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Pathogenicity of Root- Knot Nematodes *Meloidogyne incognita* on Pungam, *Pongamia pinnata* tree seedlings

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Abstract: A pot culture experiment was conducted in a glass house to assess the pathogenicity of root-knot nematode, *Meloidogyne incognita* on *Pongamia pinnata.* Poly bags (15 cm x 10 cm) were filled up with steam sterilized fine river sand mixed with red earth at the ratio of 3:1. The freshly collected, surface sterilized seeds were sown. Fifteen days after germination of seedlings, second stage larvae of *M. incognita* were inoculated in the root zone of *P. pinnata* at the rate of 0,10, 100, 1000 and 10,000 larvae pot^{-1}. Each treatment was replicated four times and arranged in a RBD in glass house at a room temperature of 27°C.The growth parameters such as root length, root weight, shoot length, shoot weight, number of galls, rhizobial nodules, number of egg masses on root, number of larvae, adult females and final soil populations were recorded at 120 DAI. The chlorophyll content and physiological parameters were also recorded at 120 DAI. The results revealed progressive reduction in length and weight of shoot and root significantly with the increase in the level of initial inoculum. The percentage of reduction in shoot length, shoot weight, root length and root weight were 38.6, 41.4, 18.3 and 34.7 per cent, respectively to the initial inoculum of 10,000 larvae when compared to uninoculated control. The percentage of reduction in plant weight was noticed due to the inoculum of *M. incognita* over uninoculated control. It was found that at an initial inoculum level of 10,000 larvae kg^{-1} soil, the collar diameter got decreased by 26.3 per cent over control. Significant reduction of rhizobial nodule was recorded for every level of larvae inoculated on *P. pinnata.* The respective per cent reduction in nodule and its diameter registered was 45.9 and 22.2, 56.4 and 26.7 and 59.2 and 40.0 to 100, 1000 and 10,000 larvae kg^{-1} soil over uninoculated control. It was found that there was significant reduction in chlorophyll 'a' chlorophyll 'b' and total chlorophyll in the inoculated pots. The data recorded on stomatal conductance, transpiration rate and net photosynthetic rate to the three levels of larvae inoculated showed significant decrease as compared to the uninoculated control.

Keywords : Pungam, root - knot nematode, pathogenicity

Introduction

Pungam *(Pongamia pinnata*) is a fast-growing, evergreen, potential oil yielding tree cultivated as an ornamental in gardens, along avenues and roadsides and as a host plant for lac insects. Pungam oil is used as a liniment for rheumatism and also used for biodiesel production. The plant parasitic nematodes are cosmopolitan in nature and cause heavy losses in the production of nursery seedlings of subtropical and tropical trees, particularly by *Meloidogyne sp* (Johnson and Fassuliotis, 1984).

Table 1. Effect of different levels of inoculum of *Meloidogyne incognita* on different plant growth parameters and rhizobial nodules of *Pongamia pinnata* at 120 DAI.

No.of Larvae/ pot	Shoot		Root		Plant weight (g)	Collar diameter (cm)	No.of branches/ plant	No.of leaves/ plant	No.of nodules/ plant	Nodule diameter (mm)
	Length (cm)	Weight (g)	Length (cm)	Weight (g)						
0	42.73	19.00	43.63	19.13	38.10	0.95	9.25	29.25	54.50	0.45
10	38.38	18.75	41.88	18.88	37.50	0.87	8.50	28.25	49.50	0.45
	(10.18)	(1.32)	(4.01)	(1.31)	(1.57)	(8.42)	(8.11)	(3.42)	(9.17)	(0.00)
100	35.88	16.50	39.25	17.63	34.10	0.84	7.75	27.00	29.50	0.35
	(16.03)	(13.16)	(10.03)	(7.84)	(10.50)	(11.58)	(16.22)	(7.69)	(45.87)	(22.22)
1000	28.50	14.75	37.25	15.00	32.70	0.78	5.75	23.50	23.75	0.33
	(33.30)	(22.37)	(14.62)	(21.59)	(14.17)	(17.89)	(37.84)	(19.66)	(56.42)	(26.67)
10000	26.25	11.13	35.63	12.50	23.60	0.70	4.25	21.75	22.25	0.27
	(38.57)	(41.42)	(18.34)	(34.66)	(38.05)	(26.31)	(54.05)	(25.64)	(59.17)	(40.00)
CD (0.05)	3.03	1.52	1.63	1.17	2.69	0.04	1.53	3.41	5.48	0.04

(Figures in parentheses are per cent increase / decrease values)

Diseases caused by nematodes have just begun to recognise as a significant forestry problem. Nematode damage, being density dependant one, becomes visible only when nematode population exceeds critical threshold of economic damage. Forest trees in nurseries are prone to severe nematode damage since nurseries with practically monoculture of species, optimum moisture and nutrient conditions provide ideal conditions for nematode build up. Forest plantations, usually planted on poor soil or abandoned farmlands, may be affected by nematode disease easily. The most classical example of nematode species causing tremendous losses in forestry is *Bursaphelenchus xylophilus* which causes pine wilt disease in Japan, Canada, U.S.A, China, Norway, Sweden, and Finland. The annual loss due to the nematode to pines during 1981 amounted to two million m^3 with the death of ten million trees in Japan (Mamiya, 1983). Information on disease and nematode population dynamics in natural forest stands in Tamil Nadu is quite scanty. Therefore, realizing the importance of nematodes in forestry, a study was carried out to assess the pathogenicity of *Meloidogyne incognita* (Kofoid & White) Chitwood on *Pongamia pinnata* (L.) Pierre.

Table 2. Number of galls, number of larvae, number of adult female, root and soil population of *Pongamia pinnata* at 120 DAI.

No.of Larvae/pot	No. of galls/ plant	Population of *Meloidogyne incognita*				Total population	P_f/P_i
		No. of larvae/ 2g of root	No.of adult female/ 2g of root	Per 2g root	Per 200 g soil		
0	1.00 (0.0000)	1.00 (0.0000)	1.00 (0.0000)	1.00 (0.0000)	1.00 (0.0000)	0.00	0.00
10	5.25 (0.7201)	2.00 (0.3100)	2.50 (0.3979)	3.50 (0.5440)	22.00 (1.3424)	152.20	15.20
100	23.25 (1.3664)	3.00 (0.6989)	9.75 (0.9890)	13.75 (1.1383)	176.00 (2.2455)	1099.00	10.90
1000	52.00 (1.7160)	32.00 (1.5051)	64.25 (1.8078)	95.25 (1.9788)	330.00 (2.5185)	3058.00	3.05
10000	88.75 (1.9481)	48.50 (1.6857)	103.00 (2.0128)	150.50 (2.1775)	484.00 (2.6848)	3709.00	0.40
CD (0.05)	0.2460	0.3874	0.7214	0.8324	0.9348		

Figures in parenthesis are log transformed values.

Table 3. Chlorophyll content and physiological parameters of *Pongamia pinnata* at 120 DAI.

No.of Larvae/ pot	Chlorophyll 'a' (mg/g)	Chlorophyll 'b' (mg/g)	Total chlorophyll (mg/g)	Stomatal conductance (m mol m^{-2} s^{-1})	Transpiration rate (m mol m^{-2} s^{-1})	Net photo synthesis (μ mol m^{-2} s^{-1})
0	1.39	1.13	2.52	113.08	23.07	7.62
10	1.39 (0.00)	1.02 (9.73)	2.41 (4.37)	111.93 (1.02)	22.63 (1.92)	7.64 (0.26)
100	1.32 (5.04)	0.90 (20.35)	2.22 (11.90)	103.06 (8.86)	19.81 (14.13)	61.3 (19.55)
1000	1.26 (9.35)	0.79 (30.08)	2.05 (18.65)	95.98 (15.12)	17.38 (24.67)	6.00 (21.26)
10000	1.15 (17.27)	0.73 (35.39)	1.88 (25.40)	89.99 (20.42)	14.13 (38.75)	5.85 (23.22)
CD (0.05)	0.06	0.07	0.11	4.95	0.83	0.25

(Figures in parentheses are per cent increase / decrease values)

Materials and Methods

A pot culture experiment was conducted in a glass house to assess the pathogenicity of root-knot nematode, *Meloidogyne incognita* on *Pongamia pinnata* for a period of five months.

The polythene bags of size 15 cm x 10 cm were filled up with steam sterilized fine river sand mixed with red earth at the ratio of 3:1. The freshly collected seeds of *P. pinnata* were surface sterilized with sodium hypochlorite at 0.1 per cent for five minutes. After surface sterilization, seeds were sown at the rate of three seeds per bag. After completion of germination, one plant was allowed to remain to grow and others thinned out. Fifteen days after germination of seedlings, second stage larvae of *M. incognita* were inoculated in the rhizosphere of *P. pinnata* @ 0, 10, 100, 1000 and 10,000 larvae pot^{-1}. Each treatment was replicated by four times and arranged in a randomized block design in glass house at a room temperature of 27°C.

The growth parameters of the seedlings such as seedling height, collar diameter, number of branches were recorded at 120 DAI. At 120^{th} day, the seedlings were carefully removed from the polythene bags by plunging the bag in a plastic bucket which was filled up with water. The observations on root length, root weight, shoot length, shoot weight, number of galls, rhizobial nodules, number of egg masses on root, number of larvae, adult females and final soil populations were recorded. The chlorophyll, 'a', 'b' and total chlorophyll content were estimated from mature fully unfurled third leaf from the growing point of the main branch adopting the method of Yoshida *et al.* (1971) and expressed as mg per gram of fresh weight. The physiological parameters such as stomatal conductance, transpiration rate and net photosynthetic rate were recorded at 120 DAI by CO_2 Gas Analyzer (CI-301 PS Photosynthesis System. CID. inc.).

Results and Discussion

The results revealed progressive reduction in length and weight of shoot and root significantly with the increase in the level of initial inoculum. The percentage of reduction in shoot length, shoot weight, root length and root weight were 18.6, 41.4, 18.1 and 14.7 per cent, respectively to the initial inoculum of 10,000 larvae when compared to uninoculalcd control. The percentage of reduction in plant weight due to the larvae of *M. incognita* at 1000 and 10,000 was 14.2 and 18.1 per cent, respectively over uninoculated control (Table 1).

It was found that at an initial inoculum level of 10,000 larvae kg^{-1} soil, the collar diameter got decreased by 26.1 per cent over control. Significant reduction of rhizobial nodule was recorded for every level of larvae inoculated on *P.pinnata*. The respective per cent reduction in nodule and its diameter registered was 45.9 and 2.2.2, 56.4 and 26.7 and 59.2 and 40.0 to 100, 1000 and 10,000 larvae kg^{-1} soil over uninoculated control (Table 1). Very minute galls were produced on *P.pinnata* roots by *M. incognitia*. The mean number of galls was observed to be 21.25, 52.00 and 88.75. respectively with 100, 1000 and 10,000 larvae inoculated. The multiplication rate of *M.incognita* on *P.pinnata* was 15.2, 10.9, 1.1 and 0.4 with 10, 100, 1000 and 10,000 larvae inoculated respectivey (Table 2).

Similarly, reduced seedling emergence or growth or survival was noticed in the inoculated plants of mulberry to *M. thamsi* (Toida, 1973); *Cryptomeria japonica* and *Pinus sylvestris* to *M. incognita* (Wang *et al.*, 1975); *Leucaena*

leucocephala to *M. incognita* (Azmi, 1985); Ixora to *M. javanica* (Davis, 1992); and *Sesbania sesban* to *M. javanica* (Desaeger and Rao, 2001). In the pathogenicity study on *Catalpa bignonioides* by *M. incognita,* the tolerance limit for fresh and dry top weight and leaf area was fixed as 0.78 eggs and juveniles (Sasanelli *et al.,* 1996). Significant reduction in seedling height, shoot and root biomass and the density of root tips per mg of root of *A. holosericea* and *A. seyal* were also reported when the population level of *M. javanica* exceeds 1000 larvae per seedling (Duponnois *et al.,* 1995). Also, Duponnois *et al.* (1997) in their studies observed decreased development of *Acacia holosericea* and *A. mangium* to *M. incognita* inoculation.

In the above pathogenicity trial, estimation of total chlorophyll, chlorophyll 'a' and chlorophyll 'b' were carried out in the leaves. It was found that there was significant reduction in chlorophyll 'a', chlorophyll 'b' and total chlorophyll in the inoculated pots. The per cent reduction in total chlorophyll to the initial inoculum of 100, 1000 and 10,000 larvae kg^{-1} soil was 11.9, 18.7 and 25.4 per cent, respectively over uninoculated control (Table 3).The data recorded on stomatal conductance, transpiration rate and net photosynthetic rate to the three levels of larvae inoculated showed significant decrease as compared to the uninoculated control. The percent reduction to 10,000 inoculum level was 20.4 in stomatal conductance, 38.8 in transpiration rate and 23.2 in net photosynthetic rate when compared to uninoculated plants (Table 3).

It was suggested that increased resistance to the diffusion of CO_2 in the leaf, inhibition of production of plant growth promoting hormones such as cytokinins and gibberellins, increased resistance by stomatal closure and subsequent water stress are responsible for the reduction in photosynthetic rate due to *Globodera rostochiensis* on potato and *M. javanica* on tomato (Bird, 1974; Fatemy *et al.,* 1985; Loveys and Bird, 1973; Wallace, 1974).

The higher loss of chlorophyll due to inoculum level of 2000 larvae kg^{-1} soil might be induced nutrient deficiency particularly nitrogen deficiency in the foliage (Trudgill *et al.,* 1975). Though roots of infected plants, contained larger quantities of N, P, K impaired translocation to foliage or mobilization of nutrients from shoot to root resulted in nutrient deficiency, which in turn affected the chlorophyll synthesis in infected plants (Hunter, 1958).

A higher reduction in stomatal conductance and the resultant reduction in net photosynthetic rate might have been caused by high diffusive resistance offered by the stomata of the infected leaves. This view was supported by Meon *et al.* (1978) who correlated the effect of infestation of *M. javanica* with restricted water flow as the result of disruption and development of abnormal vessel elements caused by the infestation. It was also concluded that infested plants respond to moisture stress by closing their stomata. Loveys and Bird (1973) also reported that high inoculum levels of *M. javanica* on tomato caused a decline in the net photosynthesis rate.

The other direct effect of stomatal closure is a greater reduction in transpiration rate. As observed in the present investigation, the population level of 10,000 larvae kg^{-1} soil on *P. pinnata* caused 38.8 per cent reduction in transpiration rate over control. Similar to these findings Odihirin (1971) reported a decreased transpiration and visible wilting in the nematode inoculated tobacco plants.

At an initial population of 10,000 *M. incognita* larvae kg soil^{-1}, the nematode was able to reduce root length, plant weight, total chlorophyll content, stomatal conductance, transpiration rate and net photosynthetic rate by 18.3, 38.1, 25.4, 20.4, 38.7 and 23.2 per cent in *P. pinnata.The* rhizobial nodule number and size on *P. pinnata* roots was found reduced by 59.2 and 40 per cent, respectively at an initial inoculum level of 10,000 *M. incognita* larvae pot^{-1}.The rate of multiplication of *M. incognita* on *P. pinnata* was found as 0.6 fold.

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