http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 3 (4): 627-629, 2000
© Copyright by the Capricorn Publications, 2000

Use of *Pseudomonas aeruginosa* with *Memnoniella echinata* in Soil Amended with Neem Cake and Chemical Fertilizers for the Management of Root-rot and Root-knot Disease in Mungbean

Imran Ali Siddiqui, Syed Ehteshamul-Haque, M. Javed Zaki and Abdul Ghaffar
Soil-borne Diseases Research Laboratory, Department of Botany, University of Karachi, Karachi-75270, Pakistan

Abstract: *Pseudomonas aeruginosa*, a plant growth-promoting rhizobacterium and *Memnoniella echinata,* a fungal antagonist used separately or in combination in soil amended with neem cake and/or chemical fertilizers significantly suppressed root infecting fungi viz., *Macrophomina phaseolina, Fusarium solani* and *Rhizoctonia solani* and the root-knot nematode *Meloidogyne javanica* in mungbean. Soil amendment increased biocontrol and growth promoting potential of *P. aeruginosa* and *M. echinata*.

Key words: *Pseudomonas aeruginosa, Memnoniella echinata,* neem cake, urea, potash, root-infecting fungi, root-knot nematode

Introduction

Fluorescent Pseudomonads are a group of bacteria that have long been found in the rhizosphere of a wide range of plants (Curl and Truelove, 1986). Many isolates from this group are known to demonstrate antagonistic activity against other micro-organisms of the soil and therefore, have been intensively used in trials for the biological control of soil-borne plant diseases. *Memnoniella echinata* (Riv.) Golloway, a cosmopolitan dematiaceous fungus is well known as a decomposer of cellulosic material (Ellis, 1971) has been reported to inhibit growth of root-infecting fungi *in vitro* (Abbas and Ghaffar, 1973) and in green house conditions (Siddiqui *et al*., 1998). Oil cakes are generally rich in mineral ingridients as nitrogen, phosphorus and potash (Akhtar, 1991). Use of oil cakes of castor (Mehta *et al*., 1994), ground nut (Ali and Mien, 1989) and neem (Goswami, 1993; Rathore, 1994) has shown good control of plant parasitic nematodes. An experiment was therefore carried out to evaluate the effects of *M. echinata* and *Pseudomonas aeruginosa*, a plant growth-promoting rhizobacterium (Siddiqui *et al*., 1999b) in soil with or without amendment of soil with neem cake and/or chemical fertilizers for the control of soil-borne root-infecting fungi viz., *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium solani* and the root-knot nematode *Meloidogyne javanica* in mungbean.

Materials and Methods

The soil used for the experiment was a sandy-loam of pH 8.1 obtained from the experimental field of the Department of Botany, University of Karachi. The soil had a natural population of 6-8 sclerotia of *M. phaseolina* as estimated by wet sieving and dilution technique (Sheikh and Ghaffar, 1975), 4.5% colonization of *R. solani* on sorghum seeds used as baits (Wilhelm, 1955) and 3000 cfu g^{-1} of soil of mixed population of *Fusarium* spp., as determined by soil dilution technique (Nash and Snyder, 1962). Powdered neem cake mixed in soil, at 1% w/w were transferred in 8-cm diam., plastic pots at 350 gm in each. In another set urea (at 0.15 g/kg soil) and potash (at 0.12 g/kg) with or without neem cake were used. The pots were watered daily and kept at 50% W.H.C. (Keen and Raczkowski, 1921). After three weeks, a 25 ml conidial or cell suspension of *M. echinata* at 2.1×10^8 cfu/ml and *P. aeruginosa* at 2.5×10^8 cfu/ml were drenched in soil. Eight seeds of mungbean (*Vigna radiata* (L.) Wilczek) were sown in each pot. Each treatment was replicated three times and the pots were randomized on a screen house bench. After germination only 4 seedlings were maintained in each pot. After one week of the seedling emergence, 2000 eggs/J2 of *M. javanica* were inoculated near the root zone by making three holes around mungbean roots. Observations on root infection caused by root-knot nematode and root-infecting fungi were recorded. To determine the incidence of root-infecting fungi, roots after washing in running tap water were surface disinfested with 1% $Ca(OCl)_2$ and one cm long root pieces from tap root were transferred on to PDA plates containing penicillin (100,000 units/l.) and streptomycin sulphate (0.2 g/l). The dishes were incubated for 5 days at 28°C to confirm infection and colonization of roots by soil-borne root infecting fungi. Infection percentage was calculated as follows:

$$\text{Infection (\%)} = \frac{\text{No. of plants infected by a pathogen}}{\text{Total no. of plants}} \times 100$$

Plant growth parameters in terms of plant height, shoot weight, root weight and number of *Rhizobium*-nodules per plant were also recorded. Data were analyzed and subjected to one way analysis of variance (ANOVA) followed by Standard Error of the Difference between means (SED) according to Gomez and Gomez (1984).

Results

Treatment showed significant difference on the development of root-knot infection in mungbean. Maximum suppression in root-knot development was achieved in the treatment where *P. aeruginosa* was used in combination with neem cake and urea followed by with neem cake-potash *P. aeruginosa* treatment which resulted in >49 percent inhibition in root-knot disease (Table 1). Treatments showed non-significant difference in the suppression of *M. phaseolina, F. solani* and *R. solani* infections. More than 50 percent inhibition in *M. phaseolina* infection was observed in the treatment where neem cake used alone, *P. aeruginosa* used with *M. echinata, P. aeruginosa* mixed with potash, *M. echinata* mixed with either neem cake, urea or potash, *P. aeruginosa* either mixed with neem cake and urea or neem cake and potash and *M. echinata* either mixed with neem cake and urea or neem cake and potash. Similarly, neem cake, urea and *P. aeruginosa* used individually, *P. aeruginosa* and *M. echinata* used together, *P. aeruginosa* used with urea, *M. echinata* used with either neem cake or urea and a *P. aeruginosa*-neem cake-potash treatment showed more than 50 percent inhibition in *F. solani* infection. Almost all the treatments suppressed *R. solani* infection by more than 50 percent (Table 1).

Neem cake used with potash produced maximum plant height whereas a neem cake-urea *M. echinata* treatment resulted in

Table 1: Effect of neem cake and chemical fertilizers on the efficacy of *Pseudomonas aeruginosa* and *Memnoniella echinata* on the development of root-knot and root-rot infection in mungbean

Treatments	Galls per root system	*M. phaseolina*	*F. solani*	*R. solani*
		Infection %		
Control	51	92	25	85
Neem cake (NC)	40	25	8	83
Urea (U)	40	67	8	8
Potash (P)	49	50	17	33
Pseudomonas aeruginosa (PA)	37	50	8	8
Memnoniella echinata (ME)	46	47	17	33
PA + ME	34	8	8	17
NC + U	34	42	17	8
NC + P	37	42	19	8
NC + PA	27	47	19	0
U + PA	34	50	0	17
P + PA	35	33	17	0
NC + ME	37	33	0	33
U + ME	41	25	8	50
P + ME	44	22	17	0
NC + U + PA	25	42	17	17
NC + P + PA	25	42	0	25
NC + U + ME	34	25	17	0
NC + P + ME	37	19	17	8
SED	3	23	14	30
Significance level (p)	<0.0001	NS	NS	NS

Table 2: Effect of neem cake and chemical fertilizers on the efficacy of *Pseudomonas aeruginosa* and *Memnoniella echinata* on the development of root-knot and root-rot infection in mungbean

Treatments	Plant height (cm)	Shoot weight (g)	Root Weight (g)	Nodules / Plant
Control	22.1	2.0	0.4	6.5
Neem cake (NC)	22.4	2.6	0.5	13.9
Urea (U)	24.5	2.7	0.5	15.0
Potash (P)	27.6	2.6	0.6	14.4
Pseudomonas aeruginosa (PA)	25.5	2.9	1.0	13.2
Memnoniella echinata (ME)	27.8	3.0	0.6	12.1
PA + ME	23.5	2.5	1.1	17.2
NC + U	26.9	3.0	0.5	13.5
NC + P	31.1	3.3	0.7	10.9
NC + PA	28.5	3.3	0.7	19.6
U + PA	25.1	2.3	0.8	19.0
P + PA	25.8	2.5	0.7	22.2
NC + ME	26.0	3.2	1.1	17.1
U + ME	27.3	2.5	1.4	22.7
P + ME	23.9	2.8	0.8	19.1
NC + U + PA	28.1	3.2	0.7	20.6
NC + P + PA	26.5	3.0	1.2	19.2
NC + U + ME	25.5	3.5	0.9	21.8
NC + P + ME	24.3	3.0	1.0	11.1
SED	1.5	0.3	0.2	4.0
Significance level (p)	<0.0001	<0.001	<0.05	<0.05

maximum fresh weight of shoot. Similarly, a neem cake-potash *P. aeruginosa* treatment produced greatest fresh weight of roots. Urea mixed with *M. echinata* produced highest number of *Bradyrhizobium*-nodules per root system (Table 2).

Discussion

The nutrition of a plant determines in large measure its resistance or susceptibility to disease, its histological or morphological structure or properties, the function of tissues to hasten or slow pathogenesis, and the virulence and ability of pathogens to survive. Non availability of nutrient elements needed to synthesize chemical and physical barriers or the diversion of elements into metabolic cul-des-sacs around infection sits, can result in susceptability to disease (Huber, 1980). In the present study, *P. aeruginosa* and *M. echinata* used in soil amended with neem cake and/or chemical fertilizers effectively suppressed root-infecting fungi and the root-knot nematode. Chemical fertilizers have been reported to suppress root-rot and root-knot diseases in mungbean (Siddiqui *et al.*, 1999a). Potassium is also known to reduce *Fusarium oxysporum* infection on tomato (Eliot, 1973) and *Rhizoctonia solani* infection on hemp (Pal and Chaudhary, 1980). Use of potash has shown reduction in *M. javanica* population (Oteifa, 1953). The deficiency of potash increases root exudation (Marschner, 1986), hence attract more micro-organisms in the rhizosphere. The mechanism involved in the control of plant-parasitic nematodes by urea was described by Huebner *et al*.

(1983) where urea when added to soil is finally converted into ammonia gas and high concentration of ammonia have shown nematicidal effect in killing nematodes (Rodriguez-Kabana *et al*. 1987). There are reports where neem cake and neem oil used as seed treatment were found effective in reducing root penetration by *M. incognita* juveniles in mungbean (Vijayalakshmi and Goswami, 1986). Reduced hatching and inhibited penetration by *M. incognita* was also observed by Mojumder and Mishra (1991) when chickpea seeds were soaked in neem cake and *Brassica rapa* extracts.

In the present study, *P. aeruginosa* and *M. echinata* used alone or in combination showed significant suppression in root-knot diseases. *P. aeruginosa* has been reported to control root-rot and root-knot diseases in chilli (Siddiqui *et al*., 1999b) and tomato (Siddiqui *et al*., 1999c). Similarly, root infection caused by *M. phaseolina, F. solani* and *R. solani* was significantly suppressed following application with *M. echinata* (Siddiqui *et al*., 1998).

It is interesting to note that biocontrol agents used in soil amended with neem cake and/or chemical fertilizers showed better results as compared to their separate use. Addition of organic matter to soil stimulates the microbial activity of bacteria, fungi, algae and other micro-organisms (Rodriguez-Kabana *et al*. 1987).

The result of the present study would therefore suggest that *P. aeruginosa* and *M. echinata* with a compatible amendment could be use in the suppression of soil-borne root-infecting fungi and plant-parasitic nematodes which may results in better plant growth.

References

Abbas, S.Q. and A. Ghaffar, 1973. Inhibition of certain fungi by *Memnoniella echinata*. Pak. J. Bot., 5: 169-169.

Akhtar, M., 1991. Studies on the management of plant parasitic nematodes with organic soil amendments. Ph.D. Thesis, Aligarh Muslim University, India.

Ali, M.R. and H. Mien, 1989. Control of potato root-knot nematode by cotton seed and mustard oil cakes. Bangladesh J. Plant Pathol., 5: 7-12.

Curl, E.A. and B. Truelove, 1986. The Rhizosphere. Springer-Verlag, Berlin, Germany, ISBN-13: 9783540158035, Pages: 288.

Eliot, C.W., 1973. Soil fertility and disease development. Better Crop Plants Food, 57: 6-8.

Ellis, M.B., 1971. Dernatiaceous Hyphomycetes. CMI Publication, USA., pp: 608.

Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agriculture Research. 2nd Edn., John Wiley and Sons Inc., New York, USA., Pages: 680.

Goswami, B.K., 1993. Effect of different soil amendments with neem cake on root-knot nematode and soil Mycoflora in Cow pea Rhizophere. Indian J. Plant Prot., 21: 87-89.

Huber, D.M., 1980. The Role of Mineral Nutrition in Defense. In: Plant Pathology: An Advance Treatise, Volume 5, Horsfall, J.G. and E.M. Cowling (Eds.). Academic Press, New York, pp: 381-406.

Huebner, R.A., R. Rodriguez-Kabana and R.M. Patterson, 1983. Hemicellulosic waste and urea for control of plant parasitic nematodes: Effect on soil enzyme activities. Nematropica, 13: 37-54.

Keen, B.A. and H. Raczkowski, 1921. The relation between the clay content and certain physical properties of a soil. J. Agric. Sci., 11: 441-449.

Marschner, H., 1986. Mineral Nutrition of Higher Plants. Academic Press, London, pp: 671.

Mehta, U.K., P. Sundararaj and N. Natesan, 1994. Effect of five oil cakes on control of *Pratylenchus* zeae in sugarcane. Nematol. Mediterr., 22: 219-220.

Mojumder, V. and S.D. Mishra, 1991. Nematicidal efficacy of some plant products and management of *Meloidogyne incognita* in pulse crops by soaking seeds in their aqueous extracts. Curr. Nematol., 2: 27-32.

Nash, S.M. and W.C. Snyder, 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology, 52: 567-572.

Oteifa, B.A., 1953. Development of the root-knot nematode Meloidogyne incognita as affected by potassium nutrition of the host. Phytopathology, 43: 171-174.

Pal, A.K. and K.C.B. Chaudhary, 1980. Effect of phosphorus and potassium on disease development in sunnhemp. Indian J. Agric. Sci., 50: 952-954.

Rathore, S., 1994. The effect of neem, mustard and karanj cakes against reniform nematode *Rotylenchulus reniformis* on cowpea. Indian J. Mycol. Plant Pathol., 24: 51-52.

Rodriguez-Kabana, R., G. Morgan-Jones and I. Clift 1987. Biological control of nematodes: Soil amendments and microbial antagonists. Plant Soil, 100: 237-247.

Sheikh, A.H. and A. Ghaffar, 1975. Population study of sclerotia of *Macrophomina phaseolina* in cotton fields. Pak. J. Bot., 7: 13-17.

Siddiqui, I.A., E. Haque and A. Ghaffar, 1998. Effect of rhizobia and fungal antagonists in the control of root infecting fungi on sunflower and chickpea. Pak. J. Bot., 30: 279-286.

Siddiqui, I.A., S. Ehteshamul-Haque and A. Ghaffar, 1999a. Effects of *Pseudomonas aeruginosa* and chemical fertilizers on root-rot and root-knot diseases of mungbean. Pak. J. Nematol., 17: 77-86.

Siddiqui, I.A., S. Ehteshamul-Haque and A. Ghaffar, 1999b. Root dip treatment with *Pseudomonas aeruginosa* and *Trichoderma* spp. in the control of root rot-root knot disease complex in Chili (*Capsicum annum* L.). Pak. J. Nematol., 17: 67-75.

Siddiqui, I.A., H. Bashir, S. Ehteshamul-Haque, V. Sultana, J. Ara, M.J. Zaki and A. Ghaffar, 1999c. Organic amendments for the control of *Meloidogyne javanica* in tomato. I. Effects on *Pseudomonas aeruginosa*. Pak. J. Nematol., 17: 173-180.

Vijayalakshmi, K. and B.K. Goswami, 1986. Effect of seed treatments with neem cake and neem oil on the germination of moong (green gram) and its vulnerability to root-knot nematode. Int. Nematol. Network Newslett., 3: 8-9.

Wilhelm, S., 1955. Longevity of the *Verticillium* wilt fungus in the laboratory and field. Phytopathogy, 45: 180-181.