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Histological Responses and Damage Potential in Roots of *Eclipta alba L* Caused by *Meloidogyne incognita*

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Abstract: The plants of *Eclipta alba* L. were inoculated with 0, 20, 200, 2,000 and 20,000 juveniles of root-knot nematode (*Meloidogyne incognita*) per pot, under green house condition. Significant and maximum reduction in plant growth and yield were noticed at the highest inoculum level. The number of galls was greatly influenced by the initial population of the nematode. The juveniles caused rupturing of root epidermis of the root of *E.alba* while penetrating into the inner tissues In young roots the juveniles migrated towards differentiating vascular tissues. Their migration were intra and inter cellular. In older roots, they migrated through the cortex.. They caused the formation of giant cells in the form of clusters. In a giant cell cluster five to twelve giant cells were observed, each having dense cytoplasm and enlarged nuclei. All the nuclei enclosed one to few nucleoli. The giant cell clusters changed the internal morphology of the affected tissue. In addition, abnormal xylem and abnormal phloem also occupied a major portion near the giant cells.

Key word: *Eclipta alba* % *Meloidogyne incognita histopathology*

INTRODUCTION

Eclipta alba L. belonging to the family *Asteraceae*, is a wildly growing annual herb. The plant contains many active ingredients which are used in preparing medicines and cosmetics. Several diseases like asthma, syphilis, diarrhoea, rheumatism and skin infections are cured by *E.alba* [1] and [2]. The economically treasured plants, including medicinal plants are attacked by a number of pests resulting in huge monetary losses [3]. Root-Knot nematodes, (*Meloidogyne* species) particularly *Meloidogyne incognita* (Kofoid and White) Chitwood and *M. Javanica* (Treub) Chitwood are widely distributed in Northern India. The degree of damage caused by nematodes depends on the species and their population densities, type of hosts and cultivars and environmental factors[4]. *M. incognita* has been reported to be existing in the rhizosphere of *E. alba* in Western Uttar Pradesh, India [5] and [6]. Yet no systemic work so far has been done to assess the damage and histopathology of infected roots. The present work illustrates the effects of *M. incognita* under the influence of different inoculation levels on the plants of *E. alba*. The effort has been made to study the histopathology of the infected plants.

MATERIALS AND METHODS

Seeds of *Eclipta alba* L. were sterilized with (0.1% $HgCl_2$). Seeds were placed on a moist sterilized filter paper kept in a sterilized petri-dish for germination. The sprouted seeds were transplanted into 15 cm diameter pots each containing one kg autoclaved loamy soil. Meanwhile, the egg masses of *Meloidogyne incognita* were collected from the infected roots of egg plants maintained through single egg mass culture. The egg masses were allowed to hatch and the second-stage juveniles were collected after regular intervals of times. The seedlings of *E. alba* were inoculated with 20, 200, 2,000 and 20,000 freshly hatched juveniles of *M. incognita.* Un-inoculated plants served as control. Each treatment was replicated five times in a sample randomized design and plants were watered as when needed. Final data were recorded and statistically analyzed 60 days after inoculation. The length fresh and dry weights of shoots and roots were measured and number of capitula and number of seeds per capitulum were counted. The disease intensity in terms of gall and egg mass index was rated on [7], scale 0=0, 1=1-2; 2=3-10; 3=11-30; 4=31-100; 5=>100.

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Table 1: Effect of different inoculum levels of *Meloidogyne incognita* on plant growth, yield and disease intensity of *Eclipta alba* (L.)

	Shoot			Root					Disease Intensity	
Treatments (Inoculum levels)	Length (cm)	Fresh weight /plant (g)	Dry weight/ plant (g)	Length (cm)	Fresh weight/ plant (g)	Dry weight/ plant (g)	No. of capitula plant^{-1}	No. of seeds capitulum^{-1}	Gall index (GI)	Egg mass index (EMI)
O (control)	28.6	8.15	2.12	22.1	2.86	1.20	22.0	103.4	-	-
20 J_2/pot	27.2	8.06	2.06	20.4	2.78	1.12	21.2	98.2	2.0	1.4
200 J_2/pot	23.1	7.21	1.64	16.7	2.46	1.05	18.2	88.6	2.8	3.0
2000 J_2/pot	17.8	6.02	1.40	13.4	2.30	0.86	16.0	71.2	4.0	3.0
20,000 J_2/pot	15.5	5.04	1.27	10.8	1.70	0.60	10.8	49.2	4.2	4.0
L.S.D. P# 0.05	5.2	0.88	0.32	2.4	0.42	0.12	4.08	12.5	-	-
L.S.D. P# 0.01	6.8	2.0	0.66	5.9	0.78	0.32	5.54	20.0	-	-

GI/EMI = index according to Taylor and Sasser (1978). J_2 = Second-Stage juveniles. Each value is an average of five replicates

For histopathlogical studies, 60 days old roots of *E. alba* were thoroughly washed with tap water and galled portion were cut and fixed in formalin acetic acid-ethyl alcohol (FAA). Selected root portions were dehydrated [8], by n-butyl alcohol method and embedded in wax. Transverse and longitudinal sections of 10-12 µm thickness of the galled roots were cut using rotary microtome and fixed on slides according to [9]. After staining with safranin and fast green [8], the sections were mounted in Canada balsam for microscopic examination and necessary photographs were taken.

RESULTS

Growth: Plant growth and yield of *Eclipta alba* in terms of length, fresh and dry weights of shoots and roots, the number of capitula and number of seeds per capitulum were decreased at all the inoculum levels, when compared with control. Significant (P#0.01). The reductions were observed at 2,000 and 20,000 juveniles per pot. The reduction was highest at the highest inoculum level. In fresh and dry weights of the shoots and the roots reductions were non-significant at lowest (20 J_2/pot), however, significant (P#0.01) reduction was observed at highest (20,000 J_2/pot) inoculum level (Table-1).

Disease Intensity: Both gall index and egg mass index indicated that the plant was susceptible towards *M. incognita* infection. The values of both parameters increased with an increase in the inoculum level (Table1).

Histopathology of the Infected Roots: Transverse and longitudinal sections of the roots of *E. alba* infected with *M. incognita* exhibited severe infestation of the nematode. The second-stage juveniles after penetrating was migrated towards vascular differentiation zone. In the zone of maturation, the juveniles take the path of cortex and then ray parenchyma. The parenchymas in the neighbourhood of the nematode, specifically near the head, was induced to enlarged (Fig-1A). In most of the instances more than one female were usually seen at one feeding site initiating complex or clusters of giant cells within the stellar region. The females, when become mature, start egg laying (Fig-1B). The giant cell complex was induced near the nematode head but hypertrophy and hyperplasic may be induced around the whole body of nematode (Fig-1C). The shape and the size of the giant cells, in a giant cell complex are not uniform. These may have ovoid, elongated or irregular shapes. The giant cells have thick walls and enclose dense and granular cytoplasm. The giant cells, in all the giant cell complexes have multinucleate condition. The nuclei were larger than their normal size. The nuclei were found robe scattered or aggregated in the cell (Fig-1D, 1E). The cortical cells exhibited compact arrangement due to the pressure developed by the nematode and the giant cell complex. In some giant cells and other hypertrophied cells, their were several nuclei but cytoplasm was less dense and less granular (Fig-1F).

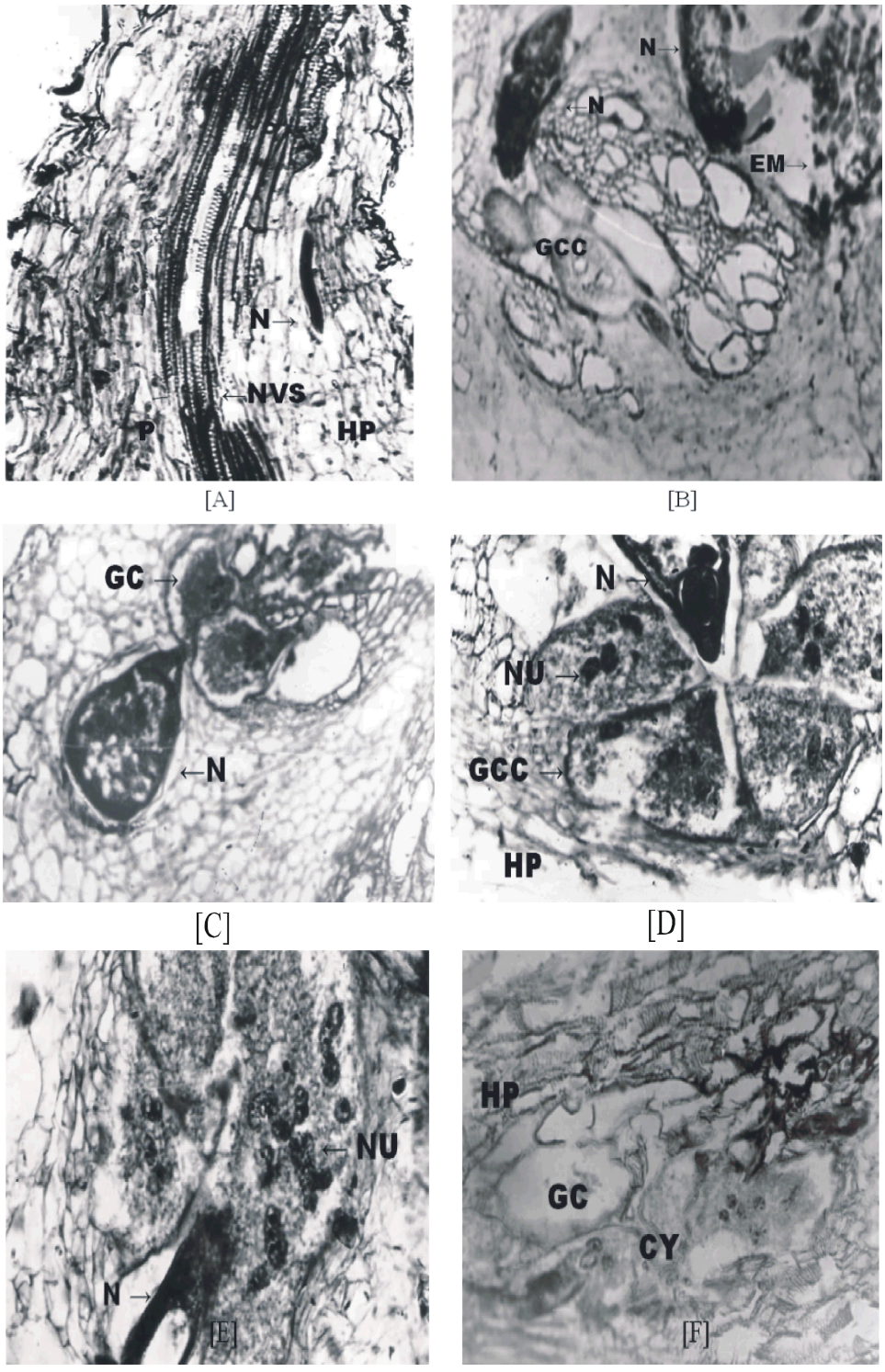

Figure Caption -1 (A-F)

[A] Showing normal xylem (NX), hypertrophic parenchyma (HP), nematode (N) and phloem (P).
[B] Showing adult females located at one feeding site (N), egg masses (EM) and giant cell complex (GCC).
[C] Showing mature female (N), feeding on giant cell (GC).
[D] Showing mature female (N), Number and shape of giant cells in a giant cell complex (GCC).
(E) Showing magnified multinucleate (NU) giant cells with granulated cytoplasm (CY).
S(F) Showing abnormal xylem (AX), giant cell (GC) and hyertrophic parenchyma (HP).

DISCUSSION

The results of the present investigations clearly indicated that *M. incognita* severely affected plant growth and resulted in stunting of *E. alba.* The deleterious effects of the disease were so intense that flowering and fruiting were also deteriorated. *Meloidogyne incognita* at all the inoculum levels produced disease symptoms. Stunting of the plant, loss in fresh and dry weight and reduction in yield might be due to harmful effects of the nematode. Alteration in root anatomy due to root-knot nematode infection resulted in impaired translocation of water and minerals towards the shoot. On the contrary the photosynthates were diverted towards the giant cells that served as a transfer cell. These two alterations in plant physiology caused stunting and yield loss. The harmful effects of the nematode on the plant might be correlated with the severity of the disease incidence that could be equated with gall index and egg mass index. The baneful effects of the root-knot nematode on the host plants have been observed and reported by Barker and Olthof, 1976; Hisamuddin 1992; Yasmeen, 2003; Youssef and E1-Nagdi, 2004; Parveen, 2006; Niyaz and Hisamuddin, 2008; Azam *et al*., 2010; Singh *et al* 2010; and Robab *et al* 2010.

The second-stage juveniles of *M. incognita* after migrating towards the stellar region causing extensive damage to the cortical cells. The juveniles immediated induced the formation of giant cells as well as proliferation of neighboring tissues resulting in formation of root galls. Hyperplastic and hypertrophy in the tissue in the close vicinity of the nematode were clearly noticed. Our studies revealed that *M. incognita* induced 4-6 multinucleate giant cells in the vascular tissues and stellar region. [21], reported 3-6 giant cells in tomato, similarly 4-9 giant cells were reported in sweet potato[22], The site of infection is usually vascular tissue but as a result of nematode development and giant cell formation, the entire complex of nematode and giant cells appear to be located in the cortex. The nematode stimulates the giant cells to synthesize cytoplasm in enormous amount. To cope with this situation, the number of nuclei are increased ,which enhance the rate of metabolism at a tremendous rate. The nucleus contains enlarged nuclei which is the indication of high rate of protein synthesis. Number of giant cells in a giant cell complex is high if two or more nematodes infect a common site. At such locations the size of the giant cell became small or when one nematode causes infection then size of a giant cell is large is evident from (Fig-1B and 1D). The depletion of giant cell cytoplasm at higher inoculum levels indicated speedy removal of metabolites by the nematode. Formation of abnormal xylem near the giant cell complex was unique phenomenon associated with the root-knot disease development of excessive abnormal xylem has been found in certain hosts which was an adaptive feature of the plants under disease stress [23-27]. Probably higher production of auxin in the infected portion leads to the transformation of abnormal xylem from normal parenchyma cells.

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