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RESEARCH PAPER

Plant parasitic nematodes associated with wheat and maize

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Key Message: This comprehensive study presents detailed morphological characters for several plant-parasitic nematodes found in abundance around the roots of wheat and maize plants in various locations in Karachi, Pakistan.

Abstract

Nematodes associated with wheat and maize cause different diseases and reduced their yield. Survey was carried out in wheat and maize fields at the University of Karachi near Department of Environmental Studies, mooti baag, Nursery (NNRC) and Crops Diseases Research Institute (CDRI). During survey a total of 16 soil samples were collected randomly from the roots of the plants. After laboratory analysis many plant parasitic nematodes were identified in which *Longidorus pisi*, *Tylenchorhynchus annulatus*, *Helicotylenchus egypteansis*, *Hoplolaimus indicus*, *Hemicriconemoides mangiferae.*, *Pratylenchus* spp, *Tylenchus* spp., *Hetrodera* spp., *Aphelenchus* spp. *and Rotylenchus* spp. are included. A large number of free-living nematodes were also found along with plant parasitic nematodes. In the CDRI wheat samples were collected the large amount of population of free living nematodes were present. Whereas the population of plant parasitic nematodes were not sufficient. From mooti baag, Department of Environmental Studies and Nursery of NNRC sample of maize were collected in which more plant parasitic nematodes were found.

Keywords: Crop diseases, Free-living nematodes, Maize, Nematode identification, Wheat, Yield reduction

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Introduction

Wheat, scientifically known as *Triticum aestivum* L., is a staple cereal grain cultivated worldwide, with its origins tracing back to the Levant region in the Near East (Khan et al., 2016; Anser et al., 2018; Shafqat et al., 2019; Mehmood et al., 2020). Wheat plays a crucial role as a primary source of vegetable protein in human diets globally, boasting a higher protein content compared to major cereals such as maize (corn) and rice (Willett et al., 2019). It played a key role in the development of city-based societies during the early stages of civilization due to its ease of large-scale cultivation and the additional benefit of long-term food storage from its harvest (Wato & Amare, 2020). In Pakistan, wheat holds a central position in agricultural politics and contributes significantly to the economy, accounting for 13.1% of value added in agriculture and 2.8% to the GDP. The Economic Survey of 2008-09 reported a cultivation area increase to 9062 thousand ha, a 5.9% rise from the previous year's 8550 thousand ha. Presently, wheat is cultivated on 9 million ha, producing 23.8 million tons annually, with a per capita consumption of 37.5 kg/annum (GOP, 2010).

Wheat comes in various forms, with white and red wheat being the most common. Additionally, natural forms such as purple wheat, a tetraploid species high in antioxidants. Other nutritionally promising, commercially minor species include black, yellow, and blue wheat. As a widely grown winter crop, wheat falls under the category of rabi crops, also known as annual crop (Fardet, 2010). The extensive variety of wheat includes dwarf, semi-dwarf, and bearded varieties, along with tall varieties for hay production. Wheat is used to manufacture various products such as maida (27%), sugi (18%), bran (15%), and flour (40%) (Farooq et al., 2001). It supplies 72% of the calories and protein in an average diet through bread, naan, and chapatti (Farooq et al., 2001). Wheat straw is crucial as fodder, and 5-10% of wheat grains are utilized as poultry and livestock feed. Wheat protein, easily digestible by nearly 99% of the human population, along with its starch, makes wheat-based meals highly nutritious (Blair, 2008; Lalman et al., 2011). Additionally, wheat contains a diverse range of minerals, vitamins, and lipids, making it a valuable dietary component when complemented with a small amount of animal or legume protein (Shewry & Hey, 2015).

Maize (*Zea mays*) is an important cereal in global agriculture, holding significant physiological efficiency since its domestication by humans. This versatile grain plays a crucial role as a staple food in various forms and serves as a vital component in livestock feed and poultry (Klopfenstein et al., 2013; Batool et al., 2019). The succulent fodder derived from green maize plants adds to its manifold utility (Klopfenstein et al., 2013). In Pakistan's agriculture, maize assumes the third position among cereals, following wheat and rice (Pakistan Bureau of Statistics, 2020). Comprising 4.8% of the total cropped area and contributing 3.5% to the value of agricultural output, maize occupies an extensive niche. The cultivation extends across an estimated 0.9 million hectares, yielding an annual production of 1.3 million tonnes. The bulk of maize production (97%) comes from two primary provinces. Khyber Pakhtunkhwa (KPK) takes the lead having 57% of the total area and contributing 68 % to the overall production. Punjab, with 38% of the acreage, plays a significant role by contributing 30 % to the total maize grain production. In contrast, the provinces of Sindh and Balochistan contribute a mere 2-3 % of the total production. Maize cultivation in Pakistan occurs on 1.02 million hectares, resulting in an annual grain production of 2.96 million tons (Government of Pakistan [GOP], 2007-08). This crop plays a significant role, contributing 6.4% to the total food grains production in the country. The maize cultivation cycle occurs twice annually, during the spring and autumn seasons. Spring maize is conventionally planted between the first week of February and the first week of March, while autumn maize is sown from the last week of July to mid-August. The recommended seeding rate for maize is 20-25 kg per hectare.

Wheat and maize production are affected by several factors, including diseases, which can reduce both the yield and quality of the grains. Of these diseases, plant parasitic nematodes (PPN) are economically important (Mueller et al., 2016). Nematode is derived from the Greek word means thread they are elongated, tube like, somewhat spindle shape that move like snakes. They are microscopic, multicellular, triploblastic and belongs to Kingdom Animalia and Phylum Nematoda and having all major physiological systems except respiratory and circulatory system (Shah & Mahamood, 2017). They are unsegmented and tapering towards the end i.e., head and tail. They are aquatic, live in marine and fresh water and in film of water within soil. Phylum Nematoda is the largest one. Most of the species are free living large number of nematodes live in soil and along the shores of marine and fresh water. They consume microorganism bacteria, fungi and algae as their food (Shah & Mahamood, 2017). Nematodes are also successful parasites. They are parasites on different species of plants, animals, agricultural crops, livestock and humans. The plant parasitic nematodes are known as Phyto nematodes. Parasitic nematodes inhibit all parts of plants including roots, stems, leaves, buds and they have great variety of feeding habits (Kumar & Yadav, 2020). Some species only feed on outermost plant tissues are known as ectoparasites they feed by inserting stylet into the host body while other penetrate in deeper tissues are known as endoparasites. Nematodes are classified depending upon the mobility they may be migratory or sedentary. Those nematodes which have ability to move place to place throughout their life cycle are known as migratory (Kumar & Yadav, 2020). Some nematodes loss their ability to move on maturity and having single feeding site are known as sedentary. So in this way they are assigned as migratory ectoparasites, migratory endoparasite, sedentary ectoparasite, sedentary endoparasite. Damage from a few nematodes s usually slight but large population severely injures or kills their hosts (Grabau et al., 2020). In addition, some nematodes reduce a plant's ability to resist fungal infection, while others transmit viruses among plants. Nematode emerging from the egg resembles the adult in its morphology of the same species (Grabau et al., 2020). Gonad are represented by a small group of cells, spicules (male) and vulva (female) which are known as secondary sex characters are absent while other characters of the species are present. As the individual grows gonads develop gradually. They shed cuticle called as molting. Mostly plant parasitic nematodes take 3-4 weeks to complete its entire life cycle.

Materials and Methods

Samples collection

Samples were collected randomly from different localities of wheat and maize fields in Karachi. Samples were collected from the rhizosphere of the plants at a depth of 10-15 cm with the help of soil scoops or trowels. Mixed the soil of 500 g and placed the sample in the polythene bag and the samples were tagged having following details about locality, date of collection, sample name, soil type and soil moisture and other relevant data.

Samples storage

In the laboratory, samples should store in a refrigerator at about 5 ºC until they were processed.

Nematodes extraction

Sample's processing consists of separating the nematode from the soil or plant material in order to identify and count them. There are numerous way of separating nematode from soil, and all have merits and demerits. The method employed in this experiment is Cobb's sieving method and modified Bearmann's funnel method.

Sieving

The process of soil analysis commenced with the careful extraction of roots, followed by thorough mixing of the remaining soil. The Bearmann funnel technique (Cobb, 1918) was employed for processing soil samples, utilizing sieving and decantation methods to isolate nematodes. Around 500 grams of soil were introduced into a plastic bucket and vigorously stirred to achieve a uniform suspension. After settling for approximately two minutes, heavier soil particles settled at the bottom while nematodes remained suspended in the water. This suspension underwent a sequential passage through a series of sieves with mesh numbers of 36, 100, and 350, with continuous manual tapping to prevent blockage. Deposits on the sieves, designated for root examination (36 mesh) and cyst isolation (100 mesh), were washed into separate beakers with a gentle stream of water. The nematode-containing water suspension was further refined by passing it through a 350-mesh sieve. Subsequently, the suspension was poured onto tissue paper affixed to a perforated plastic sheet within a funnel, equipped with a rubber tube tied at the lower end. Over a period of 48 hours, nematodes migrated through the tissue paper into the clear water below, settling at the bottom of the funnel. Following this, 100 milliliters of water, containing nematodes, were carefully transferred into a beaker for further analysis.

Isolation of nematodes

The collected aliquot was combined with water as described earlier. After settling, the majority of the supernatant liquid was carefully poured off into a Petri dish. The contents of the Petri dish were scrutinized under a stereomicroscope, and any identifiable specimens were transferred into water in a watch glass. Additional water was then introduced into the original container, thoroughly mixed, and allowed to settle once more. The liquid was decanted and re-examined for nematodes. This process was repeated until no further nematodes were detected in the decanted liquid, ensuring thorough removal of any remaining specimens.

Quantitative analysis

Nematodes were quantified using an open counting chamber, employing only 5 ml of the extracted suspension. A counter observed the nematodes under a stereomicroscope. The average of three readings provided the nematode count per unit of soil.

Temporary mounts

To conduct qualitative analysis, observations were made on temporary mounts using a stereomicroscope. Immediate preparations of temporary mounts were made for examination. The nematode suspension was allowed to settle for a minimum of 2 h. After pouring off excess supernatant water, the concentrated contents were transferred into a cavity block for examination under the stereomicroscope. Three drops of the nematode suspension were then placed on a 3 × 1 inch glass slide for further analysis. The slide is then placed over the heated plate for the killing of nematodes for getting their proper shape; care was taken not to overheat them. Then cover slips placed over the drops on the slide and then sealed with nail paint. Each slide was labeled as well.

Qualitative analysis

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

Killing, fixing and dehydration

To procure high-quality specimens for taxonomic studies, nematodes were killed by carefully placing the watch glass containing them onto a hot plate for a brief duration, ensuring they were adequately stretched without overheating. Excess water was removed using a fine dropper, and the nematodes were fixed in T.A.F. solution, left in fixative for a minimum of 24 hours. Following fixation, they underwent three washes with distilled water before being transferred to a 1.25% glycerin solution for dehydration. The nematodes remained in the glycerin solution until other components evaporated, leaving only glycerin behind. The cavity block containing the specimens was then placed in an incubator set at 35 degrees Celsius 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 19 mm diameter, cover slip 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 supports, matching the size of the nematodes, were consistently employed to ensure no pressure was exerted on the specimens. The cover slips were then securely sealed using Zut adhesive or wax and appropriately labeled.

Roots examination

The roots underwent examination for endoparasitic nematodes. To accomplish this, the roots were thoroughly washed and then sliced into pieces measuring 2-3 cm in length. These roots were examined under stereomicroscope and stained with 0.03% cotton blue lacto phenol.

Humid chamber

For conveniences and to avoid the waste of nematodes at temporary mount slides were placed inside humid chamber and humid chamber was placed inside refrigerator to avoid 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 placed inside fridge. When the qualitative analysis of each slide completed nematodes were removed from the slide by picking them.

Measurements and drawing

Measurements were conducted utilizing an ocular micrometer, and illustrations were produced using a camera lucida connected to the compound microscope. The dimensions of the nematodes were represented using the De Man (1884) formula.

Photography of eel nematodes

In light microscopy (LM), the treated nematodes were processed. Photomicrographs of eel worms, males, females, and juveniles were captured using an automatic camera connected to a compound microscope fitted with an interference contrast system.

Results

Survey

A survey of wheat and maize fields was conducted in the Karachi. Out of 16 samples, 8 samples were collected from wheat field from CDRI and 8 samples of maize were collected from mooti baag, Department of Environmental Studies and from the nursery of National Nematological Research Centre at Karachi University. The samples were taken from the infected and healthy plant roots. Many free living nematodes are found but some plant parasitic nematodes which occur frequently were listed below: *Helicotylenchus egyptiensis, Longidorus pisi, Tylenchorhynchus annulatus, Hoplolaimus indicus, Hemicriconemoides mangiferae, Heterodera* spp., *Pratylenchus* spp., *Rotylenchulus* spp., *Aphelenchus* spp., and *Tylenchus* spp.

Morphological characters

Helicotylenchus egyptiensis

Measurements

Female (n=44): L=706 µm (586-804); a=24, 4(21.6-31.2); b=4.6 (3.5-5.5); c=34.6 (26.7-47.5); c'=1.5 (1.2-1.8); o=44.3 (36.8-55.0); V=62(59-64); stylet=24.8 µm (22.1-26.5)

Description

Female: Body posture varying from an open 6 to a tight circle. Lip region slightly flattened anteriorly, not set off, with 4 indistinct annules, 5.3µm broad (4.8 to 5.9) and 3.9µm high (2.9 to 4.4). Labial framework moderately developed, stretching back 1 to 2 annules. Anterior and posterior cephalic not seen. Spear knobs rounded posteriorly and flattened to slightly hollow anteriorly, 4.9 µm broad (4.0 to 5.5) and 2.4 µm high (1.8 to 3.7). Opening of dorsal oesophageal gland 10.9 µm (9.2 to 13.3) from base of spear. Metenchium varying from very much shorter then, to slightly longer than telenchium (m=43 to 51%). median bulb 13,2µm long (12.9 to 14.0) and 10.6µm broad (9.9 to 12.1). length of oesophagus 82 µm (77 to 88) from front end of body to middle of median bulb and 76 µm (62 to 89)from middle of median bulb to end of oesophageal lobe. Excretory pore situated opposite posterior part of isthmus, 110 µm (83 to 121) from front end of the body. Hemizonid 1½ to 3 annules long, situated from directly anterior to 3 annules anterior to excretory pore. Hemizonion not seen. Width of annules on body 1.4 µm (1.1 to 1.7). Spermatheca indistinct in some specimens and small, round and empty in others. Epiptygma not clearly seen, but appears to be double and folded into the vagina in some specimens. Lateral field aerolated only from level of median bulb to front end and not on tail, 5.3 µm wide (4.4 to 7.0). phasmids located from 2 annules posterior to 5 annules anterior to anus. Caudalid not seen. Tail 20.7 µm (16.9 to 25.4) long; with a long slender, sharply pointed, ventral projection; with 9 to 14 annules.

Remarks

Specimens of *H. egyptiensis* were found in abundance around the roots of wheat and maize plants from CDRI, mooti baag field and NNRC nursery, University of Karachi, Karachi, Pakistan.

Longidorus pisi Edward

Measurements

Female: L=3.9(3.23-4.5) mm; a=115.4(103.4-126.7); b=11.8 (10.8-12.5); c=98.4(85.0-113.0); c'=1.9(1.4-2.2); V=50.1(48.91-51.4); Od. stylet= 78 µm (74-80); Od. phore= 42.8 µm (38-44); total stylet=120.8 µm (112-124)

Description

Female: Body of heat relaxed specimen slightly to strongly ventrally curved, tapering gradually at both extremities. Lip region relatively wide. Amphid pouch well developed. Cardia hemispherical. Cuticle smooth, thickened towards extremities in two layers. Hypodermal lateral field one-third to one fourth of body width. Numerous lateral pores along the body. Vulva a transverse slit. Ovary reflexed, amphidelphic. Prerectum contains objects which vary in size, number, and shape.

Remarks

Specimens of L. pisi were found in abundance around the roots of wheat and maize plants from CDRI, mooti baag field and NNRC nursery, University of Karachi, Karachi, Pakistan.

Tylenchorhynchus annulatus

Measurements

Female (n=25): L= 0.64-0.72 (0.68) mm: a = 29.2-33.4 (31): b = 4.5-5-5(4.9): c=13.3-15.5 (14.2): c'= 3.0-3.7 (3.3): V- 52.0-55.8 (54): Stylet = 17.0-17.8 (17.5) µm; annules between head and excretory pore = 60; tail annules = 22-28

Description

Female: Body cylindrical, curved ventrally forming 'c' shape, cuticle coarsely annulated. Lip region continuous, in some specimens slightly offset, bearing 3 annules. Stylet 16µm with rounded basal knobs. Oesophageal bulb slightly overlapping intestine in some specimens. Vulva transverse; vagina half way into the body: ovaries outstretched. Spermatheca absent; oocytes arranged in a single row; rectum overlapped by intestine.Tail sub cylindrical, bearing 20-25 annules with rounded and smooth terminus. Phasmid at middle of tail.

Remarks

Specimens of T. annulatus were found in abundance around the roots of wheat and maize plants from CDRI, mooti baag field and NNRC nursery, University of Karachi, Karachi, Pakistan.

Hoplolaimus indicus

Measurements

Female: n=15; L = 1.02-1.25mm; a = 22-29; b = 8.4-9.0; b' = 7.8-8.1; c = 51-67; c' = 0.66-0.68; V = 50-53; spear =33-43µm.
Male: n = 10; L = 0.94-1.09mm; a = 31-36; b = 8.9-9.9; b' = 7.2-8.4; c = 31-36; spear = 30-38µm; gubernaculum = 12-20µm; spicules = 38-48µm.

Description

Female individuals of this species exhibit an elongate-cylindrical body shape, typically assuming a C-shaped posture when relaxed, with a tapering anterior section extending beyond the esophagus to a distinctive lip region. The lip region appears hemispheroid and is characterized by 3-4 discernible annules, with basal annules displaying 6-12 longitudinal striations. The cephalic framework is notably sclerotized, featuring a hexaradiate structure, where lateral arms are bipartite while subdorsals and subventrals remain undivided. The spear is robust, often adorned with 1-3 forwardly directed processes on its knobs, with the conus comprising approximately half the length of the spear. The dorsal oesophageal gland opens 3-4 µm behind the base of the spear. Positioned centrally within the esophagus, the median oesophageal bulb is spheroid, measuring 19 × 16 µm, and hosts a well-developed central vulvular apparatus. The oesophageal glands extend dorsally and laterally, overlapping the intestine, with the anterior most dorsal gland housing 4 nuclei lying in a single plane when observed laterally, while the nuclei of the two subventral glands lie in two planes, albeit the lower one may be less distinct. The excretory pore is located 115-130 µm from the anterior end, situated above the level of the oesophago-intestinal valve. The hemizonid, typically comprising 6 annules (with variations observed in some individuals), is positioned behind the excretory pore, extending over 2 annules; sometimes appearing doubled with a hemizonion. The anterior phasmid is located on the right side, while the posterior phasmid is on the left side of the body. The nerve ring is situated 20-25 µm anterior to the excretory pore. The cuticle displays coarse annulations. The lateral field is represented by a single incisure, which becomes indistinct over most of the body, even in cross-sections. The epiptygma, if present, is typically single and anterior, occasionally double. A spermatheca containing sperm is usually present, with the ovary slightly obscured by intestinal globules. The intestine partially overlaps the rectum, with the rectum constituting approximately one-third of the anal body.

Remarks

Specimens of *H. indicus* were found in abundance around the roots of maize plants from mooti baag field and NNRC nursery, University of Karachi, Karachi.

Hemicriconemoides mangiferae

Measurements

Female: (n = 10); L = 0.47-0.51mm; a = 15-20; b = 4.9-7.1; c = 19-24; V = 92-93; spear = 65-72 µm; R = 125-137; RV = 10-13; RVan = 3-4; Ran = 7-9.

Description

Female: Body cylindrical tapering towards extremities, slightly arcuate when fixed, enclosed in a cuticular sheath which is attached at head end, excretory pore, vulva, anus and sometimes at tial end; maximum width of body and sheath c. 27 µm and 30 µm respectively. Sheath annules smooth but sometimes with numerous longitudinal indentions, 3-4 µm wide near mid-body; lateral fields absent. Total number of sheath or body annules 133-148 but may vary from 111 to 152. Lip region anteriorly flattened, with two annules of which the anterior is angular and directed outward, and the other disc slightly set off or inconspicuous, rounded on top. Spear 70-81 µm long but may be 58 to 86 µm long in other population; its conus about 87% of total stylet length; basal knobs anteriorly directed, anchor-shaped, 5-7 µm across and about 3 µm high. Orifice of dorsal oesophageal gland 5-7 µm behind spear base. *Oesophagus criconematoid* with enormous median bulb 14-16 µm wide, continuous with wide precorpus, isthmus short and broad, enveloped by nerve ring; basal bulb also short, slightly wider than isthmus, rounded at base. Excretory pore on 34-38th annule from anterior end. Vulva 11 µm wide transverse slit, usually on 12-15th annule from tail terminus; no lateral vulval flaps or vulval sheath. Vagina directed inward and forward. Ovary single, anteriorly, outstretched, may extend to oesophagus, with single row of oocytes except in multiplication zone. Spermatheca small, offset, usually with small, rounded sperms. Uterine eggs 67-71 × 16-18 µ. Anus on 3rd to 5th annule behind vulva, on 7-11th annule from tail tip. Tail conoid with terminal annule smoothly rounded or subcylindrical with hemispherical, annulated tip.

Remarks

Specimens of *H mangiferae* were found in abundance around the roots of wheat from CDRI, University of Karachi, Karachi, Pakistan.

Genus Heterodera

Description

Mature female and cyst: lemon-shaped with a short neck and terminal cone, turns into a hard-walled cyst, brown to black in color, with a lace-like or zig-zag pattern. D-layer in cuticle rudimentary or absent; sub crystalline layer present or absent. The vulva is situated terminally, marked by a slit measuring 10-60 µm in length. The anus is dorsally subterminal, positioned near the vulva but not directly on the vulval lip. Vulval fenestration is observed, typically ambifenestrate, bifenestrate, or exceptionally circumfenestrate; however, anal fenestration is absent. A vaginal remnant is commonly found beneath the bridge and bullae. While the majority of eggs are retained within the body, a portion may be laid in a gelatinous matrix.

Male

Each lateral field exhibits four incisures, with the outer band frequently showing areolation. The cephalic region typically displays an offset by constriction, bearing three to six annules; the labial disc is usually indistinct, and the basal lip annule may or may not be indented. The tail end is twisted, and the spicules are robust, measuring over 30 µm in length, often with a blunt bifid or single tip.

Second-stage juvenile

The body is slender, ranging from straight to arcuate in shape. The cephalic region typically appears continuous, with an indistinct labial disc, which appears elongated oval under SEM examination. The stylet measures less than 30 µm in length. Oesophageal glands occupy the width of the body cavity. The tail features a prominent terminal hyaline part. Phasmids are punctiform and lack a lens-like structure underneath in the muscle layer. Feeding induces the formation of a syncytium.

Remarks

Specimens of Heterodera spp. were found in abundance around the roots of wheat and maize plants from CDRI, mooti baag field and NNRC nursery, University of Karachi, Karachi, Pakistan.

Genus Aphelenchus

Description

Nematodes of medium to fairly long size (0.5-1.2 mm) exhibit characteristics such as a heat-relaxed form ranging from nearly straight to ventrally arcuate. Their cephalic region is modest in height, rounded, and slightly displaced. The cuticle displays fine annulations, while the lateral fields show a considerable number of incisures, often exceeding ten, though occasionally fewer. The stylet is slender, with minor swellings at its base. The procorpus appears cylindrical, tapering just before joining the large, ovoid median bulb, which houses crescentic valve plates centrally positioned. Oesophageal glands are situated within a dorsal lobe, with the nerve ring located just behind the median bulb, approximately opposite the excretory pore. The vulva is positioned posteriorly at around 70-80% of the body length, with slightly protruding vulval lips and the body often narrowing sharply just behind the valva. The vagina slopes anteriorly. The genital tract is monoprodelphic and stretched out, with developing oocytes typically arranged in a single row. A post-uterine sac extends for up to half the distance between the vulva and anus. The tail is short, cylindrical, occasionally slightly ventrally concave, terminating in a broadly rounded terminus. In males, when present, paired slender spicules are ventrally arcuate, slightly cephalated proximally, with a linear gubernaculum. A well-developed bursa extends from the proximal region of the spicules to the tail tip, supported by four (occasionally three) pairs of ribs, one pair preanal and the remaining three forming a subterminal group. The male tail is short, conoid, tapering to a narrowly rounded point.

Remarks

Specimens of Aphelenchus spp. were found in abundance around the roots of wheat and maize plants from CDRI, mooti baag field and NNRC nursery, University of Karachi, Karachi, Pakistan.

Genus Tylenchus

Description

This species exhibits small to medium dimensions ranging from 0.4 to 1.3 mm, with a ventral curvature observed upon relaxation. The cuticle appears moderately thick, measuring between 1 to 2 µm, and displays distinct annulations. The lateral fields feature four incisures each. Prophasmids are situated dorsosublaterally, postmedianly, just behind the vulva. The cephalic region maintains continuity and displays annulations; however, sclerotization is either light or absent within its framework. The stylet measures between 8 to 21 µm in length, with the conus constituting about half or more than one-third of the total stylet length. The knobs are either round or posteriorly sloping. The median oesophageal bulb is oval and muscular, positioned anterior to the middle of the oesophagus, with the basal bulb being pyriform. A distinct cardia is present, and the excretory pore typically lies opposite to the basal bulb. Deirids are situated just behind the level of the excretory pore. The vulva appears as a transverse slit, usually positioned at 60-70% of the body length, with unmodified lips and the absence of epiptygma or lateral membranes. The vagina typically forms a right angle to the body axis. The postvulval uterine sac is approximately a body width or less in length. The spermatheca is round to oval and offset, while the ovary is outstretched. The tail is ventrally arcuate, often hooked, and consistently tapers to a pointed or minutely rounded terminus. The bursa is adanal with crenate margins. Spicules are cephalated and arcuate, measuring between 13 to 25 µm in length. The gubernaculum is simple and fixed. Cloacal lips are slightly raised, with the anterior one pointed and the posterior usually rounded rather than tubular. Hypoptygma is absent.

Remarks

Specimens of Tylenchus spp. were found in abundance around the roots of wheat and maize plants from CDRI, mooti baag field and NNRC nursery, University of Karachi, Karachi, Pakistan.

Genus Pratylenchus

Description

Anterior sexual dimorphism is not prominently expressed. Each lateral field bears between four to six incisures, occasionally exhibiting oblique median markings. Deirids are not present, while phasmids are located near the middle of the tail. The cephalic region is characterized by a low profile, flattened anteriorly, or rarely rounded, seamlessly blending with the body contour. Sclerotization is robust, with the labial disc being inconspicuous. The stylet measures 20 µm or less in length, featuring round, anteriorly flat, or slightly basal knobs. The median bulb appears oval to round and exhibits significant muscularity. The esophagus typically spans less than two body widths, primarily ventrally over the intestine. The vulva is situated in the posterior region, usually at 70-80% of the body length. The reproductive system is pseudo-mono-prodelphic, with only the anterior ovary functional. A postvulval uterine sac may be present, with or without rudiments of the posterior ovary. The spermatheca is notably large, rounded, and usually axial. The female tail ranges from subcylindrical to conoid, typically measuring about two or three body widths in length. The terminus is smooth or annulated, lacking a distinct process or mucro. The bursa encloses the tail terminus. Spicules feature a subterminal pore on the dorsal side, while the gubernaculum is simple, trough-like, and fixed.

Remarks

Specimens of *Pratylenchus* spp. were found in abundance around the roots of wheat and maize plants from CDRI, mooti baag field and NNRC nursery, University of Karachi, Karachi, Pakistan.

Discussion

Plant parasitic nematodes feed on plant organs by puncturing the plant cells with a hollow, needle-like structure called a stylet. Digestive enzymes are injected into the cells, and the predigested cellular contents are sucked-up by the nematodes. Damage to roots is the most important effect of plant parasitic nematodes (Vieira & Gleason, 2019). The roots may be killed or stunted, which in turn, may result in poor and shallow root systems. The above ground symptoms of a nematode attack include chlorosis (yellowing) of the leaves, slow growth, gradual thinning, poor response to adequate fertilization and irrigation, rapid wilting during dry weather and weed invasion. The similar symptoms appear in periods of hot weather, drought, low fertility and other stresses. However, the estimate of crop losses due to nematodes is a matter of debate (Hartman et al., 2015). The most comprehensive estimate in this respect was obtained in a survey conducted in 1986 which incorporated the views of 371 nematologists belonging to 75 countries. According to this survey, estimated nematode damage to specific crops ranged from 3.3 to 20.6%, with a mean of 12.3%. Annual production losses at the farm gate were estimated up to US$ 121 billion globally and US$ 9.1 billion in the United States only. Developing nations reported greater yield loss percentages than did developed countries. Yield reduction in specific crops by nematodes can exceed 75% in some locations. Under these conditions, growers are commonly forced to select less profitable crops due to nematodes' threat (Sasser & Freckman, 1987). Besides direct crop losses, nematodes can vector many plant viruses or create wounds that allow the entry of other root pathogens which may, in turn, result in low and unsafe food production (Ghaderi & Karssen, 2020).

The Cereal Cyst Nematode (CCN) stands out as the most widely acknowledged and economically significant nematode affecting wheat cultivation globally. The CCN complex encompasses a group of twelve officially recognized species alongside several yet-to-be-described ones (Jones, 1981). Among these, *Heterodera avenae*, *H. filipjevi*, and *H. latipons* emerge as the primary species with substantial economic impact. One of the complexities associated with CCN is the presence of pathotypes, complicating efforts towards genetic control. Early in the growing season, above-ground symptoms induced by CCN manifest as pale green patches, varying in size from 1 to over 100 square meters ((Dababat & Fourie, 2018). Affected plants typically display yellowing lower leaves and tend to have fewer tillers. These symptoms can easily be mistaken for nitrogen deficiency or poor soil conditions. Additionally, the root damage caused by CCN exacerbates the impact of other abiotic stresses such as water or nutrient deficiencies. Below-ground symptoms may vary depending on the host plant. In wheat attacked by *H. avenae*, there is an increase in root production, resulting in a 'bushy-knotted' appearance, often with multiple females visible on each root (Dababat et al., 2014). Initially, the cysts appear glistening white-grey, later turning dark brown as they mature. Root symptoms typically become noticeable within one to two months after sowing in Mediterranean climates, and sometimes later in more temperate regions (Greco et al., 2002).

Maqbool et al. (1984) reported *Hoplolaimus columbus, H. dimorphus, H. indicus* and *H.seinhorsti* from wheat crops of Pakistan. Maqbool (1992); Maqbool & Zaki (1992) reported different species of nematodes on wheat crop which include *Aphelenchus avenae, Filenchus filiforims, Helicotylenchus dihystera, Hoplolaimus indicus, Pratylenchus penertrans,* *Criconemoids* spp. *Tylenchorhynchus* spp, and *Tylenchus* spp and *Longidorus* spp. General survey and identification of nematodes diseases in Sindh region Solangi (1981) reported *Tylenchus* spp., *Hoplolaimus* spp. and *Aphelenchus* spp. were present in the roots of the wheat crop samples. Zarina et al. (2015) reported the effect of six different cropping sequences on the soil population of *Tylenchus filformis* and found the wheat crop was the most effective in reducing population. Anwar (1989) reported *Helicotylenchus* spp., *Paratrichodorus* spp., *Pratylenchus* spp. and *Rotylenchus* spp. from wheat crops.

Maize is the largest producing field crop of the world and third largest crop of Pakistan. It is grown in all provinces specially Punjab. Following nematodes commonly associated to maize crops. Anwar et al. (1973) reported *Pratylenchus* spp. from maize and sorghum as predominant nematodes. Anwar (1989) investigated the different nematodes species including *Heterodera* spp. and *Ditylenchus* spp. and also reported *Ditylenchus dipsaci* from wheat and maize. *Meloidogyne javanica* also parasitize on maize was reported by Gul & Saeed (1990). Gul & Saifullah (1991) survey in KPK and reported different plant parasitic nematodes which severely affected maize and other field crops and they reported *Aphelenchus avenae, Helicotylenchus digonicus, Hoplolaimus geleatus, Heterodera avenae, Tylenchus butteus, Tylenchorhynchus mashhoodi* and *Xiphinema* spp. Hashmi and Hashmi (1989) studies the different hosts including maize for the purpose of economic importance of *Pratylenchus zeae* in Pakistan. *Hemicriconemoides cocophilus* found associated to maize crop was reported by Hussain & Yasmin (1976). Maqbool (1981) reported *Heterodera avenae, H. zeae* and some other cyst nematodes species along with *Meloidogyne arenaria* and *M. incognita* from maize and other field crops from Pakistan. Maqbool and Saeed (1981) reported on root knot nematodes (*Meloidogyne incognita* & *M. javanica*) on maize crop in Pakistan. reported *Merlinius brevidens, M. microdorus, M. nanus* and *Tylenchorhynchus brassicae* of Tylenchorhynchinae in Pakistan. Maqbool (1986) reported *Aphelenchoides besseyi, A bicaudatus, Ditylenchus dipsaci, Heliotylenchus digonicus, Heterodera avanae*. Maqbool (1988) study nematodes problem and reported *Aphelenchus besseyi, Ditylenchus dipsaci, Helicotylenchus dihystera, Hoplolaimus geleatus*. (Maqbool, 1992; Maqbool & Zaki 1992) reported *Aphelenchoides besseyi, A. bicaudatus, Aphelenchus avenae, Ditylenchus dipsaci*.

Maqbool & Shahina (1985) reported two new species of genus *otholenchus* from maize in Pakistan. *Bitylenchus brevilineatus* was reported on maize by Maqbool & Shahina (1987) from northern areas of Pakistan. Maqbool & Ghazala (1988) reported *Hoplolaimus galeatus* from Pakistan. Maqbool et al. (1990) reported *Boleodorus azadkashmirensis* from maize field Pakistan. Maqbool & Shahina (1995) reported *Helicotylenchus egyptiensis* and *Tylenchorhynchus annulatus* on maize. Host range of *Heterodera zeae* & *H avenae* studies by Shahina & Maqbool (1990). Shahina & Maqbool (1993) studied the effect of soil type, morphology, distribution and host range of *Heterodera cynodontis*. Shahina & Maqbool (1990) reported corn cyst nematodes and cereal cyst nematodes in Pakistan. Nasira & Maqbool (1992) reported stunt nematodes of Pakistan. Shahina & Maqbool (1995) reported cyst nematodes of Pakistan. Solangi (1981) reported *Aphelenchus* spp. *Trichodorus* spp. and *Pratylenchus* spp. during survey and identification of nematodes diseases in Sindh region. Ahmed and Qasim (1990) conducted a survey about maize cyst nematodes in KPK and northern areas. Kafi (1963) reported *Tylenchorhychus* spp. on maize crop.

Conclusion

The present research study revealed the presence of various plant parasitic nematodes, alongside free-living nematodes. The identified plant parasitic nematodes included *Helicotylenchus egyptiensis, Longidorus pisi, Tylenchorhynchus annulatus, Hoplolaimus indicus, Hemicriconemoides mangiferae,* Heterodera spp., Pratylenchus spp., Rotylenchulus spp., Aphelenchus spp., and Tylenchus spp. Morphological characterization of these nematodes exhibited varying characteristics in terms of body shape, size, stylet morphology, and reproductive systems. Additionally, the survey revealed the abundance of these nematodes around the roots of wheat and maize plants across different fields and nurseries in Karachi, indicating their potential impact on crop health and productivity. Overall, this study emphasizes the importance of understanding the diversity and distribution of plant parasitic nematodes in agricultural fields, which is essential for implementing effective management strategies to mitigate their detrimental effects on crop yields. Further research focusing on the ecological interactions and management strategies specific to these nematode species would be valuable for sustainable crop production in the region.

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