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***Research Article** (Open Access)*

HETERODERIDAE FAMILY PLANT PARASITIC ROUNDWORM "*MELOIDOGYNE INCOGNITA*" CAUSES REDUCTION IN CHLOROPHYLL CONTENT IN TOMATO

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ABSTRACT
Greenhouse tests were conducted to analyze the effect of *Meloidogyne incognita* infection in tomato cultivars (Pusa ruby and Pusa Early Dwarf) on chlorophyll content. The extent of reduction in chlorophyll content in pusa ruby and pusa early dwarf estimated after the 1000 juvenile of *M. incognita* inoculated. As a result of treatment together in three trials, cultivar pusa ruby observed as moderately resistant and pusa early dwarf susceptible to *M. incognita.* Inoculation with *M. incognita* generally resulted in the increase in number of gall and egg mass per gram of root as compared to non-inoculated treated check (control) as well as reduction in chlorophyll a, and chlorophyll b content. In all three trial *M. incognita* is associated with reduced chlorophyll content in tomato leaves. The moderately resistant tomato cultivar pusa ruby tended to have increased 1.563 ± 0.003 mg/g which was significantly higher than the pusa early dwarf variety 1.404 ± 0.004 mg/g.

Keywords: *Chlorophyll, tomato, Meloidogyne incognita, Root gall, Egg mass*

INTRODUCTION
Root knot nematodes (RKN), *Meloidogyne spp*., are sedentary endoparasites those infest nearly all crops, resulting in significant yield losses, particularly in tomato cultivation, where they cause a 35% reduction in yield in India (Ngeno 2019). Trudgill and Block (2001) assert that *Meloidogyne incognita* is among the predominant apomictic species of root-knot nematodes in numerous temperate and tropical regions. *Meloidogyne incognita* functions as a mechanical wounding agent, host modifier, rhizosphere modifier, and resistance breaker; it can incite or exacerbate fungal and bacterial diseases, leading to the potential death of the tomato plant in cases of severe infestation. They disrupt anchorage and the absorption of nutrients by crop plants (Ghahremani 2020; Thakur *et al*., 2024). The normal flow of water and nutrients to the leaves and developing galls can be disrupted by *Meloidogyne incognita*–induced galls on the tap root and lateral roots, resulting in a reduction in cotton growth and yield (EI sagheer 2019). Stunting, nutritional deficiency (chlorosis), and temporary wilting during the heat of the day are among the above-ground symptoms of *M. incognita* infection (Kavitha *et al*., 2025). Some plants may experience a decrease in photosynthetic rates as a result of *M. incognita* infection. The photosynthetic rate of inoculated tomato plants was lower than that of noninoculated plants within two days of *M. incognita* infection (Sharma and Sharma, 2017). Photosynthesis, as measured by fresh weight, leaf area, or total chlorophyll content, experienced a substantial decline during the initial phases of infection. *M. incognita's* infection of henbane (Hyoscyamus niger) resulted in a decrease in plant growth, yield, chlorophyll content, photosynthetic rate, and nutrient concentrations. The most significant reductions were observed at the highest nematode populations. The chlorophyll content and photosynthetic rate of tomato have not been documented in relation to *M. incognita* infection. Changes in nutrient concentration in tomato following infection by *M. incognita* can disrupt host metabolism and contribute to premature leaf abscission and chlorosis (Strajnar *et al*., 2012; Tsaniklidis *et al*., 2021; Sikandar *et al*., 2025).

Chlorophyll is an essential pigment in photosynthesis, and its concentration serves as a sensitive indicator of plant vitality and metabolic function. Biotic stresses, including nematode infestation, can disrupt chlorophyll biosynthesis or hasten its degradation, thus undermining the plant's photosynthetic efficiency and overall productivity (Lichtenthaler, 1996; Mishra and Misra, 2012). The decline in chlorophyll content has been documented in multiple crops subjected to nematode stress; however, the magnitude and dynamics of these effects in tomato caused by *M. incognita* are still inadequately investigated.Chlorophyll is a crucial pigment in photosynthesis, and its concentration serves as a sensitive indicator of plant vitality and metabolic function. Biotic stresses, including nematode infestation, can disrupt chlorophyll biosynthesis or hasten its degradation, thereby undermining the plant's photosynthetic efficiency and overall productivity. The decline in chlorophyll content has been documented in numerous crops subjected to nematode stress; however, the magnitude and dynamics of these effects in tomato caused by *M. incognita* are still inadequately investigated (Moustaka and Moustakas 2023; Kalariya *et al*., 2024). The diminished yield in tomato crops is attributable to numerous abiotic and biotic factors, including nematodes, bacteria, fungi, and viruses. The root-knot nematode *Meloidogyne incognita* is a detrimental pest that diminishes tomato yields by 25.0% to 49.0% (Ahmad *et al*., 2018). While nematicides can effectively address nematode infestations, their detrimental effects on the environment and pollution have prompted increasing concerns and the pursuit of safe, eco-friendly alternatives for phytonematode management. An environmentally sustainable approach to managing the root-knot nematode involves the investigation of biological pest control.

MATERIALS AND METHODS

To comprehend the fundamentals of resistance to the nematode *Meloidogyne incognita* inoculated two tomato varieties— Pusa ruby (Resistant), Pusa early dwarf (Susceptible), were cultivated in 2022-23 in pots within the net house at the Centre for Biotechnology, Maharishi Dayanand University Rohtak, Haryana, India. The 15 cm diameter clay pots were surface sterilized using a 1% formaldehyde solution and filled with aerated sterilized soil, which was autoclaved at 1.1 kg/cm² pressure for one hour daily over two consecutive days, mixed with sand and FYM in a 2:1:1 ratio, adhering to a Complete Randomized Design (CRD) with six treatments, replicated three times. The irrigation water underwent filtration through a five-hundred-mesh screen prior to application.

Figure 1: Tomato cultivars Pusa ruby and Pusa early dwarf germinated for the experimental purpose to analyze the effect of *M. incognita* in chlorophyll content.

Soil sterilization and raising nursery for maintaining M. incognita culture in tomato

The autoclave (121°C at 16 psi) sterilized sandy loam soil for 60 minutes. After sterilization, soil was stored safely and used to raise RKN nurseries. In 9-inch earthen pots, sterilized sandy loam soil was filled and transplanted tomato plants of susceptible varieties were carefully placed on the soil surface and covered with 1 cm of soil. For pure root-knot nematode culture, tomato seedlings were transplanted at 3-5 leaf stages (14 days). One egg mass from *M. incognita* infected tomato plant roots was kept in sterilized water in a Petri dish for hatching. One healthy seedling per pot was kept at the centre after 14 days. A small pipe (2cm long 5 cm bore) was inserted into the soil near each seedling's root zone for RKN inoculation holes (Fig-2). After 35 days of *M. incognita* infection, plants were carefully harvested for pure culture multiplication.

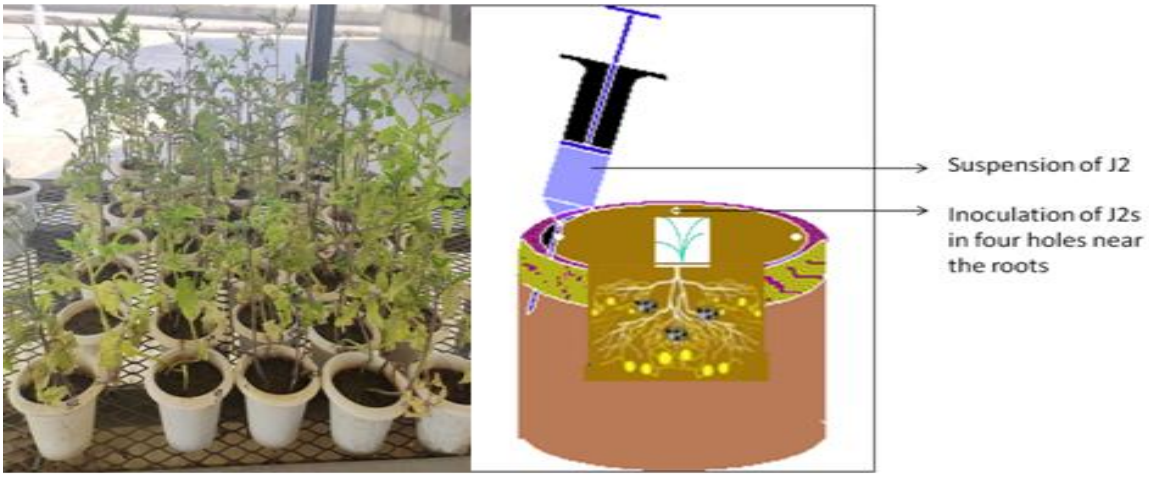

Figure 2: At the 3-5 leaf stage egg suspension of J2- *M. incognita* is inoculated in tomato and left for 35 days from the days of inoculation to complete life cycle.

Gall and egg mass formation of M. incognita in the root of tomato cultivars

Experiment conducted in 8-inch earthen pots filled with sterilized soil in triplicates. Each pot was transplanted from 3-5 leaf stage tomato seedlings; transplanted pots were inoculated with 1000 freshly hatched J2. Observations were conducted at 35th days after inoculation of J2 of M, incognita in tomato cultivars, Pusa ruby and Pusa early dwarf. One set from three replicates of all treatments was harvested at 35th days after J2 inoculation and processed in laboratory for the no. of gall formation in the root system of tomato.

Recording of observations

Thirty-Five days post-nematode inoculation, the inoculated plants were carefully extracted from the potting soil, and the subsequent parameters were assessed using various methodologies:

Chlorophyll pigment estimation

To extract the pigments (Chlorophyll a and Chlorophyll b), follow the procedure of Hiscox and Israelstam (1979) with some modifications. The leaves were finely chopped and placed in test tubes with 7.0 ml of DMSO. The amount of fresh leaves used was 100 mg. For the extraction, the test tubes were placed in an incubator set at 45ºC for 45 minutes while covered with black paper. Absorbance recorded at 645 and 663 nm using spectro-photometer. The extraction medium used is dimethyl sulphoxide (DMSO), and the chlorophyll content calculation method employed is the Arnon method (1949).

Calculation

Chlorophyll a=12.21 × (A663)-2.81(A645) ×volume/weight

Chlorophyll b =20.13× (A645)-5.03(A663) × volume/weight

STATISTICAL ANALYSIS

Statistical analyses were performed with statistical software's GraphPad Prism (version 7.04) and XLSTAT (version 2023.3.1). All measurements were performed in triplicates. Two-way analysis of variance (ANOVA) with Tukey's correction was performed with Graph Pad Prism and the difference was considered significant at p-value ≤0.05.

RESULTS AND DISCUSSION

Effect of M. incognita in gall and egg mass formation in tomato cultivars

Rate of gall formation observed under inoculation of 1000 J2 *M. incognita* alone in tomato cultivars pusa ruby and pusa early dwarf. Nematode infection results in the formation of numerous tumors on infected roots, known as galls, which contain nematode feeding sites. *P*. To study the effects of *M. incognita* on the rate of gall formation in the tomato roots was carried out and observations were recorded as presents in Table No. 1 which showed that all the treatments were observed significantly different from each other. The efficacy of parasitism and the life cycle of RKN are contingent upon their ability to induce nematode-feeding sites within the root tissues of the plant host.

Figure: 3 (A) Root Gall (B) Egg mass formation

Table:1 presents the effects of *M. incognita* inoculation on gall formation in the two tomato cultivars.

***M. incognita* inoculation**	**Cultivars**	**Gall formation**
1000J2	Pusa Ruby	*7.97 ± 1.95 A/E*
1000J2	Pusa Early Dwarf	*43.67 ± 3.06 H*

The data indicate that *M. incognita* inoculation significantly increased gall formation in both cultivars. The percentage of increase in gall formation in Pusa Ruby and Pusa Early Dwarf varies to *31.71*% and *79.84 % respectively.* The results were statistically significant at p=0.05 using Duncan's multiple range test, as indicated by different letters.

Effect of M. incognita in Chlorophyll (Chl a) and Chlorophyll (Chl a) content

Chlorophyll a content decreased significantly with increasing salt concentration in both varieties. At 0 mM salt, the Pusa Ruby variety had a Chl a content of 1.563 ± 0.003 mg/g, which was significantly higher ($p<0.01$) than the Pusa Early Dwarf variety (1.404 ± 0.004 mg/g). Chlorophyll b content followed similar pattern to Chl a, decreasing significantly with increasing salt concentration in both varieties. Pusa Ruby variety had a Chl b content of 0.664 ± 0.006 mg/g, which was significantly higher ($p<0.01$) than the Pusa Early Dwarf variety (0.524 ± 0.014 mg/g). As a result of chlorophyll pigment content in pusa ruby and pusa early dwarf, pusa ruby falls under the moderately resistant and pusa early dwarf in sensitive cultivar under the inoculation of 1000 juvenile of *M. incognita.*

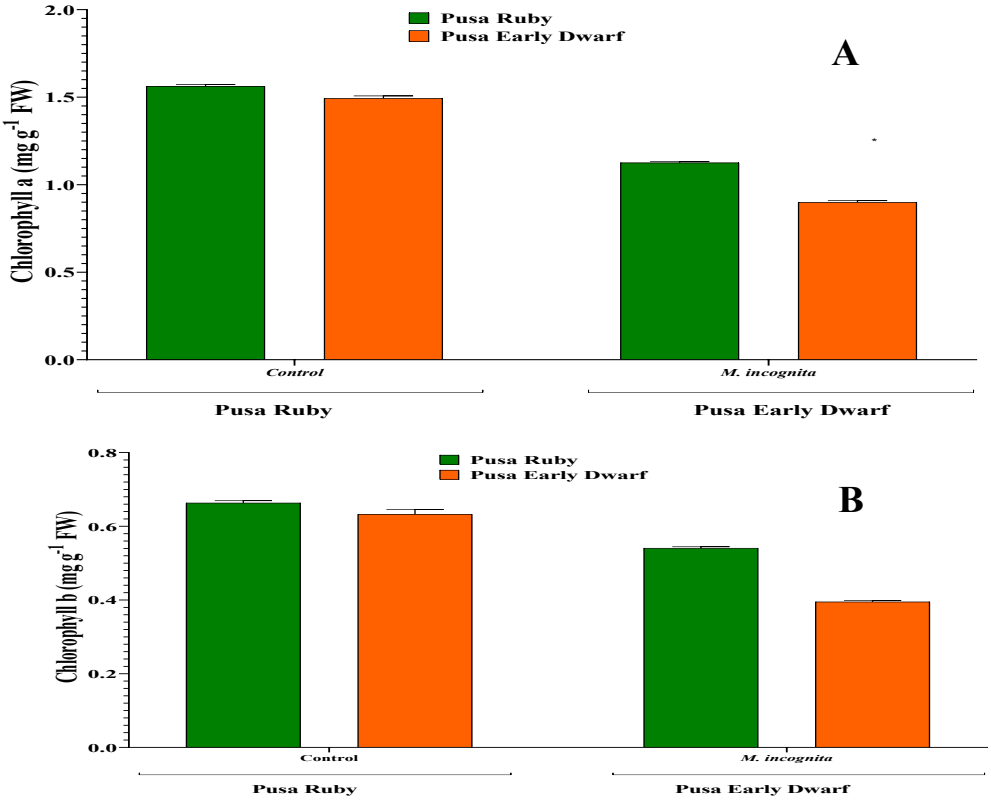

Figure 4: Effect of 1000J2 of *M. incognita* on (A) Chlorophyll a (B) Chlorophyll b content in Pusa Ruby and Pusa Early Dwarf.

DISCUSSION

The present study examined the effect of the plant-parasitic nematode *Meloidogyne incognita*, belonging to the Heteroderidae family, on chlorophyll levels in tomato *(Solanum lycopersicum*). The results unequivocally demonstrate a substantial decrease in both chlorophyll a and chlorophyll b concentrations in infected plants relative to uninfected controls. This decrease aligns with prior studies emphasizing the harmful physiological impacts of root-knot nematode infestations on host plants (Hussey & Barker, 1973; Sasser & Carter, 1985).The noted reduction in chlorophyll content is probably a result of root damage caused by nematodes, which impairs water and nutrient absorption. The development of galls and giant cells disrupts root structure and function, resulting in increased stress and diminished photosynthetic efficacy. Chlorophyll is an essential pigment in the light-dependent reactions of photosynthesis; therefore, its reduction directly correlates with decreased photosynthetic activity, reduced energy production, and inhibited growth (Kumar *et al*., 2014). Nematode infection may induce systemic biochemical responses in addition to causing physical root damage. Imbalances in stress-related phytohormones and the buildup of reactive oxygen species (ROS) in infected plants may exacerbate chlorophyll degradation or inhibit chlorophyll biosynthesis pathways (Abad *et al*., 2008). Stress signals may trigger senescence-like responses in leaves, despite the primary infection site being confined to the roots. The chlorophyll content may function as a physiological indicator of nematode stress. This indicates that monitoring chlorophyll levels could serve as an effective, non-invasive technique for assessing early nematode infection and evaluating resistance in tomato cultivars. Furthermore, these findings underscore the significance of integrated pest management (IPM) strategies in tomato cultivation. Mitigating *M. incognita* populations

via crop rotation, resistant varieties, biological control agents, or nematicides may sustain optimal chlorophyll levels, thereby enhancing overall plant vigor and productivity. This study possesses certain limitations. Although it establishes a correlation between nematode infection and diminished chlorophyll content, additional research is required to elucidate the specific molecular mechanisms underlying this interaction. Furthermore, evaluating additional physiological parameters such as photosynthetic rate, transpiration, and stomatal conductance would yield a more thorough understanding of the nematode's impact on plant physiology.

In conclusion, *Meloidogyne incognita* infestation markedly diminishes chlorophyll content in tomato plants, likely leading to compromised photosynthesis and decreased crop yield. Comprehending and alleviating their effects is crucial for sustainable tomato cultivation.

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