Histopathology of Walnut (*Juglans regia* L.) root infested with Root Knot Nematode (*Meloidogyne incognita*)

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ABSTRACT

Histopathology of the infected walnut roots (*Juglans regia* L.) with root-knot nematode *Meloidogyne incognita* showed that roots were completely destroyed because of infestation during a study in Hazara Division, KP, Pakistan. *Meloidogyne* species are potential pest of associated plants and trees. Damaged roots taken from infested host trees were fixed, sectioned and stained for histopathological study. Results showed that second stage juveniles (J2s) established themselves in the roots very successfully and resulted in the formation of giant cells. Cells with dense cytoplasm and multinuclei were observed. Feeding sites were developed and necrosis of root along with hypertrophy was seen. Cortex was completely destroyed which is a major component of ground tissue. Transverse section of the root cells showed presence of female sections and egg masses. Statistical analysis showed that *Meloidogyne incognita* had mean value 24±1.8 S.D value.

Key Words: *Meloidogyne incognita*, Giant cells, Hypertrophy, Cortex, Egg masses.

INTRODUCTION

Meloidogyne, belong to a family Heteroderidae and commonly known as root knot nematode (Diez and Dusenbery, 1988). It is one of the most important plant parasitic nematode specie as it causes great damage to the plant and ultimately results in yield loss. *Meloidogyne* sp. (root knot nematode) is an important pathogen to the plants and resulted in biomass and yield loss of different plants throughout the world (Zarina and Shahina, 2010). Root knot nematodes (*Meliodogyne*) alongwith other plant pathogens can cause yield loss upto 14. 6% in tropical regions whereas around 8.8% in developed countries globally (Ghareeb *et al*., 2022). Root knot phytonematode infestation affects both economically in the form of yield loss and poor plant growth and health. Globally *Meloidogyne* are most devastating phytonematodes among parasitic phytonematodes because of their extensive harm and damage to the host plant nematode (Wram

et al., 2022). Histopathology of the infected root to find out the damage caused because of root-knot infestation is the most successful technique throughout the world. It is the microscopic examination of infected tissue in order to study the manifestations of disease. Tissue level changes are observed which occur because of the infestation of adult and juvenile stages of root knot phytonematodes.

Many research workers from different parts of the world including Pakistan worked on histopathology to study the infestation of root knot nematodes in different plants. Fabiyi in 2021 observed through histopathological studies that effective amendments and treatments resulted in reduced root galls and egg masses and therefore found effective in the control of root knot nematode infestation. Singh *et al.,* during their study on mungbean production in South and South East Asia also identified root knot nematode Meloidogyne infestation as one of the major limiting factor in the yield. Khan *et al.* from Pakistan in 2017 observed that root knot nematode *Meloidogyne javanica* (Treub) Chitwood had adverse effects on the roots of guava seedlings. They observed that cortex was completely destroyed and hyperplasia and hypertrophy showing cells were very common. In 2015, Osunlola and Fawole worked on the histopathology of sweet potato roots infested with *Meloidogyne incognita***.** They observed that *Meloidogyne incognita* affected the sweet potato roots and resulted in cellular disorganization in endodermis, vascular cells and root cortex. Azhagumurugan and Rajan in 2015 in India worked on the histopathological responses and effects of *Meloidogyne incongita* on the roots of black gram, *Vigna mungo.* The second stage juveniles migrated all the way through the cortex in older roots and resulted in the development of giant cells in the type of clusters. These giant cell clusters were characterized with inflamed nuclei and thick cytoplasm. In the same way Akhtar and Hisamuddin, 2015; Seo *et al.,* 2014; Kayani *et al.,* 2013; Samad *et al.,* 2012; Khan *et al.,* 2010 and Sayed *et al.,* 2010 also worked on the histopathology of root-knot nematodes.

MATERIALS and METHODS

As in the Histo-pathological studies, tissues to be studied were fixed in formalin 10%. Tissues were then embedded in paraffin wax block which were manually cut into sections 4-5 μm thick by using microtome. Sections were then dewaxed and stained by using eosin and hematoxylin normally (Slaoui and Fiette, 2011). Double staining technique was used to stain the tissue section as it gives good and clear image of structure which is easy to study. Hematoxylin was used to

stain acidic structure i.e. nucleus in purplish blue color and is a basic dye while Eosin was used to dye basic structure i.e. cytoplasm as it is acidic dye and gives red or pink color.

Walnut roots infected with *Meloidogyne incognita* were washed and processed according to histopathology technique comprised of several critical steps including fixation, dehydration, embedding, double staining and permanent mounting. After that the high quality Photomicrographs were taken by Nikon photomicroscope to observe *Meloidogyne* infestation in an elaborate manner.

RESULTS and DISCUSSION:

Meloidogyne, sedentary endoparasitic root-knot phytonematodes are among the most successful parasites of the earth. They are obligate parasites and can induced specialized feeding sites which act as strong metabolic sinks to which photosynthates are mobilized (Vovlas *et al*., 2005). The histopathology of the infected roots of the walnut trees with *Meloidogyne incognita* showed that it has caused great damage to the root cells and tissues. The second stage juveniles entered the root cells through rootlets and established themselves in all possible ways. Figures showed that feeding sites were developed with giant cells showing dense cytoplasm and broken cell walls. Nucleus of the giant cells were found to be multinuclei. Cortex was found completely damaged because of the infestation of the root knot nematode juveniles. Female sections were also observed in some cells and vascular tissues were also damaged to a considerable level. Necrosis of the root with hypertrophy cells was also observed. Giant cells were seen proximal to the female of *Meloidogyne incognita*. The giant cells resulted in the aberration of the vascular region leading to the root damage.

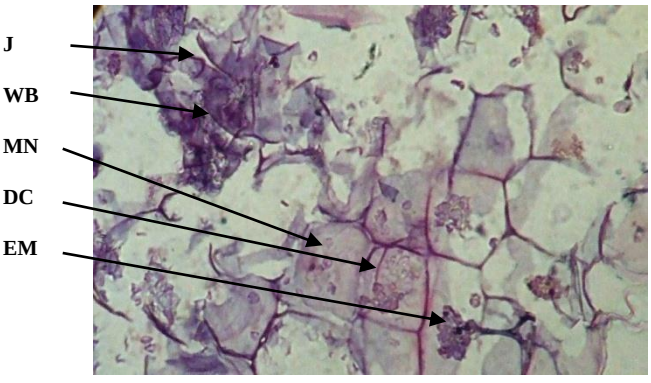

Fig. 1: Photomicrograph showing infected walnut root by *Meloidogyne* sp. (x 100)

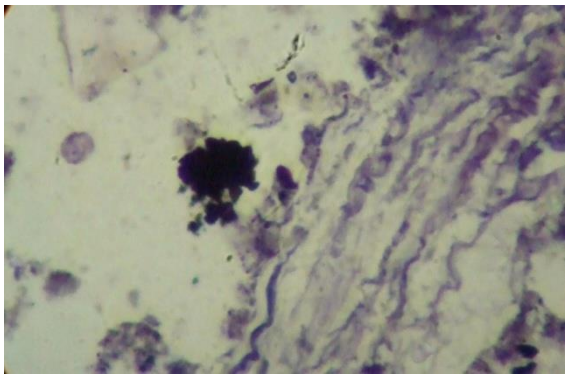

Fig. 2: Photomicrograph showing cortex of walnut root completely destroyed (x 200)

(J- Juvenile, **WB**-Wall Broken, **MN**-Multinucleated, **DC**- Dense Cytoplasm, **EM**-Egg masses**)**

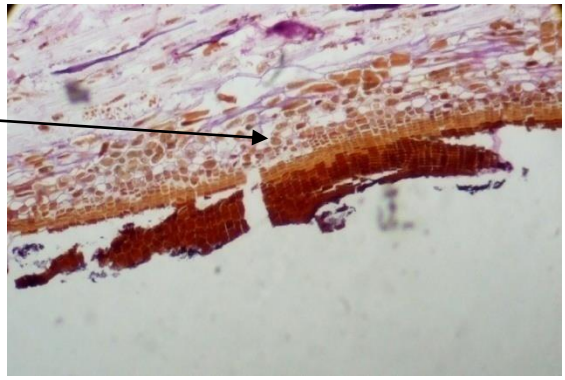

Fig. 3: Photomicrograph showing feeding sites in the cortex was completely destroyed (x 50)

(FS- Feeding Sites**)**

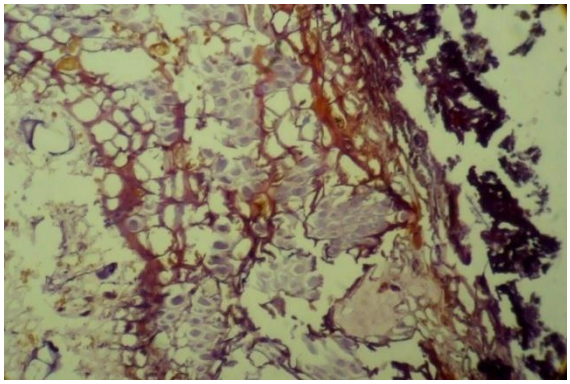

Fig.4: Photomicrograph showing Necrosis of tissue cytoplasm where infected with *Meloidogyne incognita* (x 100)

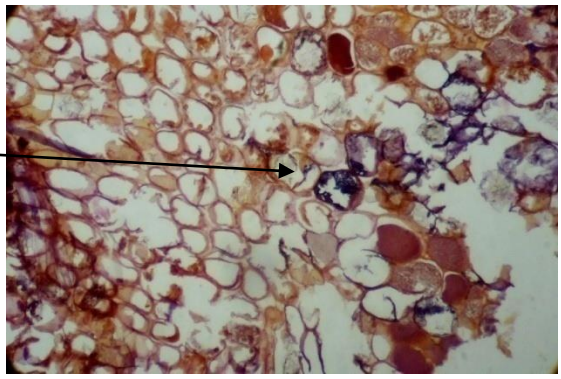

Fig. 5: Photomicrograph showing sections of second stage juveniles present in vascular area of root (x 50)

(J2s- Second stage juveniles**)**

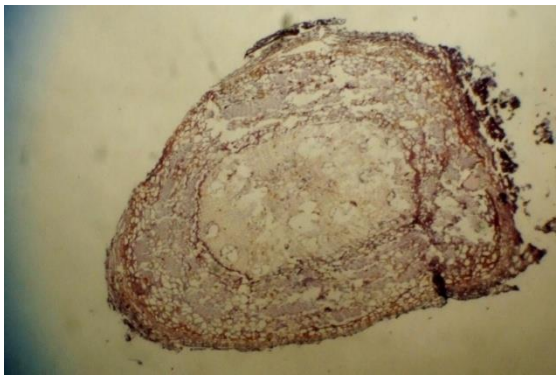

Fig. 6: Photomicrograph showing TS of infected root (x 10)

(TS-Transverse section**)**

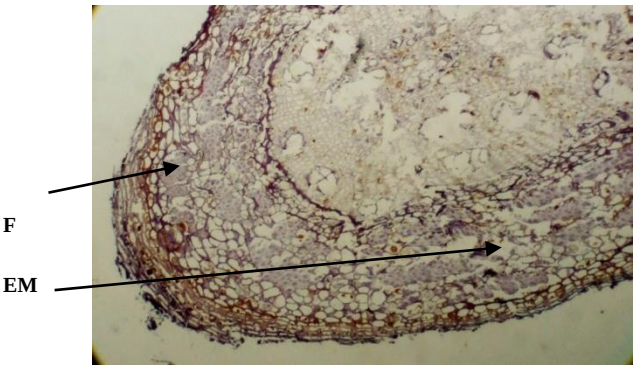

Fig. 7: Photomicrograph showing TS of infected root with egg masses and female section (x 20)

(F-Female section, **EM**-Egg masses**)**

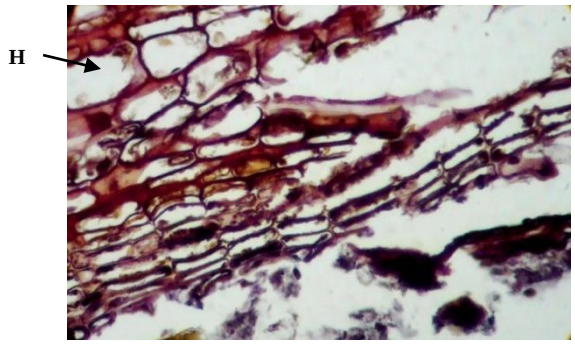

Fig. 8: Photomicrograph of root showing necrosis and blackening along with hypertrophy (x 150)

(H-Hypertrophy**)**

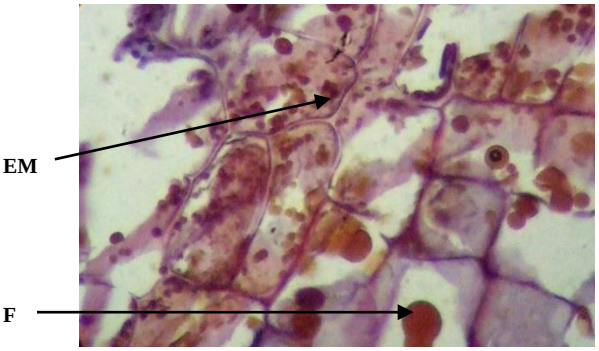

Fig. 9: Photomicrograph showing TS of *Meloidogyne incognita* female along with cells having egg masses (x 200)
(EM-Egg masses, **F**-Female**)**

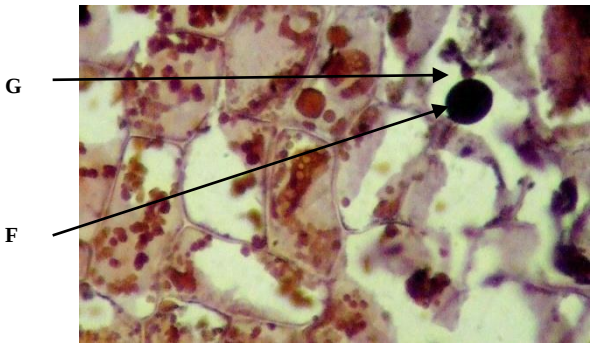

Fig. 10: Photomicrograph showing *Meloidogyne incognita* female with giant cells proximal to the nematode aberration of the vascular region (x 200)
(G-Giant cells, **F**-Female**)**

DISCUSSION:

Juveniles of the root knot phytonematode *Meloidogyne incognita* entered the root cells through rootlets and established themselves very successfully in all possible directions as shown in the above photomicrographs of infected walnut roots. Histopathology of the infected roots of walnut

trees with *Meloidogyne incognita* showed that second stage juveniles successfully established themselves and as a result of their activity giant cells were formed. With the formation of giant cells, roots cells started to deformed. Deformation of the root cells clearly evidenced the infestation of root knot nematodes in the host plant. The cytoplasm become densed and granular because of possible cell destruction as shown in Photomicrograph 1. Giant cell formation was also observed by Singh *et al*, in 2013 while doing histopathological studies on lentil (*Lens culinaris)* grown in root-knot (*Meloidogyne incognita*) infested soil.

It can be seen in photomicrograph 2 that cortex was completely destroyed as a result of juvenile penetration and successful establishment in the roots. Cortex plays an important role in the normal growth of the plant as it is meant for the storage of the water. Any damage to the cortex will ultimately results in plant health and yield damage because of poor growing conditions. After successful establishment, second stage juveniles of *Meloidogyne incognita* induced feeding sites in the infected roots of the walnut trees. Histopathology of the infected root of walnut tree by *Meloidogyne incognita* showed the feeding sites which completely destroyed the cortex tissues of infected walnut root as observed in Photomicrograph 3. The feeding sites to mobilized photosynthates act as strong metabolic sinks. The feeding sites of the root-knot nematodes are three dimensional in shape. They contain several giant cells with multinucei and dense cytoplasm therefore affecting the performance of the cortex. They resulted in the complete destruction of the cortex tissues (Vovlas *et al*, 2005). Feeding site formation resulted in tissues crushing and distortion. Root galls were formed as a result of hyperplasia of tissues nearby giant cells (Sasanelli *et al.*, 2015).

As a result cytoplasm necrosis as infection sites was observed in the walnut roots which resulted in damage to cell structure and abnormal functioning of the root as can be seen in Photomicrograph 4. Abnormal root functioning plays a critical role in the abnormal growth and ultimate death of the host plant. In photomicrograph 5 it was observed that the vascular region J2s established feeding sites with giant cells. The observation was similar to the observation of Proite *et al.*, in 2008 in which he observed that the giant cells created regular oval shape feeding sites, each one having some small nuclei and one large vacuole with several small vacuoles. Second stage juveniles were observed to be associated with hypersensitive reaction in the vascular cylinder.

Egg masses and female sections were observed in the infected cells of the walnut roots as shown in photomicrograph 6, 7 and 9. The diameter of the infected root also changed and become larger as compared to the normal root because of gall formation. Gall formation is the result of the infestation of the root gall phytonematode *Meloidogyne*. These galls contain egg masses and in some cases females were also found. Plant growth, mass and root development had adverse effects because of the root galls. As reported by Sasanelli *et al.*, in 2015 while working on the histopathology of roots of parsley with root-knot nematode *Meloidogyne javanica.*

Meloidogyne incognita resulted in necrosis of the root cells and hypertrophy was observed as a result. It can be seen in photomicrograph 8 that cell necrosis resulted in the cell deformation and malfunctioning. This observation was similar to the observation of Nemec and Morrison in 1972. They observed that because of infestation of *Meloidogyne incognita* large giant cells with thick wall were formed. Cells surrounding developing larvae resulted in necrosis. Hypertrophy was prominent in stele region.

The giant cells resulted in vascular region aberration as can be seen in photomicrograph 10. It ultimately resulted to root damage of the infected plant. It was similar to the observation of Yousif in 1979. He stated that giant cells resulted as the activity of juveniles of *Meloidogyne incognita* caused disruption in vascular tissues and injury to xylem and parenchyma cells. Because of the activity of root knot nematode, giant cell's cell walls experience the process of expansion by both loosening and thickening which supports nematode nutrient uptake (Escobar *et al*., 2015). From the cell wall defects we can easily figure out the infestation by root knot nematodes and successful giant cell formation indicates the level of damage caused by *Meloidogyne incognita*. Giant cell effects because of root knot *Meloidogyne* activity was also reported and discussed by Meidani *et al.* in 2019 while working on cell wall modifications of giant cells because of root knot nematode infestation. Root aberration is the main cause of root infestation which ultimately results in poor plant health. Root galls occur because of giant cell**.** Giant cells are enlarged multinucleated cells basically for feeding developed as a result of redifferenciation of plant cells induced because of the activity of the root knot phytonematode *Meloidogyne incognita* (Mejias *et al.*, 2021).

CONCLUSION

It was concluded that root knot nematode (*Meloidogyne incognita*) resulted in the damage to the walnut roots extensively. Presence of feeding sites, giant cells, densed cytoplasm, destroyed cortex, second stage juveniles and females in the infected roots clearly indicate that *Meloidogyne incognita* are not only potential pest of walnut trees in studied area but also resulted in great economic loss. The environment is suitable for the growth of *Meloidogyne incognita* therefore damaging roots of the host trees in the studied area which go unnoticed because of the lack of the awareness to the local farmers.

Conflict of interests

The authors declared no conflict of interest.

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