Pak. J. Bot., 49(5): 1995-2004, 2017.

EVALUATION OF ANTIBACTERIAL AND ANTIFUNGAL POTENTIAL OF ENDOPHYTIC FLUORESCENT *PSEUDOMONAS* ASSOCIATED WITH *SALVADORA PERSICA* L. AND *SALVADORA OLEOIDES* DECNE

FARZANA KOREJO[1], RUBINA NOREEN[1], SYED ABID ALI[2], FOZIA HUMAYUN[2], AFSHAN RAHMAN[1], VIQAR SULTANA[3] AND SYED EHTESHAMUL-HAQUE[1*]

[1]Agricultural Biotechnology & Phytopathology Laboratory, Department of Botany
[2]HEJ Research Institute of Chemistry, International Center for Chemical & Biological Sciences,
[3]Biotechnology & Drug Development Laboratory, Department of Biochemistry, University of Karachi, Karachi-75270, Pakistan
[*]Corresponding author's email: sehaq@uok.edu.pk"

Abstract

In the present study, cell-free culture filtrates of 35 isolates of endophytic fluorescent *Pseudomonas*, isolated from roots, shoots and leaves of *Salvadora persica* L. and *Salvadora oleoides* Decne were examined for antibacterial activity against Gram negative (*Escherichia coli, Salmonella typhimurium*) and Gram positive (*Bacillus subtilis, Staphylococcus aureus*) bacteria *in-vitro*. Most of them showed strong antibacterial activity by producing zone of inhibition. Cell free culture filtrates of *Pseudomonas* also caused growth inhibition of root rotting fungi *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* and *F.oxysporum* by producing zone of inhibition. Solvent fractions of 5 potential isolates were also evaluated for antibacterial activity. Both hexane and chloroform fractions of cell-free culture filtrates showed antibacterial and antifungal activity at varying degree. Endophytic fluorescent *Pseudomonas* offering important sources of antimicrobial compounds for pharmacological benefits, besides, their role in crop protection and plant growth.

Key words: *Salvadora*, endophytic, fluorescent *Pseudomonas*, antifungal, antibacterial

Introduction

The bacteria and fungi live inside the plant tissues or atleast part of their live, without causing any diseases are termed as endophytes (Strobel & Daisy, 2003). However, some of them may provide defense to plant against herbivores (Wang *et al.,* 2006). Among the endophytes, fluorescent *Pseudomonas*, which live around the plant roots may get entry inside the plant roots. Application of this bacteria to plant roots have been reported to suppress root diseases and induce systemic resistance in plants, resulting in improved plant growth (Shafique *et al.,* 2015; Rahman *et al.*, 2016).

The need for new and helpful compounds to supply assistance and relief in all aspects of the human health is ever growing with the passage of time. Due to occurrence of life-threatening infectious diseases, changing pattern of resistance in bacteria to certain antibiotics and tremendous increase in the occurrence of fungal infection is demanding the needs for searching for new and broad spectrum antimicrobial drugs (Liang *et al.,* 2012). The natural substances from endophytic microbes have been experimented to inhibit or kill large variety of diseases causing agents including plant pathogens and other bacteria, fungi, viruses and protozoa that have an effect on humans and animals (Strobel & Daisy, 2003; Berdy, 2005; Muzzamal *et al.,* 2012). Although endophytes are now being used in various fields, but they are mostly unstudied, particularly those associated with plants occurring in different ecological condition and able to tolerate different stresses. *Salvadora* is facultative halophytic plants, its species are able to tolerate dry environment, high soil salinity and also water logging (Ehteshamul-Haque *et al.,* 2013; Korejo *et al.,* 2014). This study describes the antibacterial and antifungal activity of some endophytic fluorescent *Pseudomonas* isolated from *Salvadora* species found in Sindh.

Materials and Methods

Collection of plant samples and isolation of fluorescent *Pseudomonas*: For the isolation of endophytic fluorescent *Pseudomonas* root, shoot and leaves from healthy *Salvadora persica* and *Salvadora oliedes* were collected from Karachi University Campus, Makle, Jampeer, Jang Shahi from Thatta, Jamshoro, Sanjar and Sehra from Moro and fluorescent Pseudomonas were isolated from root, stem and leaves of plant samples by using the method of Afzal *et al*. (2013). The presumptive Pseudomonas spp. was initially identified according to the Bergey's Manual (Garrity *et al*., 2005) and the selected isolates were further confirmed using established molecular biology techniques recently described by us (Noreen *et al*., 2015) and reported elsewhere.

Cell-free culture filtrates of bacteria and antibacterial activity: Cell free culture filtrates of test *Pseudomonas* was obtained as described by Habiba *et al*. (2016). To determine the antibacterial activity of culture filtrates, sterilized thick filter paper discs (5 mm) were impregnated with each filtrate at 20, 40 and 60 µL per disc. Bacterial lawn of test bacteria, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium* were prepared on Trypticase Soy agar (TSA) plates. Loaded discs with culture filtrates were placed at different positions in the Petri dish. Streptomycin (20µg/disc) served as positive control. Plates were incubated at 30°C for 2-3 days and diameter of zones of inhibition was recorded, averaged and expressed in mm. The experiment was repeated twice with three replicates.

Antifungal activity of culture filtrates of fluorescent *Pseudomonas*: To determine the antifungal activity the loaded discs were placed at the one side of Petri dishes containing Czapek's Dox agar. While other side of the Petri dishes a 5 mm disc of actively growing culture of *Macrophomina phaseolina, Rhizoctonia solani, Fusarium oxysporum* and *Fusarium solani* were placed. Dishes were incubated at 28°C for 7 days. Dried disc of KB broth was considered as negative control, while carbendazim at 20 µg/disc served as positive control.

The experiment was conducted twice with three replicates and zone of inhibition was measured.

Antibacterial and antifungal activity of solvent fractions of culture filtrates of endophytic fluorescent *Pseudomonas* isolated from *Salvadora* spp. (EFPS): The cell-free culture filtrates of promising isolates were extracted with *n*-hexane thrice. The hexane insoluble portion of broth was then extracted with chloroform. Each fraction was concentrated over rotary vacuum evaporator and weighed.

To determine the antibacterial activity of solvent fractions, after re-dissolving the each fraction in respective solvent (at 10 mg/mL), sterilized thick filter paper discs (5 mm) were loaded with each fraction at 20, 40 and 60 µL/disc. Lawn of test bacteria *B. subtilis, S. aureus, S. typhimurium* and *E.coli* were prepared on Trypticase soy agar plates and loaded discs with each fraction were placed at different position. To determine the antifungal activity of solvent fractions, loaded discs with each fraction were placed at different position in Petri dishes containing Czapek's Dox Agar. An actively growing 5 mm disc of test fungi viz., *M. phaseolina, R. solani, F. oxysporum* and *F. solani* were placed in the center of the plate. Dried disc of respective solvent was considered as negative control, streptomycin (20 µg/disc) served as positive control for antibacterial, while carbendazim (20 µg/disc) served as positive control for antifungal activity. The dishes were incubated at 28ºC up to 5 days and zone of inhibition (if any) were measured and averaged. The experiment was repeated twice with three replicates.

Results

***In vitro* antibacterial activity of cell-free culture filtrate of endophytic fluorescent *Pseudomonas*:** Culture filtrates of thirty five (35) endophytic fluorescent *Pseudomonas* were evaluated, all showed strong antibacterial activity against all the four test bacteria *B. subtilis, S. aureus, S. typhimurium* and *E. coli* by producing zone of inhibition. Most of the test bacteria showed activity at 20 µL/disc. However, the activity was found increased with increase in quantity of culture filtrates *i.e.*, 40 and 60 µL/disc (Table 1). The antibacterial activity of culture filtrates of fluorescent *Pseudomonas* was found comparable with commercial antibiotic streptomycin at 20 µg/disc.

***In vitro* antifungal activity of cell-free culture filtrate of endophytic fluorescent *Pseudomonas* isolated from *Salvadora* spp. (EFPS):** Culture filtrates of all the test endophytic fluorescent *Pseudomonas* showed strong antifungal activity against all the four test soil borne fungal pathogens *M. phaseolina, R. solani, F. solani* and *F. oxysporum* by producing zone of inhibition. Culture filtrates of *Pseudomonas* showed greater activity at 60 µL/disc than 20 or 40 µL/disc (Table 2). Most of the test bacteria showed activity at 20 µL/disc, but the activity was found increased with increase in quantity of culture filtrates i.e. 40 and 60 µL/disc (Table 2). The antifungal activity of culture filtrates of fluorescent *Pseudomonas* was found comparable with commercial fungicide carbendazim at 20 µg/disc (Table 2).

***In vitro* antibacterial activity of solvent fractions of culture filtrates of endophytic fluorescent *Pseudomonas*:** Out of hexane fractions of five selected *Pseudomonas* viz. EFPS-08, EFPS-19, EFPS-20, EFPS-21 and EFPS-22 were tested, all of them showed strong activity against all the four tested bacteria *B. subtilis, S. aureus, S. typhimurium* and *E. coli.* However, activity was found increased with increased in concentration of hexane fractions (Table 3). Similar antibacterial activity also showed by the chloroform fraction. Solvent fractions (hexane and chloroform) of cell-free culture filtrates of fluorescent *Pseudomonas* at 60 µL/disc produced larger sized zone of inhibition against test bacteria then streptomycin at 20 µg/disc (Table 4).

***In vitro* antifungal activity of solvent fractions of culture filtrates of endophytic fluorescent *Pseudomonas*:** Both hexane and chloroform fractions of five selected *Pseudomonas* showed strong activity against all the four tested fungi viz., *M. phaseolina, R. solani, F. solani* and *F. oxysporum.* Both fractions of all the five test bacteria were found effective at 20 µL/disc. However, activity was found increased with increasing concentrations (Table 5). Solvent fractions (hexane and chloroform) of cell-free culture filtrates of fluorescent *Pseudomonas* at 60 µL/disc produced larger sized zone of inhibition against test fungi then even control carbendazim at 20 µg/disc (Table 6).

Discussion

Natural products especially of higher plants and microbial origins have served as rich source of novel drugs. Several successful drugs originally isolated from fungi have been discovered in last 50 years (Smedsgaard & Nielsen, 2005). Among them, endophytic fungi and bacteria are important source of new compounds having broad spectrum activity (Strobel & Daisy, 2003). Endophytic bacteria are associated with healthy living tissues without causing any harm to host plants and some of them help plants against biotic and abiotic stress which are entirely different from saprophytes (Benhamou *et al.*, 1998). In this study, 35 isolates of fluorescent *Pseudomonas* were isolated from different parts of *Salvadora persica* and S. *oleoides* collected from different areas of Sindh province. Endophytic bacteria that have beneficial effect on plants, now receiving attention of researchers as biocontrol agents because they colonize internal tissues of plants (Prieto *et al.*, 2011; Cabanas *et al.*, 2014), similar ecological niche as plant pathogens (Berg *et al.*, 2005; Rosenblueth & Martínez-Romero, 2006). But in contrast to phytopathogens they do not cause harm to plant and enter inside plants for gaining residency (Kado, 1992; Kobayashi & Palumbo, 2000). Several reports are now available regarding the beneficial role of endophytic bacteria, such as biocontrol of soil borne plant pathogens and growth promotion on several crops (Siddiqui & Ehteshamul-Haque, 2001; Saunders & Kohn, 2009; Afzal *et al.*, 2013; Mercado-Blanco *et al.*, 2016; Rahman *et al.*, 2016).

Table 1. *In vitro* growth inhibition of *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Salmonella typhimurium* by culture filtrates of endophytic fluorescent *Pseudomonas* isolated from *Salvadora* species (EFPS).

Culture No.	*Pseudomonas*	*B. subtilis*	*S. aureus*	*S. typhimurium*	*E. coli*
		Zone of inhibition (mm)			
	Control	0	0	0	0
	+ ve control Streptomycin (20µg/disc)	9	10	8	9
EFPS-08	20µl/disc	7	7	10	0
	40µl/disc	0	11	9	7
	60µl/disc	8	0	10	10
EFPS-19	20µl/disc	0	10	7	7
	40µl/disc	7	9	7	10
	60µl/disc	10	10	7	10
EFPS-20	20µl/disc	7	9	9	9
	40µl/disc	0	10	9	10
	60µl/disc	8	11	10	10
EFPS-21	20µl/disc	6	7	7	7
	40µl/disc	7	8	7	7
	60µl/disc	9	8	8	10
EFPS-22	20µl/disc	7	9	7	7
	40µl/disc	8	0	8	9
	60µl/disc	10	10	9	10
EFPS-37	20µl/disc	9	8	10	8
	40µl/disc	10	9	11	9
	60µl/disc	11	11	12	10
EFPS-38	20µl/disc	7	8	7	9
	40µl/disc	10	10	7	9
	60µl/disc	11	10	9	10
EFPS-39	20µl/disc	10	9	9	9
	40µl/disc	10	8	10	7
	60µl/disc	11	10	11	10
EFPS-40	20µl/disc	9	7	8	7
	40µl/disc	8	9	9	10
	60µl/disc	10	10	10	11
EFPS-41	20µl/disc	7	7	9	9
	40µl/disc	9	8	10	10
	60µl/disc	10	10	10	11
EFPS-42	20µl/disc	7	7	8	7
	40µl/disc	9	8	10	10
	60µl/disc	10	9	11	11
EFPS-43	20µl/disc	9	8	9	10
	40µl/disc	9	9	10	11
	60µl/disc	10	9	11	11
EFPS-44	20µl/disc	9	0	8	9
	40µl/disc	10	8	10	10
	60µl/disc	10	10	11	11
EFPS-45	20µl/disc	9	7	8	8
	40µl/disc	8	8	10	9
	60µl/disc	9	9	10	9
EFPS-46	20µl/disc	9	8	8	7
	40µl/disc	10	9	8	0
	60µl/disc	11	10	9	7
EFPS-47	20µl/disc	7	0	9	9
	40µl/disc	7	8	10	10
	60µl/disc	8	10	11	10
EFPS-48	20µl/disc	8	7	8	7
	40µl/disc	9	8	9	7
	60µl/disc	10	10	10	9

Table 1. (Cont'd.).

Culture No.	*Pseudomonas*	*B. subtilis*	*S. aureus*	*S. typhimurium*	*E. coli*
	20µl/disc	6	7	8	7
EFPS-49	40µl/disc	6	7	10	7
	60µl/disc	10	10	10	11
	20µl/disc	8	9	7	7
EFPS-50	40µl/disc	0	10	9	7
	60µl/disc	9	10	10	10
	20µl/disc	7	7	7	7
EFPS-51	40µl/disc	9	9	8	11
	60µl/disc	10	10	11	11
	20µl/disc	7	7	7	10
EFPS-52	40µl/disc	10	8	9	11
	60µl/disc	10	9	12	11
	20µl/disc	0	7	9	8
EFPS-53	40µl/disc	7	9	10	10
	60µl/disc	7	10	10	12
	20µl/disc	7	7	9	8
EFPS-54	40µl/disc	10	7	10	10
	60µl/disc	10	10	11	11
	20µl/disc	10	9	7	7
EFPS-55	40µl/disc	7	10	8	9
	60µl/disc	7	10	9	11
	20µl/disc	9	7	7	9
EFPS-56	40µl/disc	7	10	10	9
	60µl/disc	10	12	10	10
	20µl/disc	9	7	10	7
EFPS-57	40µl/disc	10	7	10	10
	60µl/disc	11	10	11	10
	20µl/disc	7	8	7	7
EFPS-58	40µl/disc	7	9	8	10
	60µl/disc	10	10	9	10
	20µl/disc	9	9	9	7
EFPS-59	40µl/disc	8	10	10	10
	60µl/disc	10	11	11	10
	20µl/disc	7	7	10	9
EFPS-60	40µl/disc	7	10	0	10
	60µl/disc	10	0	0	11
	20µl/disc	7	8	9	7
EFPS-61	40µl/disc	10	9	10	9
	60µl/disc	10	10	10	10
	20µl/disc	9	8	7	10
EFPS-62	40µl/disc	10	9	9	11
	60µl/disc	11	10	11	11
	20µl/disc	7	9	8	9
EFPS-63	40µl/disc	10	10	9	10
	60µl/disc	10	12	10	11
	20µl/disc	7	8	8	7
EFPS-64	40µl/disc	9	9	10	8
	60µl/disc	10	10	10	11
	20µl/disc	8	9	8	7
EFPS-65	40µl/disc	9	10	10	10
	60µl/disc	11	11	11	11
	20µl/disc	8	7	10	8
EFPS-66	40µl/disc	10	9	11	10
	60µl/disc	11	10	12	11

Table 2. *In vitro* growth inhibition of *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* and *Fusarium oxysporum* by cell-free culture filtrates of endophytic fluorescent *Pseudomonas* isolated from *Salvadora* species (EFPS).

Culture No.	*Pseudomonas*	*M. phaseolina*	*R. solani*	*F. solani*	*F. oxysporum*
		Zone of inhibition (mm)			
	Control	0	0	0	0
	+ ve control Carbendazim (20µg/disc)	10	7	8	9
EFPS-08	20µl/disc	07	06	07	08
	40µl/disc	09	07	08	09
	60µl/disc	10	09	09	10
EFPS-19	20µl/disc	07	06	06	09
	40µl/disc	08	07	09	10
	60µl/disc	11	12	11	11
EFPS-20	20µl/disc	07	07	07	08
	40µl/disc	09	06	08	09
	60µl/disc	10	07	09	09
EFPS-21	20µl/disc	09	0	07	07
	40µl/disc	10	08	09	07
	60µl/disc	11	10	10	08
EFPS-22	20µl/disc	07	09	09	09
	40µl/disc	09	10	10	08
	60µl/disc	10	11	10	10
EFPS-37	20µl/disc	7	7	6	9
	40µl/disc	9	9	8	10
	60µl/disc	10	10	10	11
EFPS-38	20µl/disc	7	7	6	8
	40µl/disc	8	8	8	10
	60µl/disc	10	11	10	10
EFPS-39	20µl/disc	7	7	8	7
	40µl/disc	7	7	9	8
	60µl/disc	12	12	10	9
EFPS-40	20µl/disc	8	10	7	7
	40µl/disc	10	11	8	9
	60µl/disc	11	12	9	10
EFPS-41	20µl/disc	7	9	8	7
	40µl/disc	8	8	9	8
	60µl/disc	10	10	10	9
EFPS-42	20µl/disc	9	9	7	9
	40µl/disc	8	10	8	10
	60µl/disc	10	10	9	11
EFPS-43	20µl/disc	9	8	9	9
	40µl/disc	10	9	8	10
	60µl/disc	11	10	10	11
EFPS-44	20µl/disc	8	8	7	8
	40µl/disc	0	9	8	9
	60µl/disc	8	11	10	9
EFPS-45	20µl/disc	7	8	9	7
	40µl/disc	8	9	10	8
	60µl/disc	10	11	11	10
EFPS-46	20µl/disc	9	9	9	7
	40µl/disc	10	10	10	8
	60µl/disc	11	10	11	0
EFPS-47	20µl/disc	7	7	8	8
	40µl/disc	7	9	8	9
	60µl/disc	0	10	10	10
EFPS-48	20µl/disc	8	6	9	6
	40µl/disc	9	10	8	7
	60µl/disc	10	11	10	9

Table 2. (Cont'd.).

Culture No.	*Pseudomonas*	*M. phaseolina*	*R. solani*	*F. solani*	*F. oxysporum*
	20µl/disc	10	9	6	9
EFPS-49	40µl/disc	11	10	9	10
	60µl/disc	12	12	10	11
	20µl/disc	6	10	6	8
EFPS-50	40µl/disc	7	12	10	6
	60µl/disc	10	0	11	10
	20µl/disc	10	10	6	9
EFPS-51	40µl/disc	12	11	7	10
	60µl/disc	13	12	10	11
	20µl/disc	10	9	7	6
EFPS-52	40µl/disc	11	10	8	8
	60µl/disc	12	12	10	10
	20µl/disc	9	8	8	7
EFPS-53	40µl/disc	11	10	0	9
	60µl/disc	12	11	7	10
	20µl/disc	9	7	6	6
EFPS-54	40µl/disc	10	10	10	7
	60µl/disc	12	11	11	10
	20µl/disc	8	8	6	10
EFPS-55	40µl/disc	9	10	7	10
	60µl/disc	12	11	10	11
	20µl/disc	7	7	7	7
EFPS-56	40µl/disc	8	7	7	7
	60µl/disc	12	8	10	11
	20µl/disc	9	9	8	7
EFPS-57	40µl/disc	8	8	10	8
	60µl/disc	10	11	11	9
	20µl/disc	9	7	9	8
EFPS-58	40µl/disc	10	8	10	8
	60µl/disc	11	10	11	10
	20µl/disc	9	10	9	9
EFPS-59	40µl/disc	10	12	10	9
	60µl/disc	10	13	11	11
	20µl/disc	7	0	8	9
EFPS-60	40µl/disc	8	7	9	8
	60µl/disc	0	7	12	10
	20µl/disc	9	8	7	6
EFPS-61	40µl/disc	10	10	9	8
	60µl/disc	12	11	11	9
	20µl/disc	7	7	7	7
EFPS-62	40µl/disc	7	9	6	8
	60µl/disc	12	12	9	9
	20µl/disc	9	9	7	6
EFPS-63	40µl/disc	10	11	9	7
	60µl/disc	11	12	0	7
	20µl/disc	7	7	8	0
EFPS-64	40µl/disc	7	7	10	7
	60µl/disc	12	10	11	9
	20µl/disc	7	7	7	8
EFPS-65	40µl/disc	10	10	10	0
	60µl/disc	10	12	11	10
	20µl/disc	6	9	9	7
EFPS-66	40µl/disc	8	10	11	8
	60µl/disc	10	12	12	10

Table 3. *In vitro* antibacterial activity of hexane fraction of culture filtrates of endophytic fluorescent *Pseudomonas* species isolated from *Salvadora* species against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Salmonella typhimurium.*

Culture No.	*Pseudomonas*	*B. subtilis*	*S. aureus*	*S. typhimurium*	*E. coli*
		Zone of inhibition (mm)			
	Control	0	0	0	0
	+ ve control Streptomycin (20 µg/disc)	10	9	8	9
EFPS-08	20µl/disc	11	10	11	7
	40µl/disc	07	10	10	10
	60µl/disc	12	9	9	9
EFPS-19	20µl/disc	11	11	10	11
	40µl/disc	10	10	7	10
	60µl/disc	12	11	9	10
EFPS-20	20µl/disc	11	7	11	10
	40µl/disc	10	11	10	7
	60µl/disc	12	12	13	10
EFPS-21	20µl/disc	11	10	11	11
	40µl/disc	10	9	10	10
	60µl/disc	11	12	10	11
EFPS-22	20µl/disc	07	10	11	11
	40µl/disc	10	7	10	10
	60µl/disc	10	10	10	10

Table 4. *In vitro* antibacterial activity of chloroform fraction of culture filtrates of endophytic fluorescent *Pseudomonas* against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Salmonella typhimurium*.

Culture No.	*Pseudomonas*	*B. subtilis*	*S. aureus*	*S. typhimurium*	*E. coli*
		Zone of inhibition (mm)			
	Control	0	0	0	0
	+ ve control Streptomycin (20 µg/disc)	10	10	7	8
EFPS-08	20µl/disc	10	07	07	12
	40µl/disc	12	06	07	10
	60µl/disc	12	06	07	10
EFPS-19	20µl/disc	10	10	10	10
	40µl/disc	10	11	7	11
	60µl/disc	7	12	11	11
EFPS-20	20µl/disc	10	10	7	9
	40µl/disc	11	11	7	10
	60µl/disc	12	12	10	10
EFPS-21	20µl/disc	11	9	10	11
	40µl/disc	10	10	7	10
	60µl/disc	11	11	10	11
EFPS-22	20µl/disc	07	6	7	9
	40µl/disc	09	7	9	10
	60µl/disc	10	10	10	10

Table 5. *In vitro* antifungal activity of hexane fraction of culture filtrates of endophytic fluorescent *Pseudomonas* against *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* and *Fusarium oxysporum*.

Culture No.	*Pseudomonas*	*M. phaseolina*	*R. solani*	*F. solani*	*F. oxysporum*
		Zone of inhibition (mm)			
	Control	0	0	0	0
	+ ve control Carbendazim (20 µg/disc)	7	8	9	9
EFPS-08	20µl/disc	6	7	7	10
	40µl/disc	7	7	9	11
	60µl/disc	10	10	10	11
EFPS-19	20µl/disc	7	7	9	7
	40µl/disc	10	8	10	8
	60µl/disc	7	9	10	9
EFPS-20	20µl/disc	9	10	9	7
	40µl/disc	11	10	8	8
	60µl/disc	11	11	11	9
EFPS-21	20µl/disc	9	10	8	7
	40µl/disc	8	9	9	7
	60µl/disc	11	10	10	10
EFPS-22	20µl/disc	7	9	10	10
	40µl/disc	10	10	9	11
	60µl/disc	10	10	10	12

Table 6. *In vitro* antifungal activity of chloroform fraction of culture filtrates of endophytic fluorescent *Pseudomonas* against *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* and *Fusarium oxysporum.*

Culture No.	*Pseudomonas*	*M. phaseolina*	*R. solani*	*F. solani*	*F. oxysporum*
		Zone of inhibition (mm)			
	Control	0	0	0	0
	+ ve control Carbendazim (20 µg/disc)	7	9	8	10
EFPS-08	20µl/disc	8	11	10	10
	40µl/disc	9	10	9	11
	60µl/disc	11	11	10	11
EFPS-19	20µl/disc	7	7	9	7
	40µl/disc	7	8	10	8
	60µl/disc	8	9	11	9
EFPS-20	20µl/disc	10	7	10	10
	40µl/disc	11	7	10	9
	60µl/disc	10	7	11	10
EFPS-21	20µl/disc	6	9	7	7
	40µl/disc	7	10	10	8
	60µl/disc	7	10	11	10
EFPS-22	20µl/disc	10	10	10	9
	40µl/disc	10	11	11	10
	60µl/disc	11	11	12	11

Endophytic bacteria, particularly fluorescent *Pseudomonas*, besides biocontrol of plant disease also promote plant growth directly by the production of plant growth regulators (Bastian *et al.*, 1998; Spaepen *et al.*, 2009), biological nitrogen fixation (Baldani *et al.*, 2000; Oliveira *et al.*, 2002) and solubilization of bound phosphates (Verma *et al*., 2001). In low fertility soil, endophytic bacteria successfully promote plant growth by using these mechanisms (Sevilla *et al.*, 2001). Of the various soil bacteria, fluorescent *Pseudomonas* inhabit the environment surrounding the plant roots and even the root interior (Maldonado-Gonzalez *et al.*, 2013). Most of the rhizosphere bacteria generally colonized the root surface, but some of them can get entry inside the root where they established as harmless endophytes (Rosenblueth & Martinz-Romero, 2006; Prieto *et al.*, 2011). However, in this study fluorescent *Pseudomonas* were isolated from *S. persica* and *S. oleoides* and showed significant biocontrol potential against root rotting fungi which is in agreement with previous reports (Tariq *et al.*, 2009; Afzal *et al.*, 2013; Shafique *et al*., 2015).

In this study, cell-free culture filtrates and their solvent fractions of fluorescent *Pseudomonas* showed significant antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* by producing zone of inhibition in agar disc diffusion assay. There are several reports that besides root rotting fungi, fluorescent *Pseudomonas* are also effective against bacterial pathogens (Kumar & Sood, 2001; Manmeet & Thind, 2002; Tsai *et al*., 2004. *Pseudomonas aeruginosa* has also been reported to induce systemic resistance in cotton and okra (Shafique *et al.*, 2015; Rahman *et al.*, 2016,). Similarly, among the fluorescent *Pseudomonas*, *P. viridiflava* produced ecomycins were found associated with external tissues of leaves of grasses (Miller *et al.*, 1998). The ecomycins contains unusual amino acids besides, common amino acids and was found active against *Cryptococcus neoformans* and *Candida albicans.* Several new compounds have been isolated and characterized from endophytic bacteria and fungi, which could be used in modern medicine and also in agriculture (Mitchell *et al.*, 2008). Due to emerging infectious diseases and drug resistance microbes, there is a dire need to find out new resources of bioactive compounds that could be developed in to new drugs. Endophytic fluorescent *Pseudomonas* could be a new source of drugs for the treatment of infectious diseases.

Acknowledgement

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

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(Received for publication 25 September 2016)