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EFFECT OF *MELOIDOGYNE INCOGNITA* ON NODULATION AND SYMBIOTIC NITROGEN FIXATION IN PEA

by
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Summary. Inoculation experiments showed that irrespective of the time of inoculation of *Meloidogyne incognita* and *Rhizobium*, the inclusion of nematode in any of the treatments caused significant reduction in plant growth. However, the effect was less significant when *Rhizobium* was already established before the introduction of the nematode. The inclusion of the nematode in any of the treatments caused reduction in the number of nodules. The nematode infection also interfered with the symbiotic nitrogen fixation and reduced the nitrogen content of shoot and root.

Pea (*Pisum sativum* L.) is highly susceptible to root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw. Infected plants are stunted with yellow foliage. Several workers have reported that root-knot nematode causes reduced nodulation in leguminous plants, e.g. *M. javanica* on alfalfa (Nigh, 1966), white clover (Taha and Raski, 1969) and on mungbean (Bopaiah *et al.*, 1976); *M. hapla*, *M. javanica* and *M. incognita* on soybean (Balasubramanian, 1971) and *M. incognita* on mungbean (Hussaini and Seshadri, 1975).

The present work was undertaken to investigate the effect of *M. incognita* on growth, nodulation and nitrogen fixation in pea plants.

Materials and methods

The specific *Rhizobium* culture for pea was procured from the culture collection of the *Rhizobia* Culture Scheme, Durgapura Agricultural Research Station, Jaipur and multiplied on yeast extract mannitol agar (YEMA) medium on a rotatory shaker. For rhizobial treatment 10ml of three days old broth were poured near the root zone. A culture of *M. incognita* maintained on tomato plants was used for inoculation. Egg masses were isolated in sterile water and incubated at room temperature for three days. Two thousand second stage juveniles, in 5ml of H_2O, were pipetted into the root zone of each plant.

Surface sterilized pea cv. Bonnville seeds were sown singly in 15cm polythene lined earthen pots, containing steam sterilized river bed sand which had previously been acid-leached and thoroughly washed. The plants were fed on alternate days, throughout the experimental period, with 100ml of nitrogen free Long Ashton nutrient solution and watered with distilled water as required. Treatments as listed in Table I were replicated five times and applied to one week old seedlings.

TABLE I - *Interaction of* Meloidogyne incognita *and* Rhizobium *sp. and their effect on growth of pea plants.*

Treatments	Shoot length (cm)	Fresh shoot (g)	Dry shoot weight (g)	Root length (cm)	Fresh root weight (g)	Dry root weight (g)
Nematode	29.4	2.6	0.29	15.0	1.7	0.19
Rhizobium	95.0	8.6	0.93	28.4	5.2	0.55
Nematode + *Rhizobium*	43.2	3.7	0.41	14.2	3.4	0.37
Nematode followed by *Rhizobium* after two weeks	35.2	4.6	0.52	16.4	3.5	0.40
Rhizobium followed by nematode after two weeks	54.8	5.7	0.62	17.8	3.9	0.42
Control	80.0	6.8	0.74	21.0	4.4	0.51
CD (5%)	8.42	1.08	0.039	3.12	0.68	0.159
CD (1%)	11.45	1.46	0.053	4.24	0.79	NS

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Measurements were recorded sixty days from the first inoculation on height and fresh and dry weight of roots and shoots. The effect on bacterial nodulation was estimated by counting the number of nodules on the root. The rating of root-knot index of each plant was recorded on the basis of 0-4 scale (0 = no galling; 1 = light galling, 1-50 galls/plant; 2 = moderate galling, 51-100 galls/plant; 3 = heavy galling, 101-150 galls/plant and 4 = severe galling, 151 or more galls/plant). Final soil population of juveniles were extracted by Cobb's sieving and decanting technique and counted.

To study the effect of *M. incognita* on symbiotic nitrogen fixation, plants were uprooted 30, 60 and 90 days from the first inoculation. Shoots and roots were oven dried, powdered and nitrogen was estimated by Snell and Snell method (1957). Each estimate was replicated three times.

Sections of infected nodules were stained in safranin-fast green combination for histopathological studies.

Results and discussion

Bacterial nodules were observed bearing egg masses on their surface, indicating their infection with *M. incognita*. They contained a central bacteroid zone as observed in healthy nodules. Many female nematodes were observed in the cortical region but did not affect the structural integrity of the nodules. Only one female was seen per vascular bundle in which it produced giant cells. The giant cells in bacterial nodules were smaller than those present in the root galls. Others have also observed *Meloidogyne* spp. infested bacterial nodules (Taha and Raski, 1969; Hussaini and Seshadri, 1975; Sharma and Sethi, 1976). Taha and Raski (1969) found that *M. javanica* preferred the nodules in white clover. Such a preference for the nodules was not observed in pea. The infected nodules deteriorated more quickly than the healthy ones. The life of an individual nodule, unlike the root, was of short duration (Nutman, 1958) and hence it was not unusual to find infected nodules which had degenerated.

Root and shoot length and the weight of plants was significantly reduced by nematode infestation (Table I). Nematode inoculation in various combinations retarded the plant growth showing infestation by the nematode as the limiting factor irrespective of the sequence of inoculation in relation to the *Rhizobium*. However, plants showed comparatively better growth when the nematode inoculation was delayed by a fortnight. On the other hand, the delayed inoculation of *Rhizobium* was not sufficiently useful to the plants since the nematode had already established itself (Table I). Heavy damage to the tender roots of the young plants appeared to be the possible explanation for the growth retarding effect of the nematode. Bopaiah *et al.* (1976) observed significant reduction in the growth and dry weight of the shoot and root in mung in the treatments with nematode alone, and nematode followed by *Rhizobium* compared to the treatment with *Rhizobium* followed by the nematode.

Root-knot index was significantly lower in plants inoculated with nematode and *Rhizobium* either together or *Rhizobium* first and nematode afterwards or vice-versa, compared to the nematode inoculation alone. Final soil population of juveniles also showed similar trends (Table II). Thus, the inclusion of *Rhizobium* in any treatment reduced the incidence of root-knot disease.

Nematode infestation significantly reduced the number of nodules per plant (Table II). A greater reduction in number of nodules was observed when the nematode was established before the inoculation of the bacteria (51%) than the nematode and *Rhizobium* inoculated together (30%) or when the *Rhizobium* was established before the inoculation of nematode (12%).

TABLE II - *Interaction of* Meloidogyne incognita *and* Rhizobium *sp. and their effect on galling and nodulation in pea plants.*

Treatments	No. of nodules per plant	Root-knot index	Final soil population per pot
Nematode		3.3	2015
Rhizobium	57	—	00
Nematode + *Rhizobium*	40	2.4	1385
Nematode followed by *Rhizobium* after two weeks	28	2.6	73
Rhizobium followed by nematode after two weeks	51	2.6	1622
Control			00
CD (5%)	17.18	0.41	1.71
CD (1%)	NS	0.57	2.33

Reduced nodulation had been reported by other workers in different leguminous plants infected by root-knot nematodes. However, Taha and Kassab (1980) observed that inoculation of *M. javanica* with *Rhizobia* did not affect the nodulation in *Vigna sinensis* but, Hussey and Barker (1976) reported stimulated nodule formation in soybean infected with *M. hapla* and *Pratylenchus penetrans*.

In all nematode treatments the nitrogen content was significantly reduced compared with plants inoculated with *Rhizobium* only. Maximum reduction in the nitrogen content of shoot (26%) and root (35%) occurred in plants inoculated with nematodes alone (Table III). Nematode induced reduction in symbiotic nitrogen fixation in leguminous plants has been reported in white clover infected by *M. javanica* and *Heterodera trifolii* (Taha and Raski, 1969); in soybean infected by *H. glycines* (Lehman *et al.*, 1971) and cowpea infected by *M. incognita* and *H. cajani* (Sharma and Sethi, 1976). However, Baldwin *et al.* (1979)

reported that nitrogen fixation increased in *M. incognita* infected susceptible and resistant soybean cultivars. They concluded that the disease caused by the nematode in susceptible soybean cultivars was probably not primarily responsible for a net loss of fixed nitrogen but to pathogenicity similar to that which occurred in non-leguminous hosts.

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TABLE III - *Effect of* Meloidogyne incognita *on symbiotic nitrogen fixation in pea.*

Treatments	Nitrogen (%)							
	Shoot Days after inoculation				Root Days after inoculation			
	30	60	90	Mean	30	60	90	Mean
Nematode	1.20	1.36	1.00	1.19	1.10	1.20	0.72	1.01
Rhizobium	1.75	1.96	1.14	1.62	1.59	1.65	1.30	1.51
Nematode + *Rhizobium*	1.43	1.46	0.80	1.23	1.35	1.39	0.82	1.19
Nematode followed by *Rhizobium* after two weeks	35	1.48	0.76	1.20	.20	1.28	0.71	1.06
Rhizobium followed by nematode after two weeks	1.50	1.48	0.94	1.31	1.40	1.44	0.90	1.25
Control	1.37	1.20	1.14	1.24	0.80	1.20	0.70	0.90

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