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EFFECT OF DIFFERENT DOSAGES OF BACTERIA AFTER MULTIPLICATION ON SEAWEED IN THE CONTROL OF ROOT ROT OF SUNFLOWER AND MASHBEAN

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ABSTRACT

Bacteria viz., *Rhizobium meliloti*, *Pseudomonas aeruginosa* and *Bacillus subtilis* were multiplied on sea weed like *Cystoclanium purpuraeum.* After 15 days of multiplication soil was amended with different dosage of inoculum. There was a significant enhancement of plant growth and significantly control the infection of root infecting fungi viz., *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp on mash bean where soil was amended with *P. aeruginosa* inoculum on *Cystoclonium purpuraeum* used @ 1% w/w whereas *B. subtilis* inoculum on *C. purpureum* used @ 1% w/w showed greater plant growth and significant reduction of root infecting fungi on sunflower and mashbean.

Key words: Sea weeds, root rot fungi, bacteria, sunflower and mash bean.

INTRODUCTION

Many plant growth promoting rhizobacteria (PGPRS) have a beneficial effect on plants including biological control of soil borne pathogens, induced 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 *Rhizobium* spp., the root nodulating bacteria, are also known to control soil borne root infecting fungi (Zaki and Gaffar, 1987; Siddiqui *et al.*, 1998). Seaweeds provide better plant growth and received the attention of plant scientists through out the world (Atzmon *et al*., 1994, Staden *et al*., 1995). Since it contain greater potash and nitrogen as compared to farm yard manure (Chapman and Chapman, 1980).

Sea weeds also known to stimulate the growth of vegetables, fruits and other crops (Bhenden., 1991; Crouch *et al*, 1994; Washington *et al*, 1999). *Stoechospermum marginatum* and *Sargassum tenerrimum* with or without Rhizobia significantly reduced root knot nematode (*Meloidogyne javanica*) and root infecting fungi (Ehtesham-ul-Haque *et al.*, 1996).

Plant diseases causing organisms produces serious losses to crop plants and adversely affects the agricultural economy of a country (Hafeez, 1986). The primary diseases threatening crop production are due to fungi, actinomycetes, bacteria and nematodes. These are ubiquitous soil borne plant pathogens, which infect roots of plant, resulting in the death of plants. Since damage to plants by soil borne pathogens results from below ground infection, losses to crop plants from such diseases are underestimated and generally go unnoticed (Baker and Cook, 1974). The soil borne root-infecting fungus, *Macrophomina phaseolina* is reported to produces charcoal rot over 500 species of plants (Sinclair, 1982). *Rhizoctonia solani* exists as active mycelium is the soil, attacks over 2000 species of plants (Parmeter, 1970) and *Fusarium* species (Booth, 1971) are known to attack a wide range of host plants in different parts of the world.

Experiments were therefore carried out to study the effect of different dosage of bacteria after multiplication on sea weed in the control of root rot of sunflower (*Helianthus annus* L.) and mash bean (*Vigna mungo* (L.) Hepper).

MATERIALS AND METHODS

Seaweed, *C. purpuraeum*, collected from Buleji, Karachi, was washed with tap water and dried under shade and powdered in an electric grinder and was kept at room temperature. Cultures of *Rhizobium meliloti* (R5), *Pseudomonas aeruginosa* (P-58), *Bacillus subtilis* (B-35) were obtained from culture collection of 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 and Raczkowski, 1922), total nitrogen 1.5 % (Mackenzie and Wallace, 1954), total organic matter 2.4 %. Soil had natural infestation of 1-3

sclerotia of *M. phaseolina* as found by wet sieving dilution technique (Sheikh and Ghaffar, 1975), 5-10% colonization of *R. solani* on sorghum seeds used as baits (Wilhelm, 1955) and 3000cfu of *Fusarium* spp., as assessed by soil dilution technique (Nash and Snyder, 1962).

Seaweed (*C.purpuraeum*) was taken in flasks, moistened with water slightly and inoculated with cell suspension of bacteria viz., *R.meliloti* (R5), *P.aeruginosa* (P-58), and *B.subtilis* (B-35) and kept at room temperature (25 ± 2 ^{0}C). After 2 weeks of growth of bacteria, soil was amended with inoculum of *R. meliloti* ($5.4x10^9$cells/ml) *P. aeruginosa* ($7.7x10^9$ cells/ml) and *B.subtilis* ($4.2x10^9$ cells/ml) @ 0.1%, 0.5%, 1% and transferred in 8cm diam., plastic pots each containing 300gm soil. After 2 weeks of the decomposition of the organic matter 8 seeds of mash bean and sunflower were sown in each pot. Pot with out bacteria and sea weeds served as control. There were three replicates of each treatment 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 onto 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.

Data were analyzed and subjected to analysis of variance (ANOVA) following the procedure as given by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

In the present study effect of different dosage of bacteria after multiplication on sea weed in the control of root rot of sunflower (*H. annus* L.) and mash bean (*V. mungo* (L.) Hepper). Greater plant length was observed in both mash bean and sunflower where *P. aeruginosa* and *B. subtilis* inoculum on *C. purpuraeum* used @ 1% w/w ($p<0.001$). Similarly there was a significant increased in shoot weight, root weight of mash bean and sunflower ($p<0.05$). Maximum shoot and root weight were observed in sunflower where *P. aeruginosa* inoculum on *C. purpuraeum* used @ 0.5 and 1% w/w. Different dosage of bacteria after multiplication on sea weed were more effective in the suppression of *Fusarium* spp., infection on mash bean ($p<0.05$) and sunflower ($p<0.01$) (Table 1). Bacterial inoculum used @ 1% w/w showed complete suppression of *R. solani* infection on mash bean and sunflower ($p<0.01$). Similarly infection of *M. phaseolina* was significantly reduced when *P. aeruginosa* and *B. subtilis* inoculum on *C. purpuraeum* used @0. 5% w/w in mash bean and sunflower ($p<0.01$). (Table 1).

Table. 1 Effect of different dosage of biocontrol bacteria multiplied on *Cystoclonium purpuraeum* on growth parameters and infection % of mash bean and sunflower.

Mash bean

Growth parameters					Infection %		
Treatments	Shoot length (cm)	Shoot weight (gm)	Root length (cm)	Root eight (gm)	*Fusarium* spp	*Macrophomina phaseolina*	*Rhizoctonia solani*
A	17.8	0.8	7.1	0.38	100	100	100
B	21.3	0.67	6.4	0.446	100	100	100
C	19.7	0.73	5.96	0.58	100	83.33	100
D	19.6	0.66	4.16	0.58	83.33	66.66	83.33
E	20.8	0.96	5.96	0.5	833.33	83.33	83.33
F	20.6	0.86	13.6	0.49	66.66	66.66	83.33
G	24.6	1	4.3	3.4	50	100	66.66
H	19.8	0.67	4.5	0.39	66.66	83.33	83.33
I	14.2	0.68	3.66	0.35	66.66	83.33	66.66
J	21	0.67	6.2	0.37	66.66	83.33	50
K	17.7	0.86	4.86	0.34	83.33	66.66	66.66
L	22.3	0.84	4.76	1.82	83.33	50	33.33
M	28.5	1.27	7.1	0.71	33.33	50	16.66
Lsd0.05=	4.28	0.32	2.83	1.71	39.52	38.35	39.14

Sun flower **Table 1 cont'd…**

Treatments	Growth parameters				Infection %		
	Shoot length (cm)	Shoot weight (gm)	Root length (cm)	Root eight (gm)	*Fusarium* spp	*Macrophomina phaseolina*	*Rhizoctonia solani*
A	18.7	5.63	0.53	0.85	100	100	100
B	17.2	3.86	0.54	1.23	100	83.33	100
C	21.2	4.96	0.43	0.62	100	83.33	100
D	22.7	5.86	0.48	1.03	83.33	83.33	83.33
E	21.1	5.76	0.52	0.88	83.33	100	83.33
F	20.8	4.44	0.39	0.86	83.33	66.66	50
G	23.5	7.7	0.36	1.2	66.66	83.33	33.33
H	20.4	6.33	0.45	0.71	100	83.33	83.33
I	21.7	4.83	0.40	1.26	83.33	83.33	83.33
J	28.2	9.73	2.18	1.64	66.66	0	16.66
K	18.6	5.4	0.57	42.7	83.33	83.33	83.33
L	20.2	9.06	0.5	1.85	83.33	66.66	66.66
M	30.2	7.63	0.883	1.75	66.66	50	83.33
Lsd0.05=	3.73	3.21	0.31	0.77	34.39	39.71	37.35

A=Control, B=*C. purpuraeum* @ 0.1%w/w, C=*C.purpuraeum* @0.5%w/w, D=*C.purpuraeum* @ 1%w/w, E=*R.meliloti* inoculum @ 0.1% w/w, F=*R.meliloti* inoculum @ 0.5% w/w, G=*R.meliloti* inoculum @1% w/w, H=*P.aeruginosa* inoculum @ 0.1% w/w, I=*P.aeruginosa* inoculum @ 0.5% w/w, J=*P.aeruginosa* inoculum @ 1% w/w, K=*B.subtilis* inoculum @ 0.1% w/w, L=*B.subtilis* inoculum @ 0.5 % w/w,, M=*B.subtilis* inoculum @ 1% w/w.

Microorganisms that can grow in the rhizosphere are ideal for use as biocontrol agents since rhizosphere provides front line defense for roots against attack by pathogens (Weller, 1998). Present study on the efficacy of biocontrol bacteria after multiplication on sea weed substrate showed correlation between reduction in infection and population of microbial antagonists. Plant growth promoting rhizobacteria that colonize roots have been reported to improve plant growth either through direct stimulation of the plant by producing growth regulators or by suppression of pathogens (Raaijmaker *et al*., 2002 ; Weller *et al*., 2002). 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 el* (2000) and Siddiqui and Ehtesham-ul-Haque (2001) reported that species of *Pseudomonas* are to be antagonistic to soil borne plant pathogens. Raaijmaker and Weller, (1998) reported role of 2 , 4 diacetylephloroglucinol an antifungal metabolite from species of fluorescent *Pseudomonas* in plant root disease suppression. Species of *Pseudomonas* are also reported to induce systematic resistance in cucumber against *Pythium aphanidermatum* (Zhou and Paulitz, 1994). Present results would suggest that inoculum of *P. aeruginosa* and *B. subtilis* on *C. purpuraeum* (@ 1%w/w) have greater potential in controlling the soil borne root infecting fungi on mash bean and sunflower.

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