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Standardization of organic substrate based powdered Formulations of *Pseudomonas fluorescens* for viability and Rhizosphere competence

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Abstract

The present study is an attempt to standardize the agro wastes based formulations under two different storage conditions, which would result in higher shelf life of the product, and assesses the method of application that would result in greater rhizosphere competence. In Vermicompost, the population of *P. fluorescens* increased to 1206×10^8 cfu/g at 30 Days after Storage (DAS) and finally decreased to 45×10^6 cfu/g at 120 DAS when stored at room temperature. At 4°C the population load maintained at the level of 10^8 cfu/g at 120°C. Among the different methods of application of the bioformulations, seed + root+ soil technique showed the highest rhizosphere population recovery of 280.00×10^6 cfu/g at 60 days after transplanting (DAT).

Key words : *Pseudomonas fluorescens*, bioformulation, shelf life, storage temperature, method of application

The overuse of agrochemicals such as fertilizers and pesticides for management of soil borne and foliar pathogens and improving plant growth, come with high environmental cost[9]. This threat has prompted field workers to seek viable alternatives in the form of plant growth promoting rhizobacteria (PGPR). Inoculating plants with PGPR can be an effective strategy to stimulate crop growth and improve crop tolerance to the abiotic stresses (*eg*. drought, heat and salinity) likely to become more frequent as climate change conditions continue to develop[2]. Among the PGPRs, fluorescent Pseudomonads are the most exploited bacteria for crop improvement[3]. For field application of the PGPR *Pseudomonas fluorescens*, formulation development is necessary based on different carrier materials. For formulation development, it is essential to optimize mass multiplication protocols that promote product quality and quantity, preserve shelf life and aid in product delivery that would support its rhizosphere competence[7]. Therefore, the present study aims to find the appropriate carrier material for formulation, optimum storage condition and assesses the appropriate

delivery system of the bioformulation in the field which would support a higher population load of the bioagent in the rhizosphere. Present work was undertaken with the following objectives 1. To prepare powdered formulations based on agro waste as substrate carrier and *Pseudomonas fluorescens* as bioagent. 2. To study the population dynamics of the *Pseudomonas fluorescens* based bioformulations at different days after storage at room temperature and at 4°C. 3. To assess the viable population of the formulation based bioagent in rhizosphere soil at different days after transplanting for evaluation of the period with highest rhizosphere competence.

Mass culture of the isolated strain of Pseudomonas fluorescens: An indigenous strain of *Pseudomonas fluorescens* was isolated from the rizosphere soil of Brinjal Plant in Singimari of Kamrup District, Assam, characterized and tested for plant growth promoting activities.

Growth and multiplication of Pseudomonas fluorescens in different organic substrates:

Agro wastes like Rice bran (Rb), Decomposed mustard oil cake (D), Farmyard manure (F) and Vermicompost (V), were used for study as substrate carriers. The substrate carrier-adhesive-bioagent mixture was prepared by following the modified method of Kloepper and Schroth[8]. *P. fluorescens* (Pf) cell suspension @ 10^8 cfu/mL (O.D 0.5) was added into the mixture (1:10 v/w). Three sealed packets for each formulation were prepared and stored at room temperature. Same process was applied for preparation of another set for storage at 4°C for comparative study.

Population dynamics of P. fluorescens in the different substrate carrier based formulations at different days after storage (DAS) at room temperature and 4°C :

The population dynamics of the bioagent *P. fluorescens* was determined at 15, 30, 60, 90 and 120 DAS of the substrate carrier-adhesive- *P. fluorescens* based formulations at room temperature and at 4°C by the serial dilution plate technique[6]. The experiment was conducted by following the Completely Randomized design with three replications for each treatment. For assessment of population at 4°C, the formulations were first stored at room temperature for 15 days to increase the initial population of *P. fluorescens* and then population determined at subsequent DAS.

Quantitative assessment of P. fluorescens population in the rhizosphere after application of the formulation by different methods :

The *P. fluorescens* based formulations were applied to brinjal plants cv. Pusa Kranti in different methods *i.e* seed treatment (S), soil treatment (So), root treatment (R) by following the method of Sivakumar and Narayanaswamy[13]. Combination treatments of seed + soil (S+So), root+ soil (R+So), seed+ soil (S+So) and seed + root + soil (S+R+So) were also carried out. The experiment was conducted by following the Completely Randomized Block Design (CRBD), where each treatment was replicated thrice with two plants per replication.

Plant growth promoting properties of isolated P. fluorescens strain :

The *P. fluorescens* strain isolated from rhizosphere soil of brinjal plant showed positive results for plant growth promoting activities (Table-1).

Table-1. Plant growth promoting activities of the isolated strain of *P. fluorescens*

Plant growth promoting Activity	**Result (+ve: Positive; -ve: Negative)**
P- solubilisation	+ve
Ammonia production	+ve
Catalase activity	+ve
IAA production (µg/mL)	198.08

Population dynamics of the bioagent in different substrate carrier based bioformulation under two storage conditions :

The result of the population dynamics of *P. fluorescens* in powder formulations at different DAS at room temperature is presented in Table-2. The formulation VPf recorded significantly high population recovery of *P. fluorescens* as compared to other formulations sampled at all DAS indicating it as a better nutrient source and provider of congenial microenvironment required for proper growth and subsequent longer shelf life in the formulated product[11]. The population recovery in all the formulations reached its peak at 30 DAS. Thereafter during the period 60-90 DAS, the population of *P. fluorescens* showed a steady decline. However at 120 DAS, the population of *P. fluorescens* showed a highly significant decline to 10^6 cfu/g level in all the formulations. Such findings are in conformity with earlier reports that higher population densities (>10^7 cfu/g) of *P. fluorescens* were measured in talc, peat, blackgram, coirpith and skelled cobs based formulations at ambient temperatures[14]. At 4°C the performance of the substrate carriers

Table-2. Population load of *P. fluorescens* in different substrate carrier based formulations under storage at room temperature (28±2°C)

Substrate Carrier Bioagent based Formulation	**Population load of *P. fluorescens* (×10^8 cfu/g) at different days after storage (DAS)**				**Population-load (×10^6 cfu/g)**
	15 DAS	**30 DAS**	**60 DAS**	**90 DAS**	**120 DAS**
VPf	930.00 (10.97)	1206.00 (11.08)	309.33 (10.49)	60.33 (9.78)	45.00 (7.65)
FPf	890.00 (10.95)	990.00 (11.00)	302.67 (10.48)	51.67 (9.71)	42.00 (7.62)
DPf	845.33 (10.93)	953.00 (10.98)	234.00 (10.37)	45.33 (9.66)	38.33 (7.58)
RbPf	667.67 (10.82)	700.33 (10.85)	226.33 (10.35)	40.67 (9.61)	30.33 (7.48)
Effect of Substrate Carrier	S.Ed± 0.005 $CD_{0.05}$ 0.010	S.Ed± 0.003 $CD_{0.05}$ 0.006	S.Ed± 0.022 $CD_{0.05}$ 0.045	S.Ed± 0.710 $CD_{0.05}$ 1.433	S.Ed± 0.037 $CD_{0.05}$ 0.075

Figures within parenthesis indicate log transformed values

showed similar trend as shown under room temperature storage conditions (Table-3). As DAS increased at 4°C the population count declined slowly but the population count was much higher as compared to room temperature conditions. At 120 DAS the population count at 4°C was much higher as compared to the corresponding period under room temperature. The comparison of the effect of two storage conditions on the viability of *P. fluorescens* in the formulation VPf is depicted in Figure 1.

This is in conformity with the fact that 4 ° C storage temperatures is best suitable for storage of carrier based inoculants because of higher level of moisture content in the carrier inoculants[15]. Freeze drying of *Pseudomonas* strains has been explored as a method to deliver viable cells for crop protection[3].

P. fluorescens became metabolically inactive at a lower temperature (4^0C), which might have acted as a barrier for proper physico-biochemical activities essential for population build up of the bioagent in contrast to room temperature storage conditions where the population load increased and reached peak at 30 DAS.

Assessment of the methods of application of formulations for effect on rhizosphere competence :

The population of *P. fluorescens* in the rhizosphere at different days after transplanting (DAT) when the formulations were applied by the different methods are shown in Figures 2a, 2b and 2c. The results revealed that the population densities of *P. fluorescens* increased significantly from 30 DAT upto 60 DAT and decreased again at 90 DAT in all the

Table-3. Population load of *P. fluorescens* in different substrate carrier based formulations under storage at 4°C

Substrate Carrier Bioagent based Formulation	**Population load of *P. fluorescens* ($\times 10^8$ cfu/g) at different days after storage (DAS)**				
	15 DAS	**30 DAS**	**60 DAS**	**90 DAS**	**120 DAS**
VPf	930.00 (10.97)	926.33 (10.97)	920.33 (10.96)	910.33 (10.96)	890.33 (10.95)
FPf	890.00 (10.95)	880.67 (10.94)	876.33 (10.94)	870.67 (10.94)	865.67 (10.94)
DPf	845.33 (10.93)	843.67 (10.93)	838.67 (10.92)	830.33 (10.92)	820.33 (10.91)
RbPf	667.67 (10.82)	664.33 (10.82)	660.33 (10.82)	650.33 (10.81)	630.33 (10.80)
Effect of Substrate Carrier	S.Ed± 0.005 $CD_{0.05}$ 0.010	S.Ed± 0.002 $CD_{0.05}$ 0.005	S.Ed± 0.003 $CD_{0.05}$ 0.006	S.Ed± 0.002 $CD_{0.05}$ 0.005	S.Ed± 0.004 $CD_{0.05}$ 0.007

Figures within parenthesis indicate log transformed values

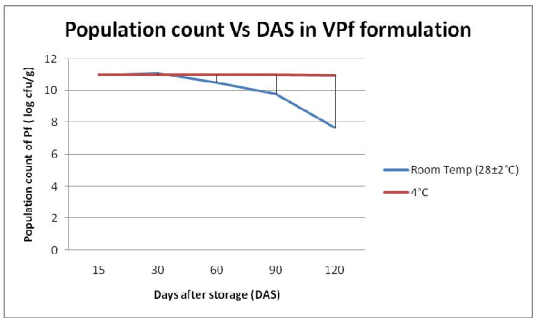

Figure 1 Comparison of population load of VPf at different DAS at room temperature and 4°C

formulations applied by different methods of application. The food base present in the formulation along with higher amount of root exudates might have provided nutrients required for initial growth and reduced nutrient competition with resident microbes which resulted in population build up and greater rhizosphere competence at 60 DAT. At 90 DAT, the population of the bioagent declined which could be justified by the change in composition of the root exudates which in turn results in temporal and spatial difference in the the rhizosphere microbial community composition and activity[5,12]. In all the formulations applied, the highest population densities were recorded in the seed + root + soil treatment (Figure 3) which is due to all round placement of the bioagent on the seed, from which the bioagent migrated to the elongating roots, the most favourable site for colonization and in soil the repertoire of both beneficial and pathogenic microbes, all of which in combination, created more favourable condition for maximum colonization giving a better competitive advantage over other rhizosphere microflora. This finding is corroborated by the dose response - population relationship, that is, high initial inoculum density, reflects higher recovery of population density of the bioagent in the rhizosphere soil[6].

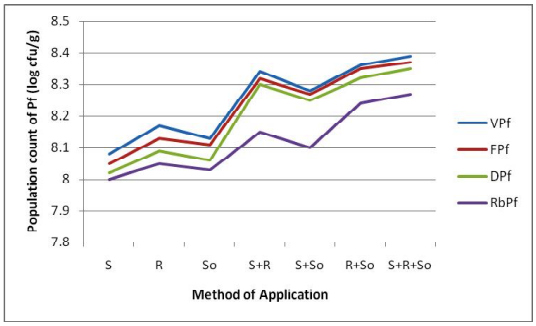

Figure 2 a

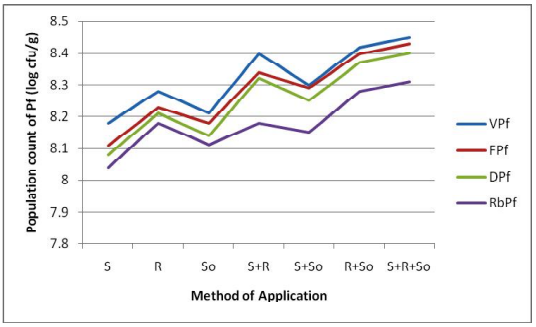

Figure 2 b

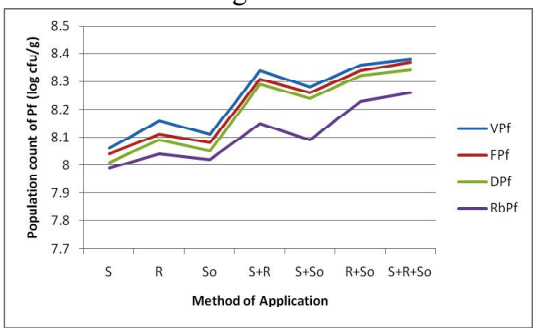

Figure 2c

Population of *P. fluorescens* in the rhizosphere soil at 30 DAT (Figure 2a), 60 DAT (Figure 2b) and 90 DAT (Figure 2c) when the formulations are applied by different methods.

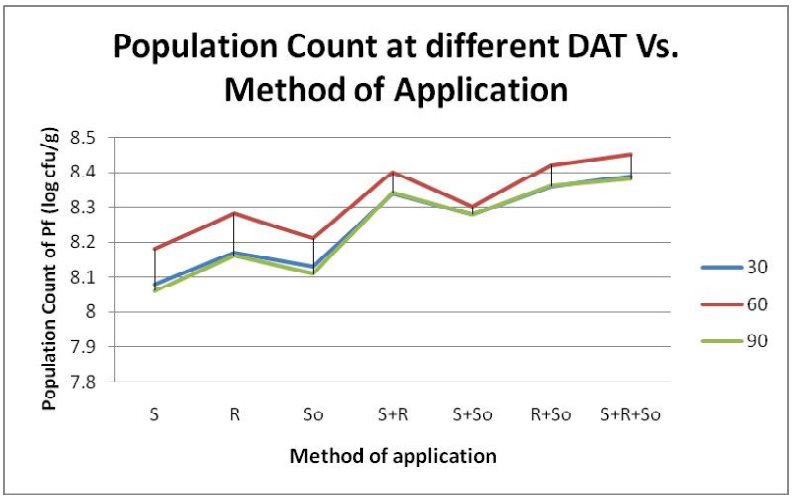

Figure 3 Rhizosphere population of *P. fluorescens* at different DAT when VPf is applied by different methods

Among individual methods of application, root treatment was more suitable for higher population densities of the bioagent, possibly due to easier colonization of the roots by the introduced bio inoculant, even in the presence of already present resident rhizosphere organisms. This is supported by the fact that niche overlap between an inoculant and resident bacteria appears to be limited, even with resident organisms that are phylogenetically closely related to the inoculant. Spatial separation and nutrient versatility are important factors contributing to this restricted overlap[4].

The present study underscores the importance of standardizing organic formulations supporting maximum shelf life and higher rhizosphere competence for making biofertilizers a commercial venture[10]. Vermicompost used as substrate carrier and a storage condition of 4°C served best in giving a higher shelf life. Again, the integration method of application of the formulation (seed+ root+ soil method) was found to give a higher rhizosphere competence to the inoculated bioagent. The period of 60 DAT was found to be the most suitable time of population build up when the rhizosphere population of the bioagent was at its peak. However, large scale field studies will be needed to study its effectiveness in promoting plant growth under different biotic and abiotic conditions.

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