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MACRONUTRIENT STATUS, GROWTH, AND PHYSIOLOGICAL PARAMETERS OF MULBERRY LEAVES INFESTED WITH THE ROOT KNOT NEMATODE, *Meloidogyne incognita*

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ABSTRACT

The nematode *Meloidogyne incognita* is notorious for its detrimental effects on mulberry plants, where it induces root knot disease and severely impairs plant growth and development. This damage disrupts leaf production, which in turn adversely affects the health of silkworms and diminishes cocoon yields, ultimately impacting the economic viability of the silk industry. To explore these impacts, a study was conducted employing a completely randomized block design with an inoculum concentration of 1000 nematode juveniles per plant. The research aimed to evaluate the effects of root knot nematodes on macronutrient levels, growth, and physiological changes in mulberry leaves, in comparison to non-infested control plants. The findings demonstrated a significant decrease in the macronutrient levels of nitrogen, phosphorus, and potassium in the leaves of infested plants. Furthermore, critical growth and physiological parameters exhibited notable adverse changes in Specific Leaf Area (cm²), Total Leaf Area (cm²), Stomatal Index (mm²), and Stomatal Conductivity (µmol of H_2O m^{-2} s^{-1}) in the infested leaves relative to the control plants.

Keywords: Leaf development, Macronutrient deficiency, Nematode infestation, Plant physiology, Silk industry impact

Mulberry (*Morus alba*) is a crop of significant economic importance to the silk industry, as the quality of its leaves directly impacts the yield of silkworms (*Bombyx mori* L.), which rely on mulberry leaves as their primary food source (Weerasak *et al.*, 2014; Nandan *et al.*, 2022). Various pathogens—including bacteria, viruses, fungi, mycoplasmas, and nematodes—adversely affect mulberry plants by reducing leaf yield and diminishing the nutritional quality of the leaves. Among these pathogens, the root knot nematode (*Meloidogyne incognita* Kofaid and White) Chitwood (Chitwood, 1951) is a major limiting factor in global mulberry crop production, causing an estimated 10-12% reduction in leaf yield (Govindaiah *et al.*, 1991). *M. incognita* is particularly problematic in sandy loam soils under irrigated conditions (Jaraba-Navas *et al.*, 2007; Gebissa Yigezu Wendimu, 2021). The root knot disease on mulberry was first identified by Bessey (1911) in the United States. *M. incognita* has a broad host range and exhibits endoparasitic behavior, making its control challenging. The nematode population proliferates rapidly under favourable conditions (Feyisa, 2022) and is recognized as one of the most pathogenic plant-parasitic nematodes (PPN) (Sikandar *et al.*, 2019).

Root-knot nematodes are economically significant and rank among the most destructive pests affecting vegetables and other crops (Youssef *et al.*, 2013; Tileubayeva *et al.*, 2021). Infested plants exhibit characteristic galls or knots on their roots and display a range of detrimental symptoms on their vegetative parts, including stunted growth, discoloration, wilting, and diminished plant vigor (D Howland *et al.*, 2023). Such infestations compromise plant health, making infected plants more vulnerable to additional diseases, and can ultimately lead to the death of severely affected plants. The present study aimed to investigate the impact of root-knot nematode infestation on the macronutrient levels, growth, and physiological parameters of leaves in the commercially important mulberry variety V1.

MATERIALS AND METHODS

The experiment was conducted from 2014 to 2018 at the Department of Sericulture, Sri Padmavati Mahila University, Tirupati, India. Seventy-day-old saplings of the V1 mulberry variety were planted using a randomized block design, arranged in small blocks with twelve plants each, spaced 3' x 3' apart, and replicated three times. Each plant was inoculated with 1000 nematode juveniles, and a control group was maintained for comparative purposes. Two months post-inoculation, the study assessed the macronutrient content—specifically nitrogen, phosphorus, and potassium—as well as growth and physiological parameters, including Specific Leaf Area (cm²), Total Leaf Area (cm²), Stomatal Index (mm²), and Stomatal Conductance (µmol of H_2O m^{-2} s^{-1}), in both infested and control plants.

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Estimation of macronutrients status of leaves

For this study three plants were selected randomly from both infested and healthy plots.

i. Estimation of nitrogen content in leaf tissue

Nitrogen estimation in leaf tissue was conducted using the micro-Kjeldahl method, as outlined by Jackson (1973) and modified by Iswaran and Marwaha (1980). The nitrogen content was measured using the following formula:

$$\text{Nitrogen content} = \frac{14.01 \text{ X (titration value-blank value)} \times \text{molarity of the acid}}{10 \times \text{weight of the sample}}$$

where:

14.01: standard value

Molarity of the acid: 1N

ii. Estimation of Phosphorous content in leaf tissue

Phosphorus content was estimated using the Vanado-molybdate yellow colour method, as described by Jackson (1973). A standard calibration curve was prepared, and the phosphorus content was calculated using the following formula:

$$\text{Phosphorus content} = \frac{\text{Graph ppm X value of the digestive sample X volume made up}}{10^6 \text{ X weight of sample X aliquot taken}} \text{ X } 100$$

iii. Estimation of Potassium content in leaf tissue

Potassium (K) content in the samples was estimated using the triple acid digestion method (Jackson, 1973). The resulting extract was directly analyzed in a flame photometer, with the filter set specifically for potassium. The percentage of potassium in the tissue was calculated based on the photometer readings, and the calculations were performed using the following formula:

$$\text{Potassium content} = \frac{\text{graph ppm X value of the digestive sample X volume made up}}{10^6 \text{ X weight of sample X aliquot taken}} \text{ X } 100$$

Growth and Physiological Parameters

i. Specific leaf area (cm²)

Specific Leaf Area (SLA), expressed in square centimeters per gram (cm²/g), was determined by measuring the leaf area of 10 leaves with a LICOR Leaf Area Meter (LI 3100C) and the dry weight of the same 10 leaves. The SLA was calculated using the following formula:

$$\text{Specific leaf area (cm}^2\text{/g)} = \frac{\text{Leaf area of (10) leaves (cm}^2\text{)}}{\text{Dry weight of (10) leaves (g)}}$$

ii. Total leaf area (cm²)

The leaf area was calculated using the dry weight method (Yoshida *et al.*, 1976). Leaves from both infested and control plants were collected separately and oven-dried at 70°C for 48 hours. After drying, the leaves were weighed, and the total leaf area was calculated using the following formula:

$$\text{Total leaf area/plant} = \frac{\text{Dry weight of leaves} \times \text{Area of 10 leaves (cm}^2\text{)}}{\text{Dry weight of (10) leaves}}$$

iii. Stomatal Index (mm²)

Stomata were observed exclusively on the lower surface of the mulberry leaf, indicating it is hypostomatous (Paul *et al.*, 2007). To assess stomatal characteristics, impressions of the abaxial leaf surface—specifically at the point of maximum leaf width near the mid-vein—were taken using Fevikwik gum and adhesive transparent cellophane tape. These impressions were mounted on glass slides and examined under a light microscope at 40x magnification. Both the number of stomata and epidermal cells were counted, and the stomatal density (SD) was calculated as the number of stomata per unit area (mm²). The Stomatal Index (SI) was determined using the formula provided by Ferris and Taylor (1994).

$$\text{Stomatal Index (\%)} = \frac{\text{Number of stomata}}{\text{No. of epidermal cell + No of stomata}} \text{ X } 100$$

iv. Stomatal conduction (μ moles of m^{-2} s^{-1})

Stomatal conductance was measured using a LICOR Portable Photosynthetic System (LI-6400XT). Measurements were taken on the central portions of the 4th, 5th, and 6th fully expanded leaves of both control and infested plants between 9 A.M. and 10 A.M. on the same day (Tuberosa *et al.*, 1994).

Statistical analysis

All experimental data, collected in three replicates, were subjected to statistical analysis using SPSS Version 2.0. Analysis of variance (ANOVA) was performed to identify significant differences between control and infested plants. Both the T-test and Two-Way ANOVA were utilized, following the procedures outlined in Agricultural Statistics (Rangaswamy, 2000).

RESULTS AND DISCUSSION

The present investigation revealed that nematode infestation in the V1 mulberry variety caused significant changes in macronutrient content, growth, and

physiological parameters (Table 1, 2).

Macronutrients status of leaves

The impact of *M. incognita* on the macronutrient status of leaves in nematode-infested mulberry plants, specifically nitrogen, phosphorus, and potassium, was analyzed and found to have an adverse effect on nutrient levels. The results are presented in Table 1.

Nitrogen content

The nitrogen content in healthy mulberry plants was recorded at 3.99%, whereas in infested plants it decreased to 3.50%, representing a reduction of 12.28%. Nitrogen is crucial for chlorophyll production and the synthesis of proteins and amino acids. A deficiency in nitrogen typically results in a pale yellow coloration of the leaves due to reduced chlorophyll levels, leading to stunted growth and diminished plant vigour. In this study, the observed decrease in nitrogen content aligns with these symptoms. Furthermore, nitrogen deficiency can increase the susceptibility of plants to diseases.

Phosphorus content

The phosphorus content was recorded at 0.196% in the leaves of healthy mulberry plants, while in nematode-infested plants, it was 0.190%, reflecting a reduction of 3.06% compared to the control (healthy) plants. Phosphorus plays a critical role in various vital processes, including photosynthesis, energy transfer (in the form of ATP), and as a fundamental component of DNA. It is also essential for flowering and seed production. In this study, the decrease in phosphorus content was observed, and phosphorus deficiency in plants is typically associated with weaker stems, poor growth, and reduced cellular activity.

Potassium content

The potassium content in healthy plants was observed to be 0.225%, while in infested plants, it decreased to 0.201%, reflecting a reduction of 10.66% compared to healthy plants. Potassium is crucial for cellular signalling and growth regulation in plants. A reduction in potassium concentration can impact CO_2 uptake and alter osmotic potential, potentially leading to decreased photosynthesis (Ferguson, 1984). Symptoms of potassium deficiency typically include necrosis on leaf margins, leaf curling and browning, and interveinal chlorosis. In this study, a notable reduction in potassium content was observed.

Nematode-infested plants typically exhibit symptoms of nutrient deficiency, particularly in essential macronutrients such as nitrogen, phosphorus, and potassium, as well as other macro- and micronutrients crucial for plant growth and development. The results (Table 1) suggest that nutrient transport may be adversely affected, potentially due to reduced water transport. This impairment in nutrient uptake and transport could be attributed to the deformation of vascular tissues in the roots or the utilization of nutrients by the nematodes.

Nutrient accumulation in nematode-infested roots may result from impaired translocation to the aerial parts of the plant or from the reallocation of nutrients from shoots to roots (McClure, 1977). Previous studies have also reported reductions in potassium (Fatemy and Evans, 1986), nitrogen, and phosphorus (Heffes *et al.*, 1991) in the leaves as a consequence of *Meloidogyne incognita* infestation.

Similar findings were reported by Paulson and Webster (1970), and Rawsthorne and Hague (1986), who observed reductions in chlorophyll, reducing sugars, nitrogen, phosphorus, and iron in rice plants infested by *Meloidogyne graminicola* and *Helicotylenchus oryzicola*. Abd-Allah *et al.* (2010) and Chandramani *et al.* (2022) also documented significant reductions in nitrogen, phosphorus, and potassium contents in plant tissues as a result of nematode infestation, attributing these decreases to the increased buildup of nematode populations and subsequent nutrient depletion.

Growth and Physiological parameters

In the present investigation, physiological parameters were analyzed, revealing a significant reduction in all growth and physiological metrics (Table 2).

Specific leaf area (cm²)

The nematode *Meloidogyne incognita* causes a substantial reduction in Specific Leaf Area (SLA) in infested mulberry leaves. Healthy mulberry plants exhibited a SLA of 97.32 cm², whereas infested plants

Table 1. Effect of *Meloidogyne incognita* on macronutrient status in mulberry leaves

Sr. No.	Parameters	Control	Infested	% of decrease over control	T- test	Level of significance
1	Nitrogen (%)	3.99	3.50	12.28	18.062	**
2	Phosphorus (%)	0.196	0.190	3.06	6.532	**
3	Potassium (%)	0.225	0.201	10.66	16.561	**

** Highly significant at $p<0.01$

Table 2. Effect of *Meloidogyne incognita* on growth and physiological parameters of mulberry

Sr. No.	Parameters	Control	Infested	% of decrease over control	t-Test	Level of significance
1	Specific Leaf area (cm^2)	97.32	87.20	10.39	186.623	**
2	Total leaf area (cm^2)	262.59	227.99	13.17	796.186	**
3	Stomatal Index (mm^2)	30.94	29.98	3.10	19.484	**
4	Stomatal conduction (μ moles of m^{-2} s^{-1})	0.19	0.14	26.31	48.067	**

** Highly significant at *p≤0.01*

showed a SLA of 87.20 cm², reflecting a percentage reduction of 10.39%.

Total leaf area (cm²)

The total leaf area was recorded as 262.59 cm²/g in healthy mulberry plants, whereas in infested plants it was reduced to 227.99 cm²/g, reflecting a percentage decrease of 13.17%. Specific Leaf Area (SLA) is a crucial parameter in plant growth analysis, defined as the leaf area per unit of dry mass (typically expressed in cm²/g). SLA offers valuable insights into a plant's resource-use strategy and physiological functioning by indicating the amount of leaf area relative to biomass production and light use efficiency. In this study, a reduction in SLA was observed. Root-knot nematodes impair water and nutrient uptake, leading to a decreased photosynthetic rate, which consequently affects growth parameters, including the number of leaves and overall leaf area.

Stomatal Index (mm²)

The Stomatal Index (SI) in healthy mulberry plants was observed to be 30.94 mm², while in nematode-infested plants it decreased to 29.98 mm², representing a reduction of 3.10%. The stomatal index is defined as the number of stomata per unit area of the leaf and is a valuable metric in plant growth analysis. It provides insights into gas exchange, water use efficiency, and environmental adaptation. Stomata play a crucial role in balancing photosynthetic performance with water availability and usage (Chaerle *et al.*, 2005). In the present study, a slight reduction in stomatal index was noted in infested plants compared to healthy ones. This reduction may be part of the plant's defense mechanism to mitigate excessive transpiration and conserve water.

Stomatal conductivity (μ mole of H_2O m^{-2} s^{-1})

Stomatal conductance in healthy mulberry plants was observed to be 0.19 μmol of H_2O m^{-2} s^{-1}, whereas in infested plants it decreased to 0.14 μmol of H_2O m^{-2} s^{-1}, reflecting a reduction of 26.31% compared to healthy plants. Stomatal conductance is a key physiological parameter that determines the rate of carbon dioxide uptake and water transpiration through the leaf stomata. This parameter is influenced by the density, size, and degree of opening of the stomatal apertures. It is closely related to water availability in plants. In this study, reduced stomatal conductance was observed, which may be attributed to impaired water absorption by the root system of infested plants.

The rate of photosynthesis is a crucial parameter influencing crop yield (Poornima and Vadivelu, 1998; Patel *et al.*, 2001). It is typically assessed based on fresh weight, total leaf area, and chlorophyll content. The data presented in table 2 show significant reductions in Specific Leaf Area, Total Leaf Area, Stomatal Index, and Stomatal Conductance in nematode-infested mulberry plants. Plants can respond to nematode infestation by altering their physiological processes, which can adversely affect photosynthesis-related activities. Root-knot nematodes induce the formation of giant cells in the roots, disrupting the root vascular system and impairing the uptake and transport of water and nutrients from the roots to the shoots (Abad *et al.*, 2003). Consequently, the plant's response to nematode parasitism results in both morphological and physiological changes that negatively impact photosynthetic processes (Melakeberhan *et al.*, 1986; Hussey and Williamson, 1998).

Several physiological processes are often implicated in the reduction of photosynthetic activity in nematode-infested plants. These include decreased chlorophyll content (Nagesh and Dhawan, 1988), changes in stomatal conductance (Saeed *et al.*, 1997), photochemical limitations (Schans and Arntzen, 1991), nutrient imbalances (Wallace, 1974), and interference with the synthesis and translocation of photosynthesis-regulating factors such as cytokinins and gibberellins produced in the roots (Loveys and Bird, 1973).

A reduced stomatal index may aid in water conservation by limiting water loss through transpiration. Root damage from nematodes can induce water stress, affecting water uptake and resulting in fewer stomata. This reduction in stomatal density can lead to diminished rates of carbon dioxide fixation and altered transpiration rates, thereby disrupting photosynthesis due to changes in enzymatic activity.

These findings are consistent with observations in other plant-nematode interactions. For instance,

Meloidogyne incognita on grapevine (*Vitis vinifera*) varieties has been reported to cause similar effects (Melakeberhan *et al.*, 1990), as has *Globodera pallida* on potato (*Solanum tuberosum*) varieties (Schans and Arntzen, 1991).

CONCLUSION

In conclusion, infestation by root-knot nematodes induces both morphological and physiological changes in crop plant tissues, primarily due to mechanical injury from nematode migration between cells. With their broad host range, these nematodes are particularly challenging to manage and can produce severe symptoms that significantly reduce crop yields. In the case of mulberry plants, root-knot nematodes lead to substantial alterations in macronutrient levels, growth, and physiological parameters. The observed reductions in nutrient content and photosynthetic efficiency reflect changes in plant physiology driven by the host-parasite interaction, ultimately contributing to a decrease in crop yield.

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Conflict of interest: Author declares that she has no conflict of interest.

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