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Population Fluctuation of Plant Parasitic Nematodes Associated With Grapevine under Hi-Tech and Low-Tech Polyhouse Conditions

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A B S T R A C T

Soil samples were collected from the rhizosphere of two months old grapevine grown in hi-tech and low-tech polyhouse. Forty samples each from hi-tech and low-tech polyhouse were withdrawn at four intervals i.e. May, June, July and August for two consecutive years 2016 and 2017 and processed in the laboratory for the determination of nematodes. Among the five nematode genera identified, four were plant parasitic *viz.*, lesion nematode, *Pratylenchus* spp., stunt nematode, *Tylenchorhynchus* spp, reniform nematode, *Rotylenchulus* spp. and *Tylenchus* spp. The free living nematode identified was *Rhabditis* spp. Nematode population was maximum in low-tech as compared to hi-tech polyhouse nursery. The population of all the nematodes increased gradually in the soil with the passage of time i.e. from May to August. Among the plant parasitic nematodes the population of *Tylenchorhynchus* spp. was maximum followed by *Pratylenchus* spp., *Tylenchus* spp. and *Rotylenchulus* spp. both in low-tech and hi-tech polyhouse. In hi-tech polyhouse the population of *Tylenchorhynchus* spp. was maximum i.e. 84 and 87 in August, 2016 and 2017, respectively whereas it was minimum for *Rotylenchulus* spp. i.e. 28 and 35 in August, 2016 and 2017, respectively. Similar was the case in low-tech polyhouse where also the population of *Tylenchorhynchus* spp. was maximum i.e. 148 and 144 and population of *Rotylenchulus* spp. was minimum i.e. 49 and 64 in August, 2016 and 2017, respectively. Because of the high population of some plant parasitic nematodes encountered around the rhizosphere of grapevines, it is assumed that the plant parasitic nematodes form an important component of soil ecosystem and may be a contributing factor in declining health of these plants.

Keywords

Grapevine, Nematode, Polyhouse, Hi-tech, Low-tech, Rhizosphere

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Introduction

Grapes (*Vitis* spp.) are considered a major commercial venture throughout the temperate region because of higher remuneration per unit area and the realization that grape consumption is essential for human health and nutrition. It is native to Asia Minor and the Caucasus region, was distributed throughout Europe, and is now extensively grown in the Mediterranean Basin, the subtropical regions of Australia, Southern Africa, and North and

South America (Brown *et al.,* 1993). During the year 2005-06, the highest percentage of world grape production was shared by Italy (12.6%), followed by USA (10.5%), France (10.0%) and China (9.7%), however India ranked first in productivity (25.4 ton/ ha), followed by USA (18.7 ton/ ha) (Kumar, 2007).

Plant parasitic nematodes are reported to be one of the major biotic constraints to grape production. These nematodes are serious concern for viticulturists as they divert nutrients from normal vine growth to supply their own needs, which in turn results in lack of vine vigour and reduced crop yield. The annual losses in the yield of grapes due to plant parasitic nematodes have been estimated 12.5% (Sasser and Freckman, 1987). Not much information is available on the distribution of plant parasitic nematodes around the rhizosphere of grape in Kashmir valley. Hence, in the present study an attempt has been made to determine the types and densities of nematodes associated with grape cultivation in SKUAST-Kashmir, Srinagar.

Materials and Methods

Collection of soil samples

Soil samples were collected from two months old grape (*Vitis vinifera*) plants grown in hi-tech and low-tech polyhouse at SKUAST-Kashmir, Shalimar campus, Srinagar. The two polyhouses were at a distance of 50 cm from each other. Soil samples were collected on monthly basis at four different intervals i.e. May-June-July-August for two consecutive years i.e. 2016 and 2017. There were 10 microplots in each polyhouse. The size of each plot was 4 x 3m and they were prepared with 0.5 m wide and 0.25 m high bund (margins). A total 80 soil samples were collected from the rhizosphere of grape plants, of which 40 from hi-tech polyhouse which was managed and necessary inputs such as fertilizers, organic manures, weeding, irrigation were given in time and another 40 from low-tech polyhouse which was unmanaged and no necessary inputs or care was taken in this polyhouse. The samples were collected at a depth of 15-20 cm from the above soil surface, randomly from three places in a microplot and a composite sample was prepared, kept in a poly bag, tagged and brought in the laboratory for isolation of nematodes.

Isolation of nematodes

Population of nematodes was determined by using Cobb's decanting and sieving method (modified) followed by Baermann funnel technique (Southey, 1986). 500 ml soil of each sample was sifted through a coarse sieve and processed separately. The soil sample (500 g) was mixed in 5 litres of water in a plastic bucket.

The soil-water mixture was stirred and then allowed to stand for 1-2 minutes. The suspension was decanted over a combination of 3 sieves (60, 200 and 500 mesh), the catch from the finest sieve was carefully washed and transferred to a beaker.

A small coarse sieve with two layers of wet paper towels was kept in a Baermann funnel filled with water. The nematode suspension from the beaker was gently poured onto the sieve and allowed to stand overnight. The nematodes because of the random and continuous movement migrate through the paper pores into the water and gradually settle down in the bottom of rubber tubing of the funnel. The nematode suspension recovered from the Baermann funnel was taken into a beaker. The population of nematode was assessed for each month with the help of Syracuse counting dish. The counting of nematode was performed under stereoscopic microscope.

Identification of nematodes

The nematodes thus collected were killed by hot water (85 ^{0}C), then fixed in TAF (Triethelene Amine Formalin) (Courtney *et al.*, 1955), water in specimens replaced by glycerin using the Seinhorst technique (Seinhorst, 1959), after which they were mounted in pure glycerin.

Permanent slides were prepared and nematodes were identified up to genera level. The study for identification of nematodes and photomicrographs were taken using Olympus CX 21i microscope.

Results and Discussion

Among the five nematode genera identified, four were plant parasitic *viz*., lesion nematode, *Pratylenchus* spp. (Fig. 1), stunt nematode, *Tylenchorhynchus* spp. (Fig. 2), reniform nematode, *Rotylenchulus* spp. (Fig. 3) and *Tylenchus* spp. (Fig. 4 and 5). The free living nematode identified, belonged to the genera *Rhabditis* spp. (Fig. 6). Total nematode population including free living was maximum in low-tech as compared to hi-tech polyhouse nursery.

Among the plant parasitic nematodes the population of *Tylenchorhynchus* spp. was maximum followed by *Pratylenchus* spp., *Tylenchus* spp. and *Rotylenchulus* spp. both in low-tech and hi-tech polyhouse (Table 1). In hi-tech polyhouse the population of *Tylenchorhynchus* spp. was maximum i.e. 84 and 87 in August, 2016 and 2017, respectively whereas it was minimum for *Rotylenchulus* spp. i.e. 28 and 35 in August, 2016 and 2017, respectively. The population of *Tylenchorhynchus* spp. was also maximum in low-tech polyhouse where it was 148 and 144 in August, 2016 and 2017, respectively but the population of *Rotylenchulus* spp. was minimum i.e. 49 and 64 in August, 2016 and 2017, respectively. The population of all the nematodes increased gradually in the soil with the passage of time i.e. from May to August. In hi-tech polyhouse the total nematode population was 446 and 391 in May, 2016 and 2017, respectively whereas it was 814 and 832 in August, 2016 and 2017, respectively. Similar was in case of low-tech polyhouse where the total nematode population was 671 and 659 in May, 2016 and 2017, respectively whereas it was 1022 and 1078 in August, 2016 and 2017, respectively (Table 1).

The low population of nematodes in hi-tech polyhouse may be attributed to maintenance such as proper weeding and application of organic manures in soil whereas the high population of nematodes in low-tech was due to lack of maintenance which provided ample opportunity for nematodes to multiply and increase their population. The above mentioned and other parasitic nematodes have also been recorded as parasites of grapes both in nurseries as well as orchards by several workers (Quader *et al.*, 2001; Téliz *et al.*, 2007; Howland *et al.*, 2014). Because of the high population of some plant parasitic nematodes encountered around the rhizosphere of grapevines, it is assumed that the plant parasitic nematodes may be a contributing factor in declining health of these plants.

Due to lack of information, diversity among the species of plant parasitic nematodes could not be compared, however it may be assumed that diversity varies considerably with habitat, area and the number of individuals (Askary *et al.*, 2013, 2014). The present study on nematode community structure associated with grape cultivation indicates the plant parasitic nematodes form an important component of soil ecosystem. However, further investigation is needed for assessing the role of related ecological parameters in the management of plant parasitic nematodes.

Table.1 Nematodes associated with the rhizosphere of grapevines at different intervals in Hi-tech and Low-tech polyhouses (Average population 500g soil)

Hi-Tech	Population of plant parasitic and other nematodes											
	***Pratylenchus* spp.**		***Tylenchorhynchus* spp.**		***Rotylenchulus* spp.**		***Tylenchus* spp.**		**Saprozoic and others**		**Total**	
	2016	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**
May	42	37	56	70	23	16	35	28	290	240	446	391
June	60	66	73	79	25	19	40	41	430	418	628	623
July	76	70	80	80	25	29	52	44	510	566	743	789
August	76	70	84	87	28	35	56	50	570	590	814	832
Low-Tech												
May	70	80	74	82	36	31	51	58	440	408	671	659
June	85	96	115	110	36	40	65	61	570	580	871	887
July	94	100	120	144	41	50	70	69	685	706	1010	1069
August	102	115	148	144	49	64	73	80	650	675	1022	1078

Each value is mean of ten replicates.

Fig.1 Anterior part of *Pratylenchus* sp. showing cephalic region flattened anteriorly

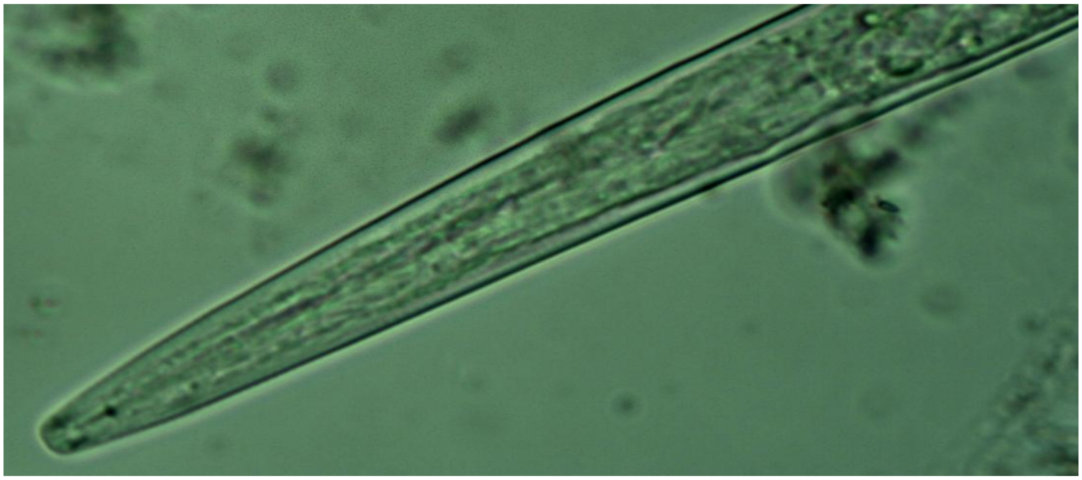

Fig.2 *Tylenchorhynchus* sp. - A full view of adult female

Fig.3 *Rotylenchulus* sp.-A full view of pre-adult female

Fig.4 *Tylenchus* sp.-A full view of adult female with filiform tail

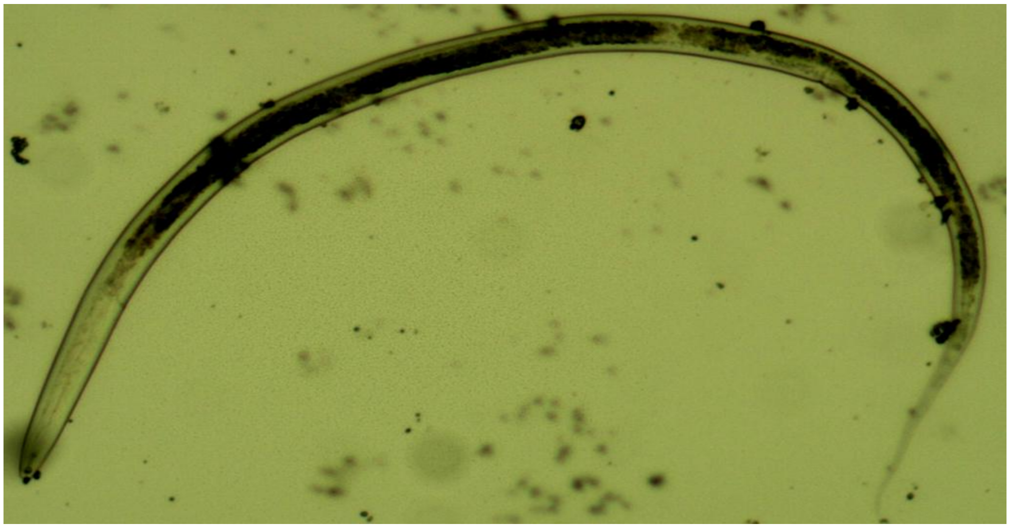

Fig.5 Posterior portion of adult male-gubernaculum and spicule present, bursa adanal (A), Variation in tail of *Tylenchus* sp. - ventrally arcuate (B), pointed (C)

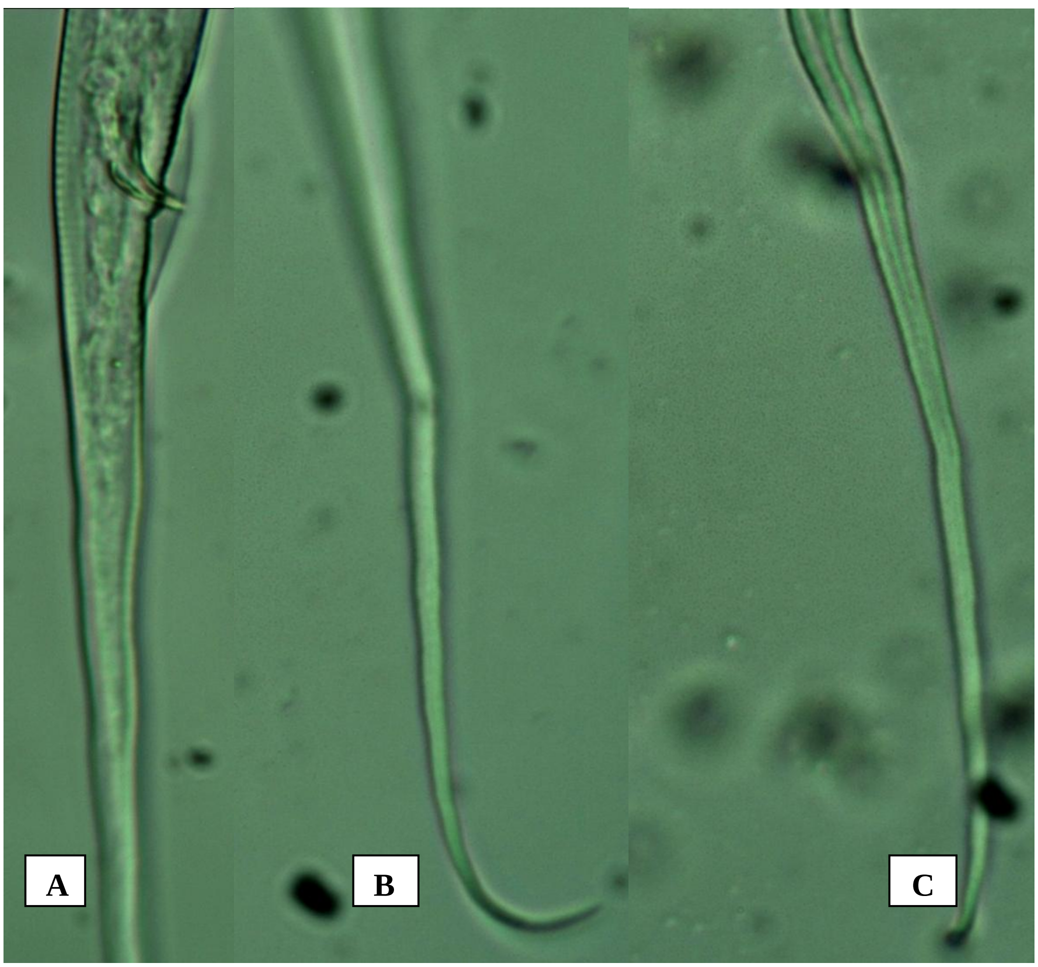

Fig.6 Anterior view of *Rhabditis* sp. with tubular stoma and bulbular oesophagous

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