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## Effect of Different Inoculum Levels of *Meloidogyne incognita* on Growth and Biochemical Parameters of *Vigna radiata*

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### ABSTRACT

The experiment was carried out to study the effect of *Meloidogyne incognita* on growth and biochemical parameters of a leguminous plant viz., *Vigna radiata* cv. PDM 139 under green house conditions by inoculating with different inoculum levels i.e., 0 (control), 200, 400, 800 and 1,600 second stage juveniles per 1.5 kg of soil/pot. With the increase in inoculum level of *M. incognita*, there was a progressive decrease in growth and biochemical parameters of the crop. Significant ( $p \leq 0.05$ ) reduction in plant length, fresh and dry weight, leaf area, chlorophyll, seed protein, nitrogenase and leghaemoglobin contents in the root nodules at 400 J<sub>2</sub>, while at higher inoculum levels i.e., 800 J<sub>2</sub> and 1,600 J<sub>2</sub>, the reduction was more pronounced and significant at  $p \leq 0.01$ , level.

**Key words:** *Vigna radiata*, inoculum level, *Meloidogyne incognita*

### INTRODUCTION

Plant parasitic nematode, *Meloidogyne incognita* alters the metabolic processes of the host which are manifested in the form of cellular, physiological and biochemical changes occurring in the infected host. The root-knot nematodes cause measurable changes in the morphology and physiology of the host (Williamson and Gleason, 2003). As a result of *Meloidogyne* infection, amounts of chlorophyll pigment, protein and oil contents are changed. Reduction in chlorophyll content of the infected plants has been reported by Vashishth *et al.* (1994), Poornima and Vadivelu (1998), Ramakrishnan and Rajendran (1998) and Parveen *et al.* (2006). In tomato and bean leaves, root-knot infection reduced the photosynthetic rates (Loveys and Bird, 1973). Mohanty and Pradhan (1989) reported that protein contents decreased and amount of free amino acids and amides increased after inoculating susceptible as well as resistant cultivars.

For the effective nematode management and advisory services information about establishment of nematode population and start of infection with respect to a particular host plant is prerequisite. Hence, the present study was undertaken to quantify the effect of different initial population densities of *Meloidogyne incognita* on plant growth and biochemical parameters under greenhouse conditions.

### MATERIALS AND METHODS

For performing the experiments, the pots of ten inch diameter were filled with steam sterilized soil at the rate of 1.5 kg of soil per pot. The seeds of *Vigna radiata* L. var. PDM 139 procured from

Indian Institute of Pulse Research, Kanpur were axenized by NaOCl method (Koenning and Barker, 1985) allowed to germinate on moist filter study and were sown in clay pots. Initially there were five seedlings per pot which were thinned to one plant per pot, when the seedlings reached three leaf stage.

*Meloidogyne incognita* was selected as test pathogen to perform experiments. Pure culture of *M. incognita* was maintained on egg plant (*Solanum melongena* L.) roots in glass house by using single egg mass. The egg masses from galled roots of egg plant were picked with the help of sterilized forceps and were allowed to hatch out  $28\pm 2^\circ\text{C}$  under aseptic conditions in the sieves, lined with tissue study and kept in petridishes containing sufficient amount of sterilized distilled water.

Three-leaf stage seedlings were inoculated with freshly hatched juveniles at the rate of 0, 200  $J_2$ , 400  $J_2$ , 800  $J_2$ , 1,600  $J_2$  per 10 mL of water by pipetting into the soil through the holes around the plant within the radius of two centimeters and plugged with the sterilized soil soon after inoculation. To maintain soil moisture in the pot, regular watering was done. Each treatment was replicated five times and the pots were arranged in complete randomized block design. Un-inoculated set of plants served as control. There were five sets of pots as given below:

- C: Control
- T1: 200  $J_2$  pot<sup>-1</sup>
- T2: 400  $J_2$  pot<sup>-1</sup>
- T3: 800  $J_2$  pot<sup>-1</sup>
- T4: 1,600  $J_2$  pot<sup>-1</sup>

After 60 days of inoculation, the experiment was terminated and following parameters were taken into account for describing the results. The data were recorded on plant length, fresh plant weight, dry weight of shoot and root, leaf area were estimated as shown in Table 1. Total chlorophyll milligram per gram leaf and seed protein content milligram per gram were also estimated. Nitrogenase activity was estimated per gm dry weight nodule and leghaemoglobin milligram per gram fresh nodules. Amount of chlorophyll was estimated by the method of MacKinney (1941). The protein content in the seeds was estimated by Folin-phenol method (Lowry *et al.*, 1951). The nitrogenase activity was assayed by the procedure of Sadasivam and Manikam (1992). The leghaemoglobin content in fresh nodules was estimated by the method described by Sadasivam and Mannickam (2008). The data obtained were analyzed statistically and significance was calculated at ( $p\leq 0.05$ ) and ( $p\leq 0.01$ ) levels of probability.

Table 1: Effect of different inoculum levels of *Meloidogyne incognita* on growth, chlorophyll pigments, seed protein content, nitrogenase activity, nodule leghaemoglobin content of *Vigna radiata*

Treatments	Plant length (cm)	Fresh weight (g)	Dry weight (g)		Leaf area (cm <sup>2</sup> )	Chlorophyll (a+b) mg g <sup>-1</sup> leaf	Seed protein (mg g <sup>-1</sup> )	Nitrogenase activity	
			Shoot	Root				( $\mu\text{m C}_2\text{H}_4 \text{ h}^{-1} \text{ g}^{-1}$ dry weight nodule)	Leghaemoglobin mg g <sup>-1</sup> fresh nodules
C (Control)	43.15	10.63	1.93	1.00	68.75	1.816	29.31	129.12	4.72
T1 (200 $J_2$ )	40.15	9.12	1.83	0.92	64.53	1.642	27.35	122.30	4.13
T2 (400 $J_2$ )	37.76	8.00	1.25	0.85	62.34	1.578	25.78	118.82	3.84
T3 (800 $J_2$ )	29.33	5.92	1.00	0.41	41.00	0.904	22.33	61.67	2.34
T4 (1600 $J_2$ )	26.73	4.87	0.96	0.32	31.73	0.885	20.45	58.34	1.00
LSD $p\leq 0.05$	3.92	2.44	0.66	0.12	4.84	0.178	2.88	7.53	0.66
LSD $p\leq 0.01$	5.43	3.35	0.90	0.16	6.51	0.240	3.94	10.41	0.92

Each value is a mean of five replicates,  $J_2$  = Second stage juveniles of *Meloidogyne incognita*

## RESULTS

From Table 1, it is evident that the plant length of *Vigna radiata* decreased non-significantly at lower initial inoculum level of  $Pi = 200 J_2$  in T1 plants, when compared with un-inoculated control (C). A significant ( $p \leq 0.05$ ) decrease in the plant length compared with control, occurred in T2 plants. In T3 and T4 plants, at higher inoculum levels of  $Pi = 800 J_2$ ,  $Pi = 1,600 J_2$ , plant length was decreased by 32.02 and 38.05% greatly and significantly ( $p \leq 0.01$ ) over the control. In comparison to control (C), fresh weight of the whole plants and dry weight of shoot and root separately decreased with an increase in initial inoculum level. No significant reduction was observed at lower initial inoculum level of  $Pi = 200 J_2$  while a significant ( $p \leq 0.05$ ) at the next higher inoculum level of  $Pi = 400 J_2$  (T2 plants). Significant ( $p \leq 0.01$ ) reductions in T3 and T4 plants were observed when compared with control (C).

The leaf area of the plant in comparison to control, decreased non-significantly at the lowest initial inoculum level of  $Pi = 200 J_2$  and significantly ( $p \leq 0.05$ ) at the next higher inoculum level of  $Pi = 400 J_2$  (T1 and T2 plants, respectively). Reductions in leaf area were higher and significant ( $p \leq 0.01$ ) at an initial inoculum levels of  $Pi = 800 J_2$  and  $Pi = 1,600 J_2$ , over the control (C). Highest (53.84%) reduction in leaf area was noticed in T4 plants. In comparison to control (C), the amount of chlorophyll in the leaves of *V. radiata* decreased slightly but non-significantly at an initial inoculum level of  $Pi = 200 J_2$  in T1 plants. There was a significant ( $p \leq 0.05$ ) decrease in the amount of chlorophyll pigments at the next higher inoculum level of  $Pi = 400 J_2$  in T2 plants. The chlorophyll (a+b) content decreased significantly ( $p \leq 0.01$ ) at higher inoculum levels i.e.,  $Pi = 800 J_2$  and  $Pi = 1,600 J_2$ , when compared with control. Maximum (51.26%) reduction in the amount of chlorophyll was observed in T4 plants.

The seed protein content of *V. radiata* exhibited reduction at all the initial inoculum levels when compared with the protein content of un-inoculated control. The reduction was non-significant in T1 plants, grown at the initial inoculum level of  $Pi = 200 J_2$  per pot. In comparison to control, there was a significant ( $p \leq 0.05$ ) decrease in T2 plants, grown at the initial inoculum level of  $Pi = 400 J_2$ . A significant ( $p \leq 0.01$ ) reduction in protein content of seeds in T3 and T4 plants was observed. Highest (30.22%) decrease was observed in T4 plants inoculated with 1,600  $J_2$  of *M. incognita*. Nitrogenase activity in nodules progressively decreased with increase in the initial inoculum levels of nematodes. Although, non-significant decrease was resulted in T1 plants at the initial inoculum level of  $Pi = 200 J_2$ , when compared with control (C). Significant decrease ( $p \leq 0.05$ ) occurred at  $Pi = 400 J_2$  in T2 plants. Significant ( $p \leq 0.01$ ) reductions in nitrogenase activity were noticed in T3 and T4 plants grown at  $Pi = 800 J_2$  and  $Pi = 1,600 J_2$ , respectively. Maximum reduction (54.81%) was observed in T4 plants. In comparison to control, the leghaemoglobin content in fresh nodules decreased at all the initial inoculum levels (T1 to T4 plants) but there was non-significant decrease in T1 plants at  $Pi = 200 J_2$  while significant ( $p \leq 0.05$ ) decrease in T2 plants at  $Pi = 400 J_2$  followed by T3 and T4 plants which showed significant ( $p \leq 0.01$ ) reduction by 50.42 and 78.81% at  $Pi = 800 J_2$  and  $Pi = 1,600 J_2$ , respectively.

## DISCUSSION

The response of *Vigna radiata* regarding the biochemical attributes was significant to various nematode treatments. The growth of the plant responded negatively towards the lower as well as higher inoculum levels of *M. incognita*. Decrease in all these parameters including plant growth, leaf area, chlorophyll, seed protein, nitrogenase activity and leghaemoglobin due to increased in number of the nematode. The effects were pronounced and significant at the higher inoculum levels

(Pi = 400 J<sub>2</sub>, Pi = 800 and Pi = 1,600) as shown in Table 1. The root knot nematode infection often reduces plant growth and yield (Sasser and Freckman, 1987; Williamson, 1998) and decreases nutrient uptake (Patel *et al.*, 1988) and infested plants show deficiencies of N, Mg, Fe, B, Cu and Zn (Good, 1968) due to root damage by RKN and subsequent prevention of water and nutrient uptake by the roots (Gaillaud *et al.*, 2008). All anatomical malformations and physiological malfunctions contributed in suppressing the plant growth and yield (Hussey, 1985).

Various forms of abiotic and biotic stresses damage plant leaf tissue and the chloroplasts (Karpinski *et al.*, 2003). The plant response to nematode parasitism thus causes morphological and physiological changes that affect photosynthetic processes (Melakeberhan *et al.*, 1986; Hussey and Williamson, 1997). Infection caused by *M. incognita* also leads to reduction in leaf area along with the reduction in height and weights as has been found by Ramakrishnan and Rajendran (1998) and Janathan and Rajendran (2002). A reduction in total chlorophyll has also been reported in French bean and rice infected with *M. javanica* (Melakeberhan *et al.*, 1986; Swain and Parasad, 1988).

Invading nematodes are considered to disturb the functioning of nodules by altering host nutrition (Doney *et al.*, 1970) and by reducing the leghaemoglobin (Sharma and Sethi, 1975; Chahal and Singh, 1984) and bacteroid (Barker *et al.*, 1972; Ali *et al.*, 1981) content of nodules. Nematodes may reduce the effective life of rhizobia nodules as found by Taha and Raski (1969) with legumes infested with *M. javanica*. As leghaemoglobin regulates the supply of oxygen and bacteroids contain nitrogenase enzyme required for the reduction of atmospheric nitrogen to ammonia then a decrease in these due to nematode infection would lead to decrease in fixation of nitrogen.

The protein content underwent a significant reduction in the seeds of mung bean after inoculation with the nematode. These findings were in agreement with Korayem *et al.* (2013) who found that crude protein and fat contents decreased in the pea nut seeds influenced by *M. arenaria* (Vaitheeswaran *et al.*, 2005). The susceptible green gram plant showed low protein content and high level of amino acids after inoculation of nematodes (Mohanty and Pradhan, 1989).

From the results of the experiment, it might be inferred that *Meloidogyne incognita* affects not only the growth of plants but biochemical parameters as well. Reduction was observed in all the parameters at all the levels in initial population density. But substantial reduction was started at 800 Pi followed by highest reduction at 1,600 Pi.

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