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Histopathological Changes Due To RKN, *Meloidogyne Incognita* in Turmeric Root System

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Abstract- **Root knot nematodes, RKN (*Meloidogyne* spp.) are important sedentary endoparasites which is a polyphagous nematodewith wide host range in many economically important crop species. Turmeric (*Curcuma longa* L.) is an important spice as well as medicinal plantgrown in many regions where root knot nematodes are major problem in production fields. Themechanismoffeedingsitedevelopedbyroot knot nematodesisnot wellunderstoodasitisavery dynamic andcomplex processboth thenematode andthehostplant root system. Therefore, the histopathological studies of root knot infested galled roots of turmeric were collected and processed for microtome sectioning with respect to treated with Carbofuran 3G against untreated control under glasshouse condition.**

Key Words: **Carbofuran 3G, Histopathology, Microtome, Root knot nematode, Turmeric.**

I. INTRODUCTION

Root knot nematodes are one of the most important economically nematode pests of many crop plants and have a diverse host range. RKN (*Meloidogyne* spp.) are sedentary root endoparasites are involved in the development of specialized feeding structures known as giant cells. The infective stage of RKN is second stage juvenile (J2). The J2 penetrate into the roots and complete three moults in the root to become adult females or males. Most of the root knot nematode species including *M. incognita* reproduce by obligate mitotic parthenogenesis [8].

The mechanism of feeding site development by RKN is not well understood because it's a very dynamic and complex process. The secretions from the oesophageal glands of the nematode are important to initiating the development of feeding structures [2],[13]. The first indication of giant cell induction by RKN is the formation of a binucleate cell. Rapid divisions of the nuclei continue in the absence of cytokinesis (acytokinetic mitosis) is responsible forthe formation of several large multinucleate cells. The surrounding cells divide to form the characteristic galls is known as root knots[4]. The xylem parenchyma cells become transfer cells by forming finger like wall invaginations [6]. This helps in water transport from the xylem to the feeding sites. [5] studied the histopathology of ginger (*Zingiber officinale* Rose.) infected by root knot nematode *Meloidogyne* sp. The authors observed that except the aerial shoot system all the other parts of the plants *viz.*, roots, rhizomes and scale were infected by nematodes. The initial target of infection was near the xylem pole. Hyperplasia of parenchyma cells was common in the infected rhizomes and roots. However, galling was conspicuous in adventitious roots. The infection induced the formation of giant cells and active division of cells surrounding the infected area. The giant cell showed akaryotic division of nuclei and thickened cell walls which may showed fine irregular wall projections. The infection resulted in discoloured spots which were seen after dissecting the roots.

The cross section of infected root portion on examination revealed that the head portion of the nematodes located near xylem (pericycle) area while the posterior region was found inside cortical parenchyma. Due to the deposition of eggmass the parenchyma cells were compressed to form a cavity. It is also observed that the adults were oriented through 5-6 layers of parenchyma cells to enable them for feeding in pericycle region[11].

Maximum strain on host plant due to root knot nematode infection was seen during the commencement of feeding by adults and continued upto to completion of egg laying [12].These giant cells were seen with thickened walls in vascular tissues including phloem and parenchyma of stelar regions. The transverse section of fresh galled roots of ginger showed that *M. incognita* entered the cortex and stelar regions and formation of giant cells in the root tissues. The existence of females within eggmass was very well observed in giant cells [9].

II. MATERIAL AND METHODS

The histopathological studies were made in root samples collected from the galled roots were carefully

removed from turmeric plant during the termination of the experiment conducted under glasshouse conditions. The steps involved in histopathology as follows [7].

A. Tissue selection and preparation

Root samples from carbofuran 3G treated and untreated plants were collected and washed gently under running tap water to remove soil particles. Then the root tissues were cut into smaller pieces (1cm) and placed into a formalin acetoalcohol (FAA) as fixative.

B. Fixation

The tissues were submerged in the fixative FAA prepared with 90 ml of 50% ethanol, 5ml of glacial acetic acid and 5 ml of 37% formaldehyde. (At least ten times greater than that of the volume of the tissue) and allowed to remain in the fixative for 48 hr.

C. Dehydration

The root tissues were dehydrated with tertiary butyl alcohol (TBA) gradually by increasing the concentration of alcohol as follows.

Step	% Alcohol	Time	Distilled water	95% Ethanol	100% Ethanol	100 % TBA
1.	50	2 hr	50	40	0	10
2.	70	Over night	30	50	0	20
3.	85	1-2 hr	15	50	0	35
4.	95	1-2 hr	0	45	0	55
5.	100	1-3 hr	0	0	25	75
6.	100	1-3 hr	0	0	0	100
7.	100	1-3 hr	0	0	0	100
8.	100	Over night	0	0	0	100

D. Infiltration

After dehydration the turmeric root tissues were replaced with 1:1 mixture of 100% TBA and paraffin oil for about 1 hr. Then the turmeric root tissues replaced with solidified paraffin in a container and covered with a layer of TBA paraffin oil solution. Then the container placed uncover in an oven slightly above the melting point of the paraffin. The melted paraffin replaced with specialized type of paraffin for histological studies and kept in oven overnight.

E. Embedding

Moulds designed for histopathological studies were coated with a thin layer of glycerine. The turmeric root tissues of both treated and untreated were placed carefully into the mould with heated forceps and additional paraffin added to fill the mould in the hot plate (60^{o}C). Then the moulds were cooled for solidification and to remove the paraffin from mould. Afterwards the mould was cut into small blocks for sectioning.

F. Sectioning

Excess paraffin surrounding the turmeric root tissues trimmed away before sectioning and leaving 1mm around the tissue. Then the trimmed block cooled in ice water for about 5 min and inserted into Spencer's rotary microtome for sectioning with 8-12 µm thickness.

G. Ribbon mounting

Ribbons were cut into shorter length and fixed on slides coated with small amount of Haupt's adhesive.

H. Staining

The sections were stained with safranin and counterstained with fast green to remove the paraffin from the sections and increase the contrast in the tissue. The turmeric root samples treated with carbofuran 3G and untreated were stained separately in a coupling jar containing staining solutions.

I. Mounting

After staining the slides were mounted with cover slip using the DPX mount. Then the slides were left flat to dry for at least 24 hr at room temperature.

III. RESULTS

Histopathological studies on the interaction of *M. incognita* with turmeric were performed by taking 12µm thickness root sectioning of plants challenged with *M. incognita* alone and in combination of chemical pesticide carbofuran (Fig. 1).

Root section of turmeric plant infected with *M. incognita* alone showed conspicuous giant cells having 4-5 nuclei with dense protoplast. The cell walls of the adjacent healthy tissues have also appeared to be slightly thicker than normal cells. Generally 3-4 giant cells were seen in a feeding site of *M. incognita* in turmeric root. The vascular bundles (xylem vessels) were distributed and pushed aside. Conversely, the root section of plants infected with *M. incognita* and treated with carbofuran exhibited smaller size giant cells numbering around 3 per feeding site (Fig. 2). However the other cell wall thickening and multinucleate conditions were the common phenomenon as seen in the turmeric roots infected with *M. incognita*. The developments of females were also slightly hampered in the plants treated with carbofuran than untreated control.

In general the giant cells were formed in the parenchymatous cells near the stelar region on both the samples. The high cytoplasmic density and smaller sized vacuoles of the giant cells was evident in matured feeding cells than giant cells formed by developing root knot nematode *M. incognita*.

IV. DISCUSSION

RKN induced giant cells were focused on acquisition and concentration of nutrients for their growth and development. The mode of giant cell formation has been subject of controversy till date with an opinion maintaining that a giant cell formation due to expansion of a single cell and other opinion is due to rupture /degradation of cell wall and merging of protoplast [1],[10].

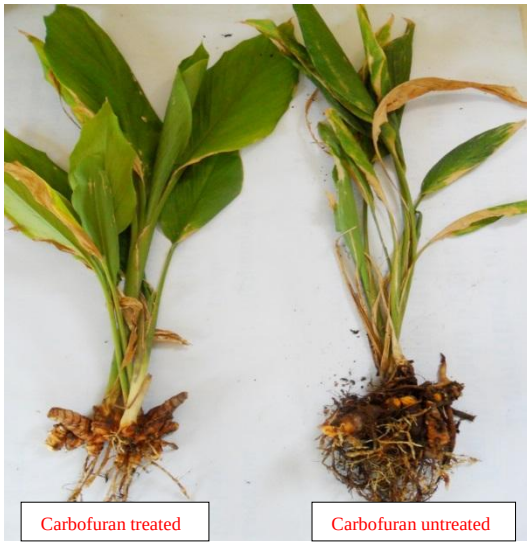

Fig.1. Difference between Carbofuran treated and untreated turmeric plants

It was not yet understood how feeding cells were induced but it was believed that pathogenicity factors secreted by the nematode might have direct effects on host cells [3]. The transformation of root cells into hypertrophied feeding cells with typical function requires extensive changes to give expression in infected root cells [4].

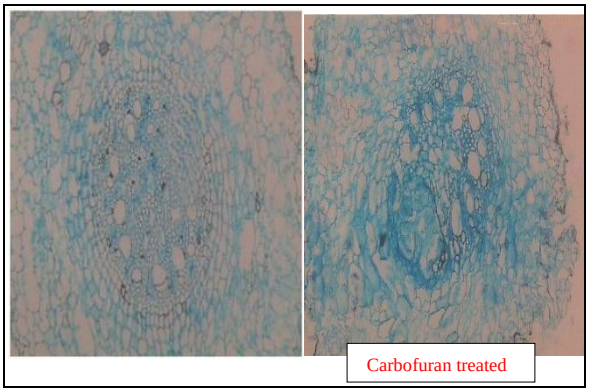

Fig. 2. Histopathological changes due to *M. incognita* in root section

V. CONCLUSION

In the present investigation, the typical structure, cell contents and morphology of giant cells formed by *M. incognita* in turmeric was confirmed with plant infected with *M. incognita* alone showed conspicuous giant cells and root section of plants treated with carbofuran exhibited smaller size giant cells numbering around 3 per feeding site.

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