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Studies on Pathogenicity of Root Knot Nematode *Meloidogyne incognita* on Mulberry in Pot and Field Conditions

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Abstract

The root knot disease is caused by microscopic, soil-born, plant parasitic nematode, *Meloidogyne incognita* Chitwood the most important nematode injuring mulberry plants. It is recognized as this nematode distributed in most of the mulberry fields throughout the world and has wide range of host plants. Mulberry is an economically important crop for silk industry. Damages in growth of mulberry plants caused possibly by root-knot nematodes reduce leaf quality and quantity which turn effect negatively on silk worm health and economy of silk industry. Hence the present study was performed to know the pathogenicity of root knot nematode *Meloidogyne incognita*, on different mulberry growth and development parameters in pot and field conditions. The observations were recorded with the respect to number of galls/g root weight, number of egg masses/g root weight, total height of plant, total number of leaves / plants, fresh weight of leaves/plant, leaf moisture percentage, leaf moisture retention percentage (6 and 12 hours), shoot fresh weight, shoot dry weight, root length, root fresh weight, root dry weight, root and shoot ratio. *Meloidogyne incognita* represents a significant threat to mulberry production because it is considered the most aggressive root-knot nematode in mulberry crops.

Key words: Mulberry, *Meloidogyne incognita,* Pathogenicity, Root knot nematode, Silk industry

Mulberry (*Morus alba* L) is an economically important fast-growing deep-rooted perennial plant that leaves are used for rearing of silkworms. Its habitat ranging is widely from subtropical to tropical regions. Mulberry is a perennial plant it is prone to various diseases caused by different types of microorganisms are the main drawback causing substantial loss in yield and nutritive values of mulberry foliage [1-3]. Silkworm rearing the infected leaves adversely affects to its health and the cocoon yield [4-6].

Among the different microbial diseases of mulberry, root knot disease produced by one of the most destructive nematode *Meloidogyne incognita* (Kofoid and White) Chitwood [7] represents a significant threat to mulberry production and was first detected from USA [8]. *Meloidogyne incognita* is widely spread in tropical and subtropical regions of all the continents of the world [9]. This root knot disease is more distributed commonly in sand soils under irrigated farming systems and caused significant yield loss in mulberry crops in terms of quality and quantity by more than 12% which causes loss of revenue for mulberry growers [10]. It has several kinds of host plants including food crops, vegetables and other cash crops. The symptoms of the disease and its effect on host plants are formation of galls/knots on the roots and shows stunted growth with reduced vigour of the plant [11]. These root knot nematodes burrow into the soft tissues of root tips and young roots, and cause the root cells to divide and enlarge induced galls that contain giant-feeding cells and also interaction with other plant-parasitic pathogens. Hence, the present study is aimed to carry out to know the effect of root knot nematode infestation on the growth and development parameters in mulberry variety V-1 both in pot as well as field conditions.

MATERIALS AND METHODS

All the pot and open field experiments pertaining to the study were conducted at the Sericulture Department of Sri Padmavathi Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India.

Pot study

For preliminary study, stem cuttings of mulberry V1 variety plants were raised in earthen pots filled with sterilized soil. Three months after the establishment of cuttings, each plant was inoculated with 1000 freshly hatched second stage juveniles by making four holes around the base of plants and simultaneously control (without nematode inoculation) was maintained. Sixty days after inoculation, the plants were carefully uprooted and the roots were washed with water to remove soil particles.

The observations were recorded with respect to number of galls/g root weight, number of egg masses/g root weight, total height of plant (cm), total number of leaves / plant, fresh weight of leaves/plant (g), leaf moisture percentage (%), leaf moisture retention percentage (%) (6 and 12 hours), shoot fresh

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weight (g), shoot dry weight (g), root length (cm), root fresh weight (g), root dry weight (g) root and shoot ratio (g).

Field study

Land preparation

For this experiment the experimental site was prepared properly and divided into three plots for three replications and was provided proper irrigation facilities. Seventy days old V1variety of mulberry saplings were planted in the field.

Selected plant variety

High yielding and popular mulberry variety V-1 (Victory-1) was selected for this experiment. This experiment was conducted to know the effect of root knot disease on the mulberry variety plants. It involved a comparison of two plots. Mulberry variety V1 saplings were planted in randomized block design with 3’ × 3’ spacing and three replications were maintained. After establishment of plants each plant was inoculated with 1000 second stage nematode juveniles (J2). In each replication two plots were separated by raising bunds to prevent contamination from infested to healthy plot.

Sixty days after inoculation of nematode, observations were made on the infestation level of nematode by recording the number of galls/g root weight and number of egg masses/g root weight simultaneously nematode pathogenicity effect on morphological parameters in mulberry plants was observed comparing with control.

Nematode pathogenicity

Number of root galls/g of root weight

Three plants were selected randomly from infested blocks and roots were collected washed with water. Then the number of galls/g of root was recorded by counting the number of galls.

Number of egg masses/g of root weight

One gram of root bits were collected from randomly selected infested plants and were washed with water thoroughly and stained with 0.1 Lacto phenol- acid fuschin for three minutes and were further washed with water. The number of egg masses/g of root weight was counted using binocular stereo microscope. The number of galls present in the root was calculated and classified following the Root knot index scale (0-5) as per Taylor and Sasser [12].

Morphological parameters of mulberry plants

For this study three plants were selected randomly from both infested and healthy plots. The data is presented in the (Table 1-4).

Total height of plant (cm)

Total plant height was observed in both infested and healthy plants. Total number of primary branches and height of primary branches was recorded and average height of plants was calculated by using the formula:

$$\text{Total plant height} = \frac{\text{Total height of branches (cm)}}{\text{Total number of branches}}$$

Number of leaves / plant

Three plants were selected randomly from both healthy and infested plots and the number of leaves was determined by counting the leaves from the base to the tip of the plant and the total number of leaves per plant was recorded.

Fresh weight of 100 leaves (g)

Hundred fresh leaves were collected from randomly selected plants during early hours of the day and their fresh weight was recorded.

Leaf moisture percentage (%)

100 leaves were collected from the selected plants and fresh weight was recorded immediately after harvesting. Then the leaves were dried in a hot air oven at 60 °C for 72 hours. The leaf moisture percentage was calculated on fresh weight basis by using the formula:

$$\text{Leaf moisture percentage (\%)} = \frac{\text{Weight of fresh leaves} - \text{Weight of dry leaves}}{\text{Weight of fresh leaves}} \times 100$$

Leaf moisture retention capacity (%)

Leaf moisture retention capacity was determined on fresh weight basis [13]. 100 matured leaves were collected separately from randomly selected plants and the leaves were wiped with a muslin cloth to remove dust particles and their fresh weight was recorded immediately. Then leaves were kept in normal environmental conditions for 6 hours and 12 hours. After 6hours and 12 hours, leaves were weighed for calculating the water retention capacity. Average values were calculated as per the procedure suggested by Mano [14] and was expressed in percentage (%).

$$\text{Leaf moisture loss (\%)} = \frac{\text{Fresh weight of leaf} - \text{Dry weight of leaf (6hrs/12hrs)}}{\text{Fresh weight of leaf}} \times \text{LMC}$$

LMC = Leaf moisture content

Leaf moisture retention (%) = 100 - Leaf moisture loss

Shoot fresh weight (g)

Shoot fresh weight was recorded from randomly selected plants and was expressed as weight in grams.

Shoot dry weight (g)

The harvested plants were dried in a hot air oven at 70 °C for four days to obtain constant weight and then the dry weight was recorded in grams.

Length of the root (cm)

The roots of individual plants were up rooted by choosing plants from both infested and control plants. After uprooting from the soil, roots were washed with water and then the length of the longest root of each plant was measured from the base of the stem to its tip and was recorded in centimeter.

Root weight (fresh and dry) (g)

Three plants were selected randomly and roots of each plant were cut from the stem and taken separately. After washing their weight was recorded as fresh weight of root. The roots were then dried in a hot air oven for 5 days at 70 °C and dry weight of roots of each plant was recorded in grams.

Root and shoot ratio (R:S)

Dry root weight was divided by shoot dry weight to obtain the Root to shoot ratio. It was expressed as grams:

$$\text{R/S (g)} = \frac{\text{Root weight}}{\text{Shoot weight}}$$

Statistical analysis

All the experimental data collected in three replicates has been subjected to statistical analysis (SPSS-2.0 Version). The analysis of variance has been done to find out the significant differences between the control and infested plants for the T-test and Two-way ANOVA test following the procedures laid down in Agricultural statistics [15].

RESULTS AND DISCUSSION

In the present investigation, observations were made on the pathogenicity of root knot nematode and also its effect on morphological parameters in mulberry sixty days after nematode inoculation. This study is important for understanding how nematodes can impact the growth and development of plants over time.

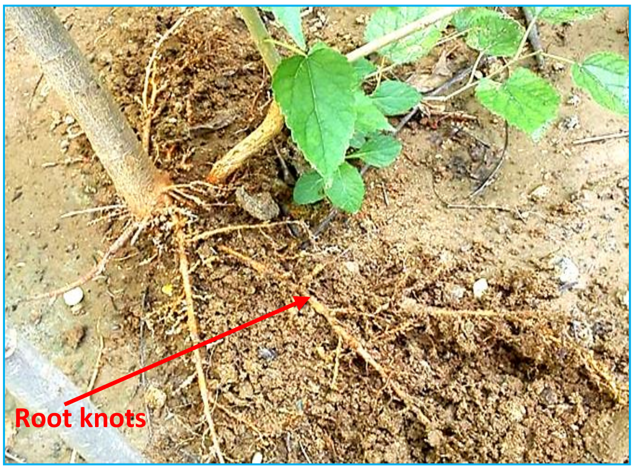
Nematode pathogenicity

Observations were made on the number of root knots/g, root weight and number of egg masses/g root weight both in pot as well as field study to know the degree of resistance by using root gall index and egg mass index (0-5) scale as per Taylor and Sasser [12].

Number of root knots/g root weight

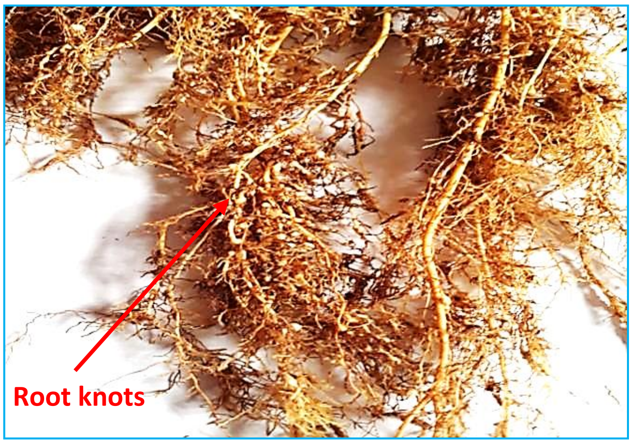
The number of root knots per gram root weight in infested plants was observed as 37.66/g root weight in pot study. In field study, the number of root knots/g root weight in infested plants was recorded as 39.12 /g root weight as mentioned in (Plate 1).

Mulberry garden (V1 variety)



Mulberry root infested with nematode showing root galls

Healthy root system



Nematode infested roots (close shot)

Plate 1 Mulberry garden (healthy and infested)

Number of egg masses/g root weight

In pot study, the number of egg masses/g root weight was observed as 31.33g in nematode infested plants. The number of egg masses/g root weight was recorded as 32.26g in nematode infested plants in the field. Presence of knots/galls on roots is a primary symptom associated with root knot nematode infestation on plants due to hyperplasia and hypertrophy reactions in the parenchymatic cells of root cortex. In the present study, the infested mulberry variety V1 has been observed as moderately susceptible to root knot nematode with gall index 4. Root knot nematodes stimulate the formation of root knots/galls which interferes with plant water supply, resulting in chlorotic and stunted growth [16]. Noling [17] reported that, under heavy nematode infestation, crop transplants may fail to develop and maintain a stunted condition causing poor plant growth. Indeed, the presence of knots or galls on roots is a common and distinctive symptom associated with root knot nematode (RKN) infestation in plants. The formation of galls is a result of complex interactions between the nematode and the plant's root system [18-19].

Morphological parameters

In the present investigation, morphological parameters were analyzed to know the effect of root knot nematode both in pot as well as field conditions and significant reductions were recorded in all the morphological parameters. The impact on morphological parameters can provide valuable insights into how nematodes affect the overall growth and health of the plants in both pot and field conditions. The results are presented in the (Table 1-4, Fig 1-2).

Total plant height (cm)

In pot study, the total plant height of healthy plants was observed as 61.33cm whereas in infested plants it was 51.00

cm. The percentage of reduction was16.84 over control. In field study, the total plant height of healthy plants was observed as 278.00cm whereas in infested plants it was 170.00 cm. The percentage of reduction was 38.84 over control. Significant difference was observed in reduction of plant height in infested plants compared to healthy plants.

Table 1 Effect of root knot nematode *Meloidogyne incognita* on morphological parameters of mulberry (pot study)

S. No.	Parameters	Control	Infested	% of decrease over control
1	Total height of plant (cm)	61.33	51	16.84
2	Total number of leaves	42.66	30.33	28.90
3	Fresh weight total leaves/plant (g)	32.43	21.62	33.33
4	Leaf moisture percentage (%)	68.79	63.45	7.76
5	Leaf moisture retention percentage (%) 6 hours	83.35	68.90	17.33
6	Leaf moisture retention percentage (%) 12 hours	69.82	49.70	28.81
7	Shoot fresh weight (g)	99.3	72.45	27.03
8	Shoot dry weight (g)	27.79	17.61	36.63
9	Root and shoot ratio (R:S)	2.12	0.90	57.54
10	Root length (cm)	35.33	27.33	22.64

Table 2 Effect of root knot nematode *Meloidogyne incognita* on root parameters of mulberry (pot study)

Parameters	Control	Infested	% of increase over control
Root fresh weight (g)	63.14	81.27	28.71
Root dry weight (g)	13.07	19.50	49.19

Total number of leaves per plant

In pot study, the average number of leaves per plant was observed as 30.33 in infested plants against 42.66 in healthy with a percentage of reduction of 28.90. In field study, the average number of leaves per plant was observed as 100.31 in infested plants against 120.74 in healthy with a percentage of reduction of 16.92.

Fresh weight of total leaves (g)

In pot study, the fresh weight of total leaves in the healthy plants was observed as 32.43g compared to 21.62g in infested plants with significant reduction of 33.33 over control.

Fresh weight of 100 leaves (g)

In field study, fresh weightof100 leaves in the healthy plants was observed as418.66g whereas in the infested plants it was 311.00g with a percentage of decrease of 25.71 over control.

Leaf moisture percentage

In pot study, the maximum leaf moisture percentage of healthy plants was observed as 68.79% whereas in infested plants it was 63.54%. The percentage of reduction was 7.76 over control. In field study, the leaf moisture percentage of healthy plants was observed as 76.69% whereas in infested plants it was 73.32%. The percentage of reduction was 4.39. Significant difference was observed in reduction of leaf moisture percentage in the infested plants compared to healthy plants.

Leaf moisture retention %

In pot study, the leaf moisture retention % after 6 hours was 83.35% in healthy plants and 68.90% in infested plants. The percentage of reduction was 17.33 compared to healthy plants. Similarly, the leaf moisture retention% after 12 hrs, in the healthy plants showed as 69.82% whereas in case of infested plants it was49.70% with a reduction percentage of 28.81over healthy plants. In field study, the leaf moisture retention% after 6 hours was 86.13% in healthy plants and 69.14% in infested plant. The percent of reduction was 19.72 compared to healthy plants. Similarly, the leaf moisture retention % after 12 hrs, in the healthy plants showed 74.96% whereas in case of infested plants it was 50.36% with a reduction percentage of 32.81 over healthy plants.

Table 3 Effect of root knot nematode *Meloidogyne incognita* on morphological parameters of mulberry (field study)

Parameters	Control	Infested	% of decrease over control	T- Test	Level of significance
Total height of plant(cm)	278.00	170.00	38.84	440.053	**
Total number of leaves	120.74	100.31	16.92	51.519	**
Fresh weight of 100 leaves (g)	418.66	311.00	25.71	486.524	**
Leaf moisture percentage (%)	76.69	73.32	4.39	14.174	**
Leaf moisture retention percentage (%) 6 hours	86.13	69.14	19.72	150.960	**
Leaf moisture retention percentage (%) 12 hours	74.96	50.36	32.81	550.073	**
Shoot fresh weight (g)	887.50	667.64	24.77	1049.733	**
Shoot dry weight (g)	230.71	180.47	21.77	269.573	**
Root and shoot ratio (R:S)	6.73	4.92	26.89	9.253	**
Root length (cm)	76.24	68.48	10.17	53.806	**

$p<0.01$, ** Highly significant at 0.01

Table 4 Effect of root knot nematode *Meloidogyne incognita* on root parameters of mulberry (field study)

Parameters	Control	Infested	% of increase over control	T- Test	Level of significance
Root fresh weight (g)	86.32	98.46	14.06	65.108	**
Root dry weight (g)	34.43	36.62	6.36	7.004	**

$p<0.01$, **Highly significant at 0.01

Shoot fresh weight (g)

In pot study, the shoot fresh weight in healthy plants was recorded as 99.3 g compared to 72.45 g in infested plants with a significant reduction of 27.03%. In field study, the shoot fresh weight in healthy plants was found as 887.50 g. compared to 667.64 g in infested plants with a significant reduction of 24.77%.

Shoot dry weight (g)

In pot study, the shoot dry weight in healthy plants was observed as 27.79g against 17.61g in infested plant with a significant reduction of 36.63%. In field study, the shoot dry weight in healthy plants was showed as 230.71 g against 180.47 g in infested plant with a significant reduction of 21.77%.

Root and shoot ratio (R:S)

In pot study, the maximum root to shoot ratio was observed as 0.90 g in infested plants against 2.12 g in healthy plants with a percentage of reduction of 57.54. In field study, the root to shoot ratio was observed as 4.92 g in infested plants against 6.73 g in healthy plants with a percentage of reduction of 26.89.

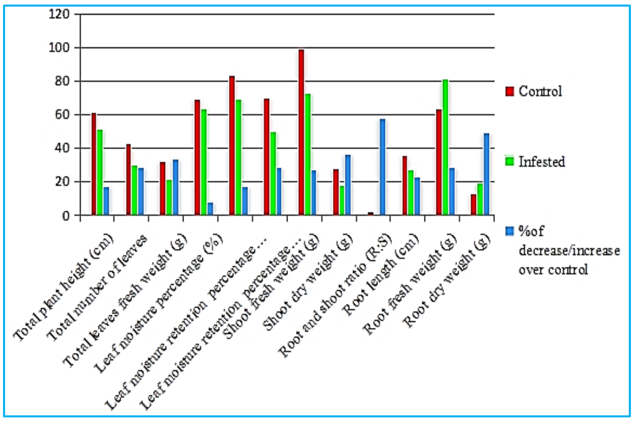


Fig 1 Effect of root knot nematode *Meloidogyne incognita* on morphological parameters of mulberry (pot study)

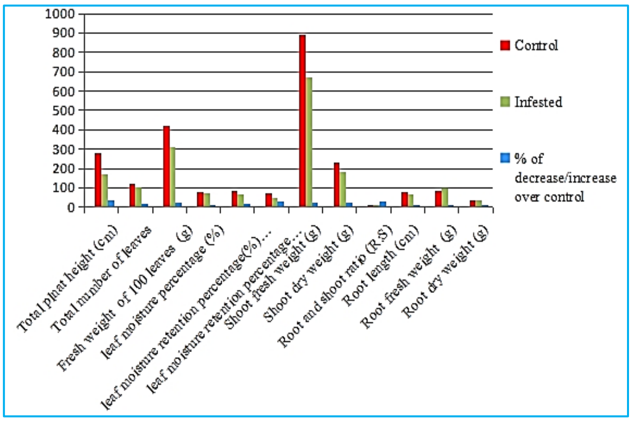


Fig 2 Effect of root knot nematode *Meloidogyne incognita* on morphological parameters of mulberry (field study)

Root length (cm)

In pot study, the maximum root length of healthy plants was observed as 35.33 cm against 27.33 cm in infested plants with a significant percentage of reduction of 22.64. In field study, the maximum root length of healthy plants was observed as 76.24cm against68.48 cm in infested plants with a percentage of reduction of 10.17 over healthy plants.

Fresh weight of root (g)

In pot study, the fresh weight of root in healthy plants was observed as 63.14 g against 81.27g in infested plants. The percentage of difference was recorded as 28.71overcontrol (healthy) plants. In field study, the fresh weight of root in healthy plants was observed as 86.32 g and 98.46 g in infested plants. The percentage of difference was recorded as14.06 over healthy plants.

Dry weight of root (g)

In pot study, the dry weight of root in healthy plants was observed as 13.07 g where as in infested plants it was 19.50 g with a percentage of increase as 49.19 over control (healthy) plants. In field study, the dry weight of root in healthy plants was observed as 34.43 g against 36.62 g in infested plants. The percentage of difference was recorded as 6.36 over control plants.

The above results of analysis in both infested and control (healthy) plant are given (Table 1-4, Fig 1-2) besides indicating the increase or decrease of the constituents between infested and control (healthy).

The results specified that root knot nematode *M. incognita* is highly pathogenic to mulberry plants and would cause significant damage to plant growth, development. The plant response to nematode parasitism thus causes morphological changes. The invasion of root knot nematode cause mechanical injury, hyperplasia and hypertrophy of root tissues which disrupts the root vascular system. The damaged vascular system reduces the water and nutrient absorption efficiency and their transport from the roots to the shoots resulting in wilting and stunting of plants and reducing plant vigour. All these factors create water stress conditions resulting in the nutrient imbalance and reduced chlorophyll content. Finally plant growth and yield is reduced. In the present study the root weight of mulberry plants increased due to the presence of galls and nematodes. The damage caused due to nematode on morphological parameters of mulberry plants is significantly both in pot and field studies. However, in pot studies it is more, which might be due to the small area, the nematode grows rapidly there and can lead to depletion of soil nutrients might be cause nematode severity.

Campos *et al*. [20] and Saha *et al*. [21] observed that root knot nematode *M. incognita* causes chlorosis of leaves, stunting of plant growth and reduction of leaf yield in mulberry. Similar results were observed by Govindaiah [22] who reported reduction in the plant height, leaf moisture percentage and leaf yield in mulberry. Reduction in root and shoot length was the result of nematode feeding on giant cells, damage vascular system, causing root growth to stop and tips to swell [23-24].

The results of present study are in conformity with the findings of Gaur and Prasad [25] who observed the decrease in the growth of brinjal with increased nematode population. Similar observations were also made by Raut and Sethi [26] in soya bean and Cho *et al*. [27] in tomato and Jagdale *et al*. [28] who recorded decrease in plant growth with increased nematode inoculums levels in betel vine.

CONCLUSION

Root knot disease originated by one of the most destructive nematode *Meloidogyne incognita* is more distributed commonly in sand soils under irrigated farming systems and caused significant yield loss in mulberry crops in terms of quality and quantity, which causes loss of revenue for mulberry growers which finally it leads to economic loss in silk industry. The above study gives a preliminary information regarding nematode pathogenicity on mulberry growth and development. Effective communication and collaboration between researchers, farmers, and industry players are key components of finding sustainable solutions.

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