Int.J.Curr.Microbiol.App.Sci (2018) *7*(1): 920-941

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 01 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.701.112

Biological Management of Vascular Wilt of Chickpea (*Cicer arietinum* L.) Incited by *Fusarium oxysporum f. sp. ciceris* by Antagonistic Rhizobacteria Co-Inoculated with Native *Mesorhizobium*

Suman Kumari* and Veena Khanna

Department of Microbiology, Punjab Agricultural University, Ludhiana-141004, Punjab, India

**Corresponding author*

Keywords

Chickpea, Rhizobacteria, *Mesorhizobium*, Fusarium wilt, Biocontrol, Phytopathogen

Article Info

Accepted:
10 December 2017
Available Online:
10 January 2018

A B S T R A C T

Sixty one out of 200 isolates, isolated from chickpea rhizospheric soils, were found to be effective to control the mycelial proliferation of the test pathogen *Fusarium oxysporum f. sp. ciceris*, maximum being with Ps16b (45.7%) and Ps45 (87.3%) in dual culture plate and liquid broth assay. Growth inhibition of the phytopathogen was also recorded by diffusible and volatile antifungal metabolites produced by the isolates. All the isolates showed ammonia production, whereas only nine isolates were recorded for Hydrogen cyanide production. Five potent antagonists (Ba1a, Ba19, Ps44, Ps45 and Ps47), selected on the basis of antagonistic traits were evaluated under greenhouse conditions to control fusarium wilt in chickpea varieties GPF-2 and JG-41. Maximum reduction in disease incidence was recorded with Ps45 (74.48±0.67 %) and (70.32±1.00%) compared to fungicide treatment (61.53±0.89% and 58.69±0.33%) in chickpea genotypes GPF-2 and JG-41 respectively. Rhizobacterial isolates Ps45, Ps47 and Ba1a inoculation alongside *Mesorhizobium* were found effective in promoting seed emergence and in controlling the disease severity.

Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop and accounts for 48% of the total pulse production in India (Anonymous, 2015). This crop is widely attacked by soil-borne diseases resulting in severe yield losses and one of them is fusarium wilt incited by *Fusarium oxysporum f. sp. ciceris* (Foc, Padwick) which is a serious soil borne disease of chickpea (Hossain *et al.*, 2013; Merkuz *et al.*, 2011). It is a major constraint to chickpea cultivation throughout the world and especially in Indian subcontinent where chickpea is a commonly grown pulse crop, as it can cause up to 100% yield loss annually (Pande *et al.*, 2010; Kumari and Khanna, 2014). This disease can affect the crop at any stage of growth. Characteristic symptoms are sudden drooping of leaves and petioles and black internal discoloration involving xylem and pith (Dubey and Singh, 2004).

Management of fusarium wilt is not a simple assignment, as no single control measure is fully effective (Butler, 1981). Several

measures including crop rotation or application of chemicals are there, but it is difficult to manage fusarium wilt by either of these, because of soil nature persistence and its capacity to survive for long time even in the absence of host (Moradi *et al.,* 2012). Thus, the use of fungicides is not usually effective as it is used mainly for the seed borne inoculum and the effect is short lived (Merkuz *et al.,* 2011). The disease can also be managed using resistant cultivars, but resistant varieties are neither available nor can be effective against different races of the pathogen prevalent in the country (Merkuz and Getachew, 2012). As a result, an alternative to the use of synthetic pesticides with the advantages of greater public acceptance and reduced environmental impact is required (Reino *et al.,* 2008).

Biological control using microbes is becoming a critically needed component of plant disease management, particularly in reducing root diseases (Nautiyal, 2000; Meki *et al.,* 2009). At present, biological control of soil and seed-borne plant pathogenic fungi has been addressed mainly by using bacterial and fungal antagonists. Strains of *Trichoderma* spp. and non-pathogenic isolates of *F. oxysporum* and some rhizobacteria especially *Pseudomonas* spp. and *Bacillus* spp., isolated from the rhizospheres of crop plants, are reported to be effective not only to control plant pathogens but also help the plants to mobilize and acquire nutrients (Gopalakrishnan *et al.,* 2011). Moreover the use of biocontrol agents is much safer and is presumed to be less polluting to the environment than the chemical pesticides (Sumeet and Mukerji, 2000).

Various mechanisms for antagonism have been implicated, like cell wall degrading enzymes (pectolytic enzymes, cellulases, xylanases and glycosidic hydrolases), siderophores that can chelate iron and other metals and contribute to disease suppression by conferring a competitive advantage to the biocontrol agent for the limited supply of essential trace minerals in natural habitats (Deshwal *et al.,* 2003). Kravchenko *et al.,* (2002) reported that microbial siderophore may also stimulate plant growth directly by competitively inhibiting iron uptake system by fungal pathogen. Biocontrol agents also produce various types of volatile and diffusible antifungal metabolites and antibiotics, capable of reducing or suppressing infection by pathogenic fungi in several pathosystems (Yang *et al.,* 2009). Furthermore, rhizobacteria have received particular attention because of their excellent root colonizing ability and their ability to induce plant's defence mechanism *via* production of various pathogenesis related proteins (Kumar *et al.,* 2010).

This research was carried out as an alternative strategy to chemical control, with the objective of evaluating the potential of rhizobacterial isolates from chickpea rhizosphere for controlling chickpea fusarium wilt. For this, the most promising rhizobacterial antagonists of Foc, were isolated and screened for *in vitro* trials. Selected potential antagonists were further evaluated for their ability to reduce fusarium wilt symptoms and to enhance the seedling emergence under greenhouse conditions.

Materials and Methods

Collection of soil samples, isolation, purification and identification of rhizobacterial isolates

Soil samples were collected randomly from different locations. Samples were collected in sterile plastic bags. From each sample, 10 g of soil was added to 90 ml of distilled sterilized water and vigorously shaken using a shaker for 20-30 minutes. From this, seven fold serial

dilutions were made by pipetting 10 ml into additional dilution water. From the final dilution (10^{-7}), aliquots of 0.1 ml each were spread on plates, containing 20 ml of Nutrient agar for *Bacillus* and *Serratia* spp. and King's B or Pigment producing medium (PsP) for *Pseudomonas* spp. (King *et al.,* 1954) and incubated at 25 ^{o}C for 24 hours.

Bacterial colonies developed on respective media, were picked and transferred to respective slants for further use. Initial characterization of all the isolates was done on the basis of colony morphology and gram's staining. Biochemical characterization of bacterial isolates was done as per the standard methods (Cappuccino and Sherman, 1992).

Pathogen culture

The fungal pathogen *Fusarium oxysporum* f. sp. *Ciceris*, procured from the Department of Plant Breeding and genetics, Punjab Agricultural University was maintained on Potato Dextrose agar slants.

Assessment of antiphytopathogenic activity of rhizobacterial isolates against the root phytopathogen

In vitro testing of rhizobacteria against mycelial growth of *Fusarium oxysporum f. sp. ciceris* (Dual culture agar plate assay)

The antagonistic rhizobacterial isolates were screened by dual culture plate assay as per the the method described by Ahmed Idris *et al.*, (2007). Ten µl drops from the 10^{8} cfu/ ml bacterial broth suspension were placed on the margin (2cm away from the fungal disc) of potato dextrose agar (PDA) plates and a 5 mm agar disc from fresh cultures of pathogenic fungi was placed at the centre of the PDA plate for each bacterial isolate and incubated at 25 ± 3 ºC for seven days. The radial growth of the fungal colony towards and away from the bacterial colony was measured. The percentage growth inhibition was calculated using the following formula:

% Inhibition = (R-r)/R×100

Where, r is the radius of the fungal colony opposite the bacterial colony and, R is the maximum radius of the fungal colony away from the bacterial colony. There were three replicate in this assay.

Fungal biomass inhibition in liquid medium (Liquid antibiosis)

One ml of 24 h old fresh bacterial culture and a disc of test fungus (5 mm) from a well-grown fungal colony on PDA plates were inoculated in 50 ml broth of sterile potato dextrose media in 250 ml conical flasks at 25^{0} C. Broth inoculated only with pathogen fungus served as control. The differences in dry weights of fungal mycelium treated with bacterium and the control cultures were recorded after 5 days through preweighed filter paper (Whatmann No.1). The filter papers were dried for 24 h at 70^{0} C and weighed. The percent reduction in weight of the test fungus was calculated using formula:

% Reduction in weight= (w1-w2)/ w1×100

Where, w1 represents the weight of the test fungus in control flasks and w2 with the bacterial antagonists.

Growth inhibition by production of Diffusible antimetabolites (covered membrane method)

PDA plates covered with a cellophane membrane were overlaid with nutrient agar and inoculated with 100 µl of antagonistic bacterial suspension. After incubation for 48 hrs at 28˚C, the membrane along with the grown bacterial isolate was removed and the

plate was inoculated in the middle with 10 mm disc of a pure culture of *F. oxysporum*. Plates were incubated at 22˚C for 48 hrs and the growth of the pathogen was measured (Kumari and Khanna, 2014).

Antagonistic activity *via* volatile antifungal compounds (sealed plate method)

One hundred µl of fresh prepared broth culture was spread on nutrient agar medium plate.

A second petri dish containing PDA was inoculated with a 6-mm bit of the test fungus and placed over the bacterial culture. The two plates were sealed together with parafilm and further incubated at 25^0 C. As a control, a petri plate containing nutrient agar medium without bacteria was placed over the PDA medium inoculated with the fungal pathogen. Radial growth of the test fungus was observed over 24 hour intervals for 5 days.

Hydrogen Cyanide (HCN) production

Petri plates containing 10% Trypticase soya agar supplemented with 4.4 g of glycine per litre were spread with 0.1 µl of 24 hrs old bacterial cultures. The plates were inverted with a lid containing filter paper, impregnated with 0.5% picric acid and 2% sodium carbonate. The plates were incubated at 28^0 C for 3 to 5 days. A change in colour from yellow to orange-brown on the filter paper indicated cyanide production (Bakker and Schippers, 1987).

Production of ammonia

Fresh (24 hrs) grown cultures were inoculated in 10 ml peptone water and incubated for 48-72 hours at 30^0 C. Nessler's reagent (0.5ml) was added in each test tube. Development of brown to yellow colour was a positive test for ammonia production (Cappuccino and Sherman, 1992).

Evaluation of plant growth promoting potential by potent antagonists

Selected antagonists were further evaluated for their potential to enhance the growth of the plants *via* production of phytohormones and iron chelating agents *in vitro* conditions. Indole acetic acid production in Luria broth by the antagonistic isolates, was performed with Van Urk Salkowski reagent using the Salkowski's method (Ehmann, 1977). The Gibberellic acid production by was determined by Borrow *et al.,* method (Borrow *et al.,* 1995). Siderophore production was detected on Chrome azurol sulphonate agar plate test (Schwyn and Neilands, 1987). Selected bacterial strains were tested by an agar assay using National Botanical Research Institute's phosphate (NBRIP) medium for phosphate solublization (Edi Premono *et al.,* 1996). The isolates were inoculated into minimal agar medium containing 0.1% insoluble zinc oxide. Twenty four hours fresh grown bacterial isolates were spotted on the Zinc containing medium and incubated at 30ºC for 48 hours for the clearing zones around the colonies.

Evaluation of antiphytopathogenic potential of antagonistic rhizobacteria under glass house conditions

Chickpea genotypes

Seeds of two chickpea genotypes "GPF-2 and JG-41" were selected and procured from Punjab Agricultural University.

Bacterial cultures and seed bacterization

Selected rhizobacterial cultures were inoculated @ 1% in 100 ml of nutrient broth and were incubated at for 24 hours with bacterial count of 10^{7-8} cfu/ml of the broth. The seeds of GPF-2 and JG-41 chickpea varieties were washed with 0.1% Mercuric

chloride followed by 70% ethanol and then repeatedly with sterile distilled water for surface sterilization. After that, seeds were soaked in selected five bacterial broth cultures (10^7 ml^{-1} broth) individually and in combination with native *Mesohizobium ciceri,* procured from department of Microbiology, Punjab Agricultural University (1:1) for 20-30 minutes before sowing the seeds.

Pathogen culture multiplication and soil inoculation

Fusarium oxysporum f. sp. *ciceris* was mass multiplied in Potato dextrose broth. Mycelial mat was used to inoculate pathogen in soil i.e. 10 g /Kg of the soil. Soil was mixed thoroughly to disperse fungal hyphae and spores properly in the soil.

Preparation for pot experiment

Selected antagonists and their co-inoculation with *Mesorhizobium* were examined for their potential to reduce wilt incidence under the glass house conditions, using sterile soil inoculated with pathogen. The experiment was designed with 13 treatments, with 5 selected culture treatments alone and in combination with *Mesorhizobium* (1:1). The absolute control with pathogen free soil and untreated seeds, negative control with sick soil and untreated seeds and Fungicide treatment with sick soil and captan treated seeds (2g/Kg seeds) were also maintained as separate treatments. Soil collected from chickpea field was autoclaved at 15 lbs (121 ℃) for 15 minutes for sterilization. Polyethylene bags (15 x 10 cm) were filled with 250 g sterilized soil inoculated with pathogen mycelial mat i.e. 10g/Kg soil. Ten seeds were sown in each pot. Pots were maintained by regular watering upto maturity and were examined for seedling emergence during initial 5-10 days. Wilt incidence was recorded up to maturity of crop plants and reduction in disease severity was recorded as: % wilt in particular treatment - % wilt in negative control / % wilt in negative control×100.

Data were statistically tested by analysis of variance (ANOVA) using CPCS1 software developed by Department of Mathematics, Statistics and Physics, PAU. Each treatment was analyzed with three replicates and standard error (SE) was calculated and data are expressed in mean ±SE of three replicates.

Results and Discussion

Two hundred rhizobacterial isolates, isolated from chickpea rhizospheric soil samples collected from different locations of Punjab, Haryana and Uttar Pradesh, were screened on the basis of antagonism test (dual culture) plates where confluent bacterial growth inhibited fungal mycelial development. Sixty one isolates were found to show inhibitory effect on the growth of the fungal pathogen.

Selected antagonists were assessed for morphological and biochemical characteristics as per Bergey's manual of Systemic Bacteriology. Twenty three isolates were found to be Gram positive and thirty eight were characterized as Gram Negative by Gram staining. Morphologically, all the isolates were found to be rod shaped. Bacterial cultures isolated on Kings B medium produced fluorescent green to blue green coloured colonies (Plate 1). Two of the isolates on Nutrient agar were observed with red coloured colonies. Selected cultures were tested for starch hydrolysis, catalase production, Methyl red test, Citrate test and Nitrate production test. On the basis of morphological and biochemical characterization, 23 cultures were observed to belong to *Bacillus* spp., 36 to *Pseudomonas* and 2 to *Serratia* spp. Bacterial cultures were maintained on Nutrient agar slants and were stored at low temperature i.e. 4° C.

Dual culture agar plate assay

Out of 200 isolates, sixty one isolates were effective in reducing mycelial growth of the pathogen. The results revealed that the PGPR strains inhibited the growth of *Fusarium oxysporum* f. sp. *Ciceris* (Foc) to a varying extent, 25.7±1.22 to 45.7±3.58%. Somewhat similar range was recorded in our earlier case study where antagonistic rhizobacterial inhibition of Foc radial growth was observed between 18.2- 41.8% (Kumari and Khanna, 2014). The application of Ps16b recorded the maximum inhibition i.e. followed by Ps47, Ps44 and Ps45 (Plate 2). However among the *Bacillus* isolates, Ba5 and Ba7 were recorded with pathogen radial inhibition of 41.4% followed by Ba1a, Ba15, Ba18, Ba19, Ba27 and Ba36 with 40% inhibition (Table 1). Observations revealed that *Pseudomonas* isolates showed more inhibitory effect than the *Bacillus* or *Serratia* spp in dual culture plate assay in support to the observations by Altinok *et al.,* 2014 where *P. aeruginosa* (P07-1 and 85A-2) and *P. putida* (P11-4) inhibited 70% of the radial growth of *Fusarium oxysporum* f. sp. *melongenae* (*Fomg*). The control was recorded with 7 cm of growth after 7 days that was referred as standard to calculate the percentage inhibition. Antagonistic activity of rhizobacterial isolates can be due to different diffusible and volatile antifungal metabolites and competitions for various nutrients (Gopalakrishnan *et al.,* 2011).

Liquid broth antibiosis

The antagonistic rhizobactera were also evaluated for their antipathogenic potential in liquid medium. In this method bacterial inhibitory effect on the mycelial proliferation in terms of dry weigh was recorded. All the isolates showed reduction in fungal biomass in a varied content compared to control. Inhibitory effect on mycelial proliferation varied between 26.4±0.72 to 87.3±0.10%. Ps45 induced maximum inhibition in liquid medium followed by Ps44 and Ba1a (Table 2). However in a similar work *Bacillus* isolate 2B inhibited the mycelial proliferation up to 93.9% and *Pseudomonas* isolates 34P, 28P and 20P were also recorded with antagonistic effect of 84.4, 79.8 and 79.8% respectively (Kumari and Khanna, 2014). As the liquid medium provides better interaction between the pathogen and the antagonist, that can be the reason of higher percentage inhibitory effect of bacterial antagonists on fungal growth than in dual culture plate assay. Such an effective antagonistic activity by these rhizobacterial isolates can be an alternate to the various chemical mechanisms to control this pathogen.

Antagonism *via* diffusible antifungal metabolite

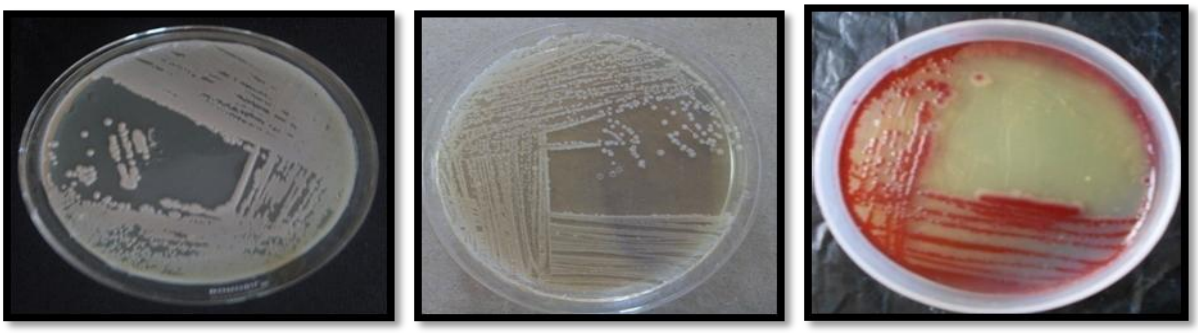
Certain diffusible antibiotics produced by PGPR include phenazine, pyoluteorin, pyrrolnitrin and cyclic lipopeptides and various enzymes that are mainly responsible for degradation of fusaric asic produced by *Fusarium* spp. and hence help in reduction of vegetative as well as reproductive growth of these pathogens (Ryan *et al.,* 2008). Inhibition varied in the range of 9.0±0.34 to 90.9±0.06% due to the production of volatile antimetabolites in membrane plate assay . Pseudomonas cultures Ps 44, Ps45 and Ps 46 were found very efficient to reduce the radial growth of test fungus to ~ 100% as the only growth recorded was the bit of 0.5 mm that was already placed during the inoculation (Plate 3). However among *Bacillus* isolates, Ba11 was found to be most efficient in pathogen inhibition i.e. 76.3% followed by Ba19 (74.5±0.21%) (Table 3). Studies have reported that *Bacillus* and *Pseudomonas* spp. produce extracellular chitinase and laminarinase which could lyse the mycelia of *Fusarium solani* that can be the main reason

for antagonistic effect (Isnansetyo *et al.*, 2003, Arias *et al.*, 2009). Furthermore flourescent *Pseudomonas* species produce extracellular metabolites like Phenazine and Di-acetyl phloroglucinol that are mainly implicated in inhibitory effect on various pathogens associated with plant diseases. In support, Giorgio and his co-workers also have reported that an array of rhizobacteria show a negative effect on the growth of various pathogens such as *Botrytis cinerea, Fusarium equiseti, F. oxysporum, F. solani, Phytophthora nicotianae, Sclerotinia* and *Verticilium* spp. (Giorgio *et al.*, 2015).

Inhibitory effect by volatile antimetabolites

Volatile antimicrobial compounds are produced by a number of rhizobacteria that can be implied to control various plant pathogens especially that incite the plants in ealier or later stages of plants (Abdeljalil *et al.*, 2016). All the twenty six antagonists variably inhibited *Foc* radial growth. Isolate Ps47 induced maximum inhibition (90.7%) via the production of volatile metabolites. Bacillus isolate Ba27 inhibited radial growth with 61.5% following Ps47 (Plate 4). Fiddman and Rossal (1993) revealed that volatiles produced by *Bacillus* spp. induce profound adversial effect on the mycelial proliferation of various fungal plant pathogens. Isolates Ba1a, Ba7, Ba8, Ba11, Ba19, Ps5, Ps11, Ps15, Ps44 and Ps45 were also recorded to inhibit ≥ 50% of the mycelial growth in sealed plate assay (Table 4). Six out of ten antagonists were found to inhibit *Fusarium oxysporum* f. sp *lycopersici* to control wilt in tomato plants with an average percentage inhibition of 31.21% (Prashar *et al.*, 2013). Some of the species of *Serratia*, *Pseudomonas* and *Bacillus* synthesize and emit complex blends of volatile compounds such as ammonia and hydrogen cyanide that inhibit growth of many phytopathogenic and non phytopathogenic fungi and play an important role in biological control (Kai *et al.*, 2007; Vespermann *et al.*, 2007).

Plate.1 Isolated hizobacteria from chickpea rhizospheric soil on



(a) Nutrient agar medium

(a) King's B medium

Plate.2 Inhibition zone produced by rhizobacterial isolates against *Fusarium oxysporum* f. sp. *ciceris*

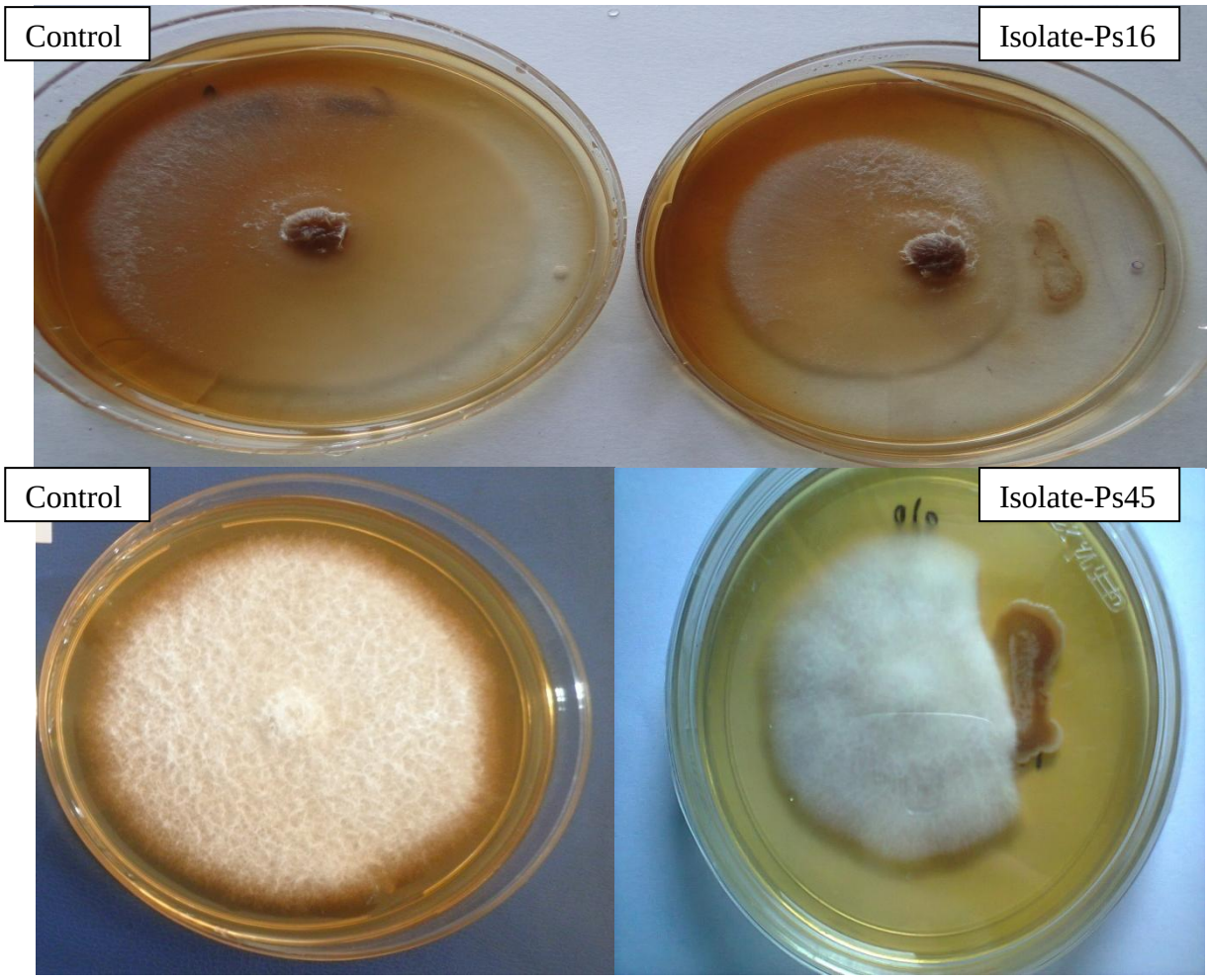


Plate.3 Relative radial growth inhibition of *Fusarium oxysporum* f. sp. *ciceris* by rhizobacterial diffusible antimetabolites

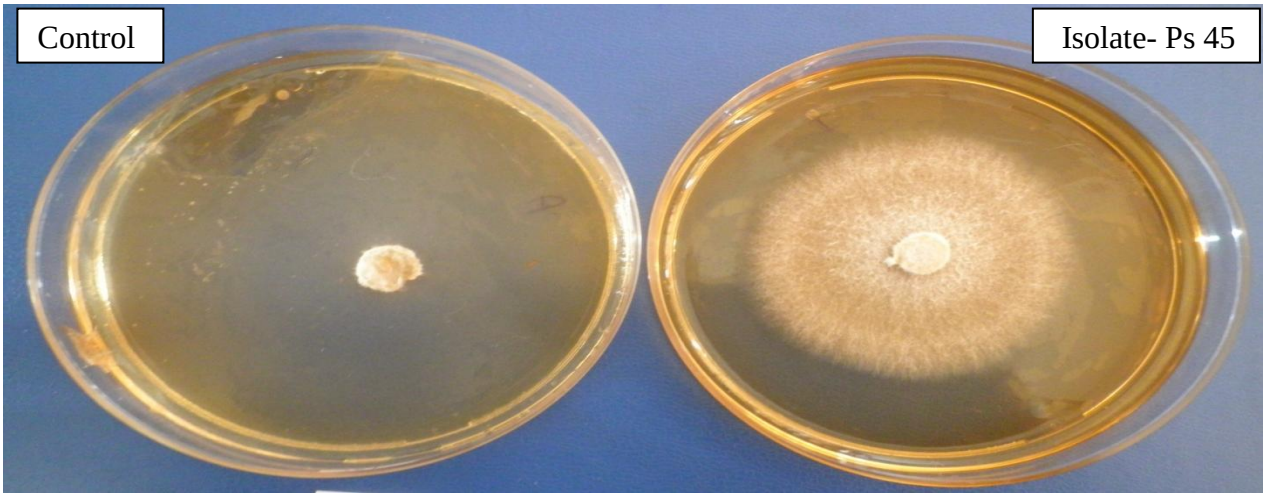


Plate.4 Relative radial growth inhibition of *Fusarium oxysporum* f. sp. *ciceris* by rhizobacterial volatiles

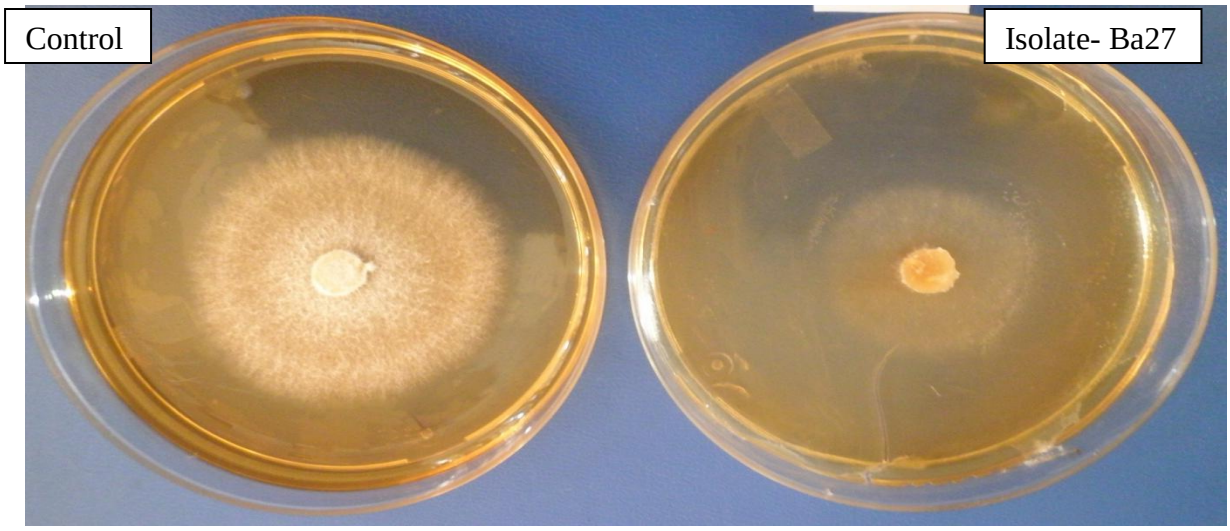


Plate.5 Ammonia production by antagonistic rhizobacteria

Yellow orange = Weak (+), Orange= Moderate (++), Orange red = Strong (+++)

Plate.6 Hydrogen cyanide production by rhizobacterial isolates

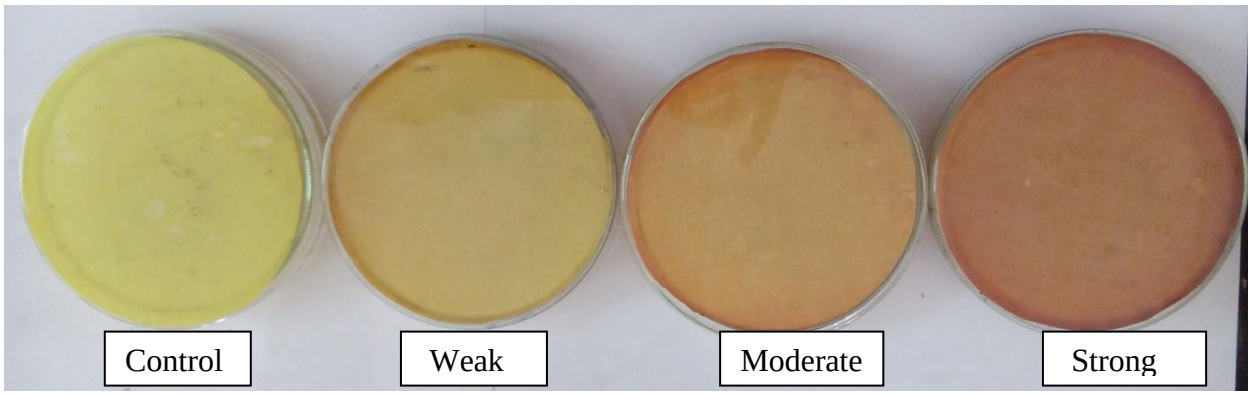
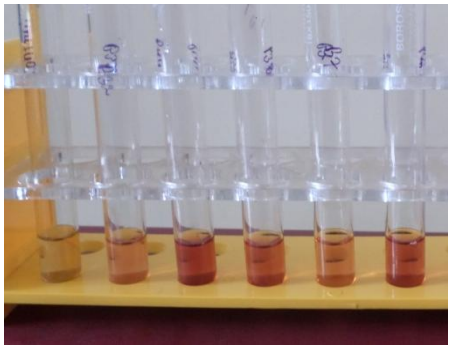
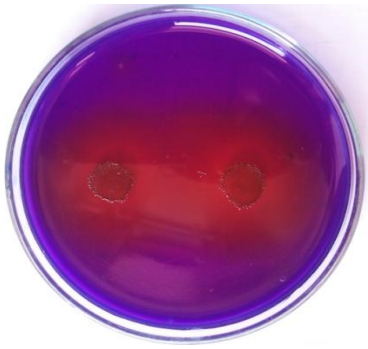


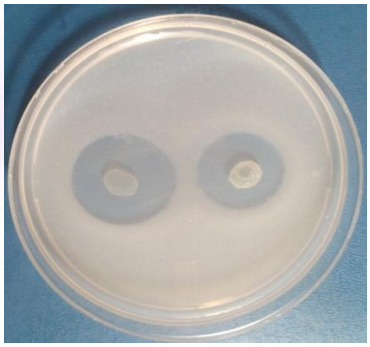
Plate.7 Plant growth promoting characteriatics of selected anatagonists



Indole acetic acid production



Phosphate solublization



Zinc Solublization

Plate.8 Pot experiment conducted to evaluate the potential of antagonistic rhizobacteria to control wilt, under glass house conditions

Plate.9 Relative seedling emergence in different treatments in chickpea under glass house conditions (GPF-2)

Absolute Control Negative control Fungicide Isolate – Ps47 Ps47+ *Mesorhizobium*

Plate.10 Relative seedling emergence in different treatments in chickpea under glass house conditions (JG-41)

Plate.11 Symptoms of wilting in chickpea plants

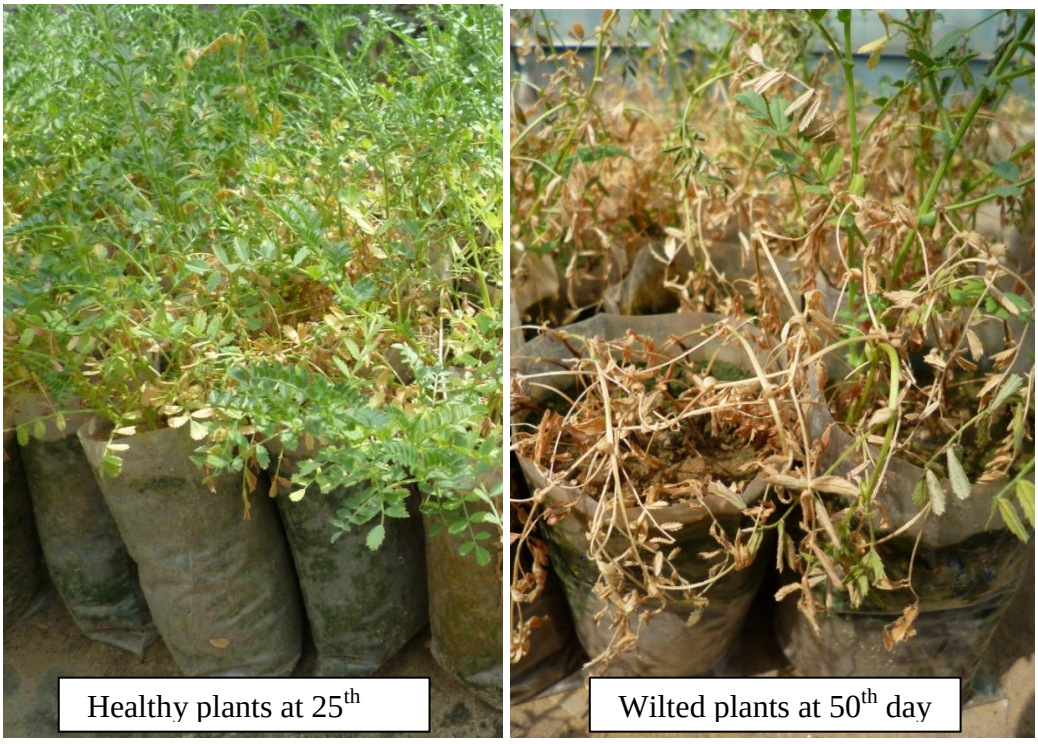


Plate.12 Relative wilt incidence in different treatments in chickpea under glass house conditions (GPF-2)

Negative control Fungicide Isolate – Ps45 Ps45+ *Mesorhizobium*

Plate.13 Relative wilt incidence in different treatments in chickpea under glass house conditions (JG-41)

Negative control Fungicide Isolate – Ps45 Ps45+ *Mesorhizobium*

Table.1 Screening of rhizobacterial isolates against the radial growth of *F. oxysporum* f. sp. *ciceris* in dual culture technique

Serial No.	Isolates	% Inhibition over control	Serial No.	Isolates	% Inhibition over control
	Control	-	31	Ps8	37.1±1.06
1	Ba1a	40.0±0.67	32	Ps9	31.4±0.43
2	Ba3	37.1±0.22	33	Ps10b	40.0±1.19
3	Ba4	40.0±0.05	34	Ps11	41.4±0.21
4	Ba5	32.8±0.72	35	Ps12	31.4±0.76
5	Ba7	32.8±0.21	36	Ps14	42.8±1.45
6	Ba8	41.4±2.22	37	Ps15	38.5±0.62
7	Ba10	41.4±1.19	38	Ps16b	45.7±3.58
8	Ba11	38.5±5.93	39	Ps17	32.8±0.67
9	Ba13	35.7±0.61	40	Ps19	25.7±1.22
10	Ba14	28.5±0.14	41	Ps20	35.7±0.59
11	Ba15	40.0±0.05	42	PS21	42.8±0.90
12	Ba17	38.5±0.62	43	Ps22	37.1±0.24
13	Ba18	40.0±0.65	44	Ps24	40.0±0.30
14	Ba19	40.0±0.74	45	Ps25	30.0±0.04
15	Ba20	28.5±0.12	46	Ps26	41.4±0.08
16	Ba21	28.5±0.11	47	Ps27	41.4±0.39
17	Ba22	31.4±0.70	48	Ps31	35.7±0.29
18	Ba24	38.5±0.35	49	Ps33	40.0±0.52
19	Ba26	31.4±0.47	50	Ps35	28.5±0.32
20	Ba27	40.0±0.16	51	Ps37	40.0±0.70
21	Ba32	35.7±0.17	52	Ps38	30.0±0.16
22	Ba34	28.5±0.67	53	Ps41	28.5±0.17
23	Ba36	40.0±0.85	54	Ps42	38.5±0.38
24	Ba42	34.2±0.21	55	Ps43	42.8±0.68
25	Ps1	28.5±0.14	56	Ps44	42.8±0.22
26	Ps2	31.4±0.21	57	Ps45	42.8±0.26
27	Ps4	35.7±0.86	58	Ps46	31.4±0.18
28	Ps5	38.5±0.60	59	Ps47	44.2±0.32
29	Ps6	34.2±0.34	60	Sm1	31.4±0.80
30	Ps7	28.5±1.16	61	Sm2	28.5±0.28

Table.2 Screenig of rhizobacterial isolates against the mycelial proliferation of *F. oxysporum* f. sp. *ciceris* in liquid medium

Serial No.	Isolates	% Inhibition over control	Serial No.	Isolates	% Inhibition over control
	Control	-	31	Ps8	64.4±0.12
1	Ba1a	83.0±0.22	32	Ps9	28.7±0.22
2	Ba3	61.0±0.51	33	Ps10b	61.0±0.91
3	Ba4	73.6±0.92	34	Ps11	60.5±0.06
4	Ba5	51.7±0.41	35	Ps12	30.0±1.70
5	Ba7	63.1±0.91	36	Ps14	43.6±0.17
6	Ba8	67.8±0.73	37	Ps15	62.8±1.60
7	Ba10	63.3±0.78	38	Ps16b	69.7±1.17
8	Ba11	64.4±6.93	39	Ps17	35.6±11.8
9	Ba13	28.7±0.15	40	Ps19	39.1±0.68
10	Ba14	37.8±0.25	41	Ps20	51.7±0.05
11	Ba15	50.6±1.27	42	PS21	55.3±0.14
12	Ba17	63.1±1.55	43	Ps22	50.8±1.23
13	Ba18	64.4±1.09	44	Ps24	49.4±1.45
14	Ba19	78.5±0.11	45	Ps25	61.1±1.19
15	Ba20	52.9±0.45	46	Ps26	67.4±1.25
16	Ba21	51.7±0.38	47	Ps27	61.0±0.72
17	Ba22	47.2±6.67	48	Ps31	28.7±0.13
18	Ba24	50.1±1.14	49	Ps33	62.0±1.85
19	Ba26	60.8±1.02	50	Ps35	50.4±1.38
20	Ba27	63.1±1.39	51	Ps37	63.1±0.19
21	Ba32	64.3±1.69	52	Ps38	54.1±0.16
22	Ba34	59.9±2.40	53	Ps41	51.7±0.34
23	Ba36	46.3±0.44	54	Ps42	50.6±1.95
24	Ba42	26.4±0.72	55	Ps43	44.7±0.96
25	Ps1	64.4±0.39	56	Ps44	86.2±0.84
26	Ps2	40.3±0.22	57	Ps45	87.3±0.10
27	Ps4	63.1±1.45	58	Ps46	52.9±1.31
28	Ps5	61.0±1.21	59	Ps47	75.4±0.14
29	Ps6	46.1±1.01	60	Sm1	28.7±0.46
30	Ps7	63.1±0.197	61	Sm2	40.0±1.47

Table.3 Effect of rhizobacterial diffusible metabolites on suppression of the radial growth of *F. oxysporum* f. sp. *ciceris.*

Serial No.	Isolates	Diameter of growth (cm)	% Inhibition over control	Serial No.	Isolates	Diameter of growth(cm)	% Inhibition over control
	Control	5.5	-		Control	5.5	-
1	Ba1a	1.8	67.2±0.06	14	Ps8	2.2	60.0±0.21
2	Ba4	3.8	30.9±0.85	15	Ps10b	0.5	90.9±0.25
3	Ba7	4.0	27.2±0.65	16	Ps11	3.5	36.3±0.42
4	Ba8	5.0	9.0±0.34	17	Ps14	1.2	78.1±030
5	Ba10	4.3	21.8±0.59	18	Ps15	1.6	70.9±0.12
6	Ba11	1.3	76.3±0.60	19	Ps16b	1.4	74.5±1.31
7	Ba17	1.9	65.4±0.18	20	Ps21	1.2	78.1±0.33
8	Ba18	4.5	18.5±0.23	21	Ps33	4.8	12.7±1.81
9	Ba19	1.4	74.5±0.21	22	Ps37	4.1	25.4±0.27
10	Ba27	2.1	61.8±1.52	23	Ps43	2.0	63.6±0.16
11	Ba32	2.0	63.6±0.95	24	Ps44	0.5	90.9±0.06
12	Ps1	4.0	27.2±023	25	Ps45	0.5	90.9±0.26
13	Ps5	4.0	27.2±0.28	26	Ps47	0.5	90.9±0.25

Table.4 Antagonistc effect of rhizobacterial volatiles on radial growth of *Fusarium oxysporum* f. sp. *ciceris*

Serial No.	Isolates	Diameter (cm)	% Inhibition	Serial No.	Isolates	Diameter (cm)	% Inhibition
	Control	6.5	-		Control	6.5	-
1	Ba1a	2.0	56.0 ±0.30	14	Ps8	3.3	49.2±0.39
2	Ba4	3.3	49.2±031	15	Ps10b	3.3	49.2±0.62
3	Ba7	3.0	53.8±0.27	16	Ps11	3.2	50.7±0.06
4	Ba8	3.0	53.8±0.12	17	Ps14	4.0	38.4±0.38
5	Ba10	3.2	50.7±0.21	18	Ps15	3.0	53.8±0.28
6	Ba11	3.0	53.8±0.10	19	Ps16b	4.5	30.7±0.24
7	Ba17	3.3	49.2±0.01	20	Ps21	4.0	38.4±0.24
8	Ba18	4.0	38.4±0.86	21	Ps33	3.3	49.2±0.19
9	Ba19	2.5	53.8±0.19	22	Ps37	3.9	40.0±0.74
10	Ba27	3.0	61.5±0.23	23	Ps43	3.8	41.5±0.18
11	Ba32	3.7	43.0±0.1	24	Ps44	2.8	56.9±0.43
12	Ps1	4.3	33.8±0.21	25	Ps45	3.0	53.8±0.58
13	Ps5	3.0	53.8±0.29	26	Ps47	0.6	90.7±0.68

Table.5 Evaluation of selected antagonists for the production of Hydrogen cyanide

Serial No.	Isolates	HCN Production		Serial No.	Isolates	HCN Production	
		Colour	Class			Colour	Class
1	Ba1a	Orange-red	Moderate (++)	14	Ps8	Orange-red	Moderate (++)
2	Ba4		-	15	Ps10b		-
3	Ba7	Yellow-orange	Weak (+)	16	Ps11	Yellow-orange	Weak (+)
4	Ba8		-	17	Ps14		-
5	Ba10		-	18	Ps15		-
6	Ba11		-	19	Ps16b		-
7	Ba17		-	20	Ps21		-
8	Ba18		-	21	Ps33		-
9	Ba19	Red	Strong (+++)	22	Ps37		-
10	Ba27		-	23	Ps43		-
11	Ba32		-	24	Ps44	Red	Strong (+++)
12	Ps1	Orange-red	Moderate (++)	25	Ps45	Red	Strong (+++)
13	Ps5	-	-	26	Ps47	Orange-red	Moderate (++)

Table.6 Ammonia production profile by selected antagonistic rhizobacteria

Serial No.	Isolates	Ammonia Production		Serial No.	Isolates	Ammonia Production	
		Colour	Class			Colour	Class
1	Ba1a	Orange	++	14	Ps8	Yellow-orange	+
2	Ba4	Orange-red	+++	15	Ps10b	Orange	++
3	Ba7	Yellow-orange	+	16	Ps11	Yellow-orange	+
4	Ba8	Yellow-orange	+	17	Ps14	Yellow-orange	+
5	Ba10	Yellow-orange	+	18	Ps15	Yellow-orange	+
6	Ba11	Orange-red	+++	19	Ps16b	Orange-red	+++
7	Ba17	Orange	++	20	Ps21	Orange-red	+++
8	Ba18	Orange	++	21	Ps33	Yellow-orange	+
9	Ba19	Orange	++	22	Ps37	Orange-red	+++
10	Ba27	Yellow-orange	+	23	Ps43	Yellow-orange	+
11	Ba32	Orange-red	+++	24	Ps44	Orange	++
12	Ps1	Orange	++	25	Ps45	Orange	++
13	Ps5	Yellow-orange	+	26	Ps47	Orange-red	+++

Weak=+, Moderate = ++, Strong = +++

Table.7 Impact of potential antagonists alone and alongside *Mesorhizobium* on germination index of two chickpea varieties (GPF-2 and JG-41) under glass house conditions

Serial No.	Treatments	Seedling emergence (%)	
		GPF-2	JG-41
1	Absolute control	86.7±1.33	73.3±1.76
2	Negative control	66.6±0.33	63.4±0.89
3	Fungicide (Captan)	86.6±1.30	76.6±1.20
4	Ps44	77.0±1.20	80.0±1.15
5	Ps45	86.6±0.88	73.3±1.33
6	Ps47	83.3±1.67	80.0±1.52
7	Ba19	76.7±1.22	74.1±1.77
8	Ba1a	73.3±1.45	76.6±1.20
9	Ps44+*Mesorhizobium*	80.0±1.00	83.0±1.20
10	Ps45+*Mesorhizobium*	93.3±0.66	80.0±1.15
11	Ps47+*Mesorhizobim*	90.0±1.00	86.7±1.33
12	Ba19+*Mesorhizobium*	83.3±1.67	83.3±1.20
13	Ba1a +*Mesorhizobium*	86.7±0.89	76.7±0.88
14	C.D. at 5%	NS	NS

Table.8 Reduction of wilt severity by antagonistic rhizobacteria in chickpea varieties (GPF-2 and JG-41) under pot conditions

Serial No.	Treatments	Reduction in disease severity (%)	
		GPF-2	JG-41
1	Absolute control	61.50±0.33	67.61±0.57
3	Fungicide (Captan)	61.53±0.89	58.69±0.33
4	Ps44	62.73±0.67	60.42±0.33
5	Ps45	67.03±0.58	56.81±0.33
6	Ps47	65.52±0.57	55.46±0.33
7	Ba19	56.52±1.00	46.01±0.57
8	Ba1a	61.03±0.69	58.69±0.33
9	Ps44+*Mesorhizobium*	64.28±0.55	66.74±0.66
10	Ps45+*Mesorhizobium*	74.48±0.67	70.32±1.00
11	Ps47+*Mesorhizobim*	73.54±0.66	68.02±0.66
12	Ba19+*Mesorhizobium*	65.71±1.15	57.20±0.00
13	Ba1a +*Mesorhizobium*	72.52±0.68	63.80±0.66
14	C.D. at 5%	NS	1.54

Elucidation of antagonistic mechanism *via* hydrogen cyanide and ammonia production

Hydrogen cyanide and ammonia belong to volatile antifungal metabolites and play a very important role in inhibiting the spore germination and mycelia growth of various fungal phytopathogens (Fernando *et al.*, 2005). All the isolates were found to produce ammonia supported by one of our earlier reports (Kumari and Khanna, 2014) (Plate 5). Seven (26.9 %), Eight (30.7%) and eleven (42.30%) were found to be strong, moderate and weak ammonia producer (Table 6). However only nine (34%) isolates were found positive for hydrogen cyanide production out of which 22.2 % were weak, 44.4 moderate and 33.3% were strong HCN producers on the basis of intensity of colour (yellow/yellowish-orange/orange-red) produced (Table 5, Plate 6). In addition to *Bacillus* and *Pseudomonas* spp. reports are there that *Mesorhizobium* spp. also produce HCN, and ammonia along with some enzymes like catalase, chitinase etc (Ahemad and Khan 2009). Ammonia inhibits cell cycle progression and thus inhibits the bacterial progression whereas Hydrogen cyanide produced by these antagonistic rhizobacteria mainly affects the respiratory chain i.e. electron transport chain of the pathogens and thus makes them ATP deficient for further growth and development. In support to this, Guo *et al.*, (2007) reported that the release of HCN by rhizospheric bacteria into the soil can be toxic to subterranean animals and phytopathogenic organisms and thus is an important mechanism in biological control of soil borne pathogens.

Other plant growth promoting traits

Five potent antagonists (Ba1a, Ba19, Ps44, Ps45 and Ps47) were selected on the basis of intensity of antagonistic traits to inhibit the growth of *Fusarium oxysporum* f. sp. *ciceris* (Foc) in laboratory conditions. These 5 selected antagonists were also evaluated for their efficiency to produce various plant growth promoting metabolites. All the isolates were found positive for the production of plant growth hormones such as Indole acetic acid (IAA), Gibberellic acid and iron chelating agent, siderophores as their excretions. Studies revealed that plant growth hormones like gibberellins, IAA and cytokinin play important role in bacterial plant interactions (Dobbelaere *et al.*, 2003). Further they were also found efficient in Zinc and Phosphate solublization (Plate 7), indicating the production and release of various organic acids responsible for the nutrient solublization, one of the mechanisms by which plant growth promoting rhizobacteria deprive the pathogen from these essential nutrients and enhance the nutrient availability to the plants (Castagno *et al.*, 2011).

Compatibility test

Rhizobacterial antagonists were evaluated for their compatibility with *Mesorhizobium*, (recommended culture of Department of Microbiology), specific for chickpea. The overlapping growth to each other on Yeast Mannitol agar plates was determined as compatible interaction between the paired microorganisms. All the antagonists showed positive interaction with *Mesorhizobium* indicating, their synergistic influence on plant growth promoting performance.

Impact of adversarial rhizobacteria alone and alongside local *Mesorhizobium*, on seedling development of chickpea

A pot experiment was conducted to evaluate the effect of potent antagonists to control wilt and enhance the growth parameters of chickpea (Plate 8). Three *Pseudomonas*

(Ps44, Ps45 nad Ps47) and two *Bacillus* (Ba1a and Ba19) antagonists selected on the basis of antagonistic parameters alone and alongside local *Mesorhizobium*, along with Captan (2g/Kg seeds) as a separate treatment were observed for their impact on the seed germination of two chickpea varieties (GPF-2 and JG41), compared to negative control, under glass house conditions. Observations revealed that highest seedling emergence was recorded in case of treatments of antagonists along with *Mesorhizobium*, indicating the synergistic effect to enhance the seed development (Table 7). Seed bacterization with Ps45 induced maximum germination, followed by Ps 47 and Ba1a co-inoculated with *Mesorhizobium* compared to Captan and negative control in GPF-2 variety (Table 7, Plate 9).

Similarly in case of JG-41, co-inoculation with *Mesorhizobium* was recorded with maximum seedling growth by Ps47 86.7%, followed by Ba19, Ps44 and Ps45 (Table 7, Plate 10). Co-inoculation with *Mesorhizobium* was recorded with positive influence on germination compared to negative control and even was found better than the fungicide, indicating the adverse effect of chemical fungicide on germination (in sterile soil containing no beneficial microbes) (Plate 9, 10).

Similar results were recorded by Kumari and Khanna in 2014. Effectively rhizobacterial seed treatment recorded percentage germination of tomato seeds in the range between 83.33 to 100% in contrast to 75% noted on the untreated control ones in *Sclerotinia sclerotiorum* affected soil under pot conditions (Abdeljalil *et al.,* 2016). Landa and his co-workers also reported that *Pseudomonas fluorescens* RG *Bacillus megaterium* RGAF enhanced seedling emergence compared to negative control (Landa *et al.,* 2004).

Elucidation of antiphytopathogenic potential to reduce disease severity in chickpea

Wilt symptoms started after 30 days of sowing, with drooping, decoloured leaves and plants became almost dry and dead in negative control after 50 days (Plate 11). Same as the seed growth, wilt incidence was noticeably reduced by rhizobacterial isolates co-inoculated with *Mesorhizobium*. Percentage disease reduction was recorded by taking total wilt in negative control as standard. Observations revealed that even absolute control containing normal non sterile soil of the field having the history of chickpea cultivation, also showed wilt symptoms. Seed treatment with Ps45+*Mesorhizobium* was recorded with maximum reduction in disease i.e. 74.48±0.67 % followed by Ps47+*Mesorhizobium* 73.54±0.66, and Ba1a + *Mesorhizobium* 72.52±0.68%, compared to fungicide treatment 61.53±0.89% in variety GPF-2 (Plate 12). Relevantly In JG-41, Ps45+*Mesorhizobium* application to the seeds showed minimum wilt incidence, with percentage reduction in disease of 70.32±1.00% and treatment of Ps47+*Mesorhizobium* reduced the disease 68.02±0.66%, compared to concision effect of fungicide (58.69±0.33%) (Table 8, Plate 13). In a similar report, Pf1-Bs16 and Pf1-Py15 recorded disease severity of16.66 and 24.99% disease incidence and reduced the disease (81.8%) and (72.7%), respectively against 91.63% disease incidence in control in mulburry (Ganeshamoorthi *et al.,* 2008). Efficacy to descend the disease by rhizobacterial treatment is not only limited to wilt, but these are also effective against other diseases such as, root rot by *Rhizoctonia solani* stem rot caused by Sclerotinia *sclerotiorum*, damping off by *Phytophthora* spp. etc by various antagonist mechanisms (Yang *et al.,* 2009). The highest disease incidence (100%) was noted on tomato plants

inoculated with *S. Sclerotiorum* in control where treatment using *B. thuringiensis* B2 (KU158884), *B. subtilis* B10 (KT921327), *B. amyloliquefaciens* B13 (KT951658), *B. amyloliquefaciens* B15 (KT923051), and *E. cloacae* B16 (KT921429) led to total suppression of disease development and using 8 out of the 25 strains tested, disease incidence did not exceeded 20% as compared to 100% recorded on pathogen-inoculated and untreated control (Abdeljalil *et al.,* 2016). All these studies emphasize on the effective role of plant growth promoting rhizobacteria to control wide range of pathogens, by various antagonistic mechanisms, so as to reduce the disease severity and enhance the germination, growth and thus yield of economically important crops.

With increasing awareness about the adverse effects of chemical fertilizers and pesticides, it is very important to explore various mechanisms by which plant growth promoting rhizobacteria can control the phytopathogenic effects in the crop plants.

In our study screened antagonistic isolates alone were also efficient in contrast to control but co-inoculation with *Mesorhizobium* has given better results in enhancing the seed germintion and controlling the wilt incidence caused by *Fusarium oxysporum* f. sp. *ciceris* in both the chickpea varieties (GPF-2 and JG-41) under glass house controlled conditions against the negative control and fungicide treatment.

They could be used as biofungicides on the condition of their similar effectiveness under field conditions. Further investigations are focussed to even enhance the self defence mechanism of plants by these antagonistic rhizobacteria and to evaluate the synergistic potential of antagonists to formulate various combinations of these so as to have better results against these phytopathogens.

Acknowledgments

The present investigation was conducted in the Pulses section, Department of Plant Breeding and Genetics, Punjab Agricultural University. Further support and assistance was provided by Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab and Department of Science and technology, New Delhi, India.

References

Abdeljalil, N.O.B., Vallance, J., Gerbore, J., Rey, P., and Remadi M.D. 2016. Bio-suppression of *Sclerotinia* Stem Rot of Tomato and Biostimulation of Plant Growth Using Tomato associated Rhizobacteria. *J. Pl. Pathol Microbiol.* 7(2): 1-11.

Ahemad, M., and Khanm, M. S. 2009. Effect of Insecticide-Tolerant and Plant Growth-Promoting *Mesorhizobium* on the Performance of Chickpea Grown in Insecticide Stressed Alluvial Soils. *J. Crop. Sci. Biotech.* 2(4): 217- 226.

Ahmed, Idris, H., Labuschagne, N., and Korsten, L. 2007. Screening rhizobacteria for biological control of *Fusarium* root and crown rot of sorghum in Ethiopia. Biol. control. 40: 97– 106.

Aktar, M.W., Sengupta, D., Chowdhuri, A. 2009. Impact of pesticides use in agriculture: their benefits and hazards. *Interdisc Toxicol.* 2(1): 1-12.

Altinok, H. H., Dikilitas, and M. Yildiz, H. N. 2014. Potential of Pseudomonas and Bacillus Isolates as Biocontrol Agents against Fusarium Wilt of Eggplant. *Biotechnol. and Biotechnol.* 47: 2952-58.

Anonymous, 2015. Commodity profile of pulses – March 2015. Department of Agriculture and Co-operation, Ministry of Agriculture, Government of India.

Arias, A.A., Ongena, M., Halimi, B., Lara, Y., and Brans, A. 2009. *Bacillus amyloliquefaciens* GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens. *Microb. Cell Fact.* 8: 63-70.

Bakker, A. W., and Schippers, B. 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. *Soil Biol. Biochem.* 19: 249-256.

Borrow, A., P., W, Brain, U., E., Chester, P,.J. Curtis, H., G., Hemming, E., C, Jeffereys, R., B., Lloyd, I., S., Nixon, G., L., F., Norris, and N., Radley. 1955. Gibberellic acids a metabolic product of the fungus *Gibberella fujikuroi* some observations on its production and isolation. *J. Sci. Food. Agric.* 6: 340-348.

Butler, E.J., 1918. *Fungi and Diseases in Plants.* Thacker Spink and Co., Calcutta, India, 547pp.

Cappuccino J. C. and Sherman, N. 1992. In: Microbiology: A Laboratory Manual, New York. Academic distributors, New Delhi. pp. 125-179.

Castagno L.N., Estrella, M.J., Sannazzaro, A.I., Grassano, A.E., and Ruiz, O.A. 2011. Phosphate solubilization mechanism and in vitro plant growth promotion activity mediated by *Pantoea eucalypti* isolated from *Lotus tenuis* rhizosphere in the Salado River Basin (Argentina). *J. Appl. Microbiol.* 110: 1151- 1165.

Deshwal, V.K, Pandey, P., Kang, K.C., and Maheshwari, D.K. 2003. Rhizobia as biological control against soil-borne plant pathogenic fungi. *Ind. J. Exp. Biol.* 41: 1160-1164.

Dobbelaere, S.J., Vanderleyden, and Okon, Y. 2003. Plant growthpromoting effects of diazotrophs in the rhizosphere. *Crit. Rev. Plant Sci.* 22: 107-149.

Dubey, S.C., Singh, and Birendra 2004. Reaction of chickpea genotypes against *Fusarium oxysporum* f. sp. *ciceri* causing vascular wilt. *Indian Phytopath. 57: 233-237.*

Edi Premono M., Moawad A. M., and Vlek P. L. G. 1996. Effect of phosphate-solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. *Indones. J. Crop Sci.* 11: 13-23.

Ehmann, A., 1977. The Van Urk-Salkowski reagent-a sensitive and specific chromogenic reagent for silica gel thin-layer chromatographic detection and identification of indole derivatives. *J. Chromatogr.* 132: 267-276.

Fernando, W.G.D., Ramarathan, R., Krishnamoorthy, A.S., and Savchuk, S.C. 2005. Identification and use of potential bacteria organic antifungal volatile isolates in biocontrol. *Soil Biol. Biochem*. 37: 955-964.

Fiddman, P. J. and Rossall. S. 1993. The production of antifungal volatiles by *Bacillus subtilis. J Appl Bacteriol* 74:119-126.

Ganeshamoorthi, P., Anand, T., Prakasam, V., Bharani, M., Ragupathi, and Samiyappan, R. N. 2008. Plant growth promoting rhizobacterial (PGPR) bioconsortia mediates induction of defense-related proteins against infection of root rot pathogen in mulberry plants. *J. Pl. Interactions.* 3: 233-244.

Giorgio, A., Stradis, A.D., Cantore, P.L., and Lacobellis, N.S. 2015. Biocide effects of volatile organic compounds produced by potential biocontrol rhizobacteria on *Sclerotinia sclerotiorum. Front. Microbiol.* 6: 1-13.

Gopalakrishnan, S., Pande, S., Sharma, M., Humayun, P., Kiran, B.K., Sandeep, D., Vidya, Deepthi, M. S.K., Rupela, O. 2011. Evaluation of actinomycete

isolates obtained from herbal vermicompost for the biological control of Fusarium wilt of chickpea. *Crop Prot.* 30: 1070-1078.

Guo, Y., Zheng, H., Yang, Y. Wang, H. 2007. Characterization of Pseudomonas corrugate strain P94 isolated from soil in Beijing as a potential biocontrol agent. *Curr. Microbiol.* 55: 247–53.

Hossain, M.M., Hossain, N., Sultana, F., Islam, S.M.N., Islam, M.S. Bhuiyan, M.K.A. 2013. Integrated management of Fusarium wilt of chickpea (*Cicer arietinum* L.) caused by *Fusarium oxysporum* f. sp. *ciceris* with microbial antagonist, botanical extract and fungicide. *Afr. J. Microbiol.* 12(29): 4699-4706.

Isnansetyo, A., Cui, L., Hiramatsu, and Kamei, K.Y. 2003. Antibacterial activity of 2,4 diacetylphloroglucinol (DAPG) produced by *Pseudomonas* sp. AMSN isolatsed from a marine alga, against vancomycin-resistant *Staphylococcus aureus* (VRSA). *Int. J. Antimicrob*. Agents 22: 545-547.

Kai, M., Effmert, U., Berg, G., and Piechulla, B. 2007. Volatiles of bacterial antagonists inhibit mycelial growth of the plant pathogen *Rhizoctonia solani*. Arch. Microbiol. 187: 351–360.

Karnwal, A., Kumar, V. 2012. Influence of plant growth promoting rhizobacteria (pgpr) on the growth of chickpea (*Cicer arietinum L*.). *Ann Food Sci. Tech.* 13(2): 1-6.

King, E.O., Ward, M.K. Raney, D.E. 1954. Two simple media for the demonstration of pyocyanin and fluorecein. *J. Lab. Clin. Med.* 44: 301-07.

Kravchenko, L.V., Makarova, N.M., Azarova, T.S., Provorov, N.A. and Tikhonovich, I.A. 2002. Isolation and phenotypic characteristics of growth-stimulating rhizobacteria (PGPR), with high root-colonizing and phytopathogenic fungi inhibiting abilities. *Microbiol.* 71(4): 521-525.

Kumar, H., Bajpai, V. K., Dubey, R.C., Maheshwari, D.K., and Kang. S.C., 2010. Wilt disease management and enhancement of growth and yield of *Cajanus cajan* (L) var. Manak by bacterial combinations amended with chemical fertilizer. *Crop Prot.* 29: 591-598.

Kumari, S. and Khanna, V. 2014. Effect of antagonistic rhizobacteria coinoculated with *Mesorhizobium ciceris* on control of fusarium wilt in chickpea (*Cicer arietinum L*.). *Afr. J. Micro. Res.* 8(12): 1255- 1265.

Landa, B.B., Cortés, J.A.N., Díaz, R.M.J. 2004. Influence of temperature on plant–rhizobacteria interactions related to biocontrol potential for suppression of fusarium wilt of chickpea. *Pl. Pathol.* 53: 341–352.

Meki, S., Ahmed S. and Sakhuja P.K. 2009. Control of chickpea wilt (*Fusarium oxysporum* f.sp. *ciceris*) using *Trichoderma* spp. in Ethiopia. Arch. Phytopathol. Pl. Protec. 44, 5.

Merkuz A., and Getachew, A. 2012. Distribution and severity of sorghum covered kernel smut in North western Ethiopia. *Int. J. Curr. Res.* 4 (4): 41-45.

Merkuz A., Seid A., Chemeda F., Sakhuja P.K. and Getachew A. 2011. Effect of mustard green manure and driedplant residue on chickpea wilt (*Fusarium oxysporum* f.sp. *ciceris*). *Arch. Phytopathol. Pl. Protec.* 44 (9), 821 – 831.

Moradi, H., Bahramnejad, B., Amini, Siosemarde, A., and Allahverdipoor, K.H. 2012. Suppression of chickpea (*Cicer arietinum* L.) *Fusarium* wilt by *Bacillus subtillis* and *Trichoderma harzianum*. *Pl. Omics J.* 5(2): 68-74.

Nautiyal, C.S., 2000. Biocontrol of plant

diseases for agricultural sustainability. In: Upadhyay R.R., Mukerji K.G., Chamola B.P., (eds.), Biocontrol potential and its exploitation in sustainable agriculture, Volume 1. Crop Diseases, Weeds, and Nematodes. Kluwer Academy Plenum, New York. pp. 9-23.

Pande, S, Desai, S., Sharma, M. 2010. Impacts of climate change on rainfed crop diseases: Current Status and Future Research Needs. National Symposium on Climate Change and Rainfed Agriculture, Hyderabad. 18(20): 55-59.

Prashar P., N. Kapoor, Sachdeva, S. 2013. Isolation and Characterization of *Bacillus sp* with *In-vitro* Antagonistic Activity against *Fusarium oxysporum* from Rhizosphere of Tomato. *Agr. Sci. Tech.* 15:1501-12.

Reino, L.R, Raul, F., Herna´ndez-Gala´n, G.R., Collado, I.G. 2008. Secondary metabolites from species of the biocontrol agent *Trichoderma*. *Phytochem. Rev.* 7: 89-123.

Ryan, R.P., Fouhy, Y., and Garcia, B.F. 2008. Interspecies signaling via the *Stenotrophomonas maltophilia* diffusible signal factor influences biofilm formation and polymyxin tolerance in *Pseudomonas aeruginosa*. *Mol. Microbiol.* 68: 75-86.

SchwynB and Neilands J B, 1987. Universal chemical assay for detection and determination of siderophore. *Anal. Biochem*. p. 47.

Vesperman A, Kai, M., and Piechulla, B. 2007. Rhizobial volatiles affect the growth of fungi and Arabidopsis thaliana. *Appl. Environ. Microbiol*. 73: 5639-5641.

Yang, J., Kloepper, J.W., and Ryu, C.M. 2009. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends. Plant. Sci.* 14: 1-4.

How to cite this article:

Suman Kumari and Veena Khanna. 2018. Biological Management of Vascular Wilt of Chickpea (*Cicer arietinum* L.) Incited by *Fusarium oxysporum f. sp. ciceris* by Antagonistic Rhizobacteria Co-Inoculated with Native *Mesorhizobium*. *Int.J.Curr.Microbiol.App.Sci.* 7(01): 920-941. doi: https://doi.org/10.20546/ijcmas.2018.701.112