Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; SP1: 61-63

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Storability Evaluation of Primed Seeds of Rice (*Oryza sativa*) *cv.*PMK-4

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Abstract
The highest seed vigour normally established during the physiological maturity and then successively declined or deterioration sequentially occurred depending on storage conditions and chemical component of the seeds. Vigour deterioration might be alleviated to some extent by priming techniques. Biopriming, a seed treatment system that integrates the biological and physiological aspects of enhancing growth, disease control and increase in yield, involves coating the seed with biological agents and incubating the seed under warm, moist conditions. In the present study, storage studies were conducted to compare two different methods of priming viz., hydropriming and biopriming with liquid formulation of biocontrol agent *Pseudomonas fluorescens* @ 20% with accomplished for 18hrs soaking durations. The unprimed seeds act as control. The storage studies were conducted for six months after imposing the priming treatment. The results revealed that germination percentage, seedling length and vigour index of primed seeds superior to control seeds. Seeds bioprimed with *Pseudomonas fluorescens* has free from insect and pathogen attack throughout the storage period. Among the protocols studied, biopriming with *Pseudomonas fluorescens* @ 20 % for rice cv.PMK 4 was established as best method of priming treatment capable of improving seed vigour as well as viability.

Keywords: storage, bio priming, vigour index, Pseudomonas fluorescens

Introduction
In general, cereals are more susceptible to storage pests and rice is no exception. Because of its high carbohydrate content rice, seed is attacked by storage pest and other microflora. The rice weevil (*Sitophilus oryzae*) in storage causes considerable damage to the seed and deteriorates the quality of seed. Apart from this, fungi associated with stored seeds are chiefly responsible for deterioration of seed quality. In order to prevent the quantitative and qualitative losses due to storage pests and diseases, several methods such as storage in safe conditions and containers with safe moisture levels and seed treatment with suitable chemicals or biocontrol agents *etc.* were adopted. Bio-priming (combined treatments between seed priming and seed coating with biocontrol agents) may be safely used commercially as substitute for traditional fungicide seed treatments for controlling seed and soil borne plant pathogens. Besides, bio-priming also improves seed germination, seedling establishment and vegetative growth. Apart from other improvements in seed performance, seed longevity was also improved by priming in some crop species, following the priming treatments (Powell *et al.*, 2000) [12]. There has been contrasting reports of storage potential of primed seed. The negative effect of priming on storage was noticed by many researchers stating that the longevity of the primed seed was considerably less compared to nonprimed seeds during storage (Lin *et al.*, 2005) [9]. Biological seed treatments for control of seed and seedling diseases offer the grower an alternative to chemical fungicides. Hence, this present study was formulated to investigate the effect of biopriming of liquid formulation of *Pseudomonas fluorescens* on rice seeds upon viability.

Materials and methods
Seeds of rice cv.PMK 4 with initial germination of 90% and 10.2% moisture were used for this study. Experiments were carried out in the Department of Seed Science and Technology, Agricultural College and Research Institute, Madurai (TNAU). Seeds were soaked in water (Hydropriming); Liquid formulation of *Pseudomonas fluorescens* @ 20% with a soaking duration of 18h at 25^0C respectively. The seeds after priming and drying back to original moisture were packed in cloth bags and kept under ambient storage for a period of six months. Unprimed seeds act as control. The seed samples were drawn at bimonthly intervals upto six months of storage and evaluated for the moisture content of the seed and germination were calculated and expressed as percentage (ISTA, 1999) [5]. Root length, shoot length were also measured at the end of 14 days.

Vigour index was calculated using the formula of Abdul-Baki and Anderson (1973) [1].

Storage fungi present on seeds were tested using blotter method (ISTA, 2010) [6]. Ten seeds were placed equidistantly on three layered moistened blotter taken in sterilized petri plates. All three treatments were replicated eight times. The plates were incubated at 20±2°C for seven days with alternate cycles of 12 h in near ultraviolet light (NUV) range and for the remaining 12 h in dark. On eighth day, the plates were examined under Stereobionocular Microscope (50X) for the presence of seed borne fungi. The number of infected seeds were counted and expressed in percentage. The percentage of insect damaged seeds was worked out as per the method proposed by Adams and Schulten (1978) [2]. The population of adult insects was counted (both living and dead) and expressed per unit by weight of the grains.

The number of colony forming units (CFU)/ml solution of seed wash of seeds bioprimed with biocontrol agent was enumerated at all periods of study using King's B medium (King *et al.*, 1954) [8]. The slimy colonies appearing after 2-3 days of incubation were counted and the population expressed as CFU ml^{-1} of seed wash.

The data obtained from different experiments were analysed by the 'F' test of significance (Panse and Sukhatme, 1985) [11]. The critical differences (CD) were calculated at 5 per cent probability level.

Result and discussion

In the present study, the moisture content did not increase in both nonprimed and bioprimed seeds over the period of six months storage. Seed biopriming with *Pseudomonas fluorescens* 20% recorded the highest germination of 92 per cent when compared to hydro and nonprimed seeds. At the end of storage period germination percentage were similar in all treatments (84%) (Fig.1). Kalaivani (2010) [7] who showed that maize seeds bioprimed with talc formulation of *Pseudomonas fluorescens* @ 80% for 12h also recorded minimal decrease in germination at sixth month of storage.

The seeds primed with *Pseudomonas fluorescens* 20% recorded highest root (24 and 20.6 cm) and shoot length (12.5 and 10.2 cm) and vigour index (3358 and 2587) from initial to end of storage period. Minimum seedling length was observed in unprimed seeds (7 and 17.2 cm) (Table.1). The minimal reduction in deterioration of primed seeds in terms of germination and seedling vigour might be attributed to the induction of repair mechanism of seed deterioration (Ellis and Butcher, 1988) [3] due to priming.

Statistically significant variation was noticed for pathogen infection due to biopriming treatment, period of storage and its interaction. Irrespective of period of storage, the pathogen infection was nil in the bioprimed seed using *Pseudomonas fluorescens* 20% and it was more in the nonprimed seed. The pathogen infection was not observed at initial months of storage in all three treatments and however, the infection was high (3.25 per cent) in control seeds at 6 months of storage and 1.75 per cent infection was observed in the hydro primed seed. At 6 months of storage, no pathogen infection was noticed in *Pseudomonas fluorescens* (20%) bio-primed seeds (Table 2). No insect damage was observed throughout the storage period in all the treatments. Mougy and Kader (2008) [10] reported that biopriming of faba bean with anyone of the strains of *Trichoderma viride*, *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens* after 3months of storage under field conditions, the antagonistic agents could protect seeds against infection by root rot pathogens at both pre and post emergence stages. However, after six months of storage, a lower protective effect was observed.

The initial population load of *Pseudomonas fluorescens* on seeds treated at 20% for 12h (6×10^7) decreased to 7×10^2 at 6 months of storage. In this present study, the *Pseudomonas fluorescens* has survived on the seed surface over six months period of storage with slight decrease in population (Table 3) when compared to initial population. This is in concordant with the results of Vidhyasekaran and Muthamilan (1995) [13] who has reported that talc formulations of the three strains of *Pseudomonas fluorescens* applied to chickpea seeds survived well throughout the experimental period of 180 days. The survivability of inoculated bacteria has been significantly reduced after a six month storage period (Falik and Okon, 1996) [4]. This reduction in the inoculated population of bacteria may probably be due to the stress that developed during storage under suboptimal conditions, such as lack of moisture, stress and available nutrients.

From the storage study, it is concluded that the storage potential of liquid formulation of *Pseudomonas fluorescens* 20% bioprimed seed was high and it could be stored for 6 months with minimal loss in vigour and viability and free of pest and pathogen infection. The survivability of *Pseudomonas fluorescens* was good upto six months of storage and its bio-control activity continues up to sixth month.

Table 1: Influence of seed priming and period of storage on shoot and root length (cm) in rice cv. PMK 4.

Storage Period(P)/Treatments(T)	Shoot Length(cm)				Root Length(cm)			
	Initial	2Month	4Month	6 Month	Initial	2Month	4Month	6 Month
Control	10.7	9.7	8.6	7	21	19.6	18.7	17.2
Hydropriming	11.3	10.8	10	8.5	21.7	21	20.1	18.6
Pseudomonas fluorescens	12.5	11.4	11.1	10.2	24	22.5	21.7	20.6
SE(D)	P	T	PXT		P	T	PXT	
	0.09	0.08	0.16		0.12	0.10	0.21	
CD (*P=0.05%*)	0.19**	0.16**	0.33**		0.24**	0.21**	0.43**	

Table 2: Influence of seed priming and period of storage on pathogen infection (%) by blotter method in rice cv. PMK 4.

Storage Period(P) / Treatments(T)	Pathogen Infection (%)			
	Initial	2Month	4Month	6 Month
Control	0	0.5	1.25	3.25
Hydropriming	0	0.25	0.75	1.75
Pseudomonas fluorescens	0	0	0	0
SE(D)	P	T	PXT	
	0.67	0.58	1.17	
CD (*P=0.05%*)	1.37**	1.19**	2.38**	

Table 3: Influence of seed biopriming and period of storage on population of biological agents (CFU/ml solution of seed wash)

Treatments(T)	Storage Period(P)			
	Initial	**2Month**	**4Month**	**6 Month**
Control	0	0	0	0
Hydropriming	0	0	0	0
Pseudomonas fluorescens	$6x10^7$	$3x10^6$	$5.67x10^4$	$7x10^2$
SE(D)	P	T	PXT	
	0.24	0.21	0.43	
CD (P=0.05%)	0.51**	0.44**	0.88**	

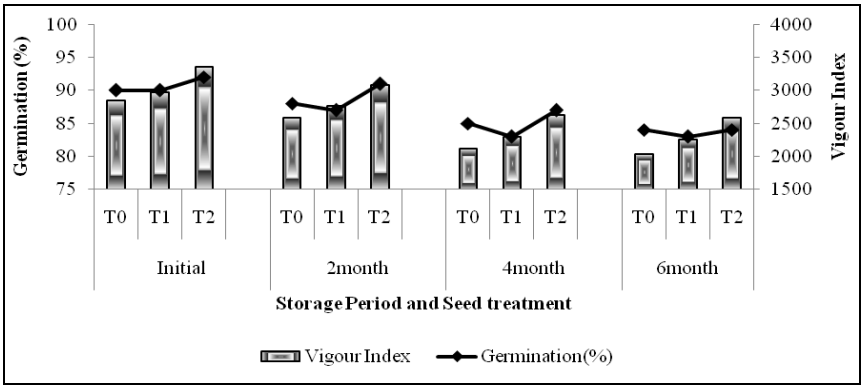

Fig 1: Influence of seed priming and period of storage on germination (%) and vigour index in rice cv. PMK 4
T0-Control, T1-Hydropriming, T2- *Pseudomonas fluorescens*

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