Sindh in 2017-2018: The values of diversity parameters for each site in relation to study year were computed; the results are given in Tables 2 and 3.

In year 2017, the highest Dominance value was for the species of Mitiari (0.069) lowest value was of for the species of Sanghar. Maximum Simpson's diversity was found at Sanghar (0.9434) which exceeded the dominance of the same locality. Simpson diversity index of the studied localities were inversely proportional to the dominance of species of the same localities. Shannon Weiner Diversity (H) was found to be highest for the locality of Sanghar (2.958) and lowest for the locality Mitiari i.e., 2.782 whereas for rest of the three localities the diversity was found to be non-significantly different. Evenness (e) for the species found during the survey was obtained maximum (0.8757) for Sanghar and second highest for Mirpurkhas (0.8722) whereas the lowest evenness value was gained for Umerkot i.e., 0.831. Brillouin Diversity (HB) was also high for the nematode fauna of Sanghar, the remaining localities data have shown almost similar values. Species richness as measured by Menhinick's index d_1 was highest in Sanghar (0.9297), second highest value was at Mirpurkhas (0.767) and lowest at Mitiari (0.686). Margalef richness index was calculated for number of individual's richness in number of taxa, this parameter of diversity was found to be significantly high in Sanghar (3.319) as compared

to the rest of the localities. Equitability was again maximum at Sanghar (0.9571), the second highest was at Mirpurkhas (0.9544) and minimum at Mitiari (0.9285) (Table 2).

In 2018, the highest Dominance value was for the species of Mitiari (0.078) lowest value was of for the species of Umerkot (0.064). Simpson's diversity was found high at Umerkot (0.935) and lowest is for Mitiari (0.921). The results for Simpson diversity index were remarkably different from the results of the previous year data. Shannon Weiner Diversity (H) was found to be highest for the locality of Umerkot (2.886) and lowest for the locality Mitiari i.e., 2.683. It has also been observed that there was no significant difference in the (H) values for the locality of Mitiari. Evenness (e) for the species found during the survey was obtained maximum (0.836) for Tando Allah Yar and second highest for Umerkot (0.814) whereas the lowest evenness value was gained for Sanghar i.e., 0.760. Evenness data was greatly fluctuated with respect to duration (year wise). Sanghar which has maximum evenness for the year 2017 had become lowest evenness for the year 2018. Brillouin Diversity (HB) was highest for the nematode fauna of Umerkot (2.823), the second highest values was for Mirpurkhas (2.814), the remaining three localities data have shown non significantly different values. Species richness as measured by Menhinick's index d_1 was obtained highest for Mirpurkhas and Umerkot (0.752), while the lowest at Mitiari (0.613). Comparably the results were similar for the locality of Mitiari in both years. Margalef richness index was found to be significantly high in Mirpurkhas (3.216) as compared to the rest of the localities whereas as lowest value was for Tando Allah Yar. Equitability was maximum at Umerkot and Tando Allah Yar (0.93), the second highest was at Mirpurkhas (0.916) and minimum at Sanghar (0.908) (Table 3). Both the species richness and evenness incorporated the diversity indices into a single value of Shannon index (H'). It is the most accepted and commonly used index in community ecology. It is evident from the Figs. 6 & 7 that Sanghar exhibited the highest overall diversity index compared to other localities in the year 2017 and Umerkot recorded the overall highest diversity in the year 2018.

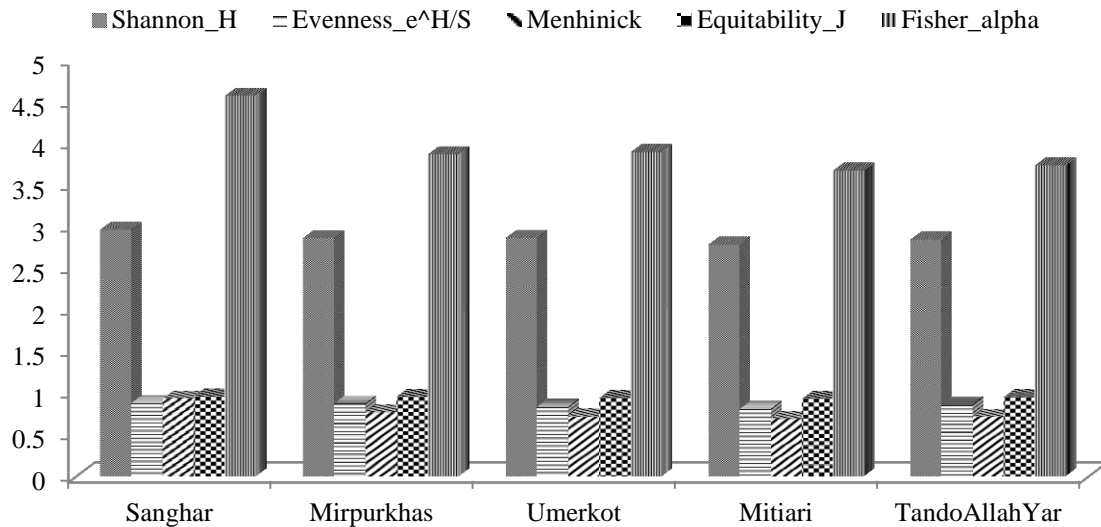


Fig. 6. Graphical presentation of Diversity Index of nematode fauna associated with cotton fields of Sindh 2017.

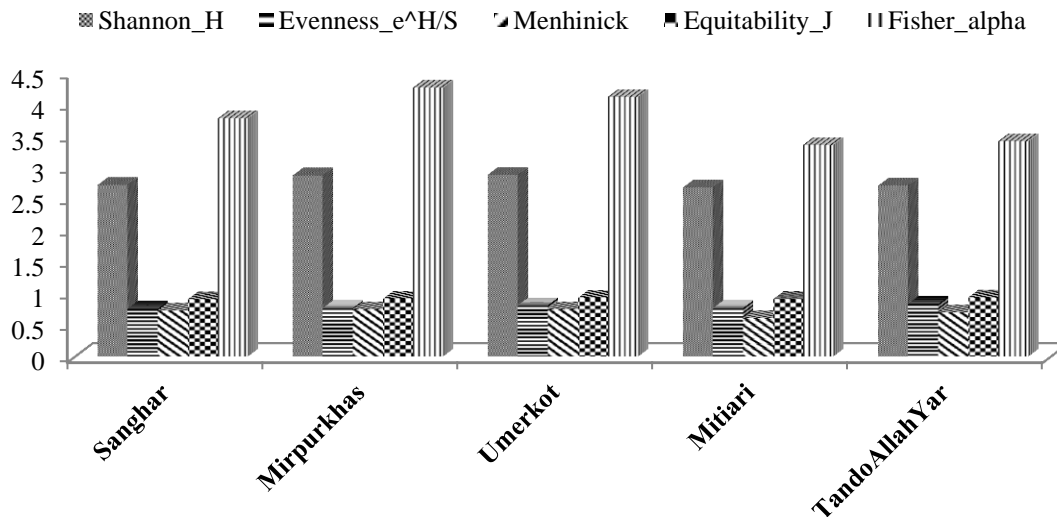


Fig. 7. Graphical presentation of Diversity Index of nematode fauna associated with cotton fields of Sindh 2018.

Among all localities, Sanghar exhibited maximum species richness and diversity in 2017. The species diversity affected by many environmental factors viz., seasonality, environmental stability, heterogeneity, habitat type, predation, competition and productivity (Rosenzweig, 1995). The species richness index values in all localities were different in both years. In 2017, the species richness index was highest for the locality of Sanghar whereas it became lowest in 2018. Evenness index seemed to be influenced by species richness and it also includes even the rare taxa in the community.

Ecologist usually used evenness. Estimating for all the localities, Sanghar has shown highest values of evenness index. This indicates that in Sanghar, the nematodes were distributed evenly. Evenness index indicates that Sanghar showed higher evenness as compared to other localities. This was supported by diversity for the locality as diversity also includes evenness. The present study clearly indicates that the rich and varied nematode diversity was of the locality “Sanghar” there by supporting the concept that the nematodes are the key components in all the ecosystems in which they lives.

Table 2. Diversity Index of nematode fauna associated with cotton fields of Sindh 2017.

	Sanghar	Lower	Upper	Mirpurkhas	Lower	Upper	Umerkot	Lower	Upper	Mitiari	Lower	Upper	TandoAllahyar	Lower	Upper
Taxa_S	22	22	22	20	20	20	21	21	21	21	20	20	20	20	20
Individuals	560	560	560	680	680	680	855	855	855	850	850	850	790	790	790
DominanceD	0.056	0.054	0.062	0.063	0.060	0.068	0.064	0.062	0.069	0.069	0.067	0.075	0.065	0.0629	0.070
Simpson1-D	0.9434	0.9378	0.945	0.9369	0.9313	0.939	0.935	0.930	0.937	0.930	0.924	0.932	0.934	0.9292	0.937
Shannon_H	2.958	2.897	2.978	2.859	2.806	2.88	2.86	2.81	2.883	2.782	2.731	2.807	2.842	2.791	2.866
Evenness_e	0.8757	0.8239	0.892	0.8722	0.827	0.890	0.831	0.7912	0.851	0.807	0.767	0.828	0.857	0.8146	0.878
Brillouin	2.869	2.81	2.888	2.789	2.737	2.809	2.8	2.751	2.823	2.724	2.675	2.75	2.78	2.729	2.803
Menhinick	0.9297	0.9297	0.9297	0.767	0.767	0.767	0.7182	0.718	0.718	0.686	0.686	0.686	0.7116	0.7116	0.7116
Margalef	3.319	3.319	3.319	2.913	2.913	2.913	2.962	2.962	2.962	2.817	2.817	2.817	2.848	2.848	2.848
Equitability_J	0.9571	0.9373	0.9633	0.9544	0.9367	0.9614	0.9394	0.9231	0.9471	0.9285	0.911	0.937	0.9486	0.9315	0.9566
Fisher_alpha	4.567	4.567	4.567	3.864	3.864	3.864	3.891	3.891	3.891	3.67	3.67	3.67	3.731	3.731	3.731

Table 3. Diversity Index of nematode fauna associated with cotton fields of Sindh 2018.

	Sanghar	Lower	Upper	Mirpurkhas	Lower	Upper	Umerkot	Lower	Upper	Mitiari	Lower	Upper	TandoAllahyar	Lower	Upper
Taxa_S	20	20	20	23	23	23	22	22	22	22	19	19	18	18	18
Individuals	750	750	750	935	935	935	855	855	855	960	960	960	660	660	660
Dominance	0.076	0.07	0.082	0.066	0.063	0.071	0.064	0.061	0.070	0.078	0.075	0.083	0.074	0.071	0.081
Simpson	0.923	0.917	0.926	0.933	0.928	0.936	0.935	0.929	0.938	0.921	0.916	0.924	0.925	0.918	0.928
Shannon_H	2.722	2.662	2.755	2.874	2.82	2.903	2.886	2.832	2.913	2.683	2.634	2.711	2.711	2.655	2.739
Evenness	0.760	0.716	0.786	0.769	0.729	0.792	0.814	0.771	0.836	0.769	0.733	0.791	0.836	0.790	0.859
Brillouin	2.659	2.601	2.692	2.814	2.761	2.843	2.823	2.77	2.849	2.634	2.586	2.661	2.646	2.591	2.674
Menhinick	0.730	0.730	0.730	0.752	0.752	0.752	0.752	0.752	0.752	0.613	0.613	0.613	0.700	0.700	0.700
Margalef	2.87	2.87	2.87	3.216	3.216	3.216	3.111	3.111	3.111	2.621	2.621	2.621	2.619	2.619	2.619
Equitability_J	0.908	0.888	0.919	0.916	0.899	0.925	0.93	0.916	0.942	0.911	0.894	0.920	0.938	0.918	0.947
Fisher_alpha	3.776	3.776	3.776	4.263	4.263	4.263	4.12	4.12	4.12	3.357	3.357	3.357	3.416	3.416	3.416

The factor of temperature in relation to nematodes diversity may be measured in *in vitro* or in confined environment but in natural occurring environment lots of factors influence the diversity pattern and could not be measured as yet. Such ecological factors viz., competition, host structural heterogeneity, predation, soil disturbance are to be considered whereas edaphic factors such as organic matter, pore space and moisture, irrigation pattern, mineral, below ground litter and root exudates also plays vital role and influence the coexistence of different nematode species which eventually support the diversity pattern of nematode in every ecosystem (Yeates, 2003).

Several attempts have been made by researchers to develop relationships between nematode community structure and succession of natural ecosystems or environmental disturbance (Liang *et al.*, 1999; Yeates, 1984). The situation of below-ground biodiversity is however, not decisively documented in relation to temperature.

The interpretation of results in relation to temperature and humidity defines that the locality District Sanghar where the diversity was maximum in 2017 seems to change significantly in the next year and same is for the District Umerkot population but in reverse order. This explains that divergence in locality conditions of soil type and rainfall pattern might be attributed to the differences. This study proved that the relative humidity also positively influenced the nematode populations of cotton fields. The high population might be due to establish well under high humidity (90%) which in turn produce more root volume that will support the nematode multiplication. Temperature is one of the most constantly changing factors that nematode come across. It directly or indirectly affects the activities of nematodes whereas; different nematode species are affected contrarily. Suitable reproduction conditions of nematodes may happen over a wide range of temperature that would give a closely flat peak on a temperature curve. Most of

nematodes reproduce best at temperatures between 25°C-30°C. In current situation, most of the soil nematodes as well as parasitic population decreased in 2018, in Sanghar after the first year observation but inversely different results obtained for District Umerkot. Such differences may be explained by the fact that different trophic groups of nematodes coexist in varying ecological conditions but their diversity, density and frequency differs.

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