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Use Of nitrogen fixing bacteria to combat root-knot nematodes

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Two types of bacteria *Azotobacter* and *Rhizobium* sp. were used in *in vivo* pot culture experiment, using okra as host plant, infested with root-knot nematodes. As the *Rhizobium* could not be associated directly to the host plants, these were inoculated in the rhizosphere of the host plants through an associated leguminous plant, grown simultaneously. The objective was to see, whether the interaction of these symbiont, nitrogen fixing bacteria, could reduce the pathogenecity, either by suppressing the activity of nematodes, or by initiating higher rate of growth, in host plants. The host plants were allowed to grow for sixty days, after which, they were harvested. The pre-harvest rate of growth and post-harvest final data of host plants and parasites, were accounted in different parameters, and were statistically compared. Among the inoculated batches of treatments, *Azotobacter* treatment showed significantly better results. *Rhizobium* proved to be less effective.

Key words : Root-knot, *Meloidogyne incognita*, okra plants, N_2 fixing bacteria

INTRODUCTION

The interaction between bacteria and other pathogens has been described by a number of authors, viz. Ark and Thomas (1936), Thorne (1961). But little work has been done to asses the feasibility of using N_2 fixing bacteria to the nematode infestation in different crops. Bopaiah (1979) investigated into the interaction between nitrogen fixing bacteria and *Meloidogyne*. He concluded that, nodulation was significantly reduced, when nematode inoculation preceded *Rhizobium* inoculation.

In the present experiment the use of nitrogen fixing bacteria in controlling the root-knot disease was tried. The objective was to see, whether the interaction of the bacteria could reduce the pathogenecity of the nematodes, either by suppressing the activity of the nematodes, or by effecting higher growth of host plants through supply of nitrogen.

MATERIALS AND METHODS

There were two experiments (repetition of the same) using okra [*Abelmoschus esculentus* (L) Moench] as the host plant. Of the two nitrogen fixing bacteria used ; one was N_2 fixing commercial bacterial manure *Azotobacter croocoum*, and the other *Rhizobium* sp. living as symbiont in the roots of leguminous plants.

The date of sowing of experiments was 10th September, 1999 and for the 2nd experiment 20th November 1999. Sixty earthen pots (25 cm X 25 cm) were filled with an autoclaved mixture of soil and compost manure, 2 : 1. The pots were grouped in five batches ; each batch consisting of 12 pots. All the batches were separately labelled with aluminium foils. The batches were (i) uninoculated untreated (uninoculated control without treatment), (ii) inoculated untreated (2nd control), (iii) uninoculated and treated with *Rhizobium*, (iv) inoculated and treated with *Rhizobium* and (v) inoculated and treated with *Azotobacter*.

Seeds of 'Pusa-sawani' variety of okra were taken, and surface sterilised with 0.1% $HgCl_2$ solution, and then were sown, 2 in each pot. When the seed-

lings were at two leaf stage, one of the plant in each pot was cut at the base of the stem and was removed (this was done to ensure, that each pot must have only one plant).

The larvae of *Meloidogyne incognita* for inoculation, were collected by sieving the infected soil of the green house pots. The screening obtained, were diluted with water, sampled for determination of the population of *M. incognita*, and then applied as inoculum, 250 ml in each pot, (Sukul *et al.*, 1974) at the time of sowing seeds. Altogether three batches of pots were inoculated at the rate of 800 larvae/pot (250 ml contained 800 larvae). The rest two batches served as uninoculated control.

1st batch of uninoculated pots served as uninoculated untreated control. Second batch uninoculated, but grown with a leguminous plant *Trigonella corniculata* (20 plants/pot) around the okra plant, carrying *Rhizobium* along with the test plants (*T. conniculata* is also an edible crop). The 3rd batch was kept as inoculated, but untreated control. The 4th batch was inoculated and was treated with *Rhizobium* (in association with *T. corniculata,* 20 plants/pot), the 5th.. batch was inoculated and was treated with a bacterial manure, *'Azotobacter'*. This contained *Azotobacter chroococum*, and is available in sealed packets of charcoal dust (a product of Nitrofix company). The dose of *Azotobacter* was 6 X 10^8/pot, (as 6 g of manure were used per pot and each g contained 10^8 bacteria).

The test plants were allowed to grow for 60 days, during which, they were regularly irrigated with clean tap water. The rate of growth of the host plants, in different parameters were taken in account, at every 10 days interval ; viz. height, number of branches, number of leaves, number of flowers and fruits. After 60 days, the plants were harvested, i.e. uprooted. The post-harvest data of final growth were recorded in different parameters, viz. root-weight, shoot-weight, root-length, shoot-length. Three types of gall, small, medium and large were counted (Sukul *et al.*, 1974). The final nematode population in the rhizospheric soil was determined. The rate of changes of growth of the host plants, i.e. the regression of the growth was established statistically. The test of significance concerning the differences in final growth between different treatments was performed mainly by the analysis of variance, and by determining the critical differences between the treatments.

OBSERVATION

There was no adverse effect of the bacterial treatments upon the plants, The changes in growth of the pre-harvest plants are shown in Table 1 ; and the post-harvest comparative record of root-weight, root-length, shoot-weight, shoot-length, number of galls and the final rhizospheric population of the parasites. The rate of change in height was maximum in uninoculated batches treated with *Rhizobium* (b=regression coefficient=0.76). Next came uninoculated untreated control (b=0.74). Among the inoculated plants, batches treated with *Azotobactor* showed maximum rate of growth (b=0.63). The rate of growth in height was minimum in inoculated untreated batches. In regard of number of branches, the uninoculated untreated (control) batch showed maximum rate of growth, followed by uninoculated batches treated with *Rhizobium* and also that treated with *Azotobacter* both being equal (b=0.68). The regressions concerning number of leaves, were maximum in two batches, namely uninoculated with *Rhizobium* and inoculated with *Azotobacter*. The minimum in growth of leaves was in inoculated batch (b=0.9). Maximum fruits and flowers were shown by the uninoculated untreated (b=0.14) followed by batch treated with *Azotobacter*. The lowest rate was found in inoculated untreated batch (b=0.08).

Though there had been differences in root weight among different treatments, the *Azotobacter* treatment showed maximum increase in root weight, but the difference was not statistically significant. The differences in root length were not at all significant between the treatments. The shoot weight was maximum in both uninoculated batches. The shoot weight in *Azotobacter* treated batch was relatively lesser but was significantly higher than inoculated untreated (control). The *Rhizobium* did not show significant result. The shoot length also, at per that of shoot weight, i.e. *Azotobacter*, occupied third position, after the two uninoculated batches. The average number of galls and final population in the

Table 1 : Effect of nitrogen fixing bacteria on the rate of growth of Okra plants inoculated with *M. incognita*

	Treatments	Days of Growth 10	20	30	40	50	60	Reg. Co-efficient	Intercept
Height of host plants (cms)	U	9.50	17.75	25.40	33.00	40.33	46.25	0.74	2.79
	UR	9.60	18.30	25.80	33.80	40.90	47.50	0.76	2.21
	I	8.00	12.90	16.75	19.90	23.00	26.08	0.35	5.38
	IR	8.30	13.66	17.80	22.33	26.50	30.00	0.43	4.61
	IA	9.25	15.40	22.60	29.50	34.40	40.40	0.63	3.21
No. of branches	U	0	0	0.33	1.50	2.50	3.10	0.069	-1.1
	UR	0	0	0.66	1.75	2.33	3.25	0.068	-1.0
	I	0	0	0.25	0.90	1.40	1.90	0.04	-0.6
	IR	0	0.16	0.50	1.60	2.40	3.10	0.066	-1.0
	IA	0	0	0.40	1.40	2.25	3.25	0.068	-1.1
No. of leaves	U	4.25	6.10	7.66	9.10	10.33	11.16	0.13	3.2
	UR	4.33	6.16	7.10	9.40	10.10	11.66	0.14	3.04
	I	4.25	5.40	6.50	7.16	8.16	8.40	0.08	3.6
	IR	4.25	6.16	7.66	8.80	10.00	10.80	0.12	3.5
	IA	4.25	6.16	7.75	9.16	10.60	11.33	0.14	3.2
No. Flowers & Fruits	U	0	0	1.16	3.40	5.00	6.50	0.14	-2.2
	UR	0	0	1.00	2.80	4.33	5.66	0.12	-2.0
	I	0	0	0.40	1.60	2.90	4.00	0.08	-1.5
	IR	0	0	0.66	2.58	4.25	5.75	0.12	-2.1
	IA	0	0	0.66	2.80	4.40	6.00	0.13	-2.2

U—Uninoculated control
I—Inoculated control
UR—Uninoculated and treated with *Rhizobium*
IR—Inoculated and treated with *Rhizobium*
IA—Inoculated and treated with *Azotobacter*

rhizospheric soil is presented in Figure 2. Galls of all size i.e. large, medium and small were significantly reduced, only in *Azotobacter* treated plants, among the inoculated batches. The treatment with *Rhizobium* and *Azotobacter*, significantly reduced the population of *M. incognita* in the soil of rhizosphere.

DISCUSSION

The rate of growth in both the uninoculated batch were maximum in most cases. Among the inoculated batches, *Azotobacter* showed maximum efficiency. The treatment with *Rhizobium* could not produce notable growth rate. As for the final growth, the treatment with *Rhizobium* could not show any significant improvement, nor did it curb galling. The treatment with *Azotobacter* produced marked growth (final) in most of growth parameters. It could also reduce the galling incidences and final population of nematodes. Thus *Azotobacter* had an edge over *Rhizobium* in providing higher plant growth and reducing nematode infestation.

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