Int. J. Biol. Res., 2(2): 115-123, 2014.

ROLE OF ENDOPHYTIC AND RHIZOSPHERIC FLUORESCENT *PSEUDOMONAS* ASSOCIATED WITH MUNGBEAN IN SUPPRESSING THE ROOT ROTTING FUNGI OF MUNGBEAAN

Naila Khatoon[1], Hafiza Asma Shafique[1], Rubina Noreen[1], Viqar Sultana[2], Rabia Badar[3] and Syed Ehteshamul-Haque[1]

[1]*Department of Botany,* [2] *Department of Biochemistry,*
University of Karachi, Karachi-75270, Pakistan.
[3]Department of Botany, Jinnah University for Women, Nazimabad, Karachi.

ABSTRACT

Efficiency of rhizosphere and endo root fluorescent *Pseudomonas* isolated from mungbean (*Vigna radiata* (L.)Wilczek) were evaluated on mungbean against soil borne pathogen *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani, Fusarium oxysporum* and root knot nematodes *Meloidogyne javanica*. Sixteen isolates of *Pseudomonas aeruginosa* were isolated from the rhizosphere soil, while four isolates were isolated from the roots of mungbean growing at Karachi University campus. *In vitro* studies, cell free culture filtrate of all isolates of *P. aeruginosa* showed nematicidal activity against *M. javanica*, root knot nematode. The cell free culture filtrate of PGPR-3, PGPR-5, PGPR-7, PGPR-14, and PGPR-15 showed 80% or more mortality after 48 hours. Antifungal activity of PGPR strains were tested against four highly destructive pathogens *M. phaseolina, R. solani, F. solani* and *F, oxysporum.* Out of 20 strains tested, six strains inhibited all the test fungi, while all other strains produced zone of inhibition against at least three test fungi with variability.

In the green house experiment two strains of *P. aeruginosa*, PGPR-3 and PGPR-13 significantly ($p<0.05$) controlled the infection of *M. phaseolina* and *R. solani.* Shoot length, shoot weight, root length and root weight of mungbean increased by PGPR-3 and PGPR-13, PGPR-17.while the number of nodules significantly ($p<0.05$) increased by the application of PGPR-3 and PGPR-5. Whereas PGPR-13, PGPR-14 and PGPR-7 showed significant ($p<0.05$) reduction in knot formation on roots.

KEYWORDS: Biologica control, PGPR, *Pseudomonas* spp., Mungbean.

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek), an important pulse crops is grown in summer as kharif crop (Yadava, 1992). The area under mungbean cultivation in Pakistan is 239.2 thousand hectares and producing about 115.4 thousand tons of grains annually (Anon., 2003). Several soil borne pathogens cause root rot diseases in mungbean and may seriously limit crop production (Siddiqui *et al*., 1999). Most of the soil borne pathogens are difficult to control by conventional control measures such as the use of resistant cultivars and synthetic pesticides (Weller, 1988; Weller *et al.,* 2002). Moreover, there is an increasing awareness that pesticides and fertilizers cause damage to the environment and effect human health; as a consequence, there is a trend toward finding ways to minimize the use of pesticides (Maas & Galletta, 1997).

The root colonizing bacteria that have a beneficial effect on plants are termed as plant growth promoting rhizobacteria (Kloepper *et al*., 1980). Plant growth promoting rhizobacteria (PGPR) have been reported to improve plant growth either through direct stimulation of the plant or by suppression of pathogens (Batool *et al.,* 2013; Weller *et al*., 2002; Compant *et al.,* 2005). Of the various rhizospheric bacteria, the bacteria belonging to the fluorescent *Pseudomonas* reported to induce systemic resistance in plants beside direct suppression of pathogens (Zhou & Paulitz, 1994; Sultana *et al.,* 2008; Ehteshamul-Haque *et al.,* 2007ab; 2013; Ramamoorthy *et al.,* 2001).

Fluorescent *Pseudomonas* are considered as rhizosphere bacteria, however several reports are now available about endophytic fluorescent *Pseudomonas* (Afzal *et al.,* 2013; Tariq *et al*., 2009; Shishido *et al.,* 1999). Considerable evidence has been accumulated in recent years to support and identify the benefits associated with the use of endophytic bacteria particularly fluorescent *Pseudomonas* in crop protection (Chen *et al.,* 1995; Ehteshamul-Haque *et al.,* 2013; Siddiqui & Ehteshamul-Haque, 2001). However, comparative biocontrol potential of rhizospheric and endophytic fluorescent *Pseudomonas* in suppressing the root diseases of mungbean was not investigated. The present report describes the comparative efficacy of rhizospheric and endophytic fluorescent *Pseudomonas* in suppressing the root diseases of mungbean.

MATERIALS AND METHODS

Collection of root and soil samples for isolation of fluorescent *Pseudomonas* from mung bean: For the isolation of fluorescent *Pseudomonas*, roots with small amount of adhering soil of mungbean plants were collected from experimental field of Department of Botany, University of Karachi. The samples were brought to the laboratory and kept at low temperature until isolation was made within 24 hours.

Isolation of fluorescent *Pseudomonas*

Isolation from soil: Soil was carefully removed from roots and collected in test tubes (1g) and different dilutions up to 10^{-3} were made. The final dilution (100 µl) was spread over Petri dishes containing S-1 medium (Gould *et al.,* 1985) supplemented with antibiotic trimethoprim (Bashan *et al.*, 1993). Petri dishes were incubated for 3 days at 28ºC. Bacterial colonies fluoresced under UV light at 366nm were purified on King B agar medium (King *et al.,* 1954).

Isolation from roots: One g roots were washed with running tap water, sterilized with 1% bleach for 3 min., rinse with 70% alcohol for 2-3 minutes and finally washed with sterilized distilled water about 1 minute. Roots were then chopped in to small pieces in a blender with 50ml of water so as to give the dilution of 1:50. After wards dilution of the root suspensions was prepared up to $1{:}10^4$ and transfer 0.1 ml suspensions into a Petri dish containing the S-1.

***In vitro* test against root infecting fungi:** Dual culture plate method was used to determine the antifungal activity of bacterial strains (Drapeau *et al*., 1973). The bacterial strains/ isolates were streaked on one side of the Petri dishes containing Czapek's Dox agar, pH 7.2. On the other side of Petri dishes, a 5mm diameter, disc of test fungus was inoculated. The dishes were incubated at 28ºC and zone of inhibition were recorded up to 5-7 days (depending upon the growth of test fungus).

Cell free culture filterate of bacteria: Bacterial strains *Pseudomonas aeruginosa* were grown on KB Broth at 30ºC for 48 hours in dark and centrifuged twice at 300rpm for 20 minutes. The pellets were discarded and cultural filtrates were collected in a beaker for use.

***In vitro* juvenile mortality test:** One ml of freshly hatched 2nd stage Juvenile suspension (20-25 juveniles) and 1 ml cell free culture filtrate of bacterial strains were transferred in glass cavity blocks and kept at room temperature ± 5^0C. There were 3 replicates of each treatment and juvenile mortality was recorded after 48 hours.

Pot experiment: The experiment was carried out to examine efficacy of rhizospheric and endophytic *Pseudomonas* (PGPR) in controlling the root rotting fungi and root knot nematode on mungbean. The soil used in this experiment had a natural infestation of 3-6 sclerotia/g of soil of *Macrophomina phaseolina* (Sheikh & Ghaffar, 1975), 5-10% colonization of *Rhizoctonia solani* on sorghum seeds used as baits (Wilhelm, 1955) and 3000 cfu/g of soil of mixed population of *F. solani* and *F. oxysporum* as assessed by soil dilution technique (Nash & Snyder, 1962). Six seeds of mungbean were sown in earthen pots containing the 1 kg soil. *Pseudomonas aeruginosa* isolates grown on King's B medium at room temperature for three days, were scrapped with the help of a sterilized bent spatula after adding some amount of water and pooled into a beaker. A 25 ml of bacterial suspension was drench in each pot @ 10^8 cfu/ml. Carbendazim (25 ml of 200 ppm) served as positive control against root infecting fungi while carbofuran (0.5 g/pot) served as positive control against root knot nematode. After germination only 4 seedling were kept in each pot and excessive were removed. Each pot were inoculated with 1000 eggs /J_2 of *M. javanica.* Each treatment was replicated four times and pots were randomized on screen house bench.

To determine the efficacy of biocontrol agents against soil borne pathogens of mungbean, plants were up rooted after 6 weeks of nematode inoculation and washed under tap water. For the incidence of root infecting fungi, one cm long root pieces, 5 from each plant after surface sterilization with 1% $Ca(OCl)_2$ for 3 minutes were transferred on PDA plates, supplemented with penicillin (100,000 units/Litre) and streptomycin (0.2g/litre). Plates were incubated for 5 days at 28ºC and the incidence of root infecting fungi *M. phaseolina*, *F. solani*, *F. oxysporum* and *R. solani* were recorded. Data on plant height and fresh weight of shoot were also recorded.

Infection percentage was calculated as follows:

$$\text{Infection \%} = \frac{\text{Total number of plants infected with a pathogen}}{\text{Total number of plants}} \times 100$$

For determination of nematode's infection, number of knots per root system was counted. Data on plant growth were also recorded. Data was statistically analyzed according to Gomes & Gomes (1984).

RESULTS

Isolation of rhizosphere and endo-root fluorescent *pseudomonas*: For the isolation fluorescent *Pseudomonas* root samples of healthy plants were collected from Karachi University campus. Sixteen isolates of *Pseudomonas aeruginosa* were isolated from rhizosphere soil while four isolates of *P. aeruginosa* were isolated from inner roots of mungbean (Table 1).

Table. 1. List of Fluorescent *Pseudomonas* isolated from rhizosphere and inner roots of mungbean, University of Karachi.

P. aeruginosa strains	Plant source	Locality
PGPR-1	Rhizospheric soil	Karachi University
PGPR-2	″″	″″
PGPR-3	″″	″″
PGPR-4	″″	″″
PGPR-5	″″	″″
PGPR-6	″″	″″
PGPR-7	″″	″″
PGPR-8	″″	″″
PGPR-9	″″	″″
PGPR-10	″″	″″
PGPR-11	″″	″″
PGPR-12	″″	″″
PGPR-13	″″	″″
PGPR-14	″″	″″
PGPR-15	″″	″″
PGPR-16	″″	″″
PGPR-17	Endo-root	″″
PGPR-18	″″	″″
PGPR-19	″″	″″
PGPR-20	″″	″″

Table. 2. *In vitro* growth inhibition of *Fusarium solani, F. oxysporum, Rhizoctonia solani* and *Macrophomina phaseolina* by rhizospheric and endophytic fluorescent *Pseudomonas*.

Treatments	*F. solani*	*F. oxysporum*	*R. solani*	*M. phaseolina*
	Zone of inhibition (mm)			
PGPR-1	0	30*	16	13
PGPR-2	16*	24	0	14
PGPR-3	8*	28	38	19
PGPR-4	6	19*	31	9
PGPR-5	15*	13*	20	0
PGPR-6	10	16	0	6
PGPR-7	0	27*	8	0
PGPR-8	9	24	0	3
PGPR-9	12	21	0	8
PGPR-10	8*	31	30	6
PGPR-11	18	18*	14	3
PGPR-12	3	0	18	7
PGPR-13	26	29*	7	16
PGPR-14	10	19	17	12
PGPR-15	12*	13	20	18*
PGPR-16	21	10*	23*	0
PGPR-17	15*	10	0	6
PGPR-18	10	13	26	4
PGPR-19	6	12	0	3*
PGPR-20	10*	0	22	10

0 = No inhibition, * = Fungal mycelium lysed

Test against root infecting fungi: Effect of different strains of PGPR against four root infecting fungi viz., *Macrophomina phaseolina, Fusarium oxysporum, Fusarium solani* and *Rhizoctonia solani* were examined- *vitro*. Out of the 20 isolates of *Pseudomonas aeruginosa,* six isolates of PGPR-3, PGPR-4, PGPR-10, PGPR-13, PGPR14, PGPR-15 inhibited the radial growth of all the four test root infecting fungi viz., *M. phaseolina, F. oxysporum, F. solani* and *R. solani*. WhilePGPR-2, PGPR-3, PGPR-4, PGPR5, PGPR-8, PGPR-9, PGPR-10, PGPR-11, PGPR-12, PGPR-13, PGPR-14, PGPR-15, PGPR-16, PGPR-17, PGPR-18, PGPR-19, PGPR-20 produced 16mm,8mm, 6mm, 15mm, 10mm, 9mm, 12mm, 8mm, 18mm, 3mm, 26mm, 10mm, 12mm, 21mm, 15mm, 10mm, 6mm, 10mm produced zone of inhibition respectively against *F. solani.* SimilarlyPGPR-1, PGPR-2, PGPR-3, PGPR-4, PGPR5, PGPR-6, PGPR-7, PGPR-8, PGPR-9, PGPR-10, PGPR-11, PGPR-13, PGPR-14, PGPR-15, PGPR-16, PGPR-17, PGPR-18, PGPR-19, produced zone of inhibition of 30mm, 24mm, 28mm, 19mm, 13mm, 16mm, 27mm, 24mm, 21mm, 31mm, 18mm, 29mm, 19mm, 13mm, 10mm, 10mm, 13mm, 12mm, respectively against the *F. oxysporum*. whereas, PGPR-1, PGPR-3, PGPR-4, PGPR5, PGPR-7, PGPR-10, PGPR-11, PGPR-12, PGPR-13, PGPR-14, PGPR-15, PGPR-16, PGPR-18, PGPR-20 produced zone of inhibition of 16mm, 38mm, 31mm, 20mm, 8mm, 30mm, 14mm, 18mm, 7mm, 17mm, 20mm, 23mm, 26mm, 22mm respectively against *R. solani***.** SimilarlyPGPR-1, PGPR-2, PGPR-3, PGPR-4, PGPR-6, PGPR-8, PGPR-9, PGPR-10, PGPR-11, PGPR-12, PGPR-13, PGPR-14, PGPR-15, PGPR-17, PGPR-18, PGPR-19, PGPR-20 produced zone of inhibition of 13mm, 14mm, 19mm, 9mm, 6mm, 3mm, 8mm, 6mm, 3mm, 7mm, 16mm, 12mm, 18mm,6mm, 4mm, 3mm, 10mm respectively against *M. phaseolina* (Table 2).

Juveniles mortality test: Culture filtrates of several bacterial isolates showed nematicidal effects by killing the second stage juveniles at varying degrees. Culture filtrates of PGPR-1 PGPR-2, PGPR-3, PGPR-4, PGPR5, PGPR-6, PGPR-7, PGPR-8, PGPR-9, PGPR-10, PGPR-11, PGPR-12, PGPR-13, PGPR-14, PGPR-15, PGPR-16, PGPR-17, PGPR-18, PGPR-19, PGPR-20 caused 73.16%, 79.73%, 87.16%, 70.66%, 85.20%, 74%, 85.20%, 74%, 79.20%, 70.06%, 59.33%, 67.66%, 76.09%, 86.29%, 82.44%, 55%, 50.33%, 531.44%, 43.12%, 51.37% juveniles mortality respectively (Table 3).

Pot experiment: No infection of *M. phaseolina* was observed where PGPR-3 or PGPR-5 was used. Similarly no infection of *F. oxysporum* was observed in PGPR-13 treated plants. Whereas, plants received PGPR-3, PGPR5, PGPR-13 or PGPR-14 showed complete suppression of *R. solani.* Application of *Pseudomonas aeruginosa* isolates PGPR-3 and PGPR-13 significantly ($p<0.05$) reduced the infection of *M. phaseolina*, *F. oxysporum, F. solani, R. solani.* While PGPR-5 significantly ($p<0.05$) reduced *M. phaseolina* and *R. solani* (Table 4).

Application of some PGPR isolates also caused a positive impact on plant growth. A significant increase in plant height was recorded in PGPR-3, PGPR-13, PGPR-17 and PGPR-5 treated plants. Greater fresh weight of shoot was observed in PGPR-3, PGPR-13, PGPR-17, PGPR-7 and PGPR-4 treated plants (Fig. 1). While root weight of mungbean were significantly ($p<0.05$) increased by PGPR-3, PGPR-17, PGPR-17, PGPR-20. Root length of mungbean plant significantly ($p<0.05$) increased by PGPR-3, PGPR-13 (Fig. 2). While number of nodules were significantly ($p<0.05$) increased by PGPR-3, PGPR-5, PGPR-13 (Fig. 3).

Number of legumes significantly ($p<0.05$) increased by application of PGPR-20. PGPR-17, PGPR-7, PGPR-14 (Fig. 3). Less infection of root knot nematode was found on all plants (Fig. 7).

Table. 3. Nematicidal activity of cell free culture filtrates of different isolates of rhizospheric and endophytic fluorescent *Pseudomonas* isolated from mungbean.

Treatment	Juvenile mortality % (48 hrs.)
Control(KB broth)	6.33
PGPR-1	73.16
PGPR-2	79.73
PGPR-3	87.16
PGPR-4	70.66
PGPR-5	85.2
PGPR-6	74
PGPR-7	85.2
PGPR-8	74
PGPR-9	79.20
PGPR-10	70.06
PGPR-11	59.33
PGPR-12	67.66
PGPR-13	76.09
PGPR-14	86.29
PGPR-15	82.44
PGPR-16	55
PGPR-17	50.33
PGPR-18	31.44
PGPR-19	43.12
PGPR-20	51.37

Table.4. Effect of *Pseudomonas aeruginosa* strains used as soil drench on the infection of *Macrophomina phaseolina, Fusarium oxysporum, Fusarium solani* and *Rhizoctonia solani* in mungbean.

Treatment	*M. phaseolina*	*F. solani*	*F. oxysporum*	*R. solani*
	Infection %			
Control	62.5	75	43.75	62.5
Carbendazim	43.75	31.25	43.75	25
PGPR-3	0	12.5	6.25	0
PGPR-4	25	31.5	56.5	25
PGPR-5	0	31.25	25	0
PGPR-7	6.25	18.75	31.25	18.75
PGPR-13	6.25	12.5	0	0
PGPR-14	18.75	25	37.5	0
PGPR-17	18.75	18.75	43.75	18.75
PGPR-20	18.75	18.75	31.25	25

$LSD_{0.05}$ = Treatment = 10.9, Pathogen = 6.9

[1]Mean values in column showing differences greater than LSD values are significantly different at p<0.05

[2]Mean values in rows showing differences greater than LSD values are significantly different at p<0.05

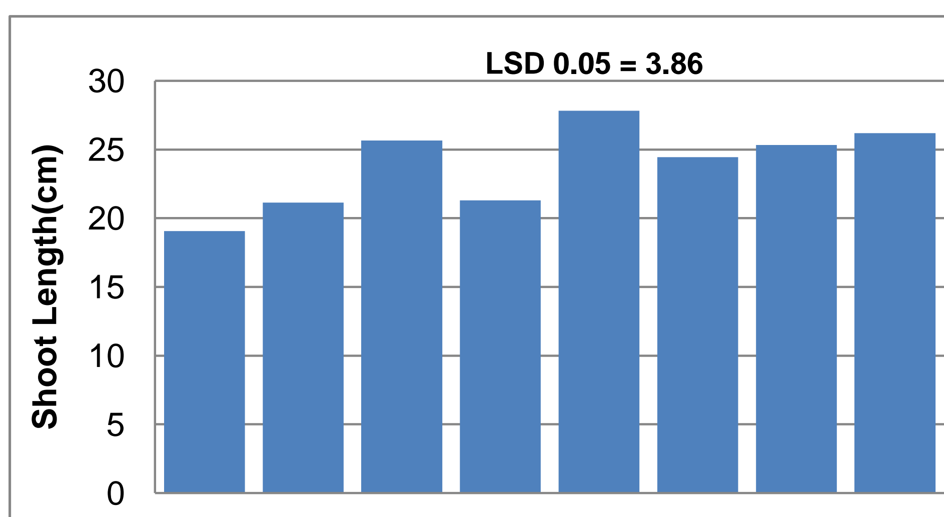

Fig.1. Shoot length

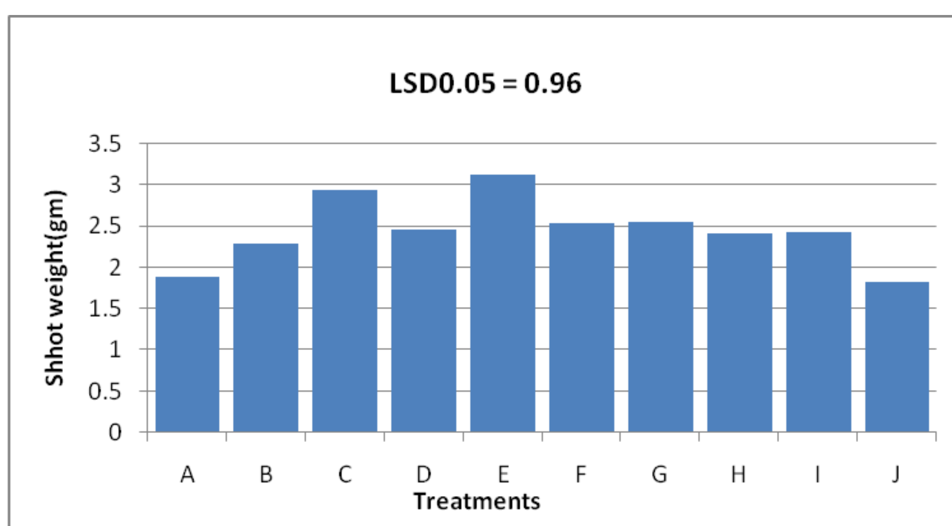

Fig. 2. Fresh shoot weight.

Fig. 1. 2. Effect of *Pseudomonas aeruginosa* isolates on the shoot length and shoot weight of mungbean
A = Control, B= Carbendazim, C= *Pseudomonas aeruginosa* -17, D= *P. aeruginosa*-20, E= *P. aeruginosa*-3, F= *P. aeruginosa*-4, G= *P. aeruginosa* -7, H= *P. aeruginosa*-5, I= *P. aeruginosa* -13, J= *P. aeruginosa* –1

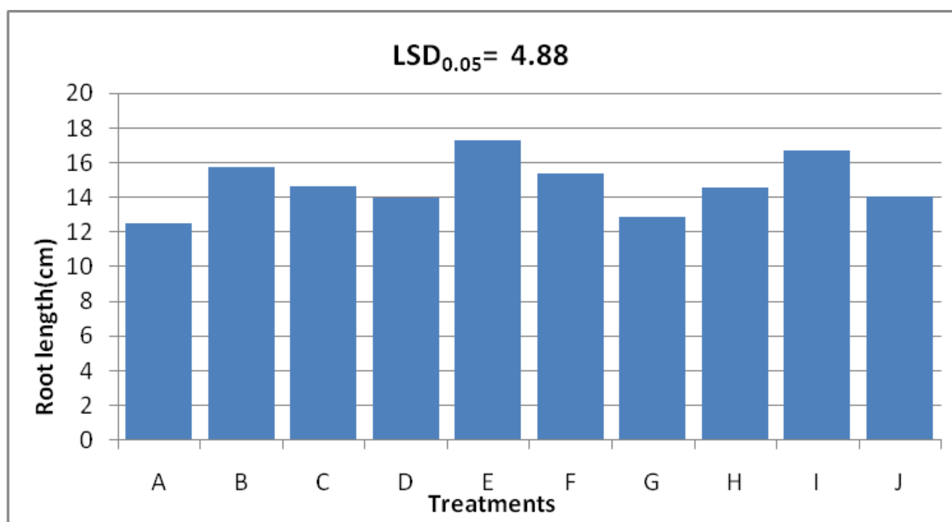

Fig. 3. Root length

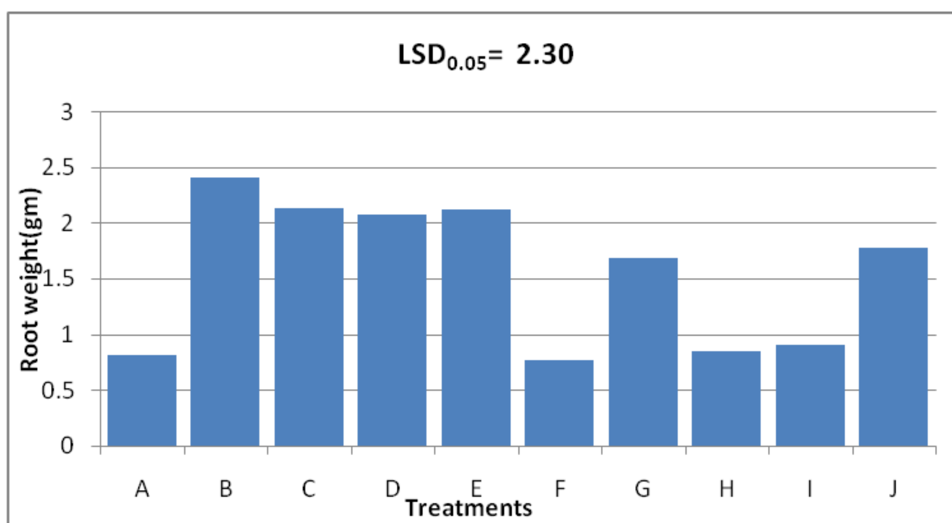

Fig. 4.fresh root weight

Fig. 3 & 4. Effect of *Pseudomonas aeruginosa* isolates on the root length and root weight of mungbean of mungbean.
A = Control, B= Carbendazim, C= *Pseudomonas aeruginosa* -17, D= *P. aeruginosa* -20, E= *P. aeruginosa* -3, F= *P, aeruginosa* -4, G= *P. aeruginosa* - 7, H= *P. aeruginosa* - 5, I= *P. aeruginosa* -13, J= *P. aeruginosa*–14

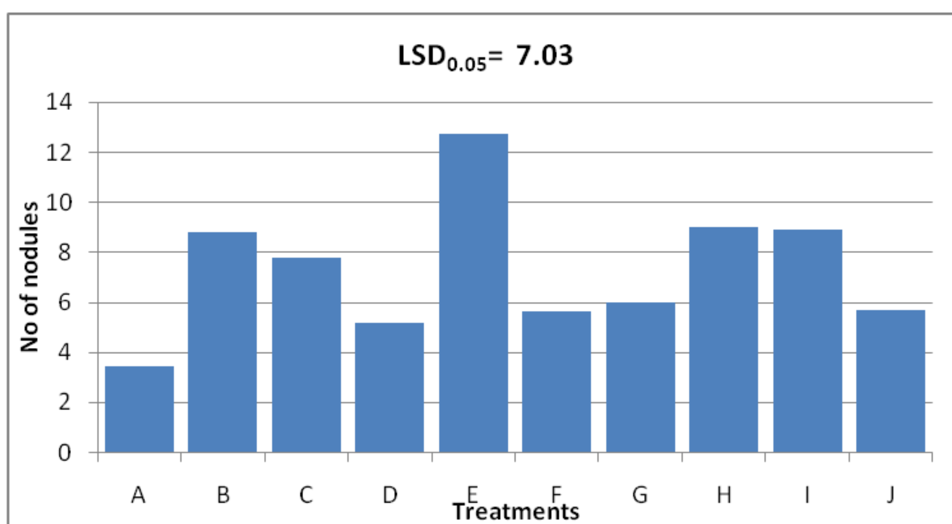

Fig. 5. Number of nodules per plant.

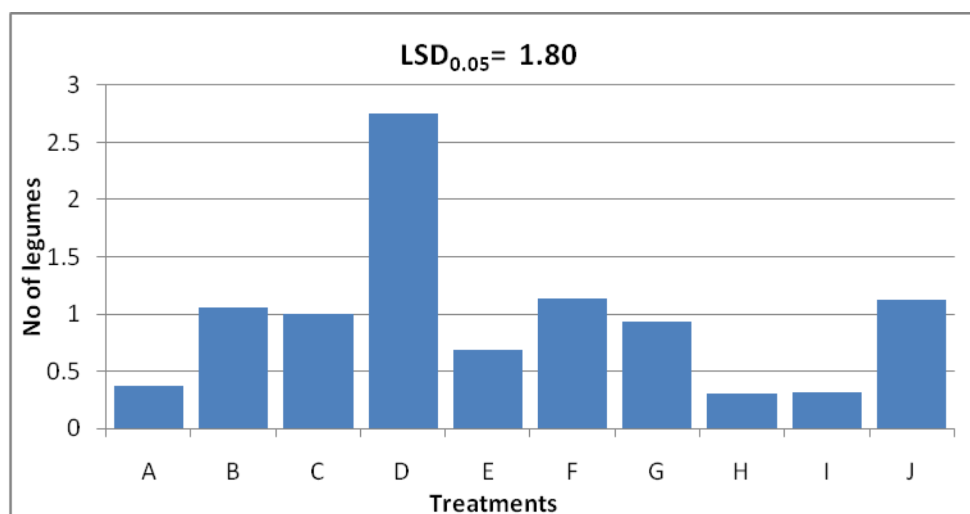

Fig. 6. Number of fruits per plant.

Fig. 5 & 6. Effect of different strains of *Pseudomonas aeruginosa* on the number of nodules and number of fruits per plant of mungbean.
A = Control, B = Carbendazim, C= *Pseudomonas aeruginosa* -17, D= *P. aeruginosa* -20, E = *P. aeruginosa* -3, F= *P, aeruginosa* -4, G= *P. aeruginosa* - 7, H= *P. aeruginosa* - 5, I = *P. aeruginosa* -13, J = *P. aeruginosa*–14

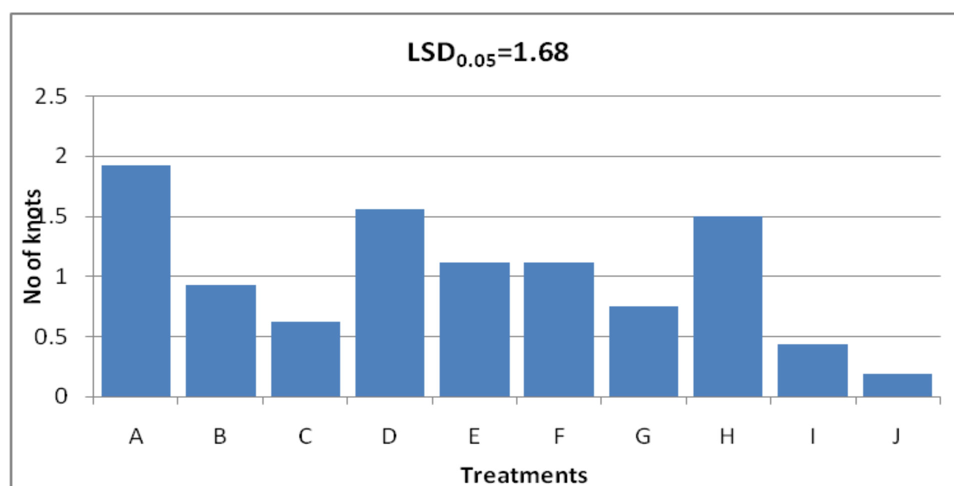

Fig. 7. Number of knots per plant.

Fig. 7.Effect of seedling treatment with different strains of *Pseudomonas aeruginosa* on number of knots on mungbean roots.
A = Control, B= Carbendazim, C= *Pseudomonas aeruginosa* -17, D= *P. aeruginosa* -20, E= *P. aeruginosa* -3, F= *P, aeruginosa* -4, G= *P. aeruginosa* - 7, H= *P. aeruginosa* - 5, I= *P. aeruginosa* -13, J= *P. aeruginosa*–14

In this study cell free culture filtrates of *Pseudomonas* also caused nematode's mortality. Root knot nematode, *Meloidogynae* spp are one of the wide spread pests limiting the world agriculture productivity (Taylor *et al*., 1982). Root-knot nematode juveniles infect plant roots, causing the development of root-knot galls that drain the plant's photosynthate and nutrients. Nematode along with soil pathogens have been found to attack every part of plant including stem, leaves, roots, seeds etc (Handoo, 1998). Selected strains of antagonistic rhizobacteria reduced nematode penetration and subsequently root knot infection in mungbean (Siddiqui *et al*., 2001). *P. aeruginosa* is proven biocontrol agent, has been found effective against the population of nematodes and specially the root knot nematodes (Siddiqui *et al.,* 2001). Three mechanism of action thought to be responsible for reduction in nematode infection (i) production of metabolite which reduces hatch and attraction, (ii) degradation of specific root exudates which control nematode behavior and (iii) enhancement of defense mechanism in plants leading to the induction of systematic resistance (Siddiqui *et al*., 2001).

DISCUSSION

Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth

(Kloepper *et al*., 1999; 1980*)*. In the present study twenty isolates of *Pseudomonas aeruginosa* were isolated from the mungbean. Out of which fourteen strains PGPR-1, PGPR-2, PGPR-3, PGPR-4, PGPR-5, PGPR-6, PGPR-7, PGPR-8, PGPR-9, PGPR-10, PGPR11, PGPR-12, PGPR-13, PGPR-14, PGPR-15, PGPR-16 were isolated from rhizosphere soil .While four strains PGPR17, PGPR-18, PGPR-19, PGPR-20, were isolated from the inside of the root of mungbean i-e endophytic. In dual plate assay, six strains of *P. aeruginosa* inhibited growth of all test fungi viz, *M. phaseolina, R. solani, F. oxysporum, F. solani*. While all other strains produced zone of inhibition against at least three test fungi with variability. The inhibitory activity of fluorescent *Pseudomonas* against plant pathogenic organisms is said to be due to production of secondary metabolites such as phenazines, acetyl phloroglucinols and cyanides (Defago & Haas, 1990). Another major mechanism involved in suppressive activity of fluorescent *Pseudomonas* is production of siderophores, which formed a complex with iron and make it unavailable to plant pathogens (Kloepper *et al*., 1980). *Pseudomonas* produce pyoverdine type siderophores, which are high affinity iron chelaters (Voisard *et al*., 1989). Besides, the aggressive root colonization character of fluorescent Pseudomonas is also reported to play an important role in rhizosphere competence and associated biocontrol activity (Neilands & Leong. 1986). However, in this study rhizospheric and endophytic *Pseudomonas* did not show any visible difference in inhibiting the root rotting fungi.

In the present study *P. aeruginosa* significantly reduced the infection of *M. phaseolina, R. solani, F. oxysporum, F. solani* on mungbean and improved plant growth. The production of certain antibiotics (Leavy *et al*., 1992) and sidospores (Buysens *et al.,* 1996) by *P. aeruginosa* has been regarded as one of the mechanism involved in antagonism. Ramette *et al*. (2003) reported that hydrogen cyanide (HCN) is a broad spectrum antimicrobial compound involved in biological control of root diseases by many plant associated fluorescent *Pseudomonas.* Raaijmakers & Weller (1998) reported the role of 2,4-diacetylphloroglucinol an antifungal metabolite from species of fluorescent *Pseudomonas* in plant root disease suppression. In addition to their potential to control plant diseases in above three experiments, most of the inoculated fluorescent *Pseudomonas* enhanced the growth parameters of mungbean plants significantly over un-inoculated control plants indicating their plant growth promotional potential. *Pseudomonas* are known to produce IAA and GA and also possess P-solubilizing ability (Megha, 2006), they might have contributed to better growth of plants. This characteristic makes the *Pseudomonas* better candidate to be utilized as biocontrol agents against soil-borne pathogens (Parveen *et al*., 1998; Haas & Defago, 2005).

ACKNOWLEDGEMENT

Financial assistance provided by the Dean Faculty of Science, University of Karachi, is sincerely acknowledged.

REFERENCES

Afzal, S., S. Tariq, V. Sultana, J. Ara and S. Ehteshamul-Haque. (2013). Managing the root diseases of okra with endo-root plant growth promoting *Pseudomonas* and *Trichoderma viride* associated with healthy okra roots. *Pak. J. Bot.,* 45: 1455-1460.

Anonymous. (2003). *Agriculture Statistics of Pakistan*. Ministry of Food, Agriculture and Livestock. Government of Pakistan, Economic Wing, Islamabad.

Bashan,Y., G. Holguin and R. Lifshitz. (1993). Isolation and characterization of plant growth promoting rhizobacteria. pp. 331-345. In: *Methods in Plant Molecular Biology and Biotechnology*. (Eds.): Glick, B.R. and J.E. Thompson. CRC Press, London.

Batool, H., N. Fatima, K. Hira, V. Sultana, J. Ara and S. Ehteshamul-Haque. (2013). Role of fluorescent *Pseudomonas* associated with root nodules of soybean in suppressing the root rotting fungi and root knot nematode of soybean in soil amended with seeds of *Vernonia antihelmenthica. Int. J. Biology Res.,* 1: 75-80.

Buysens, S., K. Heungens, J. Poppe and M. Höfte. (1996). Involvement of pyochelin and pyoverd in suppression of *Pythium* induced damping off of tomato by *Pseudomonas aeruginosa* 7NSK2. *Applied and Environmental Microbiology*, 62: 865-871.

Chen, C., E.M. Bauske, G. Musson, R. Rodriguez-Kabana and J.W. Kloepper. (1995). Biological control of *Fusarium* wilt of cotton by use of endophytic bacteria. *Biological Control,* 5: 83-91.

Compant, S., B. Duffy, J. Nowak, C. Clement and E.A. Barka. (2005). Plant growth promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action and future prospects. *Appl. Environ. Microbiol*., 71: 4951-4959.

Defago, G. and D. Haas. (1990). Pseudomonads as antagonists of soil borne plant pathogens: mode of action and genetic analysis. Soil Biochemistry. (Eds.): Bolley, J.M. and G. Stortzky. New York, Baul, 6: 249-291.

Drapeau, R., J.A. Fortin and C. Gagnon. (1973). Antifungal activity of *Rhizobium. Can. J. Microbiol.,* 51: 681-682.

Ehteshamul-Haque, S., F. Korejo, V. Sultana, S.A. Ali and J. Ara. (2013). Biocontrol potential of endophytic fluorescent *Pseudomonas* isolated from *Salvadora* species. *Phytopath.* 103 (Suppl.2): S2.38 (Abstract).

Ehteshamul-Haque, S., G. Parveen, V. Sultana and J. Ara. (2007b). Utilization of plant growth promoting and nodule producing bacteria for the management of root rotting fungi and root knot nematode. *Phytopathology,* 97: S31 (Abstract).

Ehteshamul-Haque, S., V. Sultana**,** J. Ara and M. Athar. (2007a). Cultivar's response against root infecting fungi and efficacy of *Pseudomonas aeruginosa* in controlling the root rot of soybean. *Plant Biosystems*, 141: 51-55.

Gomez. K.A. and A.A. Gomez. (1984). Statistical procedure for agriculture research. 2nd. Ed. Wiley, New York. Pg: 680.

Gould, W.D., C. Hagedorn, T.R. Bardinelli and R.M. Zablotswicz. (1985). New selective media for enumeration and recovery of fluorescent *Pseudomonas* from various habitats. *Appl. Environ. Microbiol*., 49: 28-32.

Haas, D. and G. Defago. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Reviews Microbiology*, 3(4): 307-319.

Handoo, Z.A. (1998). Plant-parasitic nematodes. (http://www.ars.usda.gov/Services/docs.htm

King, E.O., M. K. Ward and D.E. Raney. (1954). Two simple media for the demonstration of pycocyanin and fluorescin. *J. Lab. Clin. Med*., 44: 301-307.

Kloepper, J.W., J. Leong, M. Teintze and M.N. Schroth. (1980). Enhanced growth by siderophores produced by plant growth promoting rhizobacteria. *Nature,* 286: 885-886.

Kloepper, J.W., R. Rodriguez-Ubana., G.W. Zehnder., J.F. Murph., E. Sikora and C. Fernandez. (1999). Plant root-bacterial interactions in biological control of soilborne diseases and potential extension to systemic and foliar diseases. Austral. *Plant Pathol*. 28. 21-26.

Krieg, N.R. and J.G. Holt. (1984). Bergy's manual of determinative bacteriology, volume 1, Baltimore, The Williams and Wilkins Co.

Leavy, E., Z. Eyal., I. Chet and A. Hochman. (1992). Resistance mechanisms of *Septoria tritici*to antifungal products of *Pseudomonas. Physiol. Mol. Plant Pathol*., 40: 163-171.

Mass, J.L. and G.J. Galletta. 1997. Recent progress in strawberry disease research. *Acta Horticulturae,* 439: 769-780

Megha, Y.J. (2006). Functional and molecular diversity of fluorescent *pseudomonas* from the soils of Western Ghats of Uttara Kannada district, M.Sc thesis, University of Agricultural Sciences, Dharwad.

Nash, S.M. and W.C. Snyder. (1962). Quantitative estimation by plate counts of propagules of thebean root rot *Fusarium* in field soils. *Phytopathology*, 52: 567-572.

Neilands, J.B. and S.A. Leong. (1986). Siderophores in relation to plant growth and disease. *Ann. Rev. Plant Physiol*., 37.: 187-208.

Perveen, S., S. Ehteshamul-Haque and A. Ghaffar. (1998). Efficacy of *Pseudomonas aeruginosa* and *Paecilomyces lilacinus* in the control of root rot-root knot disease complex of some vegetables. *Nematol.Medit*., 26: 209-212.

Raaijmakers, J.M. and D.M. Weller. (1998). Natural plant protection by 2,4-diacetylphloroglucinol-producing *Pseudomonas* spp. in take-all decline soils. *Molecular Plant-Microbe Interactions*, 11: 144-152.

Ramamoorthy, V., R. Viswanathan, T. Raguchander, V. Prakasam and R. Samiyappan. (2001). Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pest and diseases. *Crop Protection*, 20: 1-11.

Ramette, A., M. Frapolli, G. Defago and Y. Moenne-Loccoz. (2003). Phylogeny of HCN synthase–encoding 'hcnbc' genes in biocontrol fluorescent *pseudomonads* and its relationship with host plant species and HCN synthesis ability. *Molecular Biology of Plant Microbe Interaction*, 16: 525-235.

Sheikh, A.H and A. Ghaffar. (1975). Population study of the sclerotia of *Macrophomina phaseolina* in cotton fields. *Pak. J. Bot*., 7: 13-17.

Shishido, M., C. Breuil and C.P. Chanway. (1999). Endophytic colonization of spruce by the plant growth promoting rhizobacteria. *FEMS Microbiol. Ecol*., 29: 191-196.

Siddiqui, I.A. and S. Ehteshamul-Haque. (2001). Suppression of the root rot-root knot disease complex by *Pseudomonas aeruginosa* in tomato: The influence of inoculum density, nematode population, moisture and other plant associated bacteria. *Plant & Soil,* 237: 81-89.

Siddiqui, I.A., S. Ehtesham-ul-Haque and A. Ghaffar. (1999). Effect of *Pseudomonas aeruginosa* and chemical fertilizers on root rot and root knot diseases of mung bean. *Pak. J. Nematol*, 17: 77-86.

Siddiqui, I.A., S. Ehteshamul-Haque and S.S. Shaukat. (2001). Use of rhizobacteria in the control of root rot-root knot disease complex of mungbean. *J. Phytopathology*, 149: 337-346

Sultana, V., J. Ara and S. Ehteshamul-Haque. (2008). Suppression of root rotting fungi and root knot nematode of chili by seaweed and *Pseudomonas aeruginosa. J. Phytopathology,* 156: 390-395.

Tariq, S., R. Khan, V. Sultana, J. Ara and S. Ehteshamul-Haque. (2009). Utilization of endo-root fluorescent *Pseudomonas* of chili for the management of root diseases of chili. *Pak. J. Bot.,* 41: 3191-3198.

Tariq, T., H.A. Shafiqe, V. Sultana and S. Ehteshamul-Haque1. (2014). Management of root rot disease of wheat with endophytic plant growth promoting *Pseudomonas* associated with healthy wheat roots. *Int. J. Biol. Res*., 2(1): 39-43.

Taylor, A.L., J.N. Sasser, L.A. Nelson and N.C. Raleigh. (1982). Relationship of climate and soil characteristics to geographical distribution of *Meloidogyne* species in agricultural soils. North Carolina State University Graphics.

Voisard, C., O. Keel., P. Haas and G. Defago. (1989). Cyanide production by Pseudomonas fluorescent helps to suppress black root rot of tobacco under gnotobiotic condition. *European Microbiological Journal*, 8: 351-358.

Weller, D.M. 1988. Biological control of soil borne plant pathogens in the rhizosphere with bacteria. *Ann. Rev. Phytopathol*. 26: 379-407.

Weller, D.M., J.M. Raaijmakers, B.B.M. Gardener and L.S. Thomashow. (2002). Microbial population responsible for specific soil supperssiveness to plant pathogens. *Annu. Rev. Phytopathology*, 40: 309-348.

Wilhelm, S. 1955. Longevity of the *Verticillium* wilt fungus in the laboratory and field. *Phytopathology*, 455: 180-181.

Yadava, D.S. (1992). Mungbean Pulse crops Production-Technology. 1st ed. Kalayani Publication. New Delhi, Ludhiana.

Zhou, T. and T.C. Paulitz. (1994). Induced resistance in the biocontrol of *Pythium aphanidermatum* by *Pseudomonas* spp., on cucumber. *J. Phytopathology*, 142: 51-63.

(Received July 2014; Accepted November 2014)