African Journal of Biotechnology Vol. 10(37), pp. 7174-7182, 20 July, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1209
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Comparative evaluation of organic formulations of *Pseudomonas fluorescens* based biopesticides and their application in the management of bacterial wilt of brinjal (*Solanum melongena* L.)

Gargi Chakravarty* and M. C. Kalita

Department of Biotechnology, Gauhati University, Guwahati, Assam, India.

Accepted 29 June, 2011

An experiment was conducted in the laboratory and farm of the Department of Biotechnology, Gauhati University, to explore the potentiality of various organic formulations of *Pseudomonas fluorescens* (Pf) and to manage bacterial wilt disease of brinjal (*Solanum melongena* L.) under local conditions. Different organic materials and adhesives were used in the formulation of Pf based biopesticides and stored at room temperature (30°C) and 4°C to evolve a suitable substrate carrier-adhesive based bioformulation on the basis of comparative longer shelf life of the biocontrol bacteria. Storage condition of 4°C was found to be more suitable as it yielded higher viable count of the biocontrol bacteria in all the organic formulations studied. The formulation CVPf was found to give highest viable count of 890.33 x 10^8 cfu/g at 4°C and 45 x 10^6 cfu/g at room temperature, at 120 days after storage. Five best organic formulations of Pf were selected for application 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 with 83.33% control of bacterial wilt of brinjal in field experiment conducted by completely randomized block design. Effective management of bacterial wilt of brinjal by *P. fluorescens* under local conditions signifies its potentiality and scope as a plant growth promoting rhizobacteria (PGPR) when formulated using effective substrate carrier and adhesive.

Key words: Bacterial wilt, substrate-carriers, bioformulation, biocontrol, plant growth promoting rhizobacteria (PGPR).

INTRODUCTION

The brinjal, aubergine or eggplant (*Solanum melongena* L.) is a common and popular warm weather vegetable crop grown in the subtropical and tropical regions of the world. The unripe fruit of eggplant is primarily used as a cooking vegetable. The nutritive values per 100 g of raw eggplant include carbohydrates (5.7 g), fat (0.19 g), protein (1.01 g), thiamine (0.039 mg), riboflavin (0.037 mg), niacin (0.649 mg), pantothenic acid (0.281 mg), vitamin B_6 (0.084 mg), folate (22 μg), vitamin C (2.2 mg), Calcium (9 mg), iron (0.24 mg), magnesium (14mg), phosphorus (25 mg), potassium (230 mg), zinc (0.16mg) and manganese (0.25mg) (USDA Nutrient database).

The major constraint in the production of brinjal is the bacterial wilt disease caused by *Ralstonia solanacearum*. *R. solanacearum* constitutes a serious obstacle to the cultivation of the economically important brinjal among other crops, causing total damage of plantations before as well as after bearing fruits. Bacterial wilt can account for 15 to 23% loss of brinjal crops before they bear any fruit and the average reduction in yield may be 54.6 to 62.5% due to further death of the bearing plants before full maturity (Das and Chattopadhyay, 1955).

*Corresponding author. E-mail: gargichakravarty2008@gmail.com Tel: 919706169050 or 919864015132

Abbreviation: **Pf**, *Pseudomonas fluorescens*; **PGPR**, plant growth promoting rhizobacteria; **DAS**, days after storage.

Biological control could have an important role in the management of bacterial wilt (Akiew et al., 1993). Several strains of *Pseudomonas fluorescens* have been reported to suppress soil borne diseases caused by pathogens (O'Sullivan and O'Gara, 1992). *P. fluorescens* encompasses a group of common, Gram negative, rod shaped, non pathogenic saprophytes that colonize soil, water and plant surface environments. Since they are well adapted in soil, *P. fluorescens* strains are being investigated extensively for use in biocontrol of pathogens in agriculture (Ganeshan and Kumar, 2006). It is known to enhance plant growth promotion and yield and reduce severity of many diseases (Hoffland et al., 1996; Wei et al., 1996). The cell suspensions of *P. fluorescens* should be immobilized in certain carriers and should be prepared as formulations for easy application, storage, commercialization and field use. The potential *P. fluorescens* are formulated using different organic and inorganic carriers either through solid or liquid fermentation technologies. Thus, for field application of *P. fluorescens* towards 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).

In this context, this study was undertaken to evaluate different solid organic substrates for formulation development of the potential biocontrol agent *P. fluorescens* and explore the efficacy of *P. fluorescens* based bioformulations 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 and preservation 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 on triphenyl tetrazolium chloride (TTC) agar medium (Kelman, 1954). The pathogen inoculum was stored in water (Kelman and Person, 1961) and refrigerated at 20°C for maintenance of virulence (Overbreek et al., 2004).

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 biochemically by following the guidelines described in the Bergey's Manual of Systematic Bacteriology (Garrity, 2001).

Collection and preservation of *P. fluorescens* strain

P. fluorescens strain (1749B) was collected from MTCC, Chandigarh, India. It was revived in Nutrient Agar media, enriched in nutrient broth and preserved in King's Medium B Agar slants. The slants were covered with mineral oil and refrigerated at 4°C for further use. Nutrient agar (g/L): Peptone 5 g, beef extract 3 g, sodium chloride 5 g, agar 20 g, pH 7; King's Medium B agar (g/L): proteose peptone 20 g, $K_2HPO_4.3H_2O$ 1.5 g, $MgSO_4.7H_2O$ 1.5 g, glycerol 15 ml, agar 20 g, pH 7.2 were used.

Preparation of the *R. solanacearum* and *P. fluorescens* cell suspension

Forty eight hours nutrient broth culture of *R. solanacearum* and King's B Broth culture of *P. fluorescens* were taken. Both 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).

Pathogenicity test

Seeds of brinjal (*Solanum melongena* L. cv. Pusa Kranti) were obtained from National Seeds Corporation, Pusa, India. A set of three 30 days old brinjal seedlings were inoculated with pathogen inoculum of concentration 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.

Evaluation of *P. fluorescens* as potential antagonist against the pathogen *R. 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 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. Forty eight hours *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 (1 mm 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 antibacterials were observed every 24 h for 5 to 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 h at 28 ± 1°C and the colony forming units (cfu) were counted out. The dilutions giving zones of inhibition were recorded. The cfu/ml in each dilution of the antibacterial agent was calculated as follows:

$$\text{NO. of cfu/ml} = \frac{\text{No. of cfu}}{\text{Volume plated} \times \text{total dilution used}}$$

Collection of substrates for preparation of *P. 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 (12 x 15 cm), to which 10 ml of sterile distilled water was added. Three packets of 100 g each were prepared for each organic substrate.

Growth and multiplication of *P. fluorescens* in different organic substrates

The sterilized organic substrates mentioned earlier were taken. Carboxymethyl cellulose (1% aq) (C), polyvinyl alcohol (P) and white flour gum (W) were used as adhesives for comparative study. The 48 h KMB slant culture of the biocontrol agent was washed with sterile distilled water to obtain antagonist cell suspension of concentration of 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 $CaCO_3$. The polypropylene bags were heat sealed and sterilized at 121°C for 30 min. 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 100 g formulation). Subsequently, *P. fluorescens* (Pf) cell suspension of concentration of 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-bioagent mixtures prepared were labeled with abbreviations (that is, initial letter of each of the substrate carriers and adhesives as mentioned earlier along with the biocontrol agent (Pf) example CVPf, PFPf etc.). Each bioformulation 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-Pf based formulation was divided into 3 parts, packed separately in polypropylene bags (8 x 6.5 cm), heat sealed and stored at room temperature. Another set of bioformulations was prepared for each substrate carrier-adhesive-bioagent mixture and stored at 4°C for comparative study.

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

The population dynamics of the bioagent *P. fluorescens* was determined at different days after storage (DAS) of the substrate carrier-adhesive-*P. fluorescens* formulations in the two storage conditions. Viable population of *P. fluorescens* in the powder formulations was determined at 7, 15, 30, 60, 90 and 120 DAS at room temperature. For the study of the potentiality of 4°C storage condition in the viability of the bioformulations, these were first stored at room temperature for 15 days to increase the initial population of *P. fluorescens*. Initial determination of population of *P. fluorescens* was made at 15 DAS at room temperature and later samples were made at 30, 60, 90 and 120 DAS at 4°C.

Population dynamics was examined by mixing 1 g of formulations aseptically with 10 ml sterile distilled water for 20 min in a rotary shaker. Serial dilutions were prepared and 0.1 ml aliquot from 10^{-5} to 10^{-8} dilutions were spread on KMB plates. After incubating the plates at 28 ± 1°C for 48 h, the cfu/g formulations were counted out. The population of *P. fluorescens* in powder formulations (cfu/g formulation) recorded at 7, 15, 30, 60, 90 and 120 DAS were transformed into their corresponding log values and used for analysis in this study.

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

On the basis of shelf life of *P. fluorescens* in the different carrier substrates-adhesive formulations at 120 DAS, the five best were selected out. These were done by different methods to evaluate their effectiveness in the control of bacterial wilt of brinjal.

The pot experiment was conducted during August 2007 to January 2008 and repeated during February 2008 to July 2008. The brinjal seeds cv. Pusa Kranti (a bacterial wilt susceptible cultivar) were obtained from National Seeds Corporation, Pusa, India. The earthen pots taken were 26 x 22 x 32 cm in size. A potting medium consisting of sand and potting substrate in the ratio of 1:3 was sterilized at 121°C for 30 min and filled in the pots. The potting medium was composed of humus, clay and peat in the proportion of 15:35:50, respectively.

The field experiment was done in the farm of the Department of Biotechnology, Gauhati University, India during October 2008 to March 2009 and repeated during August 2009 to January 2010. The field area in which the experiment was conducted measured 12 x 8 m. Each block measured 0.6 x 8 m^2 and space between the blocks was 0.25 x 8m^2. In each block, the space between two plants measured 0.25 x 3m^2. The space between two rows of a block was 3 m, while the space between two columns was 0.2 m. Both the pot and field experiments were conducted by following the completely randomized block design (CRBD).

The treatments for each of the five formulations were seed treatment (S), soil treatment (So), root treatment (R) and their combinations viz. S + So, So + R, S + R and S + So + R. The controls included only the pathogen inoculated treatment with no bioformulation applied (inoculated control) and no pathogen or bioformulation applied (uninoculated control).

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

Disease record

The number of wilted plants in each treatment was continuously recorded up to 90 days after inoculation with pathogen. The number of completely wilted plants was tabulated for each formulation applied by different methods. The percentage (%) wilt incidence was calculated by using the formula given below:

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

For statistical analysis, the arc sine or angular transformation of the percentage values were made.

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 negative rod, 2 to 3 µm, non acid fast, non spore former, non capsulated and motile with lophotrichous flagella; 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 Biovar3.

The strain of the potential biocontrol agent *P. fluorescens* isolated from the rhizosphere- rhizoplane of healthy brinjal plant showed distinct bluish green fluorescence 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 are presented in Tables 1 and 2. Tables 3 and 4 show the population dynamics of *P. fluorescens* in powder formulations at different DAS at 4°C. The effect of different formulations on the population trend of the bacterial antagonist (*P. fluorescens*) has been elucidated as the individual effects of substrate carrier (S) and adhesive (A) as well as combined or interaction effect of substrate carrier and adhesive (S×A) from the two way analysis of variance (ANOVA) completely randomized.

The results on the effect of different substrate carrier-adhesive based powder formulations on the population dynamics of *P. fluorescens* at room temperature (Tables 1 and 2) indicated that the formulation CVPf followed by CFPf supported the highest population of *P. fluorescens* during the DAS sampled. On the other hand, WBPf was found to be the least efficient formulation recording lowest population of *P. fluorescens* as compared to other formulations at all DAS studied.

The population of *P. fluorescens* in all the organic formulations also followed a fluctuating trend with the DAS sampled. The initial population (7 days) increased in all the formulations at 15 DAS. At 30 DAS, the population recovery in all the formulations significantly increased.

Thereafter, during the period of 60 to 120 DAS, the population of *P. fluorescens* declined progressively and at 120 DAS, a significant reduction of more than 100 to 1000 fold was recorded.

Among the substrate-carriers, vermicompost proved to be the best at all DAS, irrespective of the adhesive used. It was followed by farmyard manure, decomposed mustard oil cake, rice bran, wheat bran, rice straw and banana leaf, respectively.

Among the three adhesives viz. carboxy methyl cellulose (CMC), polyvinyl alcohol (PVA) and white flour gum (WFG) used in formulations of *P. fluorescens*, CMC consistently contributed to the significantly higher viable populations of *P. fluorescens* in the formulations during the entire study period. CMC was followed by PVA and WFG respectively.

The results of the population dynamics of *P. fluorescens* when the powder formulations were stored at 4°C (Tables 3 and 4) show that there was a slow and progressive decline of the antagonist populations in the bioformulations from 15 DAS to 30, 60, 90 and 120 DAS. Still, the population recovered was much greater as compared to that recorded during the same period under room temperature storage condition. However, the effect of the formulations, substrate carriers and adhesives on the population dynamics of *P. fluorescens* under both storage conditions followed the same pattern.

Table 5 shows the effect of the formulations (F), methods of application (M) and their combined or inter-action effect (FxM) on the percentage wilt incidence (PWI) of brinjal (*S. 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 uninoculated control with no pathogen (*R. solanacearum*) challenge. All the formulations when applied as integration methods showed lower PWI than when applied as single methods.

DISCUSSION

Vermicompost and farmyard manure used as substrate carrier in conjunction with carboxymethyl cellulose as adhesive in the formulations CVPf and CFPf, respectively might have provided better nutrient sources and congenial microenvironment required for proper growth and subsequent longer shelf life of *P. fluorescens* 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 dehy-drogenase activity which was the key factor in the suppression of bacterial wilt of tomato.

The increase in population of the antagonist at

Table 1. Population dynamics of *P. fluorescens* in powder formulations at 7, 15 and 30 DAS at room temperature.

Substrate carrier (S)	Population of *P. fluorescens* (×10^8 cfu/g)											
	7 DAS				15 DAS				30 DAS			
	Adhesive (A)											
	C	P	W	Mean	C	P	W	Mean	C	P	W	Mean
V	900.67 (10.95)	700.33 (10.85)	120.00 (10.08)	573.67 (10.63)	930.00 (10.97)	718.00 (10.85)	128.00 (10.11)	592.00 (10.64)	1206 (11.08)	780.33 (10.89)	145 (10.16)	710.44 (10.71)
F	850.67 (10.93)	252.67 (10.40)	119.67 (10.08)	407.67 (10.47)	890.00 (10.95)	682.00 (10.83)	125.00 (10.10)	565.67 (10.63)	990.33 (11.00)	710.33 (10.85)	140.00 (10.15)	613.56 (10.66)
D	830.33 (10.92)	242.00 (10.38)	100.00 (10.00)	390.78 (10.43)	845.33 (10.93)	256.67 (10.41)	112.67 (10.05)	404.89 (10.46)	953.00 (10.98)	273.00 (10.44)	133.67 (10.13)	453.22 (10.51)
Rb	660.33 (10.82)	159.00 (10.20)	90.00 (9.95)	303.11 (10.11)	667.67 (10.82)	165.33 (10.22)	106.33 (10.03)	313.11 (10.36)	700.33 (10.85)	190.33 (10.28)	118.33 (10.07)	336.33 (10.40)
W	430.33 (10.63)	140.33 (10.15)	88.00 (9.94)	219.56 (10.24)	442.67 (10.65)	156.67 (10.19)	95.33 (9.98)	231.56 (10.27)	456.67 (10.66)	168.67 (10.23)	110.67 (10.04)	245.33 (10.31)
R	252.67 (10.40)	130.67 (10.12)	80.33 (9.90)	154.56 (10.14)	270.33 (10.43)	150.67 (10.18)	89.67 (9.95)	170.22 (10.19)	300.33 (10.48)	163.67 (10.21)	100.33 (10.00)	188.11 (10.23)
B	250.33 (10.40)	120.00 (10.08)	70.00 (9.84)	146.78 (10.11)	269.33 (10.43)	139.33 (10.14)	81.33 (9.91)	163.33 (10.16)	298.67 (10.48)	150.33 (10.11)	96.67 (9.99)	181.89 (10.21)
Mean	596.48 (10.72)	249.29 (10.31)	95.43 (9.97)		616.48 (10.74)	324.10 (10.40)	105.48 (10.02)		700.76 (10.79)	348.10 (10.44)	120.67 (10.08)	

	S.Ed±	$C.D_{0.05}$	S.Ed±	$C.D_{0.05}$	S.Ed±	$C.D_{0.05}$
Effect of S	0.005	0.011	0.005	0.010	0.003	0.006
Effect of A	0.003	0.007	0.003	0.007	0.002	0.004
Interaction effect (S×A)	0.009	0.018	0.009	0.018	0.005	0.010

Figures within parentheses indicate log transformed values; C = carboxy methyl cellulose, P = poly vinyl alcohol, W = white flour gum, V = vermicompost, F = farmyard manure, D = decomposed mustard oil cake, Rb = rice bran, W = wheat bran, R = rice straw, B = banana leaf.

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 micro-environment probably caused sharp decline of the antagonist population. Similar results have been reported by Vidhyasekaran and Muthumilan (1995). Vidhyasekaran et al. (1997) reported that strains of *P. fluorescens* could survive with only a lower population in talc or peat based formulation for more than 180 DAS.

Carboxymethyl cellulose (CMC) supported significantly higher population of the antagonist as compared to PVA and WFG. Vidhyasekaran and Muthamilan (1995) used CMC as adhesive in the preparation of different *P. fluorescens* based formulations and recovered a population of more than 10^7 cfu/g as long as 240 days after storage. In this study, comparatively higher population densities of *P. fluorescens* in CMC could be attributed to the close proximity of *P. fluorescens*

Table 2. Population dynamics of *P. fluorescens* in powder formulations at 60, 90 and 120 DAS at room temperature.

Substrate carrier (S)	Population of *P. fluorescens* (×10^8 cfu/g)								Population of *P. fluorescens* (×10^6 cfu/g)			
	60 DAS				90 DAS				120 DAS			
	Adhesive (A)											
	C	P	W	Mean	C	P	W	Mean	C	P	W	Mean
V	309.33 (10.49)	295.33 (10.47)	30.67 (9.49)	211.78 (10.15)	60.33 (9.78)	40.00 (9.60)	12.67 (9.10)	37.67 (9.49)	45.00 (7.65)	36.67 (7.56)	13.00 (7.11)	31.56 (7.44)
F	302.67 (10.48)	276.33 (10.44)	28.33 (9.45)	202.44 (10.12)	51.67 (9.71)	32.00 (9.50)	10.33 (9.01)	31.33 (9.41)	42.00 (7.62)	32.33 (7.51)	11.33 (7.05)	28.56 (7.39)
D	234.00 (10.37)	150.33 (10.18)	25.33 (9.40)	136.55 (9.98)	45.33 (10.66)	29.33 (9.47)	9.67 (8.96)	28.11 (9.36)	38.33 (7.58)	21.67 (7.34)	10.67 (7.03)	23.56 (7.32)
Rb	226.33 (10.35)	143.67 (10.16)	20.67 (9.31)	130.20 (9.94)	40.67 (9.61)	20.67 (9.31)	8.33 (8.85)	23.22 (9.26)	30.33 (7.48)	20.33 (7.31)	8.67 (7.94)	19.78 (7.24)
W	205.67 (10.31)	126.67 (10.10)	16.33 (9.21)	116.22 (9.87)	30.33 (9.48)	16.00 (9.20)	5.67 (8.72)	17.33 (9.14)	27.33 (7.44)	18.33 (7.26)	7.33 (6.86)	17.67 (7.19)
R	190.67 (10.28)	110.33 (10.04)	11.67 (9.06)	104.22 (9.80)	19.33 (9.28)	12.33 (9.09)	2.33 (5.69)	11.33 (8.02)	25.00 (7.40)	16.67 (7.22)	4.33 (6.64)	15.33 (7.09)
B	142.33 (10.15)	98.33 (9.99)	8.33 (8.90)	83.00 (9.68)	18.00 (9.25)	10.00 (8.99)	1.67 (5.53)	9.89 (7.93)	22.67 (7.36)	14.33 (7.16)	3.33 (6.52)	13.44 (7.01)
Mean	230.14 (10.35)	171.57 (10.20)	20.19 (9.26)		37.95 (9.54)	22.90 (9.31)	7.24 (7.98)		34.33 (7.52)	18.95 (7.23)	9.81 (6.92)	

	S.Ed±	C.D$_{0.05}$	S.Ed±	C.D$_{0.05}$	S.Ed±	C.D$_{0.05}$
Effect of S	0.022	0.045	0.710	1.433	0.037	0.075
Effect of A	0.014	0.029	0.465	0.938	0.024	0.049
Interaction effect (S×A)	0.038	0.077	1.229	2.482	0.064	0.129

Figures within parentheses indicate log transformed values; C = carboxy methyl cellulose, P = poly vinyl alcohol, W = white flour gum; V = vermicompost, F = farmyard manure, D = decomposed mustard oil cake, Rb = rice bran, W = wheat bran, R = rice straw, B = banana leaf.

Table 3. Population dynamics of *P. fluorescens* in powder formulations at 15, 30 and 60 DAS at 4°C.

Substrate carrier (S)	Population of *P. fluorescens* (×10^8 cfu/g)											
	15 DAS				30 DAS				60 DAS			
	Adhesive (A)											
	C	P	W	Mean	C	P	W	Mean	C	P	W	Mean
V	930.00 (10.97)	718.00 (10.86)	128.00 (10.11)	592.00 (10.64)	926.33 (10.97)	715.00 (10.85)	125.33 (10.10)	588.89 (10..64)	920.33 (10.96)	710.67 (10.85)	120.33 (10.08)	583.78 (10.63)

Table 3. continues.

F	890.00	682.00	125.00	565.67	880.67	678.33	121.67	560.22	876.33	672.33	119.33	556.00
	(10.95)	(10.83)	(10.10)	(10.63)	(10.94)	(10.83)	(10.09)	(10.62)	(10.94)	(10.83)	(10.08)	(10.62)
D	845.33	256.67	112.67	404.89	843.67	254.33	110.33	402.78	838.67	250.33	104.67	397.89
	(10.93)	(10.41)	(10.05)	(10.46)	(10.93)	(10.41)	(10.04)	(10.46)	(10.92)	(10.40)	(10.02)	(10.45)
Rb	667.67	165.33	106.33	303.11	664.33	163.33	104.67	310.78	660.33	158.67	100.33	306.44
	(10.82)	(10.22)	(10.03)	(10.36)	(10.82)	(10.21)	(10.02)	(10.35)	(10.82)	(10.20)	(10.00)	(10.34)
W	442.67	156.67	95.33	231.56	440.33	148.67	92.67	227.22	437.33	140.67	87.33	221.78
	(10.65)	(10.19)	(9.98)	(10.27)	(10.64)	(10.17)	(9.97)	(10.26)	(10.64)	(10.15)	(9.94)	(10.24)
R	270.33	150.67	89.67	170.22	268.67	154.67	87.33	170.22	264.33	152.33	84.67	167.11
	(10.43)	(10.18)	(9.95)	(10.19)	(10.43)	(10.19)	(9.94)	(10.19)	(10.42)	(10.18)	(9.93)	(10.18)
B	269.33	139.33	81.33	163.33	266.33	137.67	79.33	161.11	262.33	130.67	75.33	156.11
	(10.43)	(10.14)	(9.91)	(10.16)	(10.43)	(10.14)	(9.90)	(10.15)	(10.42)	(10.12)	(9.88)	(10.14)
Mean	616.48	324.10	105.48		612.90	321.71	103.05		608.52	316.52	98.86	
	(10.74)	(10.40)	(10.02)		(10.74)	(10.40)	(10.01)		(10.73)	(10.39)	(9.99)	

	S.Ed± C.D$_{0.05}$	**S.Ed± C.D$_{0.05}$**	**S.Ed± C.D$_{0.05}$**
Effect of S	0.005 0.010	0.002 0.005	0.003 0.006
Effect of A	0.003 0.007	0.002 0.003	0.002 0.004
Interaction effect (S×A)	0.009 0.018	0.004 0.008	0.005 0.010

Figures within parentheses indicate log transformed values; C = carboxy methyl cellulose, P = poly vinyl alcohol, W = white flour gum, V = vermicompost, F = farmyard manure, D = decomposed mustard oil cake, Rb = rice bran, W = wheat bran, R = rice straw, B = banana leaf.

with the carrier particles brought about by the sticker. Moreover, the adhesive might also have played the role of bacterial preservatives for its long term viability. Green et al. (1998) and Ibrahim et al. (1999) had earlier reported that adhesives, stickers and emulsions increases the efficacy of biocontrol agents by supplying nutrients and by protecting the microbes from dessication and death. Incorporation of CMC in formulations serves as stickers in uniform seed coating of microbes. A higher PGPR (strain B4) population was found to be maintained in talc/peat based formulation using CMC or gum arabic as adhesive cum preservative (Suslow and Schroth, 1982). Again, a higher *P. fluorescens* Pf1 population has been earlier documented in the formulation prepared by mixing talc as carrier with CMC as adhesive (Vidhyasekaran and

Muthamilan, 1995). However, CMC could lead to increase in the production cost which might prevent small scale farmers from adopting it. Hence, feasibility of the technique and shelf life of the product has to be evaluated to make the technology a viable component in disease management (Nakkeran et al., 2005).

P. fluorescens became metabolically inactive at a lower temperature (4°C), which might have acted as a barrier for proper physico-biochemical activities essential for population build up of the antagonist. But the population recovered at 120 DAS (>10^8 cfu/g) was quite high for that period of storage. Earlier, Kloepper and Schroth (1981) recorded a high population of PGPR strain A1 in talc-xanthan gum formulation in a storage period of more than 10 months at 4°C.

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 elongating roots (Burr et al., 1978), on the roots, the most favourable site for colonization (Anuratha and Gnanamanickam, 1990) and on soil, the reservoir of both beneficial and pathogenic microbes (Dupler and Baker, 1984), all of which in combination, created more

Table 4. Population dynamics of *P. fluorescens* in powder formulations at 90 and 120 DAS at 4°C.

Substrate carrier (S)	Population of *P. fluorescens* (×10^8 cfu/g)							
	90 DAS				120 DAS			
	Adhesive (A)							
	C	P	W	Mean	C	P	W	Mean
V	910.33 (10.96)	707.67 (10.85)	117.33 (10.07)	578.44 (10.63)	890.33 (10.95)	672.33 (10.83)	102.67 (10.01)	555.11 (10.60)
F	870.67 (10.94)	669.33 (10.83)	112.33 (10.05)	550.78 (10.61)	865.67 (10.94)	650.33 (10.81)	107.33 (10.03)	541.11 (10.59)
D	830.33 (10.92)	245.67 (10.39)	112.67 (10.05)	391.89 (10.44)	820.33 (10.91)	238.67 (10.38)	87.33 (9.94)	382.11 (10.41)
Rb	650.33 (10.81)	145.67 (10.16)	106.33 (10.03)	296.11 (10.31)	630.33 (10.80)	132.33 (10.12)	80.00 (9.90)	280.89 (10.27)
W	431.67 (10.64)	136.67 (10.14)	95.33 (9.98)	216.56 (10.23)	420.33 (10.62)	122.67 (10.09)	68.33 (9.83)	203.78 (10.18)
R	261.33 (10.42)	140.67 (10.15)	89.67 (9.95)	160.11 (10.15)	250.67 (10.40)	128.33 (10.11)	65.33 (9.94)	148.11 (10.11)
B	269.33 (10.43)	139.33 (10.14)	81.33 (9.91)	163.33 (10.16)	242.33 (10.38)	109.33 (10.04)	48.67 (9.69)	133.44 (10.04)
Mean	616.48 (10.74)	324.10 (10.40)	105.48 (10.02)		588.57 (10.72)	293.43 (10.34)	79.95 (9.89)	
	S.Ed±	$C.D_{0.05}$			S.Ed±	$C.D_{0.05}$		
Effect of S	0.005	0.010			0.004	0.007		
Effect of A	0.003	0.007			0.002	0.005		
Interaction effect (S×A)	0.009	0.018			0.006	0.013		

Figures within parentheses indicate log transformed values; C = carboxy methyl cellulose, P = poly vinyl alcohol, W = white flour gum; V = vermicompost, F = farmyard manure, D = decomposed mustard oil cake, Rb = rice bran, W = wheat bran, R = rice straw, B = banana leaf.

favourable condition for maximum colonization, giving a better competitive advantage over other rhizosphere microflora (Loper et al., 1985).

Conclusion

The findings in this study with respect to suppression of bacterial wilt in the bioformulation treated brinjal crops as compared to inoculated control, reinforces *P. fluorescens* as a biocontrol agent of bacterial wilt in brinjal (Ramesh et al., 2008). Vermicompost and farmyard manure as substrate carriers together with carboxymethyl cellulose as adhesive and the biocontrol agent were found to be superior bioformulations with respect to shelf life of the biocontrol agent and suppression of bacterial wilt when applied in seed, root and soil together. Also, low temperature (4°C) storage condition was found to retain higher *P. fluorescens* population in the bioformulations as compared to normal storage conditions of 30°C. However, intensive screening of indigenous strains of *P. fluorescens,* development of improved carriers, suitable storage conditions and large scale field trials under different climatic conditions are necessitated for evolving formulations with better disease control activity in the field.

REFERENCES

Akiew E, Trevorrow PR, Tonells PE (1993). Management of bacterial wilt of tobacco. In: Bacterial wilt. Hartman GL and Hayward AC (eds.). ACIAR Proceedings. Australian Centre Int. Agricultural Res. Camera, 45: 270-275.

Anuratha CS, Gnanamanickam SS (1990). Biological control of bacterial wilt caused by *Pseudomonas solanacearum* in India with antagonistic bacteria. Plant Soil, 124: 109-116.

Blair JE, Lenette EH, Truant JP (1971). In: Laboratory Exercise in Microbiology (Ed. Pleczer MJ, Chan EC). Mc. Graw Hill Book Co., Berlin, p. 356.

Bora LC, Deka SN (2007). Wilt disease suppression and yield enhancement in tomato (*Lycopersicon esculentum*) by application of *Pseudomonas fluorescens* based biopesticide (Biofor-Pf) in Assam. Indian J. Agric. Sci. 77(8): 490-494.

Burr TJ, Schroth MN, Suslow T (1978). Increased potato yields by treatment of seed pieces with specific strains of *Pseudomonas fluorescens* and *Pseudomonas putida*. Phytopathology, 68: 1377-1383.

Das CR, Chattopadhyay SB (1955). Bacterial wilt of eggplant. *Indian* Phytopath. 8: 130-135.

Dupler M, Baker R (1984). Survival of *Pseudomonas putida*, a biological control agent in soil. Phytopathology, 74: 195-200.
Ganeshan G, Kumar MA (2006). *Pseudomonas fluorescens*, a potential bacterial antagonist to control plant diseases. J. Plant Interactions, 1(3): 123-134.
Garrity M George (Eds.) (2001). Bergey's Manual of Systematic Bacteriology. Second Edition. Springer-Verlag, New York.
Green S, Wade-Stewart S, Boland G, Teshler M, Liu S (1998). Formulating microorganisms for biological control of weeds. In: Plant-Microbe Interactions and Biological control. Boland G, Kuykendall L (Eds.). Marcel Dekker, Inc. New York, pp. 249-281.
Hayward AC (1964). Characteristics of *Pseudomonas solanacearum*. J. Appl. Bacteriol. 27: 265-277.
Hoffland E, Halilinen J, Van Pelt JA (1996). Comparison of systemic resistance induced by avirulent and non pathogenic *Pseudomonas* sp. Phytopathology, 86: 757-762.
Ibrahim L, Butt T, Beckett A, Clark S (1999). The germination of oil-formulated conidia of the insect pathogen, *Metarhizium anisolpliae*. Mycol. Res. 103: 901-907.
Islam Tajul MD, Toyota Koki (2004). Suppression of bacterial wilt of tomato by *Ralstonia solanacearum* by incorporation of composts in soil and possible mechanisms. Microbes. Environ. 19(1): 53-60.
Kelman A (1954). The bacterial wilt caused by *Pseudomonas solanacearum*. North Carolina Agric. Exp. Stn. Tech. Bull. 99: p. 194.
Kelman A, Person LH (1961). Strains of *P. solanacearum* differeing in pathogenicity to tobacco and peanut. Phytopathology, 51: 158-161.
Kloepper JW, Schroth MN (1981). Development of a powder formulation of rhizobacteria for inoculation of potato seed pieces. Phytopathology, 71: 590-592.
Loper JE, Haack C, Schroth MN (1985). Population dynamics of soil pseudomonads in the rhizosphere of potato *(Solanum tuberosum L.)* Appl. Environ. Microbiol. 49: 416-422.
Nakkeran S, Fernando Dilantha WG, Siddiqui AZ (2005). Plant Growth Promoting Rhizobacteria Formulations and its scope in commercialization for the management of pests and diseases. In: PGPR: Biocontrol Biofertilization. Siddiqui ZA (eds.), pp. 257-296.
O' Sullivan DJ, O' Gara F (1992). Traits of fluorescent *Pseudomonas* sp. involved in suppression of plant root pathogens. Microbiol. Rev. 56: 662-672.
Overbreek L, Bergervoet JHW, Jacobs FHH, Elsas J (2004). The low temperature induced viable but non culturable state affects the virulence of R. solanacearum biovar. 2. Phytopathology, 94: 463-469.
Ramesh R, Joshi AA, Ghanekar MP (2008). Pseudomonads: major antagonistic endophytic bacteria to suppress bacterial wilt pathogen *Ralstonia solanacearum* in the eggplant (*Solanum melongena* L.) World J. Microbiol. Biotechnol. 25(1): 47-55.
Suslow TV, Schroth MN (1982). Rhizobacteria of sugarbeets: effects of seed application and root colonization on yield. Phytopathology, 72: 199-206.
USDA Nutrient database. www.nal.usda.gov/ fnic/foodcomp/search
Vidhyasekaran P, Muthamilan M (1995). Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. Plant Dis. 79: 782-786.
Vidhyasekaran P, Rabindran R, Muthamilan M, Nayar K, Rajappan K, Subramanian N, Vasumathi K (1997). Development of a powder formulation of *Pseudomonas fluorescens* for control of rice blast. Plant Pathol. 46: 291-297.
Wei G, Kloepper JW, Tuzun S (1996). Induced systemic resistance to cucumber diseases and increased plant growth by plant growth pranoting rhizobacteria under field conditions. Phytopathology, 86: 22-224.
Winstead NN, Kelman A (1952). Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum*. Phytopathology, 42: 628-634.