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Growth Responses of *Helianthus annus* to Plant Growth Promoting Rhizobacteria used as a Biofertilizers*

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Abstract: Bacterial strains of *Pseudomonas* (Ps_1, Ps_2, Ps_3, Ps_4, Ps_5) *Azotobacter* (Ab_1, Ab_2, Ab_4, Ab_5, Ab_6) and *Azospirillum* (As_2, As_4, As_5, As_6, As_8) were used to inoculate the seeds of *Helianthus annus var* SF-187. Inoculated and non-inoculated seeds were germinated and grown under field conditions until at maturity. Two harvests were taken one after 7 days of germination and other at maturity i.e., after 4 months of sowing. All the bacterial inoculations provoked germination in *Helianthus annus*. Maximum increase was manifested by Ps_5 (223.63%). In general strains of *Pseudomonas* promoted plant growth more then *Azotobacter* and *Azospirillum*, plant height was enhanced by Ab_6 (68.66%) and number of leaves was increased by Ps_1 (63.63%) after 7 days of germination. However Ps_2 strain exhibited maximum enhancement in plant height (57.33%), while Ab_1 manifested maximum enhancement in number of leaves (49.01%) after 4 months of sowing. In case of yield parameters As_8 manifested maximum increase in number of flowers (128.57%), Ps_5 increased diameter of flowers (134.05%), Ab_1 weight of seeds (57.00%) and Ab_4 (30.35%) manifested enhancement in oil content when compared with control. Biochemical parameters were also increased with bacterial inoculations soluble protein content was enhanced by Ps_5 (321.55%), after 7 days of germination and Ab_2 (345.45%) after four months of sowing, auxin contents content was enhanced by, Ps_1 (1138%) and Ab_5 (845.50%), peroxidase activity was enhanced by Ps_5 (321.55%) and Ab_2 (345.45%) and acid phosphatase activity was increased by Ps_1 (97.13%) and As_8 (313.33%), after 7 days of germination and after four months of sowing when compared with that of control. These results demonstrate that these strains can be used for enhancing biomass as well as yield parameters.

Key words: Biofertilizer, *Azotobacter*, *Azospirilum*, *Pseudomonas*

Introduction

Every increasing population of the world demands the increase in food production which intern depends upon the improved agricultural practices. Pakistan is an agriculture based country about 60% of our population is currently related to the agriculture. Agriculture contributed 24.1% in the year 01-02 (Federal Bureau of Statistics of Pakistan, 2000-02). Sunflower as an oil crop was introduced in Pakistan during early 1960s and its commercial cultivation began in 1965. Among the three NC oilseed crops, the sunflower has been found the most successful. Pakistan is the fourth biggest edible oil importer of the world, so it spends lot of money for edible oils. Overall Pakistan expends 6, 34,630 (million Rupees) during the year 2001-02. Out of this import expenses edible oil contributes 19,045 (million Rupees) in 2000-01 and was increased to 24,034 (million Rupees) during the year 2001-2002 (Federal Bureau of Statistics Pakistan 2000-02). So Pakistan will currently spend their huge amount on their edible oil import. Modern agriculture aims to increase crop yield to satisfy the needs

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from a growing population, but to use sustainable approaches that should include the substitution of chemical inputs by a more effective use of natural resources. Biological nitrogen (N_2) fits well in this model, as it is a more environmentally clean way to satisfying plant N needs. Now a day's term PGPR is used for the bacteria's, which enhanced the plant growth and productivity in one or more then one ways. The mechanism, which plant growth attributes to promotion, enhanced plant hormones concentration, (ii) asymbiotic nitrogen fixation, (iii) antagonizing against phytopathogenic microorganisms and (iv) solubilisation of mineral phosphates and other nutrients (Burd *et al*., 2000). Inoculation must, therefore be rhizosphere competent and survive in the soil to promote plant growth. Bacteria are very important for the physical and the chemical characteristics of soil and play important roles in soil quality and plant productivity (Hill *et al*., 2000). *Acetobacter diazotoophicus, Herbaspirillum sereopedical, Azoarcus* sp. and *Azotobacter. Azospirillum* are predominantly surface colonizing bacteria while others are endophytic diazotrophs (Steenhoudt and Vanderleyden, 2000). In the free-living diazotrophs *Klebsiella pneumoniae* and *Azotobacter vinelandii*, activation of expression of genes involved in nitrogen fixation by the enhancer binding protein NIFA is controlled by the sensor protein NIFL in response to changes in levels of oxygen and fixed nitrogen *in vivo* (Money *et al*., 2001). The inhibitory activity of NIFL towards NIFA is stimulated by ADP binding to the C-terminal domain of NIFL, which bears significant homology to the histidine protein kinase transmitter domains (Money *et al*., 2001). In several diazotrophic species of *Proteobacteria*, P_{II} signal transduction proteins have been implicated in the regulation of nitrogen fixation in response to NH_4^+ by several mechanisms. In *Azotobacter vinelandii*, expression of *nifA*, encoding the *nif*-specific activator, is constitutive and thus, regulation of NifA activity by the flavoprotein NifL appears to be the primary level of nitrogen control (Rudnick *et al*., 2002). According to Raza *et al*. (2001) in all lupin cultivars plant dry weight, growth and biomass production enhanced with rhizobial inoculation. PGPR *Pseudomonas putinda* KT2440 strain colonizes the Rhizosphere of a number of agronomically important plants at high population densities (Uregel *et al*., 2000; Turnbell *et al*., 2001). Yuming *et al*. (2003) three *Bacillus* strains, *B. subtilis* NEB4 and NEB5 and *B. thuringiensis* NEB17 enhanced soybean nodulation and growth in greenhouse and field experiments. Coinoculation with non-*Bradyrhizobium* endophytic bacteria provided the largest and most consistent increases in nodule number, nodule weight, shoot weight, root weight, total biomass, total nitrogen and grain yield. Dry and wet inoculation of alginate microbeads enhanced plant growth promotion in wheat and tomato seedling growing in unfertile soil and biodegraded within 15 days in moist soil (Bashan and Hernandez, 2002). Growth promoting bacteria induces systemic resistance in host plants (Zheng *et al*., 2000; Ramammorthy *et al*., 2000). Since many trials have used gnotobiotic or greenhouse conditions to show promotion, we examined weather the addition of indigenous monoculture inoculations of *Pseudomonas, Azotobacter and Azospirillum* used as Biofertilizer has effect on *Helianthus annus var* SF-187 in field. Inoculation effects on germination, growth and yield parameters were studied in field along with biochemical analysis (auxin content, protein content, peroxidase content acid phosphatase content and oil contents) and data was statistically analyzed.

Materials and Methods

The present study was conducted in the Botanical Garden, University of The Punjab, Quaid-e-Campus Lahore, Pakistan. The experimental site was located at longitude 74° East and latitude 31° north, which is at the altitude of 644.4 feet above sea level. The research was initiated in last week of December, 2001. Seeds of *Helianthus annus* were taken from Punjab Seed Corporation Lahore, which is certified from Federal Seed Certification and Registration Department Islamabad. Fifteen bacterial strains five each of *Azotobacter*, Ab_1, Ab_2, Ab_4, Ab_5 and Ab_6, *Azospirillum*. As_2, As_4, As_5, As_6 and As_8 *Pseudomonas* i.e., Ps_1, Ps_2, Ps_3, Ps_4 and Ps_5 isolated by Aziz (2000) were used in this

study. Bacterial pellets obtained from fresh cultures (24 h incubation) of each strains and the cell density of these bacterial cultures were adjusted to 10^8 cell mL^{-1} with the help of 2-D spectrophotometer. Healthy seeds of *Helianthus annus var* were surface sterilized by soaking in 0.1% $HgCl_2$ solutions for 5 min with continuous shaking. Then seeds were left soaked in sterilized distilled water for about an hour. Sterilized seeds were then soaked in bacterial suspension with the help of sterilized forceps for about 15-20 min. For control treatment seeds were soaked in sterilized glass distilled water for same duration. Both inoculated and un-inoculated (control) seeds were then sowed in field. Germination was recorded daily for eight days and after that thinning was done in each plot. Two harvests were taken one after 7 days of germination and second at maturity i.e., after 4 months of sowing. Growth parameters such, plant height number of leaves with yield parameters were observed i.e., emergence of flowers, plant height, color of flowers and after that crop was harvested at maturity when pollination was completed and seed become mature i.e., after 4 months of sowing. For biochemical analysis at both harvests activity of peroxidase (David and Murray, 1965), acid phosphatase (Iqbal and Rafique, 1987), soluble protein content (Bhatti *et al*., 1993) and auxin content were measured by Mahadevan (1984). Data obtained was analyzed statistically following method of Steel and Torrie (1981). Mean, Standard error of the mean, least significant difference and correlation were calculated.

Results

Harvest I

Germination and Growth Parameters

In majority of cases bacterial inoculation provoked germination and 7.14% (Ps_2) to 110.74% (As_8), increases in germination of seeds were recorded (Table 1). Plant height or seedling lengths of *Helianthus annus* were enhanced significantly and it ranged from 15.85% (Ab_4) to (68.68%) Ab_6 (Table 1) when compare with that of control. Bacterial strains Ps_2, Ab_5 and As_8 showed no effect on number of leaves per seedlings. Increases in this parameter ranged from 9.09% (Ps_3) to 63.63% (Ps_1). Whereas inoculation with Ab_1 (9.09%) and As_2 (9.09%) caused some reduction, relative to control, in this parameter (Table 1).

Table 1: Effects of bacterial inoculations on percentage germination, plant height (cm) and number of leaves of *Helianthus annus* seedlings after seven days of germination

Strains	Germination (%)	Plant height	No. of leaves
Control	20.00±0.935	20.600±1.078	2.750±0.414
PS_1	32.70±1.473	25.200±1.292	4.500±0.559
PS_2	35.00±0.901	32.875±0.867	2.750±0.414
PS_3	37.20±1.197	26.875±1.407	3.000±0.612
PS_4	35.20±1.457	29.975±1.622	3.750±0.216
PS_5	45.50±1.082	32.050±3.168	3.750±0.730
AB_1	29.50±1.082	30.175±1.194	2.500±0.559
AB_2	39.50±1.620	32.800±1.218	3.500±0.559
AB_4	20.00±0.414	23.772±1.593	3.250±0.544
AB_5	27.50±0.353	25.790±2.053	2.750±0.414
AB_6	20.20±1.192	34.750±1.274	3.500±0.559
AS_2	26.70±0.353	32.975±1.741	2.500±0.559
AS_4	35.50±0.353	32.200±3.333	3.000±0.612
AS_5	38.70±0.353	33.500±3.285	3.200±0.773
AS_6	41.50±1.023	27.975±1.076	3.750±0.544
AS_8	44.55±0.216	25.700±1.124	2.750±0.414
LSD at p = 0.05	1.6860	24.881	0.8843

Harvest II

Growth and Yield Parameters

Plant height was increased significantly, with inoculation of bacterial strains, except for As_8 (6.11%), where decrease was recorded. The maximum increase in this parameter was observed with the inoculation of As_2 (64.74%) (Table 2). Among *Pseudomonas* strains 15.08% (Ps_4) to 54.38% (Ps_1) increases, in case of *Azotobacter* strains 6.19% (Ab_6)-48.48% (Ab_1), whereas in *Azospirillum* strains increases ranged from 2.416% (As_8) to 64.74% (As_2), relative to the non-inoculated control (Table 2). Bacterial inoculations increased number of leaves increase in this parameter, over control, ranged from 1.96-49.01%, however As_8 (21.56%) exhibited decreased in this parameter. Overall *Azotobacter* strains exhibited more stimulatory effects in this parameter, relative to *Pseudomonas* and *Azospirillum* strains. Number of flowers of *Helianthus annus* increased significantly, with the inoculation of bacterial strains, except As_2, which caused 28.57% reduction in this parameter, as compared to that of non-inoculated plants. The percentage increase in this parameter varied between 1.07 - 110.71%, with different strains, (Table 2). Generally *Azospirillum* strains manifested more increases then *Pseudomonas* and *Azotobacter* strains. The significant increase in diameter of flowers of *Helianthus annus* was recorded with all bacterial inoculations as compared to control. Increases in this parameter, relative to non-inoculated control, were 58.41-134.15%. The maximum increase was recorded with inoculation of As_5 (134.15%), relative to other bacterial inoculations (Table 2). With *Pseudomonas* strains percentage increases varies between 99.505% (Ps_1) to 130.71% (Ps_4), in case of *Azotobacter* strains increases were 58.24% (Ab_2) to 126.53% (Ab_6) and in *Azospirillum* strains increases ranged from 98.01% (As_2) to 134.14% (As_5), relative to non-inoculated control. All the bacterial inoculations increased weight of seeds in *Helianthus annus* plants (Table 2), when compared with control. Increase in this parameter ranged from 5.62 % (As_6) to 57.00% (Ab_1). In case of *Pseudomonas* strains maximum increase was exhibited by Ps_5 (44.85%), while Ps_1 (10.28%), showed minimum increase when compared with that of control. In case of *Azotobacter* strains increases in this parameter varies from 10.24% (Ab_5) to 57.00% (Ab_1) and in *Azospirillum* strains increases were 5.60% (As_6) to 34.11% (As_5), when compared with that of non-inoculated control. The oil contents of *Helianthus annus* seeds was increased with inoculation of most of bacterial strains, excluding Ps_1, Ab_5, As_2, As_4, As_6 and As_8, which caused reduction in this parameter, The maximum increase over non-inoculated control was recorded with inoculation of Ab_4 (30.35%) (Table 2). The increase in this parameter varied with different bacterial inoculations from 0.27% (Ps_4) to 18% (Ab_4). *Azotobacter* strains manifested more increases relative to that of *Pseudomonas* and *Azospirillum* strains *Helianthus annus var* SF-187.

Table 2: Effects of bacterial inoculations on plant height (inches), number of leaves, number of flowers, diameter of flowers and (inches) weight of seeds (g) and oil contents of *Helianthus annus* plants at mature stage (after four months of sowing)

Strains	Plant height	No. of leaves	No. of flowers	Diameter of flowers	Weight 100 seeds	Oil contents(%)
Control	33.050±16.550	12.750±1.192	10±0.935	05.050±0.167	2.14±0.414	25.59
PS_1	52.000±3.452	16.000±0.709	16±1.473	10.075±0.167	2.36±0.559	25.43
PS_2	51.100±1.076	18.250±0.789	16±0.901	11.150±0.309	2.14±0.414	25.71
PS_3	48.775±2.104	17.250±0.414	17±1.457	11.025±0.589	2.54±0.612	27.61
PS_4	38.075±7.761	16.250±1.320	14±1.082	11.651±0.528	2.61±0.216	25.66
PS_5	44.825±1.196	16.500±0.599	15±1.082	10.875±0.328	3.10±0.730	29.25
AB_1	49.150±1.177	19.000±1.118	17±1.780	09.600±0.006	3.36±0.559	30.29
AB_2	45.700±1.256	15.750±1.138	19±1.620	08.000±0.594	2.66±0.559	26.33
AB_4	44.200±2.135	13.000±0.866	13±0.414	10.700±0.050	2.79±0.544	30.35
AB_5	45.225±1.109	15.250±0.414	13±0.353	10.500±0.286	2.37±0.414	24.39
AB_6	35.200±0.340	14.250±0.739	12±1.192	11.425±0.392	2.56±0.559	25.47
AS_2	55.380±1.490	17.500±0.559	12±0.355	10.000±0.930	2.62±0.559	22.06
AS_4	33.900±2.630	14.750±0.414	13±0.353	11.650±0.419	2.74±0.612	25.29
AS_5	45.575±2.062	15.750±0.739	16±0.353	11.825±0.381	2.87±0.773	25.87
AS_6	52.675±1.194	16.000±0.707	16±0.353	10.300±0.320	2.26±0.544	20.09
AS_8	29.825±1.692	10.000±0.707	14±0.216	10.200±0.652	2.34±0.414	18.74
LSD at p = 0.05	4.1058	0.8525	1.4322	0.6767	0.0878	

Biochemical Analysis: (At Both Harvests)

Growth hormones are the substances produced in plants and these also affect the plant growth and yield, out of these distinct hormones, auxin appear to be a master hormone, exercising regulatory action over many different sorts of plant processes and probably over many of other plant hormones. Auxin content of *Helianthus annus* seedlings was enhanced significantly, over control, with the bacterial inoculations. Increase in auxin content ranged from 115.30% (As_8) to 1138% (Ps_1) after 7 days. However *Pseudomonas* strains showed relatively more increases in auxin content then *Azospirillum* and *Azotobacter* strains (Table 3). However after four months of sowing 36.36 (Ps_4)-854.35% (Ab_5) increases in auxin contents, over non-inoculated control, were recorded (Table 4). *Pseudomonas* strains Ps_2, Ps_5, *Azotobacter* strains Ab_1, Ab_4, AB_5 and *Azospirillum* strains As_5 and As_6 stimulated more than four folds increase in the auxin contents of sunflower plants (Table 4). Soluble protein contents were increased significantly over non-inoculated plants with the inoculation of many bacterial strains. after 7 days. However maximum increase was observed with the inoculation of Ps_5 (494.23%) (Table 3). The significant increase, in soluble protein contents of *Helianthus annus* was also recorded after four months of sowing, increases in this parameter, with bacterial inoculation ranged from 7.51% (Ab_2) to 41.69% (Ps_5) (Table 4). Generally *Pseudomonas* and *Azospirillum* strains

Table 3: Effect of bacterial inoculations on the auxin contents, protein contents, peroxidases and acid phosphatase activity (μg g^{-1} fresh weight) of *Helianthus annus* seedlings after seven days of germination

Strains	Auxin contents	Protein contents	Peroxidase contents	Acid phosphates contents
Control	0.650±0.035	121.50±15.192	2.364±0.041	2.650±1.992
PS_1	8.050±0.035	655.00±96.302	6.226±0.145	5.244±0.850
PS_2	4.650±0.106	381.00±66.875	6.831±0.038	3.741±0.217
PS_3	6.000±0.919	211.00±70.104	4.728±0.380	3.855±0.296
PS_4	4.250±0.176	645.00±84.158	6.303±0.975	3.781±0.455
PS_5	3.150±0.035	722.00±29.702	9.965±0.164	3.829±0400
AB_1	5.210±1.485	247.00±20.509	7.501±0.490	2.843±0.541
AB_2	4.000±0.000	307.00±16.265	10.92±0.530	3.381±0.425
AB_4	4.300±0.700	300.00±21.216	3.147±0.731	3.085±0.452
AB_5	7.750±0.459	303.00±56.873	3.886±0.052	3.369±0.211
AB_6	3.850±0.176	317.00±58.719	6.867±0.533	3.211±0.137
AS_2	7.310±0.212	482.00±8.4800	4.171±0.240	3.130±0.529
AS_4	1.900±0.919	346.00±24.045	3.421±0.353	3.519±0.298
AS_5	3.050±0.035	356.00±00.000	4.171±0.027	2.243±0.844
AS_6	1.800±0.353	277.00±54.454	2.660±0.358	3.306±0.024
AS_8	1.400±0.000	233.00±47.230	4.836±0300	2.722±0.467
LSD at p = 0.05	0.589	0.588	61.875	0.593

Table 4: Effect of bacterial inoculations on the auxin contents, protein contents, peroxidases and acid phosphatase activity (μg g^{-1} fresh weight) of *Helianthus annus* seedlings at late stage (after four months of sowing)

Strains	Auxin contents	Protein contents	Peroxidase contents	Acid phophatase contents
Control	0.011±0.0003	1.065±0.002	05.285±0.631	07.80±0.757
PS_1	0.023±0.001	1.389±0.198	12.583±0.102	25.10±1.768
PS_2	0.074±0.018	1.348±0.040	08.600±2.404	15.64±0.502
PS_3	0.037±0.001	1.232±0.042	12.479±0.338	23.49±0.615
PS_4	0.015±0.001	1.209±0.012	06.287±0.213	16.93±2.111
PS_5	0.056±0.009	1.509±0.115	12.324±0.989	17.55±1.325
AB_1	0.069±0.013	1.145±0.003	16.336±1.334	16.65±1.099
AB_2	0.019±0.006	1.201±0.074	23.520±0.706	13.83±1.782
AB_4	0.057±0.026	1.230±0.051	11.317±0.580	21.60±1.271
AB_5	0.105±0.061	1.192±0.044	07.601±1.531	11.85±1.060
AB_6	0.024±0.004	1.297±0.062	01.634±0.085	21.80±1.060
AS_2	0.029±0.010	1.291±0.062	15.430±3.163	23.13±1.286
AS_4	0.034±0.011	1.439±0.019	11.170±0.165	24.50±2.966
AS_5	0.052±0.016	1.350±0.118	08.387±0.311	27.75±2.233
AS_6	0.065± 0.034	1.234±0.086	10297±2.843	28.830±1.650
AS_8	0.033±0.905	1.468±0.076	15.967±2.384	32.240±11.003
LSD at p = 0.05	0.0280	0.1072	3.549	3.3515

exhibited more stimulation in protein contents of *Helianthus annus* plants than *Azotobacter* strains. Enzymes are the biocatalysts, which play important roles in many biochemical reactions. Two enzymes acid phosphates and peroxidase were studied in this respect. Activity of peroxidase increased with bacterial inoculations and it ranged from 104.50% (As_6) to 362% (Ab_2) (Table 3) when compared with control after 7 days. Among *Pseudomonas* strains Ps_5 (321.50%) exhibited maximum enhancement, among *Azotobacter* strains Ab_5 (64%) manifested maximum increase whereas As_8 (104.56%) showed maximum increase in *Azospirillum* strains, when compared with control. however after four months of sowing peroxidases activity was increased significantly, relative to control, except for Ab_6, 18.93% (Ps_4) to 345.45% (Ab_2), increase was recorded after 4 months of sowing (Table 4). Acid phosphatase activity of inoculated seedlings increased significantly with the exception of As_5 which showed 15.35% reduction in this parameter. The increases varied between 2.716% (As_8) to 97.13% (Ps_1), when compared with control, after 7 days (Table 3). However after four months of sowing maximum increase over non-inoculated control was recorded with the inoculation of As_8 (313.33%) (Table 4).

Discussion

Bacteria are very important for the physical and the chemical characteristics of soil and play important roles in soil quality and plant productivity and play important roles in soil quality and plant productivity (Hill *et al*., 2000). In this study all the bacterial strains used to inoculate *Helianthus annus var*. SF-187 seedlings also proven to be efficient in plant growth promotion. All the strains used increased germination. In all cases bacterial inoculations significantly enhanced the plant height, number of leaves at both harvests. In case of *Helianthus annus* plant height was enhanced by Ab_6 (68.66%) and number of leaves was increased by Ps_1 (63.63%), after 7 days of sowing (Table 1). However Ps_2 strain exhibited maximum enhancement in plant height (57.33%), while Ab_1 manifested maximum enhancement in number of leaves (49.01%), after 4 months of sowing (Table 2). Improved seedlings growth with bacterial inoculation has also been reported by Hasnain and coworkers (Mehar *et al*., 2002). Rhizospheric bacteria *Pseudomonas* sp., *Azospirillum* sp. and *Agrobacterium* sp., increased plant growth and the nutrient uptake of maize, wheat and legumes (Bashan *et al*., 2004). With increase in plant height number, diameter of flowers and weight of seeds were also increased, As_8 manifested maximum increase in number of flowers (128.57%), (4.22), Ps_5 increased diameter of flowers (134.05%),(4.23), Ab_1 weight of 100 Seeds (57.00%), (4.24) and Ab_4 (30.35%), (4.25), manifested enhancement in oil content when compared to control (Table 2). This increase might be due to increase in availability of nutrients and nitrogen fixation deareasing pathogenecity and increased in protein contents. Burd *et al*. (2000) reported that plant growth promoting rhizobacteria might enhance plant height and productivity by synthesizing phytochromes, increasing the local availability of nutrients, facilitating the uptake of nutrients by the plants decreasing heavy metal toxicity in the plants antagonizing plant pathogens and inducing systemic resistance in the plants to pathogens. *Acetobacter diazotoophicus, Herbaspirillum sereopedical, Azoarcus* sp. and *Azotobacter*. *Azospirillum* are predominantly surface colonizing bacteria while others are endophytic diazotrophs (Steenhoudt and Vanderleyden, 2000). Fixation of atmospheric nitrogen (N) by free-living soil microorganisms is considered a minor source of bioavailable nitrogen compared to systems such as the *Rhizobium*-legume and *Frankia*-alder symbioses (Kennedy and Islam, 2001). In the free-living diazotrophs *Klebsiella pneumoniae* and *Azotobacter vinelandii*, activation of expression of genes involved in nitrogen fixation by the enhancer binding protein NIFA is controlled by the sensor protein NIFL in response to changes in levels of oxygen and fixed nitrogen *in vivo* (Money *et al*., 2001). The inhibitory activity of NIFL towards NIFA is stimulated by ADP binding to the C-terminal domain of NIFL, which bears significant homology to the histidine protein kinase transmitter domains (Money *et al*., 2001). In

several diazotrophic species of *Proteobacteria*, P_{II} signal transduction proteins have been implicated in the regulation of nitrogen fixation in response to NH_4^+ by several mechanisms. In *Azotobacter vinelandii*, expression of *nifA*, encoding the *nif*-specific activator, is constitutive and thus, regulation of NifA activity by the flavoprotein NifL appears to be the primary level of nitrogen control (Rudnick *et al*., 2002). According to Raza *et al*. (2001) in all lupin cultivars plant dry weight, growth and biomass production enhanced with rhizobial inoculation. PGPR *Pseudomonas putinda* KT2440 strain colonizes the Rhizosphere of a number of agronomically important plants at high population densities (Uregel *et al*., 2000; Turnbell *et al*., 2001). Yuming *et al*. (2003) three *Bacillus* strains, *B. subtilis* NEB4 and NEB5 and *B. thuringiensis* NEB17 enhanced soybean nodulation and growth in greenhouse and field experiments. Growth promoting bacteria induces systemic resistance in host plants (Zheng *et al*., 2000; Ramammorthy *et al*., 2000). Endophytic nature of plant makes the PGPR to colonize and persist in intercellular spaces of epidermal cell (Ramammorthy *et al*., 2000). Bascones *et al*. (2000) reported that nitrogenase dependent hydrogen production is one of the major factors that influence the efficiency of symbiotic nitrogen fixation. Evidence of establishment of significant populations of these bacteria in the plant root surfaces in sprase, although many studies have demonstrated their occurrence in Rhizosphere soil surrounding the roots (Rammamooarthy *et al*., 2000). Bashan *et al*. (2000) reported that inoculation with various PGPB improved seed N, protein and P contents of *Salicornia* sp., which is an oilseed sp. In present study increase in yield of crop might be due to induced systemic resistance against pathogens. Raza *et al*. (2001) reported that analyses of plant dry weight showed that rhizobial inoculation positively influenced growth and biomass production for all lupin cultivars tested compared to with non-inoculated plants. The soluble protein contents, auxin contents, acid phosphatase and peroxidase activity were markedly increased with bacterial inoculations and this increase relates with the increase in growth and yield parameters of *Helianthus annus*. Soluble protein contents were also improved, with bacterial inoculation as compared to non-inoculation control, however in *Helianthus annus* Ps_5 (321.53%) manifested maximum increase after 7 days of germination (Table 3) and Ps_5 (41.62%) after four months of sowing as well (Table 4). PGPR improved seed N, protein and P contents of *salicornia* sp. when inoculated with PGPR (Bashan *et al*., 2000). Increase in N and protein contents of seeds 4-5 times with *Azospirillum halopraeterens* reported by Bashan *et al*. (2000). Growth hormones are the substances produced in plants and these also affect the plant growth and yield, out of these distinct hormones, auxin appear to be a master hormone, exercising regulatory action over many different sorts of plant processes and probably over many of other plant hormones. In *Helianthus annus* Ps_1 (1138%) exhibited maximum enhancement after 7 days of germination (Table 3) and Ab_5 (845.50%) after four months of sowing (Table 4). Enzymes are the biocatalysts, which play important roles in many biochemical reactions. Two enzymes acid phosphates and peroxidase were studied in this respect. Activity of enzymes, peroxidases was improved with inoculations of bacterial strains when compared with non-inoculated control, in *Helianthus annus* Ps_5 (321.55%) (Table 3) and Ab_2(345.45%) (Table 4) exhibited maximum increase, Activity of enzyme acid phosphatase also improved with inoculation of bacterial strains. Ps_1 (97.13%) (Table 3) showed maximum increase after 7 days of germination. However after four months of sowing As_8 (313.33%) manifested maximum enhancement in *Helianthus annus* (Table 4). Bashan *et al*. (2000) reported that inoculation of oil seed halophytes *Salicornia bigelovii* with *Azospirillum halopraeferens* increased N and protein contents 4-5 times. Increase in protein content with bacterial inoculations is also reported by Afrasayab and Hasnain (2000 a, b). Two PGPR *Bacillus pumilus* and *B. licheniformis* produced high amounts of C1, - GAs, G_A1, GA_3, GA_4 and G_{20} and biological data suggested that the bacterial media have the potential to elongate the stem and shoots of *Alnus glutinosa* (Manero *et al*., 2001). Bashan *et al*. (2000) reported that inoculation of oil seed halophytes *Salicornia bigelovii* with *Azospirillum halopraeferens* increased N and protein contents 4-5 times. Increase in protein content with bacterial inoculations is also reported by Afrasayab and Hasnain (2000 a,b).

Aon and Colaneri (2001) reported that enzymes have strong coorelation that enzymes activity play important role between physical, chemical and microbial soil properties, which ultimately enhanced plant growth. Two peroxidase and one Chitinase (35 k) isoforms have been induced in the PGPR treated plants inoculated with the rice sheath, blight pathogen Nandakumar (1998). It has been reported by Chen *et al.* (1999) that a novel acid phosphatase containing phosphotryrosyl phosphatase activity. Acid phosphatases from several bacterial species have been recognized as virulence factors that support intracellular survival by inhibiting the respiratory burst (Chhatwal *et al.*,1997; Reilly *et al.*, 1996).

Conclusions

It is concluded from the above discussion that in *Helianthus annus var* SF-187 all the bacterial strains stimulated significantly growth and yield parameters at both harvests in field conditions, when compared with that of control. Increase in growth parameters (shoot length, root length and plant height) as well as yield parameters (Number and diameter of flowers, weight of seed, oil contents) at both stages was associated with increase in auxin and protein contents as well as peroxidase and acid phosphatase activities. *Azotobacter* and *Azospirillum* are non-symbiotic nitrogen fixer. It appears that these strains enhanced plant growth and yield parameters by applying nitrogen and synthesizing more auxin and proteins. Hence the strains used in the present study can be used as biofertilizer for the improvement of growth and yield parameters of commercially important cash crop i.e. *Helianthus annus var.* SF-197.

References

Afrasayab, S. and S. Hasnain, 2000a. Early growth responses of *Triticum aestivum var.* Inqalab-91 under NaCl stress after inoculation with mixed culture rhizoplane and phylloplane salt tolerant bacteria. Sci. Intl., 12: 79-86.

Afrasayab, S. and S. Hasnain, 2000b. Synegistic growth stimulation effects of mixed culture bacterial inoculation on the early growth of *Triticum aestivum var.* Inqalab-91 under NaCl stress. Pak. J. Biol. Sci., 3: 1016-1023.

Aon, M.A. and A.C. Colaneri, 2001. Tamporal and spatial variation of enzymatic activity and physcio-chemical properties in an agricultural soil. Applied Soil Ecol., 18: 225-270.

Aziz, F., 2000. Growth promoting pseudomonas, azotobacter and azospirillum strains isolated from roots of different weeds. M.Sc. Thesis, University of the Punjab, Lahore, Pakistan.

Bascones, E., T. Imperial, Ruiz-Argueso and J.M. Palacios, 2000. Generation of new hydrogen recycling Rhizobiaceae strains by introduction of a novel *hup* Minitransposon. Applied Enviorn. Microbiol., 66: 4292-4299.

Bashan, Y., M. Moreno and E. Troyo, 2000. Growth promotion of sea-water irrigated oil seed halophyte *Salicornia bigelovii* inoculated with mangrove rhizosphere bacteria and halotolerant *Azospirillum* sp. Springer link. Biol. Fertile Soil, 32: 265-272.

Bhatti, G.A., A. Qureshi and K. Sultana, 1993. Studies on heat shock responces of wheat seedling using *E.coli* GroEl antibodies. Pakphyton, 5: 157-166.

Burd, G.L., D.G. Dixon and B.R. Glick, 2000. Plant growth promoting rhizobacteria that decrease heavy metal toxicity in plants. Can. J. Microbiol., 33: 237-245.

Chen, X., T. Ansai, S. Awano, T. Lida, S. Bark and T. Takehara, 1999. Isolation, cloning and expression of an acid phosphatase containing phosphotrosyl phosphate (PTPase) activity from Prevotella Intermedia. J. Bacteriol., 181: 7107-7114.

Chhatwal, G. S., M. J. Walker, H. Yan, K.N. Timmis and C.A. Guzman, 1997. Temperature depandent expression of an acid phosphatase by *Bordetella bronchiseptica*: Role in intracellular survival. Microb. Pathog., 22: 257-264

David, R. and E. Murray, 1965. Protein synthesis in dark-brown leaves. Can. J. Bot., 43: 817-824.

Federal Bureau of Statistics Pakistan, http:// www. statpak. gov. pk/ depts/ fbs/ publications/ pocket book2003/ chapter14.pdf

Hill, G.T., N.A. Motrowski, L.A. Wolfe, L.R. Emele, D.D. Jurkoine, A. Ficke, S.M. Ramirez, S.T. Lynch and E.B. Nelson, 2000. Methods for assessing the composition and diversity of soil microbial communities. Applied Soil. Ecol., 15: 25-36.

Iqbal, J. and N. Rafique, 1987. Toxic effects of $BaCl_2$ on germination, early seedling growth, soluble proteins and acid phosphatases in *Zea mays* (L). Pak. J. Bot., 19: 1-8.

Kennedy, I.R. and N. Islam, 2001. The current and potential contribution of asymbiotic nitrogen fixation to nitrogen requirements on farms: A review. Aust. J. Exp. Agric., 41: 447-457.

Mahadevan, A., 1984. Growth Regulators, Micro-organisms and Diseased Plants. Oxford and IBH Publishing Company, India, pp: 31.

Manero, G., F.J., B. Ramos-solano, A. Probanza, J. Mehouach, F.R. Tader and M. Talzon, 2001. The plant growth promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amount of physiologically active gibberellins. Physiol. Plant., 111: 206-211.

Mehar, S., S. Affrasayab and S. Hasnain, 2002. Effect of bacterial monoculture inoculation on the earlt growth of *Triticum aestivum var*. Inqalab-91 under NaCl stress. Pak. J. Biol. Sci., 5: 643-647.

Money, T., J. Barrett, R. Dixon and S. Austin, 2001. Protein-Protein Interactions in the Complex between the enhancer binding protein *NIFA* and the sensor *NIFL* from *Azotobacter vinelandii*. J. Bacteriol., 183: 1359-1368.

Nandakumar, R., 1998. Induction of systemic resistance in rice with fluorescent *Pseudomonas* for the management of sheat blight disease. M.S.c, Thesis, TNUA, Coimbatore, India.

Rammamooarthy, U., R. Vismanathan, J. Ragnchander, V. Prakasam and R. Samiyappan, 2000. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Prot., 20: 1-11.

Raza, S., Jornsgard, B. Abdel-wahab and J.L. Christiansen, 2001. Effect of combined inoculation strains on growth of lupin on newly reclaimed land in Egypt. Biol. Fertile. Soil, 4: 319-324.

Reilly, T.T., G.S. Baron, F.E. Nano and M.S. Kuhlen-schmmidt, 1996. Characterization and sequencing of a respiratory, bust-inhibiting acid phosphatase form *Francisella tublasensis*. T. Biol. Chem., 271: 10973-10983.

Rudnick, P., C. Kunz, M.K. Gunatilaka, E.R. Hines and C. Kennedy, 2002. Role of GlnK in NifL-mediated regulation of NifA activity in *Azotobacter vinelandii*. J. Bacteriol., 18: 812-820.

Steel, R.G.D. and J.H. Torrie, 1981. Principles and Procedures of Statistics, a Biometric Approach. 2nd (Edn.), McGraw Hill International Book Company.

Steenhoudt, O., J.V., 2000. *Azospirillum* a free living nitrogen fixation bacterium closely associated with grasses: Genetic, biochemical and ecological aspects. FEMS. Microbiol. Rev., 24: 487-506.

Turnbell, G.A., J.A.W. Morgan, J.M. Whipps and J.R. Saundrs, 2001. The role of bacterial motility in the survival and spread of *Pseudomonas fluorescens* in soil and in the attachment and colonization of wheat roots. FEMS. Microbiol. Ecol., 35: 57-65.

Uregel, E., Salidi., M.A. and J.L. Romas, 2000. Genetic analysis of functions involved in adeshion of *Pseudomonas putida* to seeds. J. Bacteriol., 182: 2363-2369.

Yuming, B., Z. Xiaomin and L.S. Donald, 2003. Enhanced soybean plant growth resulting from coinoculation of bacillus strains with *Bradyrhizobium japonicum*. Crop Sci., 43: 1774-1781.

Zheng, Z., W. Zeng, Y. Huang, Z.L.J. Yang and W. Su.2000. Detection of antitunor and antimicrobial activities in marine organism associated actinomycetes isolated from the Tiwan strait, China. FEMS. Microbiol. Lett., 188: 187-191.