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Studies on Effect of Root Knot Nematode *Meloidogyne incognita* (Kofoid White) Chitwood on the Growth and Development of V1 Mulberry Variety and Silk Worm *Bombyx mori* L.

P. Victoria Rani* and N. Vijaya Kumari

Research Scholar, Department of Sericulture, Sri Padmavathi Mahila University, Tirupati-517502, Chittoor (dt), AndhraPradesh, INDIA

Abstract

Root knot nematode *Meloidogyne incognita* was identified as highly pathogenic to mulberry plants and cause considerable damage to plant growth and development and reduced nutritive values in the leaves. Growth and development of silk worm *(Bombyx mori L)* and the cocoon crop yield are influenced by the nutritional quality of mulberry leaf as mulberry is the only food source (Matsumara et al, 1955, Tangamani R, Vivekananda M 1984, Li R and Sano O 1984). Hence in the present investigation was carried out to know the effect of root knot nematode on mulberry growth and development, biochemical parameters and silk worm bioassay parameters were analyzed in root knot nematode infested and healthy mulberry plants. In this study it was found that growth parameters like Total plant height (cm), Fresh weight of 100 leaves (g), Leaf moisture percentage(%) were decreased in infested plants. Biochemical analysis of both infested and control plants showed significantly changes especially in total protein, total carbohydrates, and total chlorophyll contents, which have shown significant reduction in infested plants. Whereas the phenol content was significantly increased in infested plants over control. Silk worm cocoon bioassay parameters of both infested and control treatments were analyzed for Larval weight(g), Number of cocoons harvested, Cocoon weight(g), Cocoon shell weight(g), Cocoon shell percentage(%),Total filament length(m), Non breakable filament length(m), Denier. On all the above said parameters significant reduction was observed among infested leaf fed silk worms over control.

Keywords: Biochemical parameters, Growth parameters, *Meloidogyne incognita*, Mulberry, Root knot disease, **S**ilk worm.

INTRODUCTION

Mulberry (*Morus* sps) is belongs to the family *Moraceae* and It is highly valuable plant for silk industry, because its foliage constitute the main food for growing silk worm *Bombyx mori* L. Mulberry is prone to a number of diseases caused by bacteria, virus, fungi, mycoplasma and nematodes, which cause 10-30% loss in the leaf yield and reduce nutritive values of mulberry leaves (Chanturia, 1963, Shree et al;1986, Shree and Umesh kumar, 1991). Among the different diseases of mulberry the significant economic loss is caused by the species of nematode *Meloidogyne incognita* (Kofaid and White) Chit Wood (Chit Wood. B.G. 1949), is a very serious in sandy loamy soils under irrigated conditions (Narayanan et al, 1966). It causes around 10-12% leaf yield loss in mulberry (Govindaiah et al, 1991). The root knot disease on mulberry observed first time by Bessey (1911) from U.S.A. The root knot nematode *Meloidogyne incognita* cause galls or knots on roots of its host plants. The manifestation of the disease and effect on plants are formation of galls or knots on the roots and other symptoms that can be observed above ground include stunted growth, chlorosis, wilting, leaf curling and reduced vigor of the plant (Saha.S et al, 1983).

Once the root knot nematode gets established in the mulberry garden it is difficult to control due to perennial nature of mulberry. Though there were some reports on the effect of root knot disease on various mulberry varieties, the studies on high yielding mulberry variety V1 is very scarce. Hence, the present study is aimed to carry out the effect of root knot nematode infestation on the growth and development, biochemical parameters in mulberry variety V1 and silk worm *Bombyx mori* L.

MATERIALS AND METHODS

The study was carried out at the department of Sericulture, Sri Padmavati Mahila University, Tirupati. Andhra Pradesh, during 2014-2016. Seventy days old V1 mulberry saplings were planted in randomized block design with 3’ x 3’ spacing. After three months of establishment, 1000 juveniles/plant were inoculated keeping control. Sixty days after inoculation one gram of roots with galls were collected and washed with water to remove soil particles. Then, the number of galls/gram root weight and the number of egg masses/gram of root weight were counted. Six months after mulberry plant establishment, the plants were pruned. Forty five days after pruning the growth and development, biochemical parameters and silk worm bioassay parameters were studied. Growth parameters like Total plant height (cm), Fresh weight of 100 leaves (g), Leaf moisture percentage (%), biochemical parameters were analyzed for carbohydrates, protein, phenols, and chlorophylls contents. Bioassay study was conducted on silk worm growth and development and cocoon yield such as Larval weight (g), Number of cocoons harvested, Cocoon weight (g), Cocoon shell weight(g), Cocoon shell percentage(%), Total filament length(m), Non breakable filament length(m), Denier in both infested and control plants.

Growth and development parameters of mulberry plants

For the study three plants were selected randomly from both infested and control blocks.

Total plant height (cm)

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

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

Fresh weight of 100 leaves (grams)

100 fresh leaves were collected randomly and fresh weight was recorded in the earlier hours.

Leaf moisture percentage (%)

Fresh weight of 100 leaves collected from the selected plants was recorded immediately after harvesting. Then leaves were dried in hot air oven at 60^0c for 72 hours. The leaf moisture percentage was calculated by using the formula.

$$= \frac{\text{Weight of fresh leaves} - \text{weight of dry leaves}}{\text{Weight of fresh leaves}} \times 100$$

Number of root galls/gram of root weight

Three plants were selected randomly from infested blocks and recorded the number of galls/gram of root by counting the number of galls.

Number of egg masses/gram of root weight

One gram of roots bits were collected from infested plants and washed with water thoroughly and stained with 0.1 Lacto phenol- acid-fuschin for three minutes and washed with water and counted the number of egg masses/gram of roots were recorded.

Biochemical parameters

Phenols

The total phenol content was estimated in the leaf by using Folin-ciocalteau reagent method (Malick , C P and Singh M B 1980) and it was showing as µg/g plant material.

Protein

Protein content of leaves was analyzed by using the Lowry method (Lowry et al; 1951).

Chlorophylls estimation

The total chlorophyll content in leaves was estimated by using the method of Arnon (1949) by using formula and recorded in mg/gram weight of leaf.

mg of chlorophyll- a/g tissue = 12.7(A663)–2.69(A645) X V/1000 X W

mg of chlo- b/g tissue = 22.9 (A645) – 4.68 (A663) X V /1000 X W

mg of total chlo/g tissue = 20.2 (A645) – 8.02 (A663) X V / 1000 X W

Carbohydrate

Total carbohydrate was estimated by using the Anthron method (Hedhe, J E and Hofreiter, B T (1962).

Amount of carbohydrate present in 100mg of the sample

$$= \frac{\text{mg of glucose X 100}}{\text{Volume of test sample}}$$

Silk worm bioassay studies

Two disease free layings (dfls) commercially popular double hybrid of (Bv X Bv) silk worms were selected for this study and made in to two batches of one disease free layings (dfl) each and reared separately feeding with infested and control mulberry leaf.

The following parameters were studied and results were placed in table-3 and table- 4.

Larval weight (g)

Larval weight was recorded every day after first feeding in all the three instars starting from 3rd instar till mounting. For this study ten larvae at randomly were selected from control as well as infested.

Number of cocoons harvested

Cocoons were harvested on fifth day after mounting by ensuring complete development of pupae and the number was recorded.

Assessment of post cocoon parameter

Cocoons were stifled by keeping them in hot air oven for three days at 70^0c. The following cocoon parameters will study to assess the quality.

C**ocoon weight (g) and Cocoon shell weight (g)**

Ten cocoons were selected at randomly and weight was recorded then pupae were removed and shell weight was recorded.

Cocoon shell percentage (%)

Ten cocoon were selected at randomly and shell percentage was calculated by using the formula

$$\text{Shell percentage} = \frac{\text{weight of shell X}}{\text{Weight of cocoon}} 100$$

Reeling parameters

Total filament length (m), Non breakable filament length (m) and Denier was recorded, the reeling cocoons on epprouvette.

Total filament length (m), Non breakable filament length (m) and Denier

Total filament length = number of rotations X circumference of epprouvette (1.125)

$$\text{Non breakable silk length} = \frac{\text{Total filament length (m)}}{1 + \text{number of breaks}}$$

$$\text{Denier} = \frac{\text{average weight in grams of filament}}{\text{Length in meters of filament}} \text{ X } 9000$$

RESULTS AND DISCUSSION

The present investigation revealed that nematode infestation on V1mulberry variety shows significant changes on growth and development and biochemical parameters, which were indicated in Table-1 and Table-2.

Nematode infestation studies

After nematode inoculation the number of galls/gram of root weight observed were 39.12. The number of egg masses /gram root weight was 32.26. The number of egg masses indicated that the efficacy of disease. The degree of resistance of mulberry variety V1 is moderately susceptible with gall index was 4 as per Taylor and Sasser (1978).

Root knot nematode effect on growth and development of mulberry

plant. Total plant height (cm)

Total plant height observed in infested plants was 170cm compared control 278cm. The percentage of reduction was 38.84cm.

Fresh weight of 100 leaves (grams)

Fresh weight of 100 leaves observed in infested plants was 311 grams where as in control 418.66 grams. The percentage of reduction was 25.71 grams.

Leaf moisture percentage (%)

The total leaf moisture percentage in infested plants was 73.32 observed compared to control plants 76.69. The percentage of reduction was 4.39.

Biochemical parameters

Phenols

Total phenols /g of leaf in control plants was 2.66 µg/g. Where as in infested plants 4.10 µg/g was observed. The percentage of phenols 54.13 increased in infested plant.

Protein

The present study revealed that the total protein content/g of leaf in infested plants 40.66mg/g was significantly reduced 31.08 percent over the control 59mg/g.

Chlorophylls estimation

Total chlorophylls/g of leaf in control plants were 2.80 mg/g, where as in infested plants total chlorophylls were 1.89mg/g. The percentage of reduction of total chlorophylls 32.50 was observed.

Carbohydrate

Total carbohydrates /g of leaf in control plants was shown 49mg/g against 31.33mg/g in infested plants with 36.06 percentage of reduction was observed.

Silk worm bioassay studies

Observations

During rearing of silk worm significant changes were observed in both infested and control plants.

Larval weight

In all the days of 3rd instar larval weight was shown significant reductions in the infested compared to control (3.89g and 4.55g) respectively. The percentage of reduction was 14.48.

In 4th instar the infested larvae weight was 13.92g, in control 14.53, the percentage reduction was 4.198 observed infested larval weight.

In all the days of 5th instar larval weight has shown significant reductions in the infested compared to control (21.69g, 38.662g) respectively. The percentage of reduction was 43.89.

Number of cocoons harvested

Cocoons were harvested on the fifth day of moulting and the number of cocoons spun were recorded separately for infested and control larvae formed and the number of cocoons from One dfl larvae of each infested and control were 198 (infested) 234 (control), the percentage of reduction 15.38.

Cocoon weight (g)

Cocoon weight from infested was 0.66g recorded and control was 1.5g. The percentage of reduction was 42.43

Cocoon shell weight (g)

Cocoon shell weight in infested was 0.146g, where as in control cocoon weight was 0.208g. The percentage of reduction was 29.80 observed.

Cocoon shell percentage (%)

The cocoon shell percentage in infested was 22.91. In control it was 23.52. The percentage of reduction was 2.59 over the control.

Reeling parameters

Total filament length (m)

The total length of reelable silk filament in the infested cocoons 906.97 m, where as in control cocoons 1029.59 m, the percentage of reduction was 11.90 observed.

Non breakable filament length (m)

The non breakable silk filament in infested was 129.5 m, where as in control there was 345 m. The percentage of reduction was 62.46 observed.

Denier

The denier of silk filament in infested was 1.8 and in control it was was2.2. The difference 18.18 was observed.

Data present in Table -1 revealed that the Total plant height (cm), Fresh weight of 100 leaves (g) and Total leaf moisture percentage (%) were significantly reduced over control plants by infestation of nematode.

Moisture content plays an important role in improving nutrition levels which improves digestibility of leaves for silk worms and also effects growth and development. The loss of moisture may be attributed to the disruption of root tissue due to growth of pathogen resulting roots lost their water absorbing capacity.

The present study in conformity with the reports of various scientists in different plant species infested with root knot nematode. In betelvine the plant growth was decreased with increased inoculums levels from 10-50,000 (Jag dale G.B. et.al, 1985). Similar results were recorded in soya been and tomato (Raut.S P. and Sethi C.L (1980).

Biochemical components were important for growth and development of silk worm and quality of cocoon production which indicate the nutrient values of mulberry leaf. It was considered that the quality of leaf derived by the presence of Protein, Chlorophylls and Carbohydrate contents.

The Table -2 showed that Protein, Chlorophylls, Carbohydrate contents were decreased in infested leaves over control by influence of pathogen metabolic activities.

Proteins were important for silk production. The proteins were degraded faster more in infectious leaves than healthy leaves due to utilization by pathogen as a result, the protein levels comes down (Samborski etal, 1958).

Chlorophylls are important pigments as their high quantity is advantageous for high rate of photosynthesis. The chlorophyll content of the leaves reduced in infested leaves. The Chlorophyll content of the leaf was reduced by *Meloidogyne incognita* that adversely effecting photosynthesis (Wallance, 1987).

Carbohydrates in mulberry leave directly influence to the health of silk worm and cocoon yield. Decrease of carbohydrate metabolic activity could be due to reduced photosynthetic capacity. Significant variation in carbohydrate metabolism was reported in tomato plants by (Wallace. 1974).

Phenols are the defense mechanism of the plants and responsible for disease resistance in plants against infection. In our study the phenol content was significantly increased in infested plants over control. The increase the phenols help in the formation of hyper sensitive reaction towards the nematode infection (Shukla and Chakraborty, 1988; Mazzafera etal, 1989; Balasubramanian and purushothamam, 1972; Sitaramaiah and Sing, 1978; Kerry, 2000)). Siddaramaiah and Hedge (1990) reported that with the increase in disease intensity phenol increased considerable in maize. It is reported that phenol content was found to be increased after infection of bêtelvine leaf and mango leaf (Naik et al., 1988 and Tofazzol et al.,1999.).

Vascular tissue of infested roots unable to absorption and upward translocation of water and minerals, as a result deterioration in leaf yield and quality (Dropkin, 1989; Shetty and Rudramuniyappa, 1992; Babu et al., 1996; Sharma and Gupta, 2005)

Silk worm bioassay

Data present in the Table-3 and 4 revealed that feeding with infested leaves caused significant changes on silk worm growth and cocoon formation were observed in the commercial characteristics of silk worms as compared to control. The important characters of silk worm and cocoons like Larval weight(g), Number of cocoons harvested, Cocoon weight (g), Cocoon shell weight(g), Cocoon shell percentage(%), Total filament length(m), Non breakable filament length(m) and Denier were reduced when infested leaves fed to the silk worm right from beginning to spinning. The reduced economic characters obtained in these silk worms are indicative of the leaf nutritive status.

Silk worms reared with nematode infested leaves produced poor quality of cocoons with less compactness (loose shell layer) more number of breaks while reeling and less continuity with reduced filament length. Denier of the filament was more compared to control which may be due to nutritional stress created in silk worm while spinning. Leaf moisture, Protein, Chlorophylls content deficiency leaves in nematode infested plants adversely affected the growth and silk production of silk worms (M. Muthulaksmi ; 2010). The silk worms fed infested leaves of mulberry plant suffer a significant reduction in silk ratio and silk gland weight relative to their body weight (Saha et al., 1983; Govindaiah et al., 1991; Paul et al., 1995) and result in prolonged larval period and deterioration of cocoon characters (Anonymous, 1927, Padma, 1989, Umesh kumar N.N 1991).

Table- 1 Growth and development parameters of mulberry plants

Mulberry plant	Total plant height (cm)	Fresh weight of 100 leaves (g)	Leaf moisture %	No of root gall/g of root	No of egg masses/g of root
Control	278	418.66	76.69	-	-
Infested	170	311	73.32	39.12	32.26
%of decrease after infestation	38.84	25.71	4.39	-	-

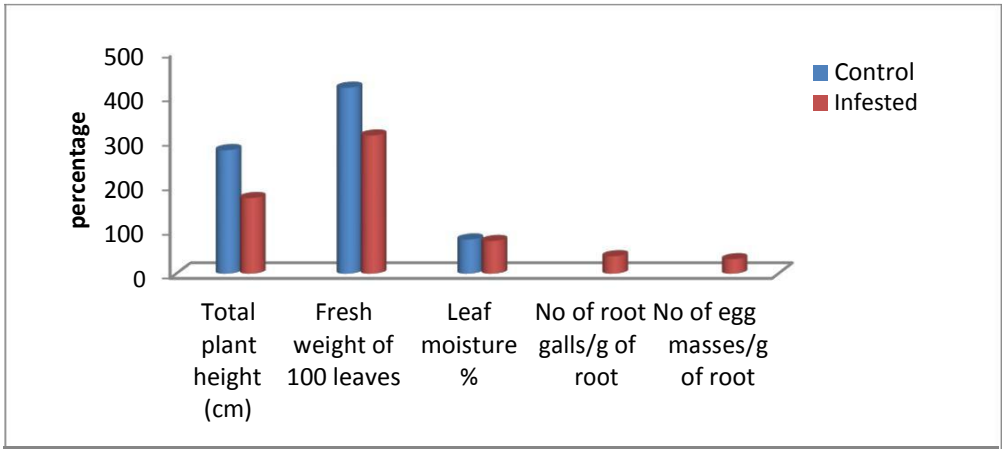


Fig-1 Growth and development parameters of mulberry plants

Table -2 Biochemical parameters of mulberry

Mulberry plant	Phenols µg/g	Protein mg/g	Carbohydrate mg/g	Total chl mg/g
Control	2.66	59	49	2.80
Infested	4.10	40.66	31.33	1.89
% of decrease	54.13	31.08	36.06	32.50

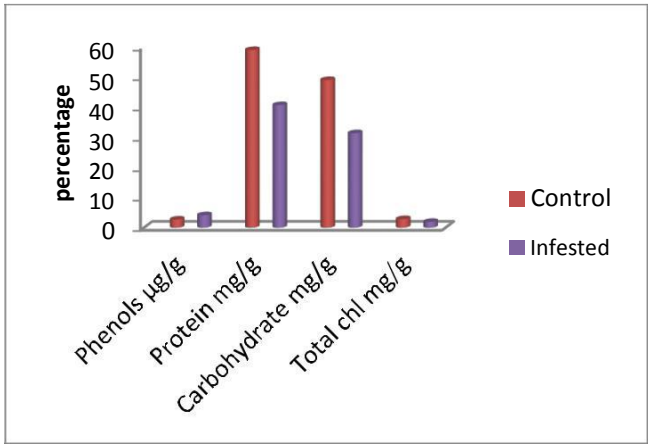


Fig-2 Biochemical parameters of mulberry

Table-3 Larval weight of infested and control silk worms

Type of larvae	Wt of larvae in 3rd instar	Wt of larvae in 4th instar	Wt of larvae in 5th instar
Infested	3.89	13.92	21.69
Control	4.55	14.53	38.66
% of decrease	14.48	4.19	43.89

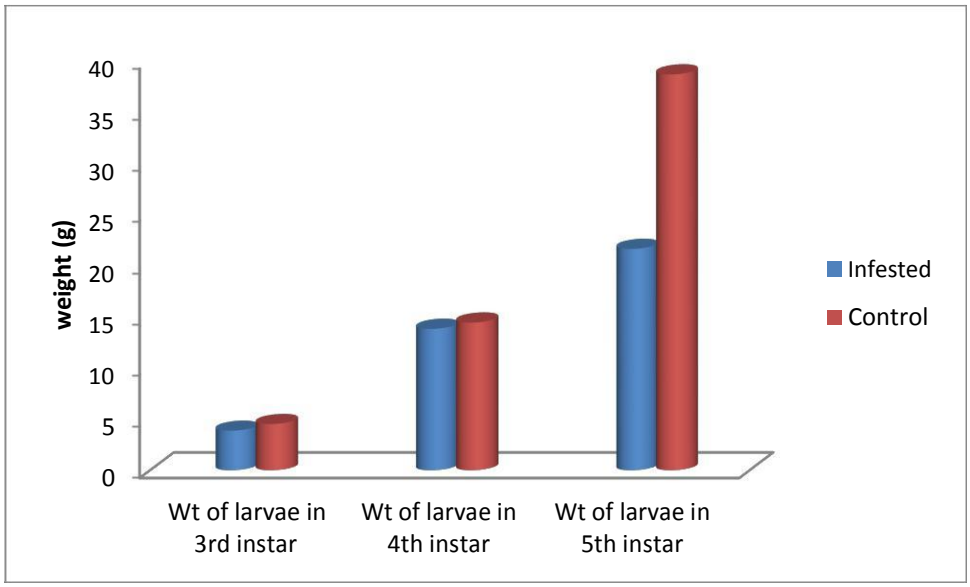


Fig-3 larval weight of infested and control silk worms

Table- 4 Post cocoon parameters

Cocoons	No of cocoons harvested	Cocoon wt (g)	Cocoon shell wt(g)	Cocoon shell percentage (%)	Total filament length (m)	Non breakable filament length (m)	Denier (u)
Infested	198	0.66	0.14	22.91	906.97	129	1.8
Control	234	1.5	0.20	23.52	1029.59	345	2.2
% of reduction	15.38	42.43	29.80	2.59	11.90	62.46	18.18

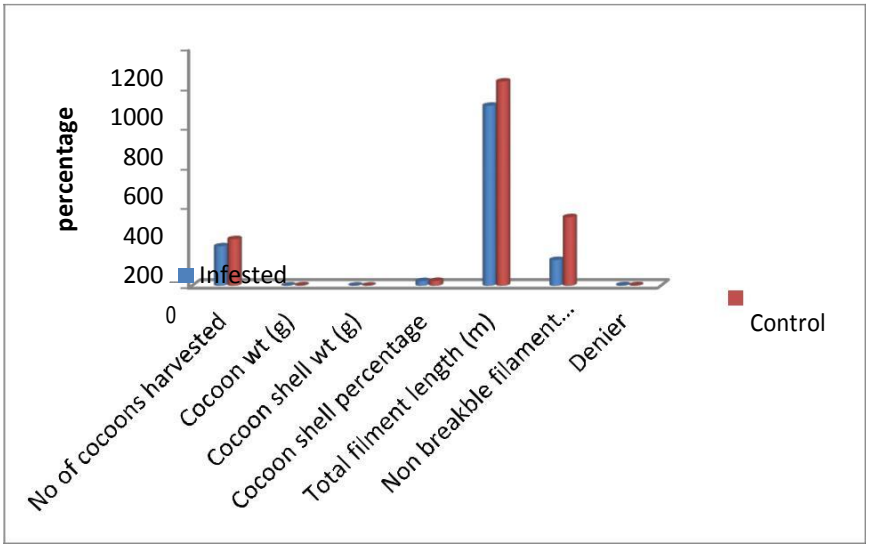


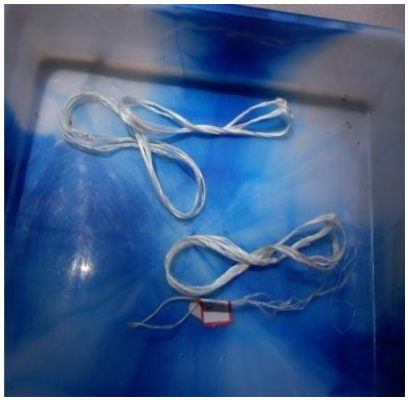
Fig -4 Post cocoon parameters

Mulberry V1

Root knot disease on mulberry

Root knots/galls on root

Cocoons on mountage



Silk of infested and control

CONCLUSION

The results concluded that the root knot nematode *Meloidogyne incognita* shows highly pathogenic on mulberry which altered nutritive, metabolic activities and growth parameters. This might be the cause of yield and quality reduction in mulberry leaves and effects on silk worm health and quality of cocoon production. Finally it leads to economic loss in silk industry.

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