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EFFECT OF PRE-, POST- AND SIMULTANEOUS INOCULATIONS OF *RHIZOBIUM*, *ROTYLENCHULUS RENIFORMIS* AND *MELOIDOGYNE INCOGNITA* ON LENTIL

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Summary. Lentil seedlings (*Lens culinaris* Medic.) suffered greater damage from *Meloidogyne incognita* and *Rotylenchulus reniformis* individually or concomitantly, when plants were not infected with *Rhizobium*. Also, nematode multiplication was greater in the absence of *Rhizobium*. Sequential nematode inoculations were time dependent i.e. prior establishment of one nematode species was mutually antagonistic to the multiplication of the other subsequently inoculated species.

In recent years a number of investigations have been carried out to study the effect of co-habitation of *Meloidogyne* spp. and *Rotylenchulus reniformis* on a number of crops, such as soybean (Singh, 1976), tomato (Kheir and Osman, 1977; Khan *et al.*, 1984; Khan *et al.*, 1985), egg plant (Khan *et al.*, 1986), cowpea (Taha and Kassab, 1979; Khan and Husain, 1988) and blackgram (Mishra and Gaur, 1981) but nothing is known about their effect on lentil in the presence and absence of *Rhizobium* although lentil has been reported parasitized by both the nematode species singly (Bernard *et al.*, 1990; Fazal and Husain, 1991a) and concomitantly (Fazal and Husain, 1991b). This prompted us to investigate the effect of test pathogens in various combinations on disease development, plant growth and nematode multiplication.

Materials and methods

Lentil (*Lens culinaris* Medic.) seedlings were raised from surface sterilized seeds in 15 cm earthen pots containing steam sterilized soil and farm yard manure mixture (4:1). Fifteen day old seedlings of uniform size were inoculated with *R. reniformis* Linford *et* Oliveira, *M. incognita* (Kofoid *et* White) Chitw. (1000 juveniles/plant) and *Rhizobium* (1 g commercial bacterial culture of lentil strain/plant) either individually, concomitantly or sequentially (with an interval of 10 days) in different combinations. Suspension of the nematode and/or the *Rhizobium* were pipetted onto exposed feeder roots which were immediately covered with a soil layer. Uninoculated bacterized plants served as a control. Each treatment was replicated three times. Two months after inoculation the experiment was terminated and plant growth, nodulation, root-knot index and reproduction factor of each nematode species were assessed. Nematode numbers in the roots were estimated by macerating 1 g root from each plant in a Waring blender. Nematode numbers in 1000 g soil samples were determined by Cobb's sieving and decanting technique followed by Baermann funnel technique.

Results

Rhizobium was beneficial for growth of both nematode inoculated and uninoculated plants (Table I). When seedlings were inoculated with *R. reniformis* and *M. incognita* in the presence of *Rhizobium*, individually or concomitantly in different combinations, there was in each case significant plant growth reduction which was significantly more in plants without *Rhizobium*. In the absence of *Rhizobium* the greatest plant reduction (35%) was caused by *M. incognita* followed by *R. reniformis* (30%) whereas the two pathogens together caused 67% reduction. In the presence of *Rhizobium* the plant growth reductions in the corresponding treatments were 28, 24 and 57%, respectively.

Inoculation of *Rhizobium* 10 days prior to inoculation with either of the two pathogens, singly or concomitantly in various combinations, resulted in significantly less plant growth reduction, poor nematode multiplication, fewer galls and improved nodulation as compared to those in which *Rhizobium* was inoculated 10 days after the inoculations. Sequential inoculation with *R. reniformis* plus *Rhizobium* prior to *M. incognita* caused 54% reduction whereas inoculation with *M. incognita* plus *Rhizobium* prior to *R. reniformis* caused 59% reduction. In other combination, sequential inoculation of *M. incognita* and *R. reniformis* prior to *Rhizobium* caused 61% plant growth reduction whereas when *Rhizobium* was inoculated 10 days prior to *M. incognita* plus *R. reniformis* the percent reduction in plant

growth was 50. When *R. reniformis* was inoculated prior to *M. incognita* plus *Rhizobium* the percent reduction was 63 whereas when *M. incognita* was inoculated prior to *R. reniformis* plus *Rhizobium* the reduction in plant growth was 65%.

The rate of multiplication of *M. incognita* and *R. reniformis* was higher on unbacterized than on bacterized plants. However, on bacterized plants the rate of multiplication of both pathogens was higher when each was present alone than present concomitantly (Table I). Also, there was a corresponding reduction in number of galls.

In the case of sequential inoculation there was a reduction in the rate of multiplication of both nematode species when *Rhizobium* was inoculated either simultaneously, prior or after the inoculation of test pathogens with any of the combinations. The reduced multiplication of *M. incognita* corresponded well with a reduction in galling.

With or without *Rhizobium*, the host infestation and rate of multiplication of both nematodes were mutually inhibited by each other in concomitant inoculation. Prior inoculation of either species was detrimental to the multiplication of the other.

The presence of either nematodes inoculated singly or concomitantly in various combinations, caused a reduction in nodulation. In the case of sequential inoculation maximum reduction in nodulation (66%) was observed when *M. incognita* was inoculated prior to *R. reniformis* + *Rhizobium.*

Discussion

Increased damage due to nematode infection on lentil and high nematode multiplication rate in the absence of *Rhizobium* than in its presence suggests that bacterized lentil plants derived protection against nematode infection (Bopaiah *et al.*, 1976; Khan and Husain, 1988).

Decrease in the rate of multiplication of both the nemtodes in case of combined inoculation shows mutually antagonistic inter-relationship between the pathogens. This might be due to the same or nearby infection sites which caused competition for food and space (Kheir and Osman, 1977; Taha and Kassab, 1980; Khan and Husain, 1988, 1990).

Reduction in nodulation might be due to the formation of *Meloidogyne* galls thus occupying space in the roots (Malek and Jenkins, 1964; Barker and Hussey, 1976) and due to suppression of lateral root formation by *R. reniformis* which might cause reduction in the number of sites for nodule initials (Taha and Kassab, 1980).

TABLE I - *Effect of pre-, post and simultaneous inoculations of* Rhizobium, Rotylenchulus reniformis *and* Meloidogyne incognita *on lentil.*

Treatments	Plant Length (cm)	Plant Fresh Weight (g)	Dry Shoot Weight (g)	Percentage reduction in dry shoot weight against control	Reniform Nematode		Root-knot Nematode		Total Number of Galls/ Plant	Total Number of Nodules/ Plant	Percentage reduction in modulation against control	Gall Index
					Total Population	Rf $\frac{Pf}{Pi}$	Total Population	Rf $\frac{Pf}{Pi}$				
UI+B	64.9	14.4	4.6	–	–	–	–	–	–	71	–	–
UI+UB	59.8	13.0	4.0	13.0	–	–	–	–	–	00	100.0	–
Rr	48.1	10.4	3.2	30.4	8695	8.7	–	–	–	00	100.0	–
Mi	45.6	9.8	3.0	34.8	–	–	10326	10.3	126	00	100.0	5
Mi+Rr	24.0	5.0	1.5	67.4	5056	5.1	6865	6.9	110	00	100.0	5
Rr+Rh	52.4	11.3	3.5	23.9	5943	5.9	–	–	–	55	22.5	–
Mi+Rh	50.0	10.9	3.3	28.3	–	–	7241	7.2	90	53	25.4	4
Mi+Rr+Rh	31.7	6.7	2.0	56.5	4358	4.4	5954	6.0	54	32	54.9	4
Rh→Rr	54.4	11.8	3.6	21.7	4862	4.9	–	–	–	59	16.9	–
Rh→Mi	52.8	11.5	3.5	23.9	–	–	6783	6.8	76	54	23.9	4
Rh→Mi+Rr	34.6	7.4	2.3	50.0	2621	2.6	4861	4.9	35	35	50.7	4
Rr→Rh	50.3	10.9	3.3	28.3	6287	6.3	–	–	–	52	26.8	–
Mi→Rh	47.7	10.3	3.2	30.4	–	–	8429	8.4	119	47	33.9	5
Mi+Rr→Rh	28.9	6.1	1.8	60.9	3760	3.8	6853	6.9	82	29	59.2	4
Rr+Rh→Mi	32.8	7.0	2.1	54.3	3264	3.3	6458	6.5	43	33	53.5	4
Mi+Rh→Rr	29.4	6.2	1.9	58.7	2966	3.0	6117	6.1	98	30	57.7	4
Rr→Mi+Rh	26.5	6.6	1.7	63.0	3668	3.7	6552	6.6	69	26	63.4	4
Mi→Rr+Rh	25.1	6.3	1.6	65.2	3415	3.4	6727	6.7	71	24	66.2	4
L.S.D. 5%	3.5	2.7	0.25							5.31		
1%	4.7	3.6	0.34							7.12		

UI+B- Uninoculated bacterized, UI+UB - Uninoculated unbacterized; Rr = Rotylenchulus reniformis, *Mi* = Meloidogyne *incognita*, Rh = *Rhizobium.*

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