

Incidence and Population Density of Plant-Parasitic Nematodes Infecting Vegetable Crops and Associated Yield Losses in Punjab, Pakistan

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Abstract. Plant-parasitic nematode population densities were determined from 325 root and soil samples collected from vegetable growing areas in Pakistan. Yield losses associated with nematode presence were quantified on 19 vegetable crops during 2007 and 2009. The most abundant plant-parasitic nematodes detected, in order of decreasing frequency of infestation (percentage of samples), were *Meloidogyne incognita* (90%), *Pratylenchus penetrans* (30.2%), *Tylenchorhynchus clarus* (29%), *Hoplolaimus columbus* (15%), *Paratrichodorus minor* (7.5%), *Xiphinema americanum* (7.1%), *M. javanica* (7%), *Belonolaimus longicaudatus* (5.6%), *Longidorus africanus* (5%), and *Helicotylenchus dihystera* (3.2%). We observed ca 23% yield losses ranged from 2% for cabbage to 45% for squash, which is 35%, 80%, and 46% higher compared to developed countries, USA, and India, respectively. The main reason for more losses in Pakistan might be related to incognizant growers about the presence of nematodes and the damage they cause. Another reason might be non-availability of resistant crop cultivars and nematicides. Another possibility is that on small-sized farms with varied cropping histories and inattention to the pest-host-status one result appears to be a larger list of available nematode species, compared to farming practices in USA where only one or two nematode species become dominant. This study provides important information for extension specialists and creates awareness among growers about these hidden crop enemies. It also suggests the need for improved management measures to avoid crop losses.

Key words: Plant-parasitic-nematodes, root-knot nematodes, vascular feeders, vegetables, yield losses.

INTRODUCTION

Vegetables are intensively grown for fresh market in Pakistan. Due to mild winters and warm summers the planting sites may be double or triple cropped each year. Root-knot nematodes, *Meloidogyne* spp. have become a major pest of these crops, impacting both quantity and quality of marketable yields in the Punjab region (Anwar *et al.*, 2007; Anwar and McKenry, 2010). The two most commonly pathogenic root-knot nematode species in Punjab are *M. incognita* and *M. javanica*. These nematodes each attack numerous host plants including vegetables, fruits, field crops, ornamentals and common weeds (Abawi and Chen, 1998; Anwar, 1989; Anwar *et al.*, 2009). Either alone or in combination with other pathogenic organisms, root-knot nematodes are a major constraint to the effective production of vegetable crops in Pakistan.

Root-knot nematodes are obligate, sedentary parasites of vascular tissues of plant roots. Infected plants usually exhibit root galling. Additional symptoms induced by nematode feeding include reduction in plant vigor, and root lesions, rotting and deformations. The plants with nematode damaged roots exhibit reduced root systems with fewer feeder roots (Anwar and Javid, 2010). Nematode root damage reduces the plants ability to extract available soil water and nutrients, the result being lack of vigor and yield loss (Trudgill, 1992).

Plant-parasitic nematodes cause estimated annual crop losses of \$8 billion in the United States and \$78 billion worldwide (Barker *et al.*, 1998). Damage caused by plant-parasitic nematodes on 24 vegetable crops in the USA was estimated to be 11% (Feldmesser *et al.*, 1971). Specific estimates of vegetable crop losses due to *M. incognita* and *M. javanica* have ranged from 17 to 20% for eggplant, *Solanum melongena*, 18 to 33% for melon, *Cucumis melo*, 24 to 38% for tomato, *Lycopersicon esculentum*, and 25% for potatoes, *S. tuberosum* (Kathy, 2000).

Eight genera of plant-parasitic nematodes

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other than root-knot have also been reported associated with vegetable crops, including *Belonolaimus* spp., *Criconea* spp., *Hoplolaimus* spp., *Helicotylenchus* spp., *Pratylenchus* spp., *Paratrichodorus* spp., *Tylenchus* spp., and *Tylenchorhynchus* spp. (Anwar and VanGundy, 1989; Maqbool *et al.*, 1988).

During a preliminary survey, several vegetable crops including bitter melon, cabbage, carrot, chilies, cowpea, cucumber, eggplant, lettuce, melon, mustard, okra, potato, pumpkin, sponge gourd, squash and tomato were found to be infected with root-knot nematodes and other plant-parasitic nematode species (Anwar *et al.*, 2007). Nevertheless, the information on the losses inflicted by these nematodes on vegetable crops is not available in Pakistan.

The objectives of this study were to (1) determine the identity, frequency, and population density of plant-parasitic nematodes associated with vegetable crops in the vegetable production area of Punjab, Pakistan, and (2) assess losses caused by these plant-parasitic nematodes.

MATERIALS AND METHODS

Assessment of plant-parasitic nematodes in vegetable fields

Nineteen vegetable crops from commercial fields located throughout Punjab were sampled during 2007 to 2009.

Crops included carrot, chilies, coriander, crucifers (cabbage and mustard), cucurbits (bitter melon, cucumber, pumpkin, sponge gourd, melon and watermelon), eggplant, lettuce, okra, pea, potato, spinach, and tomato. The surface 15 to 20 cm of these soils averaged 66.3% to 86.5% sand, 5.5% to 20.3% silt, 10% to 21.5% clay and 0.5% to 2.8% organic matter.

Presence of nematodes in vegetable roots and soil was determined at harvest of each crop. A total of 325 root and soil samples were collected over a two-year period. Ten root and soil cores were randomly collected for each crop by walking in a zigzag pattern across each field with an Oak Field tube of 2.5-cm diameter inserted to a depth of 18-20 cm. Soil cores were combined to represent each sampled field. Samples were placed into plastic

bags, placed in a cooler for transport and then stored at 4°C until processed in the lab. Roots were separated from soil and carefully washed under tap water to remove adhering soil particles and then towel dried. Nematodes were extracted from a fresh root composite sub-sample of 20g by placing them in a mist-chamber for 5 days (McKenry and Roberts, 1985). A composite soil sample of 100-cm³ was mixed with water in a bucket and sieved through #40 over #325 sieves, to capture soil-dwelling nematodes. Sieved soil was washed onto a modified Baermann funnel apparatus and misted for 3-days (McKenry and Roberts, 1985). During this period, nematodes were collected and examined in a counting dish under a stereo-binocular microscope.

Plant-parasitic nematodes were identified to genus and species using a compound microscope. Nematode identifications were based on the morphology of adults and second-stage juveniles (Eisenback, 1985; Handoo, 2000; Handoo and Golden, 1989; Mai *et al.*, 1996; Raski, 1975a,b; Sher, 1966).

Assessment of yield losses

The criteria used to assess yield loss comprised grower interviews, visual assessment based on foliage growth (necrotic, chlorotic, stunted, and wilted plants), root symptoms and educated guess to expert opinions. The number of growers interviewed was variable and ranged from 5-15 for each crop. The interview of growers included condition of the crop, quantitative and qualitative yield losses based on market value and life span of the crop. Estimates reported in this study are expressed as percentage of yield loss.

RESULTS

Nematode survey

Ten genera of plant-parasitic nematodes were found associated with 19 vegetable crops grown in the field throughout the Punjab (Table I). At least one species of plant-parasitic-nematodes was detected in samples analyzed (Table II). Eight species including *B. longicaudatus*, *H. columbus*, *H. dihystra*, *Longidorus africanus*, *M. incognita*, *M. javanica*, *P. minor*, *P. penetrans*, *T. clarus* and *Xiphinema americanum* were found in all the samples; however, their frequency and density was

Table I.- Frequency and population densities of plant parasitic nematodes in soil and roots of vegetable crops in Punjab, Pakistan

Nematode species*	Frequency**	Nematode population densities	
		100 cm ³ of soil	Per g of root
<i>Meloidogyne incognita</i>	90.0	250 ± 75.8	1275 ± 678
<i>Pratylenchus penetrans</i>	30.2	95 ± 13	11 ± 3
<i>Tylenchorhynchus clarus</i>	29.0	95 ± 30	-
<i>Hoplolaimus columbus</i>	15.0	243 ± 24	-
<i>Paratrichodorus minor</i>	7.5	38 ± 7	-
<i>Xiphinema americanum</i>	7.1	12 ± 4	-
<i>Meloidogyne javanica</i>	7.0	201 ± 29	708 ± 180
<i>Belonolaimus longicaudatus</i>	5.6	151 ± 134	-
<i>Longidorus africanus</i>	5.0	8 ± 3	-
<i>Helicotylenchus dihystra</i>	3.2	657 ± 456	-

*Nematode species are listed in decreasing frequency; -, absence of nematodes.

**Frequency, percentage of nematode infested samples.

Table II.- Nematodes associated with vegetable crops and related yield losses in the Punjab.

Name of vegetable crops		Yield loss (%)	Nematodes invading root tissues		
Common	Scientific		Epidermal	Cortical	Vascular
Bitter gourd	<i>Momordica charantia</i>	5	<i>Belonolaimus longicaudatus</i> <i>Paratrichodorus minor</i> <i>Helicotylenchus</i> spp. <i>Hoplolaimus galeatus</i> <i>Tylenchorhynchus</i> spp.		<i>Meloidogyne incognita</i>
Cabbage	<i>Brassica oleracea</i>	2	<i>B. longicaudatus</i> <i>P. minor</i> <i>Helicotylenchus</i> spp. <i>H. columbus</i>	<i>Pratylenchus</i> spp.	<i>M. incognita</i>
Carrot	<i>Daucus carota</i>	25	<i>B. longicaudatus</i> <i>P. minor</i> <i>H. columbus</i> <i>Longidorus africanus</i>	<i>P. penetrans</i>	<i>M. incognita</i> <i>M. javanica</i>
Chilies	<i>Capsicum annuum</i>	20	<i>B. longicaudatus</i> <i>H. indicus</i> <i>Helicotylenchus</i> spp. <i>P. minor</i>		<i>M. incognita</i>
Coriander	<i>Coriandrum sativum</i>	25	<i>H. columbus</i> <i>P. minor</i>	<i>Pratylenchus</i> spp.	<i>M. incognita</i>
Cowpea	<i>Vigna sinensis</i>	20	<i>B. longicaudatus</i> <i>Helicotylenchus</i> spp. <i>P. minor</i> <i>T. clarus</i> <i>Xiphinema americanum</i>		<i>M. incognita</i> <i>M. javanica</i>
Cucumber	<i>Cucumis sativus</i>	25	<i>Belonolaimus</i> spp. <i>Paratylenchus</i> spp. <i>Trichodorus</i> spp.	<i>Pratylenchus</i> spp.	<i>Meloidogyne</i> spp.
Eggplant	<i>Solanum melongena</i>	40	<i>B. longicaudatus</i> <i>Helicotylenchus</i> spp.	<i>P. penetrans</i>	<i>M. incognita</i> <i>M. javanica</i>
Lettuce	<i>Lactuca sativa</i>	10	<i>H. columbus</i> <i>Longidorus africanus</i> <i>P. minor</i>		<i>M. incognita</i> <i>M. javanica</i>
Melon	<i>Cucumis melo</i>	25			
Mustard	<i>Raphanus sativus</i>	5	<i>P. minor</i>	<i>P. penetrans</i>	<i>Meloidogyne</i> spp.

Table II Continued

Name of vegetable crops		Yield loss (%)	Nematodes invading root tissues		
Common	Scientific		Epidermal	Cortical	Vascular
Okra	<i>Hibiscus esculentum</i>	35	<i>B. longicaudatus</i> <i>H. galeatus</i>	<i>Pratylenchus</i> spp.	<i>M. incognita</i> <i>M. javanica</i>
Potato	<i>Solanum tuberosum</i>	30	<i>Helicotylenchus</i> spp. <i>P. minor</i> <i>Tylenchorhynchus</i> spp.	<i>P. penetrans</i>	<i>M. incognita</i> <i>M. javanica</i>
Pumpkin	<i>Cucurbita argyrosperma</i>	27	<i>B. longicaudatus</i> <i>P. minor</i> <i>Helicotylenchus</i> spp. <i>H. galeatus</i> <i>Tylenchorhynchus</i> spp.		<i>M. incognita</i> <i>M. javanica</i>
spinach	<i>Spinacea oleracea</i>	25	<i>Helicotylenchus</i> spp.	<i>P. penetrans</i>	<i>M. incognita</i> <i>M. javanica</i>
Sponge gourd	<i>Luffa cylindrica</i>	15	<i>B. longicaudatus</i> <i>P. minor</i> <i>Helicotylenchus</i> spp. <i>H. galeatus</i> <i>Tylenchorhynchus</i> spp.		<i>M. incognita</i>
Squash	<i>Cucurbita pepo</i>	45	<i>B. longicaudatus</i> <i>P. minor</i> <i>Helicotylenchus</i> spp. <i>Hoplolaimus. galeatus</i> <i>Tylenchorhynchus</i> spp.		<i>M. incognita</i> <i>M. javanica</i>
Tomato	<i>Lycopersicum esculentum</i>	40	<i>B. longicaudatus</i> <i>P. minor</i> <i>Helicotylenchus</i> spp. <i>H. galeatus</i> <i>Tylenchorhynchus</i> spp.		<i>M. incognita</i> <i>M. javanica</i>
Watermelon	<i>Citrullus lanatus</i>	12	<i>B. longicaudatus</i> <i>P. minor</i> <i>Helicotylenchus</i> spp. <i>H. galeatus</i> <i>Tylenchorhynchus</i> spp.		<i>M. incognita</i>
Percent losses on vegetables=		23			

highly variable from field to field and within field. Frequency of these nematode species ranged from 90 to 3.2%. The two potentially damaging nematode species to vegetables, *M. incognita*, and *M. javanica* were present in 90% and 7% of the samples, respectively (Table I).

Root-knot nematode was found in roots and soil of all the vegetable crops. These nematodes induced severe root galling of variable size and numbers on roots of vegetables, such roots had arrested root systems with few feeder roots (personal visual observations). The most common nematode species found in this study was *M. incognita* and *P. penetrans*, which were found at the frequency of 90% and 30.2%, respectively. Average population density of *M. incognita* J2 was 250 J2 per 100-cm³ and 1275 per g of soil and root, respectively, whereas the number of *P. penetrans* found in the root and soil were 11 per g and 95 per

cm³, respectively (Table I).

The migratory ectoparasitic nematodes including sting nematode, *B. longicaudatus*, spiral nematode, *H. dihystra.*, lance nematode, *H. columbus*, needle nematode, *L. africanus*, stubby root nematode, *P. minor*, stunt nematode, *T. clarus* and dagger nematode, *X. americanum* have been found to be damaging nematode pests of many vegetable crops as they cause destruction of epidermis during feeding. (Cooke, 1989; McKenry *et al.*, 2001)

Assessment of harvested yield losses

Based on our visual field observations and grower responses the average percentage of production losses due to nematode was 23% for 19 vegetable crops throughout Punjab, which ranged from 2% for cabbage to 45% for squash (Table II). Each of the eleven commercially important

vegetable crops planted in the Punjab suffered greater losses due to nematode damage individually as well as on an average (25.6%) of all eleven compared to that of planted in USA (6.63%) (Table III).

Table III.- Comparison of production losses (%) due to nematodes on eleven vegetable crops

Vegetable crops	USA*	Pakistan
Cabbage	0.05	2
Carrot	15.0	25
Cucumber	6.5	25
Eggplant	3.5	40
Lettuce	10.0	10
Okra	5.2	35
Potato	12.0	30
Pumpkin	5.2	27
Squash	3.5	45
Tomato [Fresh market]	2.0	40
Watermelon	10.0	12
Average percent losses	6.6	26

*McSorley *et al.* (1987)

DISCUSSION

Plant-parasitic-nematodes found associated with vegetable crops can be classified as epidermal, cortical, and vascular feeders. Epidermal feeders include *B. longicaudatus*, *H. dihystra*, *H. columbus*, *L. africanus*, *P. minor*, *T. clarus* and *X. americanum*. These nematode species feed at or close to root tips where they arrest root elongation and disrupt the site of plant growth factors. Root hairs, a single cell extension of the epidermis, enlarge plant surface area and improve efficiency in absorbing water while also providing sites for *Rhizobium* invasion in legumes. Invasion by ectoparasitic nematodes reduces the ability of the epidermis to absorb water by pruning root hairs, by reducing rooting depth and numbers of branched roots (Anwar and Van Gundy, 1989, Endo, 1975). Cortical feeders including *H. columbus*, *H. galeatus* and *P. penetrans*, are migratory endoparasites. Feeding activities result in cellular necrosis which interferes with radial transport of water and solutes and leakage of stored photosynthetic products (Carneiro *et al.*, 2002). Vascular feeders in these studies included two species of root-knot nematode,

M. incognita and *M. javanica*, which become sedentary endoparasites. This group of nematodes damages their hosts by redirecting large amounts of energy and nutrients from normal activities into development of the nematodes and their special feeding sites (Anwar, 1995; McClure, 1977). The altered tissues at feeding sites also disrupt the vascular system hampering the upward transport of water and dissolved nutrients by xylem and translocation of photosynthates to other regions of the plant by phloem (Hajera *et al.*, 2009). Roots severely galled by root-knot nematodes can predispose plants to root rots leading to a shorter life span of the crop. These galled tissues become succulent, poorly protected from invasion, and rich in nutrients. The result is a nutrient-rich food source which fungi can rapidly colonize (Abawi and Chen, 1998). Root-knot nematode damage results in poor growth, decline in quality and yield of the crop and reduced resistance to other stresses like drought and disease. *Meloidogyne incognita* has often been reported as a damaging nematode pest of vegetable crops including recent work by these authors (Anwar and McKenry, 2010). A high level of root-knot nematode damage can lead to total crop loss. Nematode damaged roots do not utilize water or fertilizers as effectively, leading to additional losses for the grower (Trudgill and Phillips, 1997).

In smaller commercial and backyard settings of the Punjab plants are often invaded by several different nematode genera and simultaneously cause damage at all three regions of a root: the epidermis, cortex and vessels. Such plants exhibit retarded growth, chlorotic leaves, delayed flower and fruit formation, susceptibility to fungal/bacterial/viral attack plus significant growth and yield reductions (Trudgill, 1992). They essentially exhibit all the symptoms of plants attacked by root-knot species but sporadically across the field also exhibit damage associated with the presence of other nematode species. The best example of multiple pest damage is root-knot nematode, *Meloidogyne* spp. and *Fusarium* wilt disease of tomato (Abawi and Chen, 1998).

However, when two or more nematodes attack a plant, the interaction may also be synergistic where the combined effects of the nematodes are greater than the sum of the effects of

each nematode acting alone. Multiple nematode associations appear to be causing synergistic increases in yield loss. Nematode damage experienced within relatively small-sized farms on very different soils following various cropping histories could be a major source of the greater nematode damage in the Punjab.

Reliable crop loss estimates are important for establishing research, extension and budget priorities (Dunn, 1984). Yield reductions are usually proven with studies involving nematicides, soil fumigation, or resistant and susceptible crop varieties. Unfortunately effective nematicides, soil fumigation, and data regarding resistant and susceptible crop varieties to nematodes are not available in Pakistan (senior author observations). Under such situations we have assessed losses caused by plant parasitic nematodes on our visual field observations, grower response's, educated guesses, expert opinions and population level of nematode infestation.

Crop losses due to plant parasitic nematodes are estimated to be about 12.3% in developed nations and 14.6% in the developing countries (Sasser and Freckman, 1987). However, these early estimates of nematode damage to vegetables appear underestimated when evaluating vegetable crops in third world countries. Data from the US indicate 4.5% damage for 28 vegetable crops (McSorley *et al.*, 1987) but the estimates are 12% in India (Sehgal and Gaur, 1999). We observed ca 23% loss on 19 vegetable crops commercially grown in the Punjab. These are 80% and 46% higher compared to that of USA, and India, respectively. Each of the eleven commercially important vegetable crops planted in the Punjab suffered greater losses due to nematode damage individually as well as on an average (25.6%) of all eleven compared to that of planted in USA (6.63%) In vegetable production areas of developing nations like India the experience is 43% (Bhatti and Jain, 1977; Reddy, 1980, 1985) or in Sudan the estimate is 70% (Yasin, 1974) yield loss in the tomato crop due to root knot nematode.

The present study provides important information to extension specialists, which can be used to create awareness among growers It should alert the plant scientists to consider nematodes major damaging pests of crops and start searching

for resistant cultivars as an option in plant-parasitic nematode management.

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