Use of plant growth-promoting rhizobacteria (PGPR) and soil organic amendments for the management of root diseases complex of uridbean

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Summary

Efficacy of two strains of *Pseudomonas aeruginosa* (Pa-5 and IE-2) and a *Bacillus subtilis* isolate alone or in conjunction with neem cake or *Datura fastuosa* was tested for the management of three soilborne root-infecting fungi including *Macrophomina phaseolina, Fusarium solani* and *Rhizoctonia solani* and the root-knot nematode, *Meloidogyne javanica* on uridbean. Biocontrol bacteria used in combination with either neem cake or *D. fastuosa* gave better control of the root-rot and root-knot infection with the enhancement of growth of uridbean compared to the use of either component alone. Neem cake 1% w/w mixed with *P. aeruginosa* strain IE-2 caused greatest inhibition of the root-knot development due to *M. javanica. P. aeruginosa* and *B. subtilis* used with organic amendment also increased *Bradyrhizobium*-nodules in the root system.

Key words: Pseudomonas aeruginosa, Bacillus subtilis, neem cake, Datura fastuosa, root-knot nematode, root-infecting fungi

INTRODUCTION

Control of soilborne plant diseases by the application of specific microorganisms to seed or planting material has been studied extensively over the last 2 decades. Among the plant growth-promoting rhizobacteria (PGPR), the fluorescent Pseudomonas spp., have received much attention. Certain strains of fluorescent pseudomonas have been shown to suppress various plant diseases caused by soilborne plant pathogens, including deleterious microorganisms (S c h i p p e r s, 1992; W e l l e r, T h o m a s h o w, 1994; S i d d i q u i et al., 2000). Organic amendments that are generally used for the improvement of crop plants and increasing soil fertility have also shown a suppressive effect on plant pathogenic fungi and nematodes (A 1 a m, 1990; E h t e s h a m u l-H a q u e et al., 1995). Of the organic substrates, neem cake and Datura fastuosa L. have shown promising results in the control of root-infecting fungi viz., Macrophomina phaseolina Tassi (Goid), Fusarium solani (Mart) Appel & Wollenw. emend. Snyd. & Hans., and Rhizoctonia solani Kühn (E h t e s h a m u l--H a q u e et al., 1996) and Meloidogyne javanica (Treub.) Chitwood, the root-knot nematode (A b i d, 1996; S h a h w a r et al., 1994). Since organic amendment of soil enhances the activity of biocontrol agents in the suppression of plant pathogens (Cook, 1977; Sitaramaiah, 1990), experiments were conducted to examine the effects of soil organic amendments with neem cake or Datura fastuosa on the efficacy of Pseudomonas aeruginosa (Schroeter) Migula and Bacillus subtilis Cohn in the control of root-knot nematode and root- infecting fungi on uridbean (Vigna mungo (L.) Hepper).

MATERIALS AND METHODS

Sandy loam soil (pH 8.1) obtained from the experimental field of the Department of Botany, University of Karachi was used. The soil was naturally infested with 3-9 sclerotia g⁻¹ of soil of Macrophomina phaseolina as estimated by wet sieving and dilution technique (S h e i k h, G h a f f a r, 1975); 5.8% colonization of R. solani of sorghum seeds used as baits adapted from the method of Wilhelm (1995); and 2800 cfu g¹ of soil of Fusarium spp., as estimated by soil dilution technique (N a s h, S n y d e r, 1962). The soil was mixed with powdered neem cake or air-dried leaves of Datura fastuosa at 0.5% and 1% (w/w) then transferred in 8-cm-diam. plastic pots and kept moist to allow decomposition of the plant material. After 3 weeks, aqueous cell suspension of P. aeruginosa strain Pa-5 (3.5 x 108 cfu ml1); IE-2 (2.9 x 108 cfu ml⁻¹) and B. subtilis (3.0 x 10⁸ cfu ml⁻¹) isolated from the rhizosphere of sunflower and multiplied on Nutrient Agar medium for 5 days were drenched in each pot at 25-ml/pot and kept at 50% WHC. Eight seeds of uridbean were sown in each pot and after germination only four seedlings retained in each pot. Soil without organic amendment and/or bacterial inoculum served as control. Treatments were replicated three times and pots were randomized on a greenhouse bench of Soilborne Disease Research Laboratory, Department of Botany, University of Karachi in September 1997. In another similar set 2000 eggs and J2 of M. javanica obtained from aubergine roots

were inoculated near the root zone of 5-day-old seedlings. Plants were uprooted 6 weeks after nematode inoculation and plant growth parameters such as plant height, root length and fresh weight of shoot and root and number of nodules per plant were recorded. Number of galls induced on the entire root system were counted under low magnification (x 6). To determine the incidence of fungi, roots from each plant were cut into 5-mm-long pieces, surface sterilized with 1% Ca(OCl)₂ and plated onto PDA plates supplemented with penicillin (100,000 units/l.) and streptomycin sulfate (0.2 g/l.). The plates were incubated at room temperature for 5 days and incidence of root-infecting fungi estimated as follows:

Data were subjected to analysis of variance (ANOVA) followed by least significant difference or Duncan's multiple range test in accordance with S o k a l, R o h l f (1995).

RESULTS

Soil amendment with neem cake and *D. fastuosa* 0.5% and 1.0% w/w with or without PGPR significantly (p<0.001) suppressed root-knot formation induced by *M. javanica* on uridbean roots (Table 1). Maximum suppression in root-knot development was achieved in the treatment where neem cake 1.0% w/w was used with *P. aeruginosa* strain IE-2. Use of neem cake and *D. fastuosa* 1.0% w/w had a pronounced effect in the suppression of root-knot disease when used with the bacterial antagonists (Fig. 1).

There was a significant (p<0.001) difference among treatments on root infection caused by *M. phaseolina*. Nematode-infested and non-infested soils also differed significantly as *M. phaseolina* infection was found greater in nematode-infested soil compared to non-infested soil (Table 1; Fig. 1). In nematode-infested soil, a complete control of *M. phaseolina* infection was observed in treatment where strain Pa-5, neem cake 0.5% w/w or *D. fastuosa* 1.0% w/w were used separately or where neem cake 0.5% or 1.0% (w/w) were used with Pa-5 or IE-2. Other treatments provided more than 50% reduction in *M. phaseolina* infection. In non-infested soil, *P. aeruginosa* strains Pa-5 and IE-2 when used individually showed 75% inhibition in *M. phaseolina* infection as compared to other treatments that completely prevented *M. phaseolina* infection.

In nematode-infested soil, F. solani infection was suppressed by >50% where B. subtilis used alone, strain Pa-5 mixed with neem cake 0.5 or 1% w/w, strain IE-2 used with neem cake at both the dosages and B. subtilis was used with either neem cake 1% w/w or D. fastuosa 0.5% w/w. In non-infested soil, strain IE-2 used alone, strain Pa-5 mixed with either neem cake 0.5% w/w or D. fastuosa 0.5% w/w, strain IE-2 mixed with neem cake 0.5 or 1% w/w or with D. fastuosa 1.0% w/w and B. subtilis used with all the organic substrates at all the dosages inhibited F. solani infection by >50% (Fig. 1).

Bacterial antagonists used separately or mixed with various organic substrates significantly (p<0.001) inhibited *R. solani* infection in uridbean roots (Table 1). In nematode-infested soil, *D. fastuosa* 1.0% w/w showed complete control of *R. solani* infection. Similarly, strain IE-2 or *D. fastuosa* 0.5% w/w used individually, neem cake 0.5% w/w mixed with Pa-5 or IE-2, neem cake 1.0% w/w with IE-2, *D. fastuosa* 1.0% w/w used with Pa-5 or IE-2 and *D. fastuosa* 0.5% used with Pa-5 showed more than 50% suppression in *R. solani* infection. In non-infested soil, a complete control of *R. solani* infection was observed in the treatment where *D. fastuosa* 0.5% w/w was used alone. Similarly, *D. fastuosa* 1.0% w/w used alone, neem cake 1.0% w/w used with strain Pa-5, *D. fastuosa* 1.0% w/w used with *P. aeruginosa* strains and *D. fastuosa* at both the dosages used with *B. subtilis* produced >50% suppression of *R. solani* infection (Fig 1).

Table 1.

Summary of the results from the analyses of variance (F values) of the effects of soil organic amendments on the efficacy of *Pseudomonas aeruginosa* on root-knot development, infections of *Macrophomina phaseolina*, *Fusarium solani* and *Rhizoctonia solani* on uridbean

Source	RKI	M. phaseolina	F. solani	R. solani	Plant height	Shoot weight	Root weight	Nodules/ root system
Main								
Effects								
Treatments	15.80	7.99	1.43	3.26	5.91	4.94	2.77	4.42
(T)	***	***	NS	***	***	***	***	***
Soil type†	Not	9.61	1.36	0.32	5.98	0.57	4.05	12.13
(ST)	applicable	**	NS	NS	*	NS	*	***
Interaction								
T x ST	Not applicable	1.06 NS	1.10 NS	1.68 NS	3.47	1.82	3.96	1.99

[†] Soil with and without nematode infestation.

Rhizobacterial strains used separately or in conjunction with neem cake or *Datura fastuosa* increased plant height (p<0.001), fresh weight of shoot (p<0.001) and root p<0.001 (Table 1). In nematode-infested soil, maximum plant height (>73% increase over controls) was observed where strain IE-2 of *P. aeruginosa* was used in conjunction with neem cake 1.0% w/w whereas in non-infested soil, strain IE-2 mixed with *D. fastuosa* 1% w/w showed maximum plant height of >28% over control. *B. subtilis* used alone or IE-2 mixed with neem cake 1.0% w/w showed greatest fresh weight of shoot of >56% over control in nematode-infested soil whereas in non-infested soil, strain IE-2 used in combination with *D. fastuosa* 1.0% w/w showed greatest fresh weight of shoot (>66% increase over control). Neem cake 1.0% w/w in non-infested soil and untreated control plants in nematode-infested soil produced greatest fresh weight of roots (Fig. 2).

Treatments showed significant differences in production of nodules by *Bradyrhizobia*. Lesser number of nodules per root system were produced in nematode-infested soil as compared to non-infested soil. *D. fastuosa* 1.0% w/w used alone in nematode-

^{***,} p<0.001; **, p<0.01; * p<0.05; NS, non-significant.

infested soil (>111% increase) and neem cake 1.0% w/w in non-infested soil (>105% increase) produced maximum number of *Bradyrhizobium*-nodules per plant (Fig. 2).

DISCUSSION

In the present study, use of neem cake and *Datura* with or without plant growth-promoting rhizobacteria significantly suppressed root-infecting fungi and root-knot nematode in uridbean. Neem that is well known to possess many nematicidal and larvicidal compounds (K h a n et al., 1974; R o s s n e r, Z e b i t z, 1987) was found effective in the control of plant parasitic nematodes (A l a m, 1990). Soil amendment with neem cake and *Datura fastuosa* showed control of root-infecting fungi and root-knot nematode in okra (E h t e s h a m u l-H a q u e et al., 1996; S h a h w a r et al., 1994). Addition of organic matter to the soil is reported to produce an ecological succession of microorganisms and successive phase of biochemical degradation guide and control the orderly arrangement of natural enemies of nematodes (Y a d a v, A l a m, 1993). In a previous report, addition of chitin to soil at 1% (w/w) eliminated plant-parasitic nematodes in a first planting of cotton cv. 'Rowden' and significantly reduced *Meloidogyne incognita* infestation in a second planting, confirming long-term nematode suppressiveness induced by this organic amendment. The chitin amendment was also associated with an increase in fungal and bacterial populations, especially those with chitinolytic activity (H a l l m a n n et al., 1999).

The results of the present study clearly suggest that combined use of rhizosphere bacteria and neem cake or *Datura fastuosa* have great potential in the control of root infecting fungi and plant parasitic nematodes.

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Wpływ bakterii korzystnie oddziaływujących na rośliny i dodatków organicznych wprowadzanych do gleby na kompleks chorób korzeni Vigna mungo (L.) Hepper.

Skuteczność dwóch szczepów Pseudomonas aeruginosa (Pa-5 i IE-2) oraz izolatu B. subtilis użytego osobno lub łącznie z makuchami nasion drzew Azadirachta indica lub Datura fastuosa badano w stosunku do trzech chorobotwórczych grzybów przeżywających w glebie (Macrophomina phaseolina, Fusarium solani i Rhizoctonia solani) oraz nicienia Meloidogyne javanica na roślinach Vigna mungo (L.)Hepper. Biologiczne zwalczanie bakteriami użytymi łącznie z makuchami A. indica lub D. fastuosa bardziej skutecznie ograniczały porażenie korzeni, a jednocześnie bardziej nasilały wzrost roślin V. mungo w porównaniu do zabiegów, w których użyto je osobno. 1 % makuchu A. indica zmieszany z bakterią P. aeruginosa szczep IE-2 powodował najsilniejsze zahamowanie rozwoju guzowatości korzeni powodowanego przez M. javanica. P. aeruginosa i B. subtilis zwiększały występowanie brodawek Bradyrhizobium na systemie korzeniowym V. mungo.