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## Histopathological Response of *Lens culinaris* Roots Towards Root-knot Nematode, *Meloidogyne incognita*

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**Abstract:** *Lens culinaris* (lentil) is an important pulse crop. The yield of the crop is reduced if grown in root-knot nematode (*Meloidogyne incognita*) infested field. *Meloidogyne incognita* caused infection in primary and the secondary roots leading to the anomalies in the affected part of the root. The study revealed that the second stage juveniles ( $J_2$ ) of *Meloidogyne incognita* entered the growing roots and their branches inter and intracellularly. The immediate response was hypertrophy and hyperplasia in the root tissue near the nematode head. In response to hypertrophy some cells became very large and contained dense and granular cytoplasm. Adjacent to the giant cells, the vascular tissue was found to be disturbed. Shape, size and orientation of the vascular elements was so much altered that it had become difficult to trace the normal course of vascular strands. In various sections vascular strands were found disrupted. The vessel elements had the shapes resembling the shapes of parenchyma cells. Similarly sieve tube elements of the phloem, near the giant cells were shorter and resembled with nearby parenchyma cells. Abnormalities in xylem and phloem favored transport water, minerals and metabolites towards the giant cells. From this study, it might be inferred that alteration in the cells of galled tissue was essential for the sustenance of giant cells and for the survival of the nematode.

**Key words:** Giant cells, xylem, phloem, parenchyma cells, histopathology

### INTRODUCTION

Parasitism by root-knot nematode is characterized by establishment of permanent feeding sites comprising giant cells in the cortex, endodermis, pericycle and vascular tissue of the host roots. The root cells of different plants respond quickly and characteristically to *Meloidogyne incognita* juveniles. The juveniles move intercellularly towards the region of vascular differentiation where they induce the formation of giant cells (Niyaz and Hisamuddin, 2008; Bhat *et al.*, 2009; Niyaz *et al.*, 2011). The giant cells are generally transformed from undifferentiated vessel elements or from xylem parenchyma (Christie, 1936) or from provascular strand (Krusberg and Nielsen, 1958; Littrell, 1966), or have been reported to arise from protophloem cells (Byrne *et al.*, 1977; Yasmeen, 2002).

In addition to giant cell formation, the root-knot nematode causes hypertrophy and hyperplasia in the cells adjacent to the giant cells that lead to the formation of galls. *Meloidogyne* infections accompany cortical and stelar proliferations (Davis and Jenkins, 1960),

hypertrophy and hyperplasia in the cortex, pericycle and stele of the roots (Azam and Hisamuddin, 2008; Azam *et al.*, 2011).

Histological studies appear to be the key to unraveling the association between injuries caused to plants by plant parasitic nematodes. Yet no work so far has been reported on histopathological studies of lentil roots.

Keeping in view, the damage caused by root-knot nematode, *M. incognita* to lentil roots, the present study was conducted to ascertain the histological changes that lead to the formation of galls on the roots of *Lens culinaris*, formation of giant cells and also to assess the abnormalities in orientation of xylem and phloem and to study the relationship between the giant cells and the vascular elements.

### MATERIALS AND METHODS

**Maintenance of *Meloidogyne incognita* Culture:** Pure inoculum of *M. incognita* was maintained on egg plants by using single egg mass culture. Heavily infested roots of egg plant, on which pure culture of *M. incognita* was

maintained, served as the source of nematode inoculum. The egg masses from these infected plants were handpicked with the help of sterilized forceps. The egg masses after being washed with distilled water were placed in 10 cm diameter sieves, lined with two layers of tissue paper, placed in a Petri plate containing sufficient amount of double distilled water. Freshly hatched second-stage juveniles were collected regularly after every 24 h and transferred to a beaker, incubated at room temperature 27+5°C (Den Ouden, 1958). Water suspension of second-stage juveniles was thoroughly stirred to homogenize and the total number of nematodes per milliliter was counted with the help of a counting dish (Doncaster, 1962) under stereoscopic microscope.

**Raising the test plant:** Lentil seeds (cultivar K-75) were surface sterilized with 0.1 sodium hypochlorite (NaOCl) (Koenning and Barker, 1985) poured into sterilized beaker filled with 1:1 mixture of 95% ethanol and 5.25% NaOCl. The mixture was drained off and the seeds were rinsed with sterilized distilled water. About 3-4 axenized seeds, pre-soaked in water, were sown in 15 cm clay pots, each filled with one kg steam sterilized soil (7 clay: 3 sand: 1 farmyard manure). One week after germination thinning was done to maintain single seedling per pot.

**Inoculation technique:** When the seedlings became one week old, holes of 5-7 cm depth around the plants within the radius of 2 cm from the plant were made. Through these holes the suspension of second-stage juveniles was inoculated at the rate of 1,000 J<sub>2</sub> per plant. The holes were plugged with sterilized soil. To maintain the soil moisture, the pots were regularly watered. The non-inoculated plants served as control.

**Harvesting:** Plants (one from each treated pot) were harvested after 45 days of inoculation. The roots were washed thoroughly and gently to remove soil particles. The galled roots were then cut into one cm long pieces and processed for histopathological studies.

**Processing for histopathological studies:** From infected roots, few galled portions were selected from each treatment for performing histopathological studies. The galled tissues were cut and fixed in Formalin Aceto-Alcohol (F.A.A) and then dehydrated through Tertiary Butyl Alcohol (TBA) schedule (Johansen, 1940). The galls were infiltrated with paraffin oil and then embedded in paraffin wax. The wax embedded galls were trimmed to small blocks and then fixed on wooden blocks. Sections of 10 µm thickness were obtained in the form of ribbon with the help of rotary microtome. The ribbons

were cut and mounted on the slides, which were kept in an incubator at 40°C for 24 h (Johansen, 1940). The sections were stained with safranin and fast green as described by Sass (1951) and mounted in Canada balsam. For anatomical details, the sections were observed under light microscope and necessary photographs were taken.

## RESULTS

**Anatomy of normal root:** The normal primary root of *Lens culinaris* is tetrarch. It consists of epidermis, cortex, endodermis, pericycle, radial vascular bundles and the pith. The secondary growth pattern is typically of dicotyledonous root type; secondary xylem (wood) is produced towards the periphery. Secondary xylem consists of prominent vessel elements and parenchymatous rays (Fig. 1).

**Giant cell and gall formation:** The second-stage juveniles of *Meloidogyne incognita* penetrated the young roots of lentil and moved intercellularly and settled in the region of vascular differentiation (Fig. 2). The juveniles while penetrating, pushed the root cap cells apart and made a narrow passage to move through it. The entrance formed by a juvenile was also used by others reaching at the same point (Fig. 3).

Cellular injury at the root cap was not observed but the cells were just pushed apart and thus, a narrow

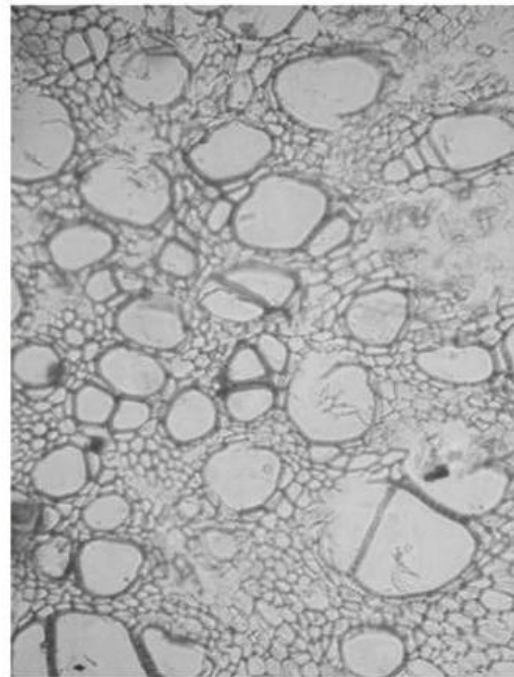


Fig. 1: Normal root anatomy

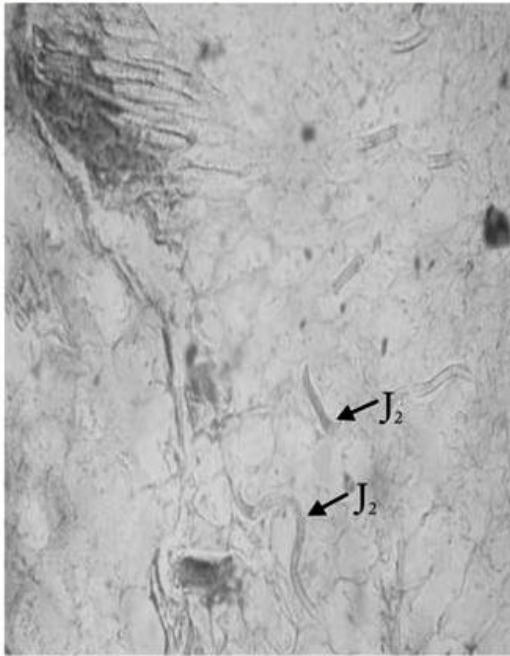


Fig. 2: (J2) second stage juvenile

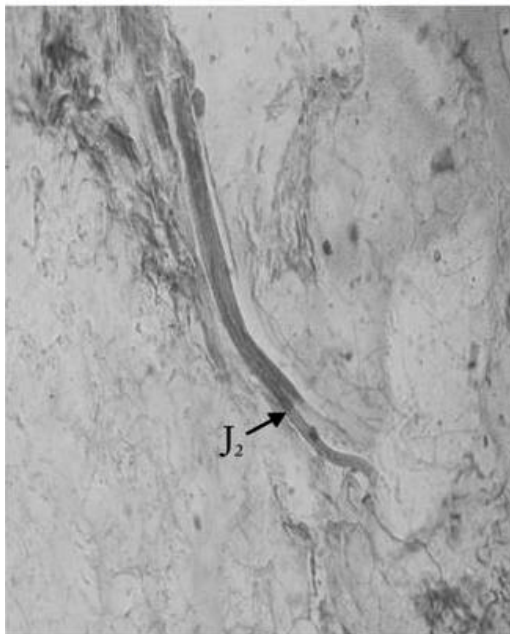


Fig. 3: Second stage juvenile moving through the path made by other juveniles

passage was established. Few cells of epidermis and outer cortex were found severely damaged at the point of entry.

The cells along the length of the nematode appeared small and compactly arranged. In some cells, two hypertrophied nuclei were noticed. In each hypertrophied two or more hypertrophied nucleoli were found.

Hypertrophy and hyperplasia occurring simultaneously were observed in the provascular region. The cells near the head of the nematode were severely hypertrophied. These incipient 'giant cells' contained a large central vacuole and parietally distributed, granular cytoplasm.

With the development of the root and formation of secondary xylem, infected zone was found pushed towards the periphery. The giant cell complexes and the nematodes occupied ray regions of the root.

### VASCULAR ELEMENTS

**Xylem:** The second-stage juveniles of *Meloidogyne incognita* penetrated the roots of *Lens culinaris* and after moving through the region of vascular differentiation settled in between xylem and phloem (Fig. 4). The vessel elements and xylem parenchyma were found distorted when observed in transverse and longitudinal sections. The vessel elements of secondary xylem, near the giant cells, were enlarged. Their diameter was larger than the normal vessel elements (Fig. 5).

Abnormalities in xylem were more prominent near the giant cell complex (Fig. 6). The giant cells containing dense cytoplasm, hypertrophied nuclei and nucleoli were observed away from the xylem elements (Fig. 7). The vessel elements exhibited abnormalities in shapes, sizes and orientation. In a transverse section longitudinally arranged abnormal vessel elements were more frequent (Fig. 8).

Abnormal xylem was found differentiated towards the cortex, in the infected part (Fig. 9). The Giant Cell Complexes (GCC) in the wood region and cortical region were observed when the same part of the root was attacked by several nematodes (Fig. 10).

In infected root the wood part and in the cortical part parenchyma exhibited abnormalities in shapes and sizes. The affected part of xylem parenchyma, was found changing into vessel like elements. The abnormal vessel elements differentiated from ray parenchyma in the affected portion were frequently seen (Fig. 7-9).

**Phloem:** Primary phloem is the product of fascicular cambium and secondary phloem of vascular cambium. The second stage juveniles were found in the zone of cell division and differentiation. The giant cells were observed in the vascular region usually at the places where xylem

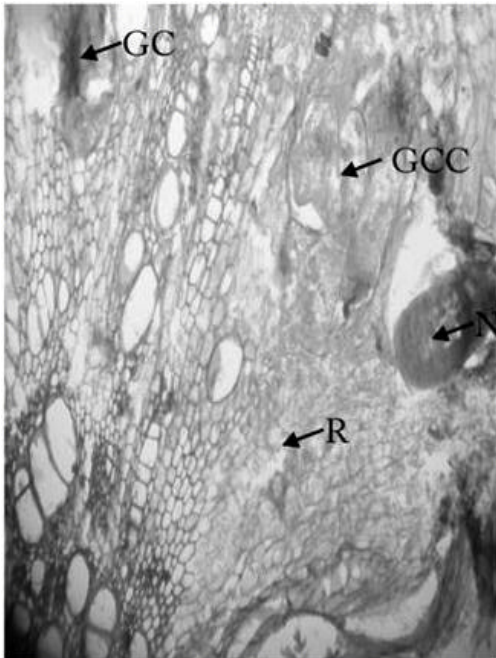


Fig. 4: Giant cell (GC), giant cell complex (GCC), mature nematode (N) and ray (R)

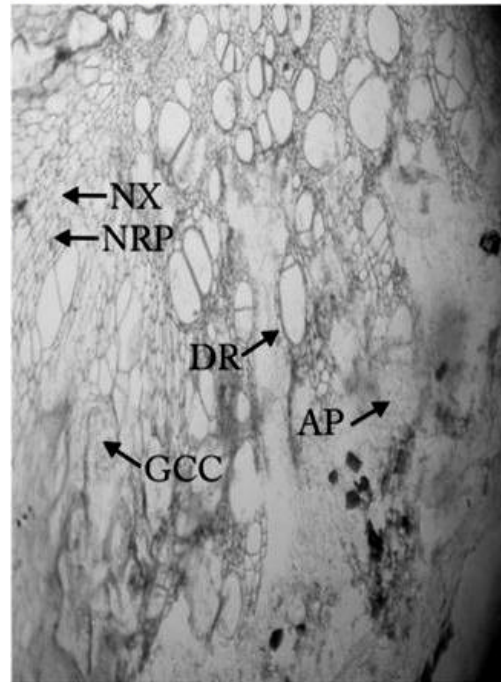


Fig. 6: Normal Xylem (NX), Normal Ray Parenchyma (NRP), distortion in ray parenchyma (DR), Abnormal Phloem (AP) and Giant Cell Complex (GCC)

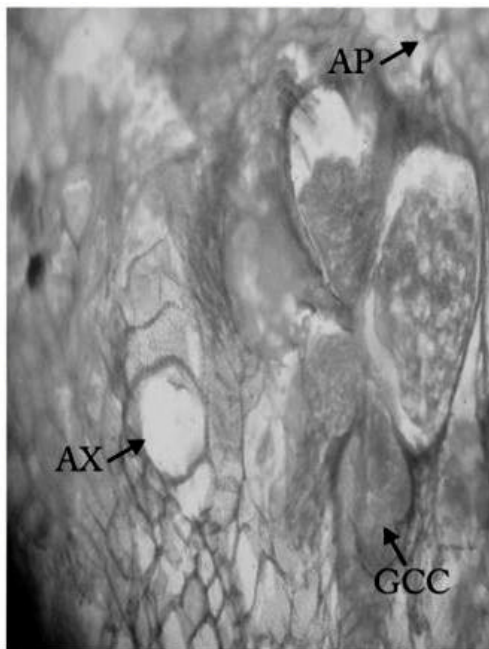


Fig. 5: Abnormal Phloem (AP), Abnormal Xylem (AX) and Giant Cell Complex (GCC)

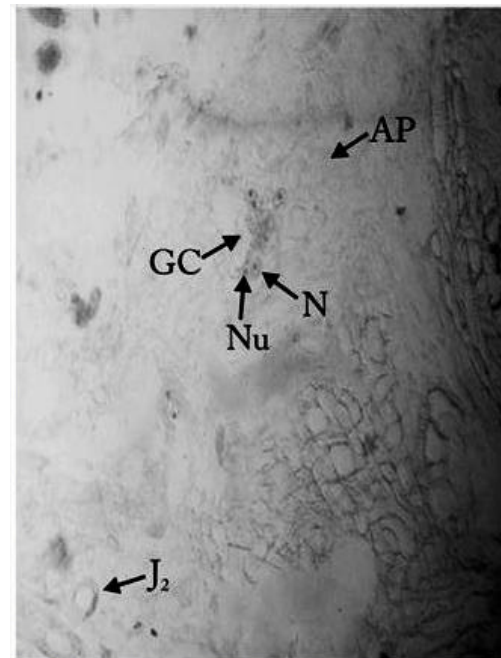


Fig. 7: Abnormal Phloem (AP), Giant Cell (GC), Nucleus (N), Nucleolus (Nu) and Juvenile (J2)

and phloem elements are synthesized and differentiated into respective elements.



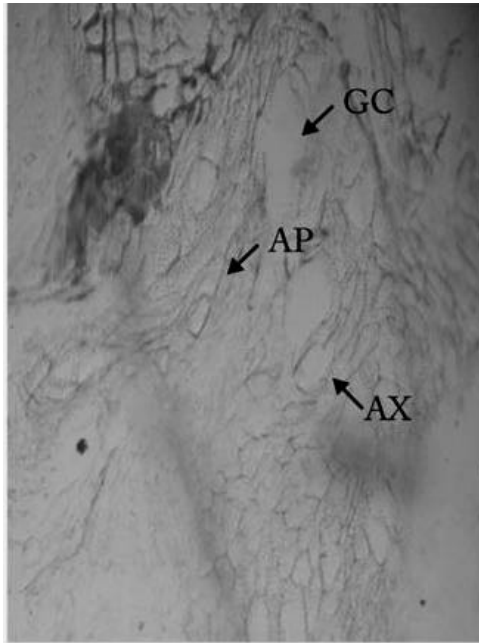


Fig. 8: Giant Cell (GC), Abnormal Phloem (AP) and Abnormal Xylem (AX)

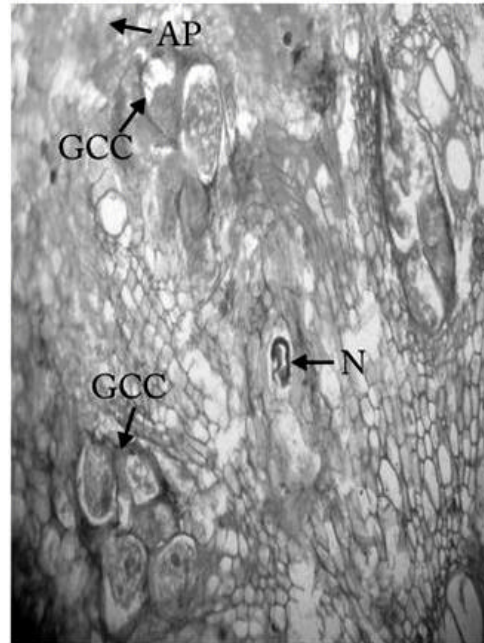


Fig. 10: Abnormal Phloem (AP), Giant Cell Complex (GCC) and mature Nematode (N)

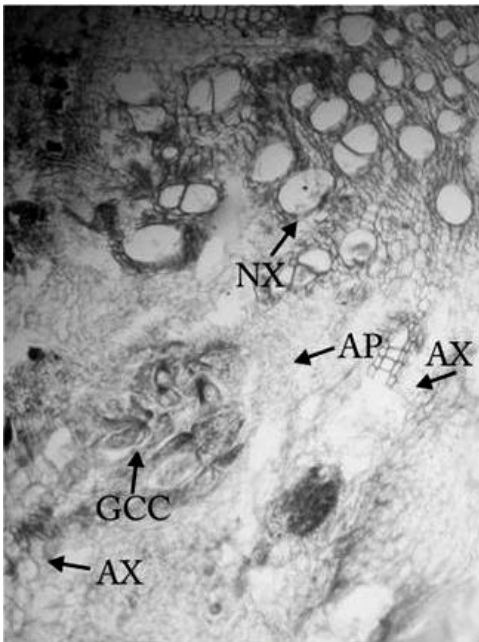


Fig. 9: Normal Xylem (NX), Abnormal Phloem (AP), Abnormal Xylem (AX), Giant Cell Complex (GCC)

Phloem elements exhibited changes in their morphology and orientation. The sieve tube elements had

larger diameter and shorter length and were termed as Abnormal Phloem (AP) (Fig. 5). Abnormal sieve tube elements adjacent to the giant cells had no any definite orientation (Fig. 8). Parenchyma cells and abnormal sieve tube elements near the giant cell complex were indistinguishable (Fig. 6, 7 and 8).

## DISCUSSION

The second-stage juveniles of *Meloidogyne incognita* are probably attracted towards the roots of lentil and gain entry into the inner tissue. The anatomical studies revealed that the juveniles entered into the cortex or the stele through the zone of cell division, elongation and differentiation. The mode of entry was axial (through the root cap), radial or tangential (through the cortex). Formation of channels in the inner tissue of the roots confirmed their intercellular migration.

Occurrence of larger cells, near the nematode, or along the passage of nematode was probably due to hypertrophic response. The hypertrophic response was also observed in the nuclei of the affected cells. Entry of the juveniles anywhere from the root cap to zone of cell differentiation has been reported earlier (Christie, 1936; Krusberg and Nielsen, 1958; Siddiqui and Taylor, 1970; Ismail *et al.*, 2004). The juveniles of root-knot nematode, *M. incognita*, migrated intercellularly by separating

the cell walls along the middle lamella (Endo and Wergin, 1973; Jones and Payne, 1978).

Movement of the nematode inside the root without damaging the inner cells and tissues was favorable for the nematode as it could be harmful for its own survival. Any injury to the root tissue may lead to death of the plant that would also be deleterious for the nematode as a consequence the nematode could die. The immediate response of the inner tissue towards the nematode was enlargement of certain cells. It is supposed that the secretions of nematode contained some enzymes and hormones, like pectinases, cellulases and auxins etc. Enlargement and fragmentation of the nucleoli of the affected cells occurred in various plants due to root-knot infection (Hisamuddin, 1992; Yasmeen, 2002; Parveen, 2006; Niyaz and Hisamuddin, 2009).

In addition to hypertrophy, the affected cells exhibited nuclear divisions without cell wall formation. Occurrence of 5-6 giant cells, indicated that the host parasite relationship has been successfully established during this period. The giant cells possessed upto six nuclei and each nucleus had one or more nucleoli (Jones and Payne, 1978; Yousif, 1979; Hisamuddin, 1992; Hisamuddin and Siddiqui, 1992).

In the affected roots of lentil (*Lens culinaris*), the root-knot nematode (*Meloidogyne incognita*) caused various abnormalities in the cortical and the stelar region (Chandel *et al.*, 2001; Sayed *et al.*, 2010). During the development, the nematode gets oriented radially. When the roots are young, then the nematode head lies in meristematic (cambial) zone and the body in the cortical region. This orientation favors the pushing out of egg masses out of the root tissues.

The most affected tissues in the roots appeared to be vascular tissues. The giant cells were found adjacent to the xylem elements which resulted in various kinds of abnormalities in xylem elements. The normal vessel elements got enlarged and disoriented. Both of these anomalies resulted in hindrance in transport of water and minerals towards the aerial part. The other alteration was that the parenchyma cells adjacent to the giant cells transformed into vessel like elements. All these changes in the xylem tissue were responsible in transport of water towards the giant cells (Singh *et al.*, 2007). The multinucleate giant cells require more amount of water for enhanced rate of synthesis of cytoplasmic materials which was fulfilled by modification of the cells of xylem strand in the galled portion.

The ordinary parenchyma cells, since are transformed into abnormal vessel like elements, therefore, their function of storage of food is taken up by the giant cells. Thus, the photosynthates that were to be stored in root

parenchyma under normal conditions, are diverted towards the giant cells. The giant cells are mimicked as storage cells, functioning as sink for the photosynthetic metabolites. This kind of modification is favorable for the nematode, as it obtains its nutrition from the giant cells (Hisamuddin, 1992) on *Lufa cylindrica*; (Sosa-Moss *et al.*, 1983) on tobacco; (Vovlas and Ekanayake, 1985) on banana; (Khan *et al.*, 2007) on jujube.

In case of severe infection a large number of galls on the roots are formed. In each gall several giant cell complexes become functional towards which mineral nutrients, water and metabolites are diverted (Senthilkumar *et al.*, 2007; Azam, 2009; Niyaz *et al.*, 2011). Transport of metabolites towards the giant cells is performed by the phloem that always remains connected with the giant cells. The phloem elements, near the giant cells become deformed and on the basis of their shapes, these cannot be identified easily. It was found that phloem strand got diverted towards the giant cells. Completely suppressed phloem was found by (Swamy and Krishnamurthy, 1971) in *Meloidogyne* infected *Basella alba* roots. Abnormal sieve tube elements with unusual orientations were formed in *Lagenaria* roots only after the destruction of primary phloem as a result of root-knot nematode infection (Siddiqui and Ghouse, 1975).

## CONCLUSION

From the study it might be concluded that in the roots of lentil plant, the nematodes modified the functions of developing cells. The parenchyma cells were differentiated into different kinds of vascular elements. The vascular tissue, in the infection court, transported water, minerals and nutrition in greater amount for sustaining the giant cells. The normal mode of translocation of water and minerals was interfered by the elements of abnormal xylem. A large amount of metabolites were translocated towards the giant cells, formation of gall and differentiation of abnormal xylem elements contributed in sustaining giant cells on which the survival of nematode is depended.

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