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Biocontrol potential of *Pseudomonas fluorescens* against bacterial wilt of Brinjal and its possible plant growth promoting effects

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ABSTRACT

The present study was undertaken to explore the possibility of using an indigenous strain of Pseudomonas fluorescens to manage one of the most devastating disease of the economically important brinjal crop, the bacterial wilt. The bacterial wilt caused by Ralstonia solanacearum has severly limited brinjal production in all parts of the world. In an attempt to evolve a biological management of the disease, the antagonistic strain of P. fluorescens was applied as suspension in pot experiment by different methods viz. seed, root, soil, and their integration methods seed+root, root+soil, seed+soil and seed+ root+soil. The control treatments were inoculated control (only pathogen inoculated) and uninoculated control (neither pathogen nor antagonist inoculated). The percent wilt incidence (PWI) was found to be lowest (33.33%) in root+soil and seed+root+soil treatment of the antagonist. The population dynamics of the pathogen and antagonist in brinjal rhizosphere soil showed that the crop receiving seed+root+soil treatment had the lowest population recovery of the pathogen 26×10^6 cfu/g (7.33) and correspondingly highest population recovery of the antagonist 179.67×10^6 cfu/g (8.25). The correlation studies established a negative correlation between PWI and population density of P. fluorescens as well as between population densities of R. solanacearum and P. fluorescens. The yield, yield attributes and physiological and biochemical parameters were also found to be best performing in the seed+root+soil treatment of the antagonist suspension indicating its potential as PGPR.

Keywords: Biocontrol, Bacterial wilt, *Ralstonia solanacearum*, *Pseudomonas fluorescens*, PGPR

INTRODUCTION

The Brinjal, Aubergine or Eggplant (*Solanum melongena* L.), of the family Solanaceae, is grown in the subtropical and tropical regions of the world. It is one of the most common, highly productive and popular vegetable crops grown in India. It is quite popular as the poor man's crop [1]. The unripe fruit of eggplant is primarily used as a cooking vegetable for the various dishes in India and China. The brinjal is also reported to possess medicinal properties. Various plant parts are used for curing ailments such as diabetes, cholera, bronchitis, dysuria, dysentery, otitis, toothache, skin infections, asthenia and haemorrhoids. It is also ascribed narcotic, anti-asthmatic and anti-rheumatic properties [2]. The major constraint, however, in the production of brinjal is the bacterial wilt disease. The bacterial wilt disease caused by *Ralstonia solanacearum* [3], [4] is primarily a soil borne disease of wide distribution in the tropics, subtropics and warm temperate regions of the world [5],[6], [7]. *Ralstonia solanacearum* is a soil borne, rod shaped, gram negative, β proteobacterium that causes bacterial wilt disease in more than 200 plant species including many economically important crops. Due to its wide geographic distribution and unusually broad host range (over 50 plant families) the pathogen is responsible for severe crops losses worldwide [6]. The disease is difficult to control. Although various control measures have been documented, bacterial wilt is still a major threat to brinjal production, because of wide host range of the pathogen and better survival of the pathogen in soil, especially

in deeper layers [8]. Crop rotation with non-host plants, although recommended, is not an efficient method, since *Ralstonia solanacearum* has its disseminating and survival stages in the soil and it remains viable for long periods of time. The race and strain diversity of the pathogen has made breeding for resistant cultivars ineffective in the control of bacterial wilt [9], [10]. Antibiotics such as streptomycin, ampicillin, tetracycline and penicillin showed hardly any effect [11]. The use of soil fumigants is environmentally destructive, expensive and largely ineffective against bacterial wilt [12]. Chemical and soil treatments such as modification of soil pH, heat treatment by solarization, and application of stable bleaching powder, as well as plant resistance inducers (eg. Acibenzolar –S-methyl), plant essential oils (eg. Thymol), or phosphorous acid have been shown to reduce bacterial populations and disease severity on a small scale [13], [14], [15], [12]. Drawbacks of these methods include environmental damage, cost and high labour inputs [16]. Thus there remains a need for pathogen control methods of bacterial wilt which are more compatible with the need for affordable and effective disease control, a high degree of food safety and minimal environmental impact. Chemical pesticides have been tested and evaluated for their ill effects such as reproductive toxicity and carcinogenesis in mammals. High doses of these agents have been proved to be fatal to animals. Therefore, biological control agents are gaining importance in the field. Another importance of these agents is their role as plant growth promoting microorganism [17]. Biological control strategies may either help development of alternative management measures or be integrated with other practices for effective disease management at the field level [18]. Many fungal and bacterial agents have been examined over a period of time for their potential as biocontrol agents [17]. Several strains of *Pseudomonas fluorescens* have been reported to suppress soil borne diseases caused by pathogens [19], [20]. Strains of *P. fluorescens* Migula have been described as 'root colonizing bacteria' to indicate its potential to colonize the rhizosphere and rhizoplane. Since they are well adapted in soil, *P. fluorescens* strains are being investigated extensively for use in applications that require the release and survival of bacteria in the soil. Chief among these applications are biocontrol of pathogens in agriculture [21]. It is known to enhance plant growth promotion and yield and reduce severity of many diseases [22], [23]. *P. fluorescens* thus, belong to Plant Growth Promoting Rhizobacteria (PGPR), the group of bacteria that play a major role in plant growth promotion, induced systemic resistance, biological control of pathogens etc. With regard to the plant growth promoting potential of *P. fluorescens*, evaluating the yield and yield attributes and the associated physiological and biochemical parameters of the treated test brinjal crops is essential.

In the above context, the present study was undertaken to isolate and characterize the bacterial wilt pathogen from the economically important brinjal crop and evaluate the potential of an isolated indigenous strain of *Pseudomonas fluorescens* applied by different methods to control the disease. The yield, yield attributes, physiological and biochemical parameters of the treated crops were also tested to evaluate the possible plant growth promoting effects of the isolated *P. fluorescens* strain.

MATERIALS AND METHODS

The experiments undertaken in the present investigation were carried out in the laboratory and fields of the Department of Biotechnology, Gauhati University. Details of the materials used and methodologies followed in conducting these experiments are:

Source of the pathogen Brinjal plants showing typical symptoms of bacterial wilt were collected from the brinjal cultivated fields of Singimari in Kamrup district' Assam and brought immediately to the laboratory. For subsequent experiments, the infected seedlings were collected from the experimental plots of Deptt. Of Biotechnology, Gauhati University.

Isolation of the pathogen The pathogen *R. solanacearum* was detected in the infected plants by ooze test. The stem pieces showing milky white ooze in water were selected for isolation of the pathogen. The pathogen was isolated on triphenyl tetrazolium chloride (TTC) Agar medium [7]. The virulent colonies in the medium characterized by dull white colour, fluidal, irregularly round with light pink centres were further streaked on TTC medium to get pure colonies of the bacterium.

Preservation of the pathogen reatment Two loopfuls of bacterium from 48 hr old colonies grown on Kelman's TTC Agar were transferred to 5 mL of sterile double distilled water [24] in screw capped vials. These were stored under refrigeration at 20^{o}C for maintenance of virulence [25]. To revive an isolate, the stored bacterium was streaked on TTC Agar medium and well separated fluidal colonies were selected.

Preparation of the pathogen reatment A bacterial suspension prepared by pouring sterile distilled water over 24 hr old bacterial growths on Nutrient agar slants was adjusted to optical density (O.D) 0.5 in Spectrophotometer (Spectronic 20) in blue filter (425nm) to obtain a bacterial population of 1 x 10^8 cfu/mL.

Pathogenicity test Seeds of brinjal (*Solanum melongena* L. cv. Pusa Kranti) were obtained from National Seeds Corporation, Pusa. These were sown on earthen pots (26cm x 22cm x 32cm) simulating nursery beds. The pots were filled with sand and potting medium in the ratio 1:3 respectively. The potting medium was composed of humus, clay and peat in the proportion 15:35:50 respectively. For the pathogenicity test, a set of three 30 days old brinjal plants were inoculated with pathogen reatment @ 10^8 cfu/mL by root inoculation technique [26]. Another set of three seedlings were inoculated with sterile distilled water to serve as control. The plants were observed for symptoms. Pathogenicity test was confirmed after Koch's postulation.

Biovar determination of the pathogen Biovar determination of the isolate was done by testing the ability of the bacterium to oxidize sugar and sugar alcohols by standard procedure [27].

Morphological, physiological, cultural and biochemical characterization of the pathogen
The pathogen *R. solanacearum* was characterized by following the guidelines described in the Bergey's Manual of Systematic Bacteriology [28]. Pure culture of the pathogenic bacterial culture was further characterized in Institute of Microbial Technology, Chandigarh, India. For morphological characterization the Gram's staining, Acid fast staining, Flagella staining, Bacterial spore (endospore) staining and Capsule staining were done. The cultural characteristics were observed by culturing the pathogen in Nutrient Agar Media, TTC Agar media [7], and King's medium B [29]. The Physiological tests performed for the bacterial pathogen are observation of growth at different temperature, pH, anaerobic conditions and on NaCl (%). The biochemical tests were also conducted.

Isolation of *Pseudomonas fluorescens*, the potential biocontrol agent for the management of bacterial wilt
Pseudomonas fluorescens, the potential biocontrol agent for the management of bacterial wilt, was isolated from the rhizosphere rhizoplane of healthy brinjal plant. The healthy brinjal plants were uprooted from the brinjal cultivated fields of Singimari in Kamrup district. The roots were severed from the plant and cut into small pieces of about 1 cm length. 5g of root pieces with the soil particles tightly adhered was weighed out and dipped in 500mL of sterile dist. Water. The suspension was shaken in a rotary shaker for 20 mins to release the rhizoplane bacteria. 0.1 mL of the suspension was then inoculated in King's Medium B (KMB) Agar plates [29], [30]. The plates were incubated at 28±1^{o}C for 48 hours and then observed under U.V. transilluminator at 366 nm for colonies with green fluorescence.

Preservation of antagonist culture Colonies showing green fluorescence under UV- transilluminator (366nm) were enriched in nutrient broth and preserved in KMB slants. The slants were covered with mineral oil and preserved in the refrigerator at 4^{0}C for further use.

Characterization of the isolated potential biocontrol agent' *P. fluorescens*
Morphological, cultural, physiological and biochemical characterization of the isolated potential biocontrol agent was carried out in the laboratory by following the guidelines described in the Bergey's Manual of Systematic Bacteriology [28] and Experiments in Microbiology, Plant pathology and Biotechnology [31]. For further confirmation, the bacterial culture was characterized in Institute of Microbial Technology, Chandigarh.

Evaluation of *Pseudomonas fluorescens* as potential antagonist against the pathogen *Ralstonia salanacerum in vitro*
In vitro test for evaluation of *P. fluorescens* as potential biocontrol agent against the pathogen *R. solanacearum* was conducted by following the paper disc plate [32].

Efficacy of the antagonist used as suspension / broth culture against wilt pathogen *R. solanacearum*
To evaluate the efficacy of *P. fluorescens* in controlling bacterial wilt of brinjal, an experiment was carried out in earthen pots experiment in the Dept. farm by following Completely Randomized Block Design (CRBD) where each treatment was replicated thrice with two plants per replication. All the recommended agronomic practices were followed during the crop period. The earthen pots taken were of size 26cm x 22cm x 32cm and the potting medium consisted of sand and potting substrate in the ratio 1:3. The potting medium used was sterilized in an autoclave at 121^{o}C for 30 min. and filled in the pots. The potting substrate was composed of humus, clay and peat in the

proportion 15:35:50 respectively. The cultivar of brinjal (*Solanum melongena* L.) taken for the experiment was Pusa Kranti. The seeds of *S. melongena* cv. Pusa Kranti were obtained from National Seeds Corporation, Pusa, India.

Preparation of the pathogen and antagonist cell suspension

48 hrs nutrient broth culture of *R. solanacearum* and King's B Broth culture of *P. fluorescens* were taken. Both the cultures were respectively harvested in 10 mL. sterile distilled water. The cell suspensions of the pathogen and antagonist were serially diluted from 10^{-1} to 10^{-5} in 9 mL distilled water taken in test tubes. The cell concentration in the final dilution was adjusted to 10^8 cfu/mL in Spectronic-20 Spectrophotometer (O.D O.5 at 425nm blue filter).

Application of *P. fluorescens* cell suspension by different methods to control bacterial wilt of brinjal caused by *R. solanacearum*

The *P. fluorescens* cell suspension was applied by different methods *viz.* seed treatment, root treatment, soil treatment and their combinations *viz*. seed + root treatment, root + soil treatment, seed + soil treatment and seed + root + soil treatment to evaluate the efficacy of the antagonist in contolling bacterial wilt of brinjal.

For seed treatment, *P. fluorescens* cell suspension @10^8 cfu/mL was applied to surface sterilized seeds. The seeds were surface sterilized by immersing in 1% sodium hypochlorite solution for 2 – 3 mins. And then rinsed with sterile distilled water and dried overnight in a sterile blotting paper. The treated seeds were sown in nursery beds and the seedling transplanted onto pots.

For soil treatment, 25mL of *P. fluorescens* cell suspension @10^8 cfu/mL was mixed with 500g sterilized potting medium (1:20 V/W) [31] for each pot one day before transplanting of seedlings. Seedlings raised from seeds treated with *P. fluorescens* cell suspension were transplanted in treated soil to achieve seed + soil treatment.

For root treatment, brinjal seedlings raised from treated as well as untreated seeds were uprooted and the soil particles adhering to it were removed. The roots were then dipped in *P. fluorescens* cell suspension (@ 10^8 cfu/mL) for 30min. and dried for 1 hr. These were then transplanted onto pots filled with the potting medium.

For root + soil treatment, the seedlings treated at the roots were transplanted onto pots filled with potting medium amended with the *P. fluorescens* cell suspension @ 10^8 cfu/mL. For root + seed treatment, the seedling raised from treated seeds were also treated with *P. fluorescens* cell suspension @ 10^8 cfu/mL at the roots and then transplanted onto sterilized potting medium. For seed + root + soil treatment, the seedlings raised from treated seeds were uprooted and after gently removing the soil particles, the roots were dipped in *P. fluorescens*, cell suspension @ 10^8 cfu/ml for 30 min. The treated roots were allowed to dry for 1 hr and then transplanted onto pots containing potting medium which was sterilized and amended with *P. fluorescens* cell suspension (@ 10^8 cfu/mL) one day before transplanting.

Each of the above treatments was replicated thrice with 2 plants / replication. 15DAT, the brinjal plants receiving treatment by different methods were challenged with *R. solanacearum* cell suspension @ 10^8 cfu/mL by following root inoculation technique [26] except uninoculated control. For control treatment a set of 3 seedlings untreated at the seed, root or soil were challenged with the pathogen cell suspensions at 15 DAT (inoculated control) whereas another set of 3 seedlings were left uninoculated by the pathogen (uninoculated control).

Disease record: The wilt incidence was recorded upto 90 DAT.

The % wilt incidence (PWI) was calculated as follows:

$$\frac{\text{No. of plants wilted in a treatment}}{\text{Total no : of plants receiving that treatment}} x100$$

Quantitative determination of pathogen and antagonist population in the rhizosphere soil

30, 60 and 90 days after transplanting of brinjal seedlings onto the pots, the pathogen and antagonist population in the rhizosphere soil were enumerated by serial dilution technique prepared into TTC agar and KMB agar plates. Three replicates were maintained for each of the dilutions. The cfu from each plate were counted out and population of *P. fluorescens* and *R. solanacearum* / g rhizosphere soil was calculated as follows:

$$\text{No. of org/g rhizosphere soil} = \frac{\text{Av. No. of colonies in a dilution x Dilution factor}}{\text{Dry wt. of soil}}$$

The **correlation studies** between percentage wilt incidence (PWI) and population of *P. fluorescens* and also between *P. fluorescens* and the pathogen *R. solanacearum* were also carried out.

Physiological and biochemical analysis of treated crops to evaluate the efficacy of the biocontrol agent as plant growth promoting rhizobacteria (PGPR)

To evaluate the efficacy of the biocontrol agent *P. fluorescens* as a PGPR, the biochemical parameters of brinjal plants were tested out 90 days after reatment. The biochemical parameters were tested in the crops which received the five best treatment methods and comparatively lower wilt incidence. The biochemical parameters tested were total carbohydrate by Anthrone method, protein estimation by Lowry's method and chlorophyll estimation by Arnon method.

Analysis of yield and yield attributes of treated crops to evaluate the efficacy of the biocontrol agent as PGPR

Data were recorded on Average fruit weight (g)/plant, No. of branches/plant, No. of fruits/plant, Yield/plant, Plant height and Mean leaf area of the treated brinjal plants to evaluate the efficacy of *P. fluorescens* as PGPR.

RESULTS AND DISCUSSION

The ooze test conducted showed milky white ooze consisting of bacterial cells and their extra cellular polysaccharides. Upon inoculation in TTC Agar plates, dull white fluidal irregular round colonies with light pink centres were observed. This showed positive test for detection of *R. solanacearum* in wilt infected plants. The pathogenicity test conducted established that the isolated bacterium from wilted brinjal plant was *R. solanacearum*, the causative agent of bacterial wilt disease. The pathogen was detected to belong to biovar 3 [27]. The colonies of the isolated potential biocontrol agent showed distinct bluish green fluorescens under U.V. light. The observation indicates positive result for *P. fluorescens* which produces a soluble greenish fluorescent pigment called fluorescein (pyoverdin), particularly under conditions of low iron availability. The isolated strain of *P. fluorescens* was tested in vitro for its inhibitory activity against *R. solanacearum* by paper disc method. Inhibition zone of 10.2 mm was found to be produced (Value is mean of 3 replication plates).

The result of the effect of *P. fluorescens* cell suspension applied by different methods on PWI of brinjal in pot experiment is recorded in **Table I.** The angular transformed values of PWI are used in analysis of the results. The results revealed that the percent wilt incidence of brinjal decreased significantly in different treatments as compared to corresponding significant increase in inoculated control. Among the antagonist treatments, application of *P. fluorescens* as seed + root + soil treatment and as root + soil treatment showed lower wilt incidence (33.35), while uninoculated control treatment showed the lowest wilt incidence (0.05). The integration methods of application, seed + root treatment and seed + soil treatment recorded lower wilt incidence (50) than single methods of application but their effectiveness was lower as compared to other integration methods of root + soil and seed + root + soil. Among the single methods of application of *P. fluorescens* cell suspension, root treatment and soil treatment showed greater efficacy (33.35% control) than seed treatment which showed only 16.70% control. The single methods of application of the antagonist suspension when compared to the integration methods of application, showed lower capacity to control bacterial wilt incidence (16.7% - 33.35% control) while the integration methods could record 50% - 66.65% control of bacterial wilt. The results are found in agreement with Kalita [34] who reported that the combined methods of application of the biocontrol agents *P. fluorescens* and *T. viride* could manage bacterial wilt of tomato to a greater degree than the single methods of application of seed, root or soil. Gohain [35] also reported that integration of seed treatment, root dip and soil application of *P. fluorescens* could demonstrate higher reduction of wilt incidence in brinjal. The delivering of rhizobacteria through combined application of different delivery systems increased the population load of rhizobacteria and thereby might suppress the pathogenic propagules [36]. The findings are also in conformity with the report of Bora and Deka [37] who found that application of *Pseudomonas*

fluorescens based biopesticide (Biofor-Pf) as combination of seed treatment, root application and soil application at transplanting showed minimum wilt incidence. For the effective management of any soil borne disease, the introduced antagonist should colonize root [38]. When applied to seeds, the effective antagonists colonize the rhizosphere at the time of seed germination, in other words, the antagonists moved from spermosphere to rhizosphere and establish there [39], [40]. Root zone application of *P. fluorescens* increased rhizosphere population of the bacteria [30]. Certain strains of fluorescent pseudomonads have been shown to provide biological control of pathogens when applied to soil [40] since some of these strains have the ability to colonize the roots [41], [20]. The result also revealed that root+ soil treatment with *P. fluorescens* exhibited the lowest wilt incidence in brinjal (33.33%) as compared to seed+ root and seed+soil treatment of the antagonist with 50% wilt incidence. This is in conformity with Peixto *et al*. [42].

Table I. Effect of *P. fluorescens* cell suspension applied in different methods on % wilt incidence (PWI) of brinjal in pot experiment

Treatments	% wilt incidence
T_1 : Seed treatment with *P. fluorescens* + *R. solanacearum*	83.33 (83.30)
T_2 : Root treatment with *P. fluorescens* + *R. solanacearum*	66.67 (66.65)
T_3 : Soil treatment with *P. fluorescens* + *R. solanacearum*	66.67 (66.65)
T_4 : Seed + Root treatment with *P. fluorescens* + *R. solanacearum*	50.00 (50.00)
T_5 : Seed + Soil treatment with *P. fluorescens* + *R. solanacearum*	50.00 (50.00)
T_6 : Root + Soil treatment with *P. fluorescens* + *R. solanacearum*	33.33 (33.35)
T_7 : Seed+ Root + Soil treatment with *P. fluorescens* + *R. solanacearum*	33.33 (33.35)
T_8 : Inoculated control (only *R. solanacearum*)	100.00 (99.95)
T_9 : Uninoculated control (No *R. solanacearum*)	0.00 (0.05)
S.Ed.±	17.55
$CD_{0.05}$	36.87

data within parentheses indicate angular transformed values

The population densities of *R. solanacearum* and *P. fluorescens* in the rhizosphere soil of brinjal were calculated at 30, 60 and 90 days after transplanting (DAT) and the results are presented in **Tables II** and **III** respectively. The population density values (cfu/g rhizosphere soil) were transformed into log values for analysis of the results. The data in **Table II** and **III** revealed that the effect of treatment (T) on *R. solanacearum* and *P. fluorescens* population was significant at 5% level of significance. However, the effect of DAT (D) and interaction effect (T×D) was non significant on *R. solanacearum* and significant on population density of biocontrol agent**.**

Among the antagonist treatments, the highest population of the pathogen recorded in brinjal rhizosphere was in seed treatment where the population density was 94.22×10^6 cfu/g (7.95) rhizosphere soil, which incidentally recorded the lowest bacterial wilt control (16.70%) and lowest population recovery of the antagonist at 68×10^6 cfu/g (7.81). The probable reason for the high population of the pathogen in treatments wherein low recovery of antagonist population was made might be due to antagonistic mechanisms like antibiosis and competition for nutrients or space in the rhizosphere or rhizoplane of brinjal, which was more favourable for the antagonist. These might have helped the antagonists to exploit their activities intensely and become more aggressive to suppress the pathogen. Similar mechanisms of antibiosis [43] as well as the combination of antibiosis and siderophore mediated nutrient competition by *P. fluorescens* [44] against *R. solani* have been reported. Among the treatments, seed + root + soil treatment recorded lowest population density of *R. solanacearum* with 26×10^6 cfu/g (7.33) and the highest population recovery of *P. fluorescens* at 179.67×10^6 cfu/g rhizosphere soil (8.25). This might be due to the better rhizosphere colonization by *P. fluorescens* and thus deprivation of the space required for invasion of roots by *R. solanacearum*. Moreover, the favourable soil environment resulting from the soil edaphic factors could have led to success of the antagonist in soil as a biocontrol agent against the pathogen *R. solanacearum*. Wuthrich and Defago [45] reported that the suppressive ability of *P. fluorescens* strain CHAO to decrease take-all of wheat and black root of tobacco was dependent on soil quality and host pathogen systems. Thus the soil quality and microenvironment of the brinjal rhizosphere might have played crucial role in establishment of the antagonist in the rhizosphere in higher numbers outcompeting the pathogen for nutrients and space.

Table II. Population dynamics of *R. solanacearum* in brinjal rhizosphere soil at different DAT

Treatments	Population of *R. solanacearum* (× 10^6 cfu/g rhizosphere soil)			
	Days after transplanting(DAT)			Mean
	30	60	90	
T_1 : Seed treatment with *P. fluorescens* + *R. solanacearum*	136.33	82.67	63.67	94.22
	(8.13)	(7.92)	(7.80)	(7.95)
T_2 : Root treatment with *P. fluorescens* + *R. solanacearum*	98.67	73.33	42.00	71.33
	(7.99)	(7.86)	(7.62)	(7.83)
T_3 : Soil treatment with *P. fluorescens* + *R. solanacearum*	106.67	78.00	49.67	78.11
	(8.03)	(7.89)	(7.70)	(7.87)
T_4 : Seed + Root treatment with *P. fluorescens* + *R. solanacearum*	70.67	61.33	26.33	52.78
	(7.85)	(7.79)	(7.42)	(7.69)
T_5 : Seed + Soil treatment with *P. fluorescens* + *R. solanacearum*	76.33	65.33	30.00	57.22
	(7.88)	(7.81)	(7.48)	(7.72)
T_6 : Root + Soil treatment with *P. fluorescens* + *R. solanacearum*	59.67	34.00	22.67	38.78
	(7.78)	(7.53)	(7.35)	(7.55)
T_7 : Seed+ Root + Soil treatment with *P. fluorescens* + *R. solanacearum*	46.67	21.33	10.00	26.00
	(7.67)	(7.33)	(6.99)	(7.33)
T_8 : Inoculated control (only *R. solanacearum*)	280.00	260.00	243.00	261.00
	(8.45)	(8.41)	(8.39)	(8.42)
T_9 : Uninoculated control (No *R. solanacearum*)	0.12	0.08	0.05	0.08
	(3.47)	(4.90)	(4.64)	(4.34)
Mean	97.24	75.12	54.15	
	(7.47)	(7.49)	(7.27)	
	S.Ed.±	$CD_{0.05}$		
Effect of treatments (T)	0.275	0.552		
Effect of DAT (D)	0.159	NS		
Interaction effect (T×D)	0.477	NS		

Figures within parentheses are logarithmic transformed values NS : Non-significant

Among the single methods of application, root treatment proved effective and could retain a mean population of 97.56×10^6 cfu/g soil (7.97) of the antagonist probably due to rapid rhizosphere colonization. Such mechanisms have been demonstrated by Bull [46] in wheat.

The population of *R. solanacearum* successively decreased from 30 DAT to 60 DAT and again decreased at 90 DAT in all the antagonist treatments (**Table II**). The population of *P. fluorescens* in the brinjal rhizosphere soil of pots receiving different treatments increased significantly from 30 DAT upto 60 DAT and then again decreased at 90 DAT (**Table III**). Similar trend of population fluctuation of antagonists in rhizosphere soil was earlier recorded by Gohain [35], who observed that the population of *P. fluorescens* and *G. virens* counted at 30 DAT also increased and reached the peak at 60 DAT. This result can be attributed to increase in amount of root exudates along with subsequent changes in the composition of the exudates at 60 DAT (flowering stage), and increase in root surface area along with the growth of the plant. Haas and Defago [47] reported that cell population of Pseudomonads depend upon the age of roots. Depending on the age and location of the microcolonies, cell densities range from 10^3 to 10^7 cfu/cm of root. The root collar – where the root joins the main stem is a site of intense exudation and is more strongly colonized by bacteria than is the root tip.

Correlation studies: To establish the relationship between the population densities of pathogen and antagonist as well as between PWI and population density of antagonist, correlation studies were carried out and the results are presented in **Table IV**. A significantly negative correlation (r= -0.485*) was found between the population densities of *R. solanacearum* and *P. fluorescens* indicating reduction of the pathogen with concomitant increase in the antagonist population. The regression curve between population of *P. fluorescens* and *R. solanacearum* is presented in **Fig. I**. A negative correlation (r= -0.150) exists between mean population of P. fluorescens and PWI. The regression curve is presented in **Fig. II**. Bora and Deka [37] observed similar phenomenon in tomato with negative correlation between *P. fluorescens* and PWI. The finding furthers supports the biocontrol properties of the antagonistic strain *P. fluorescens*. In addition to the environmental factors, the PWI is chiefly dependent on the population of *R. solanacearum* in the soil. Therefore, when the pathogen population is reduced corresponding to the higher level of the antagonist as observed in the present study, the PWI also decreased simultaneously. In correlation study, this is the most probable reason of significant negative correlation between the population densities of the pathogen and the antagonists as well as negative correlation between PWI and the population densities of the antagonists.

Table III. Population dynamics of *P. fluorescens* in brinjal rhizosphere soil at different DAT

Treatments	Population of *P. fluorescens* (× 10^6 cfu/g rhizosphere soil)			
	Days after transplanting			Mean
	30	60	90	
T_1 : Seed treatment with *P. fluorescens* + *R. solanacearum*	53.33	102.33	48.33	68.00
	(7.73)	(8.01)	(7.68)	(7.81)
T_2 : Root treatment with *P. fluorescens* + *R. solanacearum*	79.33	137.67	75.67	97.56
	(7.90)	(8.14)	(7.88)	(7.97)
T_3 : Soil treatment with *P. fluorescens* + *R. solanacearum*	77.67	110.67	70.33	86.22
	(7.89)	(8.04)	(7.85)	(7.93)
T_4 : Seed + Root treatment with *P. fluorescens* + *R. solanacearum*	92.33	156.33	89.33	112.67
	(7.97)	(8.19)	(7.95)	(8.04)
T_5 : Seed + Soil treatment with *P. fluorescens* + *R. solanacearum*	80.67	143.33	77.67	100.56
	(7.91)	(8.16)	(7.89)	(7.98)
T_6 : Root + Soil treatment with *P. fluorescens* + *R. solanacearum*	96.67	165.33	94.33	118.78
	(7.99)	(8.22)	(7.97)	(8.06)
T_7 : Seed+ Root + Soil treatment with *P. fluorescens* + *R. solanacearum*	161.33	218.33	159.33	179.67
	(8.21)	(8.34)	(8.20)	(8.25)
T_8 : Inoculated control (only *R. solanacearum*)	0.26	0.23	0.13	0.21
	(5.41)	(5.36)	(5.11)	(5.29)
T_9 : Uninoculated control (No *R. solanacearum*)	0.76	0.67	0.52	0.65
	(5.86)	(5.83)	(5.72)	(5.80)
Mean	71.37	114.99	68.41	
	(7.43)	(7.59)	(7.36)	
	S.Ed.±	$CD_{0.05}$		
Effect of treatments (T)	0.019	0.038		
Effect of DAT (D)	0.011	0.022		
Interaction effect (T×D)	0.032	0.065		

Figures within parentheses are logarithmic transformed values

Table IV: Correlation between population densities of *P. fluorescens* and *R. solanacearum* and between population density of *P. fluorescens* and PWI of brinjal

Treatments	Mean population (log cfu/g)		Mean population of *P. fluorescens* and percent wilt incidence (PWI)	
	P. fluorescens	***R. solanacearum***	***P. fluorescens* (log cfu/g)**	**PWI**
T_1 : Seed treatment with *P. fluorescens* + *R. solanacearum*	7.81	7.95	7.81	83.33
T_2 : Root treatment with *P. fluorescens* + *R. solanacearum*	7.97	7.83	7.97	66.67
T_3 : Soil treatment with *P. fluorescens* + *R. solanacearum*	7.93	7.87	7.93	66.67
T_4 : Seed + root treatment with *P. fluorescens* + *R. solanacearum*	8.04	7.69	8.04	50.00
T_5 : Seed + soil treatment with *P. fluorescens* + *R. solonacearum*	7.98	7.72	7.98	50.00
T_6 : Root + soil treatment with *P. fluorescens* + *R. solanacearum*	8.06	7.55	8.06	33.33
T_7 : Seed + root + soil treatment with *P. fluorescens* + *R. solanacearum*	8.25	7.33	8.25	33.33
T_8 : Inoculated control (only *R. solanacearum*)	5.29	8.42	5.29	100.00
Correlation coefficient(r)	- 0.485*		- 0.150^{NS}	

** Significance at 5% level of significance*
NS: Non significant
PWI: Percent wilt incidence

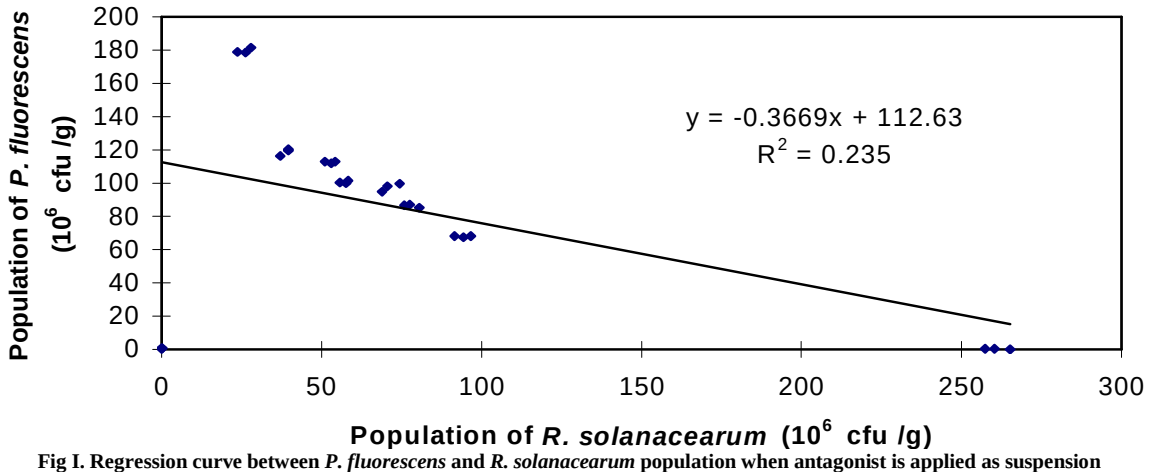

Fig I. Regression curve between *P. fluorescens* and *R. solanacearum* population when antagonist is applied as suspension

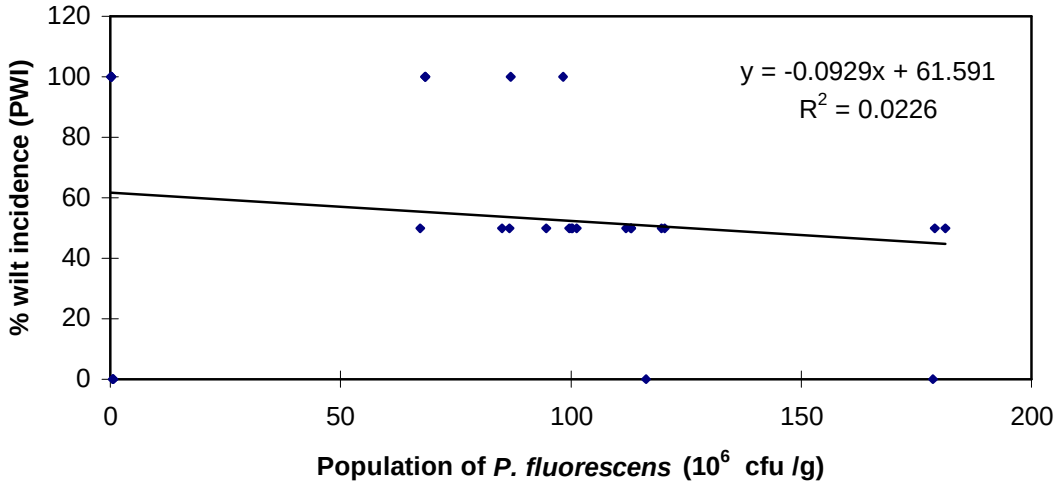

Fig II. Regression curve between *P. fluorescens* population in suspension and PWI

Performance of yield, yield attributes, physiological and biochemical characters of treated crops to evaluate the efficacy of *P. fluorescens* as PGPR

Table V reveals the variation of leaf area (cm^2), average fruit weight (g)/ plant, yield/ plant (kg), No. of fruits/ plant, No. of branches/ plant and plant height in the different crops treated with different methods and challenged with the pathogen. Among the treatments, seed+root+soil treated crops had the best yield and yield attributes. **Table VI** reveals the performance of Chlorophyll a content, chlorophyll b content, total chlorophyll content, carbohydrate content and protein content in the crops treated with different methods of application and challenged with *R. solanacearum.* Uninoculated control had significantly highest chl-a, chl-b, total chl, carbohydrate and protein content. It was followed by seed+ root+soil, root+soil, seed+ root, seed+soil and root treated crops in performance of the physiological and biochemical parameters. Thus the maximum perforfmance of the yield, yield attributes, physiological and biochemical characters observed in the brinjal crops treated with *P. fluorescens* in seed+root+soil method can be attributed to the maximum recovery of biocontrol agent in the rhizosphere and minimum wilt incidence in such crops. This is also supported by the highly significant negative correlation observed between the PWI and different yield parameters and physico-biochemical characters for each treatment method in the correlation studies carried out presently. Jinnah *et al*. [48] while studying the control of bacterial wilt of tomato by *Pseudomonas fluorescens* found that the biocontrol agent produced positive effect on the plant growth characters such as plant height, number of branches / plant and yield characters such as fruit yield, total fruit weight / plant and number of fruits/ plant. The maximum plant height, fruit yield, fruit weight / plant and highest number of fruits/ plant and branches/ plant was found in the treatment (10 times dilution of *P. fluorescens* stock suspension) which also showed the minimum wilt incidence.

Table V. Performance of yield and yield attributes of the treated crops

Treatments	Leaf area (cm^2)	Average fruit weight (g)/ plant	Yield/ plant (kg)	No: of fruits/plant	No: of branches/ plant	Plant height (cm)
T7 + *R. solanacearum*	182.00	158.33	2.066	13.00	16.00	73.00
T6 + *R. solanacearum*	170.00	142.00	1.657	11.67	15.00	73.00
T4 + *R.solanacearum*	163.00	128.00	1.277	10.00	13.00	65.00
T5 + *R. solanacearum*	159.00	125.00	1.125	9.00	12.00	63.00
T2 + *R. solanacearum*	150.00	121.33	0.931	7.67	10.00	60.00
Inoculated control (only *R. solanacearum*)	102.00	77.33	0.180	2.33	8.00	41.00
Uninoculated control (no *R. solanacearum*)	190.00	160.00	2.405	15.00	17.00	77.00
S.Ed.±	1.73	2.70	0.241	1.59	1.60	2.15
$CD_{0.05}$	3.76	5.89	0.526	3.46	3.48	4.68
$CD_{0.01}$	5.28	8.25	0.737	4.85	4.88	6.57

Table VI Performance of physiological and biochemical characters of treated crops

Treatments	Chlorophyll- a content(mg/g)	Chlorophyll-b content (mg/g)	Total chlorophyll (mg/g)	Carbohydrate content (mg/g)	Protein content (mg/g)
T7 + *R. solanacearum*	0.823	0.451	0.399	56.000	10.000
T6 + *R. solanacearum*	0.814	0.425	0.376	49.667	9.333
T4 + *R.solanacearum*	0.787	0.389	0.343	48.333	8.000
T5 + *R. solanacearum*	0.779	0.380	0.335	44.667	7.667
T2 + *R. solanacearum*	0.773	0.366	0.323	41.000	6.000
Inoculated control (only *R. solanacearum*)	0.736	0.342	0.302	36.667	4.333
Uninoculated control (no *R. solanacearum*)	0.832	0.460	0.406	56.667	10.333
S.Ed.±	0.001	0.001	0.001	1.355	0.667
$CD_{0.05}$	0.003	0.003	0.003	2.952	1.453
$CD_{0.01}$	0.004	0.004	0.004	4.139	2.036

Kumar *et al.* [49] inoculated seed with five plant growth promoting fluorescent *Pseudomonas* strains isolated from Indian and Swedish soils. They suggested that the potential use of these bacteria induce plant growth and disease suppression in sustainable agriculture production systems. In the present study leaf area and the physico-biochemical parameters carbohydrate, chlorophyll and protein content were found to increase with proportionate increase in crop yield. The finding is in agreement with Chitra and Rajamani [50], who found that the mean leaf area, starch content, protein and chlorophyll content were positively correlated with crop yield. The findings in the present study in respect of increase in yield and improvement in growth and physicochemical characters of bioformulation treated crops compared to inoculated control reinforces *P. fluorescens* as a plant growth promoting rhizobacteria.

CONCLUSION

The following conclusions were made from the present study:

a) The isolated indigenous strain of *Pseudomonas fluorescens* had potential to be used as a biocontrol agent for the management of bacterial wilt of brinjal as indicated by the reduced percent wilt incidence. The most suitable method of application of the antagonist suspension was found to be the seed+ root+ soil method and root+soil method.

b) The population densities of the pathogen and antagonist in the brinjal rhizosphere soil showed negative correlation and the percent wilt incidence and population density of the antagonist also showed negative correlation indicating further the biocontrol potential of *P. fluorescens*.

c) Besides biocontrol properties, the antagonist suspension applied by the seed+ root+ soil method also showed best performance in yield, yield attributes, physiological and biochemical parameters indicating its Plant growth promoting potential.

However, the effective biocontrol agent can be applied under field conditions or further commercialized only when immobilized in certain carriers. Thus formulations of the biocontrol agent should be prepared for easy application, storage, commercialization and field use.

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