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Research Article

NEMATODE *MELOIDOGYNE INCOGNITA* CHITWOOD INDUCED BIOTIC STRESS ON THE PHYTOCHEMICAL COMPOSITION OF MULBERRY LEAVES

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ABSTRACT

Root-knot diseased caused by nematode *Meloidogyne incognita* Chitwood cause considerable damage to the growth and development of plants and also reduced nutritive value of leaves especially in mulberry, which is the main food source of silkworm *Bombyx mori L.* The nutritional quality of the mulberry leaf has considerable effect on the silk worm cocoon crop yield. Hence, the present investigation was carried out to know the effect of root knot nematode on important phytochemicals of mulberry leaves infested with root knot nematode *Meloidogyne incognita*. After a careful study it was found that *M. incognita* induced biotic stress has effected considerable changes in biomolecule composition as well as vital enzymatic activity in mulberry leaf and also on the total qualitative yield of the leaf.

Key words: Biomolecules, Enzymes, *Meloidogyne incognita*, Mulberry, Root knot disease.

INTRODUCTION

Mulberry (*Morus* sps) belongs to the family *Moraceae,* which is a highly valuable plant for silk industry. Its foliage forms as the main food source for growing silk worm *Bombyx mori* L. Mulberry is usually subject to a number of diseases caused by bacteria, virus, fungi, mycoplasma and nematodes. These may cause 10-30% loss in the total yield of the leaf and may reduce the nutritive values of the mulberry leaves[1, 2]. Of these, the species of nematode *Meloidogyne incognita* (Kofaid and White) Chit Wood cause a great economic loss in the yield of mulberry[3]. This is often found in sandy loamy soils under irrigated conditions[4]. Bessey was the first person who identifies the effect of the root-knot disease on mulberry from U.S.A[5]. The root knot nematode *Meloidogyne incognita* cause galls or knots on roots of its host plants. Formation of galls/knots on the roots and other symptoms found above ground including stunted growth, chlorosis, wilting, leaf curling and reduced vigor of the plant are the conspicuous symptoms of the disease on the mulberry plant[6].

The perennial nature of mulberry easily succumbs to the root knot nematode once the nematode enters the field. Hence, the biotic stress of root knot nematode on the phytochemical components of mulberry plants and consequent loss of the yield have interested the researcher to take up this present study. The finding of the study are hoped to benefit the mulberry plant growers get rid of a common disease among plants and would help them avoid loss of yield.

MATERIALS AND METHODS

The study was carried out in the department of Sericulture, Sri Padmavati Mahila University, Tirupati, Andhra Pradesh, during the period 2014-2017. For this experiment, seventy days old mulberry V1 variety saplings were planted in randomized block design with 3' x 3' spacing. After three months of establishment, 1000 juveniles/plant was inoculated as ECL (Economic Threshold Level and Crop Loss)[7] keeping control. Sixty days after inoculation biomolecule compounds like Total chlorophylls, Carbohydrate, Protein, Starch and stress induced biomolecules (stress markers) such as Phenols, Free amino Acids, Proline, Ascorbic acid, Chlorogenic acid, Tryptophan were analyzed. In addition to that different non stress enzyme specific activity such as Malate dehydrogenase, Nitrate reductase, Amylase, Indole acetic acide oxidase (leaf and root) stress induced enzymes like Catalase, Super oxide dismutase, Peroxidase, Ascorbate peroxidase, Glutathione reductase, Polyphenol oxidase, Phenylalanine ammonia lyase, was studied in the leaves of mulberry plants.

Biomolecule estimations

Chlorophylls

The chlorophyll content in leaves was estimated by the method[8] and expressed as mg chl/g tissue was calculated using the following formula.

$$\text{Chl} - \text{a (mg/g tissue)} = 12.7(A663) - 2.69(A645) \times V/1000 \times W$$

$$\text{Chl} - \text{b (mg/g tissue)} = 22.9\,(A645) - 4.68\,(A663) \times V/1000 \times W$$

$$\text{Total chl (mg/g tissue)} = 20.2\,(A645) - 8.02\,(A663) \times V/1000 \times W$$

Where A = absorbance at specific wavelengths
V = final volume of chlorophyll extract in 80% acetone
W = fresh weight of tissue extracted

Carbohydrate

The total carbohydrate was estimated by anthrone method [9]. Carbohydrate was calculated in relation to fresh weight basis and expressed as mg/g. The calculation is done by using the formula.

Amount of carbohydrate present in 100 mg of the sample

$$= \frac{\text{Mg of glucose}}{\text{Volume of test sample}} \mathbf{x}\ 100$$

Protein

Protein content of leaves was estimated by Lowry's method [10]. The amount of protein mg/g sample was expressed.

Starch

The starch content was estimated by following the method [9] and the starch content was expressed as mg/g of leaf.

Stress induced biomolecules
Phenols

Phenols were estimated following the method[11]. Concentration of phenols was expressed in µg /g fresh weight material, equivalent to catechol.

Free amino acids

Free amino acids were estimated following the method[12]. Computation was done using standards and the amount of amino acids was expressed as µg/g.

Proline

Proline estimation was done by the following method[13] and expressed in µg / g fresh weight.

$$\mu g/g = \frac{\mu g \text{ proline /ml } \mathbf{X} \text{ ml toluene}}{115.5\ \mathbf{X}\ \text{g of sample}} \text{X } 5$$

Where 115.5 is the molecular weight of proline.

Ascorbic Acid

Estimation of Ascorbic acid was followed by the method[14] and expressed as mg/g.

Chlorogenic acid

Estimation of chlorogenic acid was followed by the method [15]. Chlorogenic acid content was calculated of the sample from the standard graph and it was mentioned as mg/g.

Tryptophan

The Tryptophan content was estimated by following the method[16]. It was calculated by using the following formula and which was expressed as µg/g.

$$\text{Tryptophan in leaf sample} = \frac{\text{Tryptophan value from graph in } \mu g}{\text{Percent N in the sample}} \mathbf{X}\ 0.096$$

Leaf Yield / ha/ annum (Kg)

Leaf yield was calculated on the basis of spacing given for plantation, leaf yield/ hectare/ annum was calculated by using the formula.

$$= \text{Total number of plants in an acre } \mathbf{X}\ 2.5\ \mathbf{X} \text{ Total yield / plant } \mathbf{X} \text{ Total number of crops / annum.}$$

Enzyme activity in Mulberry leaves
Non stress enzymes
Nitrate reductase

The enzyme activity was studied following the method[17]. Enzyme activity is expressed as micromole nitrite produced µ M min^{-1} mg^{-1} protein.

Malate dehydrogenase

The enzyme activity was assayed by the following method[18]. The enzyme activity was expressed as min^{-1} mg^{-1} protein.

Amylase Activity

The amylase inhibition was studied by the method[19] and expressed as unit min^{-1} mg^{-1} protein.

Stress induced enzymes
Catalase activity

Catalase activity was estimated following the method[20]. Enzyme activity was computed by calculating the amount of H_2O_2 decomposed and expressed as unit min^{-1} mg^{-1} protein.

$$\text{Unit activity} = \frac{\text{Change in Abs / min } \mathbf{X} \text{ total volume (ml)}}{\text{Ext. coefficient } \mathbf{X} \text{ volume of sample used (ml)}}$$

Where Ext. coefficient = 6.93 X $10^{-3}$$mM^{-1}$ cm^{-1}

Superoxide dismutase activity

SOD was assayed following the method[21]. Unit of enzyme activity was expressed as enzyme unit (EU) min^{-1} mg^{-1} protein.

$$\text{Unit of enzyme} = \frac{\text{Blank} - \text{Sample}}{\text{Blank}/2}$$

Ascorbate Peroxidase

Ascorbic acid was assayed by following the method[22]. Enzyme activity was calculated as concentration of ascorbic acid oxidized and expressed in unit min^{-1} mg^{-1} protein.

$$\text{Unit activity} = \frac{\text{Change in Abs / min } \mathbf{X} \text{ total volume (ml)}}{\text{Ext. coefficient } \mathbf{X} \text{ volume of sample used (ml)}}$$

Where Ext. coefficient =2.8 X $10^{-3}$$mM^{-1}$ cm^{-1}

Peroxidase

Peroxidase activity was determined specifically with Guaiacol at 436 nm following the method[23]. The enzyme activity was expressed as unit min^{-1} mg^{-1} protein.

$$\text{Enzyme activity units/litre} = \frac{3.18\ \mathbf{X}\ 0.1\ \mathbf{X}\ 1000}{6.39\ \mathbf{X}\ 1\ \mathbf{X}\ \Delta t\ \mathbf{X}\ 0.1}$$

Glutathione reductase (Gr)

Glutathione reductase activity was determined by following the method[24]. The enzyme activity was expressed as min^{-1} mg^{-1} protein.

Phenylalanine ammonia lyase (Pal)

Enzymatic assay of Phenylalanine ammonia lyase (PAL) activity was analyzed as the rate of conversion of L-phenylalanine into

trans-cinnamic acid at 270 nm in a spectrophotometer as mentioned[25] and was expressed in min^{-1} mg^{-1} protein.

$$\text{Units/ml enzyme} = \frac{(\Delta\ \text{A270nm/min Test} - \Delta\ \text{A270nm/min Blank})\ (3)\ (\text{df})}{(19.73)\ (0.1)}$$

Poly phenol oxidase (Ppo)

Polyphenol oxidase was assayed following method[26] and expressed as unit min^{-1} mg^{-1} protein.

$$\text{Units in the test} = K\ \mathbf{X}\ (\Delta A/\text{min})$$

Where, K is 0.272 for Catechol oxidase

Indole acetic acid oxidase

IAAO activity was determined by the method[27]. The enzyme activity was expressed as m mole IAA oxidized per min^{-1} mg^{-1} protein.

Statistical analysis

All results were subjected to statistical analysis. Data was analyzed by analysis variance T-test and tested for significance at 0.01 and 0.05 levels following the method[28].

RESULTS AND DISCUSSION

Effect of root knot nematode *M. incognita* on bimolecule composition and stress induced biomolecules in mulberry leaves

Plant parasitic nematode, *Meloidogyne incognita* caused considerable changes in biomolecule composition of the infested mulberry leaves. Results were presented in the Table 1 and 2.

Biomolecule compounds

A considerable changes in total chlorophyll content as well as chlorophyll-a and b, carbohydrate, protein, starch and leaf yield were observed in nematode infested leaves compared to healthy plants. The significant reductions were observed in all the above parameters against healthy plants. The results are presented in Table 1and Figures 1, 3.

Plant parasitic nematode, *Meloidogyne incognita* alters the metabolic processes of the host plant which are manifested in the form of physiological and biochemical changes occurring in the infested plants.

Hyperplasia and hypertrophy caused due to root knot disease on mulberry roots results in reduced uptake and transport of water and nutrients. Reduced water and nutrients absorptions make the plant suffer from decreased biomolecule synthesis ultimately reduces the yield. Root-knot nematodes cause giant cells to form in the roots, and this disrupts the root vascular system, reducing the uptake of water and nutrients and their transport from the roots to the shoots[29].

The present observations are in conformity with earlier reports. This was reported that protein and carbohydrates as well as chloroplast pigments are adversely affected due to root knot nematode *M. javanica* infestation in chickpea (*Cicer arietinum* L)[30]. The starch level decreased in the host plants infested by *Meloidogyne spp* as observed[31].

Stress induced biomolecules

When nematode infested mulberry leaves were analyzed for the presence of stress induced biomolecules, there was significant increase in the percentage of Phenols, Free amino acids, Proline, Ascorbic acid, Chlorogenic acid and Tryptophan.

High concentrations of Phenols, Free amino acids, Ascorbic acid, Chlorogenic acid and Tryptophan involve in the plant defense mechanism and create unfavorable conditions for the growth of pathogen and thus induce disease resistance in host plant. This might be the reason for increased amount of stress induced biomolecules.

The observations are in conformity with earlier reports that revealed that the *M.incognita* infested okra plants showed increased levels of phenolic compounds over the healthy plants[32]. It was investigated the biochemical alternation resulting from *Meloidogyne incognita* infestation in *Cicer arietinum* and reported an increased level of proline, free amino acids and total phenols in infested plants[33]. This was proposed that enzymes of root-knot nematodes liberate tryptophan from protein complexes and this compound converted to Indole acetic acid (IAA) could be responsible for galling[34].

Enzymes activity in leaves

Effect of root knot nematode, *Meloidogyne incognita* in enzyme activity of mulberry leaves.

Root knot nematode *Meloidogyne incognita* cause considerable changes in various enzyme activities in the nematode infested mulberry plants.

Non stress enzymes

Nematode infestation in mulberry plant cause reduction in Nitrate reductase and increased Malate dehydrogenase, Amylase activity and Indole acetic acid oxidase which are the major synthetic and catabolic enzymes. The reason could be the damaged root system results reduced uptake of water and nutrients. The results are presented in Table -2 and Figure-2.

Stress induced enzymes

When plants expose to any type of stress they react and try to protect them from oxidative stress which is the primary reaction. Enzymatic antioxidants like Catalase, Super oxide dismutase, Peroxidase, Ascorbate peroxidase, Glutathione reductase, Polyphenol oxidase, Phenylalanine ammonia lyase, are commonly present in the subcellular compartments which show increased activity to protect the plant system from oxidative stress. In the present study also significance increase was observed in all the stress enzyme activities. The results are presented in Table -2 and Figure-2.

Nematode induced biotic stress which leads to overproduction of Reactive oxygen species (ROS) continuously as byproducts of various metabolic pathways. They cause damage to proteins, lipids, carbohydrates and DNA resulting in oxidative stress[35]. In favor to limit oxidative damage under stress, plants have enhanced a series of detoxification systems that break down the highly toxic ROS[36].

Enzyme activity is one of the important tools to confirm the resistance to root pathogenic nematodes. When a nematode infests the host tissue, a small number of specific genes are induced to produce mRNAs that permit synthesis of similar number of specific proteins[37]. Many of these proteins are

enzymes such as phenylalanine ammonia lyase, polyphenol oxidase, peroxidase and β-1-3-glucanase. These are involved in the synthesis of low molecular weight substances such as phytoalexins, phenols and lignin which are inhibitory to the invading nematodes[38].

The results of the present investigations are in agreement with the various reports. The increased percentage of peroxidase activity, phenol content, polyphenol oxidase activity, phenylalanine ammonia lyase was found in roots of egg plant infested with nematode[39]. The investigations carried out to establish a link between the activities of catalase, peroxidase and superoxide dismutase in tomato and nematode *M. incognita* parasite interactions revealed that, increase in the enzyme activity was recorded highest after nematode inoculation for peroxidase, superoxide dismutase and catalase respectively in the susceptible cultivar[40].

Mulberry garden

Root knot diseased plant

Table 1. Effect of root knot nematode *M. incognita* on Biomolecules of mulberry leaves

S. No	Parameters	Control	Infested	% of decreased/ increased	T- Test	Significant at 0.01 level
1	Total chlorophylls (mg/g)	2.797	1.890	32.427	51.403	**
	Chl- a (mg/g)	1.860	1.200	35.483	34.467	**
	Chl- b (mg/g)	0.940	0.690	26.595	21.651	**
2	Carbohydrate (mg/g)	49.000	31.330	36.061	496.484	**
3	Protein (mg/g)	59.000	40.660	31.084	589.876	**
4	Starch (mg/g)	17.400	10.500	39.655	225.856	**
5	Leaf yield / ha/ annum (kg)	16036.370	11616.000	27.564	193.038	**
6	Phenols (µg/g)	2.660	4.100	54.135	23.568	**
7	Free amino acids (µg/g)	13.000	23.330	79.461	565.797	**
8	Proline(µg/g)	3.570	5.250	47.058	49.903	**
9	Ascorbic acid (mg/g)	82.997	92.660	11.642	346.497	**
10	Chlorogenic acid (mg/g)	42.660	56.660	32.817	588.188	**
11	Tryptophan (µg/g)	0.457	0.787	72.21	17.501	**

Note – ** Significant at 0.01 level

Table 2. Effect of root knot nematode, *M. incognita* on enzymatic activity of mulberry leaves

S.No	Parameters (min^{-1} mg^{-1} protein)	Control	Infested	% of decreased/ increased	T- Test	Significant at 0.01 level
1	Nitrate reductase	5.87	4.36	25.724	40.356	**
2	Malate dehydrogenase	0.337	0.563	67.062	11.031	**
3	Amylase	1.65	2.37	43.636	37.601	**
4a	Indole acetic acid oxidase in leaf	0.113	0.187	65.486	7.778	**
4b	Indole acetic acid oxidase in root	0.803	1.59	98.007	11.600	**
5	Catalase	7.723	9.057	17.273	33.806	**
6	Peroxidase	0.34	0.61	79.411	33.068	**
7	Ascorbate Peroxidase	1.163	2.01	72.828	35.223	**
8	Glutathione reductase	0.083	0.133	60.24	4.009	*
9	Superoxide dismutase	0.027	0.047	74.074	4.243	*
10	Polyphenol oxidase	0.34	0.66	94.117	24.787	**
11	Phenylalanine ammonia lyase	0.807	1.443	78.81	33.764	**

Note – ** = Significant at 0.01 level
*= Significant at 0.05 level

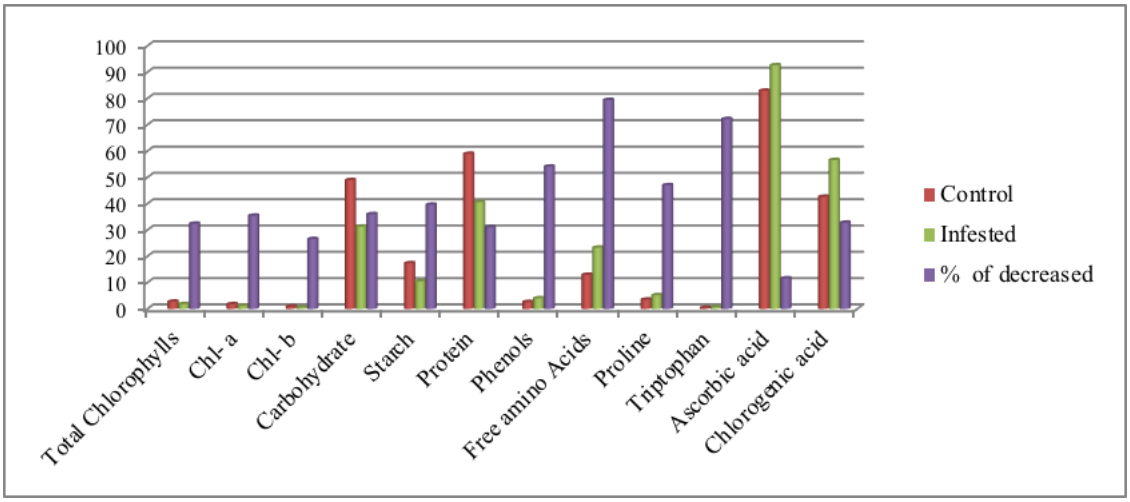

Figure 1. Effect of root knot nematode *M. incognita* on biomolecules of mulberry leaves

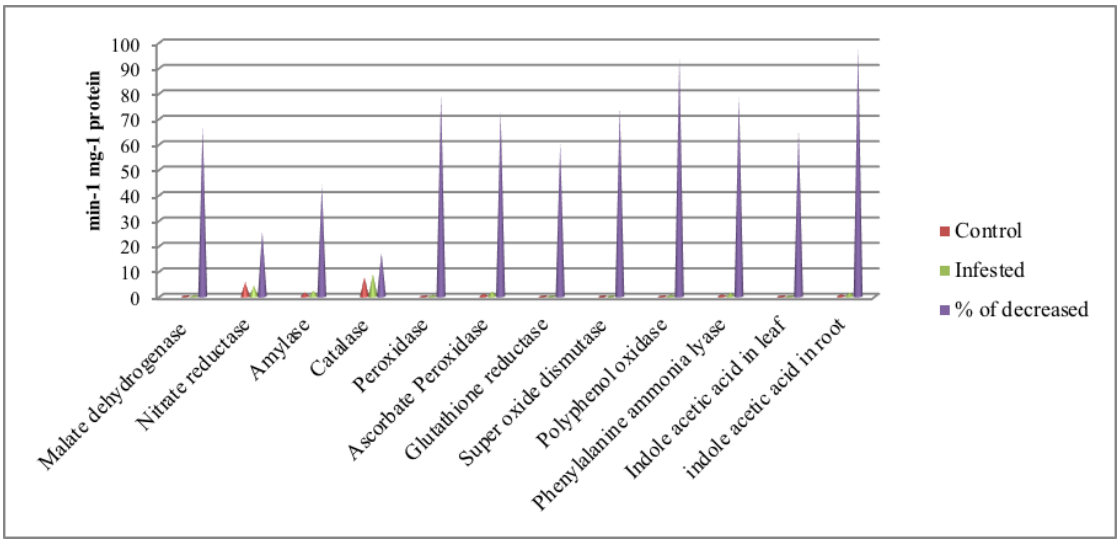

Figure 2. Effect of root knot nematode, *M. incognita* on enzyme activity of mulberry leaves

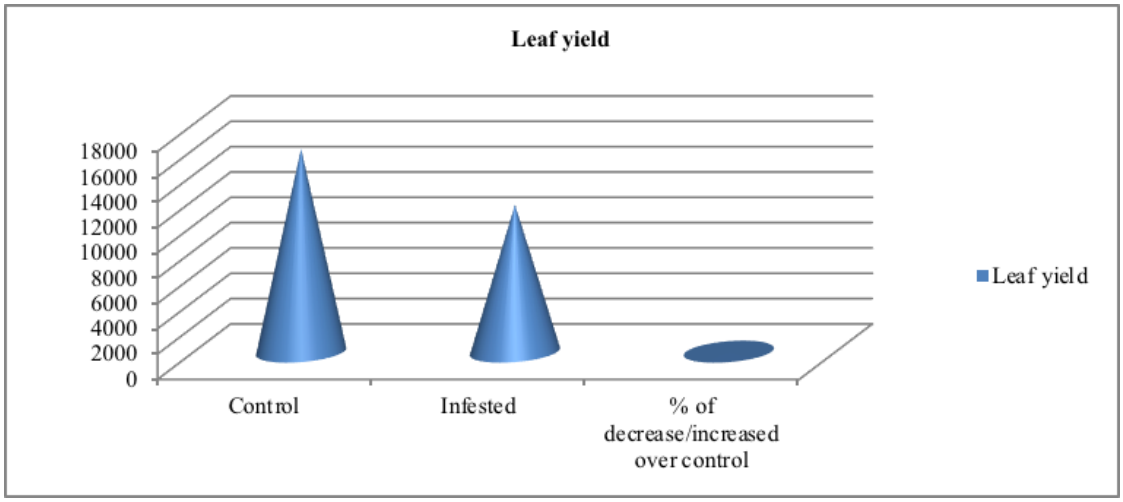

Figure 3. Effect of root knot nematode, *M. incognita* on leaf yield of mulberry

CONCLUSION

It is concluded that root-knot nematode *Meloidogyne incognita* bring about considerable phytochemical changes in infested mulberry plants may be considered as evidence of altered physiology and growth of plants due to host-parasite relationship and interaction resulting to loss in crop yield and also the increased stress enzyme activity could be resistant of plant defense mechanism against root-knot nematode *Meloidogyne incognita*.

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