



HISTOPATHOLOGICAL AND MORPHOLOGICAL STUDIES OF ROOT KNOT NEMATODE *MELOIDOGYNE* SP. ON TURFGRASS (*CYNODON DACTYLON*)

Ayoub Ibrahim Ahmed¹ and Chnar Najmaddin Fathulla²

¹Depart. of Plant Protection, Khabat Technical Institute, Erbil Polytechnic Univ., Erbil, Kurdistan Region, Iraq.

²College of Science, Salahaddin University.

Abstract

The turf grass of *Cynodon dactylon* that collected from Technical Institute Khabat-Erbil- Kurdistan region-Iraq naturally infected by the nematode (*Meloidogyne* sp.) was fixed, then sectioned and examined by the microscope. Infective juveniles of root-knot nematode, the nematode penetrated and moved within the root. Giant cells (feeding sites) were always associated with xylem tissue. The giant cells observed into the pericycle were considered by a dense cytoplasm, hypertrophy, and thicker cell walls. The second stage juvenile induces the redifferentiation of four to seven parenchymatic root cells into a multinucleate and hypertrophied feeding cell. The nematode (*Meloidogyne* sp.) cause histological changes in host plant root tissue in the form of infected cells and making swollen on the roots reflecting their symptoms.

Key words: turf grass, root knot, *Meloidogyne*, histopathology

Introduction

Natural grasslands protected millions of hectares of land throughout the world, providing sustenance to vast numbers of wild animals (Klein *et al.*, 2007). Turf grass is the most generally planted among the ornamental plants in the biosphere, helping vital resolutions in soil support and presenting safe surfaces for recreational events (Zeng *et al.*, 2012). Nematodes are phytoparasitic worms which are significant factor affecting on the health, quality, production, and maintenance of turfgrass (McClure *et al.*, 2012). Mitkowski (2001) pointed that the phytoparasitic nematodes can cause the significant damage on many turfgrasses. However, the diagnosis of parasitic plants such as nematode symptoms is still difficult to diagnose and control of these plant parasites plant can be consuming of time, expensive and difficult to control (Mitkowski, 2001). Root knot nematodes (RKN) are slowly becoming more predominant on turfgrass. These tiny nematodes can be mostly damaging to turf because of the nematodes have close relationship with plant hosts (Mitkowski, 2001). RKN, *Meloidogyne* spp., are

sedentary, endoparasitic nematodes that relate with their hosts in an interesting way. These obligate parasites have evolved the ability to manipulate host functions to their own benefit (Abad *et al.*, 2009). Root knot nematode such as *Meloidogyne* species cosmopolitan distributed around the world. Root-knot nematodes tempt their separation of parenchyma root cells into multinucleate and hypertrophied feeding cells, called giant cells. These giant cells constitute the exclusive source of nutrients for the developing nematode. Hyperplasia of the surrounding cells leads to the formation of the typical root gall, the primary visible symptom of infection. Like other plant-parasitic nematodes, root-knot nematodes have a spear, a hollow retractable needle connected to the pharynx, and three unicellular pharyngeal glands (Abad *et al.*, 2009). The infection and development of the second stage juveniles of the root knot nematode inside the root tissues which is the result of histological changes in the form of giant cells (feeding sites), (Ekanayake *et al.*, 1988). The aim of this study was to investigate the histological and morphological changes of the infected root of the *Cynodon dactylon* in the host plant.

Materials and Methods

Sample collection

The forty five samples were collected randomly in Khabat institute from April to June 2017 to investigate the infected root knot nematode as described by (Anwar *et al.*, 2013).

Staining root samples

According to Southey (1986) The root samples cut into small pieces of approximately 1-2 cm and then roots were stained with acid fuchsine lacto phenol and put the small roots put in hot (80°C) lacto phenol – acid fuchsine with forceps and left in this solution for 1-3 minutes, allowing the stain to penetrate the material. The excess stain with water and transferred to pure lacto phenol to remove the stain from the plant tissue. The root samples were left in the lacto phenol for 24 hours. The roots placed in a Petri dish filled with 1:1 glycerine/water and observed under the dissecting microscope.

Paraffin Method

The pieces of samples have been mixed in FAA (Formalin-Acetic-Acid-Alcohol) solution, prepared as a mixture of (90ml of 70% alcohol, 5ml of glacial acetic acid and 5ml of formalin) for 24hr. After that, the samples have been dehydrated using series concentrations of alcohol (95%, 100% and 100%) for 1hr. for 95% and 3-4 hours twice, for another concentrations, after that the samples were placed in xylene for 3-4 hrs. (Twice). After that the samples were embedded in a mixture of xylene and paraffin (1 xylene and 1 paraffin at 60°C) for 30 min. twice, then were transferred to pure paraffin and left at 60°C overnight. After that preparation of paraffin blocks were made and sections were prepared with the thickness of 8 micrometer using the rotary microtome. The sections were then stained using safranin (1%) and fast green or light green (1%). Finally the sections were mounted by Destrin Plastisizer Xylene (DPX) (Najmaddin and Mahmood, 2016).

Plastic method

The pieces of the samples were fixed in 2.5% glutaraldehyde for 12 hrs. The samples were post-fixed by 1% Osmium tetra oxide for two hours, then washed by distilled water, then dehydrated by using different concentration of alcohol and clearing by acetone. Infiltration has been done by using Araldite mixture in which the samples were embedded, then the samples sectioned by ultra-microtome (Reichert-Jung) (Japan), and they were stained by 1% toluidine blue (Ruzin, 1999).

Results and Discussion

This investigation shows the morphological and

histological changes and observations of infected turfgrass by second stage juvenile of root knot nematodes (Fig. 1 and 2). This indicate that the *Meloidogyne* sp. had reached the hypodermis, cortex and the root tips they inducing slightly root swelling. Formation of feeding cells in the perimeter (surrounding or environment) of, and also inside, the vascular cylinder was evident (Fig. 3). Cortical parenchyma proliferation, asymmetry of the stylet and formation feeding site giant cells induced by the swollen juveniles were observed (Fig. 4 B and D) resulting in swelling of roots compared with non-infected plants (Fig. 4 A and C). Giant cells had uniformly dense cytoplasm and contained several small nuclei, were usually concentrated in the centre of the cell. The number of giant cells at each feeding site varied from four to five



Fig. 1: Infection field, by *Meloidogyne* sp.



Fig. 2: *C. dactylon* Infected by *Meloidogyne* sp.

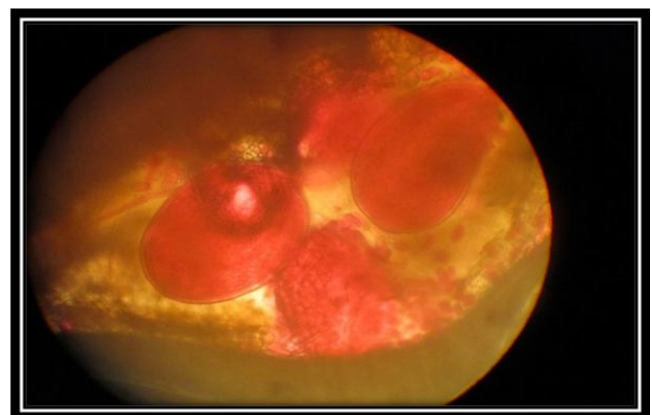


Fig. 3: Stained Roots of *C. dactylon*, stained by acid fuchsine showed the root knot nematodes with egg mass.

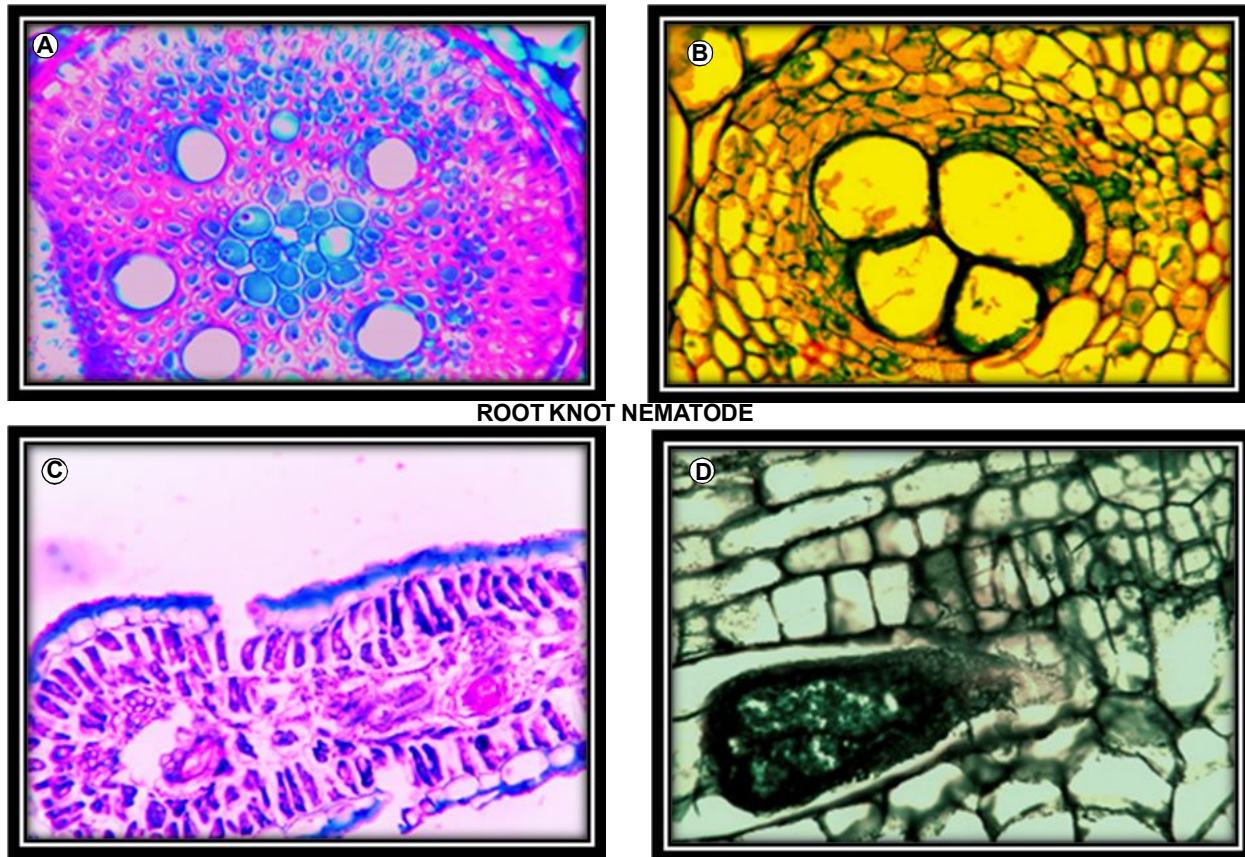


Fig. 4: Anatomical changes induced by *Meloidogyne* sp. on turfgrass roots. A- cross section of non-infected root B- cross section of infected root showing giant cells C- Longitudinal section of non-infected root D- Longitudinal section of infected root showing swollen nematodes, using paraffin methods

giant cells surrounded by a thickened cell wall. At each infection site the vascular system was enlarged because of hyperplasia and hypertrophy of the vascular tissue. Only the head and neck of nematode entered the vascular system (Fig. 5 B, D) the remaining oval shaped body of female in cortical tissue of the root. Root-knot nematodes are obligate and cosmopolitan pathogens that can only feed on living cells. They establish and preserve close relationship with their host plants. Within the root vascular cylinder, second stage juveniles induce the dedifferentiation of root cells into giant cells, which represent specialized feeding cells. Fully differentiated giant cells contain more than 100 polyploid nuclei, which have also possibly undergone extensive endoreduplication (Abad *et al.*, 2009). Giant cells may reach very big size compare to individual root vascular cells. In addition, giant cells show an increase in cytoplasmic density and a loss of normal vacuolization (Abad *et al.*, 2009). The presence of swelling on roots is a primary symptom associated with root knot nematode infection. Root knot nematodes stimulate formation of root swelling which interferes with plant water supply, resulting in stunted and chlorotic growth (Waller *et al.*, 2002; Noling, 2009). Our

investigation is similar to the results of some researchers such as Heald, 1969; Fawole, 1988; Ekanayake *et al.*, 1988; Rosso, 2004; Akhtar, 2015 but they worked on plants different of that of ours caused giant cells in vascular system of roots.

Conclusions

Results observed that *Meloidogyne* sp. was parasite and made specific nutrition site on *Cynodon dactylon*. Moreover, root knot nematode affected on the turf grass specific sit for feeding inside vascular system with several giant cells around the anterior body. Second stage juveniles penetrated to xylem tissue and made hyperplasia and hypertrophy to the cells.

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References

- Abad, P., P. Castagnone-Sereno, M.N. Rosso, J.D. Engler and B. Favory (2009). Invasion, feeding and development. In: Abad PMoens MStarr JL, eds. Root-knot nematodes.

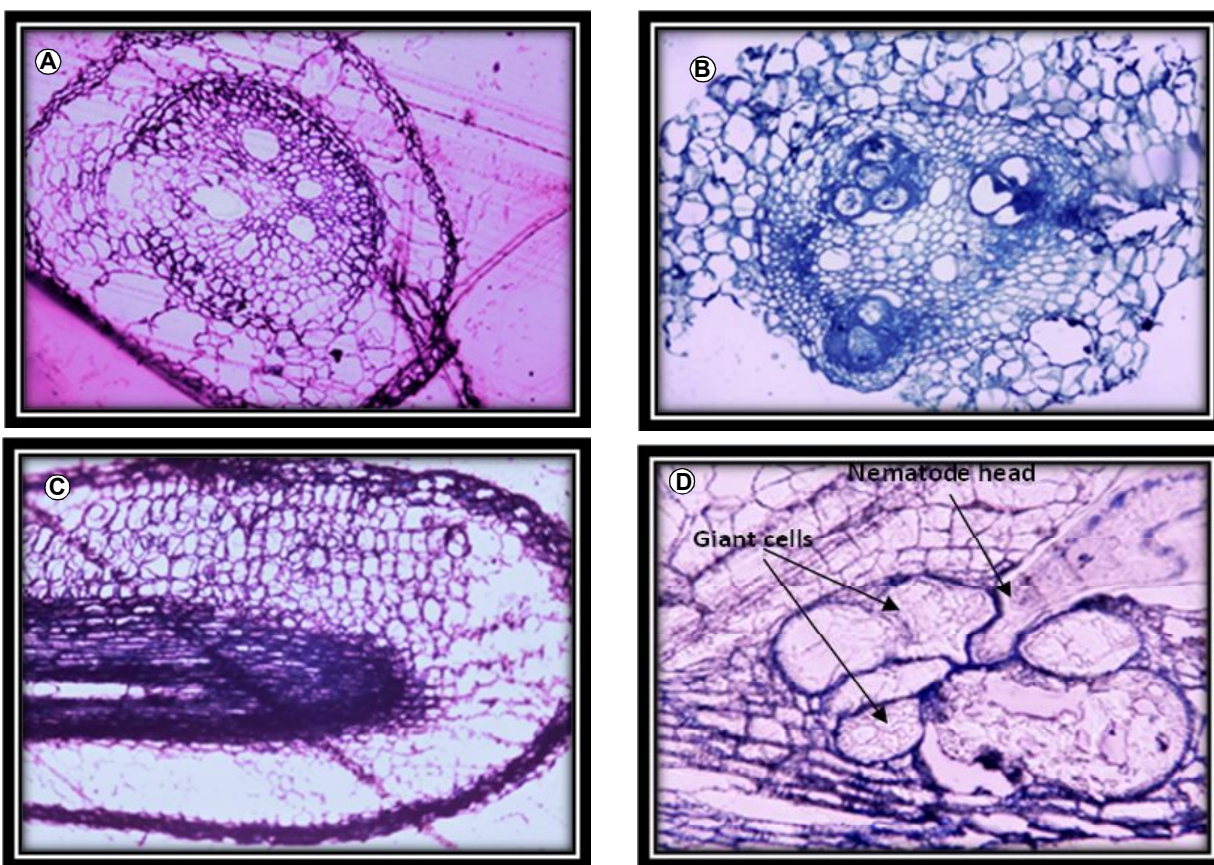


Fig. 5: Anatomical changes induced by *Meloidogyne* sp. on grass roots. A- cross section of non-infected root B- cross section of infected root showing giant cells C- Longitudinal section of non-infected root D- Longitudinal section of infected root showing giant cells with nematode heads , using plastic methods

Cambridge: CABI International Press, 163–181.

Akhtar, A. (2015). Studies on the Histopathological Responses of *Vigna Mungo* towards *Meloidogyne incognita* at Different Time Intervals. *Plant Pathology & Microbiology*. ISSN: 2157-7471.

Anwer, S.A., M.M. Mahdi, M.V. Mckenry and A. Qadir (2013). Survey of plant-parasitic nematodes associated with four vegetable crops cultivated within Tunnels. *Pak. J. Zool.*, **45(3)**: 595-603.

Ekanayake, H.M.R.K., M.D. Vito and N. Vovlus (1988). Histopathological changes caused by *Meloidogyne incognita* on tomato and egg plants roots. *Tropical Agriculturist*, **144**: 89-97.

Fawole, B. (1988). Histopathology of root-knot nematode (*Meloidogyne incognita*) infection on white yam (*Dioscorea rotundata*) tubers. *Journal of nematology*, **20(1)**: (pp. 23-28).

Heald, C.M. (1969). Pathogenicity and histopathology of *Meloidogyne graminis* infecting 'Tifdwarf' Bermudagrass roots. *J. Nematol. l.*, **1**: (pp. 31-34).

Klein, M.G., P.S. Grewal, T.A. Jackson and A.M. Koppenhöfer (2007). Lawn, turf and grassland pests. In *Field manual of techniques in invertebrate pathology* (pp. 655-675). Springer Netherlands.

McClure, M.A., C. Nischwitz, A.M. Skantar M.E. Schmitt and S.A. Subbotin (2012). Root-knot nematodes in golf course

greens of the western United States. *Plant disease*, **96(5)**: (pp. 635-647).

Mitkowski, N. (2001). Root-knot nematodes on turf in the northeastern United States. *Turfgrass Trends*, **10**: 1-4.

Rosso, L., P. de Candida, N. Leonetti and A. Ciancio (2004). Alteraciones histopatológicas causadas por *Meloidogyne incognita* en almendro (*Prunus amygdalus*). *Nematropica*, **34**: (pp. 257-261).

Najmaddin, C. and B.J. Mahmood (2016). Anatomically and palynologically studies of some *Carthamus tinctorius* genotypes. *Inter. Jo. Bio. Sci.*, **3(1)**: (pp. 1-13).

Noling, J.W. (2009). Nematode management in tomatoes, peppers and eggplant. <http://edis.ifas.ufl.edu/ng032>.

Ruzin, S.E. (1999). *Plant Microtechnique and Microscopy*. Oxford: Oxford University Press.

Southey, J.F. (1986). *Laboratory methods for work with plant and soil nematodes*. HMSO.

Waller, J.M., J.M. Lenne and S.J. Waller (2002). *Plant Pathologist's Pocketbook*. 3rd ed. CABI publishing, Wallingford.

Zeng, Y., W. Ye, S.B. Martin, M. Martin and L. Tredway (2012). Diversity and Occurrence of Plant-parasitic Nematodes Associated with Golf Course Turfgrasses in North and South Carolina, USA. *Journal of Nematology*, **44(4)**: (pp.337–347).