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Research Article

Eco-friendly management of soil borne diseases in brinjal through application of antagonistic microbial population

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ABSTRACT: In Assam, bacterial wilt, a soil borne disease caused by *Ralstonia solanacearum* Yabuchi *et al.* is the major constraint for production of solanaceous vegetables. Present study was made to evaluate the efficacy of substrate based bioformulation of a PGPR, *Pseudomonas fluorescens* against the bacterial pathogen in brinjal (*Solananum melengona*) under field condition. Inhibitory activity of *P. fluorescens* was tested against *R. solanacearum* following dual culture method. Three substrates, *viz.,* vermicompost (VC), mustard oil cake (MOC) and farm yard manure (FYM) were compared for mass multiplication of the antagonist. The highest population was recorded (105.56 x 10^8 cfu/g) when mass cultured in VC along with a standard sticker CMC and an osmoticant mannitol. Quantitative assay of population of *P. fluorescens* revealed that it could maintain high population count up to 180 days of storage at room temperature. Different method of application of the substrate based bioformulations *viz.,* seed treatment (ST), root application (RA), soil application at transplanting (SA) and soil application at 30 days after transplanting (30 DAP) and combined application of all the methods showed minimum wilt incidence and maximum disease reduction in brinjal. Minimum wilt incidence (0.25%) was recorded in the treatment comprising combination of ST, RA, SA and 30 DAP with maximum disease reduction. Following the trend of reduction in disease incidence, yield was maximum (34.40 t/ha) in this treatment and also showed the highest recovery of *P. fluorescens* strain-*Pf*-D1 in the soil rhizosphere after harvest (77.40 x 10^8 cfu/g). Vermicompost appeared to be the best nutrient source to support the antagonist for maximum multiplication and disease reduction and combined application of ST, RA, SA, 30 DAP was most effective bacterial wilt disease management in brinjal.

KEY WORDS: Antagonist, bacterial wilt, brinjal, microorganism, organic substrate, *Pseudomonas fluorescens*.

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INTRODUCTION

In Assam, brinjal is cultivated in about 26,000 hectares with productivity of 15.2 tonnes/ha (Anon., 1999). Bacterial wilt caused by *Ralstonia solanacearum* is the most important constraint for the production of this solanaceous vegetable in the state as the disease causes severe plant mortality and loss in yield. Exploitation of saprophytic antagonists as biological control agent has been considered as suitable tactic, as it is environmentally safe and economically feasible. *Pseudomonas fluorescens* is one of such active bacterial antagonists and is known as plant growth promoting rhizobacterium (PGPR) for its ability to enhance plant growth. It is also efficient in controlling many diseases of crop plants (Loper *et al.,* 2000; Bora *et al.,* 2000). The dynamics of the rhizosphere population of introduced bioagent are governed by many interacting processes, *viz.* growth, survival, lysis, and immigration depending upon the chemical, biological and physiological environment of the rhizosphere. To enhance the survival as well as effectiveness of the introduced bioagent particularly in the early stage of its establishment, various carrier materials as the nutrient sources are essential. These substrates enrich the soil with nutrients and also convert the rhizosphere more aggressive against the pathogen (Heijnen *et al.,* 1992). In context with the above background, present study was made to explore the mass culture and survivability of *P. fluorescens* in different organic substrates and management of bacterial wilt of brinjal by using *P. fluorescens* based biopesticide '*Biofor-Pf*'.

MATERIALS AND METHODS

Eexperiments were conducted in the laboratory of the Department of Plant Pathology, Assam Agricultural University, Jorhat and farmer's fields of vill. Dachamua of Golaghat district and vill. Napam

of Sonitpur district, Assam during 2007-2010. Diseased brinjal plants showing symptoms of bacterial wilt were used for the isolation of the pathogen (*Ralstonia solanacearum*) using Triphenyl Tetrazolium Chloride (TTC) medium. The inoculum concentration of the pathogen was always adjusted to a bacterial population of 1 x 10^8 colony forming units per milliliter (cfu/ml). The bacterial antagonist, *Pseudomonas fluorescens* strain used was collected from the culture stock of the Department of Plant Pathology, AAU, Jorhat. King'B agar media was used for multiplication and preservation of the *P. fluorescens*.

Different substrates used for mass multiplication of *P. fluorescens* were vermicompost, farmyard manure (FYM), and mustard oil cake (MOC). These were first air dried and passed through 350 mesh sieves separately to obtain their fine powders. Each substrate weighing 100 g each was filled into polypropylene bags separately, sealed with non-absorbent cotton plugs and sterilized at 121°C for 30 mins. Mass culture of *P. fluorescens* was prepared by transferring 24h old *P. fluorescens* growth and 15ml of the such bacterial suspension was aseptically added to 1000ml King's B broth and incubated at 28°C for 24h. From this, 10ml of the bacterial cells (10^8cfu/ml) was added to the sterilized substrates contained in the polypropylene bags. To these, 5ml of a sticker, Carboxy-methyl cellulose (1% CMC) was added in order to impart greater adherence property and 5ml of an osmoticant (3% mannitol) was added to impart higher moisture retaining property of the substrates. The inoculated substrates with *P. fluorescens* cells, CMC and mannitol were incubated at 28°C for 7 days to obtain substrate based biopesticide. The bags after complete incubation were stored at room temperature.

For determination of the population of *P. fluorescens* in different substrate based biopesticide after different days of storage, an experiment was designed following completely randomized design (CRD) with 3 replications. The different treatment combinations were : vermicompost + *P. fluorescens;* vermicompost + *P. fluorescens* + CMC + mannitol; FYM + *P. fluorescens;* FYM + *P. fluorescens* + CMC + mannitol; Control-1 :(only vermicompost); Control 2 (only FYM); MOC + *P. fluorescens*; MOC + *P. fluorescens* + CMC + Mannitol; Control-3 : (only MOC). The viable population of *P. fluorescens* in these treatments was determined after 45, 90, 135, 180, 225 and 270 days of inoculation following dilution plate technique. On the basis of the highest recovery of the population of *P. fluorescens* (cfu/g), the best biopesticide was selected for further experimentations and was named as '*Biofor-Pf'*.

The field experiment was carried out in the bacterial sick plots at villages. Dachamua (Golaghat district) and Napam of (Sonitpur district). Assam to evaluate the best method of application of the substrate based *P. fluorescens* in management of bacterial wilt of brinjal. Based on the treatments, the whole experimental fields were divided into 24 small plots arranged in randomized block design (RBD) to compare 6 treatments each replicated 4 times. The best *P. fluorescens* based biopesticide (*Biofor-Pf*) was applied following different methods, *viz*., as seed treatment, root treatment, soil treatment and their combinations at the time of transplanting and at 30 days after transplanting.

For seed treatment, brinjal seeds were first cleaned individually and treated with *'Biofor-Pf'* @1 gm/gm of seed along with a sticker/adhesive like rice glue for easy adherence. The coated seeds were then spread over a clean paper and dried overnight. For root treatment *'Biofor-Pf'* was mixed with rice gruel to form fine slurry and the roots of the brinjal seedlings were dipped in it for about 30 minutes. For 1000 seedlings, 1kg of the biopesticide was required. The treated roots of the crops were dried for 1 hour under shade before transplanting. For soil treatment at the time of transplanting, 10 g of *'Biofor-Pf'* was mixed with 100g of vermicompost (@ 10kg *'Biofor-Pf'* mixed with 1qt vermicompost/ha) was applied to the soil near the root zone of the crops. Similarly, the same treatment soil application of *'Biofor-Pf'* was repeated at 30 days after transplanting. Observations were made on wilt incidence (%, yield (t/ha) and population dynamics of *P. fluorescens* (cfu/g soil). The population dynamics of *P. fluorescens* was estimated at harvest (i.e., after 120 days after transplanting) following the serial dilution plate technique and was expressed as cfu/g of soil.

RESULTS AND DISCUSSION

Population dynamics of *P. fluorescens* (x 10^8 cfu/g) in various substrates after different days of storage

The population of *P. fluorescens* (cfu/g of substrate) in formulations prepared from the three substrates significantly increased after different days of storage (Table 1). The *P. fluorescens* multiplied in vermicompost + CMC + mannitol recorded the highest population of the antagonist after 45, 90, 135, 180, 225 and 270 days of inoculation. In the other combinations of *P. fluorescens* in FYM and MOC along with CMC + Mannitol the population of *P. fluorescens* was significantly higher over control, but was significantly less as compared to vermicompost as substrate. The population of *P. fluorescens* in both vermicompost and MOC based formulation

Table 1. Population of *Pseudomonas fluorescens* strain (x 10^8 cfu/g) assayed from different substrate based formulations after different days of storage

Treatments	Population of *P. fluorescens* (x 10^8 cfu/g) at different days of storage						
	45	90	135	180	225	270	Mean
Vermicompost + *P. fluorescens*	49.83 (8.79)	58.50 (9.16)	95.50 (9.97)	122.50 (10.09)	200.50 (14.00)	91.17 (9.91)	85.50 (9.91)
Vermicompost + *P. fluorescens* + CMC	56.50 (9.15)	62.17 (9.29)	96.50 (9.98)	124.50 (10.10)	205.17 (14.02)	92.50 (9.97)	89.22 (9.96)
Vermicompost + *P. fluorescens* + CMC + Mannitol	60.50 (9.28)	76.50 (9.48)	120.50 (10.08)	126.83 (10.10)	228.50 (15.11)	120.50 (10.08)	105.56 (10.01)
FYM+ *P. fluorescens*	27.50 (6.83)	49.50 (8.89)	61.50 (9.30)	56.50 (9.15)	54.50 (9.14)	53.17 (9.02)	50.44 (8.84)
FYM + *P. fluorescens* + CMC	43.50 (7.63)	51.50 (9.01)	62.83 (9.31)	108.83 (10.04)	91.17 (9.91)	71.50 (9.47)	69.89 (9.42)
FYM + *P. fluorescens* + CMC + Mannitol	45.50 (8.05)	53.50 (9.03)	92.50 (9.97)	110.83 (10.05)	94.83 (9.98)	75.50 (9.48)	78.78 (9.49)
MOC+ *P. fluorescens*	22.50 (6.43)	29.50 (6.61)	41.17 (7.75)	50.50 (8.85)	54.50 (8.94)	48.17 (8.40)	44.40 (7.65)
MOC +*P. fluorescens* + CMC	40.50 (7.03)	48.50 (8.41)	59.83 (9.29)	65.83 (9.34)	78.17 (9.51)	68.50 (9.45)	66.89 (9.41)
MOC+ *P. fluorescens* + CMC + Mannitol	42.30 (7.25)	50.20 (8.93)	89.20 (9.96)	66.63 (9.40)	91.63 (9.91)	73.30 (9.48)	73.59 (9.48)
Control –1 (Untreated vermicompost)	0.50 (7.70)	0.50 (7.70)	0.50 (7.70)	0.50 (7.70)	0.50 (7.70)	0.50 (7.70)	0.50 (7.70)
Control – 2 (Untreated FYM)	0.50 (7.70)	0.50 (7.70)	0.50 (7.70)	0.50 (7.70)	0.50 (7.70)	0.50 (7.70)	0.50 (7.70)
Control – 3 (Untreated MOC)	0.50 (7.70)	0.50 (7.70)	0.50 (7.70)	0.50 (7.70)	0.50 (7.70)	0.50 (7.70)	0.50 (7.70)
Mean	40.55 (9.38)	50.31 (9.47)	75.69 (9.61)	92.93 (9.69)	80.74 (9.64)	71.12 (9.59)	

S.Ed. ± = For treatment = 0.016, for days = 0.015, for treatment x days = 0.039; CD (P=0.05) = For treatment = 0.032, for days = 0.030, for treatment x days = 0.078

Figures in the parentheses are logarithm-transformed value

showed an increasing trend upto 225 days and then declined, but when FYM was used as substrate the population tend to decline after 180 days. In the best formulation *i.e*. vermicompost + *P. fluorescens* + CMC + mannitol, maximum population of *P. fluorescens* was recorded (228.5 x 10^8 cfu/g of soil). This record of higher population of *P. fluorescens* in the treatment might be due to high nutrient content of vermicompost, which is a good source of humus, possessing Vitamin B, auxin and antibiotics. On average, it contains 2.5-3.5 per cent nitrogen, 1.5-2.0 per cent phosphorous and 2.0-3.5 per cent potassium which is 3-4 times higher than that in FYM or MOC (Kohli *et al.,* 1988). Vermicompost causes a shift of pH towards neutral, a reduction in electrical conductivity, and significant reduction in water soluble chemical constituting possible environmental contaminants. Therefore, *P. fluorescens* which prefers neutral to alkaline pH tends to exhibit higher population shift in vermicompost (Alexander, 1997). CMC was used in the biopesticide as an adhesive, which might have also played a role of bacterial preservative for the long term viability of the antagonist. Earlier report suggests CMC as adhesive in biopesticide could show higher population of *P. fluorescens* (Vidhyasekaran and Muthamilan, 1995). Moreover, mannitol, which was used as osmoticant, protects desiccation of the bacterial cells and thereby

increases their survival period (Harman *et al.,* 1991). The best combination of vermicompost + *P. fluorescens* + CMC + Mannitol (*Jaiva Kiran*), was therefore used for further studies.

Effect of different methods of application of *Pseudomonas flourescens* based biopesticide (*Biofor – Pf*) on bacterial wilt incidence yield and rhizosphere population of brinjal

The wilt incidence in brinjal decreased significantly when plants were treated with *'Biofor Pf'* following different methods as compared to their controls (Table 2). However, lowest wilt incidence (0.25%) was recorded when *'Biofor-Pf'* was applied as seed treatment + root treatment + soil application at transplanting + soil application at 30 days after transplanting (DAT). The results are in agreement with the findings of Gohain (2001), who also reported that integration of seed treatment, root dip treatment and soil application of *P. fluorescens* could show higher reduction of wilt incidence in brinjal. Earlier, Rabindran and Vidhyasekaran (1996) also reported that combined application of peat based formulation of *P. fluorescens* as seed treatment + root treatment + soil application + foliar spraying effectively controlled the sheath blight disease of rice. The possible reasons for such result might be due to higher population build-up of the antagonist in the rhizosphere as a result of combined methods of application. Due to the higher population densities of *P. fluorescens*, the density dependent mechanism helped its rapid rhizosphere colonization, thereby led to rhizosphere niche exclusion in terms of space and nutrients for the pathogen and ultimately reduction wilt incidence (Bull, 1987).

The population of *P. fluorescens* in rhizosphere soil of brinjal increased significantly when plants were treated with *'Biofor-Pf'* following different methods as compared to their controls (Table 2). The biopesticide applied as seed treatment + root treatment + soil application at transplanting + soil application at 30 days after transplanting recorded the highest recovery of *P. fluorescens* (77.40 x 10^8 cfu/g soil) in rhizosphere soil. This increase in population densities might be due to increase in dose of the *P. fluorescens* based biopesticide in the brinjal rhizosphere as a result of increased number of applications. This is in conformity with Bull (1987) who investigated the effects of dosage of *P. fluorescens* on its population on the wheat roots and found that there was a direct linear relationship between the dose of *P. fluorescens* on the roots after sowing of the treated seeds. The all-round placement of the antagonist, *viz*., directly on the seed from which the antagonist migrated from the seed to the elongating roots (Burr *et al.,* 1978); on the roots, the most favourable site for colonization (Anuratha and Gnanamanickam, 1990) and on soil (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. This could be further corroborated

Table 2. Effect of different methods of application of *Pseudomonas fluorescens* based biopesticide '*Biofor-Pf*' on bacterial wilt incidence, Yield and rhizosphere population in brinjal

Treatments	Wilt incidence (%)*	Yield (t/ha)	Population of *P. fluorescens* (x 10^8 cfu/g)**
'*Biofor-Pf*' as seed treatment	40.67 (37.49)	17.60	11.30 (8.33)
'*Biofor-Pf*' as root treatment	30.05 (26.57)	18.200	36.80 (9.67)
'*Biofor-Pf*' as soil application at transplanting	20.17 (19.72)	19.40	50.20 (9.82)
'*Biofor-Pf*' as seed treatment + root treatment + soil application at transplanting	12.40 (18.54)	33.60	60.40 (9.90)
'*Biofor-Pf*' as seed treatment + root treatment + soil application at transplanting + soil application at 30 days after transplanting	0.25 (2.87)	34.40	77.40 (11.39)
T_6 = Control (Untreated)	95.24 (82.22)	0.40	0.50 (7.70)
SEd (±)	(5.82)	(2.09)	(0.075)
CD ($P = 0.05$)	(11.65)	4.18	(0.150)

* Figures in the parentheses are angular transformed value; ** Figures in the parentheses are logarithm transformed value

with the previous findings that the population density of *P. putida* was significantly higher in soil receiving the initial population density (Dupler and Baker, 1984). Vidhyasekaran and Muthamilan (1995) suggested that root zone application of a peat-based *P. fluorescens* formulation improved the survival and subsequently increased the rhizosphere population of the antagonist. The close proximity of *P. fluorescens* on the root surface resulted from the direct inoculation on the rhizosphere of tomato through root treatment might be the possible reason for better proliferation leading to higher population level in the rhizosphere soil.

The yield of brinjal increased significantly when plants were treated with *P. fluorescens* based biopesticide *'Biofor-Pf'*, following different methods as compared to their controls (Table 2). Similar to the increasing trend of the population of the antagonist, the highest yield of of brinjal (34.40 t/ha) was recorded when biopesticide was applied as seed treatment + root treatment + soil application at transplanting + soil application at 30 days after transplanting (DAT). This finding is in agreement with Rabindran and Vidhyasekaran (1996) who reported that application of *P. fluorescens* in combination of seed treatment + root treatment + soil application + foliar application against sheath blight of rice resulted in best protection as well as increase in rice yield. Such results are in concurrence with the earlier report that vermiculite-based *P. fluorescens* G32 formulation in root treatment of tomato plants could more effectively control bacterial wilt of tomato and results better yield (Mulya *et al.,* 1996). The higher population density of *P. fluorescens* due to its aggressive root colonization ability also accounted for lower level of PWI and higher yield of the crops when the biopesticide was applied as combined method. The findings are also in agreement with Howie *et al.* (1987) who related higher population density of *P. fluorescens* in wheat rhizosphere to effective suppression of take-all disease and higher yield in wheat.

Higher population densities of *P. fluorescens* resulted in rapid rhizosphere colonization after application of the biopesticide and thereby led to rhizosphere niche exclusion in terms of space and nutrients for the pathogen and ultimately reduction in wilt incidence (Bull, 1987). The increase in yields might also be due to the ability of the antagonist to decrease disease incidence and increase plant growth (Mavrodi *et al.,* 2003). More wilt incidence results in low survivability of plants and hence leads to less yield of the crop.

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