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Influence of storage period of *P. fluorescens* (TPf12) liquid formulation on *F. oxysporum* f. sp. *lycopersici*

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Abstract
Pseudomonas fluorescens strain TPf12 is being studied as an important biocontrol agent for plant disease management and growth-promoting bacteria in plants. In addition to promoting plant production, previous findings from our research group demonstrated that talc-based *P.fluorescens* (TPf12) formulation effectively reduced several plant diseases. Modernizing agro-techniques requires the development of a new formulation in which liquid inoculants can play an important part. For the production of liquid formulation, various chemicals such as trehalose, poly vinyl pyrrolidone (PVP), and glycerol were studied. Among these, the glycerol modification retained the higher *P. fluorescens* (TPf12) population level up to 210 days of storage. In addition, a study was carried out to standardize the dosage of liquid-based TPf12 formulation for seed treatment and seedling dip. For seed treatment and seedling root dip, an application of 10ml kg of seeds and 150ml ha of seedlings was found to be optimal. Without much loss in cell viability, the growth-promoting and antagonistic activities of TPf12 cultures of different ages were found to be greater than up to 180days of storage. The combined effect of seed treatment, seedling dip, and liquid formulation soil drilling documented the minimum incidence of Fusarium wilt on tomato under glasshouse conditions (14.00 per cent) and field conditions (9.54 per cent). Additionally, under glasshouse and field conditions, the liquid formulation increased the yield of tomato fruit compared with untreated control. This research thus provided effective technology for the production of a liquid-based *P. fluorescens* TPf12 bioformulation.

Keywords: *Fusarium*, liquid formulation, *Pseudomonas fluorescens*, population survival ability

Introduction
The main root colonizers are plant growth-promoting rhizobacteria (PGPR), the most powerful colonizers are from Pseudomonas, Bacillus, etc. organisms. Pseudomonas fluorescens strains are known to live in both rhizosphere as well as phyllosphere Krishnamurthy and Gnanamanickam, (1998) [14]. Some many strains of Pseudomonas have been shown to protect plants against many fungal, bacterial and viral diseases Chen *et al.* (2000) [4]; Ramama moorthy *et al.* (2001) [20]; Saravanakumar *et al.* (2009) [27]. In 1995, Vidhyasekaran and Muthamilan developed *P.fluorescens* strain Pf1 talc-based bioformulation against chick pea root rot disease. Our earlier studies have also shown effective management of various plant diseases such as rice sheath blight Radjacommare *et al.* (2002) [37], chili anthracnose Bharathi *et al.* (2004) [3], sugarcane rot Viswanathan and Samiyappan(2001) [37] and mango anthracnose Vivekananthan *et al.* (2004) [38] using Pf1 strain bioformulation centered on talc. Recently, Saravanakumar *et al.* (2007a) [24] have shown that the application of *P. fluorescens (*Pf1) talc-based bioformulation consistently decreased blister blight disease and increased the yield on tea plants. The same research group has shown that *P. fluorescens* (Pf1) in mung bean plants effectively controlled the dry root rot disease Saravanakumar *et al.* (2007b) [25]. In addition, seed treatment, soil application and seedling dip of Pf1 talc-based bioformulation effectively reduced sheath rot disease in glasshouse and field conditions on rice plants Saravanakumar *et al.* (2009) [27]. When the talc-based bioformulations is used by micro-irrigation techniques to control plant diseases in horticultural and plantation crops, the concerns are raised regarding nozzle blockage and bio-inoculant distribution. It necessarily involves the production of bioformulations dependent on liquids. However, the production of liquid formulation has been shown to have many advantages including high cell count, zero contamination, longer shelf life, increased safety against environmental stresses and increased field effectiveness Hegde (2002) [7]; Vendan and Thangaraju (2006) [26].

The microbials are present in a dormant cyst form in liquid formulation and the dormant form gives rise to active cells after application in the field. It helps to improve liquid bioformulation shelf-life for more than one year Vendan and Thangaraju (2007) [26]. The extended shelf-life of *Trichoderma asperellum* in liquid formulation has been documented recently by Kolombet *et al.* (2008) [13]. In this context, this research was carried out with the following objectives to establish and characterize the liquid-based bioformulation of *P. fluorescens* (TPf12): (1) to study the efficacy of strain TPf12 in liquid formulation at different time intervals during storage, (2) to assess the shelf life of *P. fluorescens* TPf12 in liquid formulation, (3) to assess the efficacy of liquid formulation against *Fusarium* wilt of tomato and to standardize the rate of application.

Materials and Methods

Effect of different chemical amendments on TPf12 viability

The viability of *P. fluorescens* (TPf12) in Nutrient and King's B broth (NB and KB, respectively) was tested by addition of trehalose (10mM) or polyvinylpyrrolidone (PVP) (2%) or glycerol (10mM) in 1L of broth. One milliliter of log phase culture of TPf12 was inoculated individually on to each broth and incubated at room temperature (25±2"C). The broth cultures were analyzed for viable cell population at different intervals up to decline phase. A serial dilution was prepared by transferring 1ml each of inoculum onto 9ml sterile water blanks to get 10m1 dilution. Similarly the dilutions weremade serially upto10^9. From the dilutions,10ml was pipetted out and plated on the respective media. The plates were incubated at 25±2"C and individual colonies were counted through drop plate method Somasegaran and Hoben (1994) [30]. In total, three replications were used for each dilution.

Preparation of liquid-based bioformulation of P. fluorescens TPf12 formulation on plant growth promotion:

Plant growth-promoting activity of *P. fluorescens* (TPf12) cultures of different ages were assessed based on the seedling vigor index by the standard roll towel method (ISTA, 1993). Tomato seeds (25 seeds for each replication) were bacterized with *P. fluorescens* (TPf12) cultures of different ages (2, 7, 15, 30, 45, 60, 90, 120, 150,180 and 210days) and placed over the presoaked germination paper. The seeds were held in position by placing another presoaked germination paper strip and gently pressed. The polythene she*et al.*ong with seeds were then rolled and incubated in growth chamber for 15days. Three replications were maintained for the assay of growth promotion at different time intervals. The root length and shoot length of individual seedlings were measured. The germination percentage of seeds was also calculated and was compared with that of the seeds bacterized with the talc formulation of TPf12.

The vigor index was calculated by using the formula:

Vigour Index = (Mean root length + Mean shoot length) x Germination (%) (Abdul Baki and Anderson, 1973).

In vitro assay of TPf12 cultures of different ages in liquid formulation against *Fusarium oxysporum* f. sp. *lycopersici*.

The soil borne pathogen, *F. oxysporum* f. sp. *lycopersici* was isolated by tissue segment method on potato dextrose medium. Infected tomato stems and roots were cut into small pieces of 1– 1.5cm, surface sterilized with 1% sodium hypochlorite for 1min and washed in sterile distilled water thrice and then placed in Petri-plate containing sterilized solidified potato dextrose agar (PDA) medium. The hyphal tips of fungi growing from the pieces were transferred aseptically to PDA slants for further studies. *F. oxysporum* f. sp. *lycopersici* was placed on the center of the Petri-plate. Sterile whatman No. 40 filter paper discs (6mm dia.) were placed 1cm away from the edge at four sides around the fungal disc. Twenty-five icro liters of *P.fluorescens* (TPf12) broth cultures of different ages (2,7,15,30, 45, 60, 90, 120, 150, 180 and 210days) were dropped over the filter paper discs. Assessments were made after 5days for the presence of an inhibition zone around the pathogen and nearer to the bacterial spot. Each Petri-plate constituted a single replication and three replications were maintained for each assay. Respective control was maintained with the sterile distilled water instead of bacterial inoculum.

Efficacy of TPf12 cultures of different ages in liquid

The greater viability of *P. fluorescens* (TPf12) in NB amended with glycerol enabled the development of liquid-based bioformulation of *P. fluorescens* (TPf12). The NB was prepared with addition of 2% glycerol. To this, 1ml (3"10 X 10 CFUml) of log phase culture of TPf12 was inoculated and incubated at room temperature (25±2"C). The formulation was sealed in plastic containers and used for further studies.

Characterization of *P. fluorescens* TPf12 in liquid formulation

Pseudomonas fluorescens TPf12 has been shown to produce siderophores, HCN and antibiotics Saravanakumar (2006) [23]. Thus, to test the nature of TPf12 in liquid formulation, characterization studies were carried out.

Siderophore activity of *P. fluorescens* TPf12 in liquid formulation

Siderophore production by P. fluorescens TPf12 cultures of different ages was assayed by plate assay method as described in Schwyn and Neilands (1987)[28]. The tertiary complex chromeazurol S (CAS)/Fe3+/hexadecyl trimethylammonium bromide (HDTMA) served as an indicator. To prepare 1L of the blue agar, 60.5mg CAS was dissolved in 50ml water and mixed with Fe III (1mM FeCl3'6H2Oin10mMHCl;10ml) and HDTMA solution by constant stirring. Cultures of different ages of *fluorescent pseudomonads* were streaked onto the succinate medium (Succinic acid, 4g; K2HPO4, 3g; (NH4)2SO4'7H2O, 0.2g; distilled water, 1L (pH 7.0) amended with the indicator. The plates were incubated for 3days. Production of siderophore was scored as none, little, strong and very strong up to 210days. Five replications were used for each assay at every time.

Production of HCN by P. fluorescens TPf12 in liquid formulation

TPf12 cultures of different ages in liquid formulation (2–180days) were streaked onto tryptic soya agar medium. Filter paper disc of 1.5cm diameter was soaked in picric acid solution (picric acid, 2.5g; Na2CO3, 12.5g and distilled water, 1L) and placed on the upper lid of each Petri-dish (Millerand Higgins, 1970) [16]. Dishes were sealed with parafilm and observations were made 4days after incubation. Reactions were scored as weak (yellow to light brown), moderate (brown) and strong (reddish brown). Five replications were used for each assay at every time.

Dose standardization of liquid-based formulation for seed treatment

The tomato seeds cv. PKM1 obtained from the Department of Vegetable Crops, Tamil Nadu Agricultural University, India were used in this study.

The surface sterilized seeds were bacterized at different doses *viz*., 5, 10 and 15ml (population load of 1.15"10^{10} ml) kg1 of seed.) was added to the liquid formulation and seeds were treated.

The talc-based formulation was applied at 10g (1g containing the population load of 6.5"108) kg of seed. Treated seeds were incubated at room temperature. Immediately after seed treatment with liquid and talc formulations, samples of 10 seeds were assayed to determine the number of viable bacterial cells on the seeds.

Each seed sample was placed into a 25-ml flask containing 10ml diluent (0.85% NaCl and 0.01% Tween 80) and vortexed for 5min. Samples were serially diluted and 100ml of 10^{6}-10^{9}dilutions were plated on NA agar plates. Similarly, seeds were assessed for adhering TPf12 population at 6, 12, 24, 36 and 48h after application and the results were expressed as CFU per seed.

Efficacy of *P. fluorescencs* (TPf12) bioformulation against *Fusarium* wilt of tomato under glasshouse conditions

Two sets of pot culture experiments were conducted to test the efficacy of

P. fluorescens (TPf12) separately as seed, soil, seedling dip and foliar applications in controlling *Fusarium* wilt disease incidence in tomato. The isolate of *F. oxysporum* f.sp. *lycopersici* was mass multiplied in the sand-maize medium and was mixed with the sterilized pot soil @ 15 g/kg of soil and filled in earthen pots.

The seeds were treated with PGPR bioformulations. Treated seeds were raised in pots. The treatments were compared with fungicide control (Carbendazim @ 2 g/kg of seed)**.** The seeds treated with PGPR strain served as control. *Fusarium* wilt from tomato field and cultured in lab were inoculated into plants at the time of flowering.

The experiment was conducted in a completely randomized block design three plants/pot and replicated three times. The treatments details were mentioned below

S. No	Treatments
T1-	Seed treatment with TPf12 liquid formulation @10 ml/kg of seeds)
T2-	ST +SD TPf12 liquid formulation
T3-	ST+SD+SA with TPf12 liquid formulation
T4-	ST+SD+FS with TBb8 oil formulation +TPf12 liquid formulation
T5-	Seed treatment with Carbendazim (@ 2g/Kg of seed)
T6-	Inoculated Control
T7-	Healthy control

ST- Seed treatment (10 ml/kg) SD- Seedling dip (1000 ml/ha of seedlings)

FS– Foliar spray (0.2%)

Assessment of disease incidence of wilt

$$\text{Per cent disease Incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \text{ X } 100$$

Wilt disease and yield assessment

Fusarium oxysporum f. sp. *lycopersici* on sand maize medium, multiplied (Ricker and Ricker, 1936)[22] was introduced into the 5 percent (w / w) sterilised soil attherate. The 45-day old tomato plants were inoculated with a pathogen and observed under glasshouse conditions for wilt symptoms. The disease incidence was assessed using the following formula:

Assessment of disease incidence of wilt

$$\text{Per cent disease Incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \text{ X } 100$$

The observations were made on plant height and fruit yield per plant for different treatments.

Field study

Two field trials were conducted at Thazhiyur Coimbatore (District) with three replications during from December 2012 and March 2013 to test the efficacy of PGPR bioformulations against *Fusarium* wilt under drip irrigation systems. The experiments were laid out in Randomized Block Design with seven treatments with individual plot size of 5"4m 2. The treatments and methods of application were the same as followed in the glass house study Three replications for each treatment were maintained. In the case of soil drench, liquid formulation @ 500mlha!1 was applied through drip irrigation system.

Mancozeb @ 0.2% was used for seed treatment, seedling dip followed by soil drench in field experiments. The plots were maintained without any treatment. Each treatment consisted of three replications and each replication approximately consisted of 100 plants. The disease incidence was assessed based on the number of plants infected relative to the total number of plants observed in a randomized manner. After harvesting the crop, fruit yield was recorded. Tomato PKM1 variety was used for field experiments.

Statistical analysis

The data were analyzed using IIRISTAT version 92-1 programme developed by the Biometric Unit, International Rice Research Institute, the Philippines.

Data were subjected to analysis of variance (ANOVA). Data in percent were arcsine transformed before analysis. The treatment means were compared by Duncan's multiple range test (DMRT) (Gomez and Gomez, 1984)[6].

Results

Developing liquid formulation of TPf12 with chemical amendments

The Nutrient broth was amended with chemicals *viz*., trehalose (10 mM), PVP (2 %) and glycerol (10 mM) separately to study the survival of TPf12 cells for longer period.

Except control (without any chemical amendments), glycerol and trehalose treatments maintained the population level at 10^{8} up to 210 days whereas PVP maintained up to 30 days and thereafter decreased gradually. The initial population of 10^{10} increased to 10^{12} up to 30 days and it slightly decreased during incubation after 45th day.

In contrast, in control the population was at 10^{8} during 15 days of incubation and declined sharply to 10^{2} during 60 days of incubation. Among the chemical amendments, addition of glycerol (10 mM) recorded higher level of population throughout the period of observation followed by trehalose (10 mM) (Table 1).

Table 1: Population of *Pseudomonas fluorescens* (TPf12) in Nutrient broth supplemented with different chemical amendments

Days	Population (cfu/ml)			
	*Glycerol	*Trehalose	*PVP	*Broth alone
0Th day	$5.25x10^{10}$	$5.0x10^{10}$	$5.0x10^{10}$	$5.40x10^{10}$
2nd day	$7.0x10^{10}$	$6.8x10^{10}$	$6.8x10^{10}$	$6.85x10^{10}$
5th day	$5.0x10^{12}$	$4.8x10^{12}$	$4.1x10^{12}$	$5.0x10^{11}$
15th day	$4.0x10^{12}$	$3.4x10^{12}$	$6.0x10^{11}$	$6.10x10^{8}$
30th day	$3.1x10^{12}$	$2.0x10^{12}$	$2.0x10^{10}$	$4.5x10^{5}$
45th day	$1.1X10^{11}$	$4.8X10^{11}$	$3.8X10^{9}$	$5.4X10^{4}$
60nd day	$1.1X10^{11}$	$4.8X10^{11}$	$3.75X10^{9}$	$3.2X10^{3}$
90th day	$7.8X10^{10}$	$4.0X10^{11}$	$4.5X10^{8}$	$6.2X10^{2}$
120th day	$7.5X10^{10}$	$7.0X10^{10}$	$3.7X10^{8}$	-
150th day	$7.4X10^{10}$	$5.6X10^{10}$	$2.1X10^{8}$	-
180th day	$7.3\ X10^{10}$	$5.5\ X10^{10}$	$1.1X10^{8}$	-
210th day	$7.2X10^{10}$	$5.4X10^{10}$	$1.9X10^{7}$	-

*Means of values three replications.

The King's B broth was amended with chemical amendments *viz*., trehalose (10 mM), PVP (2 %) and glycerol (10 mM) separately to study the survival of TPf12 cells.

The results revealed that population was maintained up to 30 days in amended Kings B broth whereas control (without any chemical amendments) population was maintained only up to 15 days (Table 2). Hence when compared to King'B broth, Nutrient broth found to be best only suited for addition of chemical amendment.

Table 2: Population of *Pseudomonas fluorescens* (TPf12) in Kings B broth supplemented with different chemical amendments

Days	Population (cfu/ml)			
	*Glycerol	*Trehalose	*PVP	*Broth alone
0th day	$6.45x10^{11}$	$6.0x10^{10}$	$3.5x10^{12}$	$4.0x10^{9}$
2nd day	$8.3x10^{11}$	$5.6x10^{10}$	$2.6x10^{12}$	$5.9x10^{10}$
5th day	$6.0x10^{12}$	$3.9x10^{13}$	$4.10x10^{11}$	$5.0x10^{8}$
15th day	$4.5x10^{12}$	$4.7x10^{12}$	$2.6x10^{10}$	$6.58x10^{8}$
30th day	$4x10^{12}$	$1.9x10^{12}$	$2.0x10^{11}$	$4.0x10^{5}$
45th day	$2.0X10^{12}$	$3.8.0X10^{11}$	$3.8X10^{9}$	$5.40X10^{3}$
60nd day	$1.9X10^{11}$	$3.3X10^{10}$	$3.75X10^{9}$	$3.0X10^{3}$
90th day	$1.8X10^{10}$	$3.0X10^{9}$	$4.5X10^{8}$	$2.3X10^{2}$
120th day	$1.8X10^{9}$	$3.0X10^{8}$	$3.75X10^{8}$	-
150th day	$1.8X10^{8}$	$2.9X10^{7}$	$2.10X10^{8}$	-
180th day	$1.6\ X10^{7}$	$2.5\ X10^{7}$	1.1010^{8}	-
210th day	$1.5\ x10^{7}$	$2.0\ x10^{6}$	$1.9\ x10^{8}$	-

*Means of value three replications.

Influence of storage period of *P. fluorescens*(TPf12) liquid formulation on *F. oxysoporum* f. sp. *lycopersici*

Mycelial growth of soil borne pathogen *F. oxysporum* f.sp. *lycopersici* in dual culture technique showed that carbendazim(a.i) at 0.2% significantly reduced mycelial growth (45.8mm) when compard to other treatments and control. In general, different days old cultures of TPf12 from 2nd day to 210 days significantly inhibited the growth of *F. oxysporum* f.sp. *lycopersici*. The result indicated that the cultures from 7 to 30 days recorded the least mycelial growth of 54 mm followed by the cultures.

From 2 day with the mycelial growth of 66.6 mm which is almost on par with the cultures from 60 to 120 days old. The inhibiting effect was slightly decreased from 150 to 210 days with the mycelial growth of 61.80 mm (Table 3).

Table 3: Influence of storage period of *P. fluorescens* (TPf12) liquid formulation on *F. oxysporum* f.sp. *lycopersici.*

Treatments	*F. oxysporum* f.sp. *lycopersici*	
	Mycelial growth of the Pathogen(mm)	Per cent inhibition Over control
2nd day	66.00^{b}	26.66
7th day	55.00^{ab}	38.88
15th day	55.00^{ab}	38.88
30th day	55.00^{ab}	38.88
45th day	58.00^{ab}	35.55
60th day	61.60^{ab}	31.55
90th day	60.00^{b}	33.33
120th day	62.20^{b}	31.88
150th day	62.00^{b}	31.11
180th day	61.80^{b}	30.11
210th day	61.70^{bc}	30.00
Chemical (Carbendazim 0.2%)	45.80^{a}	49.11
Control (Sterile water)	90.00^{f}	0.0

Values are mean of three replications

Means followed by a common letter are not significantly different at 1% level by DMRT

*Values in the parenthesis are arscine transformed values

Plant growth promotion

The tomato cv. PKM 1 cultivar seeds were treated with suspensions of different days liquid formulation of TPf12 tested for their growth promoting activity under *in vitro* condition. In general, different days of storage of liquid formulation of TPf12 induced the plant growth promotion significantly over untreated check. The result showed that the 2 days old bioformulation of TPf12 recorded the highest vigour index of tomato seedlings. It recorded higher germination of 95%, shoot and root length of 6.5 and 14. 23cm respectively. After 210 days of storage, the growth promotion activity has started to decline but significantly higher when compared to control (Table 4).

Table 4: Effect of storage period of liquid formulation of *P. fluorescens (*TPf12*)* on growth and seedling vigour of tomato cv. PKM1 (Roll towel method)

Treatments	Germination Percentage (%)	Shoot Length (cm)	Root Length (cm)	Vigour index
2nd day	92.00[a] (76.50)*	6.50[a]	14.23[a]	1907[a]
7th day	91.80[a](74.10)	6.17[a]	13.39[a]	1795[a]
15th day	90.40[a](72.41)	6.63[a]	12.90[a]	1765[a]
30th day	90.20[a] (72.43)	5.70[b]	11.97[a]	1593[a]
45th day	89.93[a](70.18)	5.82 [b]	11.93[a]	1596[a]
60th day	89.80[a](70.45)	5.80 [b]	11.71[ab]	1572[b]
90th day	89.68[a] (73.05)	5.91 [a]	11.84[a]	1592[a]
120th day	88.40[b] (72.68)	5.88 [b]	11.67[bc]	1551[d]
150th day	86.90[c] (72.53)	5.67[bc]	11.42[c]	1476[de]
180th day	84.54[d](60.79)	5.50[d]	11.33[d]	1422[bc]
210th day	80.36d[e](60.77)	5.43[de]	11.21[df]	1337[d]
Chemical (Carbendazim 0.2%)	90.00[a](74.02)	6.21[a]	12.20[a]	1656[a]
Control (Sterile water)	78.00[f](62.30)	4.51[f]	10.19[e]	1146[e]

Values are mean of three replications

Means followed by a common letter are not significantly different at 1% level by DMRT

*Values in the parenthesis are arscine transformed values

Siderophore and HCN production

The bioformulation containing *P. fluorescens* retained its siderophore production activity up to 210 days. It exhibited yellow coloured halo around the bacterial streak on dark blue colored agar plates indicating the production of siderophore. The siderophore produced by the TPf12 chelated the iron from the dye, chromazurol / Fe^{3+}/hexa decyl trimethyl ammonium bromide and turned dark blue to yellowish fluorescent color. The results revealed that the intensity of the siderophore production was higher up to 90 days when compared to 120 -210 days age bioformulation of TPf12 and the different age old bioformulation of TPf 12 was found to produce cyanide (HCN) which acts as an inducer of plant resistance. The results showed that the cultures up to 90 days strongly changed the yellow color of the filter paper to dark brown when compared to the cultures above 90 days old. It indicates the level of HCN production was observed up to 210th days and the results are presented in Table 5.

Table 5: Effect of storage period on the siderophore production of TPf12 liquid formulation.

Treatments	Siderophore	HCN
2nd day	+++	+++
7th day	+++	+++
15th day	+++	+++
30th day	+++	+++
45th day	+++	+++
60th day	+++	+++
90th day	+++	+++
120th day	++	+++
150th day	++	++
180th day	++	++
210th day	++	+
Control	-	-

+, low production;
++, medium production;
+ + +, strong production
-, no production

Efficacy of *P. fluorescens* (TPf12) bioformulations against Fusarium wilt under glasshouse and field conditions

Wilt incidence

Among the various treatments, seed treatment+ seedling dip+soil application of TPf 12 has significantly reduced the incidence of wilt under glasshouse conditions. The observations indicated that the wilt incidence was less in plants treated with ST+SA+SD TBb8 + TPf12 (14.0%) followed by TPf12 alone (36.0%). In chemical treatment (Carbendazim), 58.8 per cent wilt incidence was recorded and maximum incidence of 90 per cent was recorded in control (Table 6)

Table 6: In chemical treatment (Carbendazim), 58.8 per cent wilt incidence was recorded and maximum incidence of 90 per cent was recorded in control

		Glasshouse study			Field study		
	Treatments	**Plant height (cm)**	**Per cent disease incidence (%)**	**Fruit yield g/plant**	**Plant height (cm)**	**Per cent disease incidence (%)**	**Fruit yield g/plant**
T_{1}-	ST with TPf 12 liquid formulation	78.2^{bc}	36.0^{c}	420.05^{e}	81.4^{c}	24.30(29.53)^{de}	37.40b^{cd}
T_{2}-	ST+SD with TPf 12 liquid formulation	85.5^{a}	22.6^{b}	485.75^{cd}	85.4^{b}	14.52(22.39)^{a}	40.15^{bc}
T_{4}-	ST+SD+SA with TPf 12 liquid formulation	91.5^{a}	14.0^{a}	605.10^{a}	89.5^{a}	10.42(18.82)^{a}	46.54^{a}
T_{5}-	Seed treatment with Carbendazim @2g/kg of seeds	77.5^{bc}	58.8^{e}	225.55^{h}	92.5^{a}	9.54(17.99)^{a}	48.55^{a}
T_{6}-	Inoculated control	62.7^{d}	90.00^{j}	175.67^{i}	74.8^{d}	19.42(26.14)^{bc}	40.10^{b}
T_{7}-	Healthy control	67.8^{d}	-	340.50^{g}	75.4^{d}	48.41(44.09)^{f}	33.40^{d}

ST- Seed treatment (10 ml/kg)
SD- Seedling dip (1000 ml/ha of seedlings)
SA- Soil application (1000 ml/ha)
FS – Foliar spray (1000 ml/ha)
Values are mean of three replications
Means followed by a common letter are not significantly different at 5% level by DMRT

Discussion

In this sense, the production of liquid inoculants takes on added significance in the conservation of sustainable crops, which could increase the shelf-life to more than one year, in addition to providing resistance to adverse conditions. In addition, the development of quality inoculants with improved shelf-life and user-friendly formulation are important factors for bio-inoculant technology 's success. The carrier-based bio-inoculants however suffer from short shelf life, high contamination and poor performance in the field (Hegde, 2002)[7]. The effectiveness of talc-based Pf1 formulations in various agricultural and horticultural crops has already been demonstrated against pests, diseases and nematodes under glasshouse and field conditions Nandakumar *et al.* (2001) [17]; Bharathi *et al.* (2004) [3]; Ramamoorthy *et al.* (2002) [21]; Saravanakumar| *et al.* (2007a, b, c, 2009) [24]. Adding glycerol to NB in this study maintained the viability of TPf12 cells in liquid formulation for 6-month storage period. This result is in line with the findings of Singleton *et al.* (2002) [29] whom established Rhizobium's liquid formulation through incorporating various ingredients in the mannitol yeast extract media. Likewise, the improved persistence of TPf12 cells in liquid formulation may be due to the action of chemical modifications. In this analysis, the choice of glycerol and PVP as chemical modifications is justified by the fact that they retain a large amount of water and by delaying the drying process protect cells from the effect of exposure to heat. Trehalose can also enhance cell tolerance to drying, osmotic pressure and temperature stress, and stabilise enzymes and cell membranes Fillinger *et al.* (2001) [5]. In addition, this study showed that the liquid formulation has a shelf-life of 6 months compared to the carrier-based formulation that has a shelf-life of up to 3 months. Our previous findings well record the mycoparasitic ability of *P. fluorescens* TPf12 Ramamoorthy *et al.* (2002) [21]; Saravanakumar *et al.* (2007b, 2009) [27]. However, the efficacy of the bioagent in the newly formed formulation needs to be evaluated. In this sense, *P. fluorescens* (TPf12) liquid formulation at different ages showed substantial decreases in mycelial growth of A. Except on the 210th day, the liquid formulation has the ability to inhibit the pathogens' mycelial growth. TPf12 cultures of different ages significantly increased the vigour index of tomato seedlings in comparison to untreated control, close to antagonistic behavior. On the other hand, the development of siderophore, HCN, and antibiotics such as pyoleuterin was documented by *P. fluorescens* strain TPf12 and these activities were associated with its efficacy in plant disease management Ramamoorthy *et al.* (2002) [21]; Radjacommare *et al.* (2007) [18]. In pot and field experiments, the use of *P. putida* WCS358 significantly increased the growth of potato root and tuber yield, whereas the mutants faulty in siderophore biosynthesis had no such impact Bakker *et al.* (1987) [2]. Later on, the role of fluorescent siderophores in the biological control of tomato bacterial wilt was also stated by Jagadeesh *et al.* (2001) [10]. Due to its activity in situ against a variety of root and seedling pathogens, another compound, 2,4-DAPG, which is produced by certain plant-associated *fluorescent Pseudomonas* species Thomashow and Weller (1995) [33]; Keel *et al.* (1996) [12], is of particular importance to agriculture. Previous research findings have clearly shown that fluorescent pseudomonads producing siderophore and DAPG could serve as powerful biocontrol agents against plant pathogens compared to non-producing strains. Similarly, *P. fluorescens* (Pf1) cultures of various ages were able to develop antibiotics and iron chelating agents for up to 210 days in liquid formulation. This determined the liquid-based formulation efficacy of *P. fluorescens* (Pf1). The maximum viability of Pf1 cells on seeds and seedling roots respectively was reported by the application of Pf1 liquid formulation with rice gruel at 10ml/kg of seed as seed treatment and Pf1 at 150ml/ha with sterile water as seedling dip Similarly, Singleton *et al.* (2002) [29] stated that for Rhizobium liquid formulation in soybeans, the inoculum level of 5ml / kg of seed was optimal. TPf12 's liquid formulation was found to be better than the formulation dependent on the carrier. As a result of the incorporation of cell protective chemicals, this may be due to the extended survival of bacterial cells. When assessing cell numbers in the inoculant and their degree of survival after inoculation on seeds, there appears to be a broad interaction between strain and media components Singleton *et al.* (2002) [29]. The use of rice gruel liquid formulation favoured the adherence of higher cell numbers to the seeds and was due to its sticky nature and nutrient content Kundu and Gaur (1981) [15]. Liquid inoculants can therefore better promote cell survival on seeds than the formulation of the solid carrierIn addition, the efficacy of several antagonists against plant pathogens and the promotion of plant growth is directly linked to the inoculated number of antagonist cells Hofstein *et al.* (1994) [8]. Therefore, the application of highly condensed cell formulations is an easy way to improve performance. This research also showed that the efficacy of the liquid formulation of TPf12 in glasshouse and field conditions against the occurrence of Fusarium wilt was also demonstrated. In addition, contamination was prevented by

the developed formulation and counts of 10^8 CFU / ml could be maintained after 210 days. For further commercialization methods, the use of liquid formulation in the treatment of many other diseases needs to be explored in the future.

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