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EFFECT OF ORGANIC AMENDMENTS ON THE EFFICACY OF *PSEUDOMONAS AERUGINOSA* IN THE CONTROL OF ROOT ROT DISEASE OF SUNFLOWER

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Abstract

Soil amendment with neem seed cake, cotton seed cake, *Datura fastuosa* and *Steochospermum marginatum* significantly ($p<0.05$) reduced *Fusarium solani* whereas neem seed cake and cotton seed cake were effective against *Macrophomina phaseolina* and *Rhizoctonia solani*, while *Steochospermum marginatum* and *D. fastuosa* against *Rhizoctonia solani* infection on sunflower roots. *Pseudomonas aeruginosa*, the plant growth promoting bacterium significantly controlled root rot disease of sunflower caused by *M. phaseolina*, *R. solani* and *F. solani*. Use of *P. aeruginosa* with neem seed cake and *S. marginatum* produced greater fresh weight of shoot and plant height respectively as compared to their separate use.

Introduction

Sunflower (*Helianthus annuus* L.) an important oil seed crop is cultivated over an area of 25,899 hectares in Pakistan (Anon., 1990). Of the various disease causing organisms which adversely affect crop productivity, the charcoal rot fungus *Macrophomina phaseolina* has a very wide host range and attacks the root and basal stem of sunflower resulting in premature ripening of fruit leading to small head, poorly filled seed and reduction in yield (Sackston, 1981). Similarly *Rhizoctonia solani* and species of *Fusarium* also infect the roots of sunflower (Ehteshamul-Haque & Ghaffar, 1994). Organic amendment with botanical toxicants have shown promising results in the control of root infecting fungi on crop plants (Ehteshamul-Haque *et al.*, 1995; 1996). In recent past *Pseudomonas aeruginosa* the plant growth promoting bacterium also showed effective control of root infecting fungi (Ehteshamul-Haque, 1997; Izhar *et al.*, 1995). Experiments were therefore carried out to examine the effect of organic amendments on the efficacy of *Pseudomonas aeruginosa* in the control of root infecting fungi on sunflower.

Materials and Methods

Experiment were carried out in 2x1 meter microplots at the Department of Botany, University of Karachi in 1995 in randomized complete block design with three replicates. The soil had a natural infestation of 4-10 sclerotia g^{-1} of soil of *Macrophomina phaseolina* as found by using wet seiving and dilution technique (Sheikh

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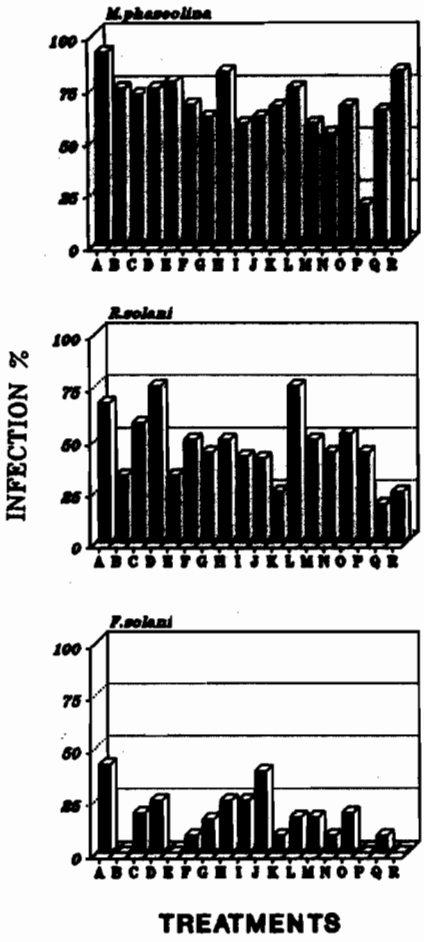

Fig.1.Effect of *Pseudomonas aeruginosa* with organic amendment in the control of *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium solani* infection on sunflower roots.

A = Control, B = *Datura fastuosa* @ 20 g/l meter (DF_1), C = D. fastuosa @ 100 g/l meter (DF_2), D = *Steochospermum marginatum* @ 20 g/l meter (SM_1), E= *S. marginatum* @ 100 g/l meter (SM_2), F = Neem seed cake @ 20 g/l meter (NC_1), G = Neem seed cake @ 100 g/l meter (NC_2). H = Cotton seed cake @ 20 g/l meter (CC_1), I = Cotton seed cake @ 100 g/l meter (CO_2), J = *Pseudomonas aeruginosa* (Pa), K = DF_1 + Pa, L = DF_2 + Pa, M = SM_1 + Pa, N = SM_2 + Pa, O = NC_1 + Pa, P = NC_2 + Pa, Q = CC_1 + Pa, R = CC_2 + Pa.

$LSD_{0.05}$ Treatments = 26.3. Pathogens = 10.7.

& Ghaffar, 1975), 5-13% colonization of *Rhizoctonia solani* on sorghum seeds used as baits (Wilhelm, 1955) and 3200 cfu g^{-1} of soil of mixed population of *F. solani* and *F. oxysporum* as assessed by soil dilution technique (Nash & Snyder, 1962). Powdered neem seed cake, cotton seed cake, *Datura fastuosa* and *Steochospermum marginatum* a seaweed were mixed in sandy loam soil @ 20 gm and 100 gm/ 1 meter row. The plots were watered at 3 day interval and after 3 weeks aqueous suspension of *P. aeruginosa* ($8.1x10^8$ cfu ml^{-1}) multiplied on Nutrient Agar were drenched @ 200 ml per row. Soil without any amendment and *P. aeruginosa* served as control. Thirty seeds of sunflower (*Helianthus annuus*) cultivar Ho-1 were sown in each row. Plants were uprooted after 30 days growth. After washing in tap water 5 cm long root pieces from each plant were cut, surface sterilized with 1% $Ca(OCl)_2$ for 3 minutes and transferred on to PDA plates containing penicillin (100000 units/litre) and streptomycin (0.2 gm/litre). Plates were incubated for 5 days at 28°C and incidence of root infecting fungi viz., *M. phaseolina, R. solani*, and *Fusarium* spp., were recorded. Data on plant height and fresh weight of shoots were also recorded. Data were analysed and subjected to factorial ANOVA followed by Least Significant Difference (LSD) according to Gomez and Gomez (1984).

Results

Soil amendments with seed neem cake (@ 20 and 100 gm/1 meter row), cotton seed cake (@ 100 gm/1 meter row) and *Pseudomonas* used alone significantly ($p<0.05$) controlled the infection of *M. phaseolina*. Combined use of *P. aeruginosa* with neem cake or *S. marginatum* (at both dosages) and *Datura* and cotton seed cake at low dosage also significantly ($p<0.05$) reduced *M. phaseolina* infection. Maximum reduction in *M. phaseolina* infection was observed where neem seed cake @ 100 gm/1 meter row was used with *P. aeruginosa* (Fig. 1). Infection of *R. solani* was significantly ($P<0.05$) reduced where soil was amended with *Datura* or *S. marginatum* (@ 100 gm/1 meter row) or *P. aeruginosa* was used alone. Maximum control of *R. solani* infection was observed where *P. aeruginosa* was used with cotton seed cake (Fig. 1). Complete control of *F. solani* infection was observed where soil was amended with *Datura* or *S. marginatum* or *P. aeruginosa* was used with neem seed cake or cotton seed cake at high dosage. *P. aeruginosa* also significantly ($p<0.05$) reduced *F. solani* infection when used with *Datura*, *S. marginatum*, neem seed cake or cotton seed cake at both dosages (Fig. 1).

Maximum fresh weight of shoot was produced by *P. aeruginosa* used with neem seed cake (@ 100 gm/1 meter row) followed by *P. aeruginosa* mixed with cotton seed cake (@ 100 gm/1 meter row). Greater plant height was produced by *P. aeruginosa* used with *S. marginatum* followed by *P. aeruginosa* mixed with *Datura* @ 100 gm/1 meter row (Fig. 2).

Discussion

In the present study soil amendment with *D. fastuosa, S. marginatum* neem seed cake and cotton seed cake used as organic substrates showed significant control of root

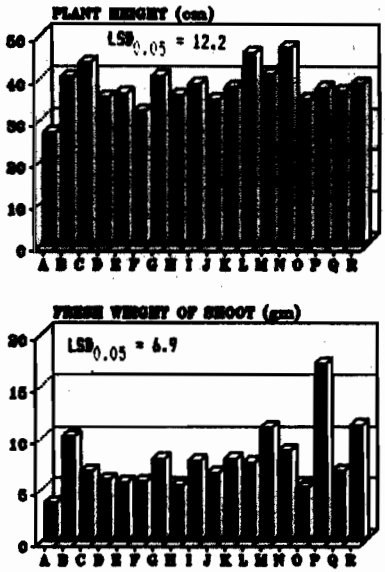

Fig.2.Effect of *Pseudomonas aeruginosa* with organic amendment on the growth of sunflowr plants: A = Control, B = *Datura fastuosa* @ 20 g/l meter (DF_1), C = D. fastuosa @ 100 g/l meter (DF_2), D = *Steochospermum marginatum* @ 20 g/l meter (SM_1), E= *S. marginatum* @ 100 g/l meter (SM_2), F = Neem seed cake @ 20 g/l meter (NC_1), G = Neem seed cake @ 100 g/l meter (NC_2). H = Cotton seed cake @ 20 g/l meter (CC_1), I = Cotton seed cake @ 100 g/l meter (CO_2), J = *Pseudomonas aeruginosa* (Pa), K = DF_1 + Pa, L = DF_2 + Pa, M = SM_1 + Pa, N = SM_2 + Pa, O = NC_1 + Pa, P = NC_2 + Pa, Q = CC_1 + Pa, R = CC_2 + Pa.

infecting fungi of sunflower. Growth inhibition of fungi by the plant extract has been reported (Agarwal, 1978; Miah *et al.*, 1990). Oil cakes have been found to inhibit the growth of *Sclerotium rolfsii* (Ramarao & Raja, 1983). Neem cake commonly used as a natural pesticide (Vyas *et al.*, 1990) inhibited the growth of *R. solani* and *M. phaseolina* and reduced pre-emergence and post emergence mortality of cotton (Jeyarajan *et al.*, 1987). Soil amendment with neem seed cake, *Datura* powder and *S. marginatum* significantly reduced root rot disease in okra caused by *M. phaseolina, R. solani* and *F. solani* (Ehteshamul-Haque *et al.*, 1996).

In the present study, *Pseudomonas aeruginosa* the plant growth promoting bacterium significantly reduced infection of root rot pathogens. *P. aeruginosa* showed better control of root infecting fungi and enhanced plant growth when used with organic amendment. A better control of root infecting fungi has been reported in mungbean by bradyrhizobia with organic substrate than either used alone (Ehteshamul-Haque *et al.*, 1995). A combined use of rhizobia with *S. marginatum* also showed better growth of okra (Ehteshamul-Haque *et al.*, 1996). Fluorescent *Pseudomonas* has also been reported to reduce *Fusarium* wilt in carnation (Yuen *et al.*, 1985), *Pythium* disease in wheat

and also significantly increased wheat yield (Weller & Cook., 1986). Among the fluorescent *Pseudomonas*, *P. aeruginosa* has also been reported to reduce growth of *R. solani, Sclerotium rolfsii, M. phaseolina, F. oxysporum* and *F. solani* (Podile, *et al.*, 1988; Ehteshamul-Haque, 1997; Izhar *et al.*, 1995). Use of plant growth promoting bacteria with organic substrate for the control of soil borne root infecting fungi holds promise.

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