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Survey of Plant-Parasitic Nematodes Associated With Four Vegetable Crops Cultivated Within Tunnels

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Abstract.- A survey was conducted during 2010-11 to determine the occurrence and population density of plant-parasitic nematodes on four vegetable crops including cumber (*Cucumis sativus*), tomato, (*Lycopersicum esculentum*), chili and bell peppers (*Capsicum frutescens*) cultivated under plastic tunnels. Major vegetable growing regions of Faisalabad, Jhang, Kasur, Lahore, N-Sahab, Sargodh, T.T. Singh, and Sheikhpur were included in our survey. The survey revealed the presence of nine species of plant-parasitic nematodes *viz.*, *Aphelenchus avenae, Helicotylenchus dihystera, Hoplolaimus columbus,, Meloidogyne javanica , M. incognita, Pratylenchus penetrans, Radopholus smilis, Tylenchorhynchus claytoni* and *Xiphinema* sp. Frequency and density of each species were highly variable from field to field and within the fields. Foliage growth was not uniform in the surveyed fields under tunnels where stunted growth, chlorotic leaves and wilted plants with fewer fruits were observed in patches. The stunted and poorly growing plants exhibited arrested root systems with reduced number of feeder roots; root galling and brown lesions were also evident.

Keywords: Plant-parasitic nematodes, *Aphelenchus avenae, Helicotylenchus dihystera, Hoplolaimus columbus,, Meloidogyne javanica , M. incognita, Pratylenchus penetrans, Radopholus smilis, Tylenchorhynchus claytoni* and *Xiphinema* sp

INTRODUCTION

Four vegetable crops including cucumber, *Cucumis sativus*, bell and chili peppers *Capsicum frutescens* and tomato, *Lycopersicum esculentum* are important agricultural commodities gown extensively for fresh market. Average annual yields of these crops in Pakistan are 59.7, 16.3 and 1000.9 tons per hectare, respectively (FAO, 2009). During recent years interest has increased to cultivate these four vegetables in plastic tunnels to take advantage of early market prices. The result has been repeated cultivations within the same tunnels for several years. As more intensive and continuous cultivation increased, soil-borne diseases and nematodes have become an important constraint in vegetable production (Anwar *et al.*, 2007; 1992). Many species of plant-parasitic nematodes are serious pests of fruit and vegetable crops (Anwar and Van Gundy, 1992). Nematode parasitism may result in secondary infection by soil-borne fungal and bacterial pathogens (Abawi and Chen, 1998; Sikora and Carter, 1987) or transmission of plant viruses (Brown *et al.*, 1995), which can negatively influence yield (Orr and Robison, 1984). Yield reductions among vegetables have reached as high as 30% for susceptible genotypes in the presence of plant parasitic nematodes in some production regions in the Punjab (Anwar *et al.*, 2007; Anwar and McKenry, 2012). Vegetable crops usually are among the most susceptible and worst affected by nematodes (Sharma *et al.*, 2006; Anwar *et al.*, 2007; Singh and Khurma, 2007). Infection of roots by nematodes alter uptake of water and nutrients and interferes with the translocation of minerals and photosynthates (Anwar, 1995; Williamson and Hussey, 1996). Such alterations can change the shoot: root ratio (Anwar and Van Gundy, 1989) leading to poor plant growth.

Chemical pesticides, crop rotation, and soil amendments are widely used by the growers to manage the soil-borne pathogens, including plant-parasitic nematodes. Use of nematicides can provide

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instant and effective control of nematodes, but several nematicides have been withdrawn from the market because of health and environmental problems associated with their production and use (Thomason, 1987). In response to increasing public and regulatory concern over pesticide use in our food supply there has been increased interest in the development of alternatives that are effective, sustainable, and environmentally friendly. Alternative methods of nematode management under study include the use of natural enemies and host plant resistance but these alternatives can be highly specific. Crop resistance or predator specifically to some nematode species but not others can be a serious limitation of their effectiveness so it is important to acquire information on the occurrence of nematode species, their host range, their damage thresholds and their pathogenic potentials. Although, recent reports in Pakistan have been published on association, distribution, and density of plant parasitic nematodes on various crops planted in field settings (Anwar and Akhtar, 1992; Anwar *et al*., 2009; Maqbool, 1992), there are no data available for vegetable crops grown within tunnels. Therefore, the objective of this study was to conduct a survey to quantify and document the occurrence, distribution, density and prevalence of nematode populations found associated with cucumber, chili pepper, bell pepper and tomato cultivated in commercial plastic tunnels in the Punjab.

MATERIALS AND METHODS

A survey was conducted in the major vegetable growing regions of Faisalabad, Toba-Tek Singh, Nankana Sahib, Lahore, Kasur, Shekhupura, Sargodha and Jhang of the Punjab (Fig. 1). Sampling of four vegetables including two peppers, tomato and cucumber grown under tunnels was conducted during 2010-11, when the crop was at harvesting stage.

Soil and root samples were collected randomly from each locality. Roots were washed free from soil, and root systems of the plants were stained with Phloxine B (Holbrook *et al*., 1983) and assessed for the presence of egg masses. The root systems were rated for galling and egg mass indices on a 0 to 5 scale (Quesenberry *et al*., 1989), where 0 = no galls or egg masses, 1 = 1 or 2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 > 100 galls or egg masses per root system. Each soil sample was a composite of 8-10 soil cores from the same tunnel to a depth of 18-20-cm with an Oakfield tube of 2.5-cm diameter. Samples were placed in labeled plastic bags, sealed and brought back to the nematology laboratory where they were stored at 4°C until processed for nematode presence.

Each soil sample was thoroughly mixed by shaking the plastic bags then a 100-cm^3 sub-sample was extracted using a combined sieving and Baermann funnel method that allowed nematode extraction in mist over a three-day period (McKenry and Roberts, 1985). In addition, mature females of root knot nematodes were dissected out from the infected roots and perineal patterns were prepared as described by Taylor and Netschler (1974). *Meloidogyne* species were identified on the basis of female perineal patterns, morphological characters of males and second stage juveniles (Eisenback, 1985; Jepson, 1987).

Collected nematodes were killed at 70^{o}C, and fixed in 4% formalin and placed in vials. Prior to counting, solutions containing nematodes were agitated thoroughly then 3-ml poured into a counting dish. Nematode populations were quantified under stereo microscope 60 × magnification. Counting of root-knot nematodes was based on second stage juveniles only. Identification of other species of plant parasitic nematodes was based on the morphology and measurements of adults (Handoo and Golden, 1989; Handoo, 2000; Choi, 2001).

Data analysis

Data on nematodes was subjected to analysis of variance using SAS (SAS Institute, Cary, NC).

RESULTS

Nine species of plant-parasitic nematodes were commonly detected in association with the four vegetables commonly cultivated within Punjab tunnels (Tables I-IV). Frequency and density of the recorded species varied greatly from tunnel to tunnel.

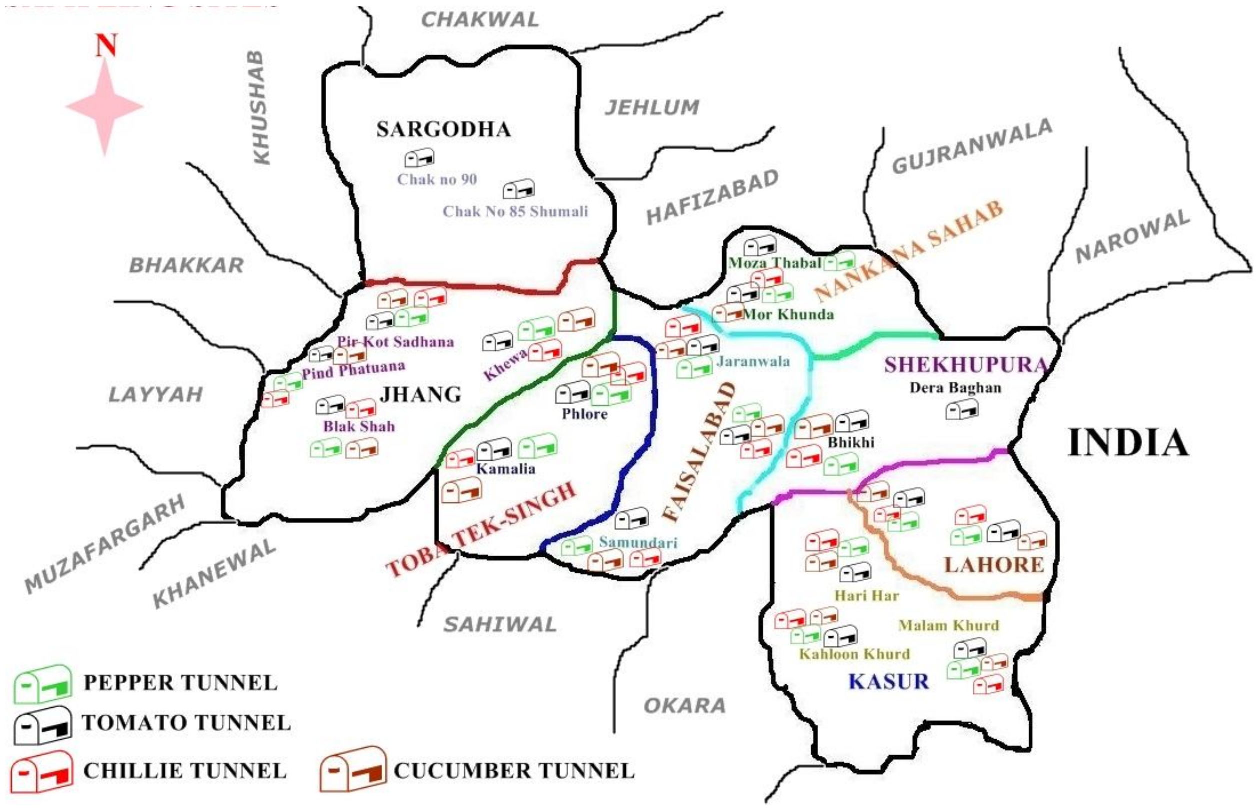

Fig. 1. Map showing the sampling sites of the eight districts of Punjab.

Two species of root knot nematode, *Meloidogyne arenaria* (Neal) Chitwood and *M. incognita* (Kofoid and White) Chitwood were common. These sedentary endoparasites of vascular tissues were recovered from all the four vegetable crops with a frequency and density that was highly variable. These nematodes induced inconsistent numbers of root galls and egg masses on roots (Table V). Galled roots exhibited arrested root systems with few feeder roots. *Meloidogyne* spp., were the predominant species in all surveyed localities. Second on the list were two migratory endoparasites: and cortical feeders including root-lesion nematode, *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans and burrowing nematode *Radopholus similis* Cobb. Five ectoparasitic nematodes (*Aphelenchus avenae* Bastian 1865, *Helicotylenchus dihystera* (Cobb), Sher, *Hoplolaimus columbus* Sher, *Tylenchorhynchus claytoni* Steiner *and Xiphinema* spp. were also recovered from each of the four vegetable crops though not at each of the field localities.

DISCUSSION

During this survey it was observed that the growth of vegetables was highly variable within most of these tunnels. It was common for plants to be severely stunted and chlorotic at latter harvests, a common indicator that nematode damage may be occurring. Arrested root systems, an inadequate number of feeder roots, root galling, and brown lesions were also seen on severely stunted and poorly growing plants.

This study provides quantification of occurrence and density of plant-parasitic nematodes associated with tunnel production of vegetables in the Punjab. Most nematodes identified in this survey can be anticipated to be a major threat to vegetable production and should be considered as serious pests (Anwar *et al.*, 2007; Anwar and Mckenry, 2012). Initial pathogenicity tests should include,

Table I.- Occurrence frequency and density of plant-parasitic nematodes associated with cucumber planted under tunnels.

Nematode species	Faisalabad (50)		Jhang (60)		Kasur (60)		Lahore (30)		N-Sahab (50)		Sargodha (20)		T.T. Singh (35)		Sheikhupur (30)*	
	RF**	MD	RF	MD	RF	MD	RF	MD	RF	MD	RF	MD	RF	MD	RF	MD
Aphelenchus avenae	-	-	25	1750	17	1232	14	740	20	833	18	132	33	1506	12	476
Helicotylenchus dihystera	20	1660	12	1110	50	1720	14	1220	-	-	17	567	17	980	25	1760
Hoplolaimus columbus	20	2000	37	2889	33	2179	28	1778	40	1269	15	58	33	1550	21	1340
Meloidogyne javanica	-	-	12	980	17	1660	28	1420	20	980	17	2011	17	1872	25	1120
Meloidogyne incognita	20	1400	50	1504	47	2001	57	2788	60	1401	18	2800	18	3921	23	1279
Pratylenchus penetrans	-	-	-	-	17	1660	14	1667	20	1167	10	58	-	-	25	1440
Radopholus smilis	40	1108	-	-	17	2000	28	1310	-	-	11	324	17	1660	17	2000
Tylenchorhynchus claytoni	-	-	25	1333.	-	-	-	-	-	-	-	-	-	-	-	-
Xiphinema spp.	20	1750	25	1346	33	913	14	620	-	-	-	-	50	1373	25	913

Localities sampled for nematodes (column groups above).

*Number of soil samples collected.
**RF = Relative frequency of occurrence (percentage of samples in which species was found); MD = Mean density ± standard deviation of nematodes in 500 cm^3 soil.

Table II.- Occurrence frequency and density of plant-parasitic nematodes associated with tomato planted under tunnels.

Nematode species	Faisalabad (60)		Kasur (40)		Lahore (45)		N-Sahab (54)		Sargodha (28)		T.T. Singh (60)		Sheikhupur (17)*	
	RF**	MD	RF	MD	RF	MD	RF	MD	RF	MD	RF	MD	RF	MD
Aphelenchus avenae	-	-	-	-	-	-	-	-	12	1750	25	1222	14	2075
Helicotylenchus dihystera	33	1268	14	1620	-	-	25	1140	25	1480	12	740	-	-
Hoplolaimus columbus	17	1420	28	1850	20	1840	50	1413	25	1416	50	1326	14	1850
Meloidogyne javanica	17	1240	15	780	-	-	12	1220	-	-	12	1870	14	980
Meloidogyne incognita	50	1177	35	2233	40	1180	50	8515	22	500	50	2222	15	1517
Pratylenchus penetrans	17	760	-	-	-	-	-	-	12	1750	25	1760	11	1334
Radopholus smilis	-	-	14	1660	20	1120	25	583	13	1600	-	-	12	2490
Tylenchorhynchus claytoni	33	1317	15	1139	20	1654	-	-	25	967	-	-	14	1660
Xiphinema spp.	`17	1110	16	1480	-	-	25	874	25	1056	-	-	13	2467

Localities sampled for nematodes (column groups above).

*Number of soil samples collected.
**RF = Relative frequency of occurrence (percentage of samples in which species was found); MD = Mean density ± standard deviation of nematodes in 500 cm^3 soil.

Table III.- Occurrence frequency and density of plant-parasitic nematodes associated with chili peppers planted under tunnels.

Nematode species	Localities sampled for nematodes													
	Faisalabad (21)		Jhang (25)		Kasur (18)		Lahore (24)		N-Sahab (26)		T.T. Sing (23)		Sheikhupur (17)*	
	RF**	MD	RF	MD	RF	MD	RF	MD	RF	MD	RF	MD	RF	MD
Aphelenchus avenae	14	1120	20	1668	-	-	15	1500	-	-	17	1114	20	560
Helicotylenchus dihystera	18	940	14	1820	12	1484	12	1254	15	1120	15	980	20	1460
Hoplolaimus columbus	18	1725	10	1445	-	-	-	-	15	1307	13	1715	10	870
Meloidogyne javanica	-	-	20	780	14	1280	12	1140	-	-	14	1660	20	1020
Meloidogyne incognita	20	1250	22	1542	15	3735	20	3117	22	1750	20	2165	28	2150
Pratylenchus penetrans	13	1273	16	1640	13	1660	15	867	14	1924	12	1334	20	1420
Radopholus smilis	15	1470	20	667	15	2282	-	-	16	1552	18	1889	14	1660
Tylenchorhynchus claytoni	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Xiphinema spp.	-	-	20	500	-	-	13	750	19	760	14	580	-	-

*Number of soil samples collected.
**RF = Relative frequency of occurrence (percentage of samples in which species was found); MD = Mean density ± standard deviation of nematodes in 500 cm^3 soil.

Table IV.- Occurrence frequency and density of plant-parasitic nematodes associated with bell peppers planted under tunnels.

Nematode species	Localities sampled for nematodes													
	Faisalabad (20)		Jhang (18)		Kasur (20)		Lahore (26)		N-Sahab (20)		T.T. Sing (16)		Sheikhupur (26)*	
	RF**	MD	RF	MD	RF	MD	RF	MD	RF	MD	RF	MD	RF	MD
Aphelenchus avenae	-	-	15	1842	-	-	14	930	12	1660	-	-	17	660
Helicotylenchus dihystera	12	740	13	1672	19	960	17	1270	-	-	12	19	-	-
Hoplolaimus columbus	14	1450	15	1016	14	1660	17	1684	15	1880	-	-	23	1530
Meloidogyne javanica	-	-	12	1470	14	770	-	-	13	920	11	17	17	880
Meloidogyne incognita	20	1260	15	2250	18	2490	19	1420	12	750	15	36.3	13	1460
Pratylenchus penetrans	20	780	-	1637	14	4980	12	880	12	1120	12	1167	14	1080
Radopholus smilis	10	1590	8	1055	14	830	13	840	10	1855	14	1828	18	1246
Tylenchorhynchus claytoni	-	-	-	-	-	-	-	-	-	-	-	22	-	-
Xiphinema spp.	-	-	12	583	14	830	-	-	15	2250	-	-	16	1110

*Number of soil samples collected.
**RF = Relative frequency of occurrence (percentage of samples in which species was found); MD = Mean density ± standard deviation of nematodes in 500 cm^3 soil.

Table V.- Host response of four vegetables to *Meloidogyne* spp. in terms of root galling and egg masses

Test crops	Number of root galling (G), gall index (I), egg masses (EM) and egg mass index (I) per root system*															
	G (I)	EM(I)	G (I)	EM(I)	G (I)	EM(I)	G (I)	EM(I)	G (I)	EM(I)	G (I)	EM(I)	G (I)	EM(I)	G (I)	EM(I)
Cumber	74 (4)	20 (3)	33 (4)	42 (4)	37(4)	32 (4)	44 (4)	37 (4)	24 (3)	29 (4)	-	-**	87 (4)	74 (4)	51 (4)	42 (4)
Tomato	52 (4)	11 (3)	-	-	65(4)	50 (4)	57 (3)	44 (4)	38 (4)	26 (3)	47 (4)	33 (4)	37 (4)	24 (3)	21 (3)	12 (3)
Chili	51 (4)	16 (3)	46 (4)	31 (4)	24(3)	18 (3)	20 (3)	15 (3)	22 (3)	19 (3)	-	-	38 (4)	31 (4)	29 (3)	27 (3)
Pepper	33 (4)	12 (3)	29 (3)	24 (3)	16(3)	11 (3)	19 (3)	14 (3)	11 (3)	7 (2)	-	-	18 (3)	23 (3)	14 (3)	6 (2)

* Gall and egg mass indices: 0-5 scale; where 0 = no galls or egg masses, 1 = 1-2 galls or egg masses; 2 = 3-10 galls or egg masses; 3 = 11-30 galls or egg masses; 4 = 31-100 galls or egg masses, and 5 = > 100 galls or egg masses per root system (Quesenberry *et al.*, 1989).
**= Plants were not sampled as there was no tunnel.

M. incognita, and *M. arenaria, P. penetrans* and *R. similis* which taken alone can be of serious economic importance in the tropics and sub tropics (Anwar *et al.*, 2007; Davide, 1988; Maqool *et al.*, 1988).

Meloidogyne spp. are common in vegetable soils world-wide where they parasitize vascular root tissues and induce their familiar root galls. Root-knot nematode, *M. incognita*, is among the most common (Anwar and Mckenry, 2010; Abawi and Widmer, 2000, Davis *et al*., 2003; Sasser, 1979; Barker and Olthof, 1976). In addition to extensive root galling leading to arrested root systems and its presence is often been associated with increased incidence and severity of *Fusarium* wilts of several field crops (Anwar and Khan, 1973; Martin *et al*., 1994). The result is reduced yield of vegetable crops due to nematode feeding that can range up to more than 40% (Anwar and Mckenry, 2012), depending on soil texture and prevailing weather conditions (Starr, 1993).

Root lesion nematode, *P. penetrans* and the burrowing nematode, *R. similis* are migratory endoparasites of roots feeding among cortical tissues. Their infections can result in necrotic brown lesions and tunneling within rootlets. This can interfere with water and nutrient movement within plant tissues as well as increased leakage of harvested, stored food due to lesions (Dorhout *et al*., 1991). *Pratylenchus penetrans* is known to enhance the severity of *Verticillium* wilt of vegetables (Vrain, 1987). Presence of these serious plant parasitic nematodes in abundance on vegetables produced within warmed tunnels should be taken seriously by growers. The association of these nematodes with vegetable crops has been reported to limit yields worldwide and there are now reports of yield losses from Punjab, Pakistan (Anwar and McKenry, 2012), India (Sehgal and Gaur, 1999), and USA (McSorley *et al*., 1987).

Other nematodes species identified during this study included *A. avenae, H. dihystera, H. columbus, T. claytoni*, and *Xiphinema* spp. These are ectoparasites of epidermal root tissues and have not been documented as dangerous pests of vegetables. Their feeding leads to pruning of root hairs and damage to epidermal tissues, which can reduce the ability of roots to absorb water and nutrients from soil leading to poor foliage growth (Endo, 1975). The occurrence of these ectoparasitic nematodes genera has frequently been found in commercially grown vegetable crops (Anwar and McKenry, 2010; Anwar and Akhtar, 1992 Maqbool, 1992; Barker *et al*., 1998). Species of *Xiphinema* in addition to the direct root damage caused by their feeding also are known to transmit viral diseases like tomato ringspot nepovirus (TomRSV), tobacco ringspot nepovirus (TobRSV) that infects tomato, tobacco, and soybean and has an economically important impact on cucurbits (Fulton, 1962; Imle and Samson, 1937; Brown *et al*., 1993, 1995). As virus vectors they can be damaging at very low population levels. Least damaging of the listed nematodes to vegetables is *A. avenae*, a nematode that derives its food from fungi and bacteria and is more associated with damage to mushroom culturing (Khanna and Kumar, 2005).

Variations in occurrence frequency and density of each nematode species surveyed from these vegetable crops appears to be influenced by cropping pattern. It is reported that plant-parasitic nematodes in cultivated soil may be affected by the planting of cover crops, the use of alternate crop sequences, soil types and length of fallow (Brodie and Murphy, 1975; Brodie *et al*., 1970).

The results of this study indicate that plant-parasitic nematodes are widely distributed on vegetable crops cultivated under tunnels in the Punjab. This information on nematode occurrence on vegetable crops will be helpful for growers for planning and administering nematode management strategies to reduce the nematode populations below their threshold levels. This study also demonstrated that the presence of many economically important plant-parasitic nematodes are associated with Punjab vegetable plantings but particularly those within tunnels where replanting is commonly on a fast cycle. This study further suggests that magnitude of nematode problem in tunnel planted crops needs serious consideration to tackle by the use of useful nematode management strategies.

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