Research Article

Histopathological Modifications in *Musa cavendishii* Roots Induced by *Radopholus similis* and *Meloidogyne incognita*

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Abstract | Several plant-parasitic nematodes have been associated with banana (*Musa cavendishii)* and some of the most economically important ones are *Radopholus similis* and *Meloidogyne* spp. the purpose of this study is mainly to clarify the pathogenic effects of both nematodes (*Radopholus similis* and *Meloidogyne incognita*, alone or in combination), as a histological modification in root tissues under natural conditions of banana cultivation. *R. similis* was observed in the cortical parenchyma as a feeding site, and for the first time, the feeding site extended to the vascular cylinder. In roots infested with *M. incognita* only, the laceration of the feeding site in the inner cortical tissue and spread to the vascular cylinder were selected as the initial feeding site, as shown. Multinucleate cells, giant cells, and thickening of cell walls were also associated with this penetration. The cytoplasmic globules as phenolic and lignified structures were shown to be associated with the feeding sites of two species. In combination with two species, the effects of *M. incognita* were noted more than those of *R. similis*. Where showed *R. similis* was protruding out or was inside the feeding site of *M. incognita*. In addition, *R. similis* was deepened within the root more than usual, with a lower number of phenolic and lignified cells. The obtained results can form the basis for understanding of the nature of nematode parasitism and be used as a basis for the establishment of field experiments that allow the creation of sustainable management strategies to suppress nematodes in infested fields.

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Introduction

Nematodes represent one of the largest group of the invertebrates, occurring in earthen and hydrous habitats, they can be parasites in animals and plants or free-living (Van Den Hoogen et al., 2019). The plant-parasitic nematodes are associated with nearly every important agricultural crop and are responsible for an approximately 10% reduction in agricultural production worldwide (McCarter, 2008). The banana is one of the most important fruits in the tropical and subtropical regions (Mohamed *et al*., 2011), however, its productivity can be affected by several pathogens; fungus and plant-parasitic nematodes. Several plant-parasitic nematodes have been associated with bananas, however, only a few species cause significant losses, among which we can mention: *Radopholus similis* and *Meloidogyne* spp. The economic losses induced by plant-parasitic nematodes on the global banana production were evaluated at about 20% (Sasser and Freckman, 1987; Jonathan and Rajendran, 2000). However, some species can cause 100% loss, depending on environmental conditions, the nematode population and the used banana cultivar (Ritzinger et al., 2007).

These nematodes are important for the damage they can cause, causing symptoms very similar to nutritional deficiency, since it hinders the absorption of water and nutrients by plants, which fundamental requirements for their growth and production (Speijer and Ssango, 1999). They can also cause negative effects on the size, shape, and structure of roots (Kajumba and Speijer, 1996). In this way, plants can have different side effects that do not consistent with the healthy plant's standards (Abawi and Widmer, 2000). The types of plant-parasitic nematodes differ in the lifestyle and the feeding sites through seven major types based on the formation of the feeding site in tissues of roots.

The species under study are divided as endoparasite; sedentary, *Meloidogyne incognita* and migratory *Radopholus similis*. Where the larvae penetrate into the tissues of the roots to settle inside in certain sites called feeding site and that in the static sedentary types, while in the migratory they feed on many neighboring cells and continuous penetration of tissues occurs causing many histological, chemical, and physiological damage (Luc *et al*., 2005).

There are several reports on the biology and host-parasite relationships of *R. similis* and *M. incognita* on banana (Oramas et al., 2006; Nithya-Devi et al., 2009), but study is lacking on the anatomical alterations induced on this host plant by either parasite alone or in combination. This study reports and illustrates the histological effects caused by the plant-parasitic nematodes; *Radopholus similis* and *Meloidogyne incognita* on alone or in combination on banana roots, cv. Hindi as the most common cultivar in Egypt.

Materials and Methods

The banana roots infected with and *Radopholus similis* and *Meloidogyne incognita* alone or combination will were collected rhizosphere of the banana farms (as split plots experiment covered a total area of 100 m^2, in a randomized complete block design with four replicates per treatment.) in cv. Hindi at age range 1-3 year from Assiut Governorate, near the coast of the Nile River and for a distance of 10 km which represented the one of the largest commercial production areas for banana in Upper Egypt.

Roots kipped in polyethylene bags and directly sent to the laboratory for examination. Fresh roots were carefully washed from soil. Roots cut into 3-5 mm long sections. Then fixed in FAA solution (2.4 parts formalin as 37% formaldehyde, 1.6 parts acetic acid, 60 parts ethanol 95 % and 80 parts of distilled water) (Johansen, 1940; Southey, 1986).

The root sections were dehydrated in tertiary butyl alcohol (TBA) depending on the protocol described by Johansen (1940) and Goodey (1949). After the dehydration process, the solution was replaced with a mixture of butyl alcohol and paraffin oil (PO) at (1:1) for one hour or more, according to the roots thickness. The root segments picked up from the previous mixture, and placed on the surface of the consolidated PO solution and placed disclosed in the oven at a bit above liquefying point of the paraffin. After two hours, the BA- PO mixture was poured down and replaced with pure melted paraffin wax and kept in the oven for two hours (Finley, 1981).

The root tissues were placed in molds and added the liquid paraffin until rises above the tissues. When the paraffin began to coherence plunged into the freezer until the solidification. The blocks were dropped from the molds and sectioned 10-12 µm thickness by rotatory microtome. Then, the paraffin strips were placed on glass slides and kept in the incubator at 40 °C until the water was evaporated. The paraffin wax was removed by placed the slides at 60 °C for about one hour until the melted of the wax. Staining process was done by using the safranin and fast green according to the protocol described by Johansen (1940) and Sass (1951).

On the slides, the mounting medium was applied and was covered carefully. The slides were placed in

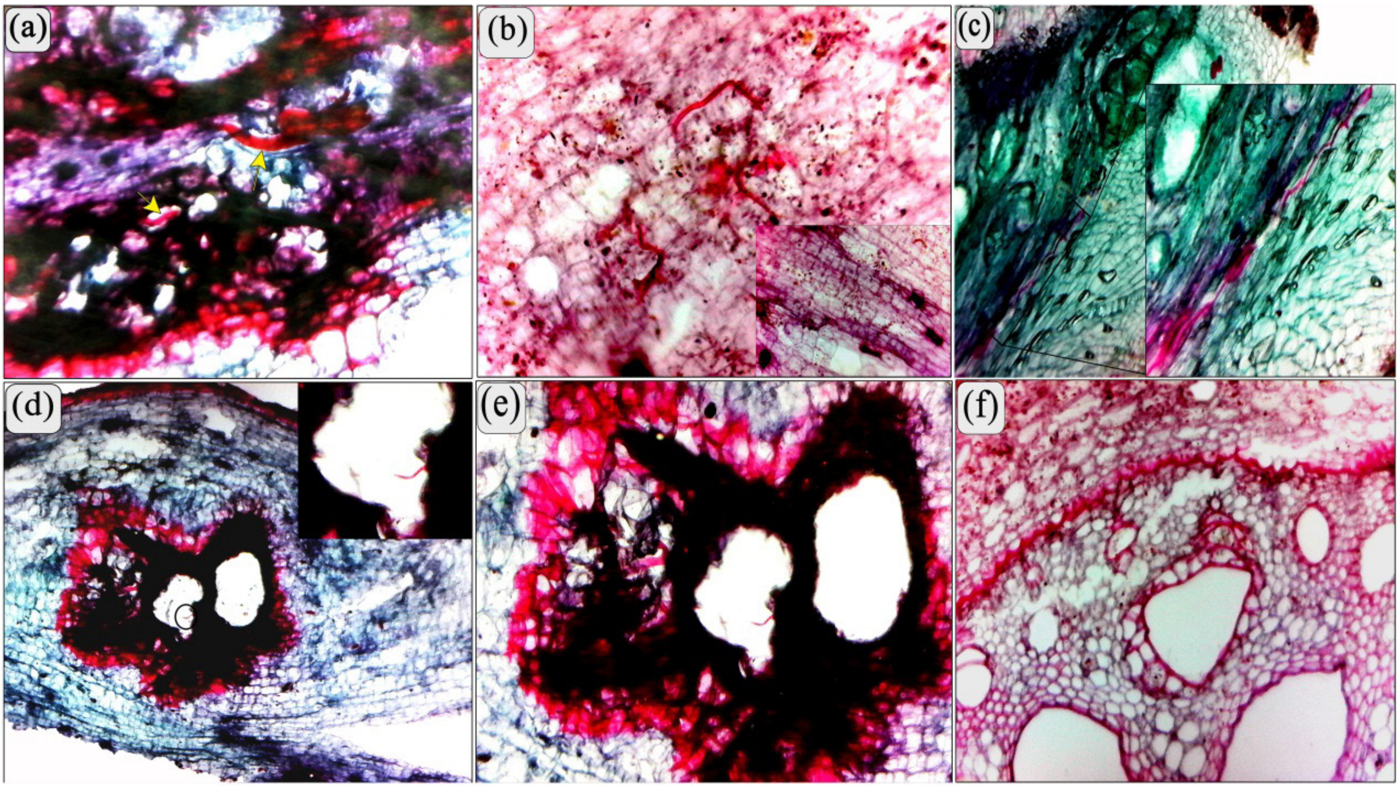

Figure 1: *(a, b, and c) Longitudinal section of a banana root showing pre-adult stages and adult stage of Radopholus similis (arrow) coiled in medial parenchyma. (b) High power represents the lignification in sub-epidermal cortical cells. (c) High power showing the pathway of pre-adult stages of R. similis in the cortical parenchyma. (d and e) showing the overlap infection between pre-adult stages of Radopholus similis through the gall formed by M. incognita. (f) Transverse section of a banana root showing the modified xylem, phloem vessels and parenchyma caused by Radopholus similis. Note a few of phenolic and lignified cells in vascular cylinder (b). (Scale bars: a = 70 µm; b-f = 200 µm).*

the incubator at 60°C to 24 for drying of mounting medium (Bybd *et al.*, 1983). The final slides were examined and photographed using Carson digital microscope, model zPix MM-940, USA.

Results and Discussion

The purpose of this study is mainly to clarify the pathogenic effect of both *Radopholus similis* alone or combination with *Meloidogyne incognita*, as histological modification in cell of roots under natural conditions of fields on banana roots, cv. Hindi the most common cultivar in Egypt.

In banana roots infested with *R. similis* only the most damages were observed in cortex and endodermis cells (Figure 1a) but with the heavy infested the damages observed in xylem and phloem vessel (stele) (Figure 1c). More of morphogenesis were observed in size and shape of medial layer of the cortical parenchyma cells. The cell walls in the root stained with safranin and fast green revealed the dark green discoloration were appeared around cells neighboring the necrosed tissues as feeding site (Figure 1d). The endodermis was also affected by the infestation, where a noticeable morphogenesis in the shape and size, as well as the thickness of the cell wall, (Figure 1f). Few of phenolic and lignified cells were showed in the vascular cylinder compared with the cortex layer, which generally contained an moderate number of phenolic and lignified bodies (Figure 1b, f). Also, the modifications appeared clearly in the longitudinal section of roots, where observed the cytoplasmic globules as dark granular structures in the cortex and extended towards the pericycle caused by pre-adult stages and adult stage of *R. similis* (Figure 1a, d, e).

In banana roots infested with *M. incognita* only, the morphogenesis was varied significantly, in terms of development and the influence value of deformation and laceration. Consequently that, the root galls formed by *M. incognita* where varied in size and shape (Figure 1b, c).

Generally, the largest galls formed near the root tips or in other words, the newly developed regions of

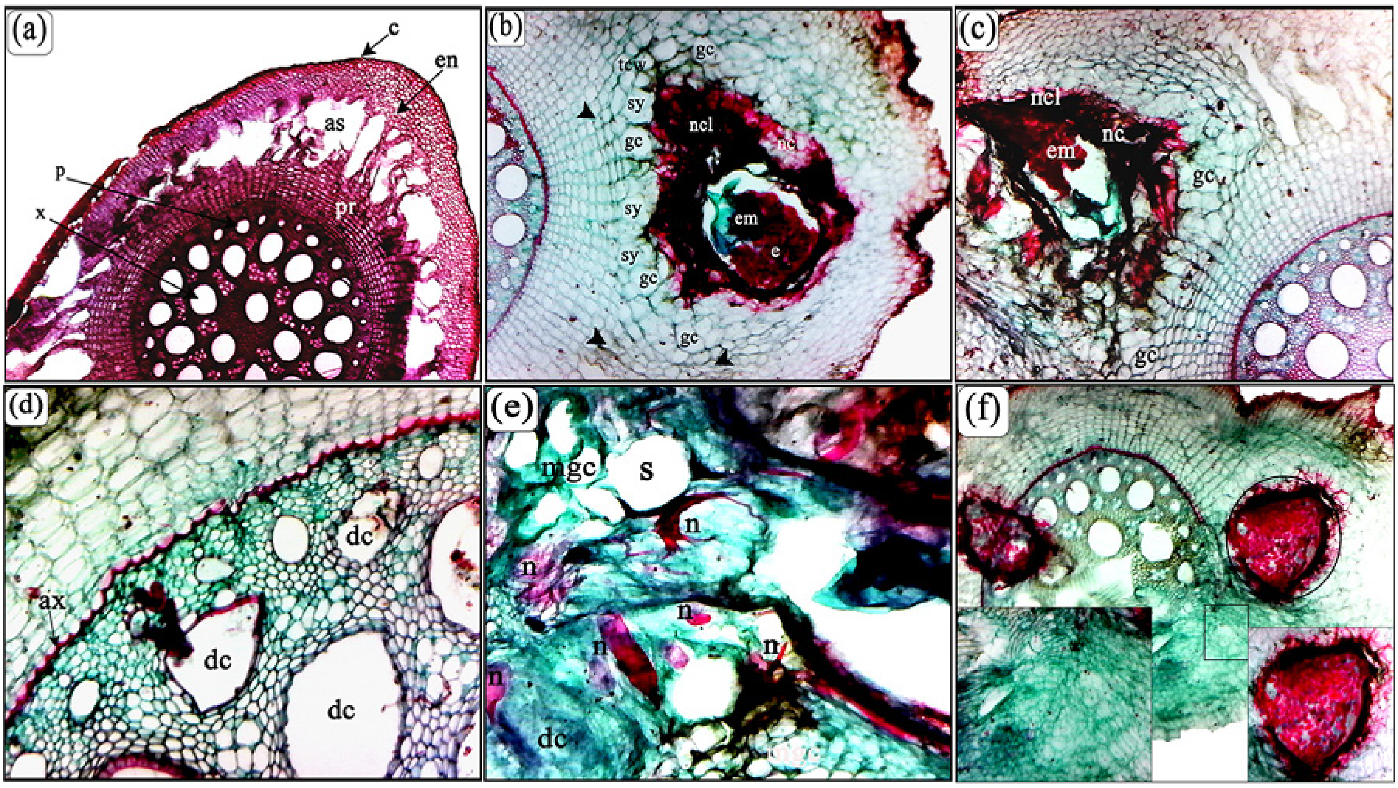

Figure 2: *(a). Transverse section of healthy banana cv. Hindi roots; Central cylindrical or stele [Phloem (p), xylem (x)], Prosenchyma (pr), Endodersmis (en), Mesodermis (parenchyma) (m), Cortex (Epidermis and exodermis) (c), Air space (as). (b) Transverse section through the gall and cortex cells of the infested banana root with Meloidogyne incognita showed the feeding site of adult female with egg masses (em), note the multinucleate cells, giant cells and lateral expansion in necrotic cell (nc) within layers (lnc) in the inner cortical tissue is beginning to spread to the stele with compressed of cells size in various layers of the of the root (head arrow) with thickness in cell walls (tcw). (c) showing lateral expansion of giant cells (gc) necrotic cell (nc) within layers (lnc) in the inner cortical tissue is beginning to spread to the stele through a passage cell in the endodermis (e). (d) The deformed cells in the parenchyma and abnormal xylem (ax). (e) Fourth stage of M. incognita (n) near of the cambium (ca). It notes a large laceration in neighboring cells of feeding site; multinucleate giant cells (mgc), note dark tissue elements and thickness of the cell wall in neighbor cells of multinucleate cells and dense cytoplasm. (f) Adult female and eggs of M. incognita near of the vascular cylinder, notes a large laceration in neighboring cells of feeding site. (Scale bars: a-f = 200 µm).*

roots and gradated along the root axes. Transverse section through the gall showed the laceration and morphogenesis of feeding site in the inner cortical tissue is beginning to spread to the vascular cylinder (stele) between a passage cell in the endodermis, where a parenchymatic xylem cells are selected as the initial feeding site. Where observed the lateral expansion in necrotic cell and thickening of the inner tangential walls of the endodermis as collapsed gradually multinucleate cells, also the hyperplasy was observed in both cells of xylem, phloem and neighboring cells of the giant cells (Figure 1d).

Later, the initial feeding site constitutes the permanent source of nutrient for *M. incognita* was aggrandized in a complex system of multinucleate transfer cells (Figure 1e, f). Generally, the depth of morphogenesis in roots caused by the feeding were varied, wherein some sections were not extended to the vascular cylinder and in others penetrated the vascular cylinder (Figure 2b), and formed the multinucleate giant cells and stellar multinucleate cells and appeared in dark tissue elements and thickness of the cell wall in neighbor cells of multinucleate cells , hypertrophy and hyperplasia with dense cytoplasm in almost of the neighboring cells of feeding site (Figure 2c).

Also, coincided with the feeding process presence of the granular structures in the cells of cortex layer near the feeding site and contain some of dispersed fibrillar and granular material in multinucleate cells and in the numerous vacuoles (Figure 2a). On the other hands, longitudinal section of a banana root through the gall showing the laceration and deformation area formed by the phenolic and lignified cortex cells and its extended towards the pericycle on the old root and less than in the young root (Figure 2d, e, f).

In banana roots infested with *Radopholus similis* combination with *Meloidogyne incognita* the transverses section of banana roots through the galls with the both plant-parasitic nematodes were showed *R. similis* induced syncytia at the periphery of the vascular cylinder area and giant cells induced by *M. incognita* in the central of the root (Figure 3a, b). In contrast to *R. similis* alone, a significant increase in population of varied stages was observed in combination with *M. incognita* especially in the permanent feeding site of in the endodermis and vascular cylinder area (Figure 3d, e), where showed that *R. similis* was protruded out or was inside the feeding site of *M. incognita*. Also, as a result of combination of two nematode species, showed a duplicated in value of the laceration and deformation area through different root tissues reigns (Figure 3f).

This study showed that *Radopholus similis* and *Meloidogyne incognita* is able to develop as affected pathogens on banana, *Musa cavendishii*, through the significantly histological modifications in roots tissues structure (Wuyts et al., 2007). The histological symptoms were varied as a result of the invasion, penetration and feeding process of *M. incognita*, where this study showed that the formation of multinucleate cells, giant cells, hypertrophic nuclei cells (Nithya et al., 2007). In giant cells were observed as normal form but we noted that the giant cells at each site of infections were varied and the number of radial cells ranges between five and six cells in one group. Our observation was contrary to Sudha and Prabhoo (1983) where mentioned that the radial cells in one group not more than five cells in *Musa cavendishii*. roots. The current study also differed with Sudha and Prabhoo (1983) in the place of the giant cell, where them indicated that the place of giant cell formation in the vascular cylinder (stele) only, while the current study showed that the giant cells was formed in along

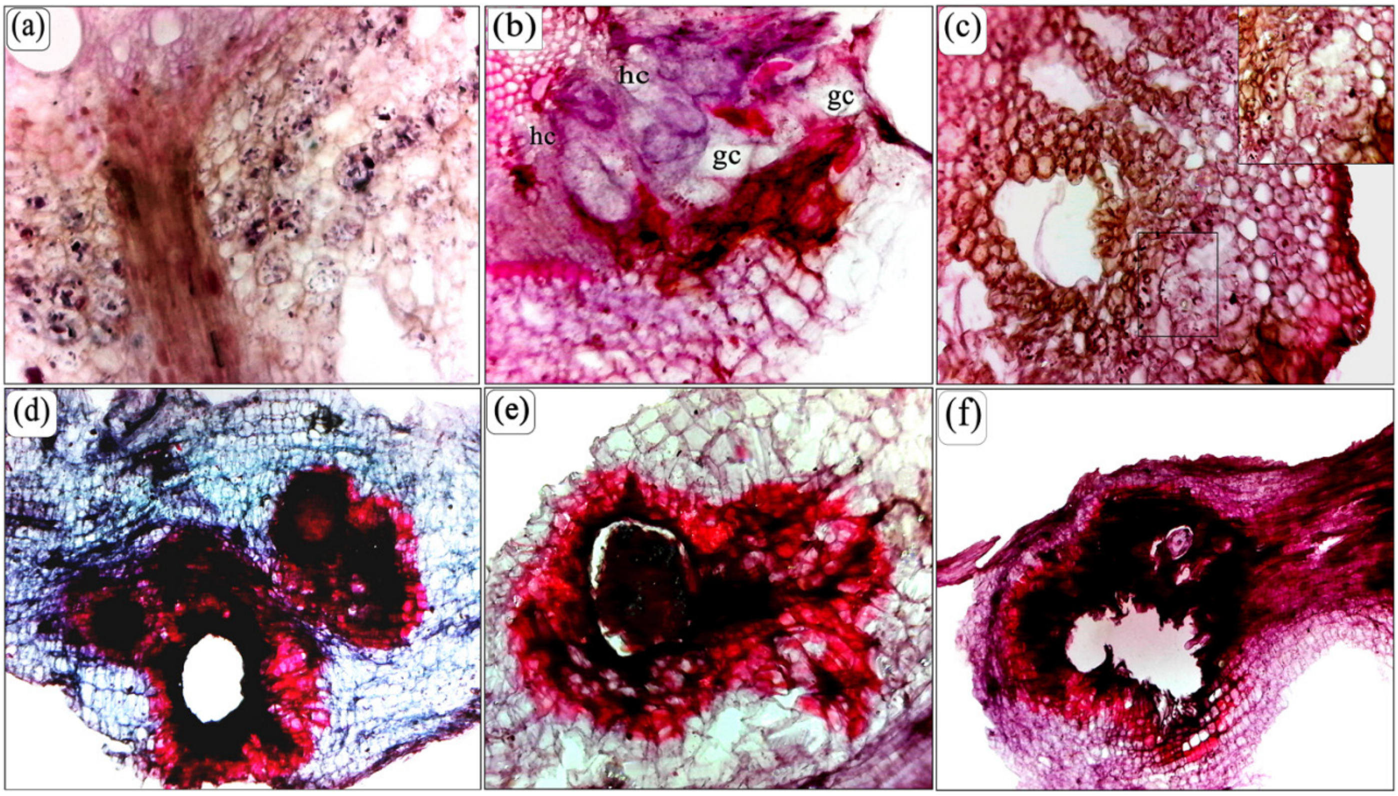

Figure 3: *(a) Transverse section of a banana root showing the granular structures in the cells of cortex layer near the feeding site of M. incognita. (b) Note the nuclei in the vicinity of nematodes either enlarged or disintegrating and either cytoplasm contracting from cell walls still intact or contracted and cell walls beginning to rupture. In addition, giant cell transforming from undifferentiated hypertrophied cells. (c) Transverse section through the cortex noted the extensive cavity in the cortex, the nematodes at the periphery of the lesion and some cell fragments towards the center necrotic and lignified cells. (d, e and f) Longitudinal section of a banana root through the gall showing the laceration and deformation area and phenolic and lignified cells of damaged cortex extended towards the pericycle on the old root and the root hair. (Scale bars: a–f = 200 µm).*

the cortex layer even inside the vascular cylinder in agreement with Chaturvedi and Khera (1979) where reported that giant cells occasionally found in the cortex layer. Cells wall were varied in thickness in both of the cell walls in modified cells and other neighboring cells. Similar observations were reported by Sudha and Prabhoo (1983) where noted that the thick and dark tissues were formed and covered all reign around the egg masses in transverse section of banana roots infested with root-knot nematode. Cytoplasm density were increased especially in the giant cells, perhaps it is due to the activating of metabolism and nematode secretions, which was approved by Bird (1974) and Sudha and Prabhoo (1983).

The most observed morphogenesis in host tissue associated with *R. similis* infested expand all along from the epidermal layer to the vascular cylinder (stele) (Araya *et al*., 2006; Moens *et al*., 2001). And the adult female was appeared coiled (Mateille, 1994). In all histological sections the cytoplasm were contained with globular structures in the in different layers were noted, same results were described by Mateille (1994) and Wuyts *et al*. (2007) where they described it as phenolic cells. The lignified cells were spread abundantly were found in the vascular cylinder. Same results reported by Nithya *et al*. (2007) and Valette *et al*. (1998).

Generally, a few of phenolic and lignified cells were showed in the vascular cylinder compared with the cortex layer, which were contained moderate number

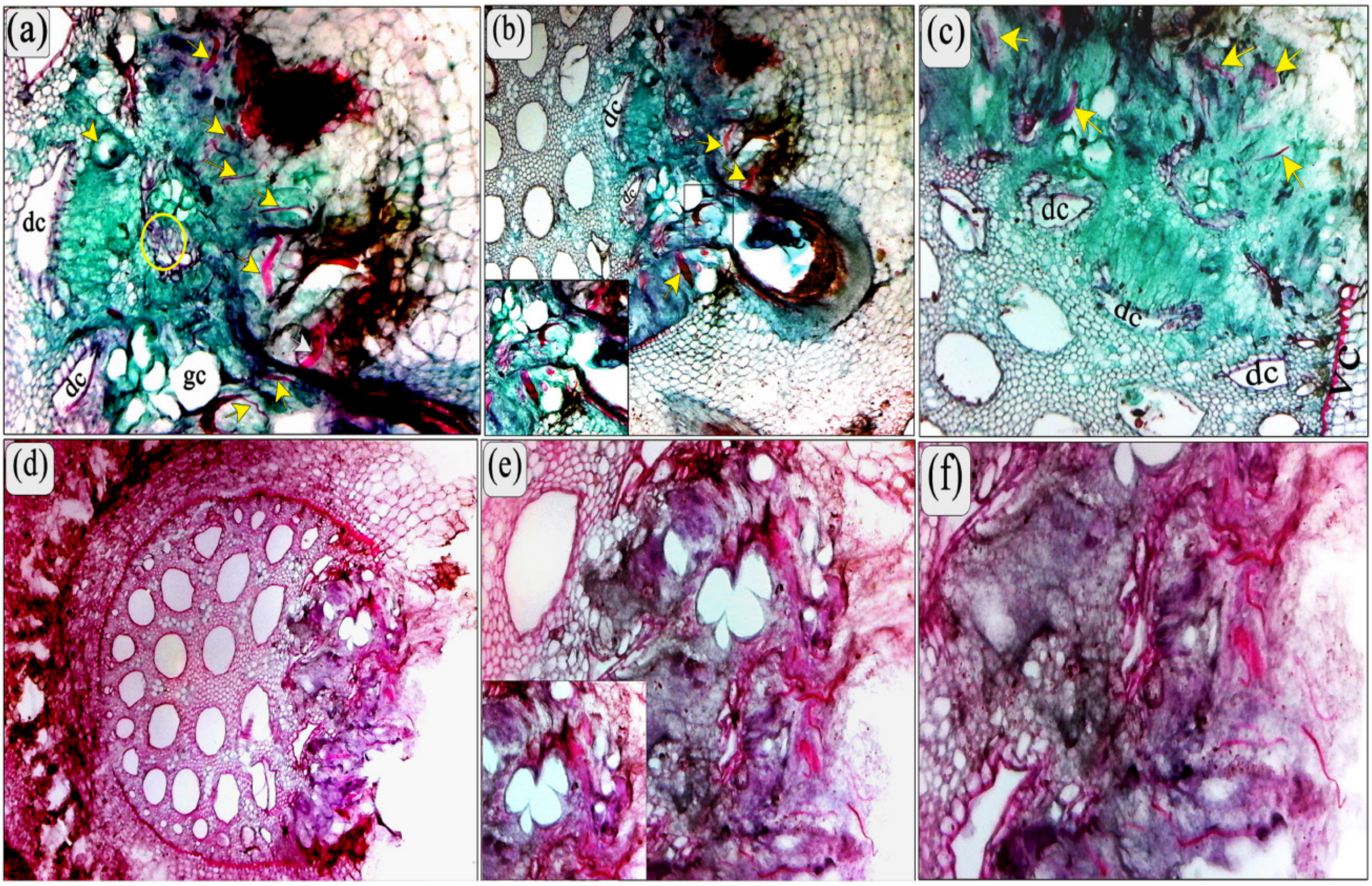

Figure 4: *(a) Transverse section of banana roots showing the adult female of M. incognita (arrows) inducing the clear multinucleate cells (cycle) and with fused syncytial cells presenting dense cytoplasm and hypertrophic nuclei, and shows some of adult and larval stages of R. similis (arrows). (b) Showing deformed cells (dc) and noted that the extension of the feeding site to the vascular cylinder. High power showing the most damage in root cells as results of a combination of both nematodes. (c and d) root infested with pre-adult stages of both M. incognita and R. similis showing the abnormal xylem, clear dissolution of endodermis and central cylinder (arrows) and note the thickness of endodermis layer compared to the normal size in the control. Also, note all the modification symptoms associated with the feeding process; multinucleate cells, giant cells, and modified cells. (e, f and d) The laceration and dissipation of the central cylinder (xylem and phloem vessel) as a result of the feeding of both species. Note the extent of the feeding sites inside the vascular cylinder. (Scale bars: a-f = 200 µm).*

Links Researchers

of phenolic and lignified cells after *R. similis* penetration. Similar observations reported by Valette et al. (1998) and Nithya et al. (2007) revealed that a few of phenolic and lignified cells were the formed by *R. similis* in the damaged cells of cortex in susceptible of banana cultivars, and suggested that this bodies had appositive correlating with cultivar resistance to nematode infestation. Where, these bodies contributed in increasing the resistance of the host against the plant-parasitic nematodes through some methods, which indicated by Ride (1975); formed in chemical modification through the cell wall lacerating by the nematode enzymes (Vaganan, 2014); normal reaction of the cell wall to the spread of nematode toxins to the host, or formed by the host to limiting the pathogen nutrition process (Nicholson and Hammerschmidt, 1992; Appel, 1993).

When the two species infested the same root concomitantly, the current study showed that *R. similis* deepened within the root more than the usual with less number of phenolic and lignified bodies. Similar results were described by Valette *et al.* (1998) where reported that a large number of these granules were impedes the movement of *R. similis*, (Croll, 1975; Ohri and Pannu, 2010), and thus decreased of this granules as a result of the destruction the cell wall by *M. incognita*, which will facilitate the movement of *R. similis* to dipper in the root.

Novelty Statement

As the first recorded, we observed a change in the feeding site of burrowing nematode (*Radopholus similis*) where extended to the vascular cylinder after it was previously reported only in the cortical parenchyma, whether alone or in interacting with the root-knot nematode (*Meloidogyne incognita*).

Author's Contribution

El-Sagheer, A. M conceived, performed and designed the experiment wrote and reviewed the manuscript. Barros, A.F. reviewed and edited the manuscript. Abd El-Aal, E,M. reviewed and edited the manuscript. Gad, M.M. and Mahmoud, D. S. commented on the figs. and reviewed the manuscript. And El-Marzoky, A. M. reviewed and edited the manuscript

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Data availability
Availability of data and materials not applicable in this study.

Consent for publication
Not applicable in this section.

Conflict of interest
The authors have declared no conflict of interest.

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