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Evaluation of Rhizobia for the Control of *Meloidogyne javanica* in *Vigna mungo*

Imran Ali Siddiqui, Amer-Zareen, S. Shahid Shaukat and M. Javed Zaki
Soil-borne Diseases Research Laboratory, Department of Botany,
University of Karachi, Karachi-75270, Pakistan

Abstract: Efficacy of different species of rhizobia including, *Rhizobium trifolii*, *R. phaseoli*, *R. meliloti*, *Bradyrhizobium japonicum* and *Bradyrhizobium* sp., were tested in the control of *Meloidogynejavanica*, the root-knot nematode on *Vigna mungo* under laboratory, greenhouse and field conditions. Cell-free culture filtrates of the rhizobium species significantly reduced egg hatching and caused mortality of *M.javanica* larvae *In vitro*. In pot experiments and under field conditions, *Bradyrhizobium* sp., gave better biocontrol along with enhanced plant growth and nodulation.

Key words: Rhizobia, culture filtrate, cell suspension, root-knot nematode

Introduction

Vigna mungo (L.) Hepper an important pulse crop which is rich in protein, is cultivated over an area of 0.57 million hectares yielding 0.28 million tones of uridbean in Pakistan (Anonymous, 1997). The root-nodule bacteria of the genus rhizobium are of considerable scientific and economic interest because of their ability to produce nitrogen-fixing nodules in leguminous plants (Hynes and O' Connel, 1990). Several plant-parasitic nematodes with different mode of parasitism have been found to cause reduction in nodulation in leguminous crops (Taha and Kassab, 1993). In the recent past, rhizobia have gained reputation of controlling soil-borne root-infecting fungi (Ehteshamul-Haque and Ghaffar, 1993) and the root-knot nematode (Siddiqui *et al*., 1998), besides fixation of the atmospheric nitrogen. This study evaluates rhizobia in the control of *Meloidogyne javanica* root-knot nematode in *Vigna mungo*.

Materials and Methods

Rhizobial strains (*Rhizobium trifolii*, Karachi University Culture Collection-842, *Trifollium alexandrianum* isolate; *R. phaseoli*, KUCC-844, *Phaseolus lanatus* isolate; *R. meliloti*, KUCC-845, *Melilotus alba* isolate; *Bradyrhizobium* sp., KUCC-819, uridbean isolate and *B. japonicum*, KUCC-569, soybean isolate), were multiplied on yeast extract mannitol broth at 30 °C for 48 h in dark. Bacterial cultures were centrifuged at (2,800 X g for 20 min) to obtain cell-free culture filtrate. Egg masses of *Meloidogyne javanica* (Treub.) Chitwood obtained from pure culture maintained in a glasshouse on the roots of aubergine, *Solanum melongena* L., were placed in distilled water and incubated at room temperature for 24h. Freshly hatched juveniles were collected and suspension of juveniles in distilled water was prepared.

To study the effect on egg hatch of *M.javanica*, one-ml of the culture filtrate was transferred into glass-cavity-slide, to which two medium sized hand-picked egg masses from the knot of aubergine were added. After 48 h, the number of hatched juveniles was counted under stereomicroscope. The egg masses from the bacterial culture filtrate were then transferred to distilled water and their hatching was recorded to ascertain whether the egg masses kept in the filtrate had been permanently or temporarily inactivated. The emergence of juveniles was recorded again after 48 h.

To test the effects of rhizobium species on mortality of *M. javanica* larvae, one-ml of the culture filtrate was transferred into cavity-glass-slides. One-ml suspension of the juveniles was added to each cavity glass slide (20-25 juveniles/cavity glass slide). Juveniles kept in sterile distilled water served as controls. Each treatment was replicated thrice. The cavity slides were incubated at room temperature ($25 \pm 3^{\circ}C$) and juvenile mortality was recorded after 48 h of exposure.

Sandy-loam soil (sand : silt : clay, 70 : 19 : 11) at pH 8.1 with a water holding capacity of 34% obtained from experimental field of Department of Botany, University of Karachi, was potted in 8-cm-diam plastic pots, (350 g /pot). Seeds of *Vigna mungo* after surface sterilization in 1% $Ca(OCl)_2$ for three minutes were rinsed several times with tap water and treated with five day old rhizobial culture using 1% gum arabic as sticking substance giving a population of 2.2-2.4 x 10^6 cfu per seed. After seed treatment, eight seeds were sown in each pot and seedlings were thinned to four per pot. Seeds treated with sterile distilled water served as controls. Each treatment was replicated thrice and pots were randomized on the greenhouse bench of Soil-borne Disease Research Laboratory, University of Karachi. One week after seedling emergence, each pot was inoculated with 2000 freshly hatched juveniles of *M. javanica*. Plants were uprooted 45 days after the addition of nematodes and plant height, fresh weight of shoot and number of nodules per root system were recorded. Numbers of galls per root system were counted under a stereomicroscope.

Rhizobial species (*Rhizobium meliloti* and *Bradyrhizobium* sp.,) which showed promising results in control of root-knot nematode both *In vitro* and in pots were used to test their biocontrol potential under field conditions. Experiment was carried in 2 x $1m^2$ microplots at the field of Department of Botany, University of Karachi in a randomized complete block design with three blocks. Seeds of *Vigna mungo* after treatment with rhizobial species were sown in 1¼ m furrows. Seeds treated with sterile distilled water served as controls. One-week-old seedlings were inoculated with 1000 freshly hatched juveniles of *M. javanica*. The juveniles were suspended in 25-ml water and poured into three holes made around the roots. The experiment was terminated 60 days of nematode inoculation and plant growth parameters, number of nodules and number of galls induced by *M. javanica* were recorded as described earlier. Number of egg masses were counted under a stereomicroscope. Nematode population density in the soil was estimated following Baermann funnel technique. Data was subjected to analysis of varaince (ANOVA) followed by least significant difference and Duncan's multiple range test to compare the means (Sokal and Rohlf,1995).

Results and discussion

Maximum inhibition of egg hatching (69%) and larval mortality (68%) were caused by *R. meliloti* while *Bradyrhizobium* sp.gave 62% inhibition of egg hatching and 47% mortality of *M. javanica* juveniles (Table 1). In the greenhouse experiments, Rhizobial spp., significantly ($p < 0.05$) reduced gall formation due to *M. javanica* in uridbean with a maximum reduction where *Bradyrhizobium* sp., was used. *R. meliloti* gave maximum plant height while the use of *Bradyrhizobium* sp. resulted in highest fresh weight of shoot and number of

Table 1: Effects of different species of rhizobium on egg hatching and juvenile mortality of *Meloidogyne javanica*.

Treatments	No. of eggs hatched		Mortality %
	Culture filtrate	Distilled water*	
Control	149a	64a	0d
Rhizobium trifolii	89b	62a	30bc
Rhizobium phaseoli	77b	49b	17cd
Rhizobium meliloti	52c	22c	69a
Bradyrhizobium sp.	84b	37b	47ab
Bradyrhizobium japonicum	90b	46b	28bc
LSD $p<0.05$	24	11	23

Means followed by the same latter in each column are not significantly different at $p<0.05$ according to Duncan's multiple range test.
*Egg masses transferred from bacterial culture filtrate into distilled water after 48 hrs.

Table 2: Effects of different species of rhizobium on the development of root-knot infection due to *Meloidogyne javanica*, plant growth and nodules production in *Vigna mungo* under greenhouse conditions.

Treatments	Galls per root system	Plant height (cm)	Shoot weight (g)	Nodules per root system
Control	64	16.4	0.79	5.0
Rhizobium trifolii	57	19.5	0.95	6.7
Rhizobium phaseoli	53	19.1	0.94	8.9
Rhizobium meliloti	46	20.9	0.99	9.4
Bradyrhizobium sp.	41	19.8	1.06	18.4
Bradyrhizobium japonicum	63	18.8	0.86	7.4
LSD $p<0.05$	9	2.9	0.4	3.2

Table 3: Effects of different species of rhizobium on the development of root-knot infection due to *Meloidogyne javanica*, plant growth and nodules production in *Vigna mungo* under field conditions.

Treatments	Galls/ Root system	Egg mass/ Root system	Nematode/ 250 cc soil	Plant height (cm)	Shoot weight (g)	Nodules / Root system
Control	83	18	2010	24.2	7.4	7
Rhizobium meliloti	67	7	1740	31.4	10.8	10
Bradyrhizobium sp.	56	5	1410	35.5	13.2	22
LSD $p<0.05$	13	6	230	4.7	3.6	4.3

nodules (Table 2).

In the field experiment, *R. meliloti* and *Bradyrhizobium* sp., significantly ($p<0.05$) inhibited root-knot development as compared to controls. Application of the bacterial antagonists although did reduce to some extent nematode population in soil but such effects were non-significant. Maximum suppression in gall formation (>32% over the controls) and nematode population in soil (>29% over controls) was achieved following seed treatment with *Bradyrhizobium* sp. which also gave maximum plant height, fresh shoot weight and number of nodules per plant (Table 3).

Of the rhizobial spp. used, *R. meliloti* and *Bradyrhizobium* sp., reduced root-knot formation, enhanced plant growth with increased number of nodules per plant. Rhizobia which are known to produce Rhizobiotoxine (Chakraborty and Purkayastha, 1984) have shown promising results in the control of soil-borne root-infecting fungi in sunflower, okra, soybean and mungbean (Ehteshamul-Haque and Ghaffar, 1993) and *Meloidogyne javanica* root-knot nematode in mungbean (Siddiqui *et al.*, 1998). Host recognition is thought to be controlled by the interaction between root surface lectins and nematode cuticular carbohydrates (Zuckerman, 1983). Rhizobia are gram-negative and may have lectin binding structures in the lipopolysaccharide layer of cell wall membrane (Lotan *et al.*, 1975). Therefore, the mechanism responsible for reduction in nematode penetration may be related to the ability of the bacteria to envelop or bind to root surface lectins, thereby interfering with the normal host recognition (Oostendorp and Sikora, 1990). Presumably, the rhizobia also produce metabolite(s) that have nematicidal activity. Further investigation is needed to find the possible mechanism whereby rhizobia suppress the root-knot nematode. The results of the present study seem to suggest that rhizobia could be used as effective biocontrol agents of the root-knot nematode besides enhancing the crop growth.

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