Chapter 8

Development of inoculum formulations

8.1 Development of phosphate-solubilizing bacterial inoculum formulations and test their shelf life and phosphate solubilization efficiency

For sustainable agriculture, substitution of high priced chemical fertilizers with eco-friendly biofertilizers is the most desired practice. The microbial inoculation in the form of seed inoculation has been proved beneficial for the maintenance of soil fertility, but the use of suitable carrier, capable of supporting high viable microbial population for a prolonged duration is most important. Keeping the above factors in view, inoculum formulations of selected phosphate-solubilizing bacterial isolates *P. cypripedii* and *P. plecoglossicida* were developed. For the inoculum formulations development, rock phosphate, fly ash, charcoal and vermiculite were used as a carrier materials. Efforts were being made to check compatibility of these carrier materials with PSB isolates, and there support as a carrier material for the viability of bacterial isolates at different temperatures for a long period of storage. The final moisture content was adjusted to 30 % of the carrier materials before packing into separate bags.

Results in Fig 8.1, 8.2, 8.3 and 8.4 showed the effect of different temperature over time and carrier material types on population of bacteria in four carrier materials. The initial population density was 10.24 - 10.51 log cfu g^{-1} of carrier material for *P. cypripedii* and *P. plecoglossicida*. As the bacterial population in logarithmic scale (Fig. 8.1, 8.2, 8.3 and 8.4) in carrier materials at 37 °C showed an upward trend up to 15 days of incubation after that bacterial population in all the carrier materials was started decreased (about one logarithmic unit in one month) while there was no increase in bacterial population stored at 4 °C.

At the end of 270 days of storage at 4 °C, the logarithm of population in carrier rock phosphate was up to 7.0 cfu g^{-1}, in fly ash 5.61 cfu g^{-1}, in charcoal 6.23 cfu g^{-1} and in vermiculite 5.59 cfu g^{-1} respectively. Similarly at 37 °C, after 270 days of storage, the mean

logarithm of population in carrier rock phosphate was 5.53 cfu g^{-1}, in fly ash 4.57 cfu g^{-1}, in charcoal 4.49 cfu g^{-1} and in vermiculite 4.43 cfu g^{-1} respectively. Results showed that viability of bacterial isolates were higher at 4 °C compared to 37 °C. Among the different carrier materials tested, rock phosphate formulations supported maximum viability of both the isolates up to 270 days of storage periods of 4 °C and 37 °C compared to other carrier materials tested. Purity of carrier materials in context to contamination was tested in control (carriers without bacterial inoculation) of all carriers after regular interval of one month and it was observed that control of each carrier material was free from any microbial load up to 270 days of storage at 4 °C and 37 °C. The loss in moisture content up 6-7 % was observed at 37 °C and there was no loss in moisture content of all the formulations at 4 °C.

Rock phosphate as a carrier material support maximum viability for bacterial isolates, at 37 °C and 4 °C, the 270 days old inoculum formulations of RP was tested for their ability to P solubilization and plant growth promotion activities and compared with fresh culture. Results in the Table 8.1 and 8.2 showed that P solubilization, and plant growth promotion activities of both the inoculums formulations at 4 °C and 37 °C was comparable to the fresh culture activities. Results suggested that long time storage of these formulations at 4 °C and 37 °C in which RP was used as carrier material were maintained their P solubilization efficiency and plant growth promotion activities and maximum shelf life.

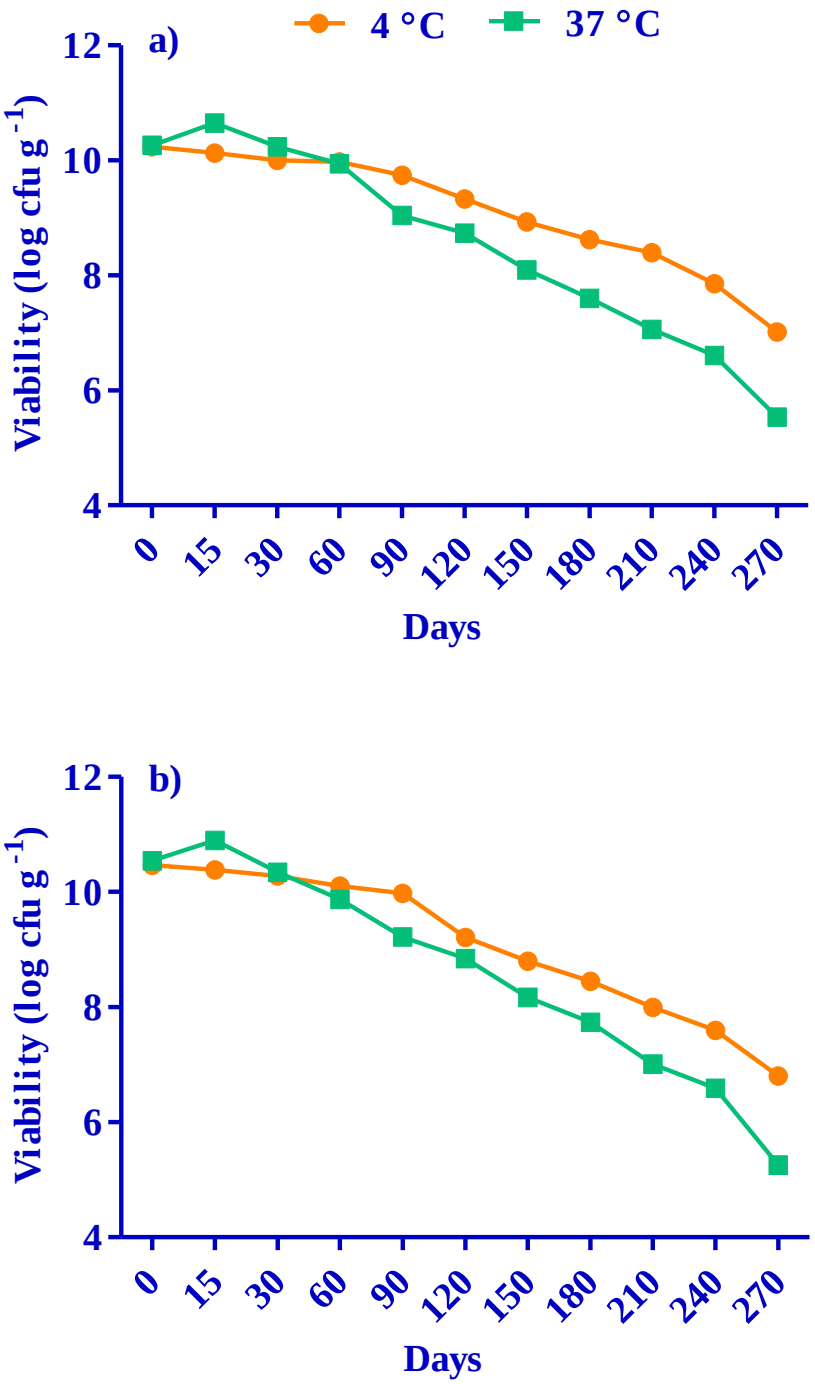

Fig. 8.1 Shelf life of inoculum formulations of a) *Pantoea cypripedii* and b) *Pseudomonas plecoglossicida* in RP at different time intervals (days), at 4 ºC and 37 ºC temperature.

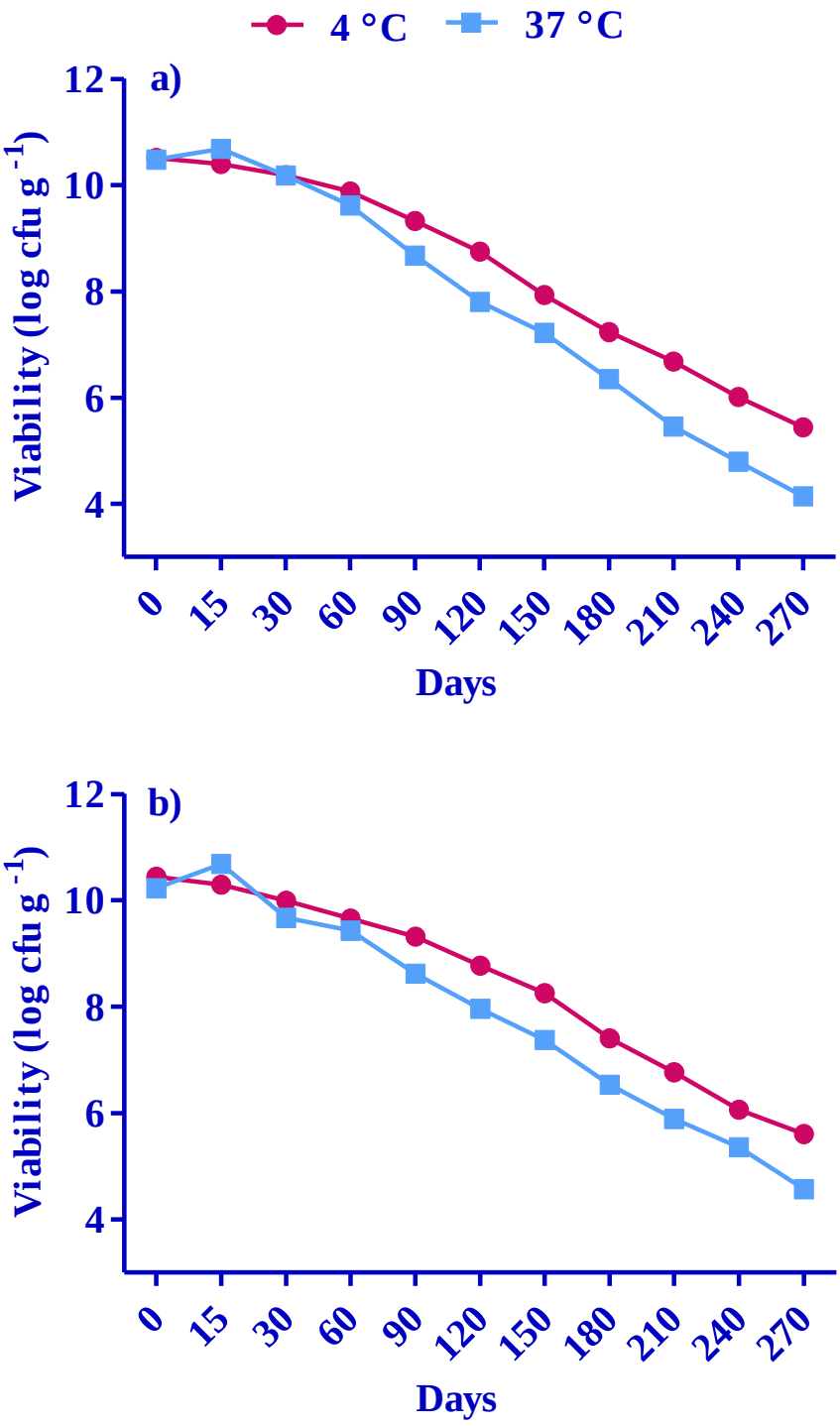

Fig. 8.2 Shelf life of inoculum formulations of a) *Pantoea cypripedii* and b) *Pseudomonas plecoglossicida* in fly ash at different time intervals (days), at 4 ºC and 37 ºC temperature.

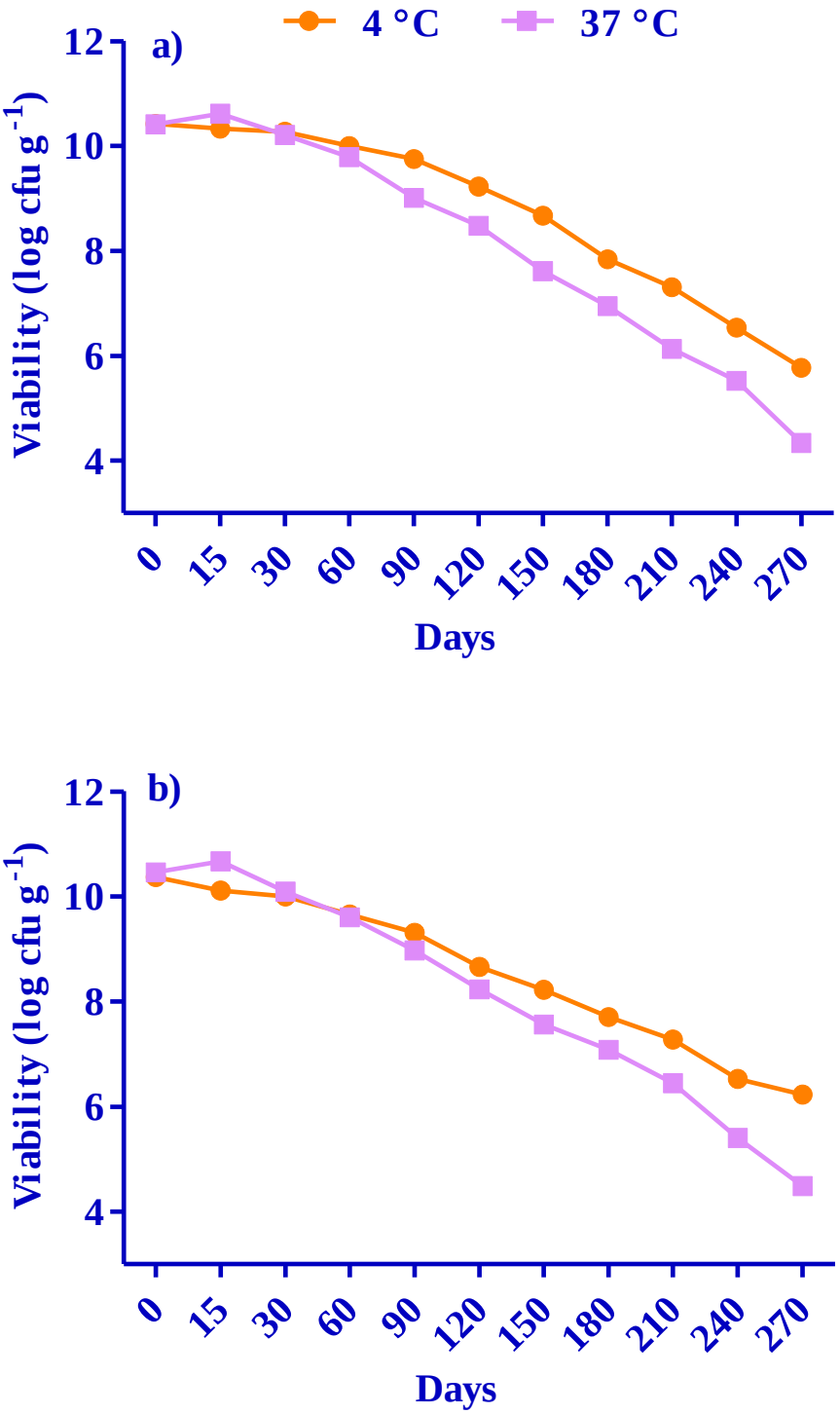

Fig. 8.3 Shelf life of inoculum formulations of a) *Pantoea cypripedii* and b) *Pseudomonas plecoglossicida* in charcoal at different time intervals (days), at 4 ºC and 37 ºC temperature.

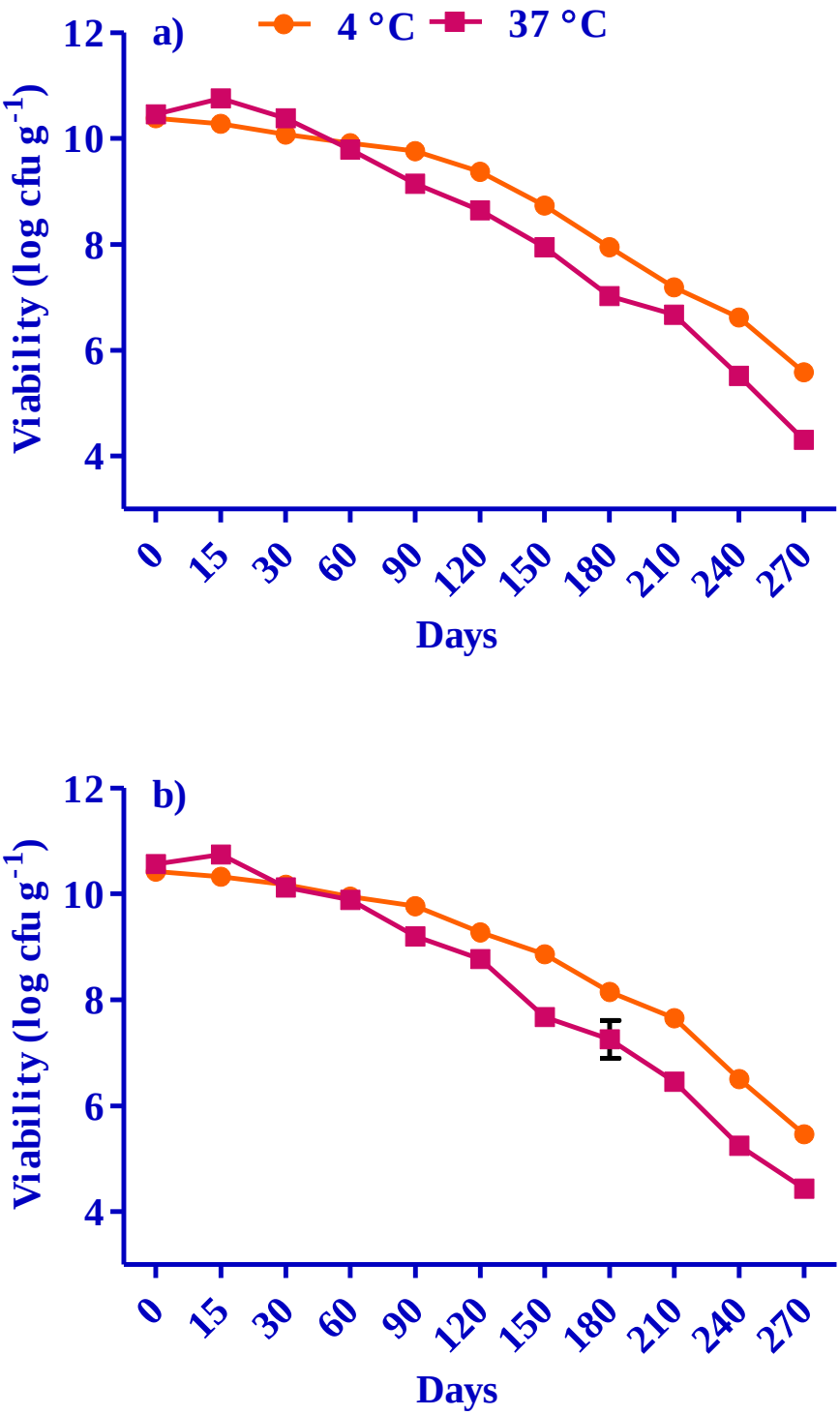

Fig. 8.4 Shelf life of inoculum formulations of a) *Pantoea cypripedii* and b) *Pseudomonas plecoglossicida* in vermiculite at different time intervals (days), at 4 ºC and 37 ºC temperature.

Table 8.1 Physiological study of bacterial inoculum formulations kept at 4 ºC after 270 days of incubation and compare with fresh culture.

Activities	Inoculums formulation		Fresh culture	
	Pantoea cypripedii	*Pseudomona Plecoglossicida*	*Pantoea cypripedii*	*Pseudomona Plecoglossicida*
pH	3.79 ± 0.13	3.73 ± 0.16	3.87 ± 0.07	3.61 ± 0.04
Soluble P (µg ml^{-1})	415 ± 4	434 ± 6	426 ± 4	429 ± 4
Enzyme activities				
Acid phosphatase (µM p.NPP/ml/hrs)	91 ± 5	95 ± 4	96 ± 3	97 ± 2
Alkaline phosphatase (µM p.NPP/ml/hrs)	55 ± 6	53 ± 3	59 ± 3	63 ± 4
Phytase (µM pi/ml/hrs)	6762 ± 92	6860 ± 44	6966 ± 50	6847 ± 24
Indole acetic acid (µg ml^{-1})				
Without tryptophan	5.8 ± 0.21	6.4 ± 0.08	7.9 ± 0.43	7.1 ± 0.33
With tryptophan	90 ± 0.7	26 ± 0.2	93 ± 0.3	27 ± 0.2
Siderophore	+	+	+	+

Values are Mean ± SD (*n* =3).

Table 8.2 Physiological study of bacterial inoculum formulations kept at 37 ºC after 270 days of incubation and compare with fresh culture.

Activities	Inoculums formulation		Fresh culture	
	Pantoea cypripedii	*Pseudomona Plecoglossicida*	*Pantoea cypripedii*	*Pseudomona Plecoglossicida*
pH	3.76 ± 0.04	3.59 ± 0.04	3.87 ± 0.07	3.61 ± 0.04
Soluble P	415 ± 12	410 ± 2	426 ± 4	429 ± 4
Enzyme activities				
Acid phosphatase (µM p.NPP/ml/hrs)	71 ± 1	81 ± 8	96 ± 3	97 ± 2
Alkaline phosphatase (µM p.NPP/ml/hrs)	46 ± 5	50 ± 5	59 ± 3	63 ± 4
Phytase (µM pi/ml/hrs)	6671 ± 72	6705 ± 83	6966 ± 50	6847 ± 24
Indole acetic acid (µg ml^{-1})				
Without tryptophan	4.8 ± 0.39	6.13 ± 0.06	7.9 ± 0.43	7.1 ± 0.33
With tryptophan	87 ± 1.8	22 ± 0.4	93 ± 0.3	27 ± 0.20
Siderophore	+	+	+	+

Values are Mean ± SD (*n* =3).

8.2 Development of phosphate-solubilizing fungal inoculum formulations and check their shelf life and phosphate solubilization efficiency

Similar to the bacterial isolates, fungal isolates were also subjected to test their viability in different carrier materials such as rock phosphate, fly ash, charcoal and vermiculite to develop the inoculum formulations. The carriers were inoculated with fungal spores and incubated at 37 °C and 4 °C. Viability of fungal inoculums in different carrier materials was tested after regular interval of 30 days. Results in Fig. 8.5, 8.6, 8.7 and 8.8 showed that both the fugal inoculums showed similar trend in decreased of viability rate at different temperature condition over a period of 270 days in different carrier materials. On day first of incubation population density of fungal inoculums in all carrier materials was 8.3 to 8.5 log cfu g^{-1} at 4 °C and 37 °C. It was observed that up to day 15 of incubation at 37 °C viability of fungal inoculums was slightly increased from its initial values in all the carrier materials. Viability of fungal inoculums was decreased rapidly at 37 °C compared to 4 °C during long period of incubation. After 270 days of incubation, logarithm of population density was reached 6.19 cfu g^{-1}, 5.00 cfu g^{-1}, 5.41 cfu g^{-1} and 4.88 cfu g^{-1} in rock phosphate, fly ash, charcoal and vermiculite respectively at 4 °C. At 37 °C, logarithm of population density was reached 4.85 cfu g^{-1}, 4.07 cfu g^{-1}, 4.25 cfu g^{-1} and 3.97 cfu g^{-1} in rock phosphate, fly ash, charcoal and vermiculite respectively. Results showed that viability of inoculums was higher at 4 °C as compared to 37 °C. It is evident from the data of present study that, *Aspergillus tubingensis* and *Aspergillus niger* can survive in the selected carrier materials but maximum viability and better survival after 270 days of storage was observed in RP compared to other carrier materials at 4 °C and 37 °C.

The 270 days old fungal inoculum that showed better survival in RP formulation was tested for P solubilization and plant growth promotion activities and compared with fresh culture. Results in the Table 8.3 and 8.4 showed that P solubilization, acid phosphatase, alkaline

phosphatase, phytase enzymes, and plant growth promotion activities such as IAA production and siderophore production of both fungal inoculum formulations at 4 °C and 37 °C were not affected and comparable to the fresh fungal culture activities. Results suggested that long time storage of these fungal inoculum formulations at 4 °C and 37 °C in which RP was used as carrier material were maintained their P solubilization potential, plant growth promotion activities and shelf life.

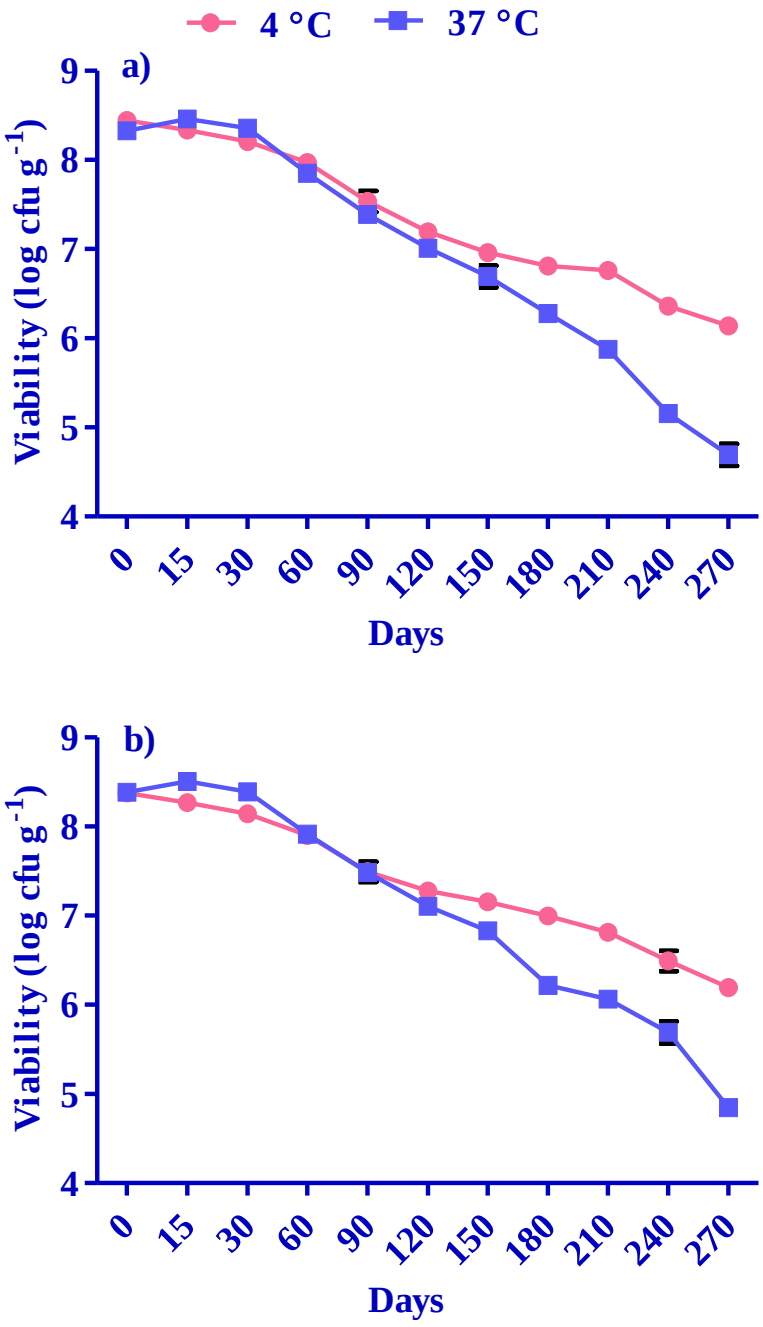

Fig 8.5 Shelf life of inoculum formulations of a) *Aspergillus tubingensis* and b) *Aspergillus niger* in RP at different time intervals (days), at 4 ºC and 37 ºC temperature.

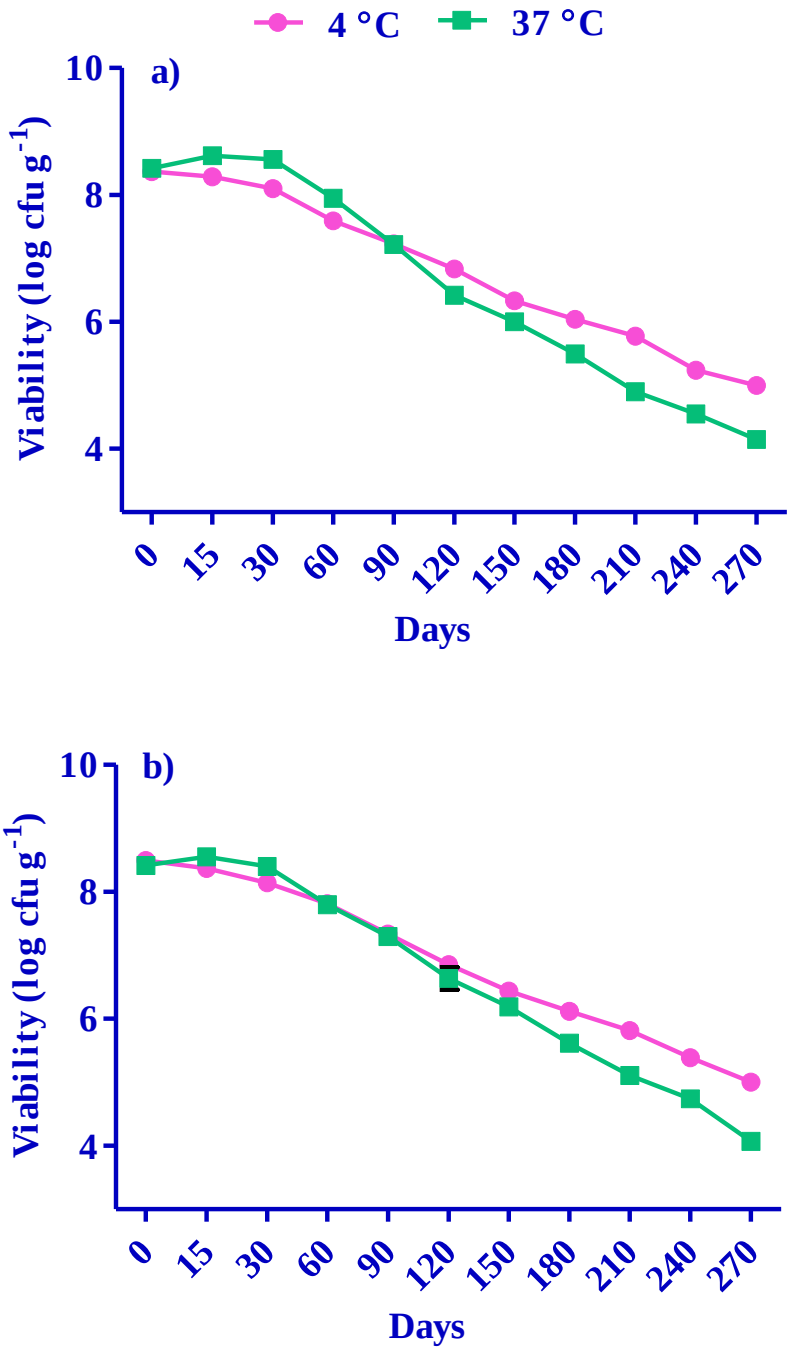

Fig 8.6 Shelf life of inoculum formulations of a) *Aspergillus tubingensis* and b) *Aspergillus niger* in fly ash at different time intervals (days), at 4 ºC and 37 ºC temperature.

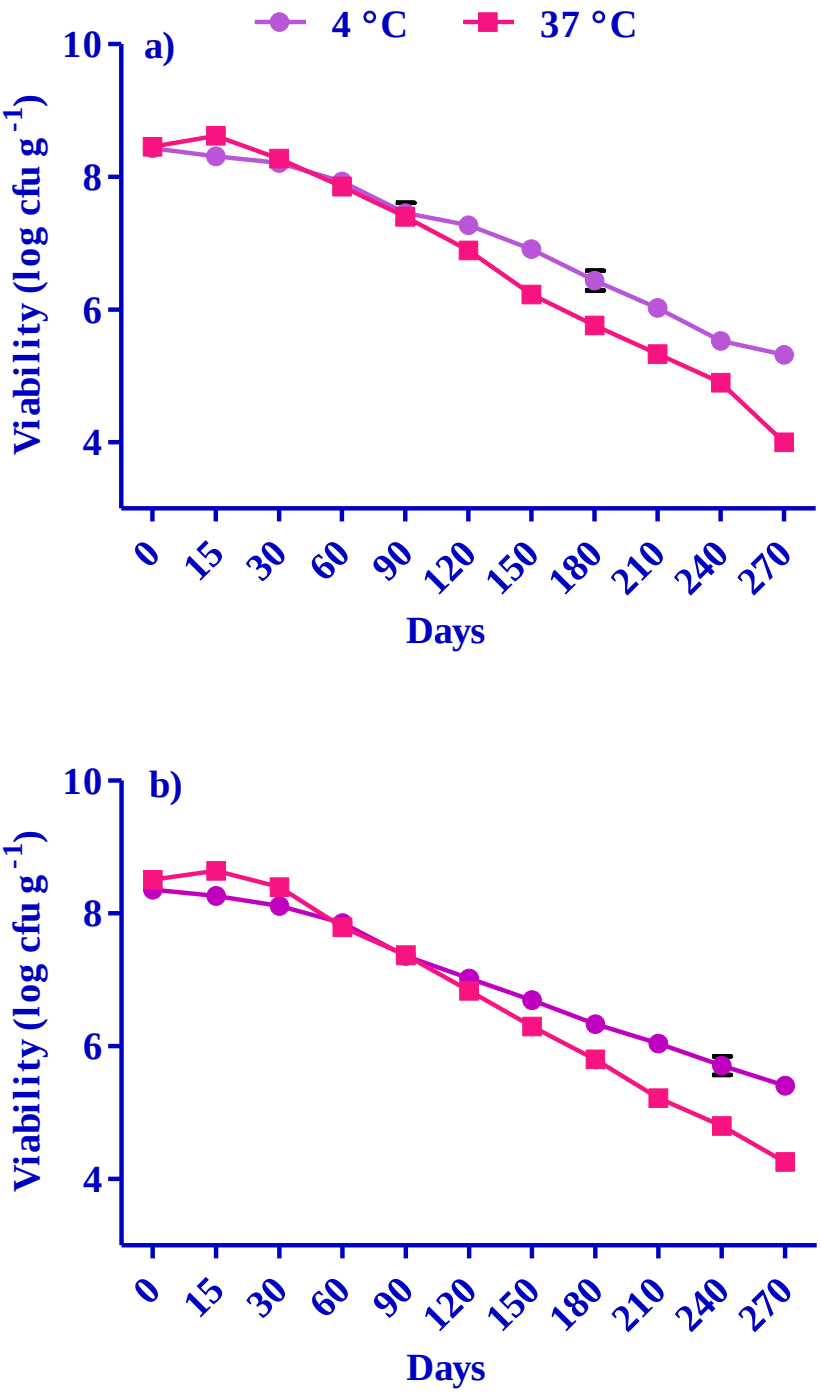

Fig 8.7 Shelf life of inoculum formulations of a) *Aspergillus tubingensis* and b) *Aspergillus niger* in charcoal at different time intervals (days), at 4 ºC and 37 ºC temperature.

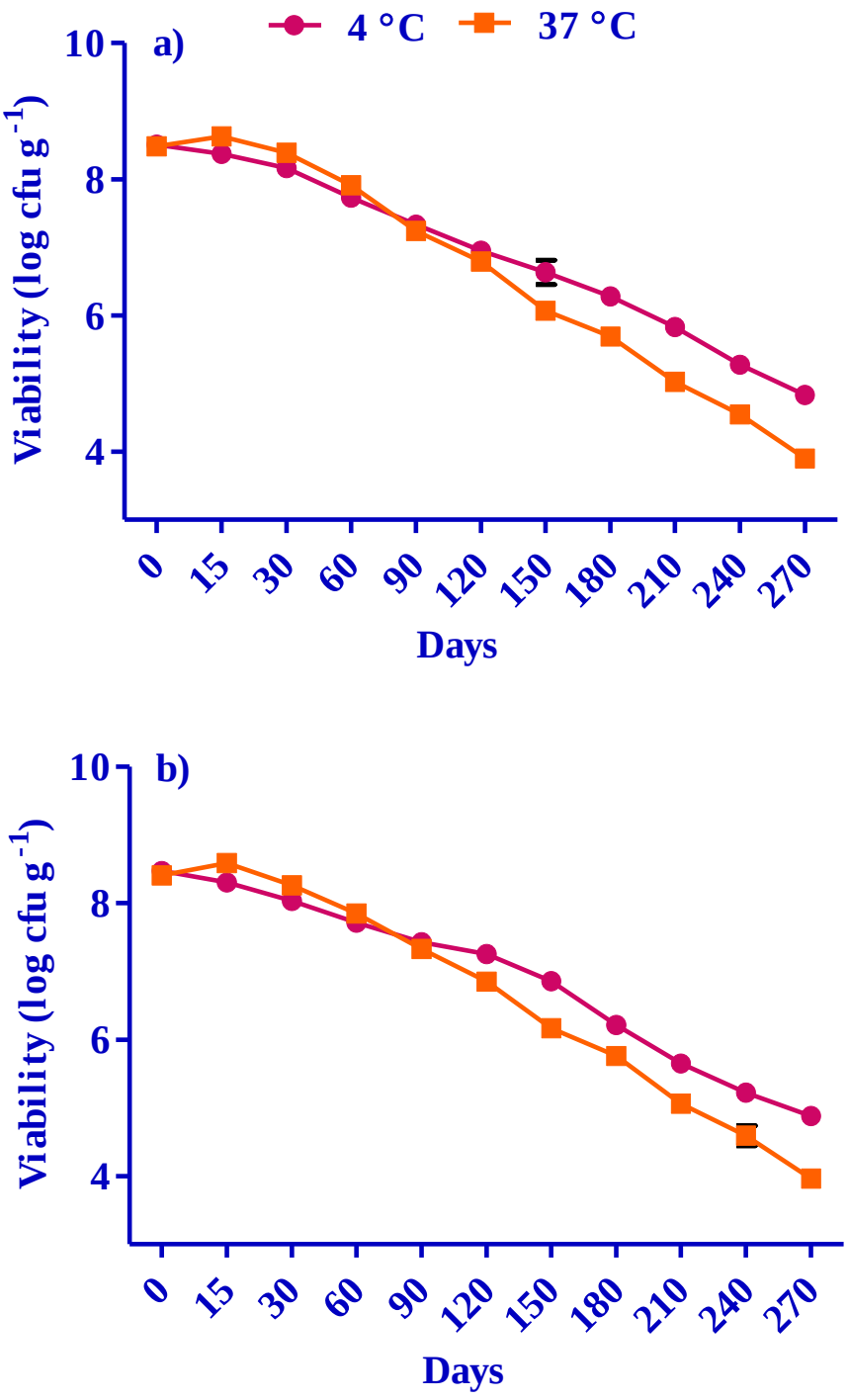

Fig 8.8 Shelf life of inoculum formulations of a) *Aspergillus tubingensis* and b) *Aspergillus niger* in vermiculite at different time intervals (days), at 4 ºC and 37 ºC temperature.

Table 8.3 physiological study of fungal inoculum formulations kept at 4 ºC after 270 days of incubation and compare with fresh culture.

Activities	Inoculum formulations		Fresh culture	
	Aspergillus tubingensis	*Aspergillus niger*	*Aspergillus tubingensis*	*Aspergillus niger*
pH	2.92 ± 0.17	2.59 ± 0.13	2.36 ± 0.15	2.30 ± 0.11
Soluble P	455 ± 6	442 ± 10	458 ± 8	440 ± 2
Dry biomass (gm/100ml)	0.430 ± 0.01	0.425 ± 0.06	0.447 ± 0.02	0.438 ± 0.065
Enzyme activities				
Acid phosphatase (µM p.NPP/ml/hrs)	148 ± 4	140 ± 3	151 ± 3	149 ± 1
Alkaline phosphatase (µM p.NPP/ml/hrs)	71 ± 2	74 ± 1	79 ± 2	76 ± 1
Phytase (µM pi/ml/hrs)	10068 ± 30	10397 ± 29	10113 ± 76	10513 ± 98
Indole acetic acid (µg ml^{-1})				
Without tryptophan	17.9 ± 0.30	19.2 ± 0.44	18.6 ± 0.21	20.5 ± 0.20
With tryptophan	47 ± 0.46	55 ± 1.0	51 ± 0.17	57 ± 0.15
Siderophore	+	+	+	+

Values are Mean ± SD (*n* =3).

Table 8.4 physiological study of fungal inoculum formulations kept at 37 ºC after 270 days of incubation and compare with fresh culture.

Activities	Inoculum formulations		Fresh culture	
	Aspergillus tubingensis	*Aspergillus niger*	*Aspergillus tubingensis*	*Aspergillus niger*
pH	2.58 ± 0.07	2.80 ± 0.06	2.36 ± 0.15	2.30 ± 0.11
Soluble P	445 ± 3	436 ± 5	458 ± 8	440 ± 2
Dry biomass (gm/100ml)	0.419 ± 0.02	0.434 ± 0.04	0.0447 ± 0.02	0.438 ± 0.065
Enzyme activities				
Acid phosphatase (µM p.NPP/ml/hrs)	143 ± 3	138 ± 8	151 ± 3	149 ± 1
Alkaline phosphatase (µM p.NPP/ml/hrs)	67 ± 6	69 ± 1	79 ± 2	76 ± 1
Phytase (µM pi/ml/hrs)	9978 ± 48	10195 ± 19	10113 ± 76	10513 ± 98
Indole acetic acid (µg ml^{-1})				
Without tryptophan	13.7 ± 0.30	16.0 ± 0.25	18.6 ± 0.21	20.5 ± 0.20
With tryptophan	45 ± 0.21	53 ± 0.24	51 ± 0.17	57 ± 0.15
Siderophore	+	+	+	+

Values are Mean ± SD (*n* =3).