Effect of Initial Inoculation of *Meloidogyne javanica* on Growth and Yield of *Lagenaria siceraria*

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Abstract: The plants of *Lagenaria siceraria* were inoculated with 02, 20, 200, 2,000 and 20,000 juveniles of root- knot nematode (*Meloidogyne javanica*) per pot, under green house condition. Significant and maximum reduction in plant growth and yield was noticed at the highest inoculum level (T_4 and T_5) plant. Non-significant reduction in the length and weight of the root and shoot of *Lagenaria siceraria* in T_1 and T_2 plants, plants were inoculated with 2 and 20 juveniles/plants. The number of galls was greatly influenced by the initial population of the nematode. The maximum number and size of galls were noticed in T_4 and T_5 plants. The juveniles caused rupturing of epidermis of the root of *Lagenaria siceraria* while penetrating into the inner tissues. In young roots the juveniles migrated towards differentiating vascular tissues. Their migration was intra and inter cellular. In older roots, they migrated through the cortex. They caused the formation of giant cells in the form of clusters. In a giant cell cluster these are five to twelve giant cells, each having dense cytoplasm and enlarged nuclei. All the nuclei enclosed one to few nucleoli. The giant cell clusters modify the internal morphology of the affected tissue. In addition, abnormal xylem and abnormal phloem also occupy a major portion near the giant cells.

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1. Introduction

Meloidogyne javanica, a plant parasitic nematode causes severe damage to a large number of plant species including the members of the family *Cucurbitaceae.* Singh and Goswami (2000) reported significant growth reduction over control in cowpea with initial population of 1,000 nematodes per 500 g soil. Maximum reduction in plant height, root length and root weight of sunflower plants recorded at $2500J_2$ (Bhatt *et al*., 2001). Parasitism by root knot nematode is characterized by establishment of permanent feeding sites comprising of giant cells in the cortex, endodermis, pericycle, and vascular tissues of the host roots. In addition, deformation and blockage of vascular tissues at the feeding sites limit the translocation of water and nutrients, which further suppresses plant growth and crop yield, (Hussey and Williamson, 1997). Low or high population densities of these nematodes produce different effects on plants.

Wallace (1971) found an increased rate of plant growth at lower, and decreased at higher population densities.

The following study was carried out to determine the effects of different inoculum levels on (i) the plant growth (ii) the plant yield, (iii) the number and size of galls (iv) the number of egg masses per plant, (v) Nematode population, (vii) reproduction factor.

2. Materials and Methods

2.1 Raising and maintenance of test plant

The seeds of *Lagenaria siceraria* were surface sterilized with 0.5% sodium hypochlorite NaOCl for four minutes and thoroughly washed five times with distilled water. The axenized

seeds were sown in 30 cm diameter clay pots containing steam sterilized soil (7 clay: 3 sand: 1 farmyard manure) and allowed to germinate. The seedlings were thinned to one seedling per pot, before inoculation.

2.2 Inoculation with nematode

Meloidogyne javanica was selected as a test pathogen. To perform experiment during the period of research, pure culture of *M. javanica* was maintained on egg plant (*Solanum melongena* L.) roots in a glass house by using single egg mass. The egg masses from the galled roots of egg plant were picked with the help of sterilized forceps and allowed to hatch. The second stage – juveniles were collected in sterilized distilled water and counted with the help of counting dish. 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 $2J_2$, $20J_2$, $200J_2$, $2{,}000J_2$, and $20{,}000J_2$ per 10 ml water, were pipette into the holes, which were covered with the soil soon after inoculation. Each treatment was replicated five times and the pots were arranged in randomized complete block design. Uninoculated set of plants served as control.

C	:	control
T_1	:	$2J_2$/pot
T_2	:	$20J_2$/pot
T_3	:	$200J_2$/pot
T_4	:	$2{,}000J_2$/pot
T_5	:	$20{,}000J_2$/pot

Watering was done regularly at an interval of two days. The plants were uprooted after 60 days of inoculation. The data for different parameters were collected and statistically analyzed.

3. Parameters

3.1 Plant growth

After 60 days of inoculation, the mature plants were uprooted with the help of hoe and gently washed with running tap water. The plants were cut at the margin of the root and the shoot. Length of the root and the shoot was measured in centimeter with the help of meter scale. Fresh weight of root and shoot was determined by physical balance. Roots and shoots were kept, separately, in bamboo paper envelopes and kept in an incubator maintained at 72 ºC temperature for 5 days. Dry weight of the root and the shoot was determined.

3.2 Number of flowers and fruits

The numbers of flowers, number of fruits per plant were counted visually.

3.3 Number and size of galls

The number of the gall was counted visually. And the size of gall was obtained by measuring in maximum length and width (in mm^2) on meter scale.

3.4 Number of mature female

For counting the number of mature females the root sample taken from each treatment were blended with 200 ml water in a warring blender for 30 seconds at low speed. The resultant suspension was passed through coarse and 100 mesh sieves in order to separate root tissue. The total female population was counted with the help of counting dish. Total number of female nematodes in the suspension was divided by the weight of each root system to derive population per gram root.

3.5 Number of egg masses

The number of egg masses per root system on infected roots was counted after staining with phloxin B, prepared by dissolving 0.12 g phloxin B per liter of water. The galled roots were placed in this solution for 15-20 minutes. The roots were gently rinsed in tap water. The egg masses were stained red and counted directly.

3.6 Nematode population (Root and Soil)

Root nematode population was determined by macerating 5g of infected root in a warring blender and suspensions were passed through 100 to 400 mesh sieves and the juvenile catch on the 400-mesh sieve was collected in a beaker. Soil of each pot was thoroughly mixed with and juveniles were extracted by Cobb's sieve and decanting and Baermann funnel methods. The number of nematodes per root system and per kilogram soil was counted using counting dish.

3.7 Reproduction factor

Reproduction factor (R_f) was calculated by the formula:

$R_f = P_f/P_i$

Where P_f is the final population and P_i is the initial population.

4. Results

4.1 Root and shoot length

In comparison to control, non-significant decrease in the root and the shoot length was observed at Pi = $02J_2$ and Pi = $20J_2$, when compared to control. In comparison to control, a significant ($P \leq 0.05$) decrease in the root and the shoot was observed at an initial inoculum level of Pi = $200J_2$. Significant ($P \leq 0.01$) and highest reduction occurred in the root and the shoot length of plant at the initial inoculum level of Pi = $2{,}000J_2$ and $20{,}000J_2$, in comparison to control plant (Table-1).

4.2 Root and shoot weight

Highest and significant ($P \leq 0.01$) reduction in the fresh and the dry weight of the root and the shoot weight was occurred at Pi = $2{,}000J_2$ and $20{,}000J_2$, when compared with control plant. A significant ($P \leq 0.05$) reduction in comparison to control was noticed at Pi = $200J_2$. Non-significant reduction in the fresh and dry weight of the root and shoot was observed at Pi = $02J_2$ and $20J_2$ (Table-1).

4.3 Number of flowers and fruits

The number of flowers and fruits decrease with an increase the initial inoculum level. The number of flowers and fruits decreased non-significantly at initial inoculum level of Pi = $02J_2$ and $20J_2$, when compared with control. The number of flowers and fruits decrease significantly ($P \leq 0.05$) decreased at initial inoculum level of Pi = $200J_2$. A significant ($P \leq 0.01$) decrease in the number of flowers and fruits was observed at an initial inoculum level of Pi = $2{,}000\ J_2$ and Pi = $20{,}000J_2$ (Table-1).

4.4 Number and size of galls

The number and size of galls were small at the initial inoculum level of Pi = $02J_2$. A non-significant increase in the number and size of galls were observed in the plants at Pi = $20J_2$, when compared to lowest inoculum level. A significant ($P \leq 0.01$) increase in the number and size of galls were observed at Pi = $2{,}000J_2$, when compared with the number and size of the gall at Pi = $02J_2$. The highest number of gall and attained maximum size at highest inoculum level of Pi = $20{,}000J_2$ which were significantly ($P \leq 0.01$) larger than the galls at all the lower inoculum levels (Table- 2).

4.5 Number of mature female

The number of mature females recovered from the roots increased with an increase in the initial inoculum level. The number of mature females per gram of root was two at initial inoculum level of Pi = $02J_2$. A non-significant increase in the number of matures female per gram of root was noticed at Pi = $20J_2$ as compared with that at Pi = $02J_2$. The number of mature females per gram of root significantly ($P \leq 0.01$) increased at the initial inoculum level of Pi = $2{,}000J_2$ than at the initial levels of Pi = $02J_2$, $20J_2$, and $200J_2$. Maximum number of mature female was collected from the roots of the plant at the highest inoculum level Pi = 20,000 followed by Pi = $2{,}000J_2$, Pi = $200J_2$, Pi = $20J_2$ and Pi = $02J_2$ (Table-2).

4.6 Number of egg masses

The number of egg masses per plant increased as the number of juveniles introduced per plant increased. A non-significant increase in the number of egg masses was observed at the initial inoculum level Pi = $02J_2$. A significant ($P \leq 0.01$) increased in the number of egg masses per plant was found at Pi =$200J_2$, $2{,}000J_2$ and $20{,}000J_2$, in comparison to the lower inoculum levels (Pi = 02 and Pi = $20J_2$) (Table-2).

4.7 Reproduction factor and rate of p opulation increase

Reproduction factor (R_f) decreased with increase in initial inoculum level. Maximum being at the lowest and minimum at highest inoculum level (Table-2).

Table-1: Effect of initial inoculum of nematode on plant growth and yield of *Lagenaria siceraria*

Treatments	Plant Length (cm)		Fresh Weight (g)		Dry Weight (g)		No of flowers plant^{-1}	No. of fruits plant^{-1}
	Root	Shoot	Root	Shoot	Root	Shoot		
Control	27.40	99.90	4.98	22.46	1.32	6.05	20.35	13.15
02J_2/pot	27.10	99.50	4.80	20.25	1.20	5.70	19.65	12.70
20J_2/pot	24.90	92.65	4.72	19.65	1.15	4.95	17.28	12.10
200J_2/pot	18.30	87.15	3.48	18.80	0.99	4.40	14.78	9.70
2,000J_2/pot	15.45	73.75	3.05	16.55	0.82	3.25	12.20	7.90
20,000J_2/pot	10.25	54.35	2.75	12.35	0.68	2.48	9.54	6.15
L.S.D≤0.05	7.16	11.30	1.24	3.26	0.24	1.36	4.13	2.42
L.S.D≤0.01	9.43	14.88	1.57	4.19	0.35	1.74	5.62	3.48

Table-2: Effect of initial inoculum of nematode on gall, egg masses, population and reproduction factor.

Treatments	No of galls plant^{-1}	Size of gall (mm^2)	No of female per (g) root	No of egg masses plant^{-1}	Root Population	Soil Population	Total Population	RF
Control	-	-	-	-	-	-	-	
02J_2/pot	2.05	3.05	5.65	2	275	555	830	415
20J_2/pot	27.55	5.32	17.15	40.68	2085	4260	6345	317.25
200J_2/pot	73.68	7.95	27.34	158.00	2670	5462	8132	40.66
2,000J_2/pot	112.35	13.10	51.55	245.25	3590	7230	10820	5.41
20,000J_2/pot	174.75	20.55	58.01	405.00	9890	16845	26735	1.33
L.S.D≤0.05	34.80	2.70	17.08	39.12	-	-	-	-
L.S.D≤0.01	47.75	4.91	23.43	53.69	-	-	-	-

5. Discussion

Tomato plants, inoculated with second stage of juveniles of *Meloidogyne javanica* caused reduction in plant length plant weight and other yield parameters. An increase in the number of juveniles per plant decreased length and weight of the plants. Increase in inoculum level from 2J_2 to 20,000J_2 per pot gradually decreased plant weight, stunting of the plant, chlorosis of the leaves, loss in plant weight are the characteristic symptoms of root-knot nematode infected plants, Which were observed in tomato infected with *M. javanica.* Reductions in plant length and weight have been reported by several workers like (Shukla and Haseeb, 1998; Jonathan and Rajendran, 2000; Nehra and Trivedi, 2002; Hisamuddin *et al*, 2005; Azam and Hisamuddin 2008; Azam *et al.,* 2008). Reduction in growth of *L. esculentum* was due to the infection caused by *M. incognita.* There was reduction in the number of flowers and fruits on nematode infected plants. Deleterious effects of the pathogen were reflected not only on plant growth but also on yield of *L. siceraria*. Highest reductions had occurred at the highest inoculum level as in evident from the finding. The loss in yield due to root – knot nematode infection has been reported by several workers on different plants (Chitwood, 2003; Hisamuddin *et al.,* 2003, 2004; Niyaz and Hisamuddin 2008; Tiyagi *et al,* 2001).

Gall formation on the root of *L. siceraria* further supports that the nematode was successfully established in the roots and had become the agent of loss of growth and yield. The size of gall indicated the magnitude of inoculum density. Smaller galls indicated low level and larger gall indicated higher level of inoculum. Maximum numbers of galls were observed at 20,000J_2 per pot. Gall number and the gall size were found proportionate to the amount of inoculum. At lower inoculum levels fewer galls were formed, on the other hand, at higher inoculum levels many galls were developed. The finding are in accordance with the earlier reports, (Niyaz and Hisamuddin 2008; Parveen 2006; Azam 2008).

From the findings it is evident that *L. siceraria* reported positively towards host-parasite relationship.

The number of egg masses per plant increased as the level of inoculum increased. Egg mass number being higher at higher inoculum level indicated that large number of juveniles entered the roots and induced gall formation. As the females matured simultaneously and laid eggs. There was a great relationship between the number of egg masses per plant and the initial inoculum level. The relationship between number of eggs per egg mass was inversely proportional. The egg masses obtained from the gall at lower inoculum level contained higher number of eggs and on the contrary, the egg masses at higher inoculum level contained lower number of eggs. From this finding it is evident that the developing females obtained sufficient amount of food from the plant at lower inoculum level. The nematode had to face a competition for food at higher inoculum level. Perveen (2006) and Niyaz and Hisamuddin (2008) had encountered the same relationship.

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