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SEED TREATMENT WITH NEMATICIDAL *RHIZOBIUM* SPECIES FOR THE SUPPRESSION OF *MELOIDOGYNE JAVANICA* ROOT INFECTION ON MUNGBEAN

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ABSTRACT

During a survey of Karachi and its suberbs from the roots of leguminous plants different *Rhizobium* and *Bradyrhizzobium* species viz., *Rhizobium sp. (Prosopis* isolate), *Bradyrhzobium* sp., (sesbania isolate), *Bradyrhizobium japonicum* (soybean isolate), *Bradyrhizobium* sp. (sesbania isolate), *Rhzobium meliloti* (*Melitotus indicus* isolate) were isolated and identified. Culture filtrates of *Rhizobium* species showed nematicidal effects reduced hatching of eggs of root knot nematode. *Rhzobium meliloti* (*Melilotus* isolate1) showed maximum reduction in egg hatching (59%) followed by *Bradyrhizobium* sp. (*Sesbania* isolate1) which showed 30% reduction in egg hatching. *Rhizobium* species (*Cymopsis* isolate) exerted maximum lethal effects (100%) followed by *Bradyrhizobium japonicum* (Soybean isolate 4) and *Rhizobium* species (*Senna oxidentalis)* resulted in the (95%) juveniles' mortality. Killing of second stage juveniles of *M.javanica* increased with an increase of exposure time. *Rhizobium* species used as seed dressing for the control of root knot nematode, significantly reduced galling as well as improved plant growth in mungbean. Among these isolates *Rhzobium meliloti (Melilotus* isolate 1) showed better growth whereas *Rhizobium* species (*Senna oxidentalis*) found effective against root knot development in all test crops.

Key-words: *Rhizobium* species, root-knot nematode, biological control, seed treatment

INTRODUCTION

Plant diseases produce serious losses to crop plant and adversely affect the agricultural economy of a country. Plant-Parasitic nematodes, are among the most widespread and important pathogens causing serious losses to crop plants. Of the various plant parasitic nematodes, root knot nematodes (*Meloidogyne* spp.) are world wide in their distribution and are known to attack a wide variety of crops (Goodey *et al.*, 1965). Of a total of 70 *Meloidogyne* species identified so far (Luc *et al.*, 1988), only 4 species viz, *M. incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood, *M. arenaria* (Neal), Chitwood and *M. hapla* Chitwood, are of major economic importance. In Pakistan root knot nematodes, *Meloidogyne* (Goeldi) spp. are recognized as important parasites of vegetable crops. About 100 host plants have been found infested with root knot nematodes from different cultivated zones of Pakistan (Zaki, 2000, Maqbool, 1988).

Since life cycle of root knot nematodes is almost completely confined to inside host plants and their enormous reproductive capacity, their control is very difficult. Chemical control of nematodes has proved generally effective but the majority of these chemicals are highly expensive and often hazardous to use. Biological control represents an attractive alternative and practicable agricultural method for the control of plant diseases caused by nematodes. In general, research on the biological control of plant parasitic nematodes has concerned the use of microbial agents, in particular the use of parasitic fungi and bacteria. These agents have been used to control cyst and root knot nematodes, which include the most important nematode pests in world agriculture. In recent years, selected rhizosphere bacteria have been demonstrated to reduce the invasion of root knot and cyst nematodes. *Rhizobium* species are of considerable scientific and economic interest because of their ability to produce nitrogen fixing nodules on leguminous plants (Hynes *et al.*, 1990). The damage caused by nematode was more in unbacterized plants than in bacterized ones. Nematode multiplication reduced in the presence of *Rhizobium* (Siddiqui *et al*., 1995). Considering the dual importance of Rhizobia as nitrogen fixer and as biocontrol agent against root knot nematode, the studies in the use of *Rhizobium* spp., as seed treatment for the control of root knot nematodes were carried out.

MATERIALS AND METHODS

In Vitro studies

After extraction of eggs of root knot nematode from roots, number of eggs per ml of suspension was determined in counting chamber. One ml of egg suspension (25 – 40 eggs) and 1 ml of culture filtrate of each rhizobial isolate was transferred in glass cavity blocks and kept in incubator. There were three replicate of each treatment and hatching of eggs was recorded for 48-96 hours.

Table 1. Effect of *Rhizobium* species on egg hatching % of *Meloidogyne javanica in vitro*.

	Egg Hatching % after		
Treaments	**48 h**	**72 h**	**96 h**
Rhizobium sp. (*Prosopis* isolate)	25	32.8	67
Bradyhizobium sp. (*Sesbania* isolate 1)	22	25	55
B. japonicum (soybean isolate 1)	19	28	61
B. japonicum (soybean isolate 2)	19	47	69
R. meliloti (*Melilotus indicus* isolate 1)	18	30	32
Bradyrhizobium sp. (*Sesbania* isolate 2)	20	33	56
B. japonicum (soybean isolate 3)	10	15	20
B. japonicum (soybean isolate 4)	2	8	12
Bradyhizobium sp. (*Sesbania* isolate 3)	30	63	80
Rhizobium sp. (*Albizia labbeck* isolate)	8	18	30
Rhizobium sp. (*Senna oxidentalis* isolate 1)	4	13	32
Rhizobium sp. (*Cymopsis tetragonatra* isolate)	1	4	10
R. meliloti (*Melilotus indicus* isolate 2)	20	55	82
Rhizobium sp. (*Senna oxidentalis* isolate 2)	7	10	18
Control	23	56	79
LSD 0.05			

Table 3. Effect of *Rhizobium* species on root knot development and growth of mungbean plants.

Treatments	**Pl. Height (cm)**	**Sh. Weight (gm)**	**No. of Galls**	**No. of nodules**
	cm	gm		
Rhizobium. meliloti (*Melilotus indicus* isolate 1)	20	2	4	15
Bradyhizobium japonicum (soybean isolate 4)	20	2	4	18
Rhizobium sp. (*Albizia labbeck* isolate)	17	1	7	15
Rhizobium sp. (*Senna oxidentalis* isolate 1)	18	1	9	14
Rhizobium sp. (*Cymopsis tetragonatra* isolate)	23	2	3	18
Rhizobium sp. (*Senna oxidentalis* isolate 2)	20	1	4	17
Control	16	1	22	13
LSD 0.05				

One ml suspension of freshly hatched juvenile (25-40 juveniles) and 1 ml culture filtrate of each bacterial isolate was transferred separately in glass cavity blocks and kept at 26 ± 5°C. There were three replicates of each treatment and juvenile mortality was recorded for 24-48 hours.

Green-house studies

The selected *Rhizobium* and *Bradyrgizobium* species were grown on yeast extract mannitol agar medium for 48 hours at room temperature. Mungbean (*Vigna radiata)* seeds after surface sterilization in 1% $Ca(OCl)_2$ for 3 minutes, were rinsed several times with tap water and treated with aqueous cell suspension of the Rhizobia using 1% gum arabic as sticker giving a population of 2.0–2.4 x 10^6 cfu per seed. After seed treatment eight seeds were sown in each pot. Seeds treated with distilled water without bacteria served as control. There were three replicates of each treatment. Pots were kept randomized on the green house bench. After germination only two seedlings were retained in each pot. After one week of seedling emergence, roots of each plant were inoculated with 2000 eggs/J_2 of *M. javanica*. Observations on plant height, shoot and root weight and length, infection of root knot nematode were estimated after 45 days of nematode inoculation. Data were analyzed and subjected to analysis of variance (ANOVA) or factorial analysis of variance (FANOVA) depending upon the experimental design according to Gomez and Gomez, (1984). The follow up of FANOVA included Least Significant Difference (LSD).

RESULTS

Culture filtrate of rhizobial strains showed nematicidal effects, reduced the hatching of eggs of *M. javanica* to a varying degree. *Rhizobium* sp. *(Cymopsis* isolate) reduced hatching (87%) of *M. javanica* followed by *Bradyrhizobium* sp. (soybean isolate 4) resulted in the (85%) as compared to control after 96 hours exposure (Table 1). Culture filtrate of rhizobial strains showed nematicidal effects. Killing. of second stage juveniles of *M. javanica* increased with an increase of exposure period. *Rhizobium* sp. (Cymopsis isolate) exerted maximum lethal effects (100%) followed by *Bradyrhizobium japonicum (s*oybean isolate 4*)*) and *Rhizobium* sp. *(Senna oxidentalis)*) resulted in the (95%) juveniles' mortality. *Rhizobium meliloti (Melilotus* isolate 1*),* and *Rhizobium* sp. (*Albizia* and *Senna* oxidentalis isolates) also produced more than 80% mortality of *M. javanica* (Table 2).

Rhizobium and *Bradyrhizobium* spp. used a seed dressing significantly ($P<.0.05$) suppressed root knot development in mungbean. Maximum inhibition in root knot development (>86%) was observed after treatment with *Rhizobium* sp. (*Cymopsis* isolate) followed by *Rhizobium* sp. *(Senna oxidentalis, Melilotus* isolates*)* and Bradyrhizobium sp. (soybean isolate 4) which reduced gall formation by more than 81% as compared to control (Table 3).

Seed treatment with selected *Rhizobium* and Bradyrhizobium species significantly ($p<0.05$) increased plant height and fresh weight of shoot and root length. Rhizobial strain (*Rhizobium* sp. *Cymopsis* soalte) showed maximum plant height (23). *Rhizobium meliloti (Melilotus* isolate 1*)* showed maximum fresh weight of shoot (1.75). While the remaining rhizobial strain showed greater plants height and fresh weight of shoot as compared with control (Table 3).

DISCUSSION

Rhizobium species are of considerable scientific and economic interest because of their ability to produce nitrogen fixing nodules on leguminous plants (Hynes *et al.,* 1990). There are many reports on interaction between nematodes and bacteria (*Rhizobium* sp.) showing diverse effects. Several plant parasitic nematodes with different modes of parasitism cause reduction in nodulation in leguminous plant (Taha, 1993, Epps and Chambers, 1962), nematodes produce stimulation of nodule formation on leguminous plants (Hussey and Barker, 1976; Verdejo *et al.,* 1988) or nematode infection had no significant effect on number and size of nodules (Caroppo and Pelagatte, 1988). The damage caused by nematode was more in unbacterized plants than in bacterized once. Nematode multiplication was reduced in the presence of *Rhizobium* (Siddiqui *et al*., 1995). In previous studies root knot galls produced by *M. javanica* decreased in the presence of *Bradyrhizobium japonicum* and *Rhizobium meliloti* in okra, tomato, chickpea and mungbean (Perveen *et al.,* 1993; Siddiqui *et al.,* 1998). Existence of Rhizobial strains capable of nodulating legumes in presence of pl. parasitic nematode is possible and the discovery and use of these bacteria would be of economic importance. Nematodes on Rhizobia – legume association are not always detrimental, as shown by the stimulation and fixation by some nematodes (Huang, 1987).

In the present studies most of the strains of *Rhizobium* spp. showed nematicidal activity against *M. javanica.* The nematicidal activity varied according to their effectiveness. Fifty percent strains caused more than 50% juvenile mortality of *M. javanica*. While thirty five percent of the total strains tested, produced more than 50% mortality. Similarly the above 21% of total strains resulted in the greater reduction of eggs hatching. Thus, the number of effective rhizobial isolates are greater than that of *Pseudomonas* strains reported by Zavaleta *et al*., (1982) who found 12% of the bacterial isolates tested active against *Meloidogyne.* Oostendorp and Sikora (1989) found only 7.2% of the tested strains antagonistic to *Heterodera schachtii.*

In the present investigation, *Rhizobium* used either as seed dressing improved plant growth and reduced disease intensity of mungbean plants due to initial colonizer of rhizosphere of test plants. It is interesting to note that

rhizobia not only showed significant control of root pathogens on leguminous plants like chickpea, mungbean as well as non-leguminous plants like okra but also increased plant height and fresh shoot weight (Zaki, 2002). The growth promoting effect appears to be direct or indirect. Direct mechanism of growth promotion includes the fixation of atmospheric nitrogen in leguminous plants only, production of plant growth regulators such as auxins, cytokinins and gibberllins like substances (Triplett *et al*., 1981; Evensong and Blevins, 1981; Sheng, 1993) which act directly on plant itself and affect growth. Indirect mechanism may involve the production of toxic metabolites (Chakraborty and Purkayastha, 1984) which have their inhibitory effect on soilborne plant pathogens, thereby increase in plant growth. Rhizobia which live as saprophyte migrate towards the root through chemotectic response and this attraction is not specific as the rhizobia are attracted to other legumes as well as to non-leguminous plants (Currier and Strobel, 1976; Dowling and Broughton, 1986). It would appear that rhizobia which is good rhizosphere organism for leguminous or non-leguminous plants presumably prevents the contact of pathogenic fungi on roots by covering the hyphal tip of the fungus and parasitizing it (Tu, 1978).

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Table 2. Effect of *Rhizobium* species on larval mortality % of *Meloidogyne javanica in vitro*.

	Mortality % after	
Treatments	**24 h**	**48 h**
Rhizobium sp. (*Prosopis* isolate)	14	23
Bradyhizobium sp. (*Sesbania* isolate 1)	20	22
B. japonicum (soybean isolate 1)	12	18
B. japonicum (soybean isolate 2)	14	27
R. meliloti (*Melilotus indicus* isolate 1)	80	90
Bradyrhizobium sp. (*Sesbania* isolate 2)	32	35
B. japonicum (soybean isolate 3)	48	92
B. japonicum (soybean isolate 4)	92	95
Bradyhizobium sp. (*Sesbania* isolate 3)	4	22
Rhizobium sp. (*Albizia labbeck* isolate)	71	87
Rhizobium sp. (*Senna oxidentalis* isolate 1)	78	87
Rhizobium sp. (*Cymopsis tetragonatra* isolate)	99	100
R. meliloti (*Melilotus indicus* isolate 2)	10	17
Rhizobium sp. (*Senna oxidentalis* isolate 2)	71	95
Control	0	1
LSD 0.05		