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USE OF SEA WEED AND BACTERIA IN THE CONTROL OF ROOT ROT OF MASH BEAN AND SUNFLOWER

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Abstract

A significant increase in growth parameters in terms of shoot length, shoot weight and a significant reduction in infection of *Fusarium* spp, *Rhizoctonia solani* and *Macrophomina phaseolina* was observed where *Rhizobium meliloti* treated seeds of mash bean and sunflower were used in *Melanothanus afaqhusainii* amended soil. Combined use of *Pseudomonas aeruginosa* with sea weeds viz., *Melanothanus afaqhusainii, Padina tetrastromatica, Cystoclonium purpuraeum* and *Hypnea valentiae* significantly reduced the infection of *M. phaseolina* and *R. solani* on mash bean and sunflower followed by *R. meliloti* and *B. subtilis* in combination with *C. purpuraeum* and *M.afaqhusainii* respectively on mash bean and sunflower. Seed treatment was found more effective method in controlling the root infecting fungi as compared to soil treatment.

Introduction

Sea weeds have received the attention of plant scientists throughout the world (Atzmon *et al*., 1994, Staden *et al*., 1995), since it contains greater potash and nitrogen as compared to farm yard manure (Chapman & Chapman, 1980) and thus provide better plant growth. Pratt *et al*., (1951) reported antimicrobial activity of some sea weeds. The marine plants constitute a prodigious source of new and structurally complex secondary metabolites (Atta-ur-Rehman *et al*., 1991). In many countries, commercial sea weed fertilizer has been marketed as soil additives and foliar spray (Paracer *et al*., 1987). Many plant growth promoting rhizobacteria (PGPRS) have a beneficial effect on plants, besides biological control of soil borne pathogens, induce systemic resistance to plant pathogens, phytoharmonic productions and improvement of nutrition and water uptake of plants (Seuk Bae *et al*., 2000). Of the *Pseudomonas* species *P. aeruginosa* has been found as an effective biocontrol agent of root rot pathogens (Izhar *et al*., 1995). Similarly *Rhizobia*, the root nodulating bacteria, are also known to control soil borne root infecting fungi (Zaki & Ghaffar, 1987; Ehteshamul-Haque & Ghaffar, 1993; Siddiqui *et al.*, 1998). Experiments were therefore carried out to study the use of sea weeds and bacteria in the control of root rot of mash bean (*Vigna mungo* (L.) and sunflower (*Helianthus annus* L.).

Materials and Methods

Seaweeds like *Melanothanus afaqhusainii, Padina tetrastromatica*, *Cystoclonium purpuraeum* and *Hypnea valentiae* were collected from Buleji, Karachi, washed with tap water, dried under shade, powdered in an electric grinder and kept at room temperature. Cultures of *Rhizobium meliloti* (R5), *Pseudomonas aeruginosa* (P-58), *Bacillus subtilis* (B-35) were obtained from Department of Botany, University of Karachi. Soil used for the experiment was obtained from the experimental plots of the Department of Botany, University of Karachi and sieved through 2mm sieve to discard particles. The soil used

was sandy loam (Sand, Silt, Clay; 70, 19, 11%), pH range from 7.5 - 8.1 with moisture holding capacity (MHC) of 24.04 % (Keen & Raczkowski, 1922), total nitrogen 1.5 % (Mackenzie & Wallace, 1954), total organic matter 2.4 %. Soil had natural infestation of 1-3 sclerotia of *Macrophomina phaseolina* as found by wet sieving dilution technique (Sheikh & Ghaffar, 1975), 5-10% colonization of *Rhizoctonia solani* on sorghum seeds used as baits (Wilhelm, 1955) and 3000cfu of *Fusarium* spp., as assessed by soil dilution technique (Nash & Snyder, 1962). Soil was amended with seaweeds viz., *M. afaqhusainii*, *P. tetrastromatica*, *C. purpuraeum*, *H. valentiae* @ 1 % w/w and transferred in 8 cm diameter plastic pots, each pot containing 300 g soil and soil moisture adjusted and maintained at 50 % MHC (Keen & Raczkowski, 1922). Pots were kept for 2 weeks for the decomposition of the organic substrate. Soil was drenched with 20 ml water containing cell suspension of *R. meliloti* (R5) ($7.48x10^8$ cells/ml), *B. subtilis* (B-35) ($12.93x10^8$ cells/ml), *P. aeruginosa* (P-58) ($6.23x10^8$ cells/ml). Mash bean and sunflower were used as test plants. Eight seeds were sown in each pot. Pots without seaweed and bacterium served as control.

In another treatment, seeds of mash bean were treated with cell suspension of *B. subtilis* containing ($5.68x10^7$ cells/seeds), *R. meliloti* ($8.98x10^7$ cells/seeds) and *P. aeruginosa* ($12.43x10^7$ cells/seeds) whereas in sunflower cell suspension containing ($9.28x10^7$ cells/seeds), ($13.46x10^7$ cells/seeds) and ($16.98x10^7$ cells/seeds) respectively were used and sown in soil amended with seaweeds. There were three replicates of each treatment and pots were randomized on a green house bench. After 30 days, roots of sunflower and mash bean were washed in a running tap water, surface sterilized in 1% Ca $(OCl)_2$ and then five 1 cm long root pieces were transferred on PDA plates containing penicillin @100,000/litre and streptomycin @ 20mg/l. Petri dishes were incubated for 5 days at room temperature to confirm infection of root infecting fungi. After 30 days of growth, plants were uprooted. Plants growth parameters in terms of root length, shoot length and fresh weights of shoot, root and incidence of root infecting fungi viz., *M. phaseolina*, *R. solani* and *Fusarium* spp., were recorded. Data were analyzed and subjected to analysis of variance (ANOVA) following the procedure as given by Gomez & Gomez (1984).

Results and Discussion

Significant increase in shoot length in mash bean and sunflower ($p<0.001$) was observed where *R. meliloti*, *B. subtilis* and *P. aeruginosa* were drenched in *P. tetrastromatica* amended soil ($p<0.01$) (Table 1). There was a significant increase in shoot length ($p<0.05$), shoot weight ($p<0.01$), root length and root weight ($p<0.01$) when *B. subtilis* were drenched in *C. purpuraeum* amended soil (Table 1) whereas combined use of *P. aeruginosa* with sea weeds viz., *M. afaqhusainii*, *P. tetrastromatica*, *C. purpuraeum* and *H. valentiae* significantly reduced the infection of *M. phaseolina* and *R. solani* on mash bean and sunflower followed by *R. meliloti* and *B. subtilis* in combination with *C. purpuraeum* and *M.afaqhusainii* respectively on mash bean and sunflower (Table 1) whereas *P. aeruginosa* in combination with *H. valentiae* significantly reduced the infection of *Fusarium* spp. Sunflower and mash bean treated seeds with *R. meliloti* when used in *M. afaqhusainii* amended soil significantly increased the growth parameters in terms of shoot length, shoot weight ($p<0.05$) and significantly reduced the infection of *Fusarium* spp., *M. phaseolina* and *R. solani* ($p<0.05$) whereas *P. aeruginosa* treated seeds of mash bean and sunflower in *C. purpuraeum* and *H. valentiae* treated soil showed a significant increase in shoot length ($p<0.05$) and reduced the infection of *Fusarium* spp., and *M.phaseolina* ($p<0.05$) (Table 2).

Table 1. Effect of soil treatment with biocontrol bacteria in seaweeds amended soil on growth and infection of mash bean and sunflower roots by *Fusarium* spp., *Macrophomina phaseolina* and *Rhizoctonia solani.*

Treatments	Plant growth parameters				Infection %		
	Shoot length (cm)	Shoot weight (gm)	Root length (cm)	Root weight (gm)	*Fusarium* spp.	*Macrophomina phaseolina*	*Rhizoctonia solani*
Mash bean							
Control	21.6	1.37	5.33	0.973	100	83.3	83.3
M. afaqhusainii	27.5	2.26	4.543	1.186	83.3	83.3	50
P. tetrastromatica	26.7	1.55	5.76	0.506	100	50	0
C. purpuraeum	23.8	1.34	5.11	0.183	50	50	50
H. valentiae	18.8	1.43	4.93	0.253	66.6	66.6	50
R. meliloti	25.3	1.296	5.773	0.706	66.6	0	83.3
P. aeruginosa	28.5	1.46	6.663	0.863	66.6	50	50
B. subtilis	23.46	0.896	4.543	0.476	100	33.3	50
M. afaqhusainii + R. meliloti	20.8	1.106	4.063	0.55	50	16.6	33.3
M. afaqhusainii + P. aeruginosa	19.55	0.886	4.186	0.263	50	66.6	50
M. afaqhusainii + B. subtilis	22.23	1.086	8.11	0.663	83.3	66.6	50
P. tetrastromatica + R. meliloti	27.03	0.91	5.986	0.343	100	66.6	50
P. tetrastromatica + P. aeruginosa	25.5	0.906	4.52	0.32	100	50	50
P. tetrastromatica + B. subtilis	27.15	1.23	4.16	0.33	83.3	50	83.3
C. purpuraeum + R. meliloti	24.26	1.543	4.866	0.573	50	0	83.3
C. purpuraeum + P. aeruginosa	21.16	1.34	5.493	0.506	33.3	50	66.6
C. purpuraeum + B. subtilis	19.36	1.51	5.94	0.416	100	50	50
H. valentiae + R. meliloti	17.46	1.13	6.776	0.586	83.3	66.6	66.6
H. valentiae + P. aeruginosa	20.96	20.1	5.966	0.43	66.6	33.3	33.3
H. valentiae + B. subtilis	21.4	1.37	4.86	0.36	100	66.6	50
LSD 0.05 =	**7.56**	**0.95**	**3.00**	**0.58**	**193.29**	**44.33**	**67.61**

Table 1. (Cont'd.).

atments	Plant growth parameters				Infection %		
	Shoot length (cm)	Shoot weight (gm)	Root length (cm)	Root weight (gm)	*Fusarium* spp.	*Macrophomina phaseolina*	*Rhizoctonia solani*
flower							
trol	28.53	0.973	3.933	0.263	100	100	100
yfaqhusainii	25.63	1.306	4.716	0.376	100	66.6	100
etrastromatica	27.63	1.43	5.553	0.276	83.3	66.6	33.3
urpuraeum	29.33	10.8	4.333	1.133	50	66.6	83.3
valentiae	28.5	0.466	3.666	0.216	83.3	66.6	66.6
neliloti	27.96	1.406	7.966	0.463	66.6	83.3	100
eruginosa	28.42	1.0	4.753	0.498	50	33.3	50
ubtilis	25.56	0.853	5.82	0.513	83.3	66.6	50
yfaqhusainii + R. meliloti	28.16	2.03	4.833	0.566	66.6	83.3	66.6
yfaqhusainii + P. aeruginosa	27.7	1.35	3.776	0.3	66.6	66.6	83.3
yfaqhusainii + B. subtilis	24.283	1.386	4.166	0.526	16.6	83.3	16.6
etrastromatica + R. meliloti	15.7	0.91	4.766	0.52	66.6	83.3	83.3
etrastromatica + P. aeruginosa	26.16	0.69	5.033	0.27	66.6	83.3	33.3
etrastromatica + B. subtilis	29.2	1.336	3.266	0.41	100	100	66.6
urpuraeum+ R. meliloti	28.43	1.3	5.433	0.266	33.3	100	16.6
urpuraeum + P. aeruginosa	26.94	1.836	4.333	0.513	100	16.6	50
urpuraeum + B. subtilis	32.1	1.866	5.553	1.066	33.3	83.3	83.3
valentiae + R. meliloti	28.56	1.55	5.553	1.243	66.6	100	66.6
valentiae + P. aeruginosa	27.63	1.386	4.943	1.42	33.3	16.6	16.6
valentiae + B. subtilis	28.26	1.85	4.776	0.58	33.3	83.3	33.3
0.05=	5.86	0.79	2.39	0.58	59.55	55.76	58.89

Table 2. Effect of seed treatment with biocontrol bacteria in seaweeds amended soil on growth and infection of mash bean and sunflower roots by *Fusarium* spp., *Macrophomina phaseolina* and *Rhizoctonia solani*.

Treatments	Plant growth parameters				Infection %		
	Shoot length (cm)	Shoot weight (gm)	Root length (cm)	Root weight (gm)	*Fusarium* spp.	*Macrophomina phaseolina*	*Rhizoctonia solani*
Mash bean							
Control	18.96	0.963	8.086	0.456	100	100	100
afaqhusainii	23.26	0.953	4.833	0.47	100	83.3	66.6
tetrastromatica	24.73	1.226	5.773	0.423	83.3	66.6	83.3
purpuraeum	21.43	1.296	5.32	0.306	50	50	50
valentiae	23.1	0.673	4.853	0.293	66.6	66.6	50
meliloti	21.4	1.216	6.22	0.586	33.3	100	66.6
aeruginosa	23.16	0.82	5.266	0.496	16.6	83.3	100
subtilis	22.3	0.973	6.633	0.543	100	100	100
afaqhusainii + *R. meliloti*	24.73	1.47	5.533	0.383	50	50	50
afaqhusainii + *P. aeruginosa*	23.86	1.48	4.653	0.513	83.3	83.3	100
afaqhusainii + *B. subtilis*	26.03	1.886	5.776	0.396	83.3	83.3	83.3
tetrastromatica + *R. meliloti*	23.73	0.97	5.2	0.23	83.3	100	100
tetrastromatica + *P. aeruginosa*	26.06	1.083	6.443	0.383	83.3	66.6	100
tetrastromatica + *B. subtilis*	25.93	1.47	6.053	0.52	66.6	50	100
purpuraeum + *R. meliloti*	24.2	1.387	8.41	0.586	66.6	83.3	83.3
purpuraeum + *P. aeruginosa*	25.5	0.853	5.443	0.36	33.3	66.6	16.6
purpuraeum + *B. subtilis*	24.16	1.196	6.41	0.393	100	50	100
valentiae + *R. meliloti*	22.61	1.2	4.943	1.303	66.6	100	100
valentiae + *P. aeruginosa*	21.4	0.916	4.996	0.52	83.3	83.3	66.6
valentiae + *B. subtilis*	22.13	0.973	3.33	0.186	83.3	83.3	100
CD 0.05 =	**5.20**	**0.77**	**2.80**	**0.455**	**54.78**	**48.77**	**41.82**

Table 2. (Cont'd.).

ıtments	Plant growth parameters				Infection %		
	Shoot length (cm)	Shoot weight (gm)	Root length (cm)	Root weight (gm)	*Fusarium* spp.	*Macrophomina phaseolina*	*Rhizoctonia solani*
flower							
trol	24.33	1.536	5.166	1.083	100	100	100
faqhusainii	23.33	1.416	5.33	1.1	100	83.3	83.3
trastromatica	24.83	1.28	3.11	0.65	83.3	83.3	100
urpuraeum	25.3	1.303	3.7	0.786	100	83.3	50
alentiae	18.75	1.35	4	0.575	100	75	75
eliloti	26.53	1.01	4.52	0.626	100	100	100
eruginosa	26.66	1.23	5.943	0.426	83.3	66.6	83.3
ubtilis	25.16	0.858	4.333	0.48	66.6	83.3	83.3
faqhusainii + R. meliloti	23.33	1.23	4.833	0.463	83.3	83.3	83.3
faqhusainii + P. aeruginosa	25.86	1.17	4.97	0.753	83.3	100	66.6
faqhusainii + B. subtilis	20.76	1.2	3.943	0.623	83.3	83.3	83.3
trastromatica + R. meliloti	27.76	1.446	4.33	0.596	50	50	66.6
trastromatica + P. aeruginosa	18.25	1.511	6.66	1.066	83.3	66.6	100
trastromatica + B. subtilis	28	1.393	4.663	0.526	100	100	100
urpuraeum+ R. meliloti	26.75	11.975	6.25	1.15	50	75	75
urpuraeum + P. aeruginosa	25.66	1.45	4.5	0.816	66.6	83.3	100
urpuraeum + B. subtilis	25	1.511	4.943	0.596	50	16.6	33.3
alentiae + R. meliloti	26.16	1.4	4.666	0.625	66.6	50	100
alentiae + P. aeruginosa	27.5	1.02	6.026	0.493	50	50	100
alentiae + B. subtilis	26.1	1.143	5.886	0.65	100	100	100
0.05 =	**8.27**	**0.66**	**2.52**	**0.57**	**56.51**	**51.57**	**49.13**

Antagonists applied to seeds not only have the potential for protecting the seeds but being the initial colonizer of the roots, may also provide protection against root infecting pathogens (Kommedahl & Windels, 1981). In the present study seed dressing with bacteria was found more effective in the control of root infecting fungi as compared to soil treatment. Seaweeds in combination with bacteria were also effective for growth of plants. Seed dressing and soil treatment with different strains of bacteria viz., *P. aeruginosa*, *R. meliloti* and *B. subtilis* showed promising results in the control of root infecting fungi. Raaijimaker *et al*., (2002) and Weller *et al*., (2002) reported that plant growth promoting rhizobacteria that colonize roots improve plant growth either through direct stimulation of the plant by producing growth regulators or by suppression of pathogens. The production of certain antibiotics (Leavy *et al*., 1992) and siderophores (Buysens *et al*., 1996) by *P. aeruginosa* has been regarded as one of the mechanism involved in antagonism. Siddiqui *et al*., (2000) and Siddiqui & Ehtesham-ul-Haque (2001) reported that species of *Pseudomonas* are antagonistic to soil borne plant pathogens. During the present study, reduction in disease intensity and improved plant growth as compared to untreated plants was observed in mash bean and sunflower. Chapman & Chapman (1980) and Colwell (1983) reported that potash, equal nitrogen and role of active biocontrol principles in seaweed may cause disease suppression resulting in plant growth enhancement. The seaweeds increased the efficacy of bacteria in the control of *M. phaseolina* on mash bean, while sea weed *H. valentiae* with *P. aeruginosa* significantly reduced the infection of *M. phaseolina*, *R. solani* and *Fusarium* spp., on mash bean and sunflower. This work suggests that seaweed alongwith plant growth promoting bacteria used either as seed dressing or soil drenching have greater potential in controlling root infecting fungi on crop plants.

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