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RESEARCH PAPER

Assessment of nematode-induced diseases in chili fields of Karachi: Implications for sustainable crop management

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Key Message: This study highlights the significant impact of nematodes on chili production in Karachi, revealing a diverse range of nematode species that cause severe damage to chili plants. The findings report the urgent need for effective and sustainable nematode management strategies to mitigate yield losses and ensure the long-term sustainability of chili cultivation.

Abstract

Nematodes represent significant pathogens affecting chili (Capsicum spp.) production, leading to various diseases that significantly reduce crop yield. This study conducted a comprehensive survey in chili fields located at the University of Karachi near the UBIT department and one field in Gadap Town to assess nematode populations. A total of fifteen soil samples were randomly collected from plant roots and subsequently analyzed in the laboratory, revealing a diverse array of nematodes. Identified species included *Longidorus elongatus, Pratylenchuscoffeae, Tylenchorhynchus elegans, Helicotylenchusdigonicus, Hoplolaimus indicus, Tylenchus spp., Ditylenchus spp., Hemicriconemoides spp., Aphelenchus spp., and Xiphinema spp.,* alongside numerous free-living nematodes. The populations of plant-parasitic nematodes (PPN) were notably high, comprising various genera and species that actively attack chili plants, resulting in symptoms such as yellowing, stunting, wilting, dieback, decline, leaf distortion, root rots, gall formation, leaf drop, and other detrimental effects. The taxonomy and systematics of these nematode genera and species are comprehensively discussed, supported by detailed descriptions, illustrations, and photographs. Understanding the diversity and impact of nematodes on chili crops is crucial for implementing effective management strategies. This study emphasizes the urgent need for sustainable nematode control measures to mitigate yield losses and ensure the long-term sustainability of chili production.

Keywords: Chili (Capsicum spp.), Diseases, Nematodes, Plant-parasitic nematodes, Taxonomy

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Introduction

In the animal kingdom, nematodes rank second to insects in terms of species diversity. However, approximately 3% of all nematode species have undergone identification and examination. Only one cubic foot of soil may harbor millions of individuals from numerous taxonomic categories. An important group of nematodes Plant-parasitic nematodes cause significant economic losses, with about 10 billion dollars lost annually in the US and over 100 billion dollars globally, making them a major concern in agriculture (Hodda, 2022; Kantor et al., 2022). Damage to plants caused by nematodes is frequently overlooked, as their symptoms such as stunted growth, yellowing, and reduced vigor can also result from issues related to nutrition or water. However, it is important to note that not all nematodes are harmful. Many species actually play beneficial roles in agriculture and the environment. For instance, some nematodes are valuable in biological pest control, managing insect populations, while others enhance soil fertility by aiding in nutrient cycling (Singh et al., 2015; Mandal et al., 2021; Topalović & Geisen, 2023).

Nematodes play crucial roles in soil ecosystems by feeding on bacteria, fungi, and other microscopic organisms, making them essential components of soil food webs. Caenorhabditis elegans, a nematode species known for its importance as a research model, feeds on various bacterial prey species, highlighting its significance in scientific studies (Kundu & Vyshali, 2023; Zhou et al., 2023). While most nematodes promote soil health and fertility, a small percentage are parasitic, causing risks to humans, livestock, and crops. Nematodes associated with bacteria have been extensively studied as indicators of soil fertility because of their capacity to enhance nitrogen level and aid in ecosystem management (Moura & Franzener, 2017; Lu et al., 2020; Renco et al., 2020). Capsicum, which is also known as chili, is a group of flowering plants belonging to the Solanaceae family and has its origin in Central and South America, and the West Indies. They have been cultivated for thousands of years and now grown globally. Due to the highly use in culinary and medicinal benefits, these plants are prized also for capsaicinoids, carotenoids, and polyphenols comprising antioxidant, anti-hypertensive and anti-inflammatory properties (Alonso-Villegas et al., 2023). Moreover, research has been conducted on capsicum species such as Capsicum frutescens to investigate their pharmacological characters like anti-diabetic, anti-bacterial and anti-cancer properties underlining their importance in traditional medicine (Nankolongo et al., 2023). Plant parasitic nematodes are significant danger to capsicum species, leading to decrease in crop production.These nematodes induce mechanical injuries, and physiological alterations, and facilitate the infection of other pathogens, leading to crop losses amounting to 21.3% in India annually (Reddy, 2021). Nematode-infected plants exhibit reduced growth, chlorophyll content, and dry matter accumulation, along with increased malondialdehyde levels and enzyme activities, inducing water stress (Khan et al., 2021).

In Pakistan, the nematode issue poses a significant challenge due to various factors. The country's tropical climate provides ideal conditions for nematode activity and reproduction year-round, particularly in sandy and warm soils prevalent in irrigated areas (Khan et al., 2021; Saeed et al., 2022). Continuous cultivation of perennial crops and the practice of growing the same crops in a field year after year contribute to heavy nematode infestations, impacting agricultural productivity. Studies in districts like Swat and Karachi have identified multiple plant-parasitic nematode species associated with crops like persimmon and grass, highlighting the economic losses caused by nematodes in these regions (Khanum et al., 2021; Ullah & Khanum, 2022). Nematode infections are estimated to cause annual losses of approximately 6% in field crops, 12% in fruit and nut crops, 11% in vegetables, and 10% in ornamental plants. In addition to these quantitative losses, nematodes can also diminish the vitamin and mineral content of edible plant parts (Reddy, 2021; Yadav & Patil, 2021; Sikora et al., 2023). Nematodes can create complex diseases when interacting with other soil-borne pathogens, amplifying the overall agricultural damage inflicted by these microscopic pests. Implementing integrated nematode management strategies is crucial to mitigate these losses and safeguard global food security (Bahadur, 2021).

In Pakistan, the plant has parasitic nematode problems that cause great damage to chilies production. Root-knot and burrowing nematodes are considered the main problem but some are other plant parasitic nematodes which also parasitize on chilies crops (Zhuge et al., 2023). Plant parasitic nematodes (PPNs) encompass a variety of species that significantly impact agricultural and horticultural crops, causing substantial yield losses globally (Khan, 2015). Among the nematodes mentioned, Root-knot nematodes (*Meloidogyne* spp.), Burrowing nematode (*Radopholus similis*), Cyst nematode (*Heterodera* spp.), Stem bulb nematodes (*Ditylenchus dipsaci*), Lesion nematodes (*Pratylenchus* spp.), Dagger nematode (*Xiphinema* spp.), Spiral nematodes (*Helicotylenchus* spp.), and Stunt nematodes (*Tylenchorhynchus* spp.) are particularly noteworthy for their damaging effects on various crops. These nematodes exhibit different feeding habits and life cycles, leading to root damage, nutrient theft, and susceptibility to secondary pathogens, ultimately resulting in economic losses. Effective management strategies, including cultural practices, biological control, and host resistance, are crucial for mitigating the impact of these nematodes on crop production. Therefore, the present research study was conducted to assess the prevalence and impact of nematode-induced diseases in chili fields across Karachi, Pakistan in order to inform sustainable crop management practices and develop effective strategies to mitigate nematode-related damage.

Materials and Methods

Collection of samples

Samples were collected randomly from different localities of onion and spinach fields in Karachi. With the use of scooping hand shovel, samples were collected from the rhizosphere of the plant about a depth of 10-15 cm. After mixing, 500 g soil from each plant was placed in a polythene bag, tied and all the samples were properly labeled on the spot with a label bearing details of locality, 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 °C until processed.

Extraction of nematodes

The processing of samples consists of separating the nematode from the soil or plant material for identification and counting. There are numerous ways of separating nematodes from soil, and all have advantages and disadvantages. The method employed in this experiment was Cobb's sieving method and modified Bearmann's funnel method.

Sieving

Roots were cautiously detached from soil for analysis. The remaining soil was thoroughly mixed. Samples were processed by Bearmann funnel method (Baermann, 1917; Cobb, 1918) sieving and decantation methods were followed for the processing and isolation of nematodes. Approximately 500 g of soil placed in a plastic container and stirred the mixture vigorously to obtain a uniform suspension which was allowed to settle for about two minutes. The heavy soil particles settled down at the bottom and nematodes remained suspended in the water. The suspension was poured out slowly and passed through a set of 36, 100, and 350 mesh numbers of sieves; sieves were continuously tapped by hand to avoid blocking. The deposits on the sieve were then washed with a gentle jet of water into a beaker, 36 for roots examination and 100 for cyst. The eel shaped nematodes holding water suspension was subsequently passed through 350 mesh sieves. Then using a funnel with a rubber tube at the bottom, nematodes suspension was poured out on a piece of tissue paper attached to a perforated plastic sheet. The tissue paper bottom was in contact with water present in funnel. The nematodes wriggled out into the clear water after 48 hours and settled at the bottom. After that, 100 ml of water containing nematodes was transferred into a beaker.

Isolation of nematodes

The collected aliquot was then mixed with water in a described manner and allowed to settle down. Following which, maximum supernatant liquid was poured off into a petri dish. After being inspected under a stereomicroscope, the contents of petri dish were collected and placed in a watch glass containing water. Water was added to the beaker again, mixed well, allowed to settle, and the liquid that was transferred and checked once more for nematodes. To ensure no nematodes were left behind, the procedure was repeated until none were discovered in the decanted liquid.

Quantitative analysis

Nematodes were counted in an open counting chamber with only 5 ml of extracted suspension by a counter under a stereomicroscope. The average of three readings gave the nematode numbers per unit of soil.

Temporary mounts

The qualitative analysis involved observing temporary mounts using a stereomicroscope. Temporary mounts were prepared for immediate examination. The nematodes suspension was permitted to settle for a minimum of 2 hours. After pouring off the excess supernatant water, the concentrated material was moved to a cavity block for observation under a stereomicroscope. Three drops of the nematode suspension were placed on a 3×1-inch glass slide. The slide was then placed on a heated plate to kill the nematodes and to get their proper shape, taking care not to overheat them. Cover slips were then placed over the drops on the slide and sealed with a sealant. Each slide was also labeled accordingly.

Qualitative analysis

For qualitative analysis, observation was made on temporary mounts up to the generic level.

Killing, fixing, and dehydration

To obtain good specimens for taxonomic studies, nematodes were killed by placing the watch glass containing them on a hot plate for a few seconds in a well-stretched condition. Caution was adopted to prevent excessive heating. Using a fine dropper, the water was carefully removed, and the nematodes were preserved in T.A.F and left in a fixative for about 24 hours. They were then washed with distilled water three times and transferred to 1.25% glycerin for dehydration. The nematodes were kept in the glycerin solution until the other components had evaporated, leaving only the glycerin. The cavity block was then placed in an incubator at 35 °C for 5-6 days.

Monitoring and sealing

Five to ten nematodes were mounted in pure dehydrated on the center of the glass slide. Paraffin wax was placed as three or four small lumps around the drop and a 19 mm diameter, coverslipwas placed on the wax lumps. The slide was then gently heated to melt the wax and filled the space between the slides and the coverslips. Glass- wool support of the same size as that of nematodes was always used to prevent any pressure on the specimens. The coverslips were finally sealed with Zut-adhesive and properly labeled as well.

Roots examination

The roots were examined for endoparasitic nematodes. For this purpose, the roots were washed thoroughly and cut into pieces of 2-3 cm length. These root pieces were examined under a stereomicroscope and stained with 0.03% cotton blue lacto-phenol.

Humid chamber

For convenience and to avoid the loss of nematodes, temporary mount slide were placed inside a humid chamber, which was then placed inside the refrigerator to avoid desiccation. A humid chamber was made by using a Petri dish with its cover. Several layers (3-4 folds) of tissue paper were placed and the tissue layers were wet with distilled water. The temporary mounts were placed over the slide, which was covered with Petri dish lid and placed inside the fridge. When the qualitative analysis of each slide was completed, nematodes were removed from the slide by carefully picking them off.

Measurements and drawing

All measurements were taken with the help of an ocular micrometer, and drawings were made with the help of camera lucida attached to the compound microscope. De Man (1884) formula was used for denoting the dimension of the nematodes.

Photography of eel nematodes

For light microscopy (LM), the fixed nematodes were processed using methods of Hopper (1970) and Golden (1978). Photomicrographs of eelworms; male, female and juvenile were made with camera (Nikon DS Fil) attached to a compound microscope equipped with an interference contrast system.

Results and Discussion

Survey

An extensive survey of nematodes associated with chili crop was conducted in fields near the University of Karachi and the department of UBIT and Gadap town. A total of 15 soil samples were collected randomly from the roots of chili plants. The laboratory analysis identified several genera and species of plant-parasitic nematodes following the identification keys of Hunt (1993) and Siddiqi (2000). The identified species included *Longidorus elongatus* (de Man, 1876) Micoletzky, 1922, *Pratylenchus coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941, *Tylenchorhynchus elegans* Siddiqi, 1961, *Helicotylenchus digonicus* Perry in Perry, Darling & Thorne, 1959, *Hoplolaimus indicus* (Sher, 1963), *Tylenchus* spp., *Ditylenchus*spp., *Hemicriconemoides* spp., *Aphelenchus* spp., and *Xiphinema* spp. Free-living nematodes were also present alongside these plant-parasitic nematodes. The quantitative and qualitative analyses of the data are shown in Tables 1 and 2.

Table 1 Quantitative analysis of nematode populations

Sample number	Calculation	Mean
1.	44+44+34/3	41
2.	42+44+60/3	49
3.	17+15+18/3	17
4.	78+86+93/3	86
5.	46+35+40/3	40
6.	$\frac{14+12+12}{3}$	13
7.	$\frac{152+136+120}{3}$	136
8.	$\frac{21+40+30}{3}$	30
9.	$\frac{17+11+17}{3}$	15
10.	$\frac{10+15+26}{3}$	17
11.	$\frac{2+4+12}{3}$	6
12.	$\frac{10+12+15}{3}$	12
13.	$\frac{10+9+11}{3}$	10
14.	$\frac{12+18+13}{3}$	14
15.	$\frac{20+16+13}{3}$	16

Discussion

The survey discovered a thriving community of nematodes associated with chili crops in the sampled fields. In these areas, potential hazards to chili agriculture are accentuated by the discovery of various plant-parasitic nematode genera and species. The existence of *Longidorus elongates*, *Pratylenchus coffeae*, and *Tylenchorhynchus elengans* stands predominantly problematic due to their documented impact on crop health. *Longidorus elongates*, a nomadic endoparasite can cause stunted growth and low yield owed to rigorous damage to root system (Brown et al., 1995).

Similarly, *Pratylenchus coffaea*, also known as migratory endoparasite is notorious for causing substantial economic losses in various crops and has a wide range of hosts (Bridge & Starr, 2007; Castillo & Vovlas, 2007). On the other side, a less aggressive parasite, *Tylenchorhynchus elegans* has a part to reduced plant vigor and productivity (Fortuner, 1987).

The high incidence of *Hoplolaimus indicus* and the high pervasiveness of *Helicotylenchus digonicus* indicate a higher concentration of sedentary endoparasite in the investigated areas. Siddiqi (2000) studied that these types of nematodes have the potential to establish feeding sites in root tissues, causing localized injury and increasing the risk of subsequent infections by other pathogen. The existence of free living nematodes which is crucial for soil health and nutrient cycling was discovered by surprisingly qualitative study. Their coexistence alongside nematodes shows a complex connection in the soil ecosystem. The results suggest that these free living nematodes could be used in biological control strategies to lower plant parasitic nematode populations (Bongers & Ferris, 1999; Ferris & Bongers, 2006).

Furthermore, geographic variability in nematode ecosystem may be due to the varying distribution of worm genera between samples. This diversity may be responsible for changes in soil properties, crop management techniques and environmental circumstances. For instance, sample 7 exhibited the highest average number of nematodes. This could indicate a key area where nematodes are common, related to specific soil features or past land use (Geraert, 2013). Integrated pest management (IPM) practices should be used to lessen the impact of plant parasitic nematodes on chili crop. Among all the possible options are crop rotation, use of resistant cultivars and biological control agents (Nicol et al., 2011). Also, for development of sustainable management practices, future study should concentrate on understanding the ecological relationships between nematode species and their environment.

Table 2 Qualitative analysis showing temporary slides observation in chillies

Sample number	No. of free-living nematodes	No. of PPN	Genera of plant parasitic nematodes	Number of genera
1.	30	09	*Longidorous* *Tylenchus* *Helicotylenchus*	02 01 06
2.	25	04	*Longidorous* *Pratylenchus*	02 02
3.	45	10	*Helicotylenchus* *Tylenchorhynchus* *Pratylenchus*	08 02 05
4.	50	40	*Helicotylenchus* *Longidorous* *Tylenchus*	35 03 02
5.	40	07	*Pratylenchus* *Helicotylenchus* *Longidorous*	01 03 03
6.	20	01	*Pratylenchus*	01
7.	70	08	*Xiphinema* *Pratylenchus*	04 04
8.	15	09	*Ditylenchus* *Hemicriconemoides* *Hoplolaimus* *Aphelenchus*	02 02 02 03
9.	20	01	*Longidorous*	01
10.	25	06	*Helicotylenchus* *Longidorous*	04 02
11.	10	00	*None*	00
12.	20	13	*Helicotylenchus* *Tylenchorhynchus*	04 02
13.	10	02	*Pratylenchus*	02
14.	25	03	*Tylenchorhynchus*	03
15.	30	00	*None*	00

Conclusion

Nematode diseases are major hindrance to chili cultivation in Karachi’s field. Nematodes are microscopic worms that inhabit the soil. They can infect the root system of chili plants, leading to stunted growth, yellowing of leaves, wilting, and overall deterioration of plant health. Among the nematodes affecting chili fields in Karachi, root-knot worms (Meloidogyne spp.) and lesion nematodes (Pratylenchus species) are the most common. The warm and humid climate of Karachi promotes the infestation of nematode and cause damage to chili crop. Nematodes are highly resilient and can survive in the soil for a long time, making their population management challenging. In addition, nematodes can rapidly spread from one plant to another. This makes the problem worse. Nematodes were previously managed by application of chemical nematicides but their use is limited due to environmental concerns and regulations. Integrated pest management (IPM) strategies which combine multiple control methods, are becoming more popular as a long term and sustainable solution. This includes cultural practices like maintaining, field cleaning, usage of clean plant material and managing optimal soil moisture and nutrient level. Regardless of these efforts, nematode diseases continue to pose a significant threat to chili farmers in Karachi. Collaborative efforts among research, agriculture experts and growers are essential to addressing this issue and ensuring the continued success of chili production in Karachi. Overall, this study provides a comprehensive survey of the nematode fauna associated with chili plantations in the surveyed field. The results emphasize the importance of continuing to monitor and manage nematode population to maintain the long term viability of chili production in these regions. To determine the effectiveness of various IPM systems and their long term impacts on nematode dynamics and crop health, additional research is needed.

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