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Histopathological Studies of Mulberry Roots Infested by *Meloidogyne incognita* (Sem Analysis)

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ABSTRACT

Background: Root knot disease caused by the nematode *Meloidogyne incognita* (Kofoid and White) Chit wood is a serious problem in mulberry and causes severe damage to growth and development of plants. Root knot nematode alter or depletes nutritional values of mulberry leaves and adversely affects to the silkworm health in turn decreases cocoon quality and quantity. The anatomical changes in the roots due to nematode infestation affects the cocoon crop negatively, because mulberry leaves main food source for silkworm *Bombyx mori* L. ultimately leads to economic loss in silk industry.

Methods: Hence the present study has been taken up to understand the histopathological changes that occur in the roots of mulberry V1 variety plants infested with nematode *Meloidogyne incognita* studied under the Scanning Electron Microscope (SEM) with an interval of 15 and 60 days after nematode inoculation.

Result: After fifteen days the root samples have shown thick starchy grains were accumulated in the parenchymatous cells and intracellular movement of juveniles. Sixty days after the infested root samples had shown a number of large sized galls or knots. Ultra- structural observation of the root gall cross sections under scanning electron microscope revealed that serious anatomical changes occur in the infested mulberry root tissues.

Key words: Histopathology, *Meloidogyne incognita*, Mulberry, Root knot nematode, Scanning electron microscope.

INTRODUCTION

Mulberry (*Morus* sps) belongs to the family Moraceae is highly valuable plant for silk industry, its foliage constitute the main food source for silk worm *Bombyx mori* L. and contribute its growth and development. India is the second largest producer of mulberry silk, next only to China, accounting more than15% of the global raw silk production. Millions of farmers were engaged in sericulture cultivating mulberry gardens (Singh, *et al*, 2010). Mulberry is attacked by different types of pathogens like bacteria, virus, fungi, mycoplasma and nematodes. Some pests which cause 10-30% loss in the leaf yield and reduce nutritive values of mulberry leaves (Chanturia *et al*., 1963; Shree and Umesh kumar, 1991). Among the different diseases of mulberry the significant economic loss is caused by the species of nematode *Meloidogyne incognita* (Kofaid and White) Chit Wood (Chit Wood, 1949) which is a very serious problem in sandy loam soils under irrigated conditions (Narayanan *et al*, 1966). It was first observed on mulberry crop by Bessey, (1911) from USA. Which distribute in many regions and affecting several plant hosts (Amal *et al*, 2020). The root-knot nematode *M. incognita* cause galls or knots on roots of its host plants. The disease manifestation and effect on the host plants are development of galls/knots on the roots and other symptoms that can be observed above ground level consist of stunted stem, chlorosis, curling leaves, wilting of plant finally reduced vigour in the plant Saha *et al*, (1983). Once the root-knot nematode gets settled in mulberry fields it is not easy to control due to perennial nature of mulberry and endoparasitic nature of nematode and has ability to survive in soil for several years. Hence, the present study is aimed to carry out to understand the histopathological changes that occur in mulberry roots infested by nematode *M. incognita* in comparison to the healthy plant roots observed by using scanning electron microscope (SEM).

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MATERIALS AND METHODS

The study was carried out in the department of Sericulture, Sri Padmavati Mahila Visva Vidyalayam, Tirupati, Andhra Pradesh, during the period 2014-2017. Scanning Electron Microscopic studies were made at Central Sericultural Research and Training Institute, Mysore. For this experiment, seventy days old mulberry V1 variety saplings were planted in randomized block design with 3' × 3' spacing. After three months of establishment, 1000 juveniles(J2)/plant were inoculated as ECL (Economic Threshold Level and Crop Loss) (Govindaiah *et al*., 1991b; Sharma, 1999a) keeping control. For Scanning Electron Microscopic studies, the nematode infested roots with an interval of 15 and 60 days

after nematode inoculation were collected and were subjected to the investigation. At the same time healthy roots were also studied.

Sample preparation

For scanning electron microscopy, the smaller transverse sections of nematode infested mulberry root and healthy roots (control) were fixed in glutaraldehyde (2.5%) prepared with 0.2 M Cacodylate buffer (pH 7.2) for 2 hrs and washed with Cacodylate buffer followed by double distilled water and thoroughly dehydrated in ethanol-acetone series. The dehydration begin in 50% ethanol, passed through 70% and 90% ethanol and then two changes occurred each through 100% ethanol, 3:1, 1:1 and 1:3 ethanol acetone combinations and subsequently through pure acetone, keeping the samples for 15 minutes at each step. Then samples were critically dried in critical point dryer (EMS-850), using CO_2 as transition fluid. The dried samples were mounted over copper stubs using double stick tape and coated by gold (20 nm thickness) in Sputter Coater (EMS-550) and Specimens were scanned under JEOL 100CX –II electron microscope at 20 Kv. (Bozzola *et al*, (1992).

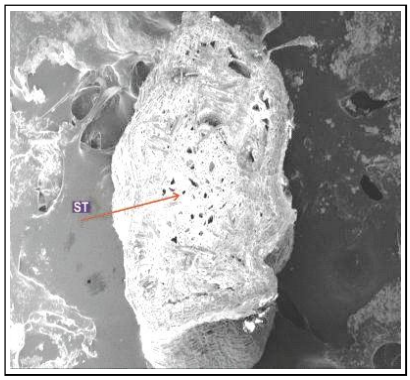
RESULTS AND DISCUSSION

Ultra-structural study of the root gall cross sections under scanning electron microscope indicated that serious anatomical changes occur in the nematode infested mulberry root tissues. Infested roots exhibited alterations in entire structure of root storage tissue and vascular system that made difficult to identification of the normal course of vascular stands. Histopathological changes indicate that the degree of pathogenicity of the root knot nematode on mulberry at the 1000 J2 inoculum level.

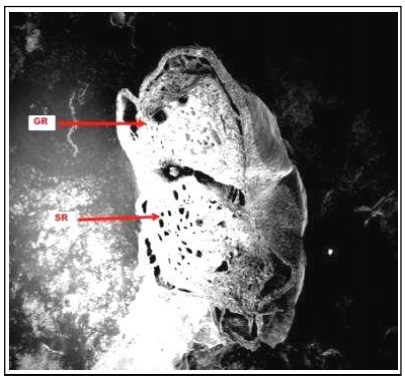
Observations

The result showed that the healthy root of mulberry has typical features of dicot root with uniseriate epidermis, parenchymatous cortex, distinct endodermis, pericycle and exarch primary xylem. Starch grains were also accumulated in parenchyma cells of cortex and stele (Fig 1a). Root knot infested root shown deformation of epidermis, cortex and vascular tissue (Fig 1b).

The root samples collected fifteen days after nematode inoculation have shown abundant number of second stage

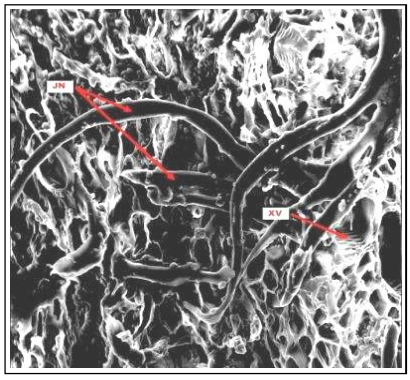


a. Transverse section of mulberry healthy root (100 × 1.666 magnification).

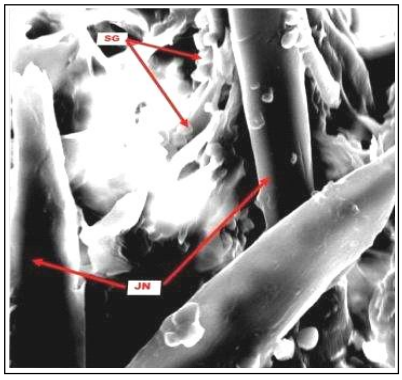


b. Transverse section of mulberry root gall (50 × 1.666).

Fig 1: Transverse section of mulberry healthy root and root gall.
ST= Stelar Region; GR= Gall Region, SR= Stelar Region.



a. Many Juvenile nematodes in cortical cells (500 × 1.666).

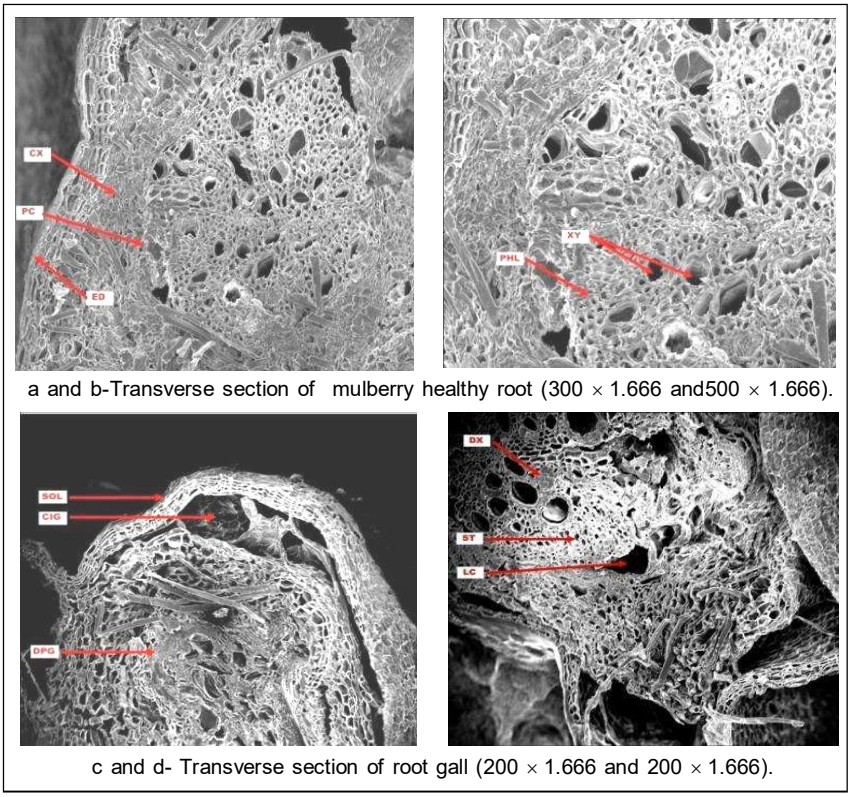


b. The close photograph of Juvenile nematodes (1500 × 1.666).

Fig 2: Mulberry root fifteen days after nematode inoculation.
SG= Starch Grain , JN= Juvenile Nematodes, XV= Xylem vessel.

juveniles in the root cross section. In parenchymatic tissue of cortex and stele of the root seemed a number of juveniles. Intracellular movement of juveniles was observed. There were slightly anatomical changes noticed in the root but thick starchy grains were accumulated in the parenchymatous cells when compared to healthy roots (Fig 2a and b). The root samples collected sixty days after nematode inoculation had shown more number of large sized galls. The cross sections of the galls showed thicker diameter and irregular circular outline.

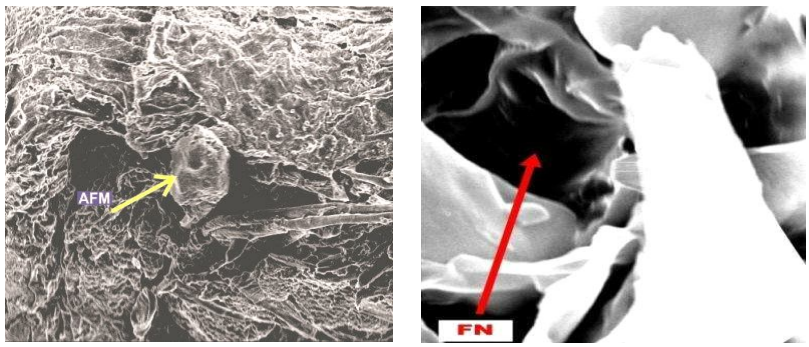
These roots showed severe anatomical alterations in the tissue. The cells were damaged with irregular cells and cavities in the cortex and most of the area was occupied with giant cells. Endodermis, pericycle, epidermal layers were ruptured. The stele was disorganised with large cavity due to deformed transport elements and was separated from



a and b-Transverse section of mulberry healthy root (300 × 1.666 and500 × 1.666).

c and d- Transverse section of root gall (200 × 1.666 and 200 × 1.666).

Fig 3: Transverse section of mulberry healthy root and root gall sixty days after nematode inoculation.
PHL= Phloem, XY= Xylem, CX= Cortex, PC= Pericycle, ED= Epidermis.
SOL= Separate Outer Layer, CIG= Cavity inside Gall, DPG= Destroyed Parenchyma in GallDX= Disorganized Xylem, ST= Stelar Region, LC= Large Cavity near Stelar Region.



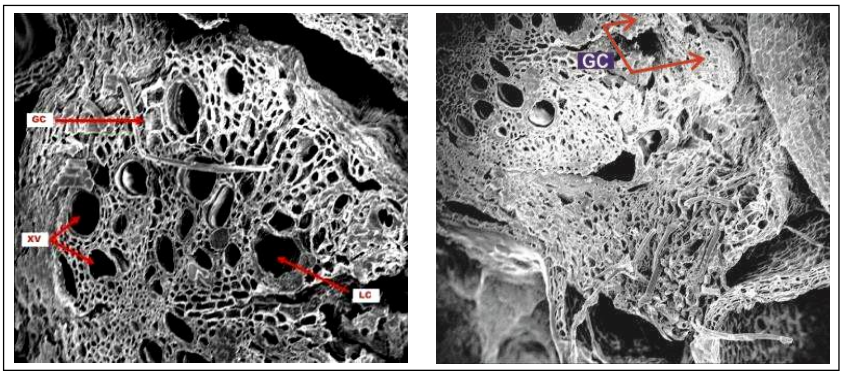
a. An adult female nematode inside a deep cavity (500 × 1.666).

b. female nematode (1500 × 1.666).

Fig 4: Transverse section of mulberry root gall.

cortex. As a result of hyperplasia and hypertrophy of the traumatic cells induced by the nematode infestation in the cortex and stele regions of the infested roots decrease shape and thickness. The healthy roots of mulberry showed that cortex was larger in size with the intact epidermis (Fig-3 a, b, c and d). More pear shaped adult female nematodes appeared in traumatic cells towards the root periphery in the cortex region. Inside the knot, cavities appeared around the females (Fig 4 a and b). Many excessively enlarged giant cells were observed in the damaged parenchyma tissue beside to adult females. The cortex region was occupied with giant cells. The 4-6 giant cells present at feeding site were bigger, thick layered. Endodermis and pericycle were damaged (Fig 5a and b).

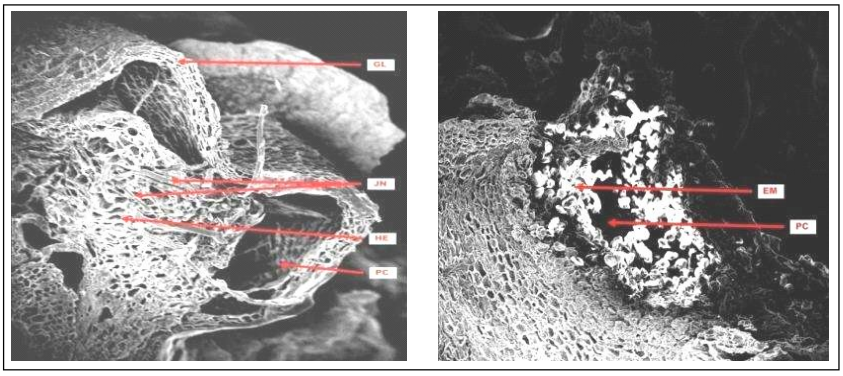
Many large cavities were noticed near the periphery just below the gall outer layer of the root filled with hatched juveniles and eggs laid by the nematode along with jelly like material (Fig-6 a and b). At the cross section of the gall across cavities, many females were found settled deep inside the gall tissue and laid the eggs. The egg masses were found embedded inside the cavities exposing the embryos. It indicates that, the eggs embedded inside the cavities can develop and repetition of the life cycle within the root tissue (Fig 7a and b). The size of gall increased the hypertrophy and hyperplasia of traumatic parenchyma cells. Mulberry which is major food material for silkworms, root knot disease is a serious problem and leads to physiological changes and decline of leaf quality. Silkworm larvae adversely affect their growth and development in turn quality of cocoon production by consumption of infested leaves. The root knot nematode *M. incognita* has always been difficult for effective management as its soil borne nature with wide host range and perennial nature of mulberry. The histopathological studies have shown anatomical alterations of root leading to the formation of galls in relation to the development of the parasite and their possible role for affecting the normal plant growth. Similar results were reported by Mishra (2008) in the histopathology of mulberry



a b

a and b= Showing giant cell of varying sizes in the gall (300X1.666 and 200 × 1.666)

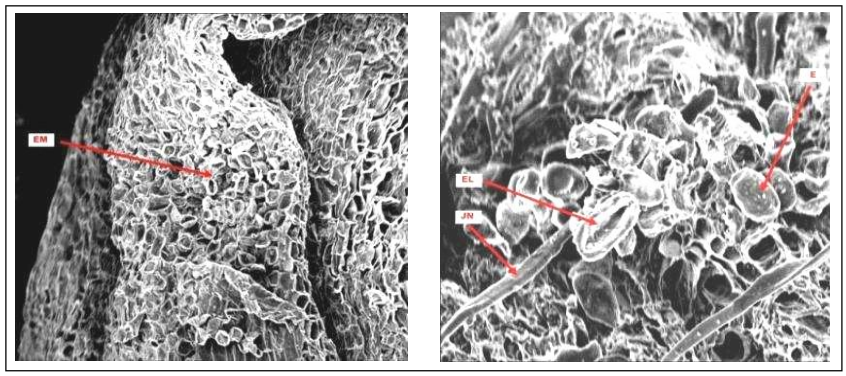
Fig 5: Scanning Electron micrograph of giant cells inside mulberry root gall.
GC= Giant cells, LC= Large Cavity, XV= Xylem Vessel.



a- Peripheral cavity with egg mass (22 × 1.666).

b- Peripheral cavity with egg mass which is opened to outside (200 × 1.666).

Fig 6: Scanning electron micrograph of T.S gall showing egg masses inside the peripheral cavities.
PC= Peripheral Cavity, GL= Gall outer layer, JN= Juvenile Nematodes, HE= Hatched eggs, EM= Egg Mass.



a- Egg mass present deep inside the cortex (300 × 1.666).

b- Embryonic larvae inside egg (200 × 1.666).

Fig 7: Scanning electron micrograph of T.S gall showing deep egg masses inside the cortex.
EM= Egg mass present in deep cortex, E= EGG, EL= Embryonic Larva inside Egg, JN= Juvenile nematode.

genotypes *viz.* S13 with moderate resistance to the root knot nematode and V1, a known susceptible variety, both of which were inoculated with root knot nematode *M. incognita*.

CONCLUSION

It is concluded that *Meloidogyne incognita* shows severe pathogenicity on mulberry and it brings about considerable anatomical changes in infested mulberry roots. Malformation of xylem and phloem in the root tissue it effects on transportation of minerals and water to plant adversely. This might be the cause of yield and quality loss in mulberry leaves and effects on silk worm health and quality of cocoon production. Finally it leads to economic loss in silk industry.

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Conflict of interest: None.

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