Journal of Agriculture and Crops
ISSN(e): 2412-6381, ISSN(p): 2413-886X
Vol. 2, No. 8, pp: 83-86, 2016
URL: http://arpgweb.com/?ic=journal&journal=14&info=aims

Penetration and Development of *Meloidogyne incognita* Race-3 on *Pseuderanthemum atropurpureum* in Presence and Absence of *Rhizoctonia solani*

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Abstract: Although lot of information on life cycle of *M. incognita* is available on several hosts but there is hardly any report available in the literature on life cycle of *M. incognita* Race-3 on *P. atropurpureum*. The present study revealed that. *M. incognita* Race-3 took 30 days to complete its life cycle on *P. atropurpure*um in absence of *R. solani*. However, the life cycle of *M. incognita* Race-3 was adversely affected in presence of *R. solani* and took 44 days to complete the life cycle. *M. incognita* Race-3 second stage juvenile (J_2) invaded the roots of *P. atropurpureum* within 12 hours after inoculation in absence of *R. solani*. However, after 12 hours no penetration of juvenile was recorded when both *M. incognita* Race-3 and *R. solani* were concomitantly inoculated. The rate of penetration gradually increased significantly, with the passage of time. The results revealed that presence of *R. solani* not only drastically reduced the percentage penetration of juveniles but also subsequently delayed the development of different stages of life cycle and decreased the fecundity of root-knot nematode in *P. atropurpureum*. Keeping in view the economic importance of *P. atropurpureum* as an ornamental as well as medicinal plant life cycle of *M. incognita* on *P. atropurpureum* was studied.

Keywords: Life cycle (Developmental stages); M. incognita Race-3; R. solani; P. atropurpureum.

1. Introduction

Nematodes are the most abundant and ubiquitous multicellular organisms on earth. They are found in almost every type of habitat, from the bottom of the deepest ocean to near the tops of the highest mountains, from the tropics to the polar regions, and from every conceivable habitat [1]. Most of the nematodes are beneficial because of their free-living and saprophytic nature and play a major role in decomposition of organic matter and nutrient recycling. Nematodes can feed on bacteria, fungi, algae, plants and they can also parasitize insects, animals and humans. Among the plant parasitic nematodes the root-knot nematodes, *Meloidogyne* spp. have been of interest to nematologists worldwide probably due to their widespread distribution and being most serious agricultural pests which are responsible for heavy losses both in quantity as well as quality [2]. The genus *Meloidogyne* comprises of about 97 described species and almost every kind of cultivated and wild plants are parasitized by one or the other species of root-knot nematodes [3].

Muller [4] was the first to describe the life cycle of *Meloidogyne* spp. Later many contributions were made on the morphology of different developmental stages and the duration of the life cycle of *Meloidogyne* spp. under different environmental conditions and with several host plants in relation to their age and nutritional status [5-9].

2. Materials and Methods

The present investigations were carried out in months of August-September and the prevailing temperature during this period was found to range between 35 to 37°C. Six week old rooted cuttings of *P. atropurpureum* were singly transplanted into 6 inch clay pots containing 1 kg sterilized soil mixture. After one week of transplantation, one set of 150 cuttings was simultaneously inoculated with freshly hatched 2000 second stage juveniles of *M. incognita* Race-3 and 3 g fungal suspension of *R. solani* and the other set of 150 cuttings was individually inoculated with same inoculum level of *M. incognita* Race-3 served as control.

Observations on penetration and developmental stages of the nematode were recorded from three cuttings of each set after every 24 hours (first being after 12 hours) and continued till the completion of life-cycle. The complete root system of the tested plants was carefully removed from the soil, washed gently in tap water and stained in boiling 0.1% acid fuchsin, in lactophenol followed by washing in tap water and keeping in plain lactophenol for further differentiation.

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The developmental stages of *M. incognita* were identified and designated as (A, B, C, D and E) as described by Triantaphyllou and Hirschmann [10].

A = pre parasitic II^{nd} stage juvenile (filiform shaped)
B = parasitic II^{nd} stage juvenile (spindle shaped)
C = III^{rd} and IV^{th} stage of juvenile (sausage shaped)
D = moulted IV^{th} stage juvenile (moulted sausage shaped)
E = adult females (sac shaped) and males (filiform shaped).

The number of juveniles (J_2) penetrated and their different developmental stages found in roots were counted under a stereomicroscope. The percentage of juveniles penetrated was calculated against the initial inoculum level of *M. incognita*. Similarly, the per cent of females and males developed against the total juveniles penetrated was calculated. The number of eggs/egg mass and the population of nematode/kg soil were also estimated.

3. Results

The data presented in Table- 1.1 clearly showed that when *M. incognita* Race-3 was inoculated alone the second stage juvenile (J_2) invaded the roots of *P. atropurpureum* within 12 hours after inoculation. However, in the same duration, the penetration of juvenile was not recorded when both *M. incognita* Race-3 and *R. solani* were concomitantly inoculated. The rate of penetration gradually increased significantly, with the passage of time. The maximum percentage of juvenile penetration into the roots of *P. atropurpureum* was recorded after 8 days of inoculation irrespective of whether nematode was either present alone or with *R. solani*. Further, results revealed that the presence of *R. solani* not only delayed the penetration of second stage juveniles but also reduced the number of juveniles penetrated. The second stage juveniles mostly penetrated the root tips, just behind the root cap, in the region of cell elongation. The terminal portions of the roots were more susceptible to the nematode entry, however, the lateral root tips were also the common sites for the penetration of juveniles. Second stage juveniles moved within the roots after penetration and migrated initially intercellularly in the cortex, away from the root tip and parallel to the root axis. The development of root galls was observed with the development of nematodes inside the roots.

It is evident from the data presented in Table- 1.2 that in absence of *R. solani*, 87.3% juveniles were found in "A" stage after 8 days of inoculation of *M. incognita* Race-3, whereas, no juveniles in "A" stage and 78.5% in "B" stage were recorded after 10 days. Moreover, after 14 days of inoculation most of the juveniles were traced in "C" stage (78.6%), only a few in "B" stage (5.2%) and no juveniles were present in "A" stage. Similarly, after 20 days of inoculation no juveniles of "A" and "B" stages were traced, but, only a few juveniles could be traced as "C" stage (1.2%) and majority of the juveniles in "D" stage (68.3%) were found. However, after 24 days of inoculation most of the developmental stages *viz.*, "A", "B", "C" and "D" were absent except the "E" stage (Adult female and male). The percentage of adult female and male of *M. incognita* was found as 70.2 and 3.7 after 24 days of inoculation.

However, on the other hand, the presence of *R. solani* along with *M. incognita* Race-3 not only decreased the percentage of penetration and occurrence of different stages in the roots but also subsequently delayed the development of different stages of the nematode in *P. atropurpureum.* In presence of *R. solani*, the highest percentage of "A" stage of juveniles (65.7%) was observed after 8 days of inoculation and no other stages of nematode were seen. Furthermore, most of the juveniles were found in "B" stage (52.6%) and few in "A" stage (13.0%) after 12 days of inoculation. The highest percentage of "C" developmental stage of juveniles (40.2%) was recorded after 19 days of inoculation, followed by "B" (15.7%) and "A" (3.2%) stages. The "A" stage of juveniles were not observed, while, very few stages of "B" (2.8%) and "C" (10.5%) and maximum percentage of "D" stage (38.6%) of development were recorded after 24 days of inoculation. The "E" stage i.e. adult male (16.0%) and female (38.2%) were traced after 31 days of inoculation, however, some juveniles were also found in "C" stage (2.1%) and in "D" stage (7.0%) and no "A" and "B" stages were observed.

The plants inoculated with *M. incognita* Race-3 alone, showed the deposition of gelatinous matrix on 26^{th} day, egg laying in egg mass was recorded on 28^{th} day and in soil, second stage juveniles were recorded on 30^{th} day of inoculation. However, on the other hand, the corresponding stages of *M. incognita* Race-3 in presence of *R. solani* were recorded on 35^{th}, 38^{th} and 44^{th} day of inoculation. Fecundity of females was also found to be reduced with an average of only 156 eggs/ egg mass in *M. incognita* Race-3 and *R. solani* inoculated plants as compared to 287 eggs per egg mass in *M. incognita* Race-3 alone inoculated plants. The highest number of juveniles (2248 J_2 / kg soil) were observed in plants inoculated with *M. incognita* Race-3 alone, whereas, by 1561 J_2 / kg soil were found in presence of *R. solani*. The percentage of female and male formation of *M. incognita* Race-3 was 80.4 and 4.2 in absence of *R. solani* respectively. While, in presence of *R. solani*, the percentage of female and male formation of *M. incognita* Race-3 was recorded as 58.1 and 24.3 respectively.

It can be concluded from the above results that life cycle of *M. incognita* Race-3 on *P. atropurpureum* was completed in 30 days, moreover, the duration of life cycle of the nematode was adversely affected in the presence of *R. solani* and it took 44 days to complete the life cycle. Hence, the presence of *R. solani* delayed the life cycle of root-knot nematode *M. incognita* Race-3 by 14 days.

4. Discussion

The results presented in Tables- 1.1 and 1.2 clearly revealed that. *M. incognita* Race-3 took 30 days to complete its life cycle on *P. atropurpure*um in absence of *R. solani*. The life cycle of *M. incognita* on different hosts has also been studied by various workers with some variation in duration of different life stages and total time period taken for completion of one generation [9, 11-13]. The variation in time required to complete the life cycle of *M. incognita* may be due to varied environmental conditions, race of root-knot nematode, host plant, temperature, soil moisture etc as also suggested by Wallace [14] and Bird [6]. Furthermore, the life cycle of *M. incognita* Race-3 was adversely affected in presence of *R. solani* and took 44 days to complete the life cycle. This showed that it was delayed by 14 days as compared to when *M. incognita* Race-3 was present alone. The results revealed that presence of *R. solani* not only drastically reduced the percentage of penetration of juveniles but also subsequently delayed the development of different stages of life cycle and decreased the fecundity of root-knot nematode in *P. atropurpureum*. My results are in agreement with the findings of Chhabra [15] who also reported that the penetration, fecundity and life cycle of *M. incognita* was adversely affected in presence of *R. solani* in okra.

The detrimental effects of *R. solani*, on penetration development of different stages and fecundity of *M. incognita* Race-3 may be attributed to the toxic metabolites produced by *R. solani* [16-18]. Which would have caused mortality of nematode, destruction of gaint cells, consequently reducing the penetration and development and also affecting the hatching and mobility of second stage juveniles of root-knot nematode. The inhibitory effect of *R. solani* on *M. incognita* has been already observed by many researchers [19-22]. Another possible reason may be ascribed to destruction of root tissue due to root-rot causing a reduction in the site available for penetration and feeding of *M. incognita* Race-3 which ultimately was not able to support the population of nematode. Further the effect of *R. solani* to reduce the number of females and increase the proportion of males could also be due to nutritional stress resulting from fungal infection.

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Table-1.1 The penetration of second stage juvenile of *Meloidogyne incognita* Race-3 in *Pseuderanthemum atropurpureum* in presence and absence of *Rhizoctonia solani*.

Treatment	Hours / Days after inoculation	Juveniles (J_2) penetrated (%)
M. incognita	12 h	10.3
M. incognita + R. solani	12 h	0.0
M. incognita	1	23.5
M. incognita + R. solani	1	12.0
M. incognita	2	31.2
M. incognita + R. solani	2	20.0
M. incognita	3	48.1
M. incognita + R. solani	3	33.6
M. incognita	4	60.5
M. incognita + R. solani	4	46.3
M. incognita	5	70.4
M. incognita + R. solani	5	58.5
M. incognita	6	75.6
M. incognita + R. solani	6	63.2
M. incognita	7	85.5
M. incognita + R. solani	7	64.5
M. incognita	8	87.3
M. incognita + R. solani	8	65.7
C.D.(*P*=0.05)		1.2
C.D.(*P*=0.01)		9.7

Table-1.2. Studies on the different developmental stages (life cycle) of *Meloidogyne incognita* Race-3 on *Pseuderanthemum atropurpureum* in presence and absence of *Rhizoctonia solani.*

Treatment/ Days after inoculation		Developmental stages of *M. incognita* (%)/ Treatment												C.D. (*P*=0.05)	C.D. (*P*=0.01)
		A		B		C		D		E					
										Adult female		Adult male			
Mi	Mi*+Rs**	Mi	Mi+Rs	Mi	Mi+Rs	Mi	Mi+Rs	Mi	Mi+Rs	Mi	Mi+Rs	Mi	Mi+Rs		
8	8	87.3	65.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.6	13.44
10	12	0.0	13.0	78.5	52.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.2	11.4
14	19	0.0	3.2	5.2	15.7	78.6	40.2	0.0	0.0	0.0	0.0	0.0	0.0	7.6	10.6
20	24	0.0	0.0	0.0	2.8	1.2	10.5	68.3	38.6	0.0	0.0	0.0	0.0	6.8	9.5
24	31	0.0	0.0	0.0	0.0	0.0	2.1	0.0	7.0	70.2	38.2	3.7	16.0	7.0	9.8

Percentage of females formed 80.4 (Mi) and 58.1 (Mi+Rs) and males formed 4.2 (Mi) and 23.4 (Mi+Rs).

Deposition of gelatinous matrix was recorded on 26th day (Mi) and 35th day (Mi+Rs) of inoculation.

Egg laying was recorded on 28th day (Mi) and 38th day (Mi+Rs) of inoculation.

Average number of eggs/ egg mass = 287 (Mi) and 156 (Mi+Rs).

The second stage juvenile (J_2) in soil was recorded on 30th day (Mi) and 44th day (Mi+Rs) of inoculation.

Number of juveniles per kg soil, 2248 (Mi) and 1561(Mi+Rs).

Mi*=*Meloidogyne incognita*, Rs**=*Rhizoctonia solani.*