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Effect of different inoculums levels of root knot nematode *Meloidogyne incognita* infecting *Vigna radiata*

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ABSTRACT

Plant-parasitic nematodes are cosmopolitan in distribution and important threat to the number of agricultural crops, and are among the difficult crop pests to be controlled. The underground symptom of the disease caused by *Meloidogyne* spp. is the formation of galls on the root. To understand host-parasite relationship between *M. incognita* and the plant for the precised assessment involving disease severity and nematode reproduction, number and size of galls, number of egg masses per root system and number of mature females per root system of *Vigna radiata.*

Keywords: *Meloidogyne* spp, nematode reproduction, ***Vigna radiata***

MATERIALS AND METHODS

Preparation and Sterilization of Soil Mixture:

For performing the experiments, soil was prepared in the ratio of 7:3:1 comprising of clay, sand and farmyard manure, respectively. The pots of 25 cm diameter were filled with soil at the rate of 1.5 kg of soil per pot. A little water was poured into each pot just to wet the soil surface before sterilization at 20 lb pressure for 20 min. Sterilized pots were allowed to cool at room temperature before use for experiments.

Raising and Maintenance of Test Plant: 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). The seeds were placed on a moist sterilized filter paper kept in a sterilized petridish for germination. The sprouted seeds 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.

Preparation of Inoculum:

Meloidogyne incognita (Kofoid and White) Chitwood was selected as test pathogen. To perform

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experiments during the period of research, 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 at 28±2^{0}C under aseptic conditions in the sieves, lined with tissue paper and kept in petri-dishes containing sufficient amount of sterilized distilled water.

Inoculation with Nematode:

The second-stage juveniles were collected in distilled water and counted with the help of counting dish. The three-leaf stage seedlings were inoculated by making holes of 5-7 cm depth around the plant within the radius of two centimeters. The second stage juveniles at the rate of 200 J_2, 400 J_2, 800 J_2, 1, 600 J_2 per 10 ml of water were pipetted into the soil through the holes. The holes were then 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 treatments as given below:-

C: Control T1:
200 J_2/pot
T2: 400 J_2/pot

T3: 800 J_2/pot
T4:1,600 J_2/pot

The plants were watered regularly whenever required. After 60 days of inoculation the experiment was terminated and following parameters were taken into account for describing the results.

PARAMETERS

Number and Size of Galls:

Total number of galls produced on the root system of each plant was recorded separately. The size of gall was measured by taking maximum length and width (in mm^2) with the meter scale.

Number of Egg Masses:

The number of egg masses per root system on infected roots was counted after staining egg masses with Phloxin B. An aqueous solution of Phloxin B 0.15g per liter of water was prepared. The galled roots were placed in this solution for 15-20 minutes. The roots were gently rinsed in tap

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water. The egg masses were stained red and were counted directly.

Number of Mature Females:

For counting the number of females in the root, infected roots were carefully washed in tap water. One gram homogenous mass of the root from each replicated treatment was stained in 0.25% acid fuschin with lactic acid and was macerated in warring blender in enough water for 30 seconds. Counts were made separately in the suspensions thus obtained, under stereoscopic microscope.

Reproduction Factor (Rf) and Rate of Population Increase (RPI):

For final nematode population (Pf), soil population was estimated by Cobb's sieving and decanting method, and root population was estimated by Blender-Baerman tray method (Hooper, 1985). Reproduction factor (Rf) was calculated by the formula-

$$Rf = \frac{Pf}{Pi}$$

Where Pf is the final population and Pi is the initial population. Rate of population increase (RPI) was calculated by the formula:

$$RPI = \frac{(Pf-Pi)}{Pi}$$

Table : Effect of different inoculum levels of *Meloidogyne incognita* on root galling, gall size, number of egg masses and number of females in *Vigna radiata*

Treatments	No. of galls root system^{-1}	Size of gall (mm^2)	No. of egg masses root system^{-1}	No. of mature females g^{-1}root	Pf	Rf	RPI
C (Control)	-	-	-	-	-	-	-
T1 (200J$_2$)	75.83	6.98	80.92	42.34	1542.93	7.71	6.71
T2 (400J$_2$)	87.62	8.73	102.56	54.34	1734.34	4.49	3.33
T3 (800J$_2$)	122.24	12.56	201.68	90.95	2089.46	2.61	1.61

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T4 (1,600J_2)	139.36	14.23	215.25	100.21	2195.50	1.37	0.37
LSD P≤0.05	10.05	1.49	16.58	9.66	172.43		
LSD P≤0.01	13.89	2.06	22.92	13.36	238.38		

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

RESULTS

Number of Galls:

The number of galls per root system increased with the increase in initial inoculum level. The number of galls on the roots of T2 plants at 400 J_2 was significantly increased, when compared with the gall number at the lowest inoculum level (Pi = 200 J_2), of T1 plants. A significant (P≤0.01) increase in gall number on T3 plants (Pi = 800 J_2), in comparison to T1, was recorded. The number of galls increased greatly and significantly (P≤0.01) at the next and highest inoculum level of Pi = 1,600 J_2 in comparison to the lower inoculum levels .

The T3 and the T4 plants, exhibited significant (P≤0.01) enhancements, as compared to T2 plants. When comparison was made with the T3 plants, the T4 plants showed a significant (P≤0.01) increase in the number of galls on the infected root

Size of Galls:

The size of the galls was found to be increased at higher inoculum levels when compared with the galls at the lower inoculum levels. A significant (P≤0.05) increase in gall size was noticed on T2 plants at Pi = 400 J_2, when compared with lowest inoculum level (T1 plants). A significant (P≤0.01) increase in size of the gall was noticed at Pi = 800 J_2, when comparison was made with the galls of T1 plants. The galls attained maximum size at the highest inoculum level of Pi = 1,600 J_2 which were significantly (P≤0.01) larger, when compared with the size of the galls on T1 plants. The gall size was significantly (P≤0.01) increased on T3 and T4, over the T2 plants. Size of the galls was found to be significantly (P≤0.05) increased on T4 as compared to T3 plants.

Number of Egg Masses:

The number of egg masses per root system was significantly (P≤0.05) increased on T2

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plants at Pi = 400 J_2, when compared with the T1 plants, inoculated with 200 J_2. A significant increase (P≤0.01) was observed at Pi = 800 J_2 in comparison to the lowest inoculum level at Pi = 200 J_2. The increase in the number of egg masses per root system was significantly (P≤0.01) higher as was observed at Pi = 1,600 J_2, in comparison to T1, T2 and T3 plants.

There was a significant (P≤0.01) increase in T3 and T4 plants, over the T2 plants. The T4 plants did not exhibit any significant enhancement at highest inoculum level i.e. 1,600 J2, when compared with T3 at 800 J_2 .

Number of Mature Females:

The number of mature females recovered from the inoculated roots increased with an increase in the initial inoculum level. The number of mature females per gram root was low at the initial inoculum level of Pi = 200 J_2 on T1 plants. A significant (P≤0.05) increase in the number of the mature females per gram root was observed at the initial inoculum level Pi = 400 J_2 (T2 plants) when compared with T1 plants. The number of mature females per gram root significantly (P≤0.01) increased at the initial inoculum level of Pi = 800 J_2 than T1 plants. Maximum number of mature females was collected from the roots of the plants at the highest inoculum level (Pi = 1,600 J_2).The Number of females was found to be significantly (P≤0.01) increased in T3 and T4 plants, over T2, while a non-significant enhancement occurred in T4 plants, when compared with T3 plants .

Reproduction Factor:

Reproduction factor (Rf) and the rate of population increase (RPI), decreased with an increase in initial inoculum level. Maximum being at the lowest, and minimum at the highest inoculum levels.

DISCUSSION

The number of galls and egg mass were observed at different inoculums levels, presented in the Table. In respect of galling, a progressive increase in galling incidence was recorded with increasing levels of inocula. Increasing inoculum levels led to an increase in the

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number of galls. This showed that when the inoculum levels were high, greater number of juveniles were able to infect the plant roots which results in reduced nutrient and water uptake by the roots and consequently poor plant growth (Karssen and Moens, 2006).

High initial population leads to the increase in the number of egg masses. Singh and Khurma (2007) showed that increasing inoculum concentration enlarges the average number of egg masses in the roots of infected plants. Amarantha and Krishnappa (1989), and Hussain and Bora (1998) reported higher galling incidence with higher inoculum levels of *M. incognita* in sunflower and French bean, respectively. Kheir *et al.,* (2004), who stated that the nematode final density of *M. incognita* on banana cultivars tested, increased proportionally with the increase of initial inoculation levels, and all inoculum levels suppressed the plant growth regardless of the cultivar. Hussain and Bora (1998) found that *M. incognita* population in french bean was found to be maximum with the maximum nematode inoculum level. It was observed that with the increase of inoculum density of *M. incognita* in fifteen-days-old seedlings of sunflower there appeared corresponding increases in the number of galls, egg masses and larval population.

With an increase in initial inoculum level, the number of mature females recovered per gram root was found to be increased. However, a limited supply of food in a limited space, probably, induced detrimental effects on the normal development of the nematode. Our results are in accordance with the studies carried out by Bhat, (1999), Jonathan and Rajendran, (2000) and Yasmeen, (2002). Our data revealed that the number of egg masses per plant increased with the increase in inoculum level and it seems quite reasonable that the lower is the inoculum level, the lower will be the number of mature females in the galled roots and consequently fewer will be the egg masses; higher is the inoculum level, correspondingly higher will be the number of mature females as well as number of egg masses.

Reproduction factor (Rf) and rate of population increase were found decreased with the increase in initial inoculum level which was highest at the lowest inoculum level and lowest at the highest inoculum level. This might be due to destruction of root system and also due to the failure of the juveniles of the subsequent generations to locate the new infection sites of subsequent generations (Ogunfowora, 1977). Jonathan and Rajendran (2000) attributed

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decrease in multiplication rate to high initial inocula that created crowded conditions, and adversely affected the rate of development of the nematode. The decrease in the rate of nematode multiplication perhaps due to destruction of the root system with high population of the nematode and due to competition for nutrition among the developing nematodes within a given root system as was reported by Chitwood (1951) for *M. hapla*; Samathanam and Sethi (1996); and Pathak *et al*., (2000) for *M. incognita*. Khan and Ashraf (2005) also reported a decrease in reproduction factor of *M. incognita* and *M. javanica* with an increase in the inoculum levels from 250 to 8,000 J_2. The variability in nematode multiplication around the root environment might be due to competition for entry sites and poor egg hatch (Nayak, *et al*.,1987; Ahmed, 1989).

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