Natural Rubber Research, **22** (1&2): 127-132, 2009

INTERACTION OF ROOT- KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* AND *BRADYRHIZOBIUM* SP. ON *PUERARIA PHASEOLOIDES*

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Thankamony, S., Kothandaraman, R., Jacob, C.K., Joseph, K. and Jose, V.T. (2009). Interaction of root-knot nematode, *Meloidogyne incognita* and *Bradyrhizobium* sp. on *Pueraria phaseoloides*. *Natural Rubber Research*, **22**(1&2): 127-132.

The effect of interaction of root-knot nematode, *Meloidogyne incognita* and *Bradyrhizobium* sp. on growth, nodulation and nitrogen content in *Pueraria phaseoloides*, a cover crop grown in rubber plantations, was studied in a pot-culture experiment by inoculating different inoculum levels of *M. incognita* and *Bradyrhizobium*. Significant reduction in nodulation due to nematode infestation was observed. Maximum gall formation was recorded in plants inoculated with nematode alone. Simultaneous inoculation of *Bradyrhizobium* and nematode or nematode inoculation after 10 days of bacterial inoculation reduced the adverse effect of nematode. Plant growth was increased by 5 to 13 per cent over control. Nitrogen content in the root and shoot of *P. phaseoloides* was considerably reduced by nematode inoculation at different inoculum levels. However, pre-inoculation of *Bradyrhizobium* could increase nitrogen content of the plants.

Keywords: *Bradyrhizobium*, Interaction, *Meloidogyne incognita*, *Pueraria phaseoloides*.

INTRODUCTION

Natural rubber (*Hevea brasiliensis)* is an important plantation crop in India. A number of agricultural practices have been introduced to improve the production of natural rubber. Establishment of leguminous cover crops in the initial stages of cultivation is an important agronomic practice adopted in rubber plantations (Chin, 1977). Leguminous cover crops enrich soil with nitrogen, prevent soil erosion, reduce soil temperature, augment organic carbon in soil and support soil microbial activity (Punnoose *et al.*, 1994). All these beneficial effects of cover crop help in increasing the growth rate and yield of *H. brasiliensis*. Among the various cover crops used in rubber plantations, *Pueraria phaseoloides* is the most popular one in India and elsewhere (Potty *et al.*, 1980). Several biotic and abiotic factors are reported to influence the growth, nodulation and nitrogen fixation in *P. phaseoloides*. Among them, an important biotic factor is the infection by the root-knot nematode, *Meloidogyne incognita* (Mammen, 1973).

The root-knot nematode, *M. incognita* has caused significant changes in growth, nodulation, nitrogen fixation and

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biochemical constituents in a variety of leguminous crops (Gupta *et al.*, 1986; Kalita and Phukan, 1993). In rubber plantations, even though there are reports on the incidence and intensity of root-knot nematode in *P. phaseoloides* (Mammen, 1973), its adverse effect on the growth of the plant and physico-chemcial activities is lacking. Hence, a pot-culture study was initiated to investigate the interaction of *M. incognita* and *Bradyrhizobium* sp. and its influence on growth of *P. phaseoloides.*

MATERIALS AND METHODS

Sterilised potting mixture (1 kg) containing fine soil, sand and well-decomposed and powdered cow dung in the ratio 2:1:1(w/w) was filled in small (20×15 cm) polythene bags. The bags were kept in another sterilized pot to prevent the entry of soil organisms. Acid-treated seeds of *P. phaseoloides* were sown in bags. One week after germination, the seedlings were thinned to five per bag and inoculated with infective larvae of root-knot nematode (*M. incognita*). The source of nematode inoculum was tomato plants. The root-nodule bacterium, *Bradyrhizobium* sp. of *P. phaseoloides* obtained from the culture collections of Plant Pathology Division, RRII was multiplied in yeast extract mannitol broth and used in the experiment.

The treatments included inoculation of *Bradyrhizobium* sp. alone (R), simultaneous inoculation of *Bradyrhizobium* and nematode (R+N), inoculation of *Bradyrhizobium* followed by nematode after 10 days (R–N), inoculation of nematode followed by *Bradyrhizobium* sp. after 10 days (N–R), nematode alone (N) and control without any inoculation. Nematode was applied at four levels *viz.* $N_1 = 1000$, $N_2 = 2000$, $N_3 = 3000$ and $N_4 = 4000$ nematodes/kg soil. The *Bradyrhizobium* was inoculated at the rate of 10 ml (10^8 cfu/ml) per bag.

All the treatments were replicated three times. Observations were recorded on various growth characters such as shoot length, shoot dry weight, root length, root dry weight, number of galls and nodules and nitrogen content of shoot and root after 60 days of inoculation.

RESULTS AND DISCUSSION

The results presented in Table 1 show that there were significant differences in the growth characteristics of the plants except shoot length between inoculated and uninoculated series. The nematode inoculation at 2000 to 4000 levels significantly reduced the shoot weight. However, the adverse effect of nematode could be reduced by *Bradyrhizobium* either by prior or simultaneous application. The application of bacteria after nematode inoculation (up to 3000 level) could also reduce the ill-effects.

Significant differences in the development of root system were recorded in inoculated and uninoculated plants. Plants inoculated with *Bradyrhizobium* alone showed 33 per cent increase of root length over control. Prior inoculation of nematodes and nematode alone inoculated series showed reduction in the root length of *P. phaseoloides*. The application of nematode @ 1000 did not affect the root length adversely. Nematode inoculation @ 3000 and 4000 significantly reduced root weight. *Bradyrhizobium* inoculation could reduce the negative effect of nematodes on root biomass. The plants inoculated with *Bradyrhizobium* alone and prior bacterial

Table 1. **Effect of *M. incognita* and *Bradyrhizobium* on the growth of *P. phaseoloides***

Treatment	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
R	80.33 (+15.86)	0.73 (+23.72)	32.22 (+32.88)	0.53 (+82.76)
R+N_1	72.66 (+4.80)	0.50 (-15.25)	23.66 (-2.75)	0.37 (+27.59)
R+N_2	65.33 (-5.70)	0.48 (-18.64)	22.33 (-8.22)	0.27 (-6.90)
R+N_3	63.66 (-8.18)	0.42 (-28.81)	19.33 (-20.55)	0.24 (-17.24)
R+N_4	52.00 (-24.94)	0.34 (-42.37)	14.00 (-42.45)	0.22 (-24.14)
R-N_1	74.00 (+6.74)	0.53 (-10.17)	29.33 (-20.55)	0.46 (+58.62)
R-N_2	62.33 (-10.09)	0.47 (-20.34)	23.66 (-2.75)	0.25 (-13.79)
R-N_3	59.00 (-14.89)	0.45 (-23.73)	21.00 (-13.69)	0.22 (-24.14)
R-N_4	51.33 (-25.96)	0.46 (-22.03)	18.66 (-31.52)	0.18 (-37.93)
N_1-R	78.00 (+12.50)	0.41 (-30.51)	16.66 (-31.52)	0.28 (-3.45)
N_2-R	72.30 (+4.32)	0.37 (-37.29)	15.66 (-36.99)	0.26 (-10.34)
N_3-R	56.33 (-18.75)	0.35 (-40.68)	15.33 (-36.99)	0.24 (-17.24)
N_4-R	54.33 (-21.15)	0.28 (-52.54)	14.66 (-39.75)	0.15 (-48.27)
N_1	59.00 (-14.89)	0.38 (-35.29)	20.00 (-17.79)	0.20 (-31.03)
N_2	61.00 (-12.01)	0.31 (-47.46)	15.00 (-38.35)	0.16 (-44.83)
N_3	59.66 (-13.95)	0.29 (-50.85)	14.33 (-41.10)	0.11 (-62.09)
N_4	48.33 (-30.29)	0.26 (-55.93)	10.66 (-56.18)	0.10 (-65.52)
Control	69.33	0.59	24.33	0.29
CD (P = 0.05)	NS	0.25	7.00	0.16

Figures in parentheses represent per cent increase (+)/decrease (-) over control

inoculation @ 1000 level of nematode significantly increased root weight.

Maximum nodulation was recorded in *Bradyrhizobium* alone inoculated plants and was found comparable to simultaneous inoculation along with nematode @ 1000 per bag (Table 2). Simultaneous inoculation showed more nodulation in plants than the prior or later inoculation of both the organisms. The nematode inoculation prior to *Bradyrhizobium* resulted in maximum reduction of nodules. On the other hand, number of galls per plant increased with an increase in nematode inoculum. Significant reduction in gall formation was recorded in prior inoculation of *Bradyrhizobium* at higher levels of nematode inoculation.

Nitrogen content in shoot was significantly higher in prior inoculation of *Bradyrhizobium* followed by nematode application up to 3000 level compared to control (Table 3). But it was significantly lower in nematode alone from 2000 level and simultaneous and post - bacterial inoculation at higher nematode levels. Nitrogen content of root was significantly higher in *Bradyrhizobium* alone inoculated and prior inoculation of *Bradyrhizobium* with nematodes up to 2000 level. Significantly lower nitrogen content was noticed in plants

Table 2. **Effect of *M. incognita* and *Bradyrhizobium* on nodulation and nematode galls of *P. phaseoloides***

Treatment	No. of nodules per plant	No. of galls per plant
R	164	Nil
R+N_1	140 (14.63)	48.00 (5.25)
R+N_2	121 (26.22)	56.33 (3.43)
R+N_3	105 (35.98)	79.33 (4.02)
R+N_4	61 (62.80)	110.66 (7.78)
R-N_1	80 (51.22)	32.00 (36.83)
R-N_2	66 (59.76)	43.66 (25.15)
R-N_3	54 (67.07)	57.00 (31.04)
R-N_4	34 (79.27)	78.88 (34.45)
N_1-R	77 (53.05)	44.33 (12.49)
N_2-R	64 (60.98)	62.66 (8.55)
N_3-R	45 (72.56)	80.33 (2.82)
N_4-R	29 (82.32)	81.66 (31.95)
N_1	Nil	50.66
N_2	Nil	58.33
N_3	Nil	82.66
N_4	Nil	120.00
Control	Nil	Nil
CD (P = 0.05)	28.33	21.64

Figures in parentheses represent per cent decrease of nodules over *Bradyrhizobium* alone inoculated plants and galls over nematode alone inoculated plants.

Table 3. **Effect of *M. incognita* and *Bradyrhizobium* on nitrogen content of *P. phaseoloides* 60 days after inoculation**

Treatment	Nitrogen content (%)	
	Shoot	Root
R	2.60 (+28.07)	2.77 (+37.81)
R + N_1	1.98 (-2.46)	1.93 (-3.98)
R + N_2	1.91(-5.91)	1.86 (-7.46)
R + N_3	1.84 (-9.36)	1.80 (-10.45)
R + N_4	1.78 (-12.32)	1.74 (-13.43)
R-N_1	2.49 (+22.66)	2.47 (+22.89)
R-N_2	2.45 (+20.69)	2.39 (+18.91)
R-N_3	2.30 (+13.30)	2.20 (+9.45)
R-N_4	2.19 (+7.88)	2.16 (+7.46)
N_1-R	2.14 (+5.42)	2.11 (+4.98)
N_2-R	1.77 (-12.81)	1.58 (-21.39)
N_3-R	1.70 (-16.26)	1.54 (-23.38)
N_4-R	1.57 (-22.66)	1.39 (-30.85)
N_1	1.91 (-5.91)	1.96 (-2.48)
N_2	1.80 (-11.33)	1.84 (-8.45)
N_3	1.57 (-22.66)	1.58 (-21.39)
N_4	1.35 (-33.50)	1.47 (-26.86)
Control	2.03	2.01
CD (P = 0.05)	0.18	0.2

Figures in parentheses denote per cent increase (+)/ decrease (-) over control

with prior inoculation of nematodes from 2000 level. Inoculation of nematodes and simultaneous application @ 3000 and 4000 levels significantly reduced nitrogen content compared to control.

Due to mechanical injury caused by nematodes to root tissues, the water absorption efficiency, mineral uptake and total biomass were found reduced in mung bean (Chahal and Chahal, 1989). The effect of interaction of *M. javanica* on growth, nodulation and nitrogen content in *Vigna radiata* has also been reported by Bopaih *et al.* (1976). According to them, the inoculation of root-knot nematode simultaneously or 2 to 7 days preceding *Bradyrhizobium* inoculation had deleterious effect on plant growth compared to plants inoculated with *Bradyrhizobium* alone. Kumar and Vadivelu (1993) reported significant decrease in the growth parameters, nodulation and nitrogen fixation by the interaction of *M. incognita* and *Rhizobium* sp. on black gram, *Vigna mungo*.

Kemenju *et al.* (1999) reported that both nodulation and nitrogen fixation processes were adversely affected especially in plants where nematode inoculation preceded rhizobial inoculation. Wardojo *et al.* (1963) and Taha and Raski (1969) also reported growth reduction in peas, lucerne and clover by the infestation of *Heterodera trifolii, Pratylenchus globulicola* Romaniko and *M. javanica* respectively. They have reported significant reduction in growth, fresh and dry weight of shoot and root in the treatments with nematode alone and nematode followed by rhizobia compared to the treatment, rhizobia followed by nematode.

Significant reduction in nodulation due to root-knot nematode, *M. incognita* observed in the present study is in accordance with earlier reports in cowpea (Sharma and Sethi, 1976) and mung bean (Chahal and Chahal, 1989; Khan, *et al.*, 2006). Nutman (1958) reported that reduced nodulation in the nematode-infested plants might be due to interruption in the translocation of certain materials particularly carbohydrates from the shoot and/or consumption of host plant materials during gall formation or directly by nematodes. Different theories like nutrient deficiency caused by nematodes on host plants (Malek and Jenkins, 1964), competition between nematode and root-nodule bacteria (Ichinohe, 1961; Epps and Chambers, 1962; Malek and Jenkins, 1964), antagonistic effect of root-rot organism upon root nodule bacteria (Epps and Chambers, 1962) and overall reduction of root system (Taha and Raski, 1969) have been put forth as possible causes for reduced nodulations. Daney *et al.* (1970) reported that nematodes can secrete hydrolytic and oxidative enzymes and growth regulators which cause reduction in nodulation, host nutrition and nitrogen fixation. The reduction in nitrogen content is reported to be due to reduced root weight, overall reduction in number of nodules, early degeneration of nodules, destruction of nodular tissue by development of nematode inside nodules and reduced leghaemoglobin content of nodules (Sharma and Sethi, 1975). It may therefore be concluded from the present study that root-knot nematode, *M. incognita* adversely affects the growth and symbiotic process of nitrogen fixation by *Bradyrhizobium* sp. on *P. phaseoloides* .

ACKNOWLEDGEMENT

The authors are thankful to Dr. James Jacob, Director, Rubber Research Institute of India for providing necessary facilities.

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