ISSN: 0974 - 0376

The Ecoscan : Special issue, Vol. IX: 627-632: 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES
www.theecoscan.com

ISOLATION, IDENTIFICATION AND UTILIZATION OF BENEFICIAL MICROORGANISMS FOR PLANT GROWTH PROMOTION AND MANAGEMENT OF CHICKPEA WILT COMPLEX

S. Kapali *et al.,*

KEYWORDS

Bio-agents
Wilt complex
P. fluorescens
B. subtilis

Proceedings of National Conference on Harmony with Nature in Context of Resource Conservation and Climate Change
(HARMONY - 2016)
October 22 - 24, 2016, Hazaribag,
organized by
Department of Zoology, Botany, Biotechnology & Geology
Vinoba Bhave University,
Hazaribag (Jharkhand) 825301
in association with
National Environmentalists Association, India
www.neaindia.org

The Ecoscan
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES

S. KAPALI, R. M. GADE, A. V. SHITOLE*, MORE YOGESHWAR AND S. ASWATHI
Department of Plant Pathology,
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola - 444 104 (M.S.) INDIA
e-mail: amolshitole1690@gmail.com

ABSTRACT

In an attempt to develop effective biocontrol system on suppression of wilt complex diseases and growth promotion in chickpea was investigated through using Plant Growth Promotion Rhizobacteria (PGPR). Antagonistic and potential growth promoting activity, *P. fluorescens* and *B. subtilis* identified based on biochemical test, were found to produce siderophore, IAA, HCN and NH_3. Biocontro lagents were found to suppress *F. oxysporum* f. sp. *ciceri*, *R. bataticola* and *S. rolfsiiin vitro*. Inoculation of two efficient PGPR alone and in combinations with different methods (seed treatment and soil application by mixing with FYM) in chickpea, promote plant health. *P. fluorescens* and *B. subtilis* alone significantly enhance germination, by 82.25% (T1) and 78.30%(T2), root length, by 9.28 cm (T1) and 10.11 cm (T5) and shoot length, by 12.11 cm (T4) and 12.75 cm (T5), over uninoculated control.Their dual inoculation resulted in significant increase in germination, root and shoot length by 88.87 % (T7), 14.89 cm (T6) and 13.50 cm (T7) over alone, respectively. This is because of growth promotion activity in *P. fluorescens* and *B. subtilis*. It may be concluded that *P. fluorescens* and *B. subtilis* are effective to manage disease as well as improve seedling health.

***Corresponding author**

INTRODUCTION

Fusarium wilt and root rot complex of chickpea is difficult to manage as the pathogens are soilborne, surviving through resistant structure *i.e.* chlamydospores and sclerotia in soil for years (Padamini *et al.*, 2015). The most common means to check the disease is by using fungicides but frequent and indiscriminate use of fungicides often leads to environmental pollution and also there is a great possibility of development of resistance within a very little span of time. Hence alternative management stratergies are most desirable. Several organisms have been successfully used as biocontrol agents such as *P. fluorescens* (Boominathan and Sivakumar, 2012) and *B. subtilis* (Christy *et al.*, 2012). Integration of seed treatment fungicides with these bioagents could gave the way of complete solution for management of the disease. Fortunately, *Bacillus* and *Pseudomonas* do exist and for several decades they have been used as biocontrol agents and promoters of plant health and development and are known to survive in the rhizosphere. *Bacillus* and *Pseudomona*s were among the first bacterial isolates to show promising biocontrol characteristics (Santoyo *et al.*, 2012). Biological control of plant diseases using antagonistic microorganisms offers a highly effective, economic and environmental friendly alternative to the use of synthetic pesticides. Common soil inhabitants like *Pseudomonas fluorescens* and *Bacillus subtilis* had been reported to manage several diseases caused by soil borne pathogens (Weisskopf, 2013). Biological control is an efficient and environmentally friendly way to prevent wilt complex diseases. Their applicability as biocontrol agents has drawn wide attention because of the production of secondary metabolites such as siderophore, antibiotics, volatile compounds, HCN, enzymes and phytohormones (Kumar *et al.*, 2004). The role of *P. fluorescens* in promoting root and shoot growth of different crops have also been demonstrated in past (Singh *et al.,* 2013). Plant growth promoting activity of *P. fluorescens* is due to its ability of producing IAA, HCN, siderophores and availability of phosphorus that may result in better plant. Production of antibiotic compound and inhibition of other microbes is the most important mechanism expressed by the antagonistic bacteria (Mina Koche *et al.*, 2013). There fore, the laboratory and greenhouse experiment was undertaken to evaluate the efficacy of PGPR against chickpea wilt complex pathogens and their effect on growth of chickpea.

MATERIALS AND METHODS

Collection of soil sample and isolaion of bioagents

Soil sample were collected from the rhizosphere of chickpea, pigeon pea and citrus. *Pseudomonas fluorescens* was isolated on King's B medium and *Bacillus subtilis* on NA medium (Shweta Sharma *et al.*, 2014). Isolation of the pathogenic fungus *F. oxysporum* f. sp. *ciceri*, *R. bataticola* and *S. rolfsii* were made by tissue isolation technique using solidified PDA media in petri plates (Walia *et al.*, 2014).

Growth inhibition test

Purified cultures of the fungus obtained from depatment of plant pthology, Dr.

P.D.K.V., Akola, were maintained on potato dextrose agar. A 5 mm disc of *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia bataticola*, *Sclerotium rolfsii* were placed separately at centre of the previously plated 90 mm diameter with 20 ml of PDA media and bioagents was streaked at opposite side of fungal culture disc (Abed *et al*., 2016). These Petri plates were incubated at 25^{0}C. Three plates were used for each replication. The per cent growth inhibition was calculated using following formula (Sreedevi and Devi, 2012).

Production of Indole acetic acid (IAA)

Indole acetic acid production tested according to Reetha *et al*., 2014).

Production of siderophore

Production of siderophore by *P. flourescens* and *Bacillus subtilis* were assessed by Plate assay method as described by Lacava *et al*., 2008).

Determination of ammonia production

Bacterial cultures were grown in peptone water (10 ml) for 24 h at 30^{0}C for 48-72 hr. 0.5 ml of Nessler's reagent was added. Conversion of brown to yellow colour indicated the production of ammonia by microorganism (Naphade and Hussain, 2014).

Determination of HCN production

HCN production was tested by the method of Ramette *et al*., 2006.

Plant growth promotion study

Chaffa, the susceptible variety was used to study disease. Treatments comprising inoculation with *P. fluorescens* and *B. subtilis*, alone and in combination by two methods *i.e.* seed treatment, soil application mixing with FYM, was set up in completely randomized design with 3 replicates. The pots were filled with inoculum (*F. oxysporum* f. sp. *ciceri*, *R. bataticola* and *S. rolfsii*) was thoroughly mixed at 1:1:1 proportion with per kg soil. Seed was treated with carrier (Talc) based efficient PGPR inoculants at the rate of 10 g kg^{-1} seed. Dual inoculation, wherever required, was done by mixing the required quantity of both the inoculants at the time of seed treatment. Soil inoculation was done using 10 g inoculant(s) in each plot of 15 cm by mixing with FYM @ 100 g kg^{-1} soil as per treatments and applied in pot prior to seed sowing. Germination, Root and shoot length were recorded at 21 DAS (Islam *et al*., 2016). The collected data were analysed statistically.

Table 1: Production of Antagonistic and PGP Traits by PGPR

Isolates	Siderophore production	HCN production	Volatile antifungal production
Pf_1	+ve	+ve	+ve
Pf_2	+ve	-ve	+ve
Pf_3	+ve	-ve	+ve
Pf_4	+ve	-ve	-ve
Bs_1	+ve	-ve	+ve
Bs_2	-ve	-ve	+ve
Bs_3	-ve	-ve	+ve
Bs_4	-ve	-ve	-ve

+ve = Possitive, -ve = Negative

RESULTS AND DISCUSSION

In the present investigation isolates of of *P. fluorescens* (4) and *B. Subtilis* (4) were isolated and characterized, named as Pf1, Pf2, Pf3, Pf4, Bs1, Bs2, Bs3 and Bs4. Identification of bacterial isolates was carried out by biochemical test. Apart from these, all isolates of bioagents were designed as potential plant growth promoting (PGP) and antagonistic rhizobacteria. Majority of the isolates (6) were able to produce ammonia, while 5 isolates were able to produce siderophore which is comparable to

Table 2: *In-vitro* antagonism of *Pseudomonas fluorescens* on *F. oxysporum* f. sp. *ciceri* (Foc) in the culture medium at 7 days after inoculation (DAI)

Isolates	Mycelial growth (mm) (DAI)			Growth inhibition (%) (DAI)		
	3	5	7	3	5	7
Pf_1	26.22	30.99	49.33	30.58	43.69	45.18(42.23)*
Pf_2	30.11	42.76	52.33	20.64	22.08	41.85(40.31)
Pf_3	34.77	46.44	66.66	7.94	15.37	25.93(30.61)
Pf_4	33.77	51.99	54.10	10.60	5.26	39.89(39.17)
Control	37.77	54.88	90.00	-	-	-
S. E. (m) ±	0.19	0.20	0.20	0.17	0.18	0.16
C.D(p = 0.01)	0.86	0.88	0.89	0.71	0.78	0.68

(Data shown are average of three replication, DAI – Days after incubation; * Values in the parenthesis arc sine transformed)

Table 3: *In-vitro* antagonism of *Pseudomonas fluorescens* on *Rhizoctonia bataticola* in the culture medium at 7 days after inoculation (DAI)

Isolates	Mycelial growth (mm) (DAI)			Growth inhibition (%) (DAI)		
	3	5	7	3	5	7
Pf_1	14.11	27.44	41.55	51.52	43.86	53.83(47.19)*
Pf_2	23.22	23.36	49.77	20.20	54.25	44.70(41.96)
Pf_3	23.66	35.08	60	18.69	28.23	33.33(35.26)
Pf_4	27.44	41.99	73	5.70	14.32	18.88(25.75)
Control	29.10	48.88	90.00	-	-	-
S. E. (m) ±	0.13	0.23	0.15	0.20	0.21	0.19
C.D.(p = 0.01)	0.58	1.0	0.65	0.89	0.95	0.82

(Data shown are average of three replication, DAI – Days after incubation; * Values in the parenthesis arc sine transformed)

Table 4: *In-vitro* antagonism of *Pseudomonas fluorescens* on *Sclerotium rolfsii* in the culture medium at 7 days after inoculation (DAI)

Isolates	Mycelial growth (mm) (DAI)			Growth inhibition (%) (DAI)		
	3	5	7	3	5	7
Pf_1	25.66	46.33	81.33	21.95	5.87	9.63(18.07)
Pf_2	23.22	34.22	47.22	29.37	30.47	47.53(43.59)
Pf_3	19.33	44.33	66.33	41.21	9.93	26.30(30.85)
Pf_4	12.22	23.22	35.66	62.83	52.82	60.37(50.99)*
Control	32.88	49.22	90.00	-	-	-
S. E. (m) ±	0.22	0.20	0.11	0.18	0.17	0.19
C.D.(p = 0.01)	0.95	0.88	0.49	0.78	0.76	0.80

(Data shown are average of three replication, DAI – Days after incubation;* Values in the parenthesis arc sine transformed)

Table 5: *In-vitro* antagonism of *Bacillus subtilis* on *Fusarium oxusporum* f. sp. *ciceri* in the culture medium at 7 days after inoculation (DAI)

Isolates	Mycelial growth (mm) (DAI)			Growth inhibition (%) (DAI)		
	3	5	7	3	5	7
Bs_1	16.55	21.67	28.33	53.55	60.51	68.52(55.87)*
Bs_2	29.50	49.82	70.35	21.89	9.22	21.83(27.85)
Bs_3	34.55	51.33	78.87	8.52	6.47	12.36(20.58)
Bs_4	35.55	52.66	75.15	5.87	4.04	16.50(23.97)
Control	37.77	54.88	90.00	-	-	-
S. E. (m) ±	0.10	0.22	0.18	0.18	0.16	0.18
C.D.(p = 0.01)	0.72	0.94	0.79	0.76	0.70	0.77

(Data shown are average of three replication, DAI – Days after incubation; * Values in the parenthesis arc sine transformed)

Table 6: *In-vitro* antagonism of *Bacillus subtilis* on *Rhizoctonia bataticola* the culture medium at 7 days after inoculation (DAI)

Isolates	Mycelial growth (mm) (DAI)			Growth inhibition (%) (DAI)		
	3	5	7	3	5	7
Bs_1	19.88	37.77	73	31.08	22.72	18.88(25.75)*
Bs_2	20.77	41.99	62.11	28.62	14.09	30.98(33.82)
Bs_3	24.50	41.99	77.66	15.80	14.09	13.70(21.72)
Bs_4	21.10	32.55	45.15	27.49	33.40	49.83(44.90)
Control	29.10	48.88	90.00	-	-	-
S. E. (m) ±	0.19	0.16	0.23	0.20	0.20	0.19
C.D.(p = 0.01)	0.80	0.69	0.98	0.89	0.87	0.80

(Data shown are average of three replication, DAI – Days after incubation; * Values in the parenthesis arc sine transformed)

study carried by Kumari *et al*. (2013). Indole acetic acid was produced by 37.5% of the isolates and only one isolate (Pf1) showed HCN production (Table 1). These findings were in agreement of Abed *et al*. (2016). A number of studies reported simultaneous production of different antagonistic and plant growth promoting properties (Sivasakthi, 2014).

In vitro results illustrated that all *P. fluorescens* isolates produced more secondary metabolites, volatile antifungal compounds and cyanide hydrogen than *B. subtilis* and inhibited pathogen growth. These results are in agreement with research previously carried out by Kandoliya and Vakharia (2013). In our study, IAA production was reported by 37.5% of the isolates in *P. fluorescens*, isolated from different rhizosphere. Reetha *et al*., (2014) reported the IAA production by *Pseudomonas* followed by *B. subtilis* which help in plant growth promotion. In the present study three different genera of the phtopathogenic fungus (*F. oxysporum* f. sp. *ciceri*, *R. bataticola* and *S. rolfsii*) were used to check the anti-microbial properties of rhizobacteria. All the isolates of *P. fluorescens* and *B. subtilis* were found to cause inhibition of the pathogen. Per cent growth inhibition recorded in the present study varies from isolate to isolate. Highest per cent growth inhibition (45.18 %) was recorded in *P. fluorescens* (Pf1) followed by Pf2 (41.85 %) against *F. oxysporum* f. sp. *ciceri* at 7 DAI (Table 2). These results are in confirmation with the finding of Khan and Gangopadhyay (2012) who also reported that, two of *P. fluorescens* (Pf-1 and Pf-P) were found to be effective in inhibition against *Fusarium oxysporum* f. sp. *ciceri* by (46 %) and (42.40%), respectively. Ardebili *et al*. (2011) also documented that production of antifungal metabolites proposed as a main mechanism of antifungal activity of *P. fluorescens* against *Fusarium* spp. Isolate Pf1 showed the highest (53.83%) reduction of the radial growth of *R. bataticola* followed by Pf2 (44.70 %) and Pf3 (33.33 %) (Table 3). The antagonistic effect of *P. fluorescens* against *R. bataticola* has already been reported by some investigators (Belkar and Gade, 2012). Khan *et al*. (2012) reported that antagonistic effect of rhizosphere *P. fluorescens* that checked the root rot disease in chickpea. Antagonism between *S. rolfsii* and *P. fluorescens* between 9.63% and 60.37% was very effective in controlling *S. rolfsii* (Table 4). These results are in line with the finding of Munde *et al*. (2013). Belkar and Gade (2012) also reported that isolate Pf3 had significant 74.70% growth inhibition of *S. rolfsii* with 22.77 mm mycelial growth against control. Among

Table 7: *In-vitro* antagonism of *Bacillus subtilis* on *Sclerotium rolfsii* the culture medium at 7 days after inoculation (DAI)

Isolates	Mycelial growth (mm) (DAI)			Growth inhibition (%) (DAI)		
	3	5	7	3	5	7
Bs_1	24.71	35.44	49.26	24.84	27.99	45.26(42.28)*
Bs_2	27.77	32.7	62.11	15.54	33.56	30.99(33.83)
Bs_3	30.55	44.55	75.04	7.08	9.48	16.62(24.06)
Bs_4	19.84	32.83	54.48	39.66	33.29	39.46(38.92)
Control	32.88	49.22	90.00	-	-	-
S. E. (m) ±	0.25	0.19	0.19	0.19	0.22	0.19
C.D.(p = 0.01)	1.09	0.83	0.85	0.85	0.98	0.86

(Data shown are average of three replication, DAI – Days after incubation,* Values in the parenthesis arc sine transformed)

Table 8: Effect of different treatments on plant growth parameters of chickpea

Tr. No.	Treatment detail	Plant growth parameters (21 days after sowing)		
		Germination %	Shoot length (cm)	Root length (cm)
T1	Seed treatment with efficient rhizobacteria 1 (10g/kg seed).	82.25(65.08)*	11.11(19.47)*	9.28(17.74)*
T2	Seed treatment with efficient rhizobacteria 2 (10g/kg seed).	78.3(62.23)	10.77(19.16)	8.26(16.70)
T3	Seed treatment with thiram (3g/kg seed).	65.67(54.13)	7.32(15.69)	7.89(16.31)
T4	Soil application of efficient rhizobacteria 1 (10g + 100g FYM /Kg soil).	72.88(77.55)	12.11(20.37)	9.20(17.66)
T5	Soil application of efficient rhizobacteria 2 (10g + 100g FYM /Kg soil).	75.31(60.20)	12.75(20.92)	10.11(18.54)
T6	T4 + T5	77.55(61.71)	14.89(22.69)	9.75(18.19)
T7	T1 + T4	88.87(70.51)	13.88(21.87)	13.50(22.63)
T8	T1 + T5	69.51(56.48)	10.55(18.96)	7.57(15.98)
T9	T2 + T4	65.87(54.25)	9.29(17.75)	10.11(18.54)
T10	T2 + T5	63.78(52.99)	9.07(17.53)	8.11(16.55)
T11	Sick soil	45.73(42.55)	6.57(14.85)	4.74(12.58)
S.E. (m) ±		0.69	0.19	0.22
CD (P = 0.01)		2.75	0.77	0.91

*Values in the parenthesis arc sine transformed. Average of three replications.

the *B. subtilis* Bs1 showed more than 68% inhibition rate against *F. oxysporum* f. sp. *ciceri* as compared to Bs1 and Bs4 showed 45.26% and 49.83% of inhibition against *S. rolfsii* and *R. bataticola* (Table 5, 6, 7). Similar studies were varied out by Munde *et al*. (2013) while carrying out screening of PGPRs from rhizosphere of different crops. Production of antibiotic compound and inhibition of other microbes is the most important mechanism expressed by the antagonistic bacteria (Mina Koche *et al*., 2013). In this study the antagonistic study expressed by the *P. fluorescens* and *B. subtilis* in dual culture method might be due to one or combination of above mechanisms. However, results are mostly depend on the ability of producing antimicrobial compounds and degradative enzymes by the tested antagonistic bacteria. It has been already reported that *P. fluorescens* has ability to produce high level of chitinase, â-l, 3-glucanace, cellulase, fungitoxic metabolites and siderophores (Jayraj *et al*., 2007) also *Bacillus* spp. have ability to produce bactericin, gramicidin, S. polymyxin, tyrotricidin, bacilysin, chlotelaine, iturin A, mycobacilin, bailomycin, mycosubtilin, fungistatin and subsporin (Meenu Saraf *et al*., 2014). This clearly indicated that in soil the antagonist competes with the pathogen and brings down the inoculums of the pathogen there by reducing the disease incidence.

The effect of different treatments of bioagents on germination, root and shoot length was significant (Table 8). Results of germination under pot condition varied from 63.78 to 82.25 % (Table 8). More than 82% germination was recorded in T7 (88.87%) and T1 (82.25%) and More than 75 % germination was recorded in T2 (78.3%), T6 (77.55 %) and T7 (75.31 %). Bharathi *et al*., (2014) found 83.10% to 94.05% increased germination in chickpea by treating seeds alone and combination of *P. fluorescens*. Root length was ranged from 7.57 cm to 13.50 cm (Table 8). The highest Root length was recorded in T7 (13.50 cm), T5 (10.11cm), T9 (10.11 cm) and T1 (9.28 cm) and the lowest was recorded in T8 (7.57 cm) and T3 (7.89 cm). Shoot length was varied from 7.32 to 14.89 cm at 21 DAS (Table 8).T6 (14.89 cm) performed best in term of maximum shoot length. The lowest shoot length 9.07 cm was observed in T10. Shoot length of T7 (13.88 cm), T5 (12.75 cm), T4 (12.11 cm) and T1 (11.11 cm) were better over the control (6.57 cm) and thiram (7.32 cm). Such positive benefits of rhizobacterial inoculation in chickpea have also been reported elsewhere (Bhattacharjya and Chandra, 2013) and may be attributed to activity PGPR alone and combination of similar or dissimilar inoculation also significantly enhance health of chickpea over control. Our results suggested that PGPR able to enhance the production of IAA, solubilisation of Phosphorus and ammonia production, thereby improving growth of chickpea.

REFERENCES

Abed, H., Rouag, N., Mouatassem, D. and Rouabhi, A. 2016. Screening for *Pseudomonas* and *Bacillus* antagonistic rhizobacteria strains for the biocontrol of *Fusarium* wilt of chickpea. *Eurasian J. Soil*

Sci. **5(3):** 1-10.

Ardebili, Z. O., Ardebili, N. O. and Hamdi, S. M. M. 2011. Physiological effects of *Pseudomonas fluorescens* CHA0 on tomato (*Lycopersicon esculentum* Mill.) plants and its possible impact on *Fusarium oxysporum* f. sp. *lycopersici*. *Aus. J. Crop Sci.* **5(12):** 1631-1638.

Belkar, Y. K. and Gade, R. M. 2012. Biochemical characterization and growth promotion activities of *Pseudomonas fluorescens*. *J. Pl. Dis. Sci.* **7(2):** 170-174.

Bharathi, V., Durga, K. K. and Rani, M. S. 2014. Potentiality of boiagents on seed quality enhancement in chickpea. *Int. J. Phytopath.* **3(3):** 111-113.

Bhattacharjya, S. and Chandra, R. 2013. Effect of inoculation methods of *Mesorhizobium ciceri* and PGPR in chickpea (*Cicer areietinum* L.) on symbiotic traits, yields, nutrient uptake and soil properties. *Legume Res.* **36(4):** 331-337.

Boominathan, U. and Sivakumar, P. K. 2012. Induction of systemic resistance by mixtures of rhizobacterial isolates against *Pythium aphanidermatum*. *Int. J. Res. in Pure and Applied Microbiol*. **2(4):** 49-53.

Christy, J. E., Tharmila, S. and Niranjan, K. 2012. Antagonistic activity of *Trichoderma* spp. and *Bacillus* spp. against *Pythium aphanidermatum* isolated from tomato damping off. *Archives of Applied Sci. Res.* **4(4):** 1623-1627.

Islam, S., Akanda, A. M., Prova, A., Islam, M. T. and Hossain, M. M. 2016. Isolation and identification of Plant Growth Promoting Rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. *Front. Microbiol.* **6:** 1360. doi: 10.3389/fmicb.2015.01360.

Jayraj, J. M., Parthasarathi, T. and Radhakrishnan, N. V. 2007. Charaterization of *Pseudomonas* strain from tomato rhizosphere and its use for integrated management of tomato damping off. *Biocontrol.* **52:** 683-702.

Kandoliya, U. K. and Vakharia, D. N. 2013. Antagonistic effect of *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *ciceri* causing wilt in chickpea. *Agril. Res. Commu. Cen.* **36(6):** 569-575.

Khan, M. A. and Gangopadhay, S. 2012. Effect of soli inhabiting anatgonostic microflora against *Fusarium oxysporum* f. sp. *ciceri*, incitant of wilt chickpea. *J. Mycol. Plant Pathol.* **42(3):** 341-346.

Khan, R. A., Bhat, T. A. and Kumar, K. 2012. Management of Chickpea (*Cicer arietinum* L.) dry root rot caused by *Rhizoctonia bataticola* (Taub.) Butler. *Int. J. R. Pharma. Bio. Sci.* **3(4):** 334-35.

Kumari, P., Khanna, V., Kaur, L. and Mukhija, B. 2013. Characterization of functionally traits of plant growth promoting rhizobacteria antagonistic to *Fusarium oxysporum* f. sp. *ciceri*. *Pl. Dis. Res.* **28(1):** 11-15.

Lacava, P. A., Silva-Stenico, M. E., Araújo, W. L., Simionato, A. V. C., Carrilho, E., Tsai, S. M. and Azevedo, J. L. 2008. Detection of siderophores in endophytic bacteria *Methylobacterium* spp. associated with *Xylella fastidiosa* subsp. *pauca*. *Pesq. Agropec. Bras.* **43:** 521-528.

Meenu Saraf, Urja Pandya and Aarti Thakkar 2014. Role of allelochemicals in plant growth promoting rhizobacteria for biocontrol of phytopathogens. *Microbiol. Res.* **169:** 18-29.

Mina, D. Koche, Gade, R. M. and Deshmukh, A.G. 2013. Antifungal activity of secondary metabolites produced by *Pseudomonas fluorescens*. *The Bioscan.* **8(2):** 723-726.

Munde, V. G., Diwakar, M. P., Thombre, B. B. and Dey, U. 2013. Evaluation of bioagents and botanicals against *Sclerotium rolfsii* causing root rot of Finger millet. *J. Pl. Dis. Sci.* **8(2):** 242-244.

Naphade, B. S. and Hussain, S. S. 2014. Isolation, characterization and evaluation of *Pseudomonas putida* as a plant growth promoting rhizobacteria. *Int. Sci. J.* **1(2):** 30-33.

Padamini, R., Mathur, K., Trivedi, A. and Rathore, S. 2015. Efficacy of different fungicides and bio-agents against wilt and root rot complex of chickpea. *Res. on Crops.* **16(2):** 304-310.

Reetha, S., Bhuvaneshwari, G., Thamizhiniyan, P. and Mycin, T.R. 2014. Isolation of indole acetic acid (IAA) producing rhizobacteria of *Pseudomons fluorescens* and *Bacillus subtilis* and enhance growth of onion (*Allium cepa*. L). *Inter. J. Curr. Microbiol. App. Sci.* **3(2):** 568-574.

Santoyo, G., Mosqueda, M. D. C. O. and Govindappa, M. 2012. Mechanisms of biocontrol and plant growth-promoting activity in soil bacterial species of *Bacillus* and *Pseudomonas*: a review. *Biol. Sci. Tech.* **22(8):** 855-872.

Shweta Sharma, Mohinder Kaur and Durga Prashad, 2014. Isolation of *Fluorescent pseudomonas* strain from temperate zone of Himachal Pradesh and their evaluation as plant growth promoting rhizobacteria (PGPR). *The Bioscan.* **9(1):** 323-328.

Singh, S. P., Singh, H. B. and Singh, D. K. 2013. Trichoderma harzianum and Pseudomonas sp. Mediated management of *Sclerotium rolfsii* rot in tomato (*Lycopersicon esculentum* Mill.). *The Bioscan.* **8(3):** 801-804.

Siva Sakthi, S., Usha Rani, G. and Saran Raj, P. 2014. Biocontrol potential of plant growth promotion bacteria (PGPR)- *Pseudomonas fluorescens* and *Bacillus subtilis*. A review, *African J. Agril. Res.* **9(6):** 1265-1277.

Walia, M., Batra, N. and Goyal, S. 2014. Isolation and characterization of plant growth promoting rhizobacteria and their application in plant growth. *Legume Res.* **37(1):** 72-78.

Weisskopf, L. 2013. The potential of bacterial volatiles for crop protection against phytophathogenic fungi. Microbial pathogens and strategies for combating them: *Sci.Tech. Edu.* **9(8):** 87-91.