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Shelf life studies on solid and liquid formulations of *Trichoderma harzianum* and *Pseudomonas fluorescens*

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Abstract
A bioformulation with increased shelf life is the essential requirement in the development of formulations of biocontrol agents. To accomplish this, the current study involved isolation of *Trichoderma harzianum* (GRT4) and *Pseudomonas fluorescens* (PF4) from the groundnut rhizosphere followed by *in-vitro* testing of above biocontrol agents each against *Macrophomina phaseolina* and *Sclerotium rolfsii*. Finally, the effective antagonistic isolates were formulated using talc, kaolinite, gypsum (solid carrier materials) and glycerol, polyvinylpyrrolidone (PVP) and soybean oil (liquid carrier materials). After development, the formulations were stored at 4 ºC and at room temperature for evaluating their shelf life periodically at 30, 60 and 90 d after storage. In our study, we found talc-based formulations of *T. harzianum* and *P. fluorescens* maintained higher inoculum densities (9.3×10^7 cfu g^{-1}, 11.9×10^7 cfu g^{-1} & 8.4×10^8 cfu g^{-1}, 10.5×10^8 cfu g^{-1}) both at room temperature and at 4ºC, respectively even after 90 d of storage when compared with other solid formulations tested. In case of liquid formulations of *P. fluorescens* tested, glycerol amended nutrient broth maintained higher inoculum densities (6.5×10^8 cfu ml^{-1}, 10.3×10^8 cfu ml^{-1}) both at room temperature and at 4 ºC respectively after 90 days of storage.

Keywords: Shelf life *Tricoderma harzianum, Pseudomonas fluorescens,* Kaolinite and Polyvinylpyrrolidone (PVP)

Introduction
Groundnut is the major oil seed crop grown under irrigated condition in India covering an area of 4.89 M ha with a production of 10.10 M t and 2.06 t ha^{-1} average productivity. In Andhra Pradesh lower production of groundnut is majorly due to soil borne diseases like stem rot (*S.rolfsii*) and dry root rot (*M. phaseolina)* among fungal diseases (Directorate of Economics and Statistics, 2019-20).

M. phaseolina is known to cause wilt as well as root, stem, peg and pod rots and leaf spots on seedlings and on older plants. It also causes seedling blight, root rot and charcoal rot diseases on more than 500 plant species from more than 100 families distributed worldwide (Rangaswami and Mahadevan, 2008) [19].

The pathogen *S. rolfsii* Sacc., commonly occurs in the tropics, sub-tropics and other warm temperate regions of the world causing root rot, stem rot, wilt and foot rot on more than 500 plant species including almost all the agricultural and horticultural crops with yield losses ranging from 25 per cent, but sometimes it reaches 80-90 per cent (Grichar and Bosweel, 1987) [12].

Biocontrol agents like *P. fluorescens* and *Trichoderma* spp. have been assessed for their efficacy against *M. phaseolina* and *S. rolfsii* by many researchers. Application of biocontrol agents for plant protection requires much care while handling without losing their efficacy. For this formulating the biocontrolagnets with suitable carrier material which could ensure longer shelf life without compromising viability of the biocontrol agent is the prime requirement. Different solid carriers that are being used to develop formulations of *Trichoderma* spp. and *P. fluorescens* are alginate prills, vermiculite-wheat bran, pesta granules, talc, coffee husk, talc with addition of glycerol, press mud, corn and sugarcane bagasse, vermicompost, farmyard manure and mustard oil cake (Bora *et al*., 2016) [6].

In addition to solid carrier materials there are several liquid carrier materials under usage which includes trehalose, polyvinylpyrrolidone and glycerol for the development of liquid bioformulation of *P. fluorescens* (Navaneetha *et al*., 2015) [16].

Materials and Methods

Isolation of Biocontrol Agents

Biocontrol agents *Trichoderma* spp. and *P. fluorescens* were isolated from rhizosphere soil of healthy groundnut plants following serial dilution technique on *Trichoderma* specific medium and King's B medium respectively. Isolates of *Trichoderma* spp. and *P. fluorescens* were purified and maintained on agar slants. Identification of *Trichoderma* spp. and *P. fluorescens* isolates was done by morphological and biochemical characters.

Testing Antagonistic Potential of Fungal and Bacterial Antagonist against Test Pathogen *In vitro*

Screening of *Trichoderma* isolates for their antagonistic potential against *M. phaseolina* and *S. rolfsii* was conducted following dual culture method. For this twenty ml of sterilized potato dextrose agar (PDA) was poured into Petri plate of 9 cm diameter aseptically. Mycelial discs measuring 5 mm diameter from four-day old cultures of both fungal antagonist and the test pathogen were inoculated at 7 cm apart leaving 1 cm from periphery. Later the plates were kept in an incubator at 25 ± 2ºC for incubation. Readings were recorded when the pathogen in the monoculture control grown fully (Morton and Straube, 1955) [15].

For finding the potential bacterial antagonist, the bacterium was streaked as 5 cm line at 1 cm away from the periphery of Petri plate containing 20 ml of equal amounts of PDA and nutrient agar (NA). Later, 5 mm mycelial disc of test pathogen was placed in the centre of the Petri plate. Inoculated plates were incubated at 25 ± 2ºC, observations were recorded as zone of inhibition after the pathogen completely grown in the control plate. Per cent inhibition of mycelial growth of test pathogen over the control was calculated using the formula given by Vincent (1927) [23].

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent reduction in growth of test pathogen.

C = Radial growth (mm) in monoculture check.

T = Radial growth (mm) in dual cultured plates.

Testing Compatibility between Fungal and Bacterial Antagonists *In vitro*

The compatibility between potential fungal and bacterial antagonists was determined by dual culture technique maintaining separate controls each for bacteria and fungi. The similar methodology was followed as described above.

Mass Multiplication of Potential Biocontrol Agents:

Potential *Trichoderma* sp. was mass multiplied on potato dextrose broth (PDB) by inoculating 4-5 discs of three-day old *Trichoderma* culture and later incubating at 25 ± 2°C for 7 days in a shaking incubator. Potential *P. fluorescens* isolate was mass multiplied on nutrient broth (NB). For this, two loops full of bacterial culture was added to the NB medium and incubated at 25 ± 2°C for three days in a shaking incubator.

Preparation of Talc, Kaolinite and Gypsum Based Formulation of Potential *Trichoderma* Isolate.

For preparing solid formulations of *Trichoderma*, one kg of solid carrier material (talc powder / kaolinite /gypsum) was taken in a metal tray under aseptic conditions and pH was adjusted to 7.0 by adding $CaCo_3$ at the rate of 15 g kg^{-1}. Later, ten g of carboxy methyl cellulose (CMC) was added to 1 kg of solid carrier material, mixed well and the final mixture was autoclaved for 30 minutes at 121°C, 15 PSI of pressure for 2 successive days. The mycelial mat of fungus along with broth was homogenized, mixed with solid carrier material @ 1:4 (w/w) and later shade dried until it attained 20 per cent moisture content under aseptic conditions. After mixing, the clumps were broken, homogenized uniformly and the mixture was packed in polypropylene bags. The packed bags were sealed and stored at room temperature and at 4ºC in refrigerator for shelf life studies (Vidhyasekaran and Muthamilan, 1995) [22].

Preparation of Talc, Kaolinite and Gypsum Based Formulation of Potential *P. fluorescens* Isolate

Formulation of potential *P. fluorescens* using solid carrier (talc / kaolinite / gypsum) was prepared by inoculating two loops full of bacteria into NB and incubated in a rotary shaker at 150 rpm min^{-1} for 48 hours at room temperature (25 ± 2ºC). One kg of solid carrier material (talc / kaolinite / gypsum) was taken in a metal tray under aseptic conditions and its pH was adjusted to 7.0 by adding $CaCo_3$ at the rate of 15 g kg^{-1}. Later, ten gm of carboxymethyl cellulose (CMC) was added to 1 kg of solid carrier material, mixed well and the mixture was autoclaved for 30 min. at 121°C, 15 PSI of pressure for 2 successive days. Bacterial suspension was mixed with solid carrier material + CMC mixture @ 400 ml kg^{-1} of carrier material under aseptic conditions. After drying to 35 per cent moisture content overnight under aseptic conditions, the mixture was packed in polypropylene bags and sealed. Later, the formulation was stored at room temperature and at 4ºC in refrigerator for shelf life studies.

Preparation of Glycerol, Soybean Oil and Polyvinylpyrrolidone (PVP) Based Formulation of Potential *P. fluorescens* Isolate

Formulation of potential *P. fluorescens* using glycerol, soybean oil and polyvinylpyrrolidone (PVP) as liquid carrier material was prepared following the method as described by Manikandan *et al*. (2010) [13]. For this, sterilized NB was prepared with the addition of two per cent liquid carrier material (glycerol / soybean oil / PVP). To this, 1 ml of log phase (3×10^{10} cfu ml^{-1}) culture of bacteria was inoculated and incubated at room temperature. The formulation was sealed in glass bottles and stored at room temperature and at 4ºC for shelf life studies.

Shelf Life Studies on Formulations of Potential *Trichoderma* and *P. florescens* isolates *In vitro*

The prepared formulations of potential fungal and bacterial antagonists using solid (talc, kaolinite and gypsum) and liquid (glycerol, soybean oil and PVP) carriers were evaluated for their viability at 30, 60 and 90 d of storage both at room temperature and at 4ºC. To do this, one gm or one ml of sample was drawn from each formulation and transferred into 10 ml of sterilized water in test tube and shaked thoroughly for 3 minutes to make 10^{-1} dilution. From this one ml suspension of stock solution was transferred into the next test tube containing 9 ml distilled water using a sterilized pipette and again shaked to make 10^{-2} dilution. The same process is repeated up to 10^{-7} dilution for fungi and 10^{-9} for bacteria. For fungal formulations, one ml of suspension was taken from 10^{-}

[7] the dilution and transferred into Petri plates containing 20 ml of sterilized PDA and gently shaken to spread evenly. For solid bacterial formulations, 100 µl of suspension was taken from the dilution of 10^{-9} and transferred into Petri plates containing 20 ml sterilized KB medium. In case of liquid bacterial formulations, 10 µl of suspension was taken from 10^{-9} dilution and transferred into Petri plates containing 20 ml of sterilized KB medium. These Petri plates were incubated at 25± 2ºC for 24 hours for development of bacterial colonies and 48 hours for the development of fungal colonies.

Later, the populations of the bacterial and fungal biocontrol agents were evaluated by serial dilution technique using formula given by Aneja (2003) [1].

$$\text{Number of cfu/g} = \frac{\text{Number of colonies}}{\text{Amount plated} \times \text{dilution}}$$

Results and Discussion

Isolation of *Trichoderma* spp.

A total of six *Trichoderma* isolates and five *P. fluorescens* isolates were isolated from rhizosphere soil samples of healthy groundnut plants obtained from different mandals of Chittoor and S.P.S.R. Nellore districts. The isolates were purified and maintained on slants for further experimental studies. The Trichoderma isolates were identified with the help of available literature (Bisset, 1984, 1991 a, b, c) [2, 3, 4, 5]. All the bacterial isolates were found Gram negative in reaction and emitted fluorescent light when visualized under U. V. light. Based on these characters the organism was identified as *P. fluorescens*. The *Trichoderma* isolates were indicated as GRT1, GRT2, GRT3, GRT4, GRT5, GRT6 and the *P. fluorescens* isolates were indicated as PF1, PF2, PF3, PF4 and PF5. Previously, Elangovan and Gnanamanickam (1990) [8] isolated eighty-five strains of *P. fluorescens* in paddy fields from Chenglepet district of Tamil Nadu.

***In vitro* Screening of Antagonistic *Trichoderma* spp. and *P. fluorescens* against *M. phaseolina*:** Results from the dual culture studies reviled that, four isolates of *Trichoderma* spp. which showed maximum mean inhibition per cent when dual cultured with four isolates of *M. phaseolina viz.*, GRT5 (59.48%), GRT2 (59.38%), GRT1 (59.27%) and GRT4 (58.96%) were considered as effective *Trichoderma* isolates and used for further studies. Similar studies were conducted by Ramezani (2008), who studied efficacy of four fungal bioagents *viz.*, *T. hamatum*, *T. harzianum*, *T. polysporum* and *T. viride* under *in vitro* conditions against the brinjal root rot pathogen, *M. phaseolina*. He reported that *T. harzianum* produced the maximum inhibition zone of 18.20 per cent compared to the minimum of 7.30 per cent by *T. hamatum*.

Two isolates of *P. fluorescens* PF3 (36.11%) and PF4 (34.45%) that showed maximum mean inhibition per cent when dual cultured with four isolates of *M. phaseolina* were considered as effective antagonistic bacterial isolates and taken for further studies. Results agreed with the findings of Shanmugam *et al*. (2002) [21] who reported that strain Pf1 significantly inhibited mycelial growth of *M. phaseolina* under *in vitro* conditions.

Screening of Antagonistic *Trichoderma* spp. and *P. fluorescens* against *S. rolfsii*

Four isolates of *Trichoderma* which showed maximum mean inhibition per cent when dual cultured with *S. rolfsii viz.*, GRT5 (74.69%), GRT2 (73.85%), GRT1 (71.56%) and GRT4 (70.42%) were considered as effective *Trichoderma* isolates and were taken for further studies. In a similar study, Rekha *et al.* (2012) had also reported that in dual culture *Trichoderma* showed maximum reduction of mycelial growth of *S. rolfsii*.

Results from the dual culture study revealed that, two isolates of *P. fluorescens* PF3 (40.93%) and PF4 (34.82%) that showed maximum mean inhibition per cent when dual cultured with *S. rolfsii* were considered as effective isolates and used for further studies. Similar results were obtained by Ganesan and Sekar (2012) [10], who evaluated biocontrol activity of eleven *Pseudomonas* isolates against *S. rolfsii*, causing stem rot disease in groundnut, by dual culture method. Among all isolates tested, seven isolates showed above 68 per cent of inhibition.

Evaluation of Compatibility between Potential *Trichoderma* sp. and *P. fluorescens*

From the dual culture studies, the antagonistic fungal isolate GRT4 (*T. harzianum*) and antagonistic bacterial isolate PF4 (*P. fluorescens*) showed minimum inhibition per cent (21.48%) which was significantly differed with the remaining treatments as shown in the table1. After prolonged incubation for ten days, the inhibition zone disappeared and mycelium of GRT4 was overgrown on bacteria. The results agree with the findings of Mishra *et al.* (2013) [14], who reported that *Trichoderma* isolate (PBAT-43) and *Pseudomonas* isolate (PBAP-27) emerged as most compatible and efficient combination in dual culture and therefore used in development of mixed formulations.

Table 1: *In vitro* evaluation of compatibility between highly potential bacterial antagonists PF3, PF4 and fungal antagonists GRT1, GRT2, GRT4 and GRT5

Bacterial antagonist	GRT1		GRT2		GRT4		GRT5	
	*Radial growth of fungal antagonist (cm)	*Percent inhibition over control	*Radial growth of fungal antagonist (cm)	*Per cent inhibition over control	*Radial growth of fungal antagonist (cm)	*Per cent inhibition over control	*Radial growth of fungal antagonist (cm)	*Per cent inhibition over control
PF3	1.93	57.04[a] (49.03)	2.17	51.85[b] (46.04)	2.53	43.70[d] (41.36)	2.73	39.26[e] (38.78)
PF4	2.30	48.89[c] (44.34)	2.37	47.41[c] (43.49)	3.53	21.48[g] (27.59)	3.23	28.15[f] (32.02)
Trichoderma monoculture	4.50	0.00 (0.00)						
SEm ±	0.08	0.56						
CD	0.25	1.69						

*Values are means of three replications; Values in the parenthesis are angular transformed values; Values with common letter are not significantly different

Preparation of Solid and Liquid Formulations of Potential *T. harzianum* Isolate GRT4

Talc, kaolinite and gypsum-based formulations of potential antagonistic *T. harzianum* isolate GRT4 were prepared. The population was estimated regularly at 30, 60 and 90 d after storage both at room temperature and at 4ºC. Previously, Gaur *et al*. (2005) [11] used the talc based formulation of *T. harzianum* multiplied on yeast molasses broth containing 2 $\times 10^6$ cfu g^{-1} for root rot control in chickpea.

Preparation of Solid and Liquid Formulation of Potential *P. fluorescens* Isolate PF4

The potential *P. fluorescens* isolate PF4 was formulated in solid (talc, kaolinite and gypsum) and liquid (glycerol, PVP and soybean oil) carrier materials following the above given procedure. The population of bacteria was periodically estimated at regular intervals (30, 60 and 90 d) both at room temperature and at 4 ºC. In a similar study, Gade *et al*. (2014) [9] developed solid formulation of *P. fluorescens* using talc, spent mushroom substrate, lignite, charcoal, farmyard manure and fly ash as carrier material. Similarly, Manikandan *et al*. (2010) [13] tested different chemicals such as trehalose, polyvinylpyrrolidone (PVP) and glycerol for the development of liquid bioformulation of *P. fluorescens*.

Shelf Life Studies on Formulations of *T. harzianum* (GRT4)

The shelf life studies of formulations of *Trichoderma* with different carrier's *viz.*, talc, kaolinite and gypsum were carried out by periodical testing at 0, 30, 60 and 90 d after preparation at room temperature (30 ºC) and at 4 ºC. The results were expressed as cfu g^{-1}/ml^{-1} of bioformulation. The treatments were replicated thrice and the data is presented in the table 2, Fig. 1a and 1b.

Initial colony counts were high in all the formulations just after preparation. Initial colony count at room temperature in talc, kaolinite and gypsum-based formulations were 28.7× 10^7, 27.3× 10^7 and 26 × 10^7 cfu g^{-1}, respectively.

At 30 d after storage, talc formulations retained higher inoculum density (24.3 × 10^7 cfu g^{-1}) at 4 ºC and lower inoculum density (23.5 × 10^7 cfu g^{-1}) at room temperature. Further, talc formulations retained higher inoculum density when compared with rest of the formulations.

After 60 d of storage, talc formulations maintained higher inoculum density (19.6 × 10^7 cfu g^{-1}) at 4 ºC and lower inoculum density (18.7 × 10^7 cfu g^{-1}) at room temperature. Here again, talc formulations only retained higher inoculum density and the others were poor in viable propagule retention with increase in storage.

Even at 90 d after storage, talc formulations retained higher inoculum density (11.9 × 10^7 cfu g^{-1}) at 4 ºC and lower inoculum density (9.3 × 10^7 cfu g^{-1}) at room temperature. Based on above observations, we found talc was the highly suitable solid carrier material for formulation development and it should be stored at lower temperatures for increased shelf life.

The results were in comparison with the findings of Prasad and Rangeshwaran (2000) [17] who studied shelf life and bioefficacy of *T. harzianum*, which was formulated in various carrier material and concluded that talc and kaolin are better carriers of *T. harzianum* and retained more than 10^6 propagules upto 90 days of storage period.

Table 2: Shelf life of *Trichoderma harzianum* (GRT4) in solid and liquid formulation using different carriers

Storage days	Population of *Trichoderma* (x 10^7cfu g^{-1}) in the bioformulation					
	Talc		Kaolinite		Gypsum	
	30 ºC	4 ºC	30 ºC	4 ºC	30 ºC	4 ºC
0	28.7	28.7	27.3	27.3	26.0	26.0
30	23.5	24.3	19.2	21.0	21.0	22.4
60	18.7	19.6	12.8	14.7	16.8	17.8
90	9.3	11.9	4.9	6.4	7.4	9.7

* Values are means of three replications

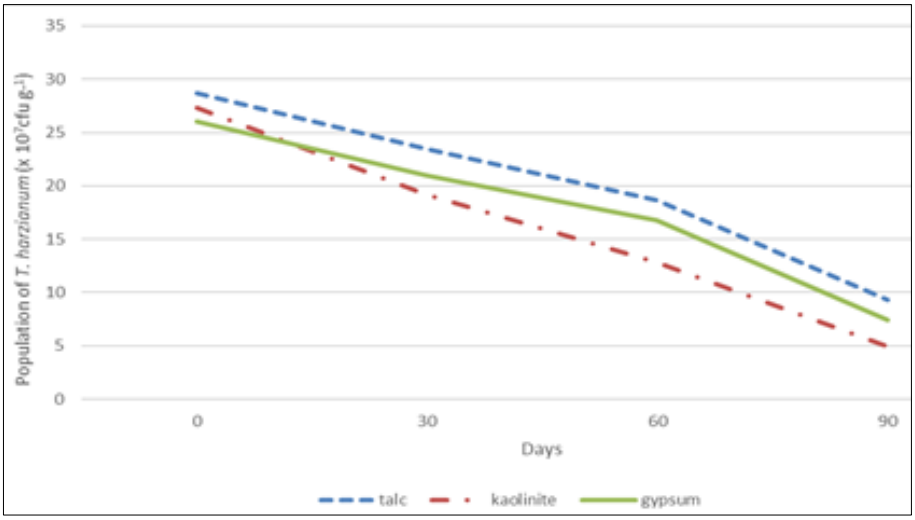

Fig 1a: Shelf life of solid formulations of *T. harzianum* (GRT4) at room temperature (30ºC)

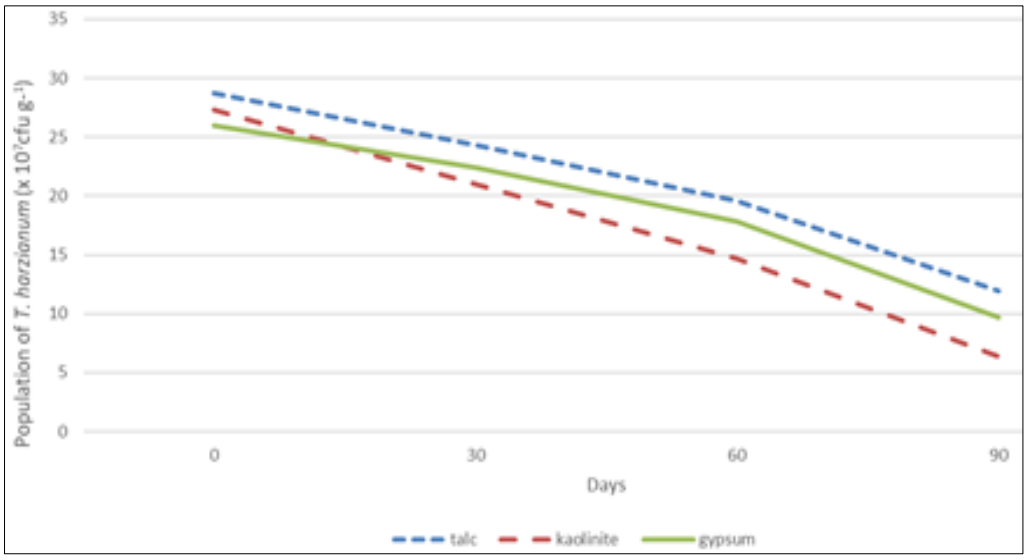

Fig 1b: Shelf life of solid formulations of *T. harzianum* (GRT4) at 4 ºC

Shelf Life Studies on Solid Formulations of *P. fluorescens* (PF4)

To check the shelf life of *P. fluorescens*, bio formulations were stored at room temperature i.e at 30°C and at 4°C. It was evident from the data showed in the table 3, Fig. 2a and 2b that just after preparation of P. *fluorescens* formulations, all the formulations retained higher inoculum density and there after started declining with increase in storage period.

Initial colony count just after preparation in talc, kaolinite and gypsum-based formulations were 21.2 × 10^8, 20.3 × 10^8 and 20.8 × 10^8 cfu g^{-1}, respectively.

At 30 d after storage, talc formulations retained higher inoculum density (18.0 × 10^8 cfu g^{-1}) at 4 ºC and lower inoculum density (16.7 × 10^8 cfu g^{-1}) at room temperature. Talc formulations retained higher inoculum density when compared with rest of the formulations.

After 60 d of storage, talc formulations maintained higher inoculum density (13.5 × 10^8 cfu g^{-1}) at 4 ºC and lower inoculum density (12.1 × 10^8 cfu g^{-1}) at room temperature. Here again, talc formulations only retained higher inoculum density and the others were poor in viable propogules retention with increase in storage.

Even at 90 d after storage, talc formulations retained higher inoculum density (10.5 × 10^8 cfu g^{-1}) at 4 ºC and lower inoculum density (8.4 × 10^8 cfu g^{-1}) at room temperature. Based on the above observations, we found that talc was the highly suitable solid carrier material for formulation development. Similar results were obtained by Gade *et al.* (2014) [9], who reported that talc based bioformulations maintained better population of the *P. fluorescens* (18 × 10^8 cfu g^{-1}) upto 6 months of storage at 25± 2ºC when compared with lignite, fly ash and spent mushroom substrate based formulations.

Table 3: Shelf life of solid formulations of *P. fluorescens* (PF4)

Storage days	Population of *P. fluorescens* (x 10^8 cfu g^{-1}) in the bioformulation					
	Talc		Kaolinite		Gypsum	
	30 ºC	4 ºC	30 ºC	4 ºC	30 ºC	4 ºC
0	21.2	21.2	20.3	20.3	20.8	20.8
30	16.7	18.0	12.9	14.5	14.6	16.1
60	12.1	13.5	7.6	8.8	9.5	10.9
90	8.4	10.5	5.8	6.1	6.2	8.4

* Values are means of three replications

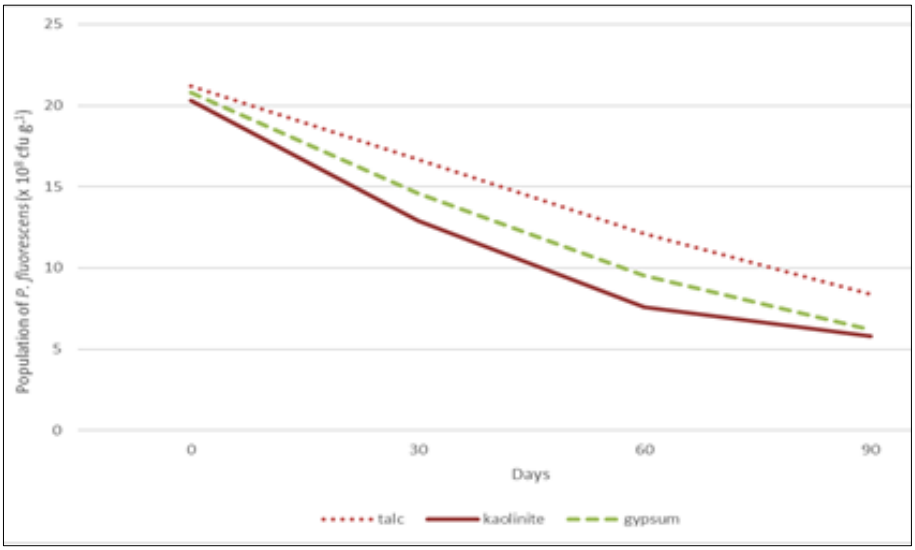

Fig 2a: Shelf life of solid formulations of *P. fluorescens* (PF4) at room temperature (30 ºC)

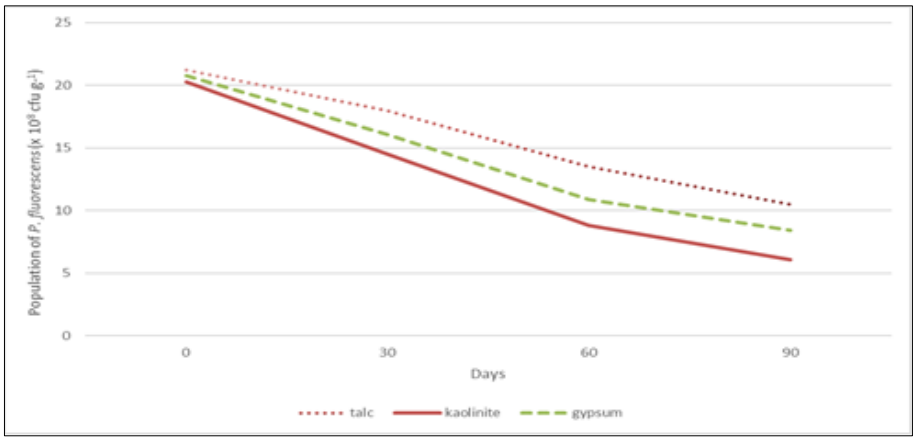

Fig 2b: Shelf life of solid formulations of *P. fluorescens* (PF4) at 4 ºC

Shelf Life Studies on Liquid Formulations of *P. fluorescens* (PF4)

To check the shelf life of liquid formulations of *P. fluorescens*, the bioformulations were stored at room temperature and at 4°C for 30, 60 and 90 days. It was evident from the data showed in the table 4, Fig. 3a and 3b that all the formulations retained higher inoculum density just after preparation and there after started declining with increase in storage period like that of solid formulations.

Initially colony count of *P. fluorescens* just after preparation in glycerol, PVP and soybean oil based formulations and nutrient broth was 41.5 × 10^8, 40 × 10^8, 38.6 × 10^8 and 38.0 × 10^8 cfu ml^{-1}, respectively.

After 30 d of storage, glycerol retained higher inoculum density (34.2 × 10^8) at 4 ºC and reduced inoculum density (30.8 × 10^8) at room temperature. Among all talc retained good number of viable propagules followed by PVP.

After 60 days of storage, again glycerol retained higher inoculum density (19.6 × 10^8) at 4 ºC and lower inoculum density (17.3 × 10^8) at room temperature. Once again talc found superior followed by PVP.

After 90 days of storage, at low temperature (4 °C) in glycerol populations of *P. fluorescens* was (10.3 × 10^8) which drastically reduced (6.5 × 10^8) at room remperature. We observed no colonies from NB formulations suggesting it to be inferior formulation among all formulations tested. By observing the above data, we found glycerol amended NB was the best liquid carrier material for formulation development followed by PVP amended NB.

The observed results were similar with the reports of Manikandan *et al*. (2010) [13], who tested the viability of *P. fluorescens* Pf1 in nutrient broth (NB) and King's B broth (KBB) by the addition of trehalose (10Mm) or polyvinylpyrrolidone (PVP) or 2 per cent glycerol (10Mm) in 1 L of broth. They reported that addition of glycerol (10Mm) to NB resulted in greater level of Pf1 viable cells (9.50 x 10^7 cfu ml^{-1}) up to 180 days of storage at room temperature compared to other amendments and KBB.

Table 4: Shelf life of liquid formulations of *P. fluorescens* (PF4)

Storage days	Population of *P. fluorescens* (x 10^8 cfu ml^{-1}) in the bioformulation							
	Glycerol		PVP		Soybean oil		Broth alone	
	30 ºC	4 ºC	30 ºC	4 ºC	30 ºC	4ºC	30ºC	4ºC
0	41.5	41.5	40.0	40.0	38.6	38.6	38.0	38.0
30	30.8	34.2	28.6	31.4	24.8	27.9	9.4	11.7
60	17.3	19.6	14.9	17.8	11.5	13.2	3.1	4.8
90	6.5	10.3	5.2	8.4	4.9	6.6	0	0

* Values are means of three replications

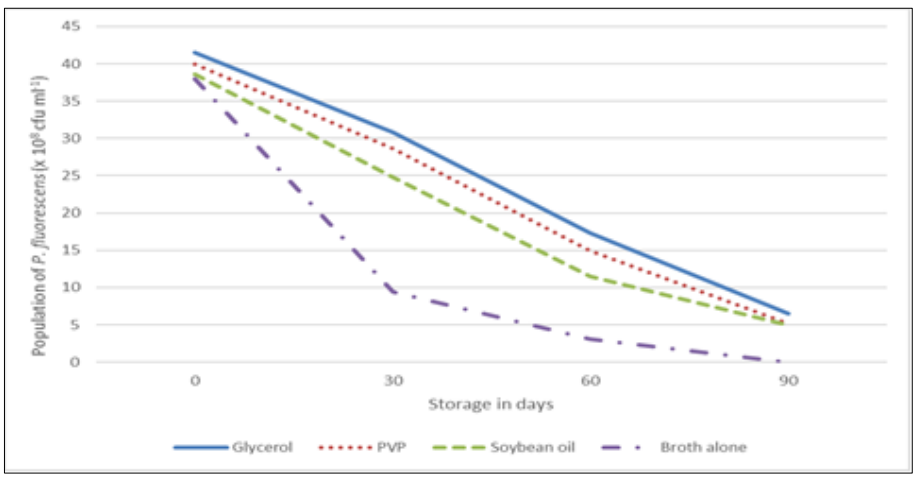

Fig 3a: Shelf life of liquid formulations of *P. fluorescens* (PF4) at room temperature (30ºC)

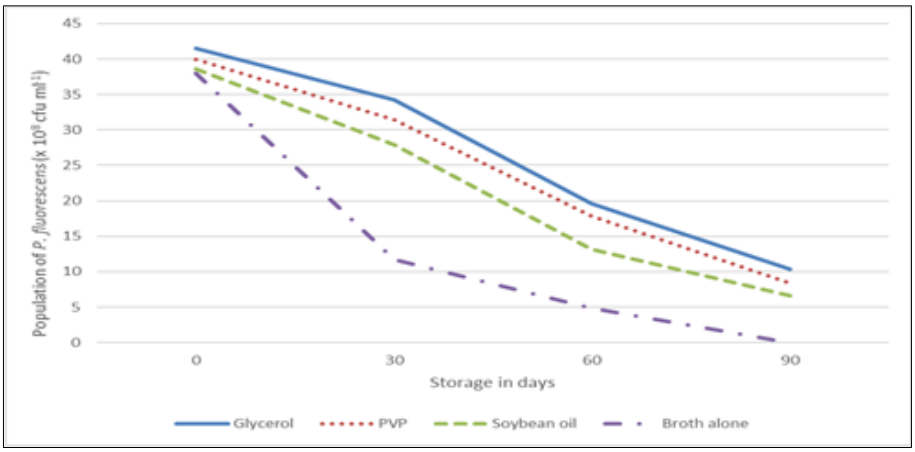

Fig 3b: Shelf life of liquid formulations of *P. fluorescens* (PF4) at 4ºC

Conclusion

A formulation with increased shelf life is must for successful application of biocontrol in plant disease management. To test the best performing carrier material, we tested three different solid (talc, kaolinite, gypsum) and liquid (glycerol, soybean oil, PVP) carriers for formulating potential *Trichoderma* sp. and *P. fluorescens* isolates. From the efficacy and compatibility studies, we obtained fungal antagonist *T. harzianum* isolate (GRT4) as promising one, which was later formulated using talc, kaolinite, and gypsum carrier materials. Similarly, the effective bacterial antagonist *P. fluorescens* isolate PF4 was also formulated using talc, kaolinite, gypsum (solid carrier materials) and glycerol, PVP and soybean oil (liquid carrier materials).

To test good storage properties of carrier materials under study, all the formulations were stored at 4ºC and at room temperature (30 ºC) for evaluating their shelf life periodically at 30, 60 and 90 d after storage. Form the shelf life studies on *Trichoderma* formulations, we found talc formulations maintained higher inoculum density even after 90 d of storage both at room temperature (9.3×10^7 cfu g-1) and at 4ºC (11.9×10^7 cfu g-1) when compared to kaolinite and gypsum formulations.

Similarly, talc formulations of *P. fluorescens* also maintained higher inoculum density even after 90 d of storage both at room temperature (8.4×10^8 cfu g-1) and at 4ºC (10.5×10^8 cfu g-1) when compared to kaolinite and gypsum formulations. When it comes to liquid formulations, glycerol amended NB liquid formulations of *P. fluorescens* maintained higher inoculums density even after 90 d of storage both at room temperature (6.5×10^8 cfu g^{-1}) and at 4ºC (10.3×10^8 cfu g^{-1}) when compared to PVP and soybean oil formulations. Finally, we found talc was best solid carrier material for formulating both fungal and bacterial formulations. In case of liquid carrier materials tested, we found glycerol amended NB was best among all and NB alone was worst in formulations development.

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