



Histopathological changes induced by *Meloidogyne incognita* in some ornamental plants



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ABSTRACT

Histopathological changes induced by the root-knot nematode (*Meloidogyne incognita*) in five ornamental plants, specifically, *Calendula* (*Calendula officinalis*), *Centaurea* (*Centaurea montana*), *Papaver* (*Papaver somniferum*), *Chrysanthemum* (*Chrysanthemum morifolium*) and *Dianthus* (*Dianthus caryophyllus*), were investigated. Based on the galling index (GI), *Centaurea* was classified as susceptible, *Calendula* as moderately susceptible, and *Papaver* as moderately resistant, while *Chrysanthemum* and *Dianthus* were highly resistant to *M. incognita* infection. The histopathology of the galled roots of *Centaurea*, *Calendula*, and *Papaver* during later stages of infection showed that nematodes were localized entirely within the cortex and generally oriented horizontally to the vascular cylinder. Most of the females were mature, and a few of them were associated with egg masses. Giant cells with a variation in cell sizes were observed in the galled roots of all three of the plant species and exhibited a granular cytoplasm and hypertrophied nuclei as a typical reaction to nematode feeding.

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1. Introduction

India is elegantly paving a route to arise as a substantial participant in the global floriculture industry and is emerging as a future floral superpower. Its multi-climatic environment provides an opportunity to cultivate almost all of the major ornamental cut flower species of the world, regardless of the flowers belonging to different climates. Nematodes represent a danger to the growth and proliferation of ornamental crops. The degree of damage caused by nematodes is dependent upon the species, their population densities, the type of host and cultivars and environmental factors (Mitkowski and Abawi, 2003). To combat the diseases associated with nematodes, it is important to diagnose the host–pathogen interaction efficiently and effectively.

The root-knot nematode *Meloidogyne incognita* (Kofoid and White, 1919; Chitwood, 1949) is a sedentary root endoparasite that causes root knot diseases of a wide variety of cultivated, ornamental and wild plants. The involvement of plant parasitic nematodes in ornamental as well as other plant productions has

remained unrecognized due to their soilborne nature, tiny size and concealed manner of life as well as non-typical deceptive feeding signs on the plants. The huge economic injury to plants by the root feeding nature of nematodes and interactions with other organisms makes the plants further susceptible to other biotic and abiotic stresses, leading to great economic losses (Sikora et al., 2005).

The nematode is in its infective stage at the second-stage juvenile (J2) stage. J2 penetrate the roots and go through three successive moults to become adult females or males. Nematodes are involved in the development of specialized feeding structures known as giant cells. However, little is known about the feeding site development and pathological anatomy of ornamental plants infected by root knot nematodes. Several attempts have previously been made to study, in detail, the histopathology of root-knot nematode-infected roots of various host plants. Krusberg and Nielson (1958); Bird (1962); Huang and Maggenti (1969) and Siddiqui and Taylor (1970) were the initial investigators to describe giant cell formation and the occurrence of abnormal xylem in root-knot nematode-infected roots of different crops. More recently, it has been documented that secretions from the oesophageal glands of the nematode are crucial in initiating the development of feeding structures (Davis et al., 2000; Williamson and Kumar, 2006). In ornamental plants, there is no detailed histological documentation

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on changes caused by nematode infection; therefore, this study was carried out to gather information on screening and histopathological changes induced in some of the economically important ornamental plants belonging to the Asteraceae, Caryophyllaceae and Papaveraceae families in relation to *M. incognita* infection.

2. Materials and methods

2.1. Test plants and pathogen

The study was carried out at the Department of Botany, Aligarh Muslim University, India. During the investigation, ornamental plants belonging to family Asteraceae (*Calendula officinalis*, *Centaurea montana*, *Chrysanthemum morifolium*), Caryophyllaceae (*Dianthus caryophyllus*), and Papaveraceae (*Papaver somniferum*) as the test plants and root-knot nematode *M. incognita* as a test pathogen were selected.

2.2. Collection and preparation of nematode inoculum

Inoculums of *M. incognita* were acquired from roots of infected egg plants collected from heavily infested field. The species was identified on the basis of taxonomic characters and perineal patterns (Eisenback et al., 1981). The inoculums consisted of second stage juveniles obtained by hatching egg masses. For obtaining the required number of inoculums, the Baermann tray method (McSorley, 1987) was opted. Large numbers of egg masses from heavily egg-infested plant roots were washed with distilled water and placed in a sieve lined with double-layered tissue paper. The sieve was placed on a tray containing water that just touched its lower portion. A series of these assemblies were retained to obtain a large number of second stage juveniles. The hatched out juveniles were collected from the trays at an interval of 24 h up to 3 d and transferred to a beaker. The water suspension of the nematode was lightly agitated to obtain a homogenized distribution of nematodes. From this suspension, 5 counts were made with the aid of a counting dish under a stereoscopic microscope and were averaged to determine the density of nematode per volume of the suspension. The volume of the suspension was adjusted so that each mL contained 200 ± 50 juveniles.

2.3. Raising of test plants

The seeds from each of the test plants were surface sterilized with a 0.5% hypochloride (NaOCl) solution for 15 min and were washed thoroughly with five changes of distilled water. The sterilized seeds were then sown in germinating trays. One week after germination, the seedlings were transplanted to pots containing approximately 1 kg of steam-sterilized potting mixture (soil + sand in a 3:1 ratio). Each of the test plant species was comprised of 40 plants and was replicated three times. All of the pots (measuring 12 cm in diameter \times 11.5 cm in height) were provided with a saucer at the base to avoid any leaching of the inoculums. The pots were placed on greenhouse benches and watered manually as required.

2.4. Inoculation with nematodes

Three-day-old transplanted seedlings were inoculated with approximately 2×10^3 *M. incognita* juveniles. Prior to inoculation, the feeder roots of the seedling were carefully exposed by removing the upper layer of soil. The required amount of nematode inoculum was then dispensed uniformly around the exposed roots using a sterilized dispenser. Soon after the inoculation, the bare roots were concealed by levelling the soil properly. Similar numbers of non-inoculated pots of each test plant served as controls.

2.5. Sampling

The sampling of roots was carried out at different time intervals, i.e., 10, 20, 40 and 60 d after inoculation (DAI). At every sampling time, ten plants were randomly selected from each test plant group. The plants were carefully uprooted, and the roots were washed of adhering soil particles while taking the utmost care to avoid loss and injury during the entire operation. The numbers of root-knot galls on infected plants were counted.

2.6. Response of plant species to *M. incognita*

The degrees of susceptibility of the respective test plants were determined on the basis of the Taylor and Sasser (1978) rating scale. In the present investigation, the gall index (GI) had been used as an indicator of plant damage. An average GI of 2 or less is interpreted as an indicator of host resistance, and an average GI of greater than 2 is interpreted as an indicator of host susceptibility (Table 1).

2.7. Histopathological studies

Galled and un-galled roots of test plants were processed for histopathological studies according to Johansen (1940). Briefly, the roots from each of the test plants were washed free of soil and fixed with triethanolamine/formalin (TAF) at 70 °C for 24 h and examined under a binocular microscope. Selected root pieces with and without galls were passed through an alcohol series and embedded in paraffin wax to obtain small blocks. Transverse longitudinal sections of 8–12- μ m thicknesses of the roots were sliced successively with the help of a rotary microtome (Microm, HM310, South Carolina, USA). The paraffin ribbons were mounted on a clean glass slide with a drop of albumin and glycerine dissolved in water. Staining was carried out with a saffranin and fast green combination as previously described (Sass, 1951). The root sections were examined under a microscope to study the anatomical changes of plant tissue and stages of nematode development. Microphotographs were taken to elucidate differences in host–plant interactions.

3. Results

3.1. Host suitability of ornamental plants to *M. incognita*

M. incognita notably affected the host plants. The infected test plants were galled between 20 and 40 DAI, and the gall index (GI) ranged between 2 and 4. The highest gall index was recorded on *Centaurea* (GI- 4) and the lowest on *Papaver* (GI- 2). Host suitability designation was assigned to the plant species based on the GI as an indicator of plant damage (Table 2). The results indicated that *Centaurea* was highly susceptible to *M. incognita* (GI = 4), *Calendula* was moderately susceptible (GI = 3) and *Papaver* was moderately resistant (GI = 2). The remaining plant species, *Chrysanthemum* and

Table 1
Taylor and Sasser's (1978) rating scale for the presence of root-knot nematode galls on roots.

Number of galls	Gall index (GI)	Host suitability designation
0	0	Highly resistant
1–2	1	Resistant
3–10	2	Moderately resistant
11–30	3	Moderately susceptible
31–99	4	Susceptible
≥ 100	5	Highly susceptible

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