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Multifarious Antagonistic Potentials of Native Pseudomonad Isolates from Rhizosphere as Biocontrol Agents for the Management of Chickpea Wilt

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

A total of nine pseudomonad isolates from different rhizosphere were isolated and screened for their antagonistic activities against *F. oxysporum* f.sp. *ciceri.* All nine isolates exhibited the ammonification property, produced HCN (Cyanide) and IAA (Indole acetic acid) and positive for phosphorus solubilization, except PGPR-PI, this found to be negative for HCN production. Among the nine isolates, pseudomonad isolates PGPR-WS were best in exhibiting multiple PGPR traits like ammonification, HCN production, IAA production (26.08 mgl^{-1}), and phosphate solubilization (306.51 mgl^{-1}) as well as best in antagonistic activity against *F. oxysporum* f.sp. *ciceri,* showed 75.00% inhibition of growth of mycelia over control and caused total lysis of mycelia in advanced stages of antagonism. Thus screening and identification of novel bioagent PGPR-WS with multifarious activities vividly reflect its potential to suppress *F. oxysporum* f.sp. *ciceri* and suggest the usefulness of this super bioinoculant as a component of integrated disease management (IDM) of chickpea wilt caused by *F. oxysporum* f.sp. *ciceri.*

Keywords: Chickpea wilt; pseudomonad; ammonification; HCN; IAA; IDM.

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1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the important legume crop grown in the Mediterranean basin and Worldwide [1]. India accounts for approximately 75 percent of global chickpea production. Chickpea contributes about 67 per cent to *Rabi* pulse production and 46 per cent of the total production of India. It occupies an area of about 8.35 million hectares with annual production of 7.17 million tons with the productivity of 859 Kg per hectare (Directorate of economics and statistics [2]). Many factors contributed towards low chickpea yield, but the pathological constraints are the most important. Chickpea wilt caused by *Fusarium oxysporum* Schlechtend Fr. f. sp. *ciceris* (Padwick) Matuo & K. Sato, [3] is the most important soil-borne disease of chickpea throughout the world and particularly in the Indian Sub-continent, the Mediterranean Basin and California [4,5,6]. In India, this disease was reported by Butler in 1918. *Fusarium oxysporum* f.sp. *ciceri* may survive in soil and on crop residues as chlamydospores for up to six years in the absence of a susceptible host and spread using both soil and infected seed [7]. Attacks of the *Fusarium* wilt pathogen can destroy the crop completely or cause a significant annual yield loss especially in low rainfall regions which is a permanent threat to the chickpea causing wilt syndrome. *Fusarium* wilt of chickpea is prevalent in almost all chickpea-growing areas of the world and its incidence varied from 14 to 32% in the different states of India [8]. This disease causes yield losses up to 100% under favourable conditions [9,10]. Characteristic symptoms of this disease develop at any stage of plant growth, and affected plants may be grouped in patches or appear spread across the field [4,6,11]. Highly susceptible cultivars can show symptoms within 25 days after sowing (designated 'early wilt'), including flaccidity of individual leaves followed by a dull-green discolouration, desiccation and collapse of the entire plant. However, the roots and stem of a plant develop a dark-brown discolouration of xylem tissues that can be seen when they are split vertically or cross-sectioned. Management of this pathogen is not possible by adopting a single approach like cultural practices, fungitoxicants, host plant resistance or bio-agents and thus shows the necessity to integrate management packages for the disease. Although fungicides have shown promising results in controlling the pathogen; phytotoxicity and fungicidal residues along with environmental contamination and human health hazards prevent their large-scale use. Therefore, replacement of fungicides with use of bio-agents and/or products has become a focus of considerable interest in the context of sustainable, economical and profitable agriculture. Currently, plant growth-promoting rhizobacteria (PGPR)-mediated ISR has received considerable attention, as a biological approach to managing pests and diseases are more ecologically sustainable. Biological control by PGPR may involve the production of bacterial metabolites which reduce the population or activities of pathogens or deleterious rhizosphere microflora [12,13,14,15]. These metabolites may include siderophores that bind Fe making it less available to harmful microflora ([16,17]) Several studies have demonstrated that production of antibiotics (e.g. pyrrolnitrin, phycocyanin, 2, 4-diacetyl phloroglucinol) by microbial inocula can cause suppression of pathogens [18,19]. Other mechanism for biological control of disease may include: competition for infection sites and nutrients parasitism on pathogens i.e. destruction of fungal pathogen by action of lytic enzymes (e.g. chitinase and β-1, 3-glucanase) and HCN that degrade fungal cell wall and uncharacterized antifungal factors (Kloepper, [12,13,15,20]). Recent work on the broad spectrum of PGPR-mediated induced systemic resistance against different pathogens in different crops has gained importance. For sustainable crop production, the components involved in IDM should be eco-friendly and cost-effective so that beneficial organisms would be safe and IDM practices would go a long way towards helping stabilise crop production. In this context, an attempt would be made to explore the multifarious antagonistic potentials of native Pseudomonad isolates from rhizosphere as biocontrol agents for the management of chickpea wilt.

2. MATERIALS AND METHODS

2.1 Isolation and Purification of Causal Organism of Chickpea Wilt

The pathogen was isolated from infected chickpea plant showing typical wilt symptoms and purified. The pathogen was sub-cultured at monthly intervals and maintained at 4 ºC in a refrigerator.

2.2 Isolation, Purification and Characterisation of PGPR

Rhizosphere isolates of pseudomonads were isolated from the soil around the roots of different

pulse and other crops grown in the campus of TCA., Dholi. For isolation of Pseudomonads, the roots with the tightly adhering soil particles were cut into small pieces, and one gram of these root pieces was vigorously shaken in 100ml of sterilised distilled water for 10-20 minutes to get the bacterial suspension. The bacterial suspensions thus obtained were used for the isolation of *Pseudomonas* rhizobacteria by dilution plate technique [21] in King's B medium (KMB) [22]; supplemented with benomyl, 20 mg/ml to avoid the growth of fungi. The organisms were allowed to grow at 28 ± 2 ºC. Dilutions of 10^{-4} and 10^{-6} were used, and the typical different colonies of *Pseudomonas* spp. were further purified. The different isolates of pseudomonad were maintained on KMB slants at 4 ºC in a refrigerator and further characterised for Plant growth-promoting attributes (e.g. Indole Acetic Acid (IAA), Phosphorus-solubilization, Ammonia production, HCN production etc.).

2.3 Plant Growth-promoting Attributes

2.3.1 Ammonia production

Detection of ammonia production was done by adding 1 ml Nessler's reagent to a 72-h-old culture grown in peptone broth (peptone broth: 10.0 g peptone, 5.0 g NaCl, 1,000 ml distilled water, 7.0 pH) and recording the presence of the yellowish brown colour [23]. An uninoculated control was kept for comparison of results. The experiment was conducted with three replications for each isolate.

2.3.2 Hydrogen cyanide (HCN) production

HCN production was tested according to the method described by Bakker and Schipper [24] on King's B medium ((Protease peptone 20 g + $K_2HPO_4.3H_2O$ 1.908 g + $MgSO_4.7H_2O$ 1.5 g + glycerol 15 ml + agar 15g + distilled water 985 ml) amended with 4.4 g l^{-1} glycine. Discolouration of the filter paper from yellow to orange/ light brown after incubation was considered as microbial production of cyanide. An uninoculated control was kept for comparison of results. The experiment was conducted with three replications for each isolate.

2.3.3 Estimation of Indole Acetic Acid (IAA)

IAA produced by bacteria was assayed colourimetrically using ferric chloride-perchloric acid reagent ($FeCl_3$-$HClO_4$) [25]. This method estimated the quantities of indole compounds produced by bacteria in LB (Luria Bertani medium) amended with 50 µg/ml tryptophan. The LB medium contained (in 1000 ml distilled water), 5.0 g Tryptone, 3.0 g Yeast extract and, 5.0 g NaCl. The pH of LB medium was adjusted to 7.0 before autoclaving. The amount of IAA was estimated using a standard curve. The experiment was conducted with three replications for each isolate.

2.3.4 Phosphorus-solubilization

Solubilization of tri-calcium phosphate was quantified in Pikovskaya's [26] broth. The medium consisted of 10.0 g glucose, 5.0 g tri-calcium phosphate, 0.5 g $(NH_4)_2SO_4$, 0.2 g KCl, 0.1 g $MgSO_4.7\ H_2O$, a trace of $MnSO_4$ and $FeSO_4$ and 0.5 g Yeast extract, in 1,000 ml distilled water. Quantitative phosphate solubilization was estimated by Fiske and Subbarow method [27]. The amount of soluble phosphorus was estimated using a standard curve. The experiment was conducted with three replications for each isolate.

2.4 Identification of Potential Antagonists (PGPR) against the *Fusarium oxysporum* f.sp. *ciceri* for Management of Chickpea Wilt

The *Fusarium oxysporum* f.sp. *ciceri* was used in the present study. Initial *in vitro* screening of Pseudomonads spp. against the *Fusarium oxysporum* f.sp. *ciceri* was performed in KMB medium. The actively growing mycelial disc (8 mm diameter) of the respective isolate of *Fusarium oxysporum* f.sp. *ciceri* was placed at the centre of the Petri plate, and the respective bacterial isolate was streaked 4 cm away from the pathogen in a rectangular fashion. Plates without bacterial streaks served as control and the experiment was replicated four times. The plates were incubated at room temperature (28 ± 2 ºC) and the percent inhibition of mycelial growth was calculated using this formula; $I = 100 (C - T) / C$

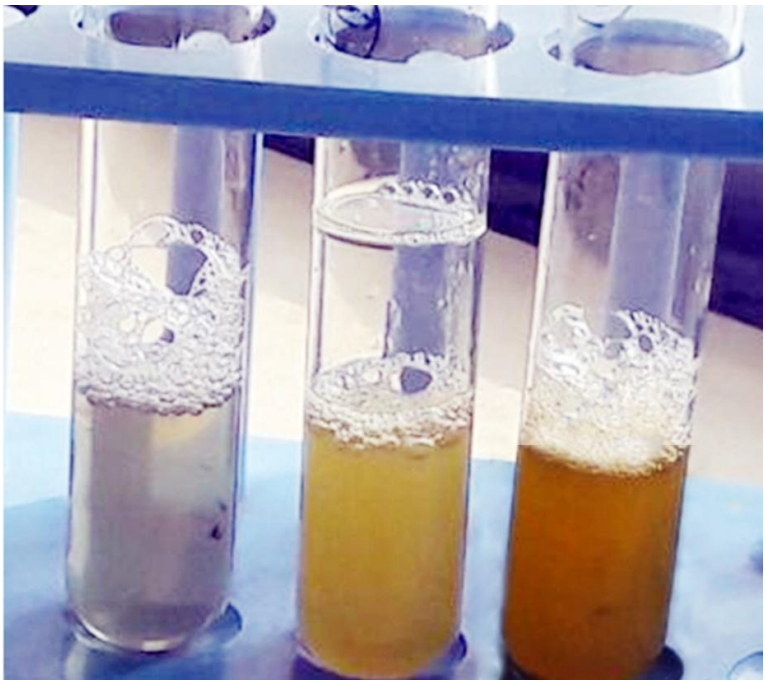
Where I = Percent inhibition of mycelial growth, C = growth of pathogen in control plate and T = growth of pathogen in dual cultures.

3. RESULTS

Pseudomonads were isolated from rhizosphere of different pulse crops and soil, were tested for a wide array of traits associated with biocontrol as well as growth promoting attributes, like HCN

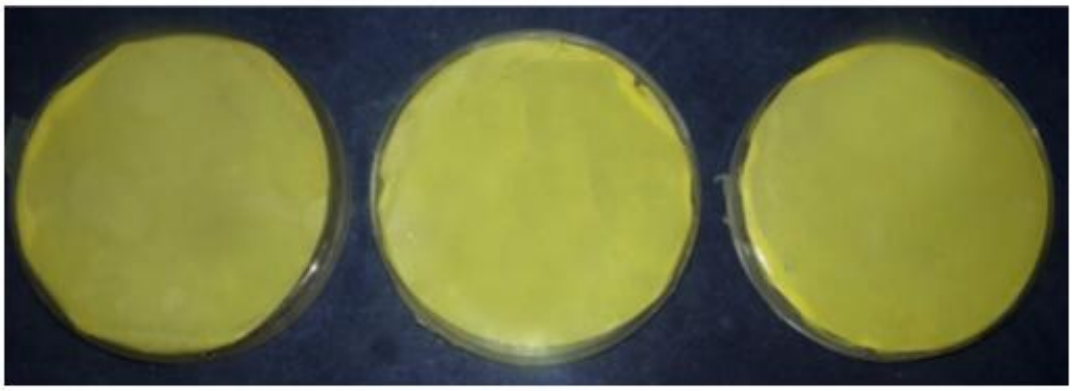
production, ammonification, IAA production and solubilization of tri-calcium phosphate *in vitro*, besides studying their antagonistic activity against the soilborne fungal pathogen *Fusarium oxysporum f.sp. ciceri*. Results indicated that all nine isolates exhibited the ammonification property, produced HCN (Cyanide) and IAA (Indole acetic acid) and positive for phosphorus solubilization, except PGPR-Pl, this found to be negative for HCN production (Table 1, Figs. 1, 2, 3 and 4).

Our experimental result revealed that all nine pseudomonad isolates exhibited the ammonification property. However, PGPR-WS, PGPR-Ra, PGPR-Bn and PGPR-lit were found to produce the maximum amount of ammonia, 2 isolates (PGPR-Ch, & PGPR-Mb) produced medium amount of ammonia whereas, PGPR-Chs, PGPR-Pl and PGPR-Mg showed the lowest response for ammonification activity (Table 1, Fig. 1).

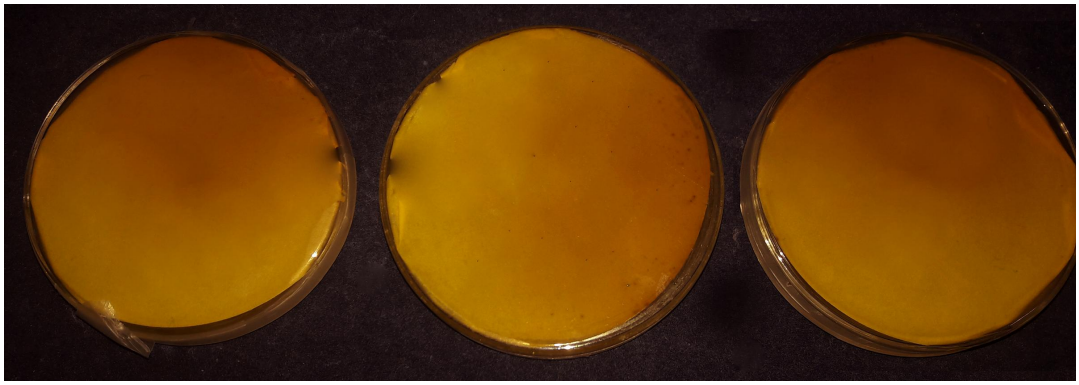


(a) **(b)** **(c)**

Fig. 1. Ammonia production. a) No ammonia production, b) low amount of ammonia production c) high amount of ammonia production

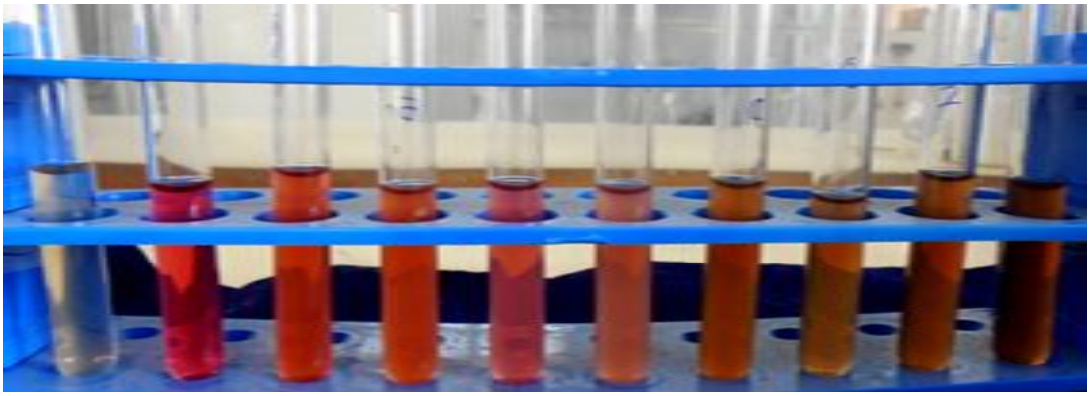


(a)

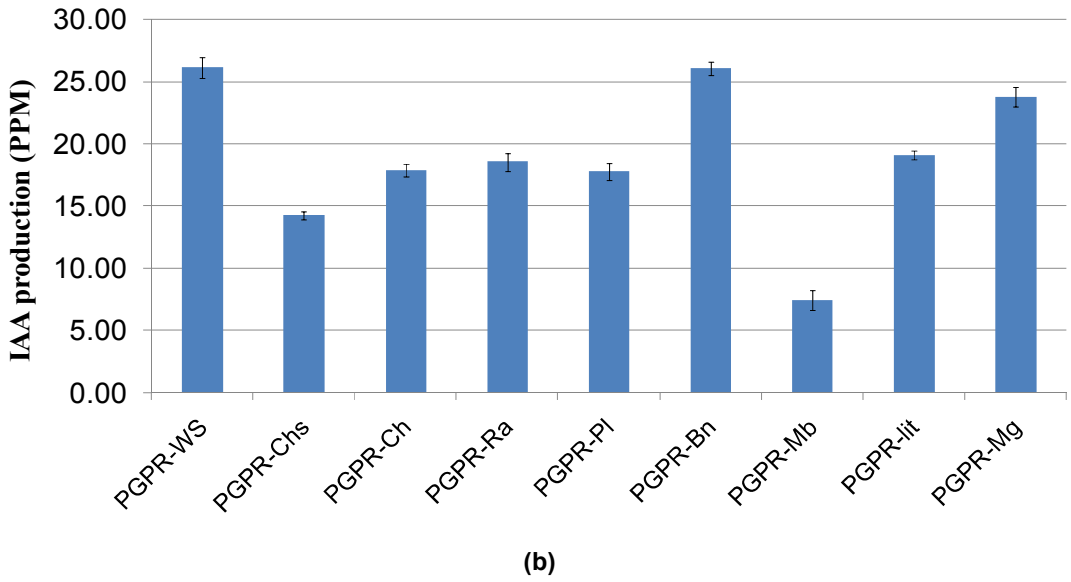


(b)

Fig. 2. HCN production. (a) no HCN production and (b) high amount of HCN production



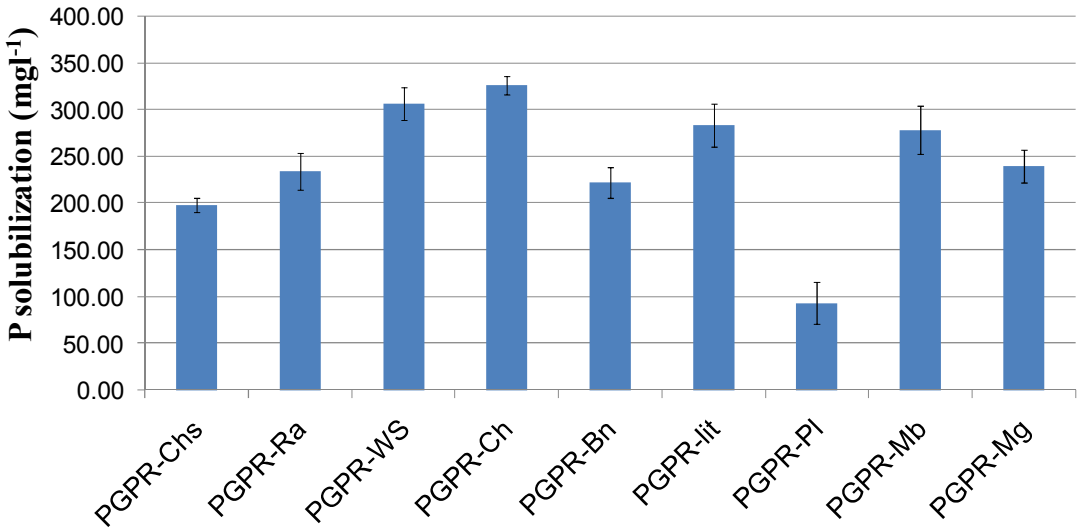
(a)



(b)

Fig. 3. IAA (Indole Acetic Acid) production assay. (a) Intensity of colour indicates the amount of IAA production. (b) Graph indicates quantification of IAA production by different isolates

(a)



(b)

Fig. 4. Phosphate solubilization assay. (a) Intensity of blue colour indicates the amount of phosphate solubilization by different isolates. (b) Graph indicates the quantification of phosphate solubilization by different isolates

Out of nine isolates, eight isolates viz., PGPR-WS, PGPR-Chs, PGPR-Ch, PGPR-Ra, PGPR-Bn, PGPR-Mb, PGPR-lit and PGPR-Mg were found to produce volatile cyanide, except PGPR-Pl, this found to be negative for HCN production. Among the nine isolates, only one isolate PGPR-Mb was strongly cyanogenic, three isolates viz., PGPR-WS, PGPR-Chs, PGPR-Ch, were moderately cyanogenic, whereas, four isolates namely PGPR-Ra, PGPR-Bn, PGPR-lit and PGPR-Mg were weakly cyanogenic. (Table 1, Fig. 2 a-b).

All the nine isolates were able to produce IAA in the range 7.43 to 26.08 mgl^{-1}. Among the nine isolates for IAA producers, PGPR-WS and PGPR-Bn were the best in producing IAA (26.08 and 26.03 mgl^{-1}, respectively). Whereas, all the pseudomonads used in this study were found to solubilized tri-calcium phosphate in the range of 93.04 to 326.02 mgl^{-1}. However, among the nine isolates positive for phosphorus solubilization, only two isolates, PGPR-Ch and PGPR-WS solubilised highest amount of tri-calcium phosphate (326.02 and 306.51 mgl^{-1}, respectively) (Table 1, Figs. 3 and 4 a-b).

Among the nine isolates, pseudomonad isolates PGPR-WS were best in exhibiting multiple PGPR traits like ammonification, HCN production, IAA production (26.08 mgl^{-1}), and phosphate solubilization (306.51 mgl^{-1}). The data clearly suggested the wide spectrum of biological activities performed by these strains under *in vitro* conditions.

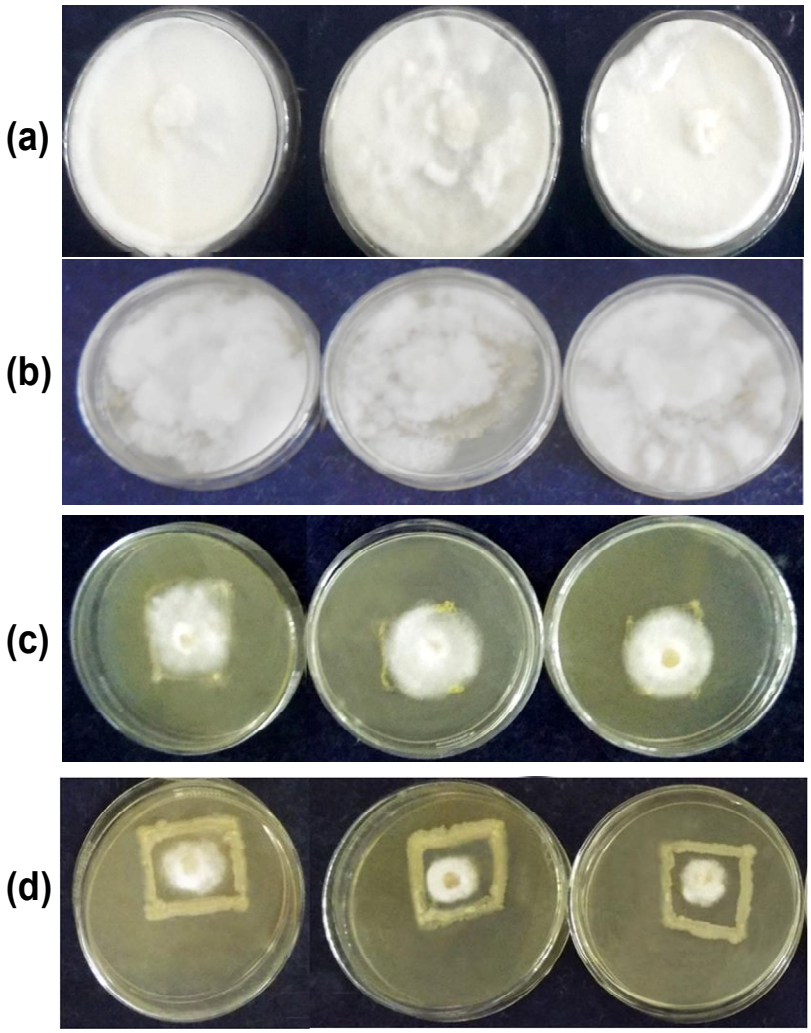


Fig. 5. Interaction of Foc with pseudomonad isolates. a) Control, b) Foc+ PGPR-Pl, c) Foc+ PGPR-Bn and d) Foc+ PGPR-ws

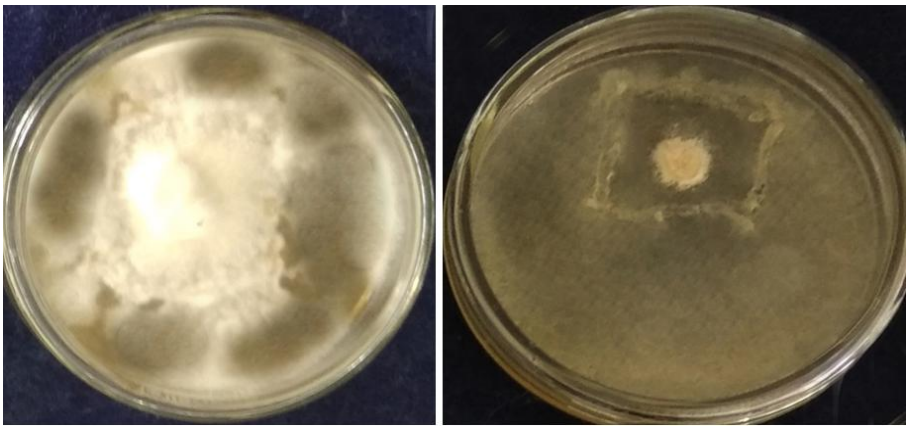


Fig. 6. Lysis of mycelia of pathogen against pseudomonad isolate. a) Incomplete Lysis and b) Complete Lysis

Table 1. Growth-promoting traits of pseudomonad isolates

Sl.No.	Pseudomonad isolates	Habitat (Host rhizosphere)	(NH_4 production)a	(HCN production)b	IAA production (mgl^{-1})) ‡	P solubilization (mgl^{-1}) ‡
1	PGPR-WS	Chick pea wilt sick soil	+++	++	26.08 ± 0.81 [a]	306.51± 17.60 [a]
2	PGPR-Chs	Chickpea field soil	+	++	14.22 ± 0.33 [b]	197.49± 7.55[b]
3	PGPR-Ch	Chickpea	++	++	17.85 ± 0.50 [c]	326.02± 9.97 [c]
4	PGPR-Ra	Rajma	+++	+	18.54 ± 0.72 [cd]	234.02± 19.81[abd]
5	PGPR-Pl	Pipal tree	+	-	17.75 ± 0.71 [c]	93.04± 22.07 [e]
6	PGPR-Bn	Banyan tree	+++	+	26.03 ±0.56 [a]	221.92± 16.71 [bf]
7	PGPR-Mb	Mungbean	++	+++	7.43 ± 0.76 [e]	278.82± 25.46 [adf]
8	PGPR-lit	Litchi orchard soil	+++	+	19.08 ± 0.34 [d]	283.11± 23.18 [adf]
9	PGPR-Mg	Mango orchard soil	+	+	23.75 ±0.76 [f]	239.61± 17.64 [adf]

a± indicates degree of reaction for ammonia production in peptone water broth; - indicates no ammonia production, + indicates low amount of ammonia production, ++ indicates medium amount of ammonia production, +++ indicates high amount of ammonia production., b± indicates degree of HCN production; - indicates no HCN production, + indicates low amount of HCN production, ++ indicates medium amount of HCN production., +++ indicates high amount of HCN production; ‡ = Mean of three replication, IAA= indole acetic acid; P solubilization = phosphorus solubilization

Table 2. Inhibition pattern of *Fusarium oxysporum* f.sp*. ciceri* by pseudomonad isolates by dual culture technique

Pseudomonad isolates	Interaction with pathogen	Mycelia of pathogen	Inhibition zone (mm) ‡	Percent inhibition of mycelial growth over control	Lysis pattern
PGPR-WS	**P_i**	NM	**22.5**	**75.00**	**TL**
PGPR-Chs	I_0	TM	**-**	**-**	IL
PGPR-Ch	I_0	NM	**-**	**-**	IL
PGPR-Ra	C_g	DM	**-**	**-**	IL
PGPR-Pl	I_0	DM	-	-	IL
PGPR-Bn	C_g	NM	**-**	**-**	NL
PGPR-Mb	I_0	NM	**-**	**-**	IL
PGPR-lit	C_g	NM	**-**	**-**	IL
PGPR-Mg	I_0	DM	**-**	**-**	IL

P_i = Pathogen inhibited by pseudomonad isolate; C_g = Cessation of growth of pathogen at line of contact; I_0 = Pseudomonad isolate overgrow by pathogen; NM = Normal mycelia; TM = Thin mycelia; DM = Deform mycelia; TL = Total lysis; IL = Incomplete lysis; NL = No lysis ; ‡ = Mean of three replication

3.1 Identification of Potential Antagonists (PGPR) against the *Fusarium oxysporum* f.sp. *ciceri* for Management of Chickpea Wilt

All the nine pseudomonad isolates were evaluated for their potential as a biocontrol agent against *Fusarium oxysporum* f.sp. *ciceri*. They were screened for their antagonistic efficiency by dual culture technique [21] (Table 2). *F. oxysporum* f.sp. *ciceri* showed differences in mycelial growth pattern during interaction with different pseudomonad isolates (Fig. 5). In some cases, *F. oxysporum* f.sp. *ciceri* produced deform or thin mycelia instead of producing normal mycelia during interaction with different pseudomonad isolates.

It produced deform mycelia during interaction with pseudomonad isolates PGPR-Ra, PGPR-PI, and PGPR-Mg, whereas, it produced thin mycelia during interaction with pseudomonad isolates PGPR-Chs. Our results showed that pseudomonad isolates varied in their ability to inhibit *F. oxysporum* f.sp. *ciceri in vitro*. It showed differences in inhibition and lysis pattern during interaction with different pseudomonad isolates (Table 2 and Fig. 5). Five pseudomonad isolates were found to overgrow by *F. oxysporum* f.sp. *ciceri*, e.g. PGPR-Chs, PGPR-Ch, PGPR-PI, PGPR-Mb, and PGPR-Mg (Fig. 5 b) followed by incomplete lysis of mycelia in advanced stages of antagonism (Fig. 6) except PGPR-Bn, this showed no lysis of mycelia with the increase incubation period. Whereas, three pseudomonad isolates restricted the growth of *F. oxysporum* f.sp. *ciceri* at the point of interface, e.g. PGPR-Ra, PGPR-Bn and PGPR-lit (Fig. 5 c) followed by incomplete lysis of mycelia in advanced stages of antagonism. However, only one of the pseudomonad isolate, PGPR-WS showed inhibition at a distance with a clear inhibition zone from the beginning of antagonism against *F. oxysporum* f.sp. *ciceri* (Fig. 5 d) having 75.00% inhibition of growth of mycelia over control and caused total lysis of mycelia (Table 2 and Fig. 6) in advanced stages of antagonism.

Our result of the present investigation clearly indicates that pseudomonad isolates varied in their antagonistic activity against *F. oxysporum* f.sp. *ciceri*. They showed differences in inhibition pattern, inhibition zone, and lysis pattern. Among the nine pseudomonad isolates, PGPR-WS was found to be the best in antagonistic activity against *F. oxysporum* f.sp. *ciceri,* showed 75.00% inhibition of growth of mycelia over control and caused total lysis of mycelia in advanced stages of antagonism.

4. DISCUSSION

All the pseudomonad bio-agents were effective against test pathogen, as they showed high mycoparasitism. It may be due to the lack of availability of nutrients for growth of the pathogen and production of certain inhibitory toxic metabolites by antagonists in the culture medium. Production of volatile ammonia was also implicated as a possible mechanism to control soil-borne pathogens (Baligh et al. [28,29] and [30,31]). In our experiment, all pseudomonad isolates produced ammonia. Among these PGPR-WS, PGPR-Ra, PGPR-Bn and PGPR-lit were found to produce the maximum amount of ammonia. PGPR-WS showed strong antibiosis against *F. oxysporum* f.sp. *ciceri*, while PGPR-Ra, PGPR-Bn and PGPR-lit did not show strong antibiosis. Therefore, antagonistic activity of pseudomonad isolates against *F. oxysporum* f.sp. *ciceri* could not be directly linked with their ammonification trait, although ammonification seems to be one of the traits associated with antagonistic activity of pseudomonad against *F. oxysporum* f.sp. *ciceri*.

The production of volatile cyanide is very common among the rhizosphere pseudomonad ([24]; Dowling and O'Gara, 1994). HCN production has been postulated to play an important role in biocontrol of pathogens [32]. Other workers have also reported HCN production by antagonistic bacteria [25] and are known to inhibit the electron transport, disturbing the energy supply to the cells, ultimately leading to the death of the pathogen [33]. Production of HCN by pseudomonad is associated with biological control of the black root of tobacco [34]. Flaishman et al. [35] also reported that overproduction of cyanide might control fungal diseases in wheat seedlings. However, some other workers observed that it could have a detrimental effect on plant growth [36]. In this investigation, eight HCN producing strains viz., PGPR-WS, PGPR-Chs, PGPR-Ch, PGPR-Ra, PGPR-Bn, PGPR-Mb, PGPR-lit and PGPR-Mg were found to be deleterious and caused differential lysis and deformation of mycelia. Thus our results also indicated that antagonistic activity of pseudomonad isolates against *F. oxysporum* f.sp. *ciceri* is not solely associated with the HCN production trait. PGPR-WS was moderately cyanogenic but showed strong antagonistic against *F. oxysporum* f.sp. *ciceri.* In

contrast, PGPR-Mb was strongly cyanogenic but caused partial lysis. Therefore, HCN production has not a strong link for biocontrol of *F. oxysporum f.sp. ciceri.*

Thus the antagonistic activity of pseudomonad isolates against *F. oxysporum* f.sp. *ciceri* may not be described solely by the production of ammonia and HCN production. However, we do not rule out, the involvement of these traits in the antagonistic activity of pseudomonad isolates against *F. oxysporum* f.sp. *ciceri.* It can thus be assumed that besides these traits, other chemical compounds produced by pseudomonad, which were not investigated here, affected *F. oxysporum* f.sp. *ciceri* and thus could be involved in the impairment of *F. oxysporum* f.sp. *ciceri g*rowth. It might be attributed by a combination of one or more biological control mechanism like the production of antibiotic or antifungal metabolites such as pyoluteorin, pyrroluitrin, phenazines and 2, 4-diacetyl phloroglucinol or production of siderophores or lytic enzymes like chitinase, β-1, 3 glucanase etc. The coordinated expression of one or more of the plant growth-promoting trait might have contributed to the suppression of *F. oxysporum* f.sp. *ciceri.*

Indole acetic acid is one of the most physiologically active auxins. IAA is a common product of L-tryptophan metabolism by several microorganisms including PGPR [37]. All the nine isolates were able to produce IAA in the range 7.43 to 26.08 mgl^{-1}. Out of nine, two isolates PGPR-WS and PGPR-Bn were the best in producing IAA (26.08 and 26.03 mgl^{-1}, respectively). This observation revealed that IAA production varies in different isolates of pseudomonad. Tien et al. [38] established that rhizobacteria that produce IAA could responsible for increasing the number of lateral roots and root hairs in pearl millet. But there are some reports that rhizobacteria that overproduce IAA inhibit root elongation, and this was attributed to the stimulation of ethylene synthesis by IAA [39,40].

Rhizospheric bacteria are known to play a very significant role in plant growth promotion by different mechanisms, one of them being the ability to solubilise phosphorus in soil and making it available for plant uptake [41,42]. The mineral phosphate solubilising property is associated primarily with the production of low molecular weight organic acids which forms complexes with the metal ions such as Fe, Al, and Ca of the phosphate ore. The metabolic and genetic basis for the high-efficiency solubilization of P by the majority of the gram-negative PSB studied so far, has been attributed to the production of gluconic acid by direct oxidation of glucose via, membrane-bound quinoprotein glucose dehydrogenases (Gcd) enzyme. This enzyme is known to act not only on glucose but also on several other aldo sugars such as xylose, arabinose, maltose, etc. and oxidises them to their corresponding acids. All the pseudomonads used in this study were found to solubilise complex form of P to the plant available form in the range 93.04 to 326.02 mgl^{-1}. However, among the nine isolates positive for phosphorus solubilization, only two isolates, PGPR-Ch and PGPR-WS solubilised highest amount of tri-calcium phosphate (326.02 and 306.51 mgl^{-1}, respectively). These results indicate that the solubilization of phosphorus varies in different pseudomonad isolates. Some P solubilising organisms have been reported as plant growth promoters [43,44]. The ability to convert insoluble P to an accessible form like orthophosphate is an important trait for a PGPR for increasing plant yields [45]. The present study indicated that many isolates of pseudomonad found to be phosphorus solubiliser and thereby expected to enhance uptake by the plants, resulting in increased plant biomass. Apart from phosphorus solubilization, phytohormone production like IAA is another mechanism that directly influences plant growth [15].

The initial analysis of the pseudomonad isolates for their antagonistic activity against *F. oxysporum* f.sp. *ciceri in vitro*. It was observed that some isolates inhibited the growth of *F. oxysporum* f.sp. *ciceri*. This suggested that some pseudomonad isolates can produce inhibitory metabolites against *F. oxysporum* f.sp. *ciceri* that checked the growth of *F. oxysporum* f.sp. *ciceri* isolates. The inhibitory property of the isolates reflects the inherent potential of the pseudomonads to produce inhibitory metabolites against *F. oxysporum f.sp. ciceri*. Several workers reported that many bacteria produce antibiotics or antifungal proteins for their survival. Some of these antifungal factors are effective inhibitors of phytopathogens and play important roles in the biological control of plant diseases [46,36,19].

Our results clearly indicate that different pseudomonad isolates showed differences in inhibition pattern against *F. oxysporum f.sp. ciceri* isolate, and it might be attributed due to

variable antifungal activity possessed by different pseudomonad spp. It is known that the extent of inhibition zone formation is related to the ability of the organism to produce inhibitory metabolites against the test organism [47].

Our findings indicated that the period of incubation played a highly significant role with inhibition at the beginning followed by maximum differential lysis of *F. oxysporum f.sp. ciceri* in the advanced stage of antagonism. As a result, the natural fluffy growth of the fungal pathogen was suppressed and lead to total lysis or partial lysis of mycelia.

Among the nine pseudomonad isolates, PGPR-WS was found to be the best in antagonistic activity against *F. oxysporum* f.sp. *ciceri,* showed 75.00% inhibition of growth of mycelia over control and caused total lysis of mycelia in advanced stages of antagonism. Results from the present investigation reveal interesting observation that in addition to inhibiting the pathogen *in vitro*, the PGPR-WS has also other growth promoting attributes like IAA production, phosphorus solubilization and ammonification, which may contribute to the enhancement of growth, yield and nutrient uptake of the plant. Thus screening and identification of novel bioagent PGPR-WS with multifarious activities vividly reflect its potential to suppress *F. oxysporum* f.sp. *ciceri* and suggest the usefulness of this super bioinoculant as a component of IDM of *F. oxysporum* f.sp. *ciceri.*

5. CONCLUSION

Thus screening and identification of novel bioagent PGPR-WS with multifarious activities vividly reflect its potential to suppress *F. oxysporum* f.sp. *ciceri* and suggest the usefulness of this super bioinoculant as a component of integrated disease management (IDM) of chickpea wilt caused by *F. oxysporum* f.sp. *ciceri.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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