RESEARCH PAPER

Survey and characterization of nematode populations affecting onion and spinach crops in Karachi, Pakistan

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Key Message: This study conducted a comprehensive survey of nematode populations affecting onion and spinach crops in Karachi, Pakistan, identifying ten different genera of plant parasitic nematodes, including previously unreported species. The findings emphasize the critical need for effective management strategies to mitigate nematode-induced yield losses and ensure sustainable agricultural practices in the region.

Abstract

Vegetables are an important source of micronutrients, which can help malnourished people throughout the world and improve their health. In many countries, spinach and Onion are popular vegetables that help to overcome micronutrient deficiencies. Current technology and advances have greatly aided agricultural productivity, while most of the producers have overlooked several critical aspects that might help to reduce yield losses. Particularly, root-knot (Meloidogyne spp.) and cyst (Heterodera spp.) nematodes are thought to be among the most significant plant pests on a variety of crop plants. They have a wide host range, a brief life cycle and a rapid rate of multiplication. This research study conducted an extensive survey of nematode populations affecting onion and spinach crops in Karachi, Pakistan. Soil samples were collected from farmers' fields and the UBIT field at the University of Karachi. The survey identified a total of 10 different genera of plant parasitic nematodes including *Longidorus elongatus*, *Hemicriconemoides communis*, *Rotylenchulus reniformis*, *Hoplolaimus columbus*, *Bitylenchus brevilineatus*, *Aphelenchus avenae*, *Pratylenchus coffeae*, Seinura spp., Filenchus spp., and Helicotylenchus spp., along with free-living soil nematodes. Notably, some genera, such as *Longidorus elongatus*, *Bitylenchus brevilineatus*, *Rotylenchulus reniformis*, *Hemicriconemoides communis*, and Seinura spp., were reported for the first time in onion and spinach crops in Pakistan. The study also provided systematic information, morphometric data, and illustrations of these genera. The findings highlight the importance of understanding and managing nematode populations in onion and spinach crops to ensure sustainable agricultural practices in Karachi, Pakistan. © 2022 The Author(s)

Keywords: Diseases, Nematodes, Onion, Plant parasitic nematodes, Spinach

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Introduction

In Pakistan, vegetables are cultivated continuously for the fresh market. The location of planting can be doubled or tripled due to warm summers and mild winters. Meloidogyne spp. is a serious parasite in many plants affecting market production both in quality and quantity (Nicol et al., 2011). *M. incognita* and *M. javanica* are types of two most prevalent root knot pathogenic nematode species. These nematodes target a variety of crops including fruits and vegetables (Anwar, 1989; Anwar et al., 2009). Root-knot nematodes, either alone or in combination with other pathogens, significantly limit vegetable crop yield in Pakistan (Khan et al., 2023). The indications of nematode feeding include rotting, deformities, lesions in the roots, and a decrease in plant vitality (Enyiukwu et al., 2021). Crops have a small system of fruit and few root feeders which are damaged by root nematodes (Anwar & Javid, 2010). Plant vigor and yield loss are caused by damage of root nematodes which decrease the crop capacity for take up of present water and nutrition in soil (Trudgill, 1992). Losses of crops due to parasite plant nematodes are estimated to be seventy-eight billion dollars globally per year (Barker et al., 1998). It was estimated that plant-parasitic nematodes caused 11% of the damage to 24 different vegetable crops of US (Feldmesser et al., 1971). Vegetable plants are linked to density, population and frequency of parasite plant nematode. The majority of nematode species are thought to be advantageous. They consume fungus, bacteria, and other aquatic or soil-dwelling creatures for food. While some are classified as omnivores and may eat a variety of foods, others are more selective about the kinds of meals they consume. Certain nematode species parasitize both plants and animals (Maule & Curtis, 2011).

Three traits are shared by plant-parasitic nematodes. Initially, all of them are little, with adults measuring between 1/60th and 1/4th of an inch in length. Secondly, they are obligatory plant parasites which mean that in order for them to proliferate and develop; they need to feed on live plant tissues. Lastly, these nematodes have needle-like sucking mouthparts called stylets, which pierce plant cells and withdraw their contents (Jones, 1961). While certain plant-parasitic nematodes are primarily found in root or leaf tissues, all of them are at least partially soil dwelling across their life cycle (Neher, 2010). There is the presence of at least one plant-parasitic nematode for every plant species. Approximately 95% of the reported species are nematodes, primarily plant-parasitic, predominantly feeding on roots (Kumar & Yadav, 2020). They inhabit soil, residing either externally or internally within root tissues. The larvae of nematodes can feed on leaves. Plant-parasitic nematodes spread out in search of other plants or perish because to lay eggs and grow into an adult. Specific secondary symptoms do not arise from feeding by plant-parasitic nematodes. Nematode issues are hence frequently misdiagnosed. Typically, nematode infections cause wilting, yellowing, and stunting as above-ground symptoms. Yields might be drastically decreased under some circumstances. Certain worm species do exhibit distinctive symptoms or indicators; however, they will be covered in the section on particular nematodes. Nematodes frequently lower yields without exhibiting any signs that are visible above ground (Nicol et al., 2011).

One of the major condiments that is used year-round in all homes is the onion (*Allium cepa* L.). The young leaves and both mature and immature bulbs can be eaten raw or added to vegetable preparations (Lawande, 2012). Onions are used as a condiment and in soups and sauces. According to recent studies, eating onions may help avoid heart disease and other conditions. Carbohydrates, calcium, and phosphorus are abundant in onion bulbs. Allyl-propyl disulphide, a volatile oil, is the cause of onion pungency. Over 9,000,000 acres (3,642,000 hectares) of onions are thought to be cultivated annually throughout the world. Approximately 170 nations grow onions for home consumption, and 8% of the world's production is exported. China is the largest country of the world in onion production. China produced onion about 20817295 MT in 2008. Other major producer countries of the world are India, USA, Egypt and Turkey respectively. In Pakistan Onion is cultivated on 129600 ha producing over 1.7 million tons of onions (MINFA, 2008-09). A member of the Amaranthaceae family, spinach (*Spinacia oleracea* L.) is an edible flowering plant. It is indigenous to Asia's center and southwest regions. It is an annual plant that can reach a height of up to 30 cm (occasionally biannual). In temperate climates, spinach can withstand the winter months. Microscopic roundworms known as "plant parasitic nematodes" inhabit soil and plant tissues. The stem and bulb nematodes feed on the stems, leaves, and bulbs of the plant that it inhabits. They can survive for several years without water and can withstand desiccation. The juveniles of the second stage of root knot nematodes are motile, while the other stages are stationary; they reside within the roots. The soil is home to the stubby root nematode, which feeds on roots.

Growers utilize chemical pesticides, crop rotation, and soil additives extensively to control soil-borne infections, which include worms that parasitize plants. Nematicides can be used to produce rapid and efficient worm management; nevertheless, due to health and environmental concerns raised by their use and manufacture, several nematodes have been taken off the market (Thomason, 1987). The public's and regulators' growing concern over the use of pesticides in our food supply has sparked a renewed interest in the creation of ecologically nonthreatening, sustainable, and effective alternatives. The utilization of natural enemies and host plant resistance are two additional nematode management strategies that are currently being researched, however they can be very particular. It's critical to learn about the prevalence of nematode species, their host range, damage thresholds, and pathogenic potentials because crop resistance or predators that selectively target some nematode species while sparing others might seriously limit their efficacy. Data for vegetable crops cultivated in tunnels are nonexistent, despite recent findings in Pakistan (Anwar & Akhtar, 1992; Anwar et al., 2009) on the association, distribution, and prevalence of plant parasitic nematodes on a variety of crops planted in field settings. Thus, the purpose of this study was to examine the occurrence, distribution, density, and prevalence of nematode populations linked with spinach and onion crops in Karachi, Pakistan, and to characterize and record these data.

Materials and Methods

Collection and storage of samples

Random samples were taken from several locations at Karachi's onion and spinach farms. A scooping hand shovel was used to gather samples from the plant's rhizosphere at a depth of 10 to 15 cm. Following mixing, 500 g of soil from each plant was put in a polythene bag, knotted, and all the samples were immediately labeled with information about the location, the date of collection, sample name, soil type, soil moisture and other relevant data. Samples were collected at three different sites. In the laboratory, samples were stored in a refrigerator at about 5 ^{o}C until processed.

Extraction of nematodes

In order to identify and count the nematodes in a sample, they were separated from the soil or plant material. There are numerous ways of separating nematode from soil, and all have advantages and disadvantages. The method employed in this experiment is Cobb’s sieving method and modified Bearmann’s funnel method.

Sieving

For analysis, the roots were carefully taken out of the ground. The leftover dirt was well-combined. The samples were subjected to the Bearmann funnel procedure (Cobb, 1915; Bearmann, 1917) which involved screening and decantation techniques to isolate and process the nematodes. After adding roughly 500 g of soil to a plastic bucket and giving it a good shake to create a uniform suspension, the mixture was left to settle for about two minutes. Nematodes which are still afloat in the water while the heavier soil particles have sank to the bottom. The suspension was gradually passed through a series of 36, 100, and 350 mesh sieves; to prevent clogging, the sieves were manually tapped on a regular basis. A mild jet of water was then used to wash the residues on the sieve, 36 for roots examination and 100 for cyst. After that, 350 mesh sieves were used to filter the water suspension that contained nematodes shaped like eels. Then, using a funnel with a rubber tube and a clam at the bottom, the nematode suspension was poured over a piece of tissue paper that was fastened to a perforated plastic sheet. The bottom of tissue paper was in contact with the water within the funnel. The nematodes wriggled out into the clear water in the funnel after 48 hours and settled at the bottom. 100 cc of the nematode-containing water was then poured into a beaker.

Nematodes isolation

The aliquot obtained in the previous step was mixed with water. After allowing it to settle, the majority of the supernatant liquid was poured into a Petri dish. The contents of the Petri dish were rinsed with water and examined under a stereomicroscope. Additional water was added to the beaker, thoroughly mixed, allowed to settle, and the liquid was decanted again for further nematode inspection. This process was repeated to ensure thorough removal of nematodes until none were detected in the decanted liquid.

Quantitative analysis

Under a stereomicroscope, nematodes were counted in an open counting chamber using just 5 ml of extracted solution by a counter. The nematode counts per unit of soil were determined by averaging three readings.

Temporary mounts

For qualitative analysis, observations were done on temporary mounts with the use of stereomicroscope. Temporary mounts were prepared for immediate examination. The suspension was allowed to settle for at least two hours. The surplus supernatant water was drained off, and the remaining concentrated contents were placed to a hollow block for observation under a stereo microscope. Three droplets of nematode suspension were put on a glass slide measuring 3 × 1 inch. The slide was placed over a heated plate to effectively kill the nematodes and preserve their proper shape, ensuring not to overheat them. Cover slips were then placed over the drops on the slide and sealed with Zut. Each slide was carefully labeled for identification.

Qualitative analysis

On temporary mounts, observations were made up to the general level for the qualitative analysis.

Killing, fixing and dehydration

To obtain good specimens for taxonomic studies, nematodes were killed by placing the watch glass containing nematodes on the hot plate for a few seconds just enough to kill the nematodes. Care was taken to avoid overheating. The water was drawn out with the help of a fine dropper and nematodes were fixed in T.A.F. fixative left to soak for a minimum of 24 hours. These were then washed with the distilled water 3 times and then these were transferred to 1.25% glycerin for dehydration. The glycerin solution was used to keep the nematodes alive until the remaining ingredients vanished and only glycerin remained. Finally, the prepared specimens were kept in an incubator at 35 degrees Celsius for five to six days.

Monitoring and sealing

On the glass slide's middle, five to ten nematodes were put in a state of pure dehydration. Around the drop, three or four tiny lumps of paraffin wax with a 19 mm diameter were inserted, and a cover slip was placed on top of the wax lumps. After that, the slide was slowly heated to melt the wax, filling the area between the coverslips and the slides. To ensure that the specimens were not under any pressure, glass-wool supports the same size as nematodes were always employed. Zut-adhesive was finally used to seal the cover slips and properly labeled as well.

Roots examination

The roots were inspected for endoparasitic nematodes; roots were carefully cleaned and sliced into 2-3 cm length sections. The roots were examined under stereomicroscope and stained with 0.03% cotton blue lacto phenol.

Humid chamber

To ensure convenience and prevent the loss of nematodes during temporary mounting, slides were placed inside a humid chamber stored in a refrigerator to prevent desiccation. Humid chamber was made by using Petri dish with its cover, 3-4 folds of tissue papers were placed, and the tissue layer were wet with distilled water, then the temporary mount was placed over the slide, covered with Petri dish lid, and then stored in the fridge. When the qualitative analysis of each slide was completed, nematodes were carefully picked off the slide.

Measurements and drawing

All measurements were done using an ocular micrometer and drawings. The images were created with a camera lucida mounted to a compound microscope. The De Mans (1884) formula was employed to denote the dimension of the nematode.

Photography of eel nematodes

For light microscopy (LM), the fixed nematodes were processed following the methods outlined by Hopper (1970) and Golden (1978). Photomicrographs of eel worms, male, female, and juvenile were taken using an automated camera mounted to a compound microscope equipped with an interference contrast system.

Results

Survey

A survey of onion and spinach field was conducted in Karachi. Out of 15 samples, 6 samples were taken from the onion fields of Allah Baksh Farm, Gadap Town, and 9 samples were gathered from the UBIT field located at Karachi University. The samples were taken from the infected & healthy plant roots. Frequently occurring nematodes such as *Helicotylenchus*, *Longidorus*, *Pratylenchus*, *Bitylenchus*, *Rotylenchulus*, *Aphelenchus*, *Hoplolaimus*, *Hemicriconemoides*, *Filenchus and Seinura* are given in Tables 1 and 2.

Quantitative analysis

Table 1 consists of a total of 15 samples. Each sample has three sub samples taken randomly from the main sample. Different number of nematodes were found in sub samples including free living and plant parasitic nematodes. The details of each sample is mentioned below:

Table 1 Quantitative analysis of nematode populations in soil samples

Sample Number	Calculation	Mean
1.	$\frac{10+5+15}{3}$	10
2.	$\frac{15+20+28}{30}$	21
3.	$\frac{55+53+38}{3}$	49
4.	$\frac{38+105+107}{3}$	83
5.	$\frac{18+15+25}{3}$	19
6.	$\frac{28+55+25}{3}$	36
7.	$\frac{97+39+44}{3}$	60

8.	$\frac{65+29+28}{3}$	41
9.	$\frac{53+38+24}{3}$	38
10.	$\frac{60+15+57}{3}$	44
11.	$\frac{20+23+23}{3}$	22
12.	$\frac{38+35+33}{3}$	35
13.	$\frac{50+80+18}{3}$	49
14.	$\frac{20+23+23}{3}$	22
15.	$\frac{20+42+23}{3}$	28

Nematode population of onion and spinach

After further detail analysis of each sample, plant parasitic and free-living nematodes were isolated from one another in the sample and found various genera of plant parasitic nematodes. In each sample as mentioned below:

Table 2 Nematode population analysis in onion and spinach samples

Sample #	No. of free- living nematodes	No. of PPN	Nematode genera that parasitize plants	Number of genera
1.	40	10	*Longidorous* *Pratylenchus*	04 06
2.	42	03	*Longidorous* *Tylenchus*	01 02
3.	57	05	*Tylenchorhynchus* *Pratylenchus* *Helicotylenchus*	02 01 02
4.	70	01	*Filenchus*	01
5.	28	05	*Pratylenchus* *Helicotylenchus* *Longidorous*	03 01 01
6.	30	08	*Filenchus* *Pratylenchus* *Longidorous (100 mesh)*	04 02 02
7.	18	05	*Pratylenchus* *Hoplolaimus*	03 02
8.	18	10	*Pratylenchus* *Longidorous (100 mesh)* *Aphilenchus* *Helicotylenchus*	04 02 02 02
9.	33	15	*Pratylenchus* *Longidorous*	12 03
10.	65	20	*Aphilenchus* *Helicotylenchus* *Filenchus*	05 12 03
11.	50	13	*Pratylenchus* *Helicotylenchus*	3 10

12.	38	28	*Helicotylenchus* *Hoplolaimus*	23 05
13.	47	13	*Helicotylenchus* *Pratylenchus* *Filenchus*	09 02 02
14.	45	00	No	00
15.	20	18	*Helicotylenchus* *Filenchus*	12 06

Spinach reaction to cyst nematode H. schachtii

Results from 2019 trials were comparable to those from 2020. Table 3 shows how the examined spinach responded to infection by *H. schachtii*. Spinach 'Balady', 'Barkly', 'Orient', and 'Solanicy' were very susceptible to *H. schachtii* (Rf = 1.11 - 4.95), whilst 'Pacific' was moderately susceptible. Swiss chard 'Balady' and 'Ford Hook' exhibited sensitive reactivity to *H. schachtii* (Rf = 1.12 - 3.93). Nematode infection caused considerable declines in shoot and root dry weights in sensitive and highly susceptible spinach.

Table 3 Spinach reaction to cyst nematode H. schachtii during the year 2019 and 2020

	Year 2019		Year 2020	
Plants type	Number of the pots	Rf(x)	Number of pots	Rf(x)
Balady spinach	79	5	73	5
Barkly spinach	19	1.2	18	1.2
Orient spinach	31	2	29	2
Salonicy spinach	25	2	24	1.5
Pacific spinach	13	1	14	1

Discussion

Vegetables are vital sources of micronutrients that can significantly benefit undernourished populations globally, enhancing overall health. Spinach, a widely consumed vegetable, is particularly rich in various micronutrients and helps address micronutrient deficiencies (Hedges & Lister, 2007). However, vegetable yields are often compromised by pests and diseases (Wilson, 2014). Spinach, in particular, is highly susceptible to nematode infestations (Premachandra & Gowen, 2015). Despite advancements in agricultural technology improving productivity, many producers have neglected essential measures to mitigate yield losses. Root-knot (Meloidogyne spp.) and cyst (Heterodera spp.) nematodes are among the most significant plant pests affecting a wide range of crops (Mandal et al., 2021). These nematodes can spread through contaminated planting materials and seeds, persist in soil for extended periods, and exhibit a broad host range, short life cycle, and rapid reproduction rate. Research study conducted an extensive survey of nematode populations affecting onion and spinach crops. The survey identified 10 different genera of plant parasitic nematodes, including *Longidorus elongatus*, *Hemicriconemoides communis*, *Rotylenchulus reniformis*, *Hoplolaimus columbus*, *Bitylenchus brevilineatus*, *Aphelenchus avenae*, *Pratylenchus coffeae*, Seinura spp., Filenchus spp., and Helicotylenchus spp., along with free-living soil nematodes. Notably, some genera, such as *Longidorus elongatus*, *Bitylenchus brevilineatus*, *Rotylenchulus reniformis*, *Hemicriconemoides communis*, and Seinura spp., were reported for the first time in onion and spinach crops in Pakistan.

The study provided detailed information, morphometric data, and illustrations of these genera, emphasizing the importance of understanding and managing nematode populations in onion and spinach crops to promote sustainable agricultural practices in Karachi. Crop rotation is commonly practiced, where onion and spinach are rotated with non-host crops, such as legumes or cereals to disturb the nematode's life cycle (Bahadur, 2021). Additionally, the use of resistant varieties that show tolerance or resistance to nematodes is an effective approach (Starr & Mercer, 2009). Soil polarization, a technique that involves covering the soil with transparent plastic to increase soil temperature and kill nematodes, has shown promise in managing nematode populations. However, the success of soil polarization depends on factors such as duration, timing, and soil moisture (Riga et al., 2020).

Chemical nematicides have been used in the past, their usage is being more limited owing to environmental concerns and regulations (Chen et al., 2020). Integrated pest management (IPM) practices, including cultural practices, such as field sanitation, using clean planting material, and maintaining optimal soil fertility and moisture levels, are gaining popularity as sustainable alternatives to chemical control (Duncan & Noling, 1998). The findings of this study show that plant parasitic nematodes are widely dispersed on vegetable crops grown beneath tunnels in Punjab. This information on nematode incidence on vegetable crops will assist producers in planning and implementing nematode management measures to reduce nematode populations below threshold levels. This study also found that the prevalence of several economically significant plant-parasitic nematodes relates to Punjab vegetable plants, particularly those in tunnels where replanting is typically done on a quick cycle. This study further reveals that the scale of the nematode problem in tunnel planted crops deserves careful consideration to handle through the adoption of appropriate worm control measures.

The harm produced by root-knot nematodes, Meloidogyne species, to diverse crops is determined by species or physiological race, as well as the size of nematode population densities in soil at planting time (Anwar & Mckenry, 2010). To ensure improved crop performance, plant growers primarily want information on the pre-planting (initial) nematode population densities in soil prior to establishing their crops. Furthermore, crop response to changing initial infective juvenile (IJ) densities of Meloidogyne nematodes is critical for implementing control actions ahead of time to reduce yield losses. The results of this study revealed that the variance in growth and root infection of spinach plants in response to a range of initial population densities of *M. incognita*. The response of growth to various nematode inocula was assessed using four parameters: shoot and root length, shoot and root weight. Meloidogyne nematode root infestations are frequently examined using the gall index, which is a measure of the number of galls per root system. However, such a measure does not offer an accurate assessment, owing to the fact that gall size fluctuates with the density of nematodes in the root tissue of the plant species that they infected (Eisenback & Triantaphyllou, 1991). Previous studies found that the galls formed by *M. incognita* on spinach roots varied in size (Di Vito et al., 2004).

This study found that the majority of the chenopodiaceous plant cultivars evaluated were both sensitive and suitable hosts for *H. schachtii*. The findings complement previous research suggesting that *H. schachtii* may infect and proliferate on certain cabbage, cauliflower, and turnip cultivars (Ibrahim et al., 2013). *Heterodera schachtii* considerably reduced the shoot and root dry weights of sensitive and highly susceptible spinach varieties. Ibrahim et al. (2013) discovered that infection with *H. schachtii* affected the development of some cabbage, cauliflower, and turnip varieties. Losses in marketable yields of spinach were 30% and 36%, respectively, at densities of 6,500 and 18,500 *H. schachtii* J2/kg of soil (Olthof et al., 1974).

Losses in marketable yields of spinach were 29% and 35%, respectively, at densities of 6,000 and 18,000 *H. schachtii* J2/kg of soil (Olthof et al., 1974). The examined spinach was either moderately sensitive or moderately resistant to this nematode, except for spinach 'Balady'. Infection with *H. trifolii* considerably lowered the shoot and root dry weights of sensitive spinach. The current findings suggest that *H. trifolii* infection has harmful effects on the tested spinach. Infection with *H. schachtii* clearly caused higher damage to the dry weight of infected spinach shoots and roots. Furthermore, it was noticed that on the examined cultivars with the name 'Balady' of spinach, *H. schachtii* showed high reproduction rates (Rf = 4-8) as opposed to *H. trifolii* (Rf = 2-2.2). The current study also demonstrated that both *H. schachtii* and *H. trifolii* have high damage potential and reproductive capacities on susceptible and highly susceptible spinach. The study of determining the host reaction of certain spinach cultivars to *H. schactii* and *H. trifolii* concluded that resistance to these nematodes is significant and can be useful to incorporate in breeding programs when planning cyst nematode control measures. More study is needed to generate resistant or tolerant varieties of these food crops.

Conclusion

The occurrence and distribution of nematodes in onion and spinach fields at Karachi pose a significant concern for crop production. Nematodes are microscopic worms that can cause detrimental effects on plant health and yield. The common nematode species at Karachi affecting onion and spinach crops are root-knot nematodes (Meloidogyne species), lesion nematodes (Pratylenchus species), and dagger nematodes (Xiphinema species). The nematodes can infest the root systems of onion and spinach plants, leading to inhibited growth, staining leaves, wilted plant leaves, and reduced plant vigor. The warm and humid climate of Karachi provides favorable conditions for nematode reproduction and spread, making nematode infestation a recurrent problem for farmers. Despite these management efforts, nematodes continue to be a persistent challenge in onion and spinach fields in Karachi. Continued research, extension services, and farmer education are crucial for developing effective and sustainable nematode management strategies. Collaborative efforts between researchers, agricultural experts, and farmers are necessary to address the occurrence and distribution of nematodes in onion and spinach fields, ensuring the long-term productivity and sustainability of these crops in Karachi.

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