J. Agr. Sci. Tech. (2018) Vol. 20: 633-645

Study on Morphological and Biochemical Characteristics of Babchi (*Psoralea corylifolia*) Infected with the Root-Knot Nematode, *Meloidogyne incognita.*

M. Danish[1]*, H. Seikh [1], and M. I. Robab[2]

ABSTRACT

A pot experiment was carried out to determine the effects of *Meloidogyne incognita* on *Psoralea corylifolia* at different inoculum levels *viz*., 100, 500, 1,000, 2,000 J2 per plant in each pot containing 2.5 kg sterilized soil with complete randomized block design. Results indicated significant reduction in all the growth and yield parameters of the plants in comparison to non-inoculated control plants. Biochemical parameters such as photosynthetic pigments viz., Chlorophyll a, Chlorophyll b and carotenoid contents and enzymatic activity like nitrate reductase and carbonic anhydrase were significantly decreased when inoculum levels were increased. Highest and significant (P≤ 0.05) reduction was noticed at 2,000 J_2, in comparison to healthy plants. The number of nematodes in the infected root was higher and the size of the galls was larger at high inoculums levels. The root and the soil populations of second stage-juveniles indicated that *M. incognita* reproduced successfully on the roots of *P. corylifolia.* The damages caused due to severe infection might lead to death of the affected plant.

Keywords: Enzymatic activity, Inoculum level, Morphology, Photosynthetic pigments.

INTRODUCTION

Psoralea corylifolia L. is a medicinally important plant indigenous to the tropical and the sub-tropical regions of the world. It is a small, erect, annual herb, 30-180 cm tall and is grown throughout the world. Every part of this amazing plant is used: root, stem, leaf, and seeds. The plant is used to treat a variety of skin problems, like leukoderma, skin rashes, infections, and various others (Krishnamurthi, 1969, Khushboo *et al*., 2010). The essential oil obtained from the seeds contains limonene, α-elemene, γ-elemene, *β*-caryophylenoxide, 4 terpineol, linalool, and geranylacetate (Kapoor, 2001). Leaves of *P. corylifolia* also contain raffinose, psoralen, and isopsoralen (Krishnamurthi, 1969). The seed extract of *P. corylifolia*, was found to exert anti-oxidative, anti-microbial, anti-inflammatory, anti-tumour, anti-mutagenic effects and inhibit insect hormonal activities (Bapat *et al*., 2005; Haraguchi *et al*., 2002; Khatune *et al*., 2004).

The plant is also used in indigenous medicine such as laxative, aphrodisiac, anthelmintic, diuretic and diaphoretic in febrile conditions (Rastogi and Mehrotra, 1990). Many Indian pharmaceutical companies have used *P. corylifolia* as a raw material in the production of medicines and Ayurvedic skin care soaps (Baskaran and Jayabalan, 2007). Leaves are used in curing diarrhea and roots are useful in the treatment of caries of teeth (Anonymous, 1989).

[1] Department of Botany, Section of Plant Pathology and Nematology, University of Aligarh Muslim, Aligarh, 202002, UP, India.
[2]Department of Botany, School of Sciences, University of Maulana Azad National Urdu, Hyderabad, 500032. Telangana State, India.
*Corresponding author; e-mail: danish.botanica@gmail.com

[Downloaded from jast.modares.ac.ir on 2024-10-12]

[DOR: 20.1001.1.16807073.2018.20.3.13.9]

Plant parasitic nematodes are soil borne pathogens that attack a wide range of economically important crops and affect both yield and quality (Noling, 2005). Susceptibility of nematodes to different medicinal, aromatic and spice plant species has been reported by Haseeb (1994). Damage caused by plant parasitic nematode has been estimated up to 10% of world crop production (Whitehead, 1998). Root-knot nematodes (Meloidogyne) are the most widely distributed nematode in agriculture, attacking over 2,000 different plant species, including cultivated crops and wild plants, causing an estimated monetary loss of $100 billion per annum worldwide (Oka *et al.,* 2000). To date, more than 100 *Meloidogyne* species have been described (Karssen and Moens, 2006), of which four species, namely, *Meloidogyne javanica*, *Meloidogyne arenaria*, *Meloidogyne hapla*, and *M. incognita* are responsible for approximately 90% of the nematode damages in tropical and subtropical environments (James, 1991; Taylor and Sasser, 1978). *Meloidogyne incognita* has also been found associated with *P. corylifolia* (Sultan *et al*., 2010; Khan *et al*., 2014).

Root-knot nematode, *M. incognita* induces morphological and biochemical changes in host plants. Hisamuddin *et al*. (2005) reported significant reduction in dry weight and chlorophyll content of *Phaseolus mungo* L. inoculated with 1,000 second stage Juveniles (J2) of *M. incognita.* Azam (2008) reported significant and greatest reduction in plant growth and yield at highest inoculum level of 5,000 J2 on tomato plant. Niyaz and Hisamuddin (2008) found that an increase in nematode inoculum level caused a decrease in plant growth and yield of *Eclipta alba.* At higher inoculum levels of *M. incognita,* in case of *Solanum nigrum,* all the growth parameters were found drastically reduced (Robab *et al*., 2009). Danish *et al*. (2016) observed significant reduction in growth and physiological parameters in *Dianthus caryophyllus* at different inoculum levels of *M. incognita.*ost.

For successful cultivation and crop management, it is necessary to know the crop is a host of the nematodes and the degree of resistance or susceptibility. Hence, we aimed to examine the development and susceptibility of *M. incognita* at different inoculum levels on *P. corylifolia* and to explore the relationship between the nematode and biochemical aspects of the host plant.

MATERIALS AND METHODS

Collection and Preparation of Inoculum

Roots of eggplant (*Solanum melongena)* infected with the root-knot nematode (*M. incognita*) were collected from an eggplant field. Root-knot species *M. incognita* was identified on the basis of North Carolina differential host test and perennial pattern morphology (Hartman and Sasser, 1985). Single species populations were maintained on local cultivar of eggplant in greenhouse. After 90 days of inoculation, the egg masses were picked from the galled roots with the help of sterilized forceps and were allowed to hatch. The infective second-stage Juvenile (J2) that comprised primary inoculums were collected in sterilized distilled water and counted with the help of counting dish under the stereomicroscope. The suspension was standardized to 1,000 J2 10 mL^{-1} of suspension (Khan, 2008).

Test Plant

Seedlings of *P. corylifolia* were obtained from Botanical Garden (Department of Botany) of the Aligarh Muslim University and were planted in a fully autoclaved 38 cm clay pot filled with 2.5 kg steam sterilized soil.

Inoculation

One week after the seedlings were transplanted, 5-7 cm deep holes were made about 2 cm from the stem of the plants. The infective nematode juveniles were pipette in

[Downloaded from jast.modares.ac.ir on 2024-10-12]

[DOR: 20.1001.1.16807073.2018.20.3.13.9]

these holes at the rate of 100, 500, 1,000, 2,000 J2 (Pi= Initial Population) per plant in each pot. The holes were filled with the soil soon after inoculation. Six replicates were maintained for each treatment. Three replicates were used within fifteen days of inoculation for biochemical analysis. Remaining three replicates were harvested hundred and twenty days post inoculation for growth and yield parameters. Pots were arranged in randomized complete block design. Non-inoculated set of plants served as control. Watering of plants was done regularly as required by drip irrigation.

Estimation of Growth and Yield Parameters

Hundred and twenty days after inoculation, shoot length was measured using meter scale. The numbers of seeds per plant were counted and the weight of 100 seeds of each treatment was compared with the seeds of control plants. The leaves area was determined using graph paper method. The plants were uprooted with the help of a hoe and cut at the margin of the root and shoot. The roots were gently washed free of soil with tap water. Fresh weights of the roots and the shoots were determined with the help of a balance. Root and shoots were kept in an incubator maintained at 72 ^{o}C for 5 days. The dry weight of the roots and shoots was determined.

Estimation of Photosynthetic Pigment

The amount of chlorophyll pigment was calculated by following the method of MacKinney (1941). Within fifteen days after inoculation, fresh leaves (100 mg) of each plant of each treatment from three replicates were homogenized in a mortar with the sufficient quantity of 80% acetone. The extract was filtered and the supernatant was collected in the volumetric flask. Finally, the volume was made up to 1,000 mL with 80% acetone. Three mL of the leaf extract sample was transferred to cuvette and the absorbance was read at 645, 663 nm on spectrophotometer for estimation of chlorophyll “a” and “b”, and at 480 and 510 nm for carotenoid.

Formulas used were the following:

Chl a= [12.7 (D 663)–2.69 (D 645)] $X \frac{V}{1000\,W}$ (mg g^{-1}) (1)

Chl b= [22.9 (D 645)–4.68 (D 663)] $X \frac{V}{1000\,W}$ (mg g^{-1}) (2)

Carotenoid= [7.6 (D 480)–1.49 (D 510)] $X \frac{V}{1000\,W}$ (mg g^{-1}) (3)

Where, *V*= Total volume of the solution, *W*= Weight of the tissue used for extraction of the pigment, and *D*= Optical Density of sample at 645 and 663 nm.

Biochemical Parameter

Total Phenol Content

The adopted method of Bray and Thorpe (1954) was used for the extraction and determination of total phenolic contents.

Determination of Nitrate Reductase Activity

Nitrate reductase activity in the leaf was determined according to the procedure given by Jaworski (1971). One hundred mg of fresh chopped leaves were transferred into tubes containing 1.25 mL of 0.1M phosphate buffer (pH 7.4). To each tubes, 0.25 mL of 0.2M potassium nitrate were added in each tubes followed by 1.25 mL of 5% isopropanol. These mixtures were kept in BOD incubator at 25±2°C for 2 hours. Later, 0.2 mL of this incubated solution was taken into separate test tubes and 0.15 mL of each 1% sulphanilamide and 0.02% NED-HCL was added. The mixture was left for 20 minutes at room temperature for maximum color development. Finally, the mixture was diluted by adding distilled water to make

[Downloaded from jast.modares.ac.ir on 2024-10-12]

[DOR: 20.1001.1.16807073.2018.20.3.13.9]

volume 5 mL and OD was read at 540 nm by spectrophotometer against a blank. A standard curve was plotted using the known concentration of sodium nitrite. Nitrate reductase activity was expressed in µmole NO_2 g^{-1}fw hr $^{-1}$ after comparing the OD of sample with Standard curve.

Determination of Carbonic Anhydrase Activity

The CA activity was determined, in the fresh leaves, according to the procedure given by Dwivedi and Randhawa (1974). One hundred mg of Fresh leaves from each treatment was separately cut into small pieces and transferred to test tube, followed by addition of 5 mL of 0.2M cystein hydrochloride solution. This mixture was incubated at 4°C for 20 minutes. To each test tube, 2 mL phosphate buffer and 2 mL of 0.2M sodium bicarbonate solution followed by 0.1 mL of 0.02% bromothymol blue was added into the mixture and shaken properly. Again, this mixture was left for 20 minutes at 4°C. The reaction mixture was titrated against 0.05 N HCl using methyl red as indicator. Reading was noted as red pink color developed. A control sample, without leaf tissue, was also titrated against 0.05N HCL. The CA activity was expressed as µM CO2 kg^{-1} leaf FW S^{-1}

Number and Size of Galls

The number of galls per plants was counted visually, and the size of gall was obtained by measuring its maximum length and width (in mm) using a micrometer.

Number of Egg Masses

The number of egg masses per root system on infected root was counted after staining with phloxin-B (Holbrook *et al*., 1983). The galled roots were placed in the solution for 15-20 minutes and the roots gently rinsed in tap water and the red stained egg masses counted.

Nematode Population (Root and Soil)

Root population of the nematode was determined by macerating 5 g of infected root in Waring blender; the suspension was passed through 100 to 400 mesh sieves and the juveniles were washed from the 400 mesh sieves into a beaker. The population level of nematode in the soil was determined using Cobb's sieving and Baermann funnel methods (Southey, 1986). The number of nematodes per root system and per kilogram soil was counted using a de Grisse counting dish under a stereomicroscope at 40X magnification.

Reproduction factor (Rf) was calculated as formula given by Ferris (1985):

$Rf= Pf/Pi$ (4)

Where, *Pf* is the final Population and *Pi* is the initial Population of the nematode.

Statistical Analysis

All data were subjected to Analysis Of Variance (ANOVA) using SPSS 17.0. Least Significant Differences (LSD) were calculated at probability level of $P\leq 0.05$. Linear correlation curve was plotted between number of galls and nitrate reductase activity, carbonic anhydrase activity.

RESULTS

Plant Length (Root and Shoot)

The effect of *M. incognita* on the height of *P. corylifolia* is presented in Table 1. At all the different inoculum levels, the infected plants, in all the treatments, exhibited stunted growth, when compared with the non-inoculated control plant. Non-significant reductions in plant height were observed when the plants were inoculated at

[Downloaded from jast.modares.ac.ir on 2024-10-12]

[DOR: 20.1001.1.16807073.2018.20.3.13.9]

[DOR: 20.1001.1.16807073.2018.20.3.13.9] [Downloaded from jast.modares.ac.ir on 2024-10-12]

Table 1. Effect of *M. incognita* on shoot and root morphology of *P. corylifolia*.

Treatment	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
Control	65.9±1.8[a]	81.5±1.5	29.3±0.4	36.6±0.8	15.6±0.3	6.9±0.1
100 J2	62.7±1.8	78.0±1.6	28.6±0.3	35.4±0.3	14.6±0.3	6.7±0.1
500 J2	52.5±1.3	72.6±1.2	24.0±0.4	28.9±0.4	12.0±0.5	5.2±0.1
1000 J2	47.3±1.7	49.3±1.3	14.4±0.3	23.3±0.7	9.1±0.1	3.7±0.5
2000 J2	30.4±1.6	39.9±1.5	13.2±0.3	16.1±0.6	6.0±0.3	2.1±0.0
LSD (P≤ 0.05)	8.2	7.19	2.2	3.1	1.8	1.3

[a] **Note:** Each value is a mean of three replicates±Standard error.

an initial inoculums level of 100 J2, but significant reduction was noticed in all the other treatments, in comparison to the non-inoculated plants. Highest and significant reductions in shoot (53.8%) and root (55.9%) length were observed in the plants inoculated with 2,000 J2, when compared with the control plants.

Fresh Weight (Shoot and Root)

The fresh weight of shoot and roots were not decreased significantly at initial inoculum level of 100 J2 over the non-inoculated control plants. As expected, the most reduction in the fresh weight of the shoots (51.0%) and the roots (61.2%) were observed in the plants inoculated with highest number of infective juveniles. Significant (P≤ 0.05) reductions were observed among the plants inoculated with 500, 1,000 and 2,000 J2 (Table 1).

Dry Weight (Shoot and Root)

In comparison with the control, non-significant reduction was found in the dry weight of the shoot and the root at the initial inoculum level of 100 J2, when compared with control. In comparison with the non-inoculated control plants, the rest of the treatments (500, 1,000 and 2,000 J2) exhibited significant reduction. Maximum reductions in the dry weight of the shoot (54.8%) and the root (69.0%) were observed in the plants inoculated with the highest inoculum level of 2,000 J2 per pot (Table 1).

Number of Branches and Number of Seeds per Plant

The number of branches and seeds per plant decreased with an increase in initial inoculum levels. Maximum (50.0, and 50.3%) and significant (P≤ 0.05) reductions were observed in the plants inoculated with 2,000 J2 followed by 1,000 J2, in both

parameters, when compared with the control plants (Table 2).

Leaf Area

At higher inoculum levels of *M. incognita,* significant (P≤ 0.05) reduction was found in leaf area (58.6%), when compared with the control plant. Reduction in the plants inoculated with initial inoculums level of 100 J2 was not significant in comparison to non-inoculated control plants. Significant reductions in leaf area were found between the plants inoculated with 500, 1,000 and 2,000 J2 (Table 2).

Seed Weight

After maturation of the plants, 100 seeds were collected randomly from each treatment and compared with the seeds of control plants. At an initial inoculum level of 100 J2, reduction in weight of 100 seeds was non-significant, in comparison to the control plants. Highest and significant decreases in the weight of seeds were observed in the plants inoculated with highest inoculums level of 2,000 J2 followed by 500 and 1,000 J2 inoculated plants, on comparing with the control plants (Table 2).

Photosynthetic Pigments (Chlorophyll a, b and Carotenoid)

In response to inoculation with *M. incognita,* chlorophyll a, chlorophyll b, and carotenoid contents of leaves did not decrease significantly at the initial inoculum level of 100 J2, but significantly decreased at the next higher inoculum levels, when compared with the control plants. Maximum and significant decrease in the amount of chlorophyll ‘a’ (68.1%), chlorophyll ‘b’ (42.2%) and in carotenoid (55.0%) were found at higher inoculum levels (2,000 J2) over non-inoculated plants (Table 3).

Total Phenol

An increase in total phenol content was noticed in the infected plants with second-stage juveniles, in comparison to the control plants. The maximum (66.7%) and significant (P≤ 0.05) increase was observed in the plants inoculated with the highest inoculum level of 2,000 J2, when compared with the rest of the treatments (100, 500, 1,000 J2) and non-inoculated control plants (Table 3).

Nitrate Reductase Activity

The nitrate reductase activity decreased significantly at an initial inoculum level of 100 J2, compared with the control plants. The reduction was highest (64.5%) and significant in the plants inoculated with 2,000 J2 followed by 1,000 J2 inoculated plants, in comparison to the control plants (Table 3).

Carbonic Anhydrase Activity

The lowest reduction in carbonic anhydrase activity was observed at initial inoculums level of 100 J2. The plants treated with 500, 1,000, and 2,000 J2 showed significantly (P≤ 0.05) reduced content, compared with the control plants. Moreover, significant reduction was also observed in between the plants inoculated with 500, 1,000 and 2,000 J2. The highest reduction (37.6%) was observed in the plants inoculated with 2,000 J2 in comparison to the control (Table 3).

Number and Size of Galls

The galls were observed in all the treated plants. The lowest numbers and size of galls were observed at initial inoculum level of 100 J2 (Table 4). Numbers and size of galls increased with the increase in inoculum level. Highest number and maximum size of

[Downloaded from jast.modares.ac.ir on 2024-10-12]

[DOR: 20.1001.1.16807073.2018.20.3.13.9]

[DOR: 20.1001.1.16807073.2018.20.3.13.9] [Downloaded from jast.modares.ac.ir on 2024-10-12]

Table 2. Effect of different inoculum levels of *M. incognita* on growth and yield of *P. corylifolia.*

Treatments	Leaf area (cm^2)	Number of branches $plant^{-1}$	Number of seeds $plant^{-1}$	Weight of 100 seeds (g)
Control	9.6±0.3[a]	14.3±0.1	231±2.6	1.4±0.0
100 J2	9.0±0.1	14.0±0.2	225.3±2.3	1.4±0.0
500 J2	8.5±0.1	11.0±0.2	201.6±2.1	1.3±0.0
1000 J2	5.8±0.1	8.0±0.4	145.3±2.6	0.9±0.0
2000 J2	4.0±0.2	7.0±0.2	114.6±2.1	0.8±0.0
LSD ($P \leq 0.05$)	1.1	1.3	11.8	0.14

[a] Note: Each value is a mean of three replicates.

Table 3. Effect of *M. incognita* on photosynthetic pigments and enzymatic activities of *P. corylifolia.*

Treatment	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Carotenoid (mg g^{-1})	Total phenol (mg g^{-1} Catechol equivalent)	Nitrate reductase (µmol No_2 kg^{-1} FW^{-1} h)	Carbonic anhydrase (µM Co_2 kg^{-1} FW^{-1} S)
Control	1.9±0.0[a]	1.3±0.0	1.51±0.0	8.5±0.2	1.5±0.0	156.0±2.3
100 J2	1.7±0.0	1.2±0.0	1.5±0.0	8.6±0.4	1.3±0.0	154.0±2.5
500 J2	1.4±0.0	1.0±0.0	1.1±0.0	11.0±0.5	1.1±0.0	142.6±2.1
1000 J2	1.1±0.0	0.8±0.0	0.8±0.0	12.0±0.2	0.8±0.0	115.8±1.9
2000 J2	0.6±0.0	0.7±0.0	0.6±0.0	14.1±0.4	0.5±0.0	97.3±2.3
LSD ($P \leq 0.05$)	0.1	0.1	0.14	1.9	0.12	11.1

[a] Note: Each value is a mean of three replicates.

[DOR: 20.1001.1.16807073.2018.20.3.13.9]
[Downloaded from jast.modares.ac.ir on 2024-10-12]

Table 4. Nnumber of galls, size of galls, number of egg masses per plant, and reproduction factor on *P. corylifolia.*[a]

Treatments	No. of galls	Size of galls (mm^2)	No. of egg masses $plant^{-1}$	Root population	Soil population	Total population	Reproduction factor (Rf= Pf/Pi)
Control	0.00	0.0	0.0	0.0	0.0	0.0	0.0
100 J2	16.0	4.2	74.0	1548.0	2962.0	4510.0	45.1
500 J2	43.0	9.1	164.0	1926.0	3789.0	5915.0	11.8
1000 J2	51.0	14.5	200.0	2159.0	4361.0	6520.0	6.5
2000 J2	110.0	15.8	266.0	3074.0	6281.0	9356.0	4.6
LSD (P≤ 0.05)	15.4	2.6	16.7	354.2	657.0	918.5	--

[a] **Note:** Each value is a mean of three replicates. mm^2= Maximum width and length of gall; *Rf*= Reproduction factor; *Pf*= Final Population, *Pi*= Initial Population.

galls were observed on the plants which were inoculated with the highest number of second-stage juveniles (2,000 J2).

Number of Egg Masses per Plant

The number of egg masses per plant increased significantly (P≤ 0.05) in all the treatments (Table 4). Maximum numbers of egg masses were observed in the plants inoculated with the highest number of second-stage juveniles (2,000 J2). The lowest significant increase was observed in the plant inoculated with the initial inoculum level of 100 J2.

Nematode Final Population and Reproduction Factor (Rf)

Final population (root population + soil population) of the nematode was smallest at the initial inoculum level of 100 J2 per pot and maximum in the plants that were inoculated with the highest number of infective juveniles (2,000 J2). The Reproduction factor (Rf) decreased with an increase in the initial level of inoculum, the maximum being associated with the lowest, and the minimum with the highest inoculum level (Table 4).

DISCUSSION

The experiment showed that babchi (*P. corylifolia*) was highly susceptible towards *Meloidogyne incognita.* The nematode not only caused stunting of the plant growth but also changes in biochemical parameters.

From the results of the experiment, it was found that the plant growth parameters, like shoot and root lengths and fresh and dry weights of the plants infected with *M. incognita*, were adversely affected as the inoculums level increased from 100 to 2,000 J2 per plant. The damage caused to the plants on infection by *Meloidogyne incognita* involves several mechanisms. The

impairment in growth of *P. corylifolea* caused by *M. incognita* might be due to one or more or all of the following reasons: (1)Obstruction in proper translocation of water and mineral elements from the roots to the shoots due to abnormalities and deformities occurring in the galled roots. (2) Departure in translocation path of metabolites from shoots towards the newly formed sinke "the giant cells" in the galled roots instead of normally moving towards growing regions, (3) Deceleration in the rate of synthesis of metabolites as a result of scarcity of water and nutrients in the leaves, (4) Withdrawal and consumption of metabolites in significant amount diverted towards giant cells by few nematode, in case of primary infection, and in large amount, in case of secondary and subsequent infection.

Contribution of the above mentioned phenomenon in suppression of plant growth has been reported by Hisamuddin *et al*. (2005); Robab *et al*. (2010). Reduction in the plant height and weight of the plant, as the initial inoculum level increased has been found in several plants (Azam *et al*., 2010; Robab, 2012).

Significant reduction in chlorophyll a, chlorophyll b, and carotenoid contents at higher inoculum levels hampered the physiological process of photosynthesis and, consequently, growth, as was evident from reduction in fresh and dry weight of the infected plants. Photosynthetic pigments are essential for carrying out the event of light dependent phase. Any deficit in their amount would affect CO2 fixation, metabolite synthesis, and growth and development of plant (Wallace, 1987). Increase in inoculum level causes reduction in the chlorophyll and carotenoid contents of different plants was observed by different authors (Shukla and Haseeb, 1998; Kheir *et al*., 2004; Hisamuddin *et al.*, 2005).

From the results (Table 3) it is evident that phenolic content was high in nematode infected plants than healthy plants. An increase in the primary inoculum level leads to an increase in phenolic content. The highest phenolic content was observed at the treatment with the highest inoculum level. Bhargava *et al*. (2007) and El-Sherif *et al*. (1973) reported that the total phenol was higher in infected plants in comparison to healthy plants.

Nitrogen assimilation in the plant cell first reduce nitrate to nitrite that is catalyze by the enzyme nitrate reductase. Reversible hydration of carbon dioxide is catalyzes by carbonic anhydrase. The enzymatic activity was directly related to photosynthetic carbon assimilation because infection of *M.inocognita* on *P. corylifolia* affected Activities of Nitrate Reductase (NRA) and Carbonic Anhydrase (CA). Increase in inoculums level decreased the efficiency of nitrate reductase and carbonic anhydrase activity. The study has revealed that both enzymatic activities were highly sensitive to alteration in biochemical pathway of host plant induce by *M. incognita.* The resulting biochemical changes were due to interruption in translocation of water and mineral elements from root to shoot and formation of galls caused by nematode. Decrease in nitrate reductase activity in the plant infected with nematode has been reported by James (2004) and Pavaraj (2007).

An increase in inoculum levels of *M. incongita* caused more and larger galls, with largest and highest number of galls on the plants inoculated with 2,000 J2. This trend might be due to the fact that at higher inoculum level more feeding sites were explored by larger number of juveniles which resulted in increased number of galls on the infected roots. In several plants, increase in size and number of galls have been observed with increase in initial inoculums levels (Yasmeen, 2002; Parveen, 2006; Niyaz and Hisamuddin, 2008; Azam, 2008; Robab, 2012).

The number of egg masses per plant increased on increasing initial inoculums level. From the results, it seems that the number of egg masses per plant was proportional to the number of nematodes present in the gall. The lower is the amount of inoculums, the fewer would be the gall

[Downloaded from jast.modares.ac.ir on 2024-10-12]

[DOR: 20.1001.1.16807073.2018.20.3.13.9]

number, while the higher is the inoculums level, the greater would be the gall number. Thus, number of egg masses per gall corresponds to the number of nematode causing infection of the gall. At lower inoculum level, the nutrients are sufficient for maximum growth of the nematode. At higher inoculum levels, the number of nematodes present in the gall is higher, which compete for the same amount of food. From the results, it was observed that Reproduction factor (Rf) was high at lower inoculums levels, but decreased with an increase in the amount of inoculum: being lowest at the highest inoculum level. The reproduction factor decreased with the increase in inoculums level due to competition for nutrients and space. The likelihood of proper development, maturation and competition of life cycle is higher at lower inoculum level due to abundant food supply and available space. At higher inoculum level, proper development and maturation of the nematode was severely affected due to limited amount of nutrients and confined space. This contribution has been reported by Chitwood (1951); Pathak *et al*. (2000); Khan *et al*. (2004); and Robab (2012).

CONCLUSIONS

From the results of the experiment, it may be concluded that root-knot nematodes reproduced on roots of *P. corylifolia* and brought about morphological, physiological, and biochemical changes (chlorophyll a, chlorophyll b, carotenoid content, total phenol content, nitrate reductase and carbonic anhydrase activity) in infected plants, which caused stunting or retardation in the growth of infected plant. The damage caused due to severe infection might lead to death of the plant.

ACKNOWLEDGEMENTS

Authors are thankful to the chairman of Department of Botany, Aligarh Muslim University, Aligarh for providing laboratory and necessary facilities.

REFERENCES

1. Anonymous, 1989. *Wealth of India, Raw Material*. Vol. III, Council of Scientific and Industrial Research, New Delhi, PP. 295-298.
2. Ardakani, A. S., Mafi, Z. T., Hesar, A. M. and Goltappeh, E. M. 2014. Relationship between Soil Properties and Abundance of *Tylenchulus semipenetrans* in Citrus Orchards, Kohgilouyeh va Boyerahmad Province. *J. Agr. Sci. Tech.*, **16:** 1699-1710
3. Azam, T. 2008. Histopathological Study of the Roots of Tomato Infected with Meloidogyne incognita. Abstract 31st All India Botanical Conference and International Symposium on Plant Biology and Environment Changing Scenario, University of Allahabad, 67 PP.
4. Azam, T., Hisamuddin, and Robab, M. I. 2010. Effect of Initial Inoculation of *Meloidogyne javanica* on Growth and Yield of *Lagenaria sicenaria. J. Amer. Sci.*, **6:** 617-622.
5. Bapat, K., Chintalwar, G. J., Pandey, U., Thakur, V. S., Sarma, H. D. and Samuel, G. 2005. Preparation and *In Vitro* Evaluation of Radioiodinated Bakuchiol as an Antitumour Agent. *Appl. Radiat. Isot.*, **62:** 389 393.
6. Baskaran, P. and Jayabalan, N. 2007. Rapid Micropropagation of *Psoralea corylifolia* L.Using Nodal Explants Cultured in Organic Additive-Supplemented Medium. *J. Hortic. Sci. Biotechnol*. **82**: 908-913.
7. Bhargava, S., Sharma, M. K. and Dhasora, P. K. 2007. Histopathological and Biochemical Changes Induced by Root-Knot Nematode, *Meloidogyne incognita* of Resistance and Susceptible Cultivars of Cowpea. *J. Mycol. Pl. Pathol*., **37:** 112-116.
8. Bray, H. G. and Thorpe, W. V. 1954. Analysis of Phenolic Compounds of Interest in Metabolism. *Meth. Biochem. Analy.*, **52:** 1-27.
9. Chitwood, M. D. 1951. Notes on the Physiology of *Meloidogyne javanica. J. Parasitol*., **37:** 96-98.
10. Danish, M., Robab, M. I. and Hisamuddin, 2016. Influence of Differential Inoculum Levels of *Meloidogyne incognita* on Morphology and Photosynthetic Pigments of

[Downloaded from jast.modares.ac.ir on 2024-10-12]

[DOR: 20.1001.1.16807073.2018.20.3.13.9]

Dianthus caryophyllus. *Acad. J. Sci. Res*., **4:** 016-021.

11. Dwivedi, R. S. and Randhawa, N. S. 1974. Evaluation of Rapid Test for the Hidden Hunger of Zinc in Plants. *J. Plant Soil Sci*., **40:** 445-45.
12. El-Sherif, M. A., Hafiz, S. L. and Otiefa B. A. 1973. Effect of *Rotylenchulus reniformis* Infection on Phenolic Contents of Cotton, *Gossypium barbadense*. *Nemtaol. Medit.,* **1:** 111-114.
13. Ferris, H. 1985. Density-Dependent Nematode seasonal Multiplication Rates and Overwinter Survivorship: A Critical Point Model. *J. Nematol.,* **17:** 93-100
14. Haraguchi, H., Inonui, J., Tamura, Y. and Mizutani, K. 2002. Antioxidative Components of *Psoralea corylifolia* L. (Leguminosae). *Phtytother. Res.,* **16:** 535–544
15. Hartman, K. M. and Sasser, J. N. 1985. Identification of *Meloidogyne* Species on the Basis of Differential Host Test and Perineal Pattern Morphology. 2. In: "*Advanced Treatise on Meloidogyne".* North Carolina State University Graphics, North Carolina, PP. 69-77.
16. Haseeb, A. 1994. Plant Parasitic Nematodes of Medicinal and Aromatic Plants. In: "*Vistas in Seed Biology"*, (Eds.): Singh, T. and Trivedi, P. C. Printwell Publisher, Allahabad, India.
17. Hisamuddin., Parveen, R. and Niyaz, T. 2005. Studies on the Interactive Effect of *Meloidogyne incognita* and *Pythium aphanidermatum* on *Phaseolus mungo*. *Ind. J. App. Pure Biol.,* **20:**1-4.
18. Holbrook, C. C., Knauft, D. A. and Dickson, D. W. 1983. A Technique for Screening Peanut for Resistance to *Meloidogyne arenaria*. *Plant Dis.,* **57:** 957- 985.
19. James, J. A. 2004. Efficacy of the Seed Extract of *Nerium, Thevetia neriifolia* on the Root-Knot Nematode, *Meloidogyne incognita* Affecting the Green Gram, *Phaseolus aureus* (Roxb). M. Phil. Dissertation, Ayya Nadar Janaki Ammal College (Autonomous) Sivakasi.
20. James, W. C. 1991. Estimated Losses of Crops from Plant Pathogens. In: "*Hand book of Pest Management in Agriculture*", (Ed.): Pimentel, J. CRC Press, Boca Raton, FL, PP. 15-51
21. Jaworski, E. G. 1971. Nitrate Reductase Assay in Intact Plant Tissues. *Biochem. Biophys. Comm*., **43:** 1247-1279.
22. Kapoor, L. D. 2001. *Handbook of Ayurvedic Medicinal Plants*. CRC Press, Boca Raton, FL, USA, PP. 274–275.
23. Karssen, G. and Moens, M. 2006. Root-Knot Nematodes. In: "*Plant Nematology"*, (Eds.): Perry, R. N. and Moens, M. CABI Publishing, Wallingford, UK, PP. 59–90.
24. Khan, M. R. 2008. Plant Nematodes. In: *"Methodology, Morphology, Systematics, Biology and Ecology"*. New Hampshire, Science Publishers, USA.
25. Khan, T. A., Nasir, S. and Ashraf, M. S. 2004. Effect of Population Levels of *Meloidogyne javanica* on Plant Growth and Nematode Multiplication on Cucurbits. *Pak. J. Nemato.,l* **22:** 88-89.
26. Khan, Z., Kumar, A., Mahamood, M., Gawade, B. and Gautam, N. K. 2014. The Root-Knot Nematode, *Meloidogyne incognita*, on *Psoralea corylifolia* in India. *Nematro.,* **44:** 81-84.
27. Khatune, N. A., Islam, M. E., Haque, M. E., Khoudhkar, P. and Rahman, M. M. 2004. Antibacterial Compounds from Seeds of *Psoralea corylifolia* L. *Fitoterapia.,* **75:** 228–300
28. Kheir, A. M., Amin, A.W., Hendy, H. H. and Mostafa, M. S. 2004. Interrelationship between banana cultivars and *Meloidogyne incognita* under stress of different inoculums levels. *Pak. J. Nematol*. **22:** 91-102.
29. Khushboo, P. S., V. M. Jadhav, V. J. Kandam, and N. S. Sathe. 2010. *Psoralea orylifolia* Linn. "Kushtanashini". *Pharmacognosy Rev.,* **4:** 69–76.
30. Krishnamurthi, A. 1969. *The Wealth of India*. Vol. III, Ph-Re, National Institute of Science Communication and Information Research, Council of Scientific and Industrial Research, New Delhi, 394 PP.
31. MacKinney, G. 1941. Absorption of Light by Chlorophyll Solutions. *J. Biol. Chem*., **140:** 315–322.
32. Niyaz, T. and Hisamuddin. 2008. Cellular responses in roots of Eclipta alba by Meloidogyne incognita. Abstract, All India Botanical Conference and International Symposium on Plant Biology and Environment Changing Scenario, University of Allahabad, 42 PP.
33. Noling, J. W. 2005. *Nematode Management in Tomatoes, Pepper and Egg Plants*.

[Downloaded from jast.modares.ac.ir on 2024-10-12]

[DOR: 20.1001.1.16807073.2018.20.3.13.9]

Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, PP. 1-16

34. Oka, Y., Koltai, H., Bar-Eyal, M., Mor, M., Sharon, E., Chet, I. and Spiegel, Y. 2000. New Strategies for the Control of Plant-Parasitic Nematodes. *Pest Manage. Sci.,* **56:** 983–988
35. Parveen, R. 2006. Studies on *Ocimum sanctum* (L) Infected with Root-Knot Nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood. PhD. Thesis, Aligarh Muslim University, Aligarh.
36. Pathak, K. N., Keshari, N. and Haider, M. G. 2000. Effect of Population Levels of *Meloidogyne incognita* on Seed Germination, Seedling Emergence and Plant Growth of Cauliflower. *Ind. J. Nematol.,* **20:** 8-12
37. Pavaraj, M. 2007. Efficacy of the Leaf Extract of *Ageratum conyzoides*, the Root-Knot Nematode, *Meloidogyne incognita* Affecting the Black Gram, *Vigna mungo*. M. Phil. Dissertation, Ayya Nadar Janaki Ammal College, Tamilnadu.
38. Rastogi, R. and Mehrotra, B. N. 1990. *Compendium of Indian Medicinal Plants*. Vol. 1, Central Drug Research Institute, Lucknow and National Institute of Science Communication, New Delhi, India.
39. Robab, M. I. 2012. Studies on *Solanum nigrum* Infected with Root-Knot Nematode (*Meloidogyne incognita*). PhD. Thesis, Aligarh Muslim University, Aligarh.
40. Robab, M. I., Hisamuddin, and Azam, T. 2010. Histopathology of Roots of *Glycine max* (L.) Merrill Induced by Root-Knot Nematode (*Meloidogyne incognita*), *Arch. Phytopathol. Plant Protect*., **43(18):** 1758-1767. DOI: 10.1080/03235400802678360
41. Robab, M. I., Hisamuddin, and Azam, T. 2009. Effect of Flyash Amended Soil on the Plant Growth, Yield, and Chlorophyll Contents of *Solanum nigrum* L. *Proceeding of International Conference of Emerging Technologies in Environmental Science and Engineering*. October 26-28, AMU, Aligarh, PP. 1559-1570.
42. Shukla, P. K. and Haseeb, A. 1998. Relationship between Different Inoculums Density of Plant Parasitic Nematodes and Growth/Oil Yield *Mentha citrate. Proceedings of the Third International Symposium of Afro-Asian Society of Nematologists (TISAASN)*, Coimbatore, PP. 57-62.
43. Southey, J. F. 1986. *Laboratory Methods for work with Plant and Soil Nematodes*. Ministry of Agriculture Fisheries and Food, Her Majesty's Stationery Office, London, UK.
44. Sultan, M. S., Sharma, S. K. and Dhillon, N. K. 2010. Identification of Nematode Problems in Medicinal, Aromatic, and Spice Plants in Punjab, India. *Tren. Biosci.,* **3:** 56-57.
45. Taylor, A. L. and Sasser, J. N. 1978. Identification and Control of Root-Knot Nematodes (Meloidogyne Species). In: "*Biology*". A Cooperative Publication of the Department of Plant Pathology, North Carolina State University, and the United State Agency for International Development. North Carolina State University Graphics, Raleigh.
46. Wallace, H. R. 1987. Effect of Nematode Parasites on Photosynthesis. In: "*Vistas on Nematology"* (Eds.): Veech, J. A. and Dickson, W. D. Society of Nematologists, Hyattsville, MD, USA, PP. 253–259.
47. Whitehead, A. G. 1998. *Plant Nematode Control*. CAB International, Wallingford, UK, 384 PP.
48. Yasmeen, N. 2002. Histopathological Studies on *Lagenaria leucantha* Infected with *Meloidogyne incognita* and *Pythium aphanidermatum* in Fly Ash Amended Soil. PhD. Thesis, Aligarh Muslim University, Aligarh, 215 PP.

[Downloaded from jast.modares.ac.ir on 2024-10-12]

[DOR: 20.1001.1.16807073.2018.20.3.13.9]

مطالعه ویژگی های مورفولوژیکی و بیوشیمیایی گیاه Babchi (*Psoralea corylifolia*) آلوده به نماتد ریشه گرهی *Meloidogyne incognita*

م. دانیش، ح. سیخ، و م. ا. رباب

چکیده

به منظور تعیین اثرات Meloidogyne incognita روی Psoralea corylifolia با غلظت های مختلف مایه تلقیح شامل مقادیر100، 500، 1000، و 2000 J2 در هر گیاه، پژوهشی با استفاده از طرح بلوک های کامل تصادفی در گلدان هایی انجام شد که حاوی 2/5 کیلو گرم خاک استرلیزه شده بود. نتایج حاکی از کاهش معنادار همه پارامترهای رشد و عملکرد گیاهان مایه زنی شده در مقایسه با گیاهان شاهد تلقیح نشده بود. با افزایش مقدار مایه تلقیح، پارامترهای بیوشیمیایی مانند رنگدانه های فتو سنتزی شامل کلروفیل a و bمحتوای کاروتینوئید و فعالیت آنزیمهایی از قبیل رداکتاز نیترات و carbonic anhydrase به طور معناداری کاهش یافت. بیشترین کاهش که معنادار ($P\leq0.05$) هم بود در تیمار 2000 J2 و در مقایسه با گیاهان سالم مشاهده شد. در سطوح بالای مایه تلقیح، تعداد نماتدها در ریشه های آلوده بیشتر، و اندازه گال ها بزرگتر بود. جمعیت second stage-juveniles در ریشه و خاک چنین اشارت داشت که M. incognita روی ریشه P. corylifolia به طور موفقیت آمیزی تولید مثل کرده بود. صدمات وارده به گیاه شدیدا آلوده ممکن است منجر به نابودی گیاه شود.

[Downloaded from jast.modares.ac.ir on 2024-10-12]

[DOR: 20.1001.1.16807073.2018.20.3.13.9]