SKUAST Journal of Research 26(4): 588-594; (2024)
(*DOI No.10.5958/2349-297X.2024.00065.X*); NASS rating: 4.88

Research Paper

Abundance and distribution of plant-parasitic nematodes in high density apple orchards within the temperate agro-ecological zone of the Kashmir Valley

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(Received October 16, 2026; accepted December 15, 2024)

ABSTRACT

The Apple growing industry has witnessed a major shift from low density to medium and high density plantations in the recent past. The soil micro-fauna associated with the roots of apple varieties suitable for high density plantations remains unknown in Kashmir valley.Among the many organisms found in association with apple plant roots, plant-parasitic nematodes are responsible for causing several severe diseases including, yield reduction, apple decline and apple replant disease. Considering the importance of knowledge about abundance and distribution of plant parasitic nematodes, this experiment was carried out in the major fruit growing belts of Kashmir valley (Baramulla, Anantnag and Srinagar) during the year 2018. In all the composite samples from three different districts, nine nematode genera were isolated from the rhizosphere of high density apple orchards in the three districts of Kashmir valley *viz*., *Helicotylenchus* sp., *Pratylenchus* sp., *Tylenchorhynchus* sp., *Tylenchus* sp., *Xiphinema insine*, *X.index*, *X.americanum*, *Longidorus elongatus* and *Hoplolaimus* sp. The absolute density, relative density, absolute frequency and relative frequency of each different taxonomical group was calculated and compared. Community analysis of predominant plant parasitic nematodes in high density apple plantation of Kashmir valley on various parameters revealed that *Helicotylenchus* sp. followed by *Pratylenchus* sp., *Tylenchus* sp. and *Tylenchorhynchus* sp. showed highest mean absolute density in all the three districts. The mean performance of parameters *viz* relative frequency, relative density and prominence value also showed similar trend as that of the mean absolute density.

Key words: Ecology, community analysis, orchard, plant parasitic nematodes, prominence value, relative density, relative frequency.

Apple (*Malus* × *domestica* Borkh.) is one of the most ubiquitous and well-adapted species of temperate fruit crops, grown particularly in North-Western Himalayas at an elevation range of 1,500 – 2,700 m above mean sea level. Known as the "King of temperate fruits", apple production forms the backbone of economy in the Union Territory of Jammu and Kashmir. However, due to low productivity of the traditional apple cultivars the high density planting (HDP) system has being conceived as an alternative production system with an aim to improve the potential of productivity and profitability. HDP gives higher yield as well as return per unit area as there are more trees in the unit area. (Lauri *et al*., 2004). However, like the traditional apple orchards several abiotic and biotic factors result in reduced productivity. Among the numerous agents, plant parasitic nematodes are a significant threat to apple production in India, substantially impacting both quality and quantity of apple crop (Askary *et al*., 2011).

These parasitic nematodes which are members if the distinct phylum Nematoda in the kingdom Animalia, are a global source of concern for farmers because of their variable feeding habits and symptomatology.Nematodes that parasitize plants have been classified into over 4100 species (Gheysen and Jones, 2006). According to estimates, they harm crops to the tune of \$80-118 billion annually (Nicol *et al*., 2011). The most economically significant nematode species, which make about 15% of all known species, directly attack the plant roots of essential agricultural crops, preventing them from absorbing water and nutrients, lowering agronomic performance, overall quality, and yields.According to the research, PPNs, are the main cause of most complex diseases such as, dieback, replant disease, root and rhizome rot, and reduced bearing period of fruit trees. They are also one of the key factors restricting the horticulture industry's profitability. Because they generate significant losses due to an increased rate of tree mortality, disease complexes like apple replant disease and apple proliferation are of great concern to fruit growers (Bridge and Starr, 2007). However, there is dearth of knowledge on the distribution, identification and pathological investigations of plant parasitic nematodes in the Kashmir valley, as well as their detailed and varied state. This prompted the present research with the objectives of, 1) identification of the plant parasitic nematodes associated with high density apple plantations in Kashmir valley, and 2) determination of the community analysis of predominant plant parasitic nematodes in high density apple plantation of Kashmir valley.

MATERIALS AND METHODS

Localities of the three major apple growing districts of the state viz. Srinagar, Anantnag and Baramulla comprising the central, southern and northern fruit belts of the Kashmir valley were surveyed during the summer of year 2019 (May-August)and from each district twenty seven composite samples were collected. Selection of prominent locations with extensively apple cultivations were made, having same kind and same aged fruit plants. From each district three different locations were chosen. In Baramulla, Ladoora, Zangam and Arampora were the three selected villages. Similarly, in Anantnag, Gopalpora, Achabal and Kokernag were selected. Following the similar trend three different locations, SKUAST-K, Habak and Zakura were selected in district Srinagar. About 500-1000 cc soil samples were selected from each district from the feeder zone of apple orchards. Sampling was done during peak growing season (May to July).The samples were collected manually up to a depth of 30-40 cm covering 60-90cm radius. The samples were collected in polybags and were tied with rubber band and the samples were stored in laboratory at a temperature of 7ºC. Nematode extraction was performed by following modified Cobb's sieving and decanting technique from each thoroughly mixed 250 cc of composite soil sample (Cobb, 1918). For extraction of nematodes a set of sieves were used (18,60,100,200, 300 and 400 mesh). Then the resultant suspension of each sieve with residue was separately spread on the double layer tissue paper placed on aluminum wire mesh and same was placed on petri-plates containing fresh water/distilled water. The nematodes were then heat relaxed in test tubes dipped in metallic container filled with boiling water. Suspension was constantly shaken to apply uniform distribution of heat till all the nematodes were killed. Suspension was fixed with 4% formaldehyde solution and the volume was reduced to 20 ml and stored in McCartney vials at 10-15ºC for further studies.

For the staining and counting purpose, about 1g of root cutting was collected and stained with 0.1% acid fuchsin lacto phenol for 30s for counting of PPNs using a stereoscopic microscope (40 x) (Hooper, 1970). The extracted nematodes were morphologically identified up to genus and species level using standard identification keys. The number of nematodes were counted in one ml of nematode suspension (after thorough bubbling) with the help of a counting dish and an average of five such counts were taken as the number of nematodes per ml. The volume of the suspension was multiplied with average count of nematodes per ml to estimate total number of nematodes per 250 cc of soil sample. Nematode community structure with reference to relative frequencies, relative densities, prominence value, biomass and importance value of nematodes was figured out using the formulae proposed by Norton (1978).

$$\text{Absolute frequency ofsp./genusx} = \frac{\text{No. of samples containing species/genus}}{\text{No. of samples collected}} \times 100$$

$$\text{Relative frequency of sp./genus x} = \frac{\text{Frequency of species/ genus}}{\text{Sum of frequencies of all spp/genus present in samples}} \times 100$$

$$\text{Absolute density of sp./genus x} = \frac{\text{No. of individuals of x in a sample}}{\text{Volume or mass or unit of sample}} \times 100$$

$$\text{Relative density of sp./genus x} = \frac{\text{No. of individuals of sp. / genus in a sample}}{\text{Total no. of individuals of all spp/ genus. in a sample}} \times 100$$

Prominence value of sp. /genus x = Absolute density x √Frequency

Biomass of sp. /genus x = $a^2.b/16 \times 10^5$ µg.

(a = average greatest body width, b = average body length of a nematode in µm)

Importance value = Relative frequency + Relative density + Relative biomass

Population density with range and frequency of occurrence of each nematode genus/ species was determined at each location and for total samples collected from the particular area. The Shannon Weiner density index formula was applied to find diversity of plant parasitic nematodes from locality to locality and from one belt to other belt.

$$H = \sum^{s} i{=}1 \; Pi \log_e Pi$$

Where,

s = Number of species/ genus at each location,

Pi =Relative abundance of i^{th} species/ genus.

RESULTS AND DISCUSSION

In all the composite samples from three different districts, nine nematode genera were isolated from the rhizosphere of high density apple orchards in the three districts of Kashmir valley *viz*., *Helicotylenchus* sp., *Pratylenchus* sp., *Tylenchorhynchus* sp., *Tylenchus* sp., *Xiphinema insine*, *X.index*, *X.americanum*, *Longidorus elongatus* and *Hoplolaimus* sp. But as with 95% of taxonomically characterized adenophoreans, parthenogenesis was probably widespread as males were rarely found. Among the major nematodes found during study the *Helicotylenchus* sp. was having the maximum number followed by *Pratylenchus* sp., *Tylenchus* sp., *Tylenchorhynchus* sp. The maximum absolute density was recorded for *Helicotylenchus* sp. followed by *Pratylenchus* sp., *Tylenchus* sp. and *Tylenchorhynchus* sp.Similarly the mean performance ofrelative density, 95 per cent confidence interval, absolute frequency, prominence value and relative prominence value followed the same trend as that of absolute density in all the three districts under study. However, the mean biomass showed maximum value for *Longidorus elongatus* followed by *Xiphinema index*, *Xiphinema insigne* and *Xiphinema americanum. Xiphinema index* was having the highest mean total biomass followed by *Longidorus elongatus, Xiphinema insigne* and *Helicotylenchus* sp. The mean relative biomass followed the same trend as that of mean total biomass. The importance value showed maximum value for *Helicotylenchus* sp. followed by *Xiphinema index, Longidorus elongatus* and *Pratylenchus* sp (Table 1, Fig. 1).

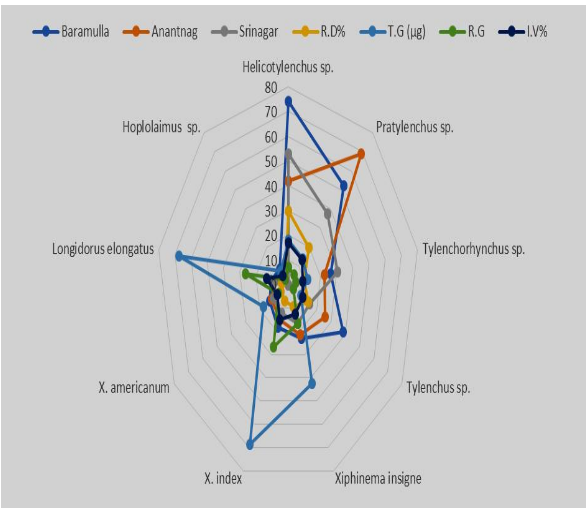

Fig. 1: Cumulative diversity indices for different nematode species identified

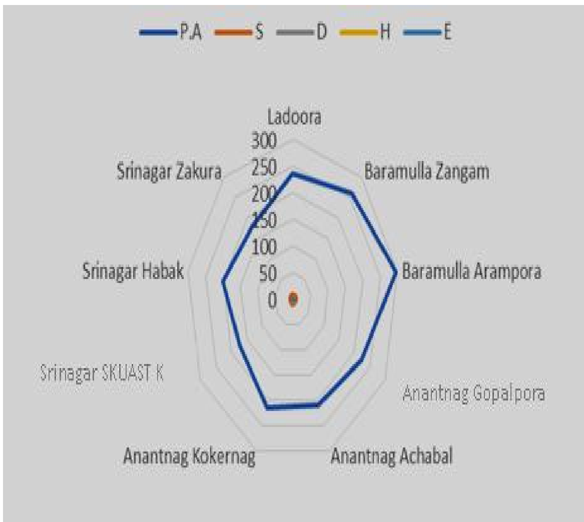

Fig. 2: Village wise Shannon Weiner density index measures in the three surveyed districts.

Table 1: Diversity and community population of plant parasitic nematodes associated with district Baramulla, Anantnag and Srinagar

S	Nematodes	Districts A.D			A.D mean	Cummulative mean for all the districts								
		Baram-ulla	Anant-nag	Sri-nagar		R.D %	95% C.I	A.F %	P.V	R.PV %	G/Nem (µg)	T.G (µg)	R.G	I.V %
1	*Helicotylenchus sp.*	73.95	41.81	52.85	**56.20**	29.63	54.92-75.76	100.00	562.00	24.98	0.32	17.98	7.02	16.78
2	*Pratylenchus* sp.	52.29	69.22	37.18	52.89	19.72	33.73-53.79	100.00	528.90	23.51	**0.26**	13.75	5.37	12.92
3	*Tylenchorhynchus* sp.	26.29	22.31	30.52	26.37	9.47	13.80-28.66	91.34	251.83	11.19	0.46	12.13	4.74	8.89
4	*Tylenchus* sp.	38.48	25.71	15.11	26.43	14.24	21.29-41.85	95.05	257.42	11.44	0.33	8.72	3.41	10.22
5	*Xiphinema insigne*	23.22	21.63	16.48	20.44	9.27	11.81-29.07	86.41	204.4	9.09	2.08	42.52	16.60	12.56
6	*X. index*	18.44	15.04	12.33	15.27	6.9	8.22-22.32	82.70	152.7	6.79	4.50	68.72	26.83	15.00
7	*X. americanum*	12.96	11.89	10.63	11.82	5.41	5.90-17.74	81.47	118.2	5.25	1.49	17.61	6.88	7.80
8	*Longidorus elongatus*	10.74	8.48	8.55	9.25	4.22	3.35-15.15	72.83	92.5	4.11	**7.30**	67.53	26.36	13.50
9	*Hoplolaimus* sp.	8.17	-	-	**8.17**	3.06	2.77-13.57	74.06	81.7	3.63	0.88	7.19	2.81	5.00

A.D: Absolute mean, **R.D:** Relative density, **C.I:** Confidence interval**, A.F:** Absolute frequency,
P.V: Prominence value, **R.PV:** Relative prominence value,
G: Biomass of nematodes, **T.G:** Total Biomass, **R.G:** Relative Biomass, **I.V:** Importance value.

A comparison of plant parasitic nematode density and their population abundance on the basis of number and biomass per 250 cc soil, the Shannon Weiner Density Index revealed that no significant difference in energy flow of plant parasitic nematodes was found from locality to locality and from district to district. The highest Shannon Weiner Density Index was recorded for district Baramulla followed by Anantnag and Srinagar. Population abundance in all the three districts *viz.* Baramulla, Anantnag and Srinagar revealed that the diversity index ranged from 1.813 (Srinagar) to 1.957 (Baramulla) with the difference in energy flow of 0.144 which is almost nearer to the diversity index at Anantnag (1.864) fruit belt (Table 2, Fig. 2**)**. The diversity and status of each species in detail is as follows:

Table 2: Shannon Weiner density index measures (village wise) based on population abundance in district Baramulla, Anantnag and Srinagar of Kashmir valley

S. No.	District	Village	P.A	S	D	1-D	1/D	H	E
1	Baramulla	Ladoora	237	09	0.172	0.828	5.813	1.954	0.889
		Zangam	260	09	0.160	0.840	6.251	1.982	0.902
		Arampora	296	09	0.170	0.830	5.881	1.937	0.881
2	Anantnag	Gopalpora	223	08	0.181	0.819	5.525	1.880	0.904
		Achabal	209	08	0.190	0.810	5.263	1.849	0.889
		Kokernag	215	08	0.184	0.816	5.432	1.863	0.896
3	Srinagar	SKUAST-K	172	08	0.174	0.826	5.745	1.901	0.914
		Habak	200	08	0.182	0.818	5.491	1.857	0.893
		Zakura	178	08	0.176	0.824	5.680	1.681	0.808

P.A: Population abundance, **S:** Species richness, **D:** Simpson's index, **1-D:** Simpson's index of diversity, **1/D:** Simpson's reciprocal index, **H:** Shannon Wiener index, **E:** Evenness.

***Xiphinema insigne* (Loos, 1949)**: The status of *Xiphinema insigne* with respect to mean population per 250 cc soil in the fruit belt of northern district of the valley, Baramulla, was recorded as 23.22 ranging from 14.11-32.33 at 95 per cent confidence interval. The per cent relative frequency and per cent relative prominence value were recorded as 10.95 and 8.48 (Table 1, Fig.1). Similarly, in the southern district of Anantnag the status of *Xiphinema insigne* with respect to mean population per 250 cc soil in the belt was recorded as 21.63 ranging from 13.09-30.17.The per cent relative frequency and per cent relative prominence value were recorded as 12.63 and 9.77 (Table 1). The central district, Srinagar, recorded the absolute density mean for the species *Xiphinema insine* as 16.48 ranging from 8.23-24.73 at 95 per cent confidence interval. Moreover, the per cent relative frequency and per cent relative prominence value recorded were 11.29 and 6.15, respectively (Table 1, Fig. 1). The femalebody was anteriorly tapering from base of stylet, then more noticeable from a point four to five lip region widths behind oral opening. Posteriorly, body tapering evenly from anus to tail terminus. Lip region hemispherical set off from rest of body by slight constriction. Stylet almost straight, 25 µm long. Flanged portion of stylet 12 µm wide. Vulva non- protruding, vagina transverse. Tail uniformly elongate-conical, about five anal-body-widths in in length of 110 µm long.

***Helicotylenchus* Steiner, 1945:** The data presented in Table 1 reveals that the mean population of *Helicotylenchus* sp., in district Baramulla was recorded as 73.95 ranging from 61.83-86.07 at 95 per cent confidence interval.The per cent relative frequency and per cent relative prominence value of *Helicotylenchus* sp., in the district Baramulla were recorded as 12.32 and 28.69, respectively and ranked as first dominant plant parasitic nematode in the entire district on the basis of population density and frequency of occurrence. *Helicotylenchus* sp., the Spiral nematode was recorded as second dominant specie with mean population in the fruit belt as 41.81 ranging from 33.49-50.13 at 95 per cent confidence interval. The per cent relative frequency and per cent relative prominence value of *Pratylenchus* sp., in the district Anantnag were recorded as 14.21 and 33.21, respectively. The absolute density mean per 250 cc soil for *Helicotylenchus* sp., in the belt as 52.84 with range as 43.19-62.50 at 95 per cent confidence interval with 100.00 per cent frequency and thus indicating this species as first dominant plant parasitic nematode among other plant parasitic nematodes in the entire Srinagar district. Female body is usually in the form of spiral with lip region resembling a cup with 3-4 indistinct annules. Cephalic framework well developed. Spear 25.01-29.6 µm in length. Median oesophageal bulb oval to round with distinct vulval apparatus. Excretory pore 121-125 µm from anterior end. Vulva a depressed slit, located at 58.6-64.04 % of the body. Ovaries paired, amphidelphic, outstretched. Tail curved dorsally with slight ventral projection, marked by 5-11 annules on the ventral side.

***Pratylenchus* Filipjev, 1936:** *Pratylenchus* sp., the lesion nematode was recorded as second dominant species with mean population in the fruit belt as 52.29 ranging from 38.36-66.22 at 95 per cent confidence interval. The per cent relative frequency and per cent relative prominence value of *Pratylenchus*sp., in the district Baramulla were recorded as 12.32 and 28.69, respectively. The mean population of *Pratylenchus* sp., in district Anantnag was recorded as 69.22 ranging from 59.74-78.70 at 95 per cent confidence interval. The per cent relative frequency and per cent relative prominence value of *Pratylenchus* sp., in the district Anantnag were recorded as 14.21 and 33.21, respectively and ranked as first dominant plant parasitic nematode in the entire district on the basis of population density and frequency of occurrence.In district Srinagar, Pratylenchus sp. emerged as the second important plant parasitic nematodes in terms of absolute density that was recoded as 37.18 (29.33-45.02) with per cent frequency as 100.00 as can be deduced by studyingthe data presented in Table 1 and Fig.1.

Female body almost straight anteriorly and slightly curved ventrally in posterior half when fixed. Lip region with two annules, offset by slight constriction. Cephalic framework well developed, strongly sclerotized, cup shaped. Stylet well developed with rounded basal knobs. Excretory pore 110- 115µm from anterior end. Lateral field with 4 incisures. Oesophagus is typically paratylenchoid type, oesophageal lobe overlapping the intestine ventrally for about two to two and half times widths long. Vulva located posteriorly 77-83% from anterior end. Ovary single, prodelphic, stretched anteriorly and oocytes are arranged in single file. Spermatheca broadly oval to nearly rounded, almost filled with sperms. Tail 27-40µm long with 18-23 annules. Shape of tail terminus smooth, broadly rounded to truncate annulate.

Other plant parasitic nematodes of importance isolated and identified during the study include*Tylenchus* sp. with a mean absolute density of 26.43 and *Tylechorhynchus* sp. with a mean absolute density of 26.37. Remaining less plant parasitic nematodes identified were, *Xiphinema index*, *Xiphinema americanum*, *Longidorus elongatus* and *Hoplolaimus* sp. with the respective mean absolute densities of 15.27, 11.82, 9.25 and 8.17.

A large number of plant parasitic nematodes feed on the roots of fruit trees and other plants causing direct damage while many species of different plant parasitic nematodes have also been found responsible for transmission of serious viral diseases from one plant to another in various countries. These virus vector nematodes cause damage to many economically important crops both by direct feeding on the roots (Christie and Perry, 1951 and Bernard *et al*., 2017) and by vectoring plant diseases (Hewitt *et al*., 1958 and Singh *et al*., 2020).

In the present study nine plant parasitic nematodes *viz*. *Helicotylenchus* sp., *Pratylenchus* sp., *Tylenchus* sp., *Tylenchorhynchus* sp., *Xiphinema insine*, *X.index*, *X.americanum*, *Longidorus elongatus* and *Hoplolaimus* sp. were isolated from the rhizosphere of high density apple orchards in all the three districts (Baramulla, Anantnag and Srinagar). Among the major nematodes found during study the *Helicotylenchus* sp. was having the maximum number followed by *Pratylenchus* sp., *Tylenchus* sp., *Tylenchorhynchus* sp. Similar results were obtained by Sharma and Kaur, 1985. Consensus in the data can also be drawn from a recent study conducted in high density citrus orchards where *Helicotylenchus, Pratylenchus, Tylenchulus* and *Tylenchus* were found to be the most abundant genera (Lavhe *et al*., 2024). Much the same way similar results were also obtained by Lone *et al*. (2018). The identity of major nematodes revealed in our study are in close conformity with the results of (Hoestra 1967, McElory, 1972 and Kiryanova and Krall 1980). The nematode genera *Helicotylenchus*, *Pratylenchus*, *Tylenchorhynchus* and *Hoplolaimus* as recovered from the high density apple orchards of Kashmir valley (Baramulla, Anantnag and Srinagar) in the present study have also been isolated from the apple orchards in different parts of the world (Kaul, 1985; Wallace and MacDonald, 1979; Lan, 1993; Khan *et al.,* 1997 and Lone *et al*., 2012). Maqbool & Quasim (1988) and Tan & Kilic (2012) also confirmed that high frequency of *Pratylenchus* and *Helicotylenchus* species occurs in apple orchards of different parts of the world. Forge *et al.* (2021) found similar results while investigating the nematode population of apple orchards amongst various other fruit orchards investigated. However, the *Hoplolaimus* nematode was found only in orchards of Baramulla district of Kashmir valley. The results of our studies are also supported by the findings of (Khan and Sharma, 1990).

CONCLUSION

Apple industry being the backbone of agrarian economy, which Kashmir valley largely is, is the chief crop cultivated in monoculture for decades altogether. Consequentially that will lead to the development of negative plant–soil feedbacks leading to yield decline of crops. With high density plantation overtaking most of the traditional apple orchards in the valley, it is becoming appallingly important to study the effect of large populations of different plant parasitic nematodes, associated with the dwarfing and semi-dwarfing apple rootstocks, that has already been proven with this study. The role of plant parasitic nematodes in severe diseases like apple decline and apple replant disease need to be ascertained in high density apple plantations and appropriate mitigation strategies can then be devised.

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