J. Mycopathol. Res. ***51(2)*** *: 295-299, 2013;* (ISSN 0971-3719)

Effect of sodium chloride, streptocycline sulphate and different agrochemical on growth of *Pseudomonas fluorescens*

***MINA D. KOCHE AND R.M. GADE**
Department of Plant Pathology, Dr. PDKV., Akola 444104, Maharasthra

Received : 01.06.2013 Accepted : 06.09.2013 Published : 28.10.2013

Experiment was conducted *in vitro* to see the effect of sodium chloride, streptocycline sulphate and different agrochemicals on growth of *Pseudomonas fluorescens*. Soil sample were collected from Vidarbha region for isolation of *Pseudomonas fluorescens*. On the basis of morphological (staining), physiological and biochemical test (oxidase test, arginine dihydrolysis test, starch hydrolysis, gelatin liquefaction, nitrate reduction test, citrate utilization, urease test, H_2S, catalase test and KOH test) they were identified as by *P. fluorescens*. The isolates of *P. fluorescens* were inoculated in nutrient broth and incubated having different concentration (g/l) of Nacl (5, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) and streptocyclin sulphate (100 ppm, 200 ppm,300 ppm and 400 ppm). Growth of the bioagent was measured turbidometrically. Most of the isolates of Pf preferred 5.5 g/l salt for multiplication. The isolates Pf_9 and Pf_{10} showed an ability to tolerate 6 g/l sodium chloride concentration. In case of streptocycline sulphate, all isolates were efficiently grow at 100 ppm concentration but the growth was restricted with the increase in the concentration of streptocycline sulphate. Incorporation of metalaxyl, Fosetyl–Al, COC and B.M. in growth medium of *P. fluorescens* affected its growth. COC and B.M. was less compatible with all *Pseudomonas* isolates. Maximum growth of Pf_{15} was observed in metalaxyl (0.2%) (35.33 cfu/ml). whereas Pf_9(37.33 cfu/ml) was most compatible to Fosetyl-Al followed by Pf_{10} (35.00 cfu/ml).

Key words: Sodium chloride, streptocycline sulphate , *Pseudomonas fluorescens*

INTRODUCTION

Salinity is one of the major constraints, which hamper agricultural production. However, profitable utilization of salt affected soils for agricultural production is also dispensable. In addition to the use of traditional breeding and plant genetic engineering approaches of developing salt tolerance of transgenic plants, the use of PGPR may prove useful in developing strategies to facilitate plant growth in saline lands. PGPR inoculants are inexpensive, simple to use and have no adverse effects. However, the degree of efficacy of the PGPR to enhance growth may vary with crops, varieties or species, cultural conditions and inoculants strains. *Pseudomonas fluorescens* is found to tolerate salt in addition with growth promoting ability of the crop (Kausar and Shahzad, 2006). Streptocycline mostly is recommended for management of foliar diseases caused by bacteria in most of the crops. It is beneficial if any bioagent can have resistance to this effective antibiotic so that this can be used in combination for management of the diseases. Fungicides may have deleterious effects on the pathogen as well as the

*mdkoche@gmail.com

antagonist. The effect of fungicides on the pathogen and the antagonist would provide information on the selection of particular fungicide which is effective against pathogen and compatible to antagonist. Objective of the present study is to see the effect of sodium chloride, streptocycline sulphate and different agrochemicals on growth of *Pseudomonas fluorescens.*

MATERIALS AND METHODS

Soil samples were collected from rhizosphere of citrus in Vidarbha region. Thirty isolates were obtained through serial dilution using King's B medium (King *et al.* 1954). Colonies that showed fluorescens were selected and further purified. The isolated bacteria was confirmed by morphological (staining), physiological and biochemical test (oxidase test, arginine dihydrolysis test, starch hydrolysis, gelatin liquefaction, nitrate reduction test, citrate utilization, urease test, H_2S, catalase test and KOH test).

Different concentration (g/l) of NaCl (i.e. 5, 5.5, 6.0, 6.5g, 7.0, 7.5 and 8.0) and were made in nutrient broth medium. Ten ml of broth medium of each salt concentration was poured in sterilized test tubes. The test tubes were plugged with cotton and autoclaved. The test tubes were inoculated with fourteen isolates of *P. fluorescens* isolates and incubated for 3 days. After 72 h the optical density was measured at 620 nm with the help of spectrophotometer. The maximum optical density indicated more growth of *P. fluorescens.*

Different concentrations (ppm/l) of streptomycin sulphate i.e. 100, 200, 300 and 400 were added in sterilized nutrient broth medium. Ten ml of broth medium of each streptomycin sulphate concentration was poured in sterilized test tubes. The test tubes were plugged with cotton and inoculated with fourteen isolates of *P. fluorescens* and incubated for 3 days. After 72 h the optical density with the help of spectrophotometer was measured at 620 nm. The maximum optical density indicated more growth of *P. fluorescens.*

Biocontrol bacteria were also tested *in vitro* for their compatibility to the fungicides. The bacterial suspensions, at the concentration of 10^8 cfu/ml, were pipetted out in Petri dishes containing King's B agar amended with the fungicides like metalaxyl (0.2%), fosetyl-Al (0.2%), COC-(0.3%) and Bordeaux mixture (B.M.) (1.0%). The suspensions were dispersed over the medium. The Petri plates were then incubated at 25°C for 48 h. After 48 h. no. of colonies counted and compared with control.

RESULTS AND DISCUSSION

Thirty strains of *Pseudomonas fluorescens* were isolated from 44 soil samples collected from the rhizosphere of citrus. All the isolated bacterial antagonistic were gram negative and rod shaped and produced round to irregular colonies with greenish yellowish, water soluble pigment (Gate, 2009) (Table 1). All were positive for oxidase, arginine dihydrolysis, citrate, urease, H_2S production, catalase and KOH test (Ipper *et al.* 2005; Gate 2009; Nisharani Urkade 2010). While all isolates were negative for nitrate reduction (Gade, 2009) and only two isolates show positive reaction against starch hydrolysis and remaining show negative reaction (Siddiqui and Shakeel, 2009) and in case of gelatin liquefaction only two isolates show negative reaction and remaining all isolates show positive reaction (Tiwari and Thrimurthy, 2007). On the basis of morphological, physiological and biochemical characteristic they conformed the *P. fluorescens.*

Generally the bacterial cells remain in a higher osmotic pressure than out side. Salt is good preservative because most of the bacteria cannot grow in high salt concentration. The rhizobacteria can tolerate salt concentration up to certain level. With this view, the different concentrations of salt were tried (Table 3).

The growth was measured turbidometrically after 72 h of incubation. All isolates of *Pseudomonas fluorescens* were observed to prefer 5.5 g/l salt for multiplication. There was reduction in growth from 6 to 8 g/l supplementation. Pf_6 (1.693), Pf_{15} (1.505), Pf_{18} (1.198), Pf_{20} (1.312) and Pf_{26} (1.761) preferred 5 g while Pf_9 (1.413) and Pf_{10} (1.329) preferred 6.0 g/l of salt for maximum growth.

Minimum growth of almost isolates was found due to supply of 7, 7.5 and 8 g/l, which indicated that these concentrations might not be tolerated by the isolates as observed by their poor growth. Membre

Table 1 : Morpholoical, physiological and biochemical characters of *Pseudomonas fluorescens.*

Isolates	Physiological and bochemical Test									
	Oxidase test	Arginine dihydrolysis	Starch hydrolysis	Gelatine liquifaction	Nitrate reduction	Citrate utilization	Urease test	H_2S production	Catalase test	KOH test
Pf_1	+	+	-	+	-	+	+	+	+	+
Pf_4	+	+	-	+	-	+	+	+	+	+
Pf_5	+	+	-	+	-	+	+	+	+	+
Pf_6	+	+	-	-	-	-	+	+	+	+
Pf_9	+	+	-	+	-	+	+	+	+	+
Pf_{10}	+	+	+	+	-	+	+	+	+	+
Pf_{14}	+	+	+	+	-	+	+	+	+	+
Pf_{15}	+	+	-	+	-	+	+	+	+	+
Pf_{16}	+	+	-	+	-	+	+	+	+	+
Pf_{18}	+	+	-	-	-	+	+	+	+	+
Pf_{19}	+	+	-	-	-	+	+	+	+	+
Pf_{20}	+	+	-	+	-	+	+	+	+	+
Pf_{26}	+	+	-	+	-	+	+	+	+	+
Pf_{30}	+	+	-	+	-	+	+	+	+	+

and Burlot (1994) reported the growth of *Pseudomonas* in five per cent concentration of NaCl. In our study also, all isolates of *P. fluorescens* can grow up to 8g/l. Kausar and Shahzad (2006) observed that *Pseudomonas fluorescens* produce 1-aminocyclo-propane 1-carboxylic acid (ACC)

Table 2 : *Pseudomonas fluorescens* isolates obtained from soil samples of Citrus orchards in Vidarbha region.

District	Soil samples collected	Isolates no.
Akola	Akola	Pf_1
	Akola	Pf_4
	Akot	Pf_5
	Akot	Pf_6
	Akoli Jahagir	Pf_9
	Akoli Jahagir	Pf_{10}
Amravati	Warud	Pf_{14}
	Warud	Pf_{15}
	Warud	Pf_{16}
	Amravati	Pf_{18}
Nagpur	RFRS Katol	Pf_{19}
	RFRS Katol	Pf_{20}
	Savandri	Pf_{26}
	Nagpur	Pf_{30}

deaminase which can play an important role in the process of plant growth and resistance to salt. The beneficial attributes of these organisms also include improved nutrient recycling. In the rhizosphere, microorganism and root colonization are some of the challenges under stress(Paul and Nair, 2008).

Generally streptocycline sulphate is used as bactericide for control of bacterial infection. Rhizobacteria can tolerate streptocycline sulphate concentration up to certain level. The different concentration of streptocycline sulphate was tried and revealed varied tolerance (Table 4).

The growth was measured turbidometrically after 72 h. Each concentration of streptocycline sulphate exhibited differences in sensitivity to individual isolates. It was revealed from Table 3 that all isolates were sensitive at 400 ppm concentration. Pf_4 (1.372), Pf_9 (1.302), Pf_{10} (1.132), Pf_{14} (1.175), Pf_{15} (0.732), Pf_{16} (1.303), Pf_{18} (1.127), Pf_{19} (1.215) and Pf_{30} (1,714) tolerated the 100 ppm concentration while only two isolates i.e. Pf_6 (1.393) and Pf_{20} (0.843) grew well at 200 ppm as compared to other concentration. Pf_1 (1.657), Pf_5 (1.701), and Pf_{26} (1.200) showed good growth at 300 ppm concentration of streptocycline sulphate. Jayaswal *et al.* (1990) reported that *Pseudomonas* strain RJ2 was resistant to streptomycin. Present findings corroborate with these results.

Incorporation of metalaxyl, Fosetyl–Al, COC and B.M. in growth medium of *P. fluorescens* affected its growth. COC and B.M. was less compatible with all *Pseudomonas* isolates. This may be due to the presence of antibacterial properties in both fungicides as compared to metalaxyl and Fosetyl-Al. Maximum growth of Pf_{15} was observed in metalaxyl (0.2%) (35.33 cfu/ml). Whereas Pf_9 (37.33 cfu/ml) was most compatible to Fosetyl-Al followed by Pf_{10} (35.00 cfu/ml) (Table 5). Mean of fungicides would give isolate variability.

In vitro studies clearly established that bacterial antagonist was able to tolerate the recommended

Table 3 : Sensitivity of *Pseudomonas fluorescens* with salt concentration

Isolates no.	Control	Concentration of salt g /l (Optical Density at 620 nm)						
		5	5.5	6.0	6.5	7.0	7.5	8.0
Pf_1	1.921	1.535	1.758	1.383	1.354	1.270	1.335	1.315
Pf_4	1.810	1.348	1.663	1.282	0.989	0.925	0.918	1.016
Pf_5	1.844	1.588	1.663	1.240	1.319	1.069	1.128	1.334
Pf_6	1.825	1.693	1.452	1.230	1.247	1.132	1.453	1.056
Pf_9	1.672	0.985	1.284	1.413	1.015	0.959	1.137	0.832
Pf_{10}	1.532	1.192	1.028	1.329	1.264	1.069	1.298	1.049
Pf_{14}	1.985	1.561	1.585	1.266	0.946	1.163	1.097	0.851
Pf_{15}	1.705	1.505	1.113	0.812	0.826	0.912	0.531	0.937
Pf_{16}	1.954	1.693	1.780	0.915	1.085	1.365	1.231	0.898
Pf_{18}	1.328	1.198	1.159	0.995	1.149	1.098	0.961	0.945
Pf_{19}	1.935	1.561	1.727	0.976	1.126	0.984	1.019	0.971
Pf_{20}	1.653	1.312	1.402	0.733	0.909	1.065	0.746	0.849
Pf_{26}	1.939	1.761	1.583	1.223	1.492	1.437	0.987	1.441
Pf_{30}	2.026	1.415	1.956	1.555	1.347	0.971	1.386	1.401
Mean	1.795	1.453	1.511	1.168	1.148	1.101	1.088	1.064
...	0.033	0.060	0.131	0.054	0.090	0.085	0.071	0.077
CD(P=0.01)	0.110	0.207	0.401	0.180	0.315	0.289	0.243	0.269

Table 4 : Sensitivity of *P. fluorescens* to streptocycline sulphate concentration

Isolates No.	Control	Concentration of streptocycline sulphate (ppm) (Optical Density)			
		100	200	300	400
Pf_1	1.810	1.048	1.485	1.657	0.909
Pf_4	1.532	1.372	1.039	0.987	0.878
Pf_5	1.921	1.201	1.399	1.701	0.825
Pf_6	1.538	0.711	1.393	1.319	0.524
Pf_9	1.640	1.302	0.574	0.496	0.450
Pf_{10}	1.370	1.132	0.969	0.816	0.780
Pf_{14}	1.321	1.175	0.871	0.780	0.658
Pf_{15}	0.952	0.732	0.661	0.528	0.402
Pf_{16}	1.428	1.303	1.272	0.995	0.874
Pf_{18}	1.453	1.127	0.922	0.576	0.493
Pf_{19}	1.515	1.215	0.910	0.809	0.702
Pf_{20}	0.905	0.321	0.843	0.562	0.120
Pf_{26}	1.521	1.075	1.399	1.200	1.005
Pf_{30}	1.978	1.714	1.598	1. 379	0.997
Mean	1.492	1.102	1.095	0.956	0.687
S.E. (m)±	0.035	0.021	0.033	0.013	0.019
CD (P=0.01)	0.114	0.068	0.110	0.044	0.064

fungicidal dose. Although the strains exhibited minor variation among them, their multiplication was influenced at lower concentration of fungicides. Singh and Dubey (2010) observed that the growth of *Pseudomonas fluorescens* was not inhibited by fungicides like metalaxyl alone and in combination with mancozeb. The earlier findings of Mahesh (2007) corroborate the present findings that, metalaxyl and Fosetyl-Al were compatible with *P. fluorescens* except Bordeaux mixture. Yildiz *et al.* (2007) observed no decline in the colonial growth of *P. fluorescens* with low doses of fungicides. Mallikarjuniah (1995) tested the compatibility of different fungicides with rhizobacteria and found that all tested fungicides showed stimulatory action against rhizobacteria. However, so far not many attempts have been made on the combined efficacy of *P. fluorescens* with fungicide against

Table 5 : Compatibility of *Pseudomonas fluorescens* with different fungicides (cfu/ml broth)

Isolate no.	Fungicides (cfu)				Control
	Metalaxyl (0.2%)	Fosetyl-Al (0.2%)	COC (0.3%)	B.M. (1.0%)	
Pf_1	34.67	32.33	21.00	23.67	38.33
Pf_4	33.00	32.33	21.33	22.33	36.00
Pf_5	32.33	30.00	22.00	21.00	38.00
Pf_6	33.00	33.00	19.00	22.00	35.67
Pf_9	35.00	37.33	20.00	21.33	40.00
Pf_{10}	34.33	35.00	18.33	20.67	39.00
Pf_{14}	34.33	33.33	21.00	22.33	38.00
Pf_{15}	35.33	33.00	20.33	21.33	39.33
Pf_{16}	32.33	33.33	19.33	22.33	37.67
Pf_{18}	32.67	32.33	18.67	21.33	38.00
Pf_{19}	33.33	34.00	22.33	22.00	39.00
Pf_{20}	34.33	33.00	21.33	22.33	38.67
Pf_{26}	34.00	33.33	22.00	22.33	37.33
Pf_{30}	33.00	30.00	22.33	21.00	37.67
SE(m)±	0.48	0.65	0.60	0.41	0.50
CD(P=0.01)	2.20	3.06	2.93	1.93	2.30

plant diseases, while a few reports are available on the effect of pesticides on plant diseases suppressing bacteria other than *Pseudomonads* .

This study demonstrates that utilization of combining bacterial strains with recommended dose of fungicides could be an effective strategy in reducing gummosis and root rot in Nagpur mandarin. In the present investigation, it is clearly established that integration of chemical and biological treatments has high potentials for success especially for fungicides with bacterial antagonists. Hence further studies need to understand combined efficacy of metalaxyl, fosetyl-Al, COC, B.M. and *Fluorescent Pseudomonads* under field conditions for managing the gummosis and root rot in citrus. It requires the development of tolerant biotypes of the biocontrol agents to be utilized in the integrated approach (Malathi *et al.* 2002).

REFERENCES

Gade, R.M. 2009. Biological and chemical management of *Phytophthora* root rot/collar rot in citrus nursery. AFPP-9th International conference on Plant Diseases Tours, France.

Gate, V. L. 2009. Characterization of *Pseudomonas fluorescens* and siderophore mediated antibiosis against soil borne fungal pathogens. M.Sc. Thesis (Unpub.) Dr. P.D.K.V., Akola (M.S.). Ipper, N.S., Jung Eun Kim, Jun Halk Koo, Jang Hyun Hur and Chun Keun Lim. 2005. Inhibitory effect of Korean strain Gpf 01 identified as *Pseudomonas fluorescence* on cucumber mosaic virus. *Plant Pathol. J.* **2**: 262-269.

Jayaswal, K. R. Marcel, A. Fernandez and Schroeded, R. G. 1990. Isolation and Characterization of a *Pseudomonas* strain that restrict growth of various Phytopathogenic fungi. *Appl.and Environ. Microbiol.* **56**: 1053-1058.

Kausar Rizwana and Sher Muhammad Shahzad. 2006. Effect of ACC- deaminase containing rhizobacteria on growth promotion of maize under salinity stress. *J. of Agri. Soc. sci.* **2**: 216-218.

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

Mahesh, G. M., 2007. Growth promotion and disease suppression ability of *Pseudomonas fluorescens* on acid lime. M. Sc. Thesis (Unpub) Dr. P. D. K. V. Akola.

Malathi, P., R. Viswanathan, P., Padmanaban, D. Mohanraj and A. Ramesh, 2002. Compatibility of biocontrol agents with fungicides against red rot disease of Sugarcane. *Sugar tech*, 4: 131-136.

Mallikarjuniah, R.R. 1995. Effect of some fungicide and insecticide on growth and nitrogen fixation in *Azotobactor. Mysore J. Agric. Sci.* **29:** 36-42.

Membre, J. M. and Burlot, P.M. 1994. Effect of temperature, ph and Nacl on growth and pectinolytic acitivity of *Pseudomonas marginallis. Appl. and Envorn. Microbiol.* **60**: 2017-2022.

Nisharani Urkade. 2010. Efficacy of *Pseudomonas fluorescens* in management of wilt and root rot of Chickpea. M.Sc. Thesis (Unpub.) Dr. P.D.K.V. Akola (M.S.).

Paul, D. and Nair, S. 2008. Stress adaptions in a plant growth promoting *Rhizobacterium* (PGPR) with increasing salinity in the coastal agricultural soils. *J. Basic Microbiol.* **48**:1-7

Siddiqui, Z. A. and Shakeel, U. 2009. Biocontrol of wilt disease complex of pigeonpea (*Cajanus cajan* L. Millsp.) by isolates of *Pseudomonas* spp. *African Journal of Plant Science* **3** : 1-12.

Singh, Birendra and S.C. Dubey, 2010. Bioagent based integrated management of Phytophthora blight of pigeonpea. *Archives of Phytopathology and Pl. Protection.* **43:** 922-929.

Tiwari, P. K. and Thrimurthy, V.S. 2007. Isolation and characterization of the *Pseudomonas fluorescens* from rhizosphere of different crops. *J. Mycol. Pl. Pathol.* **37**: 231-234.

Yildiz, F., Yildiz, M. Delen, N. Coskuntuna, A. Kinay, P. and Turkusay, H. 2007. The effect of biological and chemical treatment on gray mold disease in tomatoes grown under greenhouse conditions. *Turk J Agric.* **31:** 319-325.