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MANAGEMENT OF BACTERIAL WILT OF BRINJAL BY *P. fluorescens* BASED BIOFORMULATION

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ABSTRACT

An experiment was conducted in the laboratory and farm of the Department of Biotechnology, Gauhati University to explore the potentiality of an indigenous strain of *Pseudomonas fluorescens* as a bio-control agent in the management of bacterial wilt disease of brinjal under local conditions. Different organic materials were evaluated for their potential as substrate carriers in the formulation of *P. fluorescens* based biopesticide. Vermicompost and farmyard manure were found to support significantly longer shelf life of the biocontrol bacteria. Carboxy methyl cellulose and substrate carrier based formulations of *P. fluorescens* were applied by different methods in pot and field experiments to study their effectiveness in the control of bacterial wilt of brinjal. CVPf formulation and seed + root + soil method of application performed significantly better than others providing 83.33% control of bacterial wilt of brinjal in field experiment conducted by Completely Randomized Block Design. CVPf treated brinjal plants also recorded significantly higher yield and yield attributes than other formulation treated brinjal crops. Effective management of bacterial wilt of brinjal and improvement of yield and yield attributes by the *P. fluorescens* based organic formulation under local conditions signifies its potentiality and scope as a PGPR.

Keywords: brinjal, bacterial wilt, *P. fluorescens*, bioformulation, biological control.

INTRODUCTION

The Brinjal (*Solanum melongena* L.), of the family Solanaceae, is a common and popular warm weather vegetable crop grown extensively in India and is a major source of income for the small and marginal farmers as well. The unripe fruit of eggplant is primarily used as a cooking vegetable for the various local dishes. It is also reported to possess medicinal properties.

The major constraint in the production of brinjal is the bacterial wilt disease. Bacterial wilt caused by *Ralstonia solanacearum* Yabuuchi *et al*., (1996), is primarily a soil borne disease of wide distribution in the tropics, subtropics and warm temperate regions of the world (Buddenhagen 1962). *R. solanacearum* is a rod shaped, gram negative, β proteobacterium that causes bacterial wilt in more than 200 plant species including many economically important crops. It survives long term in water, soil and latently infected plants and can be transmitted by soil, water, equipment and infected plant materials (Hayward 1991). The pathogen invades through wounds in roots, lateral root emergence points or stomata and colonizes the intercellular spaces of the root cortex and vascular parenchyma, eventually entering the xylem vessels (Vasse, Frey and Trigalet 1995). In the xylem vessels the pathogen dissolves the cell walls and produce highly polymerized polysaccharides that increase the viscosity of the xylem and results in plugging. Blocking of vessels by bacteria is the major cause of wilting.

Bacterial wilt is a major threat to brinjal production. Crop rotation with non-host plants has not beeneffective, since *R. solanacearum* has its disseminating and survival stages in the soil. The race and strain diversity of the pathogen has made breeding for resistant cultivars ineffective (Wang *et al*., 1998). Chemical and soil treatments such as modification of soil pH, heat treatment by solarization, application of stable bleaching powder, as well as plant resistance inducers (eg. Acibenzolar -S-methyl), plant essential oils (eg. Thymol), or phosphorous acid (Norman *et al*., 2006) have been shown to reduce bacterial populations and disease severity on a small scale. Drawbacks of these methods include environmental damage, cost and high labour inputs (Champoiseau *et al*., 2009).

Thus there remains a need for control methods of bacterial wilt which are more affordable, effective and provide high degree of food safety and minimal environmental impact. In this context, 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 (Lwin and Ranamukhaarachchi 2006).

Several strains of *Pseudomonas fluorescens* have been reported to suppress soil borne diseases caused by pathogens (O'Sullivan and O'Gara 1992). *Pseudomonas fluorescens* enhances plant growth promotion and yield, induce systemic resistance and reduce severity of many diseases (Hoffland *et al*., 1996). For field application of *P. fluorescens* for the management of the bacterial wilt disease of brinjal, development of commercial formulations with suitable carriers that support survival of the bacteria for a considerable length of time is necessary (Nakkeeran *et al*., 2005).

The present study was undertaken for isolation of a local strain of *P. fluorescens* and evaluation of solid organic substrates for formulation development of the potential biocontrol agent *P. fluorescens* for studying the efficacy of *P. fluorescens* based bio formulations in suppressing bacterial wilt of brinjal in pot experiment and field conditions.

MATERIALS AND METHODS

Source of the pathogen

Brinjal plants showing typical symptoms of bacterial wilt were collected from the brinjal cultivated fields of Singimari in Kamrup district of Assam, India during the winter season (December 2006 and January 2007).

Isolation of the pathogen

Stems of infected brinjal plants were cut obliquely at the base and placed in sterile distilled water. 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 (Kelman 1954).

Preservation of the pathogen inoculums

The pathogen inoculum was stored in water (Kelman and Person 1961). These were stored under refrigeration at 20^{o}C for maintenance of virulence (Overbreek *et al*., 2004).

Preparation of the pathogen inoculums

A bacterial suspension of 24 hr Nutrient Agar (NA) slant growth was prepared in sterile distilled water. The suspension obtained was adjusted to optical density (O.D) 0.5 in Spectrophotometer (Spectronic 20) in blue filter (425nm) to obtain a bacterial population of 10^8 colony forming units (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. On germination, the seedlings were transplanted into pots filled with the same potting substrate and sand. For the pathogenicity test, set of three 30 days old brinjal seedlings were inoculated with pathogen inoculum @ 10^8 cfu/mL by root inoculation technique (Winstead and Kelman 1952). A set of three seedlings were inoculated with sterile distilled water to serve as control. The plants were observed for the symptoms. Pathogenicity test was confirmed after Koch's postulation. The pathogen was isolated from the inoculated plants and cultured on TTC media and cultural characteristics observed. It was reinoculated on healthy brinjal seedlings and observed for the same symptoms.

Characterization 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 (Hayward 1964).The pathogen *R. solanacearum* was characterized morphologically, physiologically, culturally and bio chemically by following the guidelines described in the Bergey's Manual of Systematic Bacteriology (Garrity G. 2001).

Isolation of *pseudomonas fluorescens*, the potential bio control agent for the management of bacterial wilt

The healthy brinjal plants were uprooted from the brinjal cultivated fields of Singimari in Kamrup district, Assam, India. The roots were severed from the plant and cut into small pieces of about 1 cm length. The root pieces with the soil particles tightly adhered was placed in sterile dist. water (1:100 w/v). The suspension was shaken in a rotary shaker for 20 minutes to release the rhizoplane bacteria. 0.1mL of the suspension was then taken and inoculated in King's Medium B (KMB) Agar plates (King *et al*., 1954; Vidhyasekaran and Muthamilan 1995). 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 picked up and enriched in nutrient broth. Thereafter these were streaked in KMB plates and preserved in KMB slants. The slants were covered with mineral oil and preserved in the refrigerator at 4^{o}C for further use.

Characterization of the isolated potential bio control agent, *P. fluorescens*

Morphological, cultural, physiological and biochemical characterization of the isolated potential bio control agent was carried out in the laboratory by following the guidelines described in the 8th edition of Bergey's Manual of Systematic Bacteriology (Garrity G. 2001).

Evaluation of *Pseudomonas fluorescens* as potential antagonist against the pathogen *Ralstonia salanacerumin vitro*

In vitro test for evaluation of *P. fluorescens* as potential bio control agent against the pathogen *R. solanacearum* was conducted by following the paper disc plate method of Blair *et al*., (1971).

Determination of Minimum Inhibitory Concentration (MIC) of the biocontrol agent

The MIC was determined by standard tube dilution procedure. 48 hr *P. fluorescens* nutrient broth culture was serially diluted up to 10^{-10} dilution. 0.1 mL of each dilution was plated on the surface of NA plates. Sterilized paper discs (1mm diameter) dipped in each dilution of the antibacterial agent were placed carefully on the surface of another set of pathogen inoculated NA plates labeled with the respective dilutions. The plates with paper discs of the anti bacterial were observed every 24 hrs for 5-7 days for any zone of inhibition. The other set of NA plates inoculated with each dilution of the antibacterial agent were incubated for 48 hrs at 28± 1^{o}C and the cfu counted out. The dilutions giving zones of

inhibition were recorded. The cfu / mL in each dilution of the antibacterial agent was calculated as follows:

$$\text{No. of cfu/mL} = \frac{\text{No. of cfu}}{\text{Volume plated x total dilution used}}$$

Collection of substrates for preparation of *pseudomonas fluorescens* based bioformulation

For mass production of the bacterial agents, solid state fermentation was done. Rice bran (Rb), wheat bran (W) and decomposed mustard oil cake (D) were collected from the local markets of Guwahati, Assam, India. Farmyard manure (F), Vermicompost (V), and rice straw (R) were collected from the local farmers of Greater Guwahati and banana leaf (B) was collected from the farm of Department of Biotechnology, Gauhati University.

Preparation of substrates

The substrates were air dried for 14 days, grinded properly and filled into polypropylene sleeves (12cm x 15cm) to which 10mL of sterile distilled water was added. Three packets of 100g each were prepared for each organic substrate.

Growth and multiplication of *pseudomonas fluorescens* in different organic substrates

The sterilized organic substrates viz. rice bran (Rb), wheat bran (W), vermicompost (V), decomposed mustard oil cake (D), rice straw (R), banana leaf (B) and farmyard manure (F) were taken. Carboxymethyl cellulose (1% aq) (C) was used as adhesive and Mannitol (3% aq) was used as an osmoticant. The 48 hrs KMB slant culture of the bio control agent was washed with sterile distilled water to obtain antagonist cell suspension @ 10^8 cfu/mL. The experiment was conducted in Completely Randomized Design. Adhesive was mixed with the substrate carrier contained in polypropylene bags (1:10 v/w). The pH was adjusted to 7 by adding suitable amount of $CaCO_3$, which varied with the type of substrate carrier adhesive mixture. The polypropylene bags were heat sealed and sterilized at 121^{o}C for 30 minutes. The mixture was then spread in a sterilized non sticky disposable plate under sterile conditions with the help of a sterilized spoon. Mannitol was added as osmoticant (@ 8.5 mL of 3% mannitol for 100g formulation).Subsequently *P. fluorescens* (Pf)cell suspension @ 10^8 cfu/mL was pipetted into the mixture (1:10 v/w) and thoroughly mixed with the help of sterilized spoon. The substrate carrier-adhesive-bio agent mixture was thinly spread on the non sticky plates, covered with another sterilized plate and incubated and allowed to dry for 3 days at room temperature (Kloepper and Schroth 1981).Each substrate carrier- adhesive- *P. fluorescens* based formulation was divided into 3 parts and packed separately in polypropylene bags (8cm x 6.5cm) and heat sealed.

Population dynamics of *P. fluorescens* in the different substrate carrier based formulations

The population dynamics of the bio agent *P. fluorescens* was determined at 7, 15, 30, 60, 90 and 120 days after storage (DAS) of the substrate carrier-adhesive-*P. fluorescens* formulations at room temperature by following the serial dilution plate technique of Waksman (1922). On the basis of highest recovery of cfu/g of *P. fluorescens*, the five best formulations were selected and evaluated for management of bacterial wilt of brinjal in pot and field experiments.

Evaluation of five best *P. fluorescens* based bio formulation against bacterial wilt of brinjal in pot and field experiments

The five bio formulations were applied by different methods in pot and field experiments to evaluate their effectiveness to control bacterial wilt of brinjal. The pot experiment was conducted during August 2007 - January 2008 and repeated during February 2008 - July 2008 and the field experiment was done in the Department farm during October 2008 - March 2009 and repeated during August 2009 - January 2010.

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 seeds of *S. melongena* cv Pusa Kranti were obtained from National Seeds Corporation, Pusa and used in the experiments. The Completely Randomized Block Design was used for carrying out the experiments. For each formulation, three plants were treated with a single method of application i.e., formulation treatment combination was replicated thrice. The field area for the field experiment was 96 sqm; total no. of blocks for each bio formulation was 3; area of each block was 0.6 m x 8m; No. of plots in each block was 9; Space between two plants within a block was 0.25 m x 3 m.

The methods of application of the bio formulations were seed, root and soil treatments and their combinations seed + root , root + soil, seed + soil and seed + root + soil treatments.

For seed treatment (S), formulation containing *P. fluorescens* @ 10^8 cfu/g was mixed with rice gruel to form fine slurry (30g formulation mixed with 30 mL rice gruel to treat 1 kg seed) and applied for 30 minutes to coat the seeds. The coated seeds were spread in a Petri plate and dried overnight. Care was taken to avoid clumping of the seeds. Another set of seeds were treated with the slurry prepared by mixing rice gruel with sterile distilled water to serve as untreated seeds. The treated as well as untreated seeds were sown on earthen pots simulating nursery beds.

For root dip treatment (R), 30 days old brinjal seedlings raised from bio formulation untreated seeds were uprooted. The soil particles loosely adhered to roots were gently removed. The roots of these seedlings were dipped into the respective formulation rice gruel slurry for 30 minutes and dried for 1 hr in shade before transplanting.

For soil treatment (So), each formulation was mixed with sand (1:20 w/w) (Sivakumar and Narayanaswamy 1998) and formulation sand mixture was incorporated into each pot or plot one day before transplanting of seedlings.

Following the above methods the combination treatments seed + soil + root, seed + soil, seed + root and root+ soil were carried out.

The above procedures were followed for each of the five *P. fluorescens* based formulations. Two control treatments were kept where no bio formulations were applied.

At 15 days after transplanting (DAT), all brinjal plants were challenged with cell suspension of *R. solanacearum* (@ 10^8 cfu/mL) by following root inoculation technique (Winstead and Kelman 1952) except in uninoculated control treatment.

Quantitative estimation of antagonist and pathogen population in rhizosphere soil

At 30, 60 and 90 DAT, the population dynamics of *P. fluorescens* as well as of the pathogen *R. solanacearum* in the rhizosphere soil of treated and control brinjal plants were estimated by the serial dilution plate technique of Waksman (1922). Since populations of bacteria approximate a log normal distribution (Loper *et al*., 1985), values were log transformed before analysis to normalize variance.

Disease record

The no. of wilted plants in each treatment was continuously recorded up to 90 days after inoculation with pathogen. The no. of completely wilted plants was tabulated for each formulation applied by different methods. The % wilt incidence was calculated out as follows:

$$\% \text{ wilt incidence} = \frac{\text{No. of plants wilted in each treatment-formulation} \times 100}{\text{Total no. of plants receiving that treatment}}$$

Analysis of yield and yield attributes of bio formulation treated crops to evaluate the efficacy of the bio control agent as PGPR

Data were recorded on Plant height (cm), No. of branches/plant, Average fruit weight (g)/plant, Yield/plant (kg), No. of fruits/plant and Mean leaf area (cm^2) of the bio formulation treated brinjal plants at 90 DAT to evaluate the efficacy of *P. fluorescens* as PGPR.

RESULTS

The stem of wilted plant showed milky white ooze consisting of bacterial cells and their extra cellular polysaccharides in sterile distilled water. Upon inoculation in TTC Agar plates, dull white fluidal irregular round colonies with light pink centers were observed. The pathogenicity test established the isolated bacterium from wilted brinjal plant as *R. solanacearum*, the causative agent of bacterial wilt disease.

The pathogen was found to be Gram -ve rod 2-3 µm, non acid fast, non spore former, non capsulated and motile with lophotrichous flagell; microaerophilic; positive to oxidase, catalase, nitrate reduction, KOH solubility and citrate utilization tests; negative to tween 80 hydrolysis, gelatin liquefaction, starch hydrolysis, indole production, methyl red and Voges Proskauer tests; produces acid from glucose and sucrose aerobically.The pathogenic strain was detected as Biovar 3.

The strain of the potential bio control agent *P. fluorescens* isolated from the rhizosphere- rhizoplane of healthy brinjal plant showed distinct bluish green *fluorescens* under U.V. light (366 nm) in KMB media. In morphological and biochemical characters it showed similar reactions as the pathogenic bacterium.

In dual culture assay of the strains of the pathogen and *P. fluorescens*, inhibition zone of 10.2 mm/diameter was found to be produced (Value is mean of 3 replications). The maximum dilution of the antibacterial agent (*P. fluorescens*) which produced inhibition zone was 10^{-5}. The MIC was calculated as 10^8 cfu/mL.

The results of the population dynamics of *P. fluorescens* in powder formulations at different days after storage (DAS) at room temperature is presented in Table-1. The substrate carriers differed significantly in supporting the population of *P. fluorescens* in the powder formulations during the storage period. The significantly highest and lowest population of *P. fluorescens* was recorded in CVPf and CBPf, respectively. The initial population count at 7 DAS increased at 15 DAS and reached its peak at 30 DAS in all the formulations. Thereafter during the period60-90 DAS, the population of *P. fluorescens* showed a steady decline and at 120 DAS, the populations of *P. fluorescens* reduced more than 100 to 1000 fold.

Table-1. Population dynamics of *P. fluorescens* in powder formulations at different days after storage (DAS) in room temperature.

Adhesive-substr. carrier *P. fluorescens* Bioformulation	**Population of *P. fluorescens*** ($\times 10^8$cfu/g)					**Population of *P. fluorescens*** ($\times 10^6$cfu/g)
	7 DAS	**15 DAS**	**30 DAS**	**60 DAS**	**90 DAS**	**120 DAS**
CVPf	900.67 (10.95)	930.00 (10.97)	1206.00 (11.08)	309.33 (10.49)	60.33 (9.78)	45.00 (7.65)
CFPf	850.67 (10.93)	890.00 (10.95)	990.33 (11.00)	302.67 (10.48)	51.67 (9.71)	42.00 (7.62)
CDPf	830.33 (10.92)	845.33 (10.93)	953.00 (10.98)	234.00 (10.37)	45.33 (9.66)	38.33 (7.58)
CRbPf	660.33 (10.82)	667.67 (10.82)	700.33 (10.85)	226.33 (10.35)	40.67 (9.61)	30.33 (7.48)
CWPf	430.33 (10.63)	442.67 (10.65)	456.67 (10.66)	205.67 (10.31)	30.33 (9.48)	27.33 (7.44)
CRPf	252.67 (10.40)	270.33 (10.43)	300.33 (10.48)	190.67 (10.28)	19.33 (9.28)	25.00 (7.40)
CBPf	250.33 (10.40)	269.33 (10.43)	298.67 (10.48)	142.33 (10.15)	18.00 (9.25)	22.67 (7.36)
Mean	596.48 (10.72)	616.48 (10.74)	700.76 (10.79)	230.14 (10.35)	37.95 (9.54)	34.33 (7.52)
Effect of Substrate-carrier	S.Ed.± 0.005 $C.D_{0.05}$ 0.011	S.Ed.± 0.005 $C.D_{0.05}$ 0.010	S.Ed.± 0.003 $C.D_{0.05}$ 0.006	S.Ed.± 0.022 $C.D_{0.05}$ 0.045	S.Ed.± 0.710 $C.D_{0.05}$ 1.433	S.Ed.± 0.037 $C.D_{0.05}$ 0.075

Figures within parentheses indicate log transformed values.

Table-2 shows the effect of the formulations (F), methods of application (M) and their combined or interaction effect (F x M) on the percentage wilt incidence (PWI) of brinjal (*Solanum melongena* L.) in field experiment. As a whole, the formulation CVPf applied as seed + soil + root method recorded the lowest PWI of 16.67% (15.85) statistically and numerically at par with the un inoculated control with no pathogen (R. *solanacearum*) challenge. All the formulation when applied as integration methods showed lower PWI than when applied as single methods.

The trend of population dynamics of P. *fluorescens* in the rhizosphere soil of the bio formulation treated brinjal plants at three different time intervals after transplanting is presented in Table-3. The population densities of P. *fluorescens* at 30 DAT increased significantly up to 60 DAT and decreased again at 90 DAT in all the formulations applied by different methods of application. The study of the population dynamics of the pathogen R. *solanacearum* in the rhizosphere of brinjal treated with various bio formulations during the same time intervals showed that the population of the pathogen successively decreased as DAT increased. The formulation CVPf applied by seed + root + soil method showed significantly lowest population recovery of the pathogen at 30,60 and 90 DAT compared to other formulation treatments and thus proved as the most effective bio formulation and method of application in the management of bacterial wilt disease. On the other hand, the formulation CWPf applied as seed treatment showed the maximum population recovery of the pathogen compared to other formulations applied by various methods and thus exhibited as least effective formulation and method of application in controlling the disease in brinjal.

Table-2. Effect of five best formulations applied in different methods on % wilt incidence (PWI) of brinjal in field experiment.

Formulations	% wilt incidence (PWI)							
	Method of application							
	S	R	So	S+R	R + So	S + So	S+So+ R	Mean
CVPf + Rs.	66.67 (59.57)	66.67 (59.57)	66.67 (59.57)	33.33 (30.43)	33.33 (30.43)	50.00 (45.00)	16.67 (15.85)	47.62 (42.92)
CFPf + Rs	83.33 (74.15)	66.67 (59.57)	83.33 (74.15)	50.00 (45.00)	33.33 (30.43)	50.00 (45.00)	33.33 (30.43)	57.14 (51.25)
CDPf + Rs	83.33 (74.15)	83.33 (74.15)	83.33 (74.15)	66.67 (59.57)	50.00 (45.00)	66.67 (59.57)	50.00 (45.00)	69.05 (61.65)
CRbPf + Rs	100.00 (88.72)	83.33 (74.15)	100.00 (88.72)	66.67 (59.57)	66.67 (59.57)	83.33 (74.15)	66.67 (59.57)	80.95 (72.06)
CWPf + Rs	100.00 (88.72)	83.33 (74.15)	100.00 (88.72)	83.33 (74.15)	66.67 (59.57)	83.33 (74.15)	66.67 (59.57)	83.33 (74.15)
Inoculated control (only Rs.)	100.00 (88.72)	100.00 (88.72)	100.00 (88.72)	100.00 (88.72)	100.00 (88.72)	100.00 (88.72)	100.00 (88.72)	100.00 (88.72)
Un inoculated Control (No Rs.)	16.67 (15.85)	16.67 (15.85)	16.67 (15.85)	16.67 (15.85)	16.67 (15.85)	16.67 (15.85)	16.67 (15.85)	16.67 (15.85)
Mean	78.57 (69.98)	71.43 (63.74)	78.57 (69.98)	59.52 (53.33)	52.38 (47.08)	64.29 (57.49)	50.00 (45.00)	
	S.Ed.±	$CD_{0.05}$						
Formulations (F)	6.299	12.522						
Method of application (M)	6.299	12.522						
Interaction effect (F×M)	16.666	NS						

S = Seed; R = Root; So = Soil; S+ R = Seed + Root; R + So = Root + Soil; S + So = Seed + Soil; S+ so + R = Seed + Soil + Root; Rs = *R. solanacearum*
Figures within parentheses indicate angular transformed values
NS: Non-significant

Table-3. Population dynamics of *P. fluorescens* in brinjal rhizosphere soil at different days after transplanting (DAT) treated with five best formulations applied in different methods in field experiment.

Formulations	Population of *P. fluorescens* (10^6 cfu /g)							
	30 DAT							
	Method of application							
	S	R	So	S+R	R + So	S + So	S+ So+ R	Mean
CVPf + Rs.	195.33 (8.29)	218.33 (8.34)	203.33 (8.31)	241.00 (8.38)	250.33 (8.40)	229.33 (8.36)	262.67 (8.42)	228.62 (8.36)
CFPf + Rs	183.33 (8.26)	207.33 (8.32)	196.00 (8.29)	220.00 (8.34)	231.33 (8.36)	216.67 (8.34)	247.33 (8.39)	214.57 (8.33)
CDPf + Rs	150.67 (8.18)	166.67 (8.22)	159.33 (8.20)	187.33 (8.27)	198.67 (8.30)	178.33 (8.25)	209.33 (8.32)	178.62 (8.25)
CRbPf + Rs	123.33 (8.09)	142.33 (8.15)	131.33 (8.12)	162.33 (8.21)	175.33 (8.24)	154.33 (8.19)	186.00 (8.27)	153.57 (8.18)
CWPf + Rs	108.33 (8.03)	126.33 (8.10)	118.67 (8.07)	146.33 (8.17)	159.33 (8.20)	134.67 (8.13)	171.00 (8.23)	137.81 (8.13)
Inoculated control (only Rs.)	1.50 (6.18)	1.50 (6.18)	1.50 (6.18)	1.50 (6.18)	1.50 (6.18)	1.50 (6.18)	1.50 (6.18)	1.50 (6.18)
Uninoculated control (No Rs.)	1.80 (6.26)	1.80 (6.26)	1.80 (6.26)	1.80 (6.26)	1.80 (6.26)	1.80 (6.26)	1.80 (6.26)	1.80 (6.26)
Mean	109.17 (7.61)	123.46 (7.65)	115.98 (7.63)	137.17 (7.68)	145.46 (7.70)	130.93 (7.67)	154.22 (7.72)	
	S.Ed.±	$CD_{0.05}$						
Formulations (F)	0.001	0.003						
Method of application (M)	0.001	0.003						
Interaction effect (F×M)	0.004	0.007						
	60 DAT							
	S	R	So	S+R	R + So	S + So	S+So+ R	Mean
	(8.36)	(8.38)	(8.38)	(8.43)	(8.45)	(8.41)	(8.47)	(8.41)
CFPf + Rs	200.67 (8.30)	222.33 (8.35)	212.33 (8.33)	243.67 (8.39)	260.00 (8.41)	231.33 (8.36)	273.33 (8.44)	234.81 (8.37)
CDPf + Rs	183.33 (8.26)	204.67 (8.31)	196.67 (8.29)	224.67 (8.35)	235.00 (8.37)	216.00 (8.33)	250.00 (8.40)	215.76 (8.33)
CRbPf + Rs	159.67 (8.20)	180.00 (8.26)	170.33 (8.23)	201.33 (8.30)	209.67 (8.32)	190.00 (8.28)	204.00 (8.31)	187.86 (8.27)
CWPf + Rs	126.67 (8.10)	143.00 (8.16)	134.33 (8.13)	160.67 (8.21)	168.33 (8.23)	151.33 (8.18)	202.00 (8.31)	155.19 (8.19)
Inoculated control (only Rs.)	1.26 (6.10)	1.26 (6.10)	1.26 (6.10)	1.26 (6.10)	1.26 (6.10)	1.26 (6.10)	1.26 (6.10)	1.26 (6.10)
Un inoculated Control (No Rs.)	1.70 (6.23)	1.70 (6.23)	1.70 (6.23)	1.70 (6.23)	1.70 (6.23)	1.70 (6.23)	1.70 (6.23)	1.70 (6.23)
Mean	128.82 (7.66)	141.91 (7.69)	136.63 (7.67)	157.48 (7.72)	165.53 (7.73)	149.91 (7.70)	175.39 (7.75)	
	S.Ed.±	$CD_{0.05}$						
Formulations (F)	0.001	0.002						
Method of application (M)	0.001	0.002						
Interaction effect (F×M)	0.003	0.006						

	90 DAT							
	S	**R**	**So**	**S+R**	**R + So**	**S + So**	**S+So+ R**	**Mean**
CVPf + Rs.	184.33 (8.27)	206.33 (8.31)	190.33 (8.28)	225.00 (8.35)	238.33 (8.38)	215.67 (8.33)	250.67 (8.40)	215.81 (8.33)
CFPf + Rs	152.33 (8.18)	180.00 (8.26)	165.33 (8.22)	205.00 (8.31)	216.33 (8.34)	195.33 (8.29)	228.33 (8.36)	191.81 (8.28)
CDPf + Rs	136.67 (8.14)	141.33 (8.15)	150.33 (8.18)	170.33 (8.23)	160.67 (8.21)	172.33 (8.24)	179.33 (8.25)	158.71 (8.20)
CRbPf + Rs	119.67 (8.08)	138.33 (8.14)	126.33 (8.10)	150.00 (8.18)	168.00 (8.23)	150.33 (8.18)	170.00 (8.23)	146.10 (8.16)
CWPf + Rs	102.33 (8.01)	122.33 (8.09)	112.33 (8.05)	132.33 (8.12)	145.33 (8.16)	131.33 (8.12)	167.00 (8.22)	130.43 (8.11)
Inoculated control (only Rs.)	0.80 (5.90)	0.80 (5.90)	0.80 (5.90)	0.80 (5.90)	0.80 (5.90)	0.80 (5.90)	0.80 (5.90)	0.80 (5.90)
Un inoculated control (No Rs.)	1.66 (6.22)	1.66 (6.22)	1.66 (6.22)	1.66 (6.22)	1.66 (6.22)	1.66 (6.22)	1.66 (6.22)	1.66 (6.22)
Mean	99.68 (7.54)	112.97 (7.58)	106.73 (7.56)	126.45 (7.62)	133.02 (7.63)	123.92 (7.61)	142.54 (7.66)	
	S.Ed.±	$CD_{0.05}$						
Formulations (F)	0.001	0.003						
Method of application (M)	0.001	0.003						
Interaction effect (F×M)	0.004	0.008						

Figures within parentheses are logarithmic transformed values.

The yield and yield attributes of bio formulation treated crops were assessed and recorded in Table-4. The leaf area (cm^2), average fruit weight (g)/ plant, yield/ plant (kg), No. of fruits/ plant, No. of branches/ plant and plant height (cm) of the uninoculated control plant was significantly greatest while the inoculated control plant had significantly lowest yield and yield attributes. Among the bio formulations, CVPf treated crops performed best while CWPf treated crops performed least.

Table-4. Performance of yield and yield attributes of bio formulation treated crops to evaluate the efficacy of *P. fluorescens* as PGPR

Treatments	Leaf area (cm^2)	Average fruit weight (g)/ plant	Yield/ plant (kg)	No. of fruits/plant	No. of branches/ plant	Plant height (cm)
CVPf + *R. solanacearum*	182.00b	158.33a	2.066ab	13.00ab	16.00ab	73.00a
CFPf + *R. solanacearum*	170.00c	142.00b	1.657bc	11.67abc	15.00abc	73.00a
CDPf + *R.solanacearum*	163.00d	128.00c	1.277cd	10.00bcd	13.00bcd	65.00b
CRbPf + *R. solanacearum*	159.00e	125.00cd	1.125cd	9.00cd	12.00cd	63.00bc
CWPf + *R. solanacearum*	150.00f	121.33d	0.931d	7.67d	10.00de	60.00c
Inoculated control (only *R solanacearum*)	102.00g	77.33e	0.180e	2.33e	8.00e	41.00d
Un inoculated control (no *R. solanacearum*)	190.00a	160.00a	2.405a	15.00a	17.00a	77.00a
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

Figures within columns followed by the same letters are not significantly different.

DISCUSSIONS

The effect of different substrate carrier-adhesive based powder formulations on the population dynamics of *P. fluorescens* indicated that vermicompost and farmyard manure used as substrate carriers in conjunction with carboxymethyl cellulose as an adhesive might have provided better nutrient sources and congenial microenvironment required for proper growth and subsequent longer shelf life in the formulated product. Bora and Deka (2007) also found that the biopesticide combination of vermicompost, *P. fluorescens*, carboxymethyl cellulose and mannitol showed best shelf life as it maintained highest population recovery at different DAS. Islam and Toyota (2004) reported higher microbial activity in Farmyard manure due to increased rates of CO_2 evolution and high dehydrogenase activity which was the key factor in the suppression of bacterial wilt of tomato. The increase in population of the antagonist at 30 DAS might be due to the fact that the formulations contain easily available carbon or other forms of nutrients required for subsequent growth and population build-up of antagonist. In contrast, in the later part of evaluation (120 days), the nutrients exhausted due to prolonged utilization and the altered unfavourable microenvironment probably caused sharp decline of the antagonist population. Similar results have been reported by Vidhyasekaran and Muthumilan (1995). The formulations when applied in seed, root and soil were more effective in reducing PWI of brinjal possibly due to the all round placement of the antagonist *viz*. on the seed, from which the antagonist migrated to the elangating roots (Burr *et al*., 1978), on the roots, the most favourable site for colonization (Anuratha and Gnanamanickam 1990) and on soil, the repertoire of both beneficial and pathogenic microbes (Dupler and Baker 1984), all of which in combination, created more favourable condition for maximum colonization giving a better competitive advantage over other rhizosphere microflora (Loper *et al*., 1985). The population build-up of the antagonist in the brinjal rhizosphere is supported by the fact that niche overlap between an inoculant and resident bacteria appears to be limited. Spatial separation and nutrient versatility are important factors contributing to this restricted overlap (Castro- Sowinski *et al*., 2007). Complex reciprocal interactions between soil, plant and microorganisms that occur could also account for the population dynamics of the bio control agent (Costa *et al*., 2006). The population density of the antagonist increased 100-1000 fold in the rhizosphere of the bio formulation treated crops than the population recovered in the rhizosphere of either inoculated or uninoculated controls. The residual food base present in the formulations might have provided the nutrients required for initial growth without involving in nutrient competition with other resident soil microorganisms. The higher inoculum level developed in the formulations further enhanced the population build up of the antagonist in the subsequent days. The probable reason for the lower pathogen population corresponding to

the higher recovery of the antagonist population, might be due to the antagonistic mechanisms such as antibiosis (Bull *et al*., 1991), siderophore mediated nutrient competition (Kloepper *et al*., 1980), higher rhizosphere and rhizoplane colonization ability in the brinjal plants, specifically favourable for *P. fluorescens*. Competitive exclusion of other microbes including pathogens as a result of rapid colonization ability of the rhizosphere by *P. fluorescens*, induced systemic resistance and specific and subtle pathogen antagonist interactions has been reported to be important factors in disease control (Hass and Defago 2005). The maximum performance of the yield and yield attributes observed in the CVPf treated brinjal crops can be attributed to the maximum recovery of biocontrol agent in the rhizosphere and minimum wilt incidence in such crops. Jinnah *et al*., (2002) found that the bio control agent *Pseudomonas fluorescens* produced positive effect on the plant growth characters. The findings in the present study in respect of suppression of bacterial wilt with concomitant improvement in yield and yield attributes of bio formulation treated crops compared to inoculated control reinforces *P. fluorescens* as a bio control agent of bacterial wilt in brinjal as well as plant growth promoting rhizobacteria (Ramesh, Joshi and Ghanekar 2008). However, intensive screening of indigenous strains of *P. fluorescens*, development of improved carriers and large scale field trials under different climatic conditions are necessitated for evolving formulations with better disease control activity in the field.

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