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An Assessment of Cosplay of Microbes Treated Seeds on Storability of Rice Seeds

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ABSTRACT

Background: Warm, humid weather can hasten the deterioration of seeds in the context of sustainable agriculture due to changing climatic circumstances. For rice, therefore, ideal growing conditions are incompatible with storage conditions. Thrissur's tropical environment makes seed storage in warehouses after harvest particularly difficult. In order to determine the impact of various seed priming treatments on the longevity of seeds under accelerated ageing, the current study was undertaken.

Methods: Rice seeds of variety Jyoty were treated with *Pseudomonas florescens* (10 g/kg), *Trichoderma viride* (4 g/kg) and coconut water 75% in different combinations. Treated seeds were packed in butter paper bags with pin holes to absorb moisture for ageing and placed in the wire gauge/mesh above the water well such that seeds are not in touch with water inside BOD incubator from 0 to 7 days and received a temperature of 40±1°C and a relative humidity of 98%. Accelerated aged samples were taken at daily intervals and subjected to further tests to determine its quality and longevity.

Result: At the end of the ageing period, coconut water (75%) and *P. fluorescens* (10 g/kg) + *T. viride* (4 g/kg) + coconut water (75%) recorded the longest root length (14.12 cm and 14.09 cm, respectively) and was on par with *T. viride* @4 g/kg (13.78 cm), coconut water treatments (T_7 14.94 and T_3 14.32) have shown early emergence. Furthermore, dry dressing with bio agents has been found superior in performance compared to wet treatments under accelerated ageing.

Key words: Accelerated ageing, *Pseudomonas florescens,* Seed priming, *Trichoderma viride*.

INTRODUCTION

Rice [*Oryza sativa* (L.) Poaceae] is a staple cereal crop that serves as a crucial source of carbohydrates, proteins, vitamins, minerals and dietary fibre. Its cultivation thrives in humid, tropical and subtropical climates, where optimal conditions promote high yields. However, these same conditions characterized by high temperatures and humidity accelerate seed deterioration during storage, posing challenges for long-term *via*bility and quality maintenance.

Physiochemical transformations during storage

Rice undergoes notable physicochemical changes during storage, which become evident within approximately three months (Saikrishna *et al.,* 2018). These changes are governed by factors such as:

Varietal differences

Rice variety determines physical and compositional variations.

Storage conditions

Temperature and humidity significantly impact the rate of biochemical transformations.

Amylose content

This influences the texture and retrogradation behaviour of stored rice.

Modifications in protein and lipid composition, interactions between proteins and lipid oxidation products and protein-starch interactions collectively influence processing behaviour. Additionally, oxidative reactions in stored rice contribute to decline in nutritional quality and functional properties.

Seed ageing and deterioration dynamics

Seed ageing is associated with reduced germination rates and vigour, while increasing susceptibility to environmental stresses. Seed degeneration manifesting as loss of quality, *via*bility and metabolic stability is exacerbated in developing regions where ambient storage conditions lack humidity and temperature control.

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Mechanisms of accelerated ageing

Accelerated ageing methodologies artificially simulate storage-induced deterioration by subjecting seeds to elevated temperatures and high relative humidity (Kapoor *et al.,* 2011). These stress conditions amplify: Membrane permeability defects, increasing solute leakage. Oxidative stress, hastening lipid peroxidation and protein degradation. Enzymatic dysfunction, impacting metabolic pathways essential for germination.

Interestingly, studies indicate that primed seeds, particularly onion seeds, exhibit improved performance and extended shelf life under unfavourable storage conditions, as priming mitigates accelerated ageing effects (Yalamallae *et al.,* 2019).

MATERIALS AND METHODS

Experiment was carried out in the Department of Seed Science and Technology, College of Agriculture, Vellanikkara (KAU). Freshly harvested seeds were allowed to age naturally for nine months in 700-gauge polythene bags sealed tightly and stored under ambient conditions. The moisture content of seed sample after treatment were set to around 8.1%. The seed quality parameters were assessed as per ISTA guidelines (ISTA, 2019).

Seed priming

There were eight treatments with seed priming treatments. Seeds were dry dressed with *Pseudomonas fluorescens* (10 g/kg), *Trichoderma viride* (4 g/kg), *P. fluorescens* + *T. viride* are considered as dry treatments. As for wet treatments seeds were soaked in water (Hydropriming); coconut water (75%); *P. fluorescens* + coconut water; *T. viride* + coconut water; *P. fluorescens* + *T. viride* + coconut water in rice variety Jyoty, the seeds were soaked in solution for 16 h respectively. Un-primed seeds were used as control.

Accelerated aging

Artificial aging of seeds was done by considering two parameters temperature and RH as per the method explained by Delouche and Baskin, (1973). The treated seeds were packed in butter paper bags with pin holes to absorb moisture for ageing and placed in the wire gauge / mesh above the water well such that seeds are not in touch with water inside BOD incubator from 0 to 7 days and received a temperature of 40±1°C and a relative humidity of 98%. Accelerated aged samples were taken at daily intervals and received further tests to determine a variety of quality parameters.

Germination (G%) and seed vigour (VI)

These were estimated as per the procedure prescribed by ISTA, (2019) using between paper method (Roll towel).

Moisture content (MC %) and seedling dry weight (g/10 seedlings)

Estimated as per the procedure of high constant temperature protocol of ISTA (2010).

Speed of germination (SPG)

Estimated using procedure of Maguire (1962) which is known as top of paper method.

Mean germination time (MGT)

Estimated using the equation formulated by Ellis and Robert (1981).

Time taken for 50% germination (T_{50})

Estimated using the procedure given by Coolbear *et al.* (1984) formulated the 50% germination time equation (T50) as modified by Farooq *et al.* (2006).

Electrical conductivity (ISTA, 2010)

The electrical conductivity of the leachate from the grains was measured with a digital conductivity meter.

Estimation of ROS scavengers

SOD enzyme activity

Using Beauchamp and Fridovich's (1971) method of photochemical inhibition of Nitro Blue Tetrazolium (NBT) reduction, the activity of SOD was assessed. At 560 nm, the absorbance of the reaction mixture was measured.

Dehydrogenase enzyme activity (Kittock and Law, 1968)

The enzyme dehydrogenase activity is valued at 470 nm using spectrophotometer is the OD value.

Analysis of the data on various seed quality parameters was performed following the Completely Randomized Design (CRD) with three replications using OPSTAT, a Statistical software package.

RESULTS AND DISCUSSION

Seed priming is a pre-sowing technique used to improve germination speed and uniformity under adverse environmental conditions. The process involves controlled hydration followed by dehydration, ensuring optimal moisture levels for storage while retaining the physiological benefits of priming (Arvindkumar *et al.,* 2015). Primed seeds exhibit enhanced quality traits compared to unprimed seeds (Table 1). Germination rates increased by 2.01% to 23.48%, with the highest germination (83.69%) recorded in seeds treated with a combination of *Pseudomonas, Trichoderma* and Coconut water, which performed on par with dry treatment using Pseudomonas.

This improvement can be attributed by several factors, Microbial enhancement plays a crucial role, as beneficial microbes suppress harmful pathogens and promote overall seed health. Additionally, hormonal stimulation by these microbes contribute to gibberellin production, regulating α-amylase synthesis, which mobilizes starch reserves for early seedling growth. Furthermore, enzymatic activation leads to increased activity of proteases and nucleases, facilitating the digestion of nucleic acids and efficient nutrient translocation to the endosperm, enhancing seed vigour and development (Saudi, 2017).

Seedling performance and stress tolerance are significantly enhanced through seed priming treatments. The combined application of *Pseudomonas, Trichoderma* and Coconut water resulted in the highest germination speed (18.99), closely followed by dry-treated *Pseudomonas* (18.89) and *Pseudomonas* combined with *Trichoderma* (19.02). Additional improvements were observed in seedling length, with the maximum elongation (19.66 cm) recorded in the combined treatment, which was comparable to Coconut water-treated seeds (19.07 cm). The vigour index peaked at 3674, confirming enhanced seedling establishment, while the highest dry weight (0.242 g) was observed in primed seeds, similar to *Trichoderma*-treated seeds (0.240 g) (Table 2) which are

Table 1: Impact of biopriming on different parameters of rice seeds after nine months natural ageing.

Treatments	MC	G%	SL	VI	MGT	T_{50}	EC	SPG	DWT
T_1: *Pseudomonas fluorescens*	10.71^{g}	83.23^{ab}	18.66^{bc}	3459^{bc}	5.22^{b}	4.70^{d}	32.60^{g}	18.89^{a}	0.229^{c}
T_2: *Trichoderma viride*	10.91^{f}	82.89^{b}	18.55^{bc}	3526^{b}	5.19^{bc}	4.56^{e}	36.28^{c}	17.61^{cd}	0.240^{ab}
T_3: Coconut water	11.02^{e}	81.84^{c}	19.06^{ab}	3451^{c}	5.15^{d}	4.9^{c}	33.94^{e}	18.3^{b}	0.231^{c}
T_4: *P. fluorescens* + *T. viride*	10.61^{h}	83.15^{ab}	18.10^{d}	3332^{d}	5.13^{d}	4.42^{f}	37.85^{b}	19.02^{a}	0.230^{c}
T_5: *P. fluorescens* + coconut water	11.22^{d}	81.10^{d}	18.54^{c}	3432^{cd}	5.08^{e}	4.54^{e}	30.83^{h}	17.52^{cd}	0.228^{c}
T_6: *T. viride* + coconut water	11.32^{c}	79.08^{e}	18.90^{b}	3328de	5.19^{bc}	5.24^{a}	33.70^{f}	16.49^{d}	0.232^{c}
T_7: *P. fluorescens* + *T. viride* + coconut water	11.02^{e}	83.69^{a}	19.66^{a}	3674^{a}	5.08^{e}	5.00^{b}	34.73^{d}	18.99^{a}	0.242^{a}
T_8: Hydro priming	11.55^{b}	77.85^{f}	18.66^{bc}	3176^{e}	5.18^{bc}	5.01^{b}	33.99^{e}	15.80^{e}	0.222^{d}
T_9: Control	11.62^{a}	77.62^{e}	18.03^{de}	3284de	5.36^{a}	5.29^{a}	38.86^{a}	17.84^{c}	0.223^{d}
C.D	0.027	0.702	0.323	69.288	0.044	0.058	0.144	0.38	0.004
SE(m)	0.009	0.234	0.107	23.141	0.015	0.019	0.048	0.127	0.001

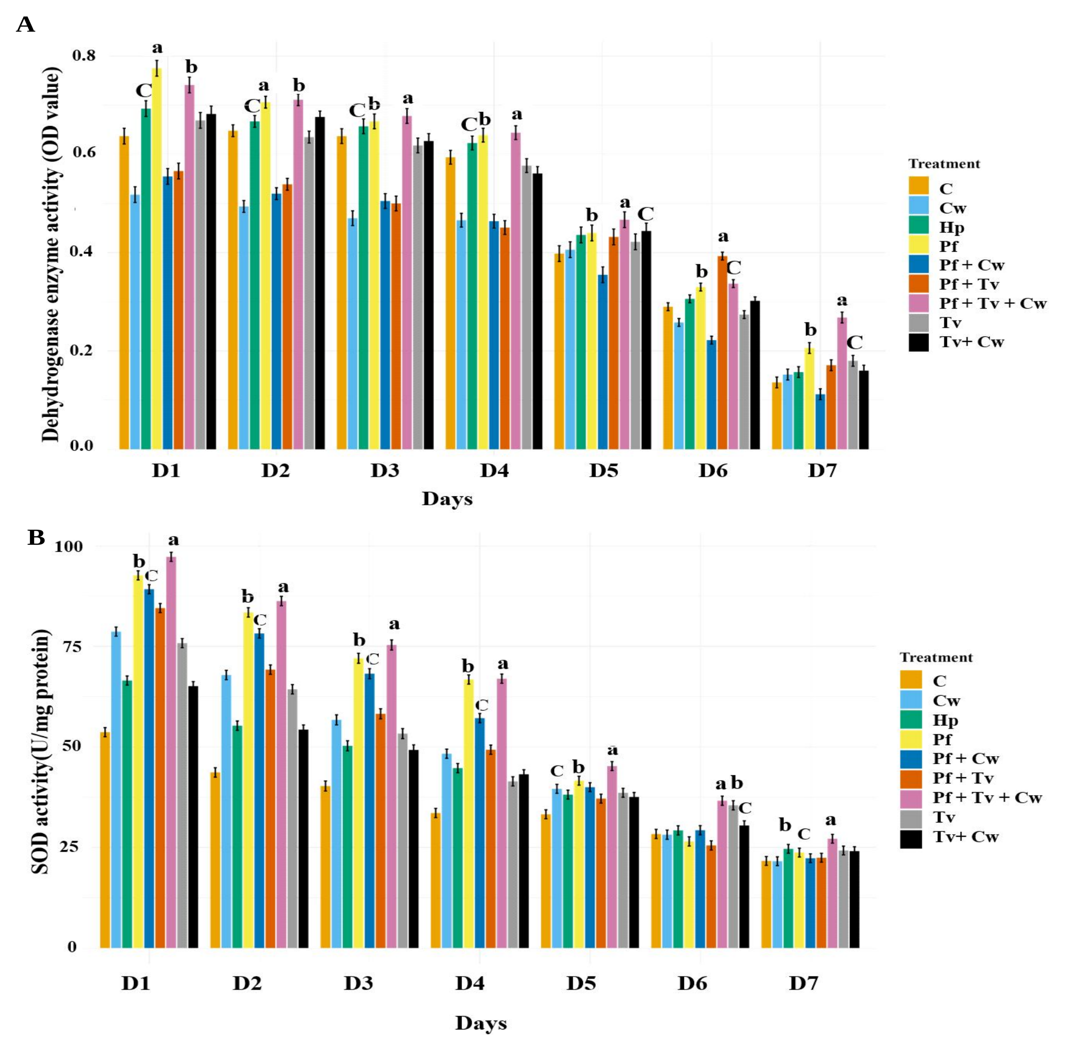
Table 2: Impact of biopriming on different parameters of rice seeds before artificial ageing.

Treatments	MC	G%	SL	VI	MGT	T_{50}	EC	SPG	DWT
T_1: *Pseudomonas fluorescens*	9.60^{bc}	89.56^{a}	23.85^{a}	4294^{a}	2.95^{ab}	4.12^{a}	25.85^{de}	18.50^{a}	0.249^{a}
T_2: *Trichoderma viride*	8.74^{d}	86.67^{a}	22.84^{ab}	4008^{bc}	2.93^{ab}	4.16^{a}	27.05^{cd}	17.87^{bc}	0.226^{b}
T_3: Coconut water	9.75^{ab}	86.72^{a}	21.07^{cd}	3946^{bc}	2.85^{bc}	4.06^{a}	22.95^{e}	18.38^{ab}	0.248^{a}
T_4: *P. fluorescens* + *T. viride*	10.50^{a}	89.26^{a}	21.20^{cd}	4046^{b}	3.01^{a}	4.00^{a}	27.65^{cd}	17.69^{cd}	0.243^{a}
T_5: *P. fluorescens* + coconut water	8.90^{cd}	85.61^{a}	20.27^{de}	3849^{c}	2.80^{c}	4.04^{a}	26.45^{cd}	16.71^{e}	0.248^{a}
T_6: *T. viride* + coconut water	8.90^{cd}	85.24^{a}	20.51^{de}	3813^{c}	2.63^{d}	4.02^{a}	29.35^{bc}	17.29^{d}	0.248^{a}
T_7: *P. fluorescens* + *T. viride* + coconut water	9.10^{bc}	88.55^{a}	21.43^{bc}	4164^{b}	3.03^{a}	3.95^{ab}	29.25^{bc}	17.37^{cd}	0.248^{a}
T_8: Hydro priming	9.90^{ab}	79.95^{b}	23.16^{bc}	3818^{bc}	2.59^{d}	3.67^{c}	24.85^{de}	16.20^{e}	0.248^{a}
T_9: Control	9.15^{bc}	86.72^{a}	21.88^{cd}	4007^{b}	2.95^{ab}	3.7^{bc}	31.25^{a}	17.88^{bc}	0.248^{a}
C.D	0.733	3.91	0.86	241.91	0.1	0.266	3.149	0.531	0.006
SE(m)	0.245	1.30	0.28	80.79	0.034	0.089	1.052	0.177	0.002

Table 3: Impact of biopriming on different parameters of rice seeds after artificial ageing.

Treatments	MC	G%	SL	VI	MGT	T_{50}	EC	SPG	DWT
T_1: *Pseudomonas fluorescens*	14.75^{d}	83.11^{a}	20.16^{bc}	3427^{b}	6.42^{a}	7.35	47.95^{d}	14.69^{a}	0.23
T_2: *Trichoderma viride*	14.25^{e}	83.58^{a}	22.51^{a}	3636^{a}	6.37^{ab}	7.18	44.55^{f}	13.31^{cd}	0.24
T_3: Coconut water	15.25^{c}	73.15^{d}	20.30^{cd}	3262^{cd}	6.22^{e}	7.47	54.65^{b}	14.32^{b}	0.243
T_4: *P. fluorescens* + *T. viride*	14.35^{de}	82.93^{ab}	21.12^{c}	3628^{ab}	6.27^{cde}	7.14	46.45^{f}	15.04^{a}	0.241
T_5: *P. fluorescens* + coconut water	14.75^{d}	74.69^{cd}	19.88^{e}	3210^{cd}	6.29^{cd}	7.03	51.50^{c}	13.32^{cd}	0.231
T_6: *T. viride* + coconut water	14.25^{e}	76.62^{c}	16.5^{f}	3288de	6.24^{de}	7.14	48.15^{e}	12.37^{d}	0.242
T_7: *P. fluorescens* + *T. viride* + coconut water	14.50^{de}	81.06^{b}	22.18^{b}	3534^{b}	6.32^{bc}	7.03	46.65^{de}	14.94^{a}	0.233
T_8: Hydro priming	18.35^{b}	73.24^{d}	20.55^{b}	3117de	6.04^{f}	7.25	53.40^{bc}	11.83^{e}	0.234
T_9: Control	19.40^{a}	72.55^{e}	17.63^{ef}	3159^{ef}	6.52^{a}	7.54	68.65^{a}	11.76^{e}	0.237
C.D	0.453	2.11	0.75	137.9	0.057	N/A	1.594	0.26	N/A
SE(m)	0.151	0.70	0.25	46.05	0.019	0.122	0.533	0.124	0.005

MC - %; G - %; SL- cm; EC- μScm^{-1}; DWT- g; SOD- U/mg protein.



a. Dehydrogenase enzyme (OD value) b. SOD (U/mg protein) across the ageing period.

Fig 1: Impact of priming treatments on enzyme activity.

supported by previous studies of Maurya *et al.* (2024) in Chick pea, Negi *et al.* (2021) in French bean.

Oxidative stress mitigation plays a crucial role in maintaining seed viability. Seeds exposed to oxidative stress accumulate reactive oxygen species (ROS), leading to physiological damage. Protective enzymes such as superoxide dismutase (SOD) and dehydrogenase neutralize these harmful radicals, thereby improving stress resistance (Chatnaparat *et al.,* 2009; Hemsanit *et al.,* 2010). Among treatments, T_7 exhibited the highest enzymatic activity, followed by dry-dressed *Pseudomonas*, reinforcing the role of microbial priming in oxidative stress defense (Table 3). Enzyme activity correlated with previous studies in rice and maize, supporting the beneficial effects of microbial priming (Prathuangwong *et al.,* 2012; Wattanakulpakin *et al.,* 2012).

Seed aging is primarily associated with the gradual inactivation of enzymes due to molecular modifications (Radha *et al.,* 2014). Long-term storage leads to reduced SOD and dehydrogenase activity (Fig 1A), consistent with findings in paddy (Vijayan, 2005) and black gram (Manimekalai, 2006). Towards the end of the aging process, all treatments exhibited equal reductions in SOD activity (Fig 1B), indicating uniform oxidative conversion (O, to H, O) across samples.

Adding to, seed priming significantly enhances germination, seedling vigour and enzymatic defense, mitigating the negative effects of storage deterioration. This study provides valuable insights into seed longevity, biochemical stability and storage optimization, contributing to improved agricultural practices.

CONCLUSION

Seed treatment with root-colonizing organisms such as *Pseudomonas fluorescens* and *Trichoderma viride* is an effective strategy for managing rhizosphere-resident microbial antagonists. These biocontrol agents are particularly valued for their strong root colonization ability, their capacity to produce a diverse range of antimicrobial compounds and their role in inducing systemic resistance in plants (Vanitha *et al.,* 2009; Erdogan and Benlioglu, 2010). The application of P. *fluorescens,* either alone or in combination with other biocontrol agents or organic treatments, has been shown to enhance seed longevity and quality. This effect is largely attributed to the presence of endogenous plant growth regulators, including gibberellins, cytokinins and indole-acetic acid, which contribute to increased mineral availability and improved water uptake. These factors collectively support seed vigour and resilience during storage.

Furthermore, studies indicate that seven days of continuous accelerated aging is equivalent to nine months of natural aging, providing a rapid method for assessing seed deterioration and *via*bility under controlled conditions. This correlation between accelerated and natural aging highlights the effectiveness of biological seed treatments in mitigating storage-related stress and preserving seed quality over extended period.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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