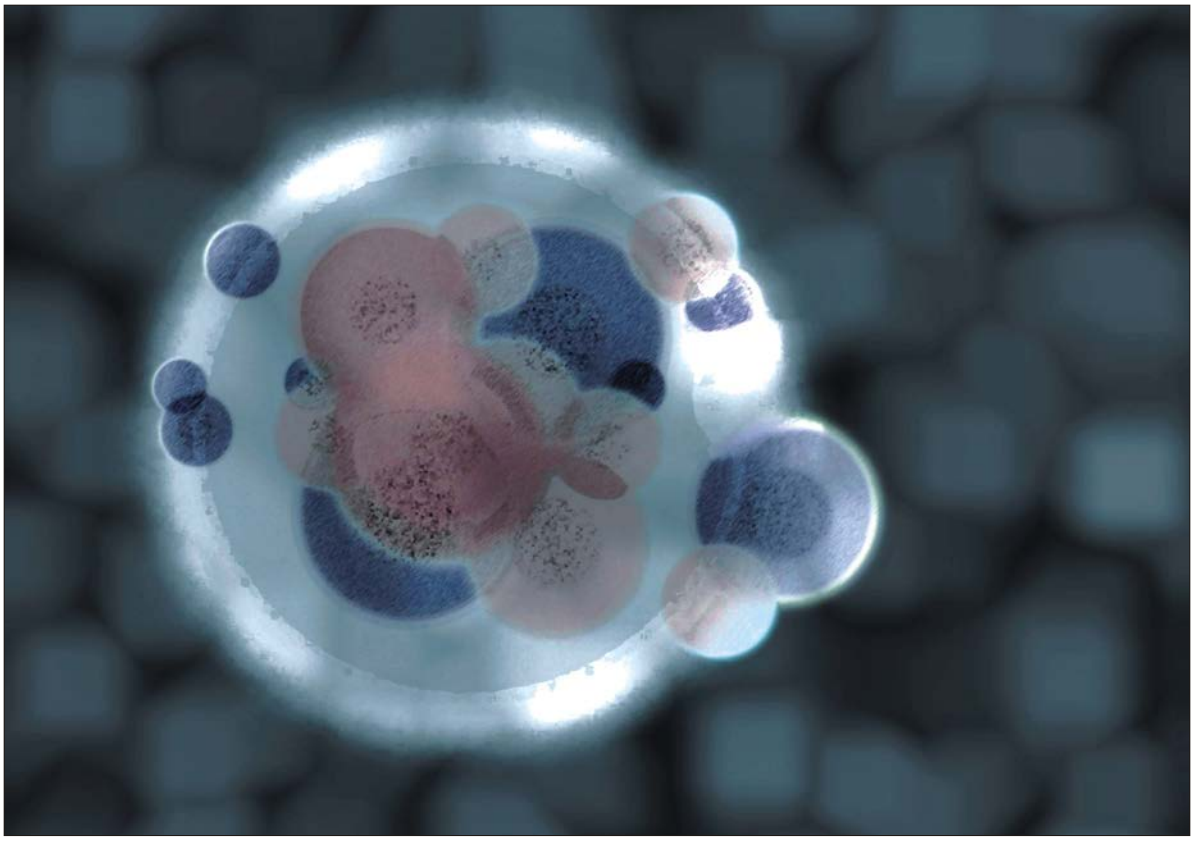

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 6 (3): 610-615, 2006
ISSN 1727-3048

Growth Promoting of Some Ornamental Plants by Root Treatment with Specific Fluorescent *Pseudomonads*

M. Erhan Göre and Nedim Altin
Plant Protection Research Institute, 35040 Bornova, Izmir, Turkey

Abstract: The objective of the present study was to evaluate the ability of *Pseudomonads,* to promote plant growth on *Pelargonium*, *Chrysanthemum* and *Dahlia* under commercial greenhouse conditions. The screening procedure consisted of two stages. In the first stage all the strains were tested in the two ornamental plants, *Pelargonium* and *Chrysanthemum*. In the second stage the most promising strain selected in the first stage was tested in *Dahlia*. Analysis of variance was done with the GLM (General Linear Models) procedure of SAS. Results of the first trial showed that *Chrysanthemum* plants treated with *Pseudomonas fluorescens* strain 51 increased plant height and number of flowers by 10 and 19%, respectively. Besides, increases as high as 13, 56 and 60% were obtained by *P. fluorescens* strain 148 applied by soil treatment, respectively for plant height, leaf surface area and number of flowers of *Pelargonium*. In the second trial, performed on *Dahlia,* plant raised from strain 51 treated pots recorded 27% increase in root length, 107% more flowers, 29 and 14% enhancement in fresh and dry weight, respectively. Moreover, the compatibility of the most efficient strain 51 with some fungicides, which are commonly sprayed on greenhouse was investigated under *in vitro* conditions. Fungicides did not adversely affect the colonial development of bacteria at concentrations as high as 100 μg mL^{-1} *in vitro*.

Key words: *Ornamental plants, PGPR, Pseudomonads*

INTRODUCTION

Agriculture is increasingly dependent on the use of chemical fertilizers, growth regulators and pesticides to increase yield. This dependency is associated with problems such as environmental pollution, health hazards, interruption of natural ecological nutrient cycling and destruction of biological communities that otherwise support crop production. Hence, crop improvement and disease management have to be achieved in shorter intervals of time with fewer detrimental inputs. The use of bioresources to replace chemical pesticides, growth regulators and fertilizers is growing. In this context, Plant Growth Promoting Rhizobacteria (PGPR) are often novel and potential tools to provide substantial benefits to agriculture.

PGPR are naturally occurring soil bacteria that are able to colonize plant roots and improve plant growth when applied to roots, tubers or seeds (Enebak *et al*., 1998; Kloepper *et al*.,1980; Weller, 1998). In recent years, the concept of PGPR-mediated plant growth promotion is gaining worldwide importance and acceptance. The beneficial effects of PGPRs fall into two categories: direct plant growth promotion and plant disease suppression. The exact mechanisms of PGPR action are not yet fully understood, but they are reported to enhance plant growth by production of hormones, phosphate solubilization or nitrogen fixation. Disease suppression may be due to iron sequestration, production of antibiotics or induction of systemic resistance (Alstrom, 1991; Liu *et al*., 1995; Reddy *et al*., 1999; Vidyasekharan *et al*., 2001; Zehnder *et al*., 2000). The utilization of natural PGPR strains as inducers of plant defense responses may increase their applicability and offer a practical way to deliver growth promotion. Large numbers of bacteria, mainly *Pseudomonas fluorescens* and other sp.; *Bacillus* sp. and *Serratia marcescens* have been reported previously to act as PGPRs. In the present study, five plant growth-promoting rhizobacteria were evaluated under greenhouse conditions for their capacity to promote the growth of *Pelargonium peltatum* F1, *Chrysanthemum* (Virunga Yellow) and *Dahlia variabilis* Figaro plants.

MATERIALS AND METHODS

Bacterial strains and inoculum preparation: The PGPR strains chosen, *Pseudomonas putida* strain 17, *Pseudomonas fluorescens* strains 51, 116, 122 and 148, were obtained from Ege University, Faculty of

Corresponding Author: M. Erhan Göre, Plant Protection Research Institute, 35040 Bornova, Izmir, Turkey
Tel: 90 232 3880030 Fax: 90 232 3741653

Agriculture, Department of Plant Protection. All of the selected PGPR strains for evaluation have significantly promoted plant growth and reduced ascochyta blight of pea in the field conditions (Göre and Bora, 2004).

Fluorescent *Pseudomonads* were grown in Erlen-meyer flasks (250 mL) containing 100 mL of King' s medium B (KB) for 20 h on a rotary shaker at 24°C. At this stage, bacterial cells were active and were found in the exponential growth phase. The cells were pelleted by centrifugation at 10, 000×g for 15 min at 4°C, suspended in sterile phosphate buffer (70 mM potassium phosphate buffer at pH = 7) and the concentration adjusted to 10^9 CFU mL^{-1} using a viable versus absorbance at 600 nm curve specific for *P. fluorescens* and *P. putida* strains. Appropriate dilutions from the original concentration were performed as described by Thompson (1996).

Experimental design and cultural practices: The experiments were conducted at the commercial greenhouse conditions, Inciralti, Izmir, Türkiye, during the years 2004 to evaluate 5 PGPR strains and a control. The experimental design consisted of 10 replications per treatment arranged in a randomized block design. Three week old plants were transplanted into larger 600 mL pots containing planting mix and drenched with 100 mL of a 10^9 CFU mL^{-1} bacterial suspension per plant. Bacterial inoculation was repeated one week later, in the same conditions. A water/buffer solution was applied to control plants. Plants were grown under 50% soil water holding capacity, 85-90% relative humidity, temperature of 18-23°C during the daytime and 14-16°C the nighttime.

The screening procedure consisted of two stages. In the first stage all strains were tested in the two ornamental plants, *P. peltatum* F1 and *Chrysanthemum* (Virunga Yellow). In the second stage, the most promising strain selected in the first stage was tested in *D. variabilis* Figaro. The first trials were ended 50 sec was ended 30 days after first inoculation. Observations were made all along the experiments, measuring days from inoculation to first flower, diameter of each plant's leaf canopy, leaf surface area, plant height, root length, fresh and dry weight, number of leaves and flowers.

Mechanisms of PGPR: The phosphate solubilizing test was done using Pikovskaya's medium (Pikovskaya, 1948). Agar plates were prepared and the bacterial strains were spot inoculated at the centre of the plates and incubated for 5-6 days. The plates were observed for a clearing zone around the colony and the diameter of the clearing zone was measured. Hydrogen cyanide production was assessed by the picrate method (Sneath, 1966). Tubes containing LB agar (Luria Bertani agar medium) and a filter paper strip impregnated with 0.5% picric acid and 2% $NaCO_3$ introduced into the test tube screw cup, were inoculated with the bacteria. Upon incubation at 24°C for 3 days production of cyanide was detected as a change of the filter paper color from yellow to orange-brown. Indole Acetic Acid (IAA) and related compounds were identified upon colony growth in LB agar amended with 5 mM l-tryptophan and overlaid with an 82 mm dia nitrocellulose membrane disk (Brito Alvarez *et al*., 1995). Agar plates were inoculated with bacterial cultures and incubated at 24°C for 3 days. Then, the membranes were removed and placed on Whatman filter paper impregnated with the Salkowski reagent. Strains producing IAA or analogs were identified by a pink to red color in the filter surface. Siderophore production was determined using Chrome Azurol S (CAS) medium (Schwyn and Neilands, 1987). CAS agar plates were inoculated and incubated at 24°C for 5 days. Strains exhibiting an orange halo were positive for siderophore production.

***In vitro* fungitoxicity tests:** The fungicides; myclobutanil, captan and tebuconazole were kindly supplied by Hektas Crop Protection AG (Gebze, Turkey), diniconazole and thiophanate methyl by Sumitomo (Osaka, Japan), iprodione by Bayer Crop Science AG (Leverkusen, Germany) and benomyl by DuPont (Wilmington, USA). Stock solutions of these fungicides were made in ethanol, with exceptions of benomyl and iprodione which were dissolved in acetone. The sensitivity of strain 51 to the fungicides was assessed on KB plates containing a range of concentrations of each fungicide (0, 1, 3, 10, 30 and 100 μg mL^{-1}). The fungicides were added aseptically to sterilized growth medium from stock solutions, prior to inoculation. At least six concentrations with ten replicates for each fungicide were used in order to obtain the respective fungitoxicity curves. The experiments were conducted in 9 cm petri dishes, inoculated with 0.1 mL bacterial suspensions. The effect of the fungicide on growth was determined by measuring the number of colonies, after incubation for 3-4 days at 24°C in the dark.

Statistical analysis: The statistical analyses were performed with SAS software (SAS Institute, 2000 Inc, Cary, NC, USA). Data collected were subjected to analysis of variance using PROC, ANOVA and treatment means were separated using Duncan's multiple range test at $p = 0.05$.

RESULTS

To examine the effect of the *Pseudomonas* strains on plant growth promoting activity, in the first trial, 5 PGPRs were inoculated on *Chrysanthemum* and *Pelargonium* plants at 3 weeks old. Table 1 showed that

Table 1: Effect of the strains of *Pseudomonads* on growth promotion of *Pelargonium peltatum* F1[1]

Treatments	Height (cm)	Percent increase over control	Leaf surface area (cm^2)	Percent increase over control	No. of flowers	Percent increase over control
P. putida 17	13.1^{c}	-11	166.7^{bc}	22	0.4	-20
P. fluorescens 51	12.4^{c}	-16	160.5^{c}	18	1.0	100
P. fluorescens 116	12.0^{c}	-18	172.3^{b}	27	0.2	-60
P. fluorescens 122	12.9^{c}	-12	149.7^{d}	10	0.6	20
P. fluorescens 148	16.6^{a}	13	212.1^{a}	56	0.8	60
Control	14.7^{b}	0	136.2^{e}	0	0.5	0

[1]Values are the means of ten plants. Means followed by the same letter are not significantly different according to the Waller-Duncan test ($p = 0.05$). Plants were treated by watering two times with a suspension of 10^9 CFU mL^{-1}. Measurements were done 50 days after the first inoculation

Table 2: Effect of the strains of *Pseudomonads* on growth promotion of *Chrysanthemum* Virunga Yellow[1]

Treatments	Height (cm)	Percent increase over control	No. of days required for flowering (%50)	Decrease over control	Days from inoculation to first flower	Decrease over control	No. of flowers	Percent increase over control
P. putida 17	16.1	5	64	6	33	1	35.8^{b}	0
P. fluorescens 51	16.9	10	63	7	28	6	42.9^{a}	19
P. fluorescens 116	15.0	-3	69	1	29	5	30.8^{b}	-14
P. fluorescens 122	16.5	7	64	6	32	2	36.5^{b}	2
P. fluorescens 148	15.3	-1	67	3	34	0	36.5^{b}	2
Control	15.4	0	70	0	34	0	35.9^{b}	0

[1]Values are the means of ten plants. Means followed by the same letter are not significantly different according to the Waller-Duncan test ($p = 0.05$). Plants were treated by watering two times with a suspension of 10^9 CFU mL^{-1}. Measurements were done 50 days after the first inoculation

Table 3: Effect of *Pseudomonas fluorescens* strain 51 on growth promotion of *Dahlia variabilis* Figaro[1]

Treatment	Root length (cm)	Percent increase over control	Fresh weight (g)	Percent increase over control	Dry weight (g)	Percent increase over control	No. of flowers	Percent increase over control
51	14.0	27.3	64.4	29.6	6.2	14.8	5.6	107
Control	11.0	0.0	49.7	0.0	5.4	0.0	2.7	0

[1]Results are the means of 10 replicates consisting of one plants per replicate in a randomized block trials Plants were treated by watering two times with a suspension of 10^9 CFU mL^{-1}. Measurements were done 30 days after the first inoculation

Pelargonium plants exhibited significant increase in growth, mainly in the production of more leaf surface area and more flowering when *P. fluorescens* strain 148: 51 and 122 were applied to soil. 148: 51 and 122 augmented, respectively 56, 18 and 10% of leaf surface area as well as 60, 100 and 20% of number of flowers. Also, highest plant height was observed in 148 treated plants with an increase of 13% over the control. The lowest efficacy was recorded in strain 17 and 116. Treatment with these isolates enlarged the leaf surface area but decreased the number of flowers and plant height.

In *Chrysanthemum*, significant increase in plant growth parameters was registered in *P. fluorescens* strain 51 giving 10 and 19% increase in plant height and number of flowers, respectively (Table 2). In addition, it significantly decreased the No. of days required for flowering (50%), increased the number of basal branches (branches from the rosette) and decreased the number of aerial branches (branches above the rosette). Moreover, plants treated with 51 flowered on average 6 day faster than control plants. This was followed by 17 and 122 which resulted in significant reduction in number of days required for 50% flowering and augmented, respectively 5 and 7% of plant height (Table 2).

Based on the plant growth promoting activity on *Pelargonium* and *Chrysanthemum*, fluorescent pseudomonas strain 51 was selected for the second greenhouse trial which was performed on *Dahlia*. Also, *P. fluorescens* 51 treatment was found to be very effective in improving the plant growth characters of *Dahlia* compared to non-treated control. Plant raised from 51 treated pots recorded 27% increase in root length, 107% more flowers, 29 and 14% enhancement in fresh and dry weight, respectively (Table 3). It also decreased the number of days required for flowering (7 days).

Characterization of the strains: Of the 5 growth-promoting isolates, 5 produced siderophores, 2 produced auxins, 3 solubilized tricalcium phosphate and 4 produced HCN *in vitro* (Table 4).

Fungicide sensitivity of the most efficient strain: Among the PGPRs, significant increase in plant growth parameters was registered in *P. fluorescens* strain 51. To be integrated into most production systems, strain 51 must be compatible with other management practices. For this purpose, we assessed the compatibility of strain 51 with seven fungicides recommended for ornamental plants (Table 5). Fungicides tested did not kill strain 51 at the concentrations tested in the *in vitro* experiment. Visual observations on fluorescent pigmentation, growth and population counts of the bacteria revealed that benomyl, thiophanate methyl and tebuconazole gave the

Table 4: Secondary metabolite production of the fluorescent *Pseudomonads in vitro*

		Origin		Secondary metabolites[1]			
Species	Strain	Plant	Organ	HCN	IAA	Sid.	Phos.
P. putida	17	Tobacco	Root	+	-	+	-
P. fluorescens	51	Pea	Root	-	+	+	+
	116	Pea	Root	+	+	+	+
	122	Pea	Root	+	-	+	-
	148	Pea	Root	+	-	+	+

[1]Secondary metabolite production: Hydrogen Cyanide (HCN), Indole Acetic Acid (IAA), Siderophores (Sid.) and Phosphate solubilization (Phos.)

Table 5: *In vitro* effects of some fungicides on colonial development of *P. fluorescens* strain 51

		Mean bacterial count[1]				
Proprietary name	Active ingredient	(10 ppm)	(30 ppm)	(100 ppm)	(300 ppm)	(1000 ppm)
Nimbus 24 EC	Myclobutanil	27.5	30.3	21.8	19.0	9.6
Spotless 5 EC	Diniconazole	22.8	29.0	18.8	24.2	1.2
Rovral 50 WP	Iprodione	35.0	31.4	28.4	29.6	16.2
Benlate 50 WP	Benomyl	24.0	27.8	22.4	39.2	28.0
Captan'H 50 WP	Captan	33.0	26.4	18.6	6.8	12.4
Miracle 25 WP	Tebuconazole	25.2	32.0	26.8	26.6	32.6
Sumitop 70 WP	Thiophanate methyl	32.2	25.25	29.3	30.7	35.0
	Control			28.6		

[1]All values are means of two experiments with 10 replications in each experiment

best compatibility. Iprodione was found to be partly compatible with the strain 51 and reduced final count only at 100 μg mL^{-1}. However, diniconazole was found to be most toxic to strain 51 and significantly reduced growth rate and final colony count at 100 μg mL^{-1} compared to growth on unamended medium. Captan and myclobutanil reduced final colony count at 30 μg mL^{+} or greater.

DISCUSSION

Present results clearly demonstrate that PGPRs can be used under greenhouse conditions to enhance growth of *Pelargonium*, *Chrysanthemum* and *Dahlia*. Growth stimulation effects reported in the literature by the use of PGPR strains are similar to those reported by us (Dedej *et al*., 2004; Scherm *et al*., 2004). However most reports deal with industrial and vegetable plants, including chick-pea, soybean, sugarcane, pea and canola (Dileep Kumar and Dube, 1992; Dileep Kumar *et al*., 2001; Gore and Bora, 2004; Kloepper *et al*., 1988; Mirza *et al*., 2001).

The effect of *Pseudomonads* was dependent on the rootstock type. According to analysis of variance, the interaction between strain and rootstock was significant ($p<0.001$). Among the strains, 51 was effective on three plants rootstock, 148 was effective only in *Pelargonium* and others were partly effective in some plant systems. Similar results describing strain-host plant specificity have been reported in other plant systems such as several herbaceous crops (Howie and Echandi, 1983; Kloepper, 1996). This specificity appears to be related to the different composition of rhizosphere exudates depending on the plant species which affect the levels of colonization and subsequently the efficacy of the PGPR strains (Latour *et al*., 1996), or to the specific compounds present in the exudates that may stimulate in the bacteria the synthesis of secondary metabolites implicated in the plant growth promotion (Van Overbeek and Van Elsas, 1995).

The best strains were very diverse in secondary metabolite production. This is in agreement with other studies that have found a wide functional and genetic diversity between PGPR strains (Ellis *et al*., 2000; Rainey *et al*., 1994). However, in some strains certain phenotypic characteristics could be associated to rootstock growth promotion. Production of IAA *in vitro* by strain 51 may be implicated in the promotion of rootstock growth. Other reports indicate that while many plant growth-promoting bacteria can produce at least small amounts of IAA, high IAA producers are often inhibitory to root growth (Barazani and Friedman, 1999). Therefore, the role of IAA is not always consistent with plant growth promotion among bacteria and may depend on the plant species because plant responses to hormones vary considerably (Gutierrez-Manero *et al*., 1996). However, more specific research should be conducted to prove its direct implication. The production of cyanide by strain 17 and by most of the *P. fluorescens* strains greatly contrasts with its ability to promote growth. Cyanide production can increase the fitness of the PGPR strains and enhance the suppression of deleterious rhizobacteria (Mazzola *et al*., 1992) or can stimulate the exudation of nutrients from plants (Åström, 1991). However, cyanide production has been reported to cause deleterious effects on plant development by inhibition of root growth on some

plant-PGPR systems (Schippers *et al*., 1991). All strains produced siderophores. Siderophores can sequester iron in the rhizosphere making it unavailable to other rhizosphere microorganisms, then increasing the fitness of the PGPR strains (Kloepper *et al*., 1990). In addition strains 51, 116 and 148 were efficient phosphate solubilizers. As reported by Richardson (2001) and Diby Paul *et al*. (2001), P solubilization by rhizosphere microorganisms may play significant roles in plant growth promotion.

It is a general belief that fungi or bacteria used as biocontrol or plant growth promoting agents should not be mixed together with any chemical pesticides and farmers are advised in the same way. Such a recommendation will limit the use of these agents in crops like ornamental plants where chemical pesticides are frequently applied as combined spray. Hence, it was felt necessary to study the compatibility of *P. fluorescens* strain 51 with commonly used fungicides. Strain 51 was found to be compatible with benomyl, tebuconazole, thiophanate methyl and partly with iprodione. This is supported by the findings of Laha and Venkataraman (2001) that carbendazim was compatible with *P. fluorescens*. Significantly higher bacterial population was obtained in 100 μg mL^{-1} carbandazim amended KB than in 50 μg mL^{-1} amended KB or KM alone. In the Mathew (2003) studies, *P. fluorescens* was highly found compatible with 100 μg mL^{-1} carbendazim, mancozeb and imidacloprid and maximum bacterial count was recorded with these treatment.

The screening procedure used in this study was performed in a pathogen-free environment in a commercial nursery and no artificial inoculation of root pathogens was done. Thus, the theoretical benefits of certain antagonistic properties or antimetabolite production, may not be expressed in the environmental conditions used. It is expected that growth-promoting strains with high antimicrobial potential will provide additional advantages to *Chrysanthemum*, *Pelargonium* and *Dahlia* plants in the presence of root pathogens or deleterious bacteria. However, further studies are required to show if the suppression of specific pathogens of *Chrysanthemum*, *Pelargonium* and *Dahlia* rootstock from the root system increase the effect of growth promotion. To investigate the involvement of bacterial metabolites in rootstock growth promotion, experiments using reductionist molecular techmiques will be performed.

Finally, the fact that *P. fluorescens* strain 51 was effective and consistently enhanced development of *Chrysanthemum*, *Pelargonium* and *Dahlia* rootstock after irrigation with relatively diluted bacterial suspension opens the possibility of its use in commercial ornamental nurseries. The effect of this strain on plant rootstock development is particularly interesting because an optimal growth during the first month is essential for good establishment in the greenhouse and also it is an advantage to shorten the time required for plant production. However, additional work has to be done to set-up suitable methods of mass production and formulation of the bacteria to be used in practice.

ACKNOWLEDGMENTS

We are grateful to Prof. T. Bora and Prof. H. Özaktan (Ege University, Faculty of Agriculture, Department of Plant Protection) for the critical review of the manuscript.

REFERENCES

Alstrom, S., 1991. Induction of disease resistance in common bean susceptible to halo blight bacterial pathogen after seed bacterization with rhizosphere *Pseudomonads*. J. Gen. Applied Microbiol., 37: 495-501.

Åström, B., 1991. Role of bacterial cyanide production in differential reactions of plant cultivars to deleterious rhizosphere *Pseudomonads*. Plant Soil, 133: 93-100.

Barazani, O. and J. Friedman, 1999. Is IAA the major root growth factor secreted from plant-growth-mediating bacteria? J. Chem. Ecol. 25: 2397-2406.

Brito, Alvarez, M.A., S. Gagné and H. Antoun, 1995. Effect of compost on rhizosphere microflora of the tomato and on the incidence of plant growth-promoting rhizobacteria. Applied Environ. Microbiol., 61: 194-199.

Dedej, S., K.S. Delaplane and H. Scherm, 2004. Effectiveness of honey bees in delivering the biocontrol agent *Bacillus* subtilis to blueberry flowers to suppress mummy berry disease. Biol. Control, 31: 422-427.

Diby Paul, A., M. Kumar, M. Anandaraj and Y.R. Sarma, 2001. Studies on the suppressive action of fluorescent *Pseudomonads* on *Phytophthora capsici*, the foot rot pathogen of Black pepper. (Abstract) Ind. Phytopathology, 54: 515.

Dileep Kumar, B.S. and H.C. Dube, 1992. Seed bacterization with a fluorescent *Pseudomonas* for enhanced plant growth, yield and disease control. Soil Biol. Biochem., 24: 539-542

Dileep Kumar, B.S., I. Berggren and A.M. Mårtenson, 2001. Potential for improving pea production by co-inoculation with fluorescent *Pseudomonas* and *Rhizobium*. Plant Soil, 229: 25-34.

Ellis, R.J., T.M. Timms-Wilson and M.J. Bailey, 2000. Identification of conserved traits in fluorescent *Pseudomonads* with antifungal activity. Environ. Microbiol., 2: 274-284.

Enebak, S.A., G. Wei and J.W. Kloepper, 1998. Effects of plant growth promoting rhizobacteria on loblolly and slash pine seedlings. For. Sci., 44 : 139-144.

Göre M.E. and T. Bora, 2004. Microplot field experiments on the role of fluorescent *Pseudomonads* In biological control of ascochyta blight of peas. Proc. 1st Plant Prot. Cong. Turkey, Samsun, pp: 49.

Gutierrez-Mañero, F.J., N. Acero, J.A. Lucas and A. Probanza, 1996. The influence of native rhizobacteria on European alder (*Alnus glutinosa* (L.) Gaertn.) growth. Plant Soil, 182: 67-74.

Howie, W.J. and E. Echandi, 1983. Rhizobacteria: Influence of cultivar and soil type on plant growth and yield of potato. Soil Biol. Biochem., 15: 127-132.

Kloepper, J.W., 1996. Host specificity in microbe-microbe interactions. Bioscience, 46: 406-409.

Kloepper, J.W., D.J. Hume, F.M. Scher, C. Singleton and B. Tipping *et al*., 1988. Plant growth-promoting rhizobacteria on canola (rapeseed). Plant Dis. 72: 42-46.

Kloepper, J.W., M.N. Schroth and T.D. Miller, 1980. Effects of rhizosphere colonization by plant growth promoting rhizobacteria on potato yield and development. Phytopathology, 70: 1078-1082.

Kloepper, J.W., R.M. Zablotowýxz, E.M. Tipping and R. Lifshitz, 1990. Plant Growthpromotion Mediated by Bacterial Rhizosphere Colonizers. In: Keister, D.L. and P.B. Cregan, (Eds.). The rhizosphere and plant growth. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp: 315-326.

Laha, G.S. and S. Venkataraman, 2001. Sheath blight management in rice with biocontrol agents. Ind. Phytopathol., 54: 461-464.

Latour, X., T. Corberand, G. Laguerre, F. Allard and P. Lemanceau, 1996. The composition of fluorescent pseudomonad population associated with roots is influenced by plant and soil type. Applied Environ. Microbiol., 62: 2449-2456.

Liu, L., J.W. Kloepper and S. Tuzun, 1995. Induction of systemic resistance in cucumber against *Fusarium* wilt by plant growth-promoting rhizobacteria. Phytopathology, 85: 695-498.

Mathew, A.V., 2003. Pseudomonas Fluorescens-antagonism, compatibility with pesticides and alternate media for mass multiplication. 6th Intl. PGPR Workshop, Calicut, India, pp: 159-164.

Mazzola, M., R.J. Cook, L.S. Thomashow, D.M. Weller and L.S. Pierson III, 1992. Contribution of phenazine antibiotic biosynthesis to the ecological competence of fluorescents *Pseudomonads* in soil habitats. Applied Environ. Microbiol., 58: 2616-2624.

Mirza, M.S., W. Ahmad, F. Latif, J. Haurat, R. Bally, P. Normand and K.A. Malik, 2001. Isolation, partial characterization and the effect of Plant Growth-Promoting Bacteria (PGPB) on micro-propagated sugarcane *in vitro*. Plant Soil, 237: 47-54.

Pikovskaya, R.E., 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Microbiologia, 17: 362-370.

Rainey. P.B., M.J. Bailey and I.P. Thompson, 1994. Phenotypic and genotypic diversity of fluorescent *Pseudomonads* isolated from field grown sugarbeet. Microbiology, 140: 2315-2331.

Reddy, M.S., R. Rodriguez-Kabana, D.S. Kenney, C.M. Ryu, S. Zhang, Z. Yan, N. Martinez-Ochoa and J.W. Kloepper, 1999. Growth promotion and Induced Systemic Resistance (ISR) mediated by a biological preparation. Phytopathology, 89: S65.

Richardson, A.E., 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Aust. J. Plant Physiol., 28: 8797-906.

Scherm, H., H.K. Ngugi, A.T. Savelle and J.R. Edwards, 2004. Biological control of infection of blueberry flowers caused by *Monilinia vaccinii-corymbosi.* Biol. Control, 29: 199-206.

Schippers, B.A, W. Bakker, P. Bakker and R. Van Peer, 1991. Beneficial and deleterious effects of HCN-producing *Pseudomonads* on rhizosphere interactions. Plant Soil 129, 75-83.

Schwyn, B. and J.B. Neilands, 1987. Universal chemical assay for the detection and the determination of siderophores. Anal. Biochem., 160: 47-56.

Sneath, P.H.A., 1966. Identification Methods Applied to *Chromobacterium.* In: Identification methods for Microbiologist, Part A. Eds B Mgibb and F A Skinner. Academic Press, London. pp: 15-20.

Thompson, D.C., 1996. Evaluation of bacterial antagonists for reduction of summer patch symptoms in Kentucky blue grass. Plant Dis., 80: 856-862.

Van Overbeek, L.S. and J.D. Van Elsas, 1995. Root exudate-induced promoter activity in *Pseudomonas fluorescens* mutants in the wheat rhizosphere. Applied Environ. Microbiol., 61, 890-898.

Vidyasekharan. P., N. Kamala, N. Ramanthan, K. Rajappan, V. Paranidharan and R. Velazhahan, 2001. Induction of systemic resistance by *Pseudomonas fluorescens* Pf1 against *Xanthomonas oryzae* pv.*oryzae* in rice leaves. Phytoparasitica, 29: 1-12.

Weller, D.M., 1998. Biological control of soil-borne pathogens in the rhizosphere with bacteria. Ann. Rev. Phytopathol., 26: 379-407

Zehnder, G.W., C. Yao, G. Wei and J.W. Kloepper, 2000. Influence of methyl bromide fumigation on microbe-induced resistance in cucumber. Biocont. Sci. Technol., 10: 687-693.