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Research Article

Limitation of Improvement in Germination by Osmopriming of Differentially Aged Non-Orthodox Neem (*Azadirachta indica*) Seeds

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Abstract

This work involves physiological and biochemical features of seed ageing gauged from seed viability and vigour over the period of storage. Both conventional storage (natural ageing) and controlled deterioration (accelerated ageing) are resulted in loss of germination capacity and vigour as well as poor seedling establishment. Present fndings indicate that both natural and accelerated ageing sustain similar pattern, except their mortality curve. In natural ageing, prior to entering sigmoidal type decline a period of relative stability exists; whereas in accelerated aging, such relative stability is absent. It is also observed that rate- controlling process of ageing (natural ageing slow whereas accelerated ageing fast) was dependent upon moisture content and temperature. These physical factors have negative linear correlation with seed viability. Membrane integrity and lipid peroxidation are associated with seed ageing, however peroxidation does not hold exact with accelerated ageing. Additionally, these aged seeds were exposed to

Keywords: Natural ageing; Accelerated ageing; Osmopriming; Germination improvement; Azadirachta indica; Membrane integrity; Lipid peroxidation

Abbreviations: PEG: Polyethylene Glycol; MC: Moisture Content; TTC: Tri-phenyl Tetrazolium Chloride; LSMC: Lowest Safe Moisture Contents; MDA: Malondialdehyde; TBARS: iobarbituric Acid-Reactive Substances; BHT, Butylated Hydroxy Toluene; DM: Dry Mass; FW: Fresh Weight

Introduction

e importance of tropical tree species is well recognized. Many tropical trees propagate through seeds. It is a matter of concern, when the seeds of some of the tropical trees have low to very low storage longevity. ese seeds generally display intermediate or recalcitrant storage behavior. Unlike orthodox, seeds [intermediate or recalcitrant] shed at very high moisture contents do not survive below critical moisture content (depend upon drying rate and condition). Intermediate seeds survive drying and/or moderate low moisture content, but are o en injured by low temperature. is is attributed to their sensitivity to desiccation and/or low temperatures. Storage of such seeds results in loss of seed viability under natural condition by way of decline in the moisture content. ese seeds are to be conserved (stored) for sake of reforestation as well as ex situ conservation as forest genetic resources. Neem (Azadirachta indica), is a valuable and economically important tropical tree species. e seeds of Azadirachta indica have been characterized as having intermediate storage longevity. ey lose viability within 3 months a er harvesting [1,2]. Loss of germinability occurs during dry storage over the time. According to Heydecker et al. [3] seed ageing exhibits deteriorative changes that lead to decreased viability, poor germinability and weak seedling establishment. Besides natural ageing (NA), accelerated ageing (AA) under high temperature and high humidity have a great potential for understanding the mechanism of ageing and associated deterioration processes of seed [4]. e process of deterioration under accelerated ageing conditions is considered fundamentally similar to those under normal condition but not so far veri ed. However, the major di erence is that the rate of deterioration is much faster during accelerated ageing. A number of studies have been carried out in the past to analyze the physiological and biochemical changes associated with accelerated ageing in di erent seeds [5-7]. Membrane integrity is important marker to determine seed longevity. It is most probable site of biochemical and biophysical changes. Membrane chemical stability is determined by degree of peroxidation of membrane lipids leading to irreversible gel phase domains and loss of membrane function. During last few decades, several priming treatment established to improve germination time, rate, homogeneity and synchrony of aged seeds. Osmopriming is a pre-sowing treatment that exposes seeds to such osmoticum that treated solution with an inert osmoticum. Such primed seeds tend to have an improved seed performance indicated by better germination rate and uniformity [9]. Polyethylene glycol-6000 is o en used as the osmopriming reagent [10]. If the seeds are not used immediately a er treatment, then they must be dried back to lowest safe moisture contents (LSMC) at which they can be stored without deterioration. Present work depicts ageing dynamics in both natural and accelerated aged seeds. It is not known whether mechanisms of seed ageing are alike under accelerated ageing and natural ageing. Neem seeds behave as intermediate storage longevity. at gives the opportunity to execute

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natural and accelerated ageing at static point. For the rst time to the best of our knowledge, this article is also an attempt to determine the

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mg) were homogenized at 26 ± 2°C with 0.5% (w/v) 2-thiobarbituric acid (TBA) dissolved in 20% (w/v) trichloroacetic acid (TCA). 1% (w/v) Butylated hydroxy toluene (BHT) was included in the reaction mixture to eliminate artifactual peroxidative damage to the samples during processing. The sample homogenates were heated in a 95°C water bath for 30 min, followed by 15 min on ice to remove the proteins and centrifuged (4°C) at 10000 g for 5 min. The clear supernatants were collected and the amount of malondialdehyde (MDA)-thiobarbituric acid (TBA) complex in the supernatant was measured by absorbance at 540 nm (TBARS products) and corrected for the non-specific absorbance by subtracting the value obtained at 600 nm (turbidity). Interfering absorbance were removed by recording absorbance at 440 nm (sugars) to eliminate the interference by sucrose. The amount of MDA was calculated with the extinction coefficient of 155 mM^{-1} cm^{-1} and expressed in µmol MDA g^{-1} FW and corresponded to means of measurements carried out with four extracts ± SE.

$$MDA\ g^{-1}\ FW = \{[A_{532} - A_{600}] - [A_{440} - A_{600}] \times 0.057\}/157000 \times 10^{6}$$

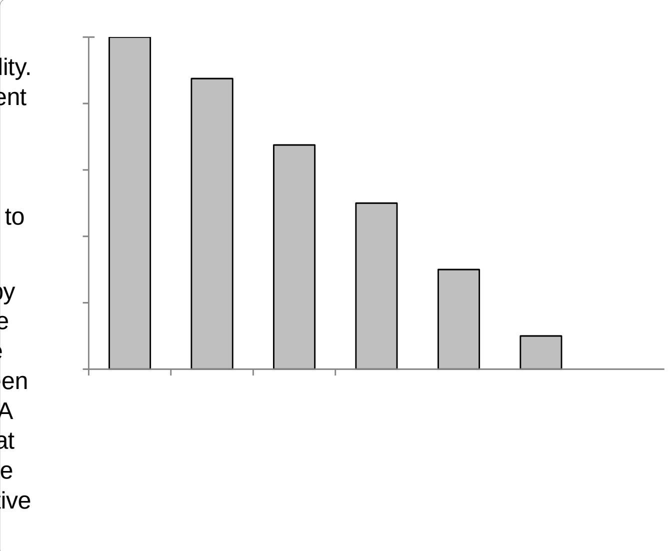
Statistical analysis

Data were analyzed by one-way and three-way ANOVA in combination with the Duncan's multiple-range tests at 5% level of significance ($p \leq 0.05$) for post-hoc comparisons of means. The bars/lines having similar alphabets were statistically non-significant at p<0.05 level, according to Duncan's multiple-range tests. Statistical tests were carried out using SPSS (version-16) for Microsoft Windows. Data given in percentage were subjected to arcsine transformation before analysis.

Results

Seed viability represents a trait that is important for the conservation of seed resources. To test viability of Neem (*Azadirachta indica*) seeds need to be stored for a long time and assessed by germination ability. Post-harvest matured Neem seeds displayed high moisture content (0.53 ± 0.017 g H_2O g^{-1} DM) with 100% germinability. The moisture content rapidly declined up to 0.23 ± 0.023 g H_2O g^{-1} DM within 14 days after harvesting and thereafter gradually decreased under natural storage condition (Figure 2). Seeds exhibited 100% germination up to 33 days of storage.

Figure 2 demonstrates natural ageing (NA) characterized by statistically significant (<0.001) decline in germination percentage during storage. Neem seeds lost total viability within 129 days. The controlled deterioration test, defined as accelerated aging (AA), has been developed as an alternative to analyze this property more efficiently. AA conditions are utilized to speed up the loss of viability. We found that treatment at 45°C and 100% humidity could artificially accelerate the aging of Neem seeds (Figure 3). Loss of viability showed linear positive correlation with accelerated ageing (R^2=0.994). AA treatment for 15 h exhibited total loss of germination. Evaluation of seed viability by TTC reduction assay revealed that viable seeds showed by red stained. Viability gradually decreased in axes, however drastically reduction observed in cotyledon of NA and AA seeds (Figure 4). We observed that 12-17% improvement takes place in germination of differentially NA and AA aged seeds after priming treatment (Table 1). The germination improvement is limited at 50% germination level of ageing, below 50% failed. Accordingly, priming exhibited germination index (seed vigour) improvement and exhibited rapid germination (Figure 5). Loss



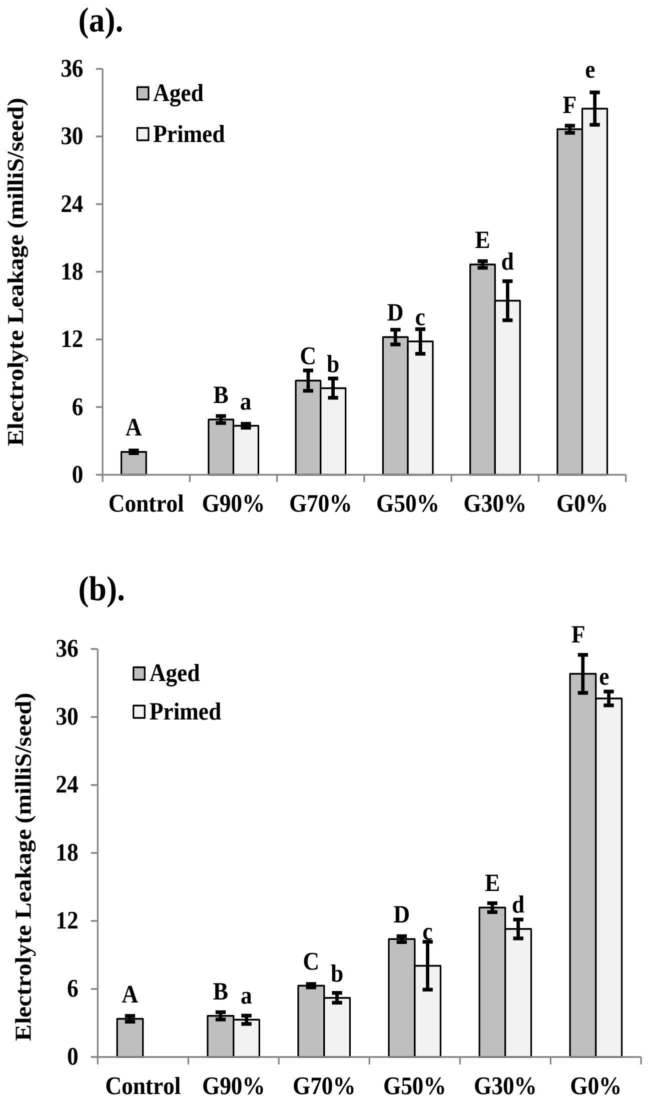
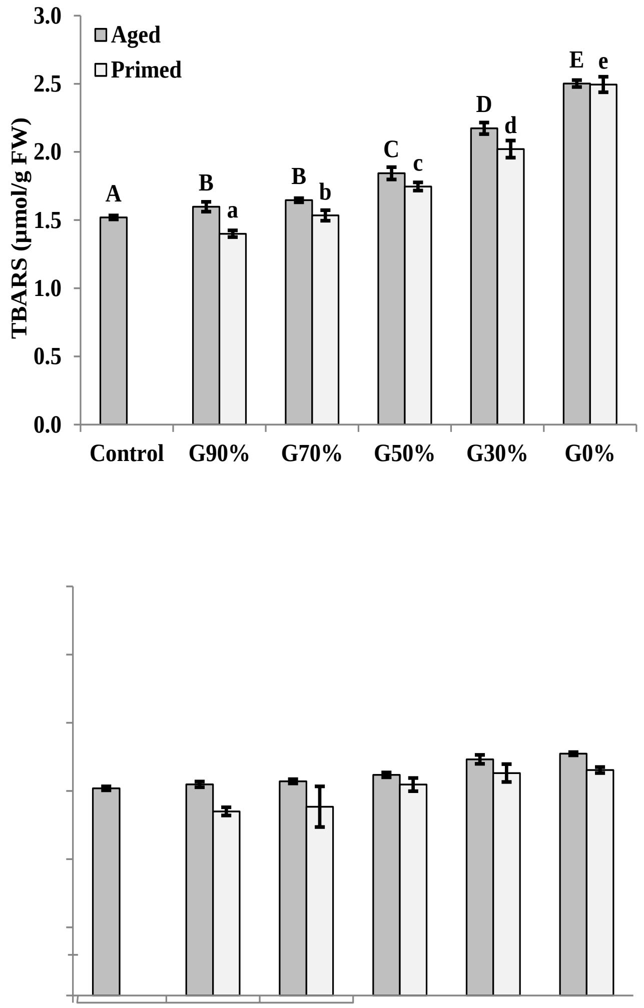


Figure 6: Effect of osmopriming on electrolyte leakage of (a) natural and (b) accelerated aged Neem seed at different germination percent. Vertical bar represent mean of fve independent replicates ± SE. Membrane damage exponentially increased with time of ageing and improved with osmopriming.



treatment) is widely used to enhance seed performance with respect to rate and uniformity of germination [3,22,23]. ere are many factors associated with the e ects of seed priming, but the concentrations of priming solutions as well as the time and temperature during priming were crucial [24]. Osmopriming optimized with -0.78 MP PEG-6000 concentrations at 30°C for one day are the best with reference to germination response and germination index (Supplementary Table 1). e results of this study indicate that osmopriming of aged seeds has signi cant positive e ect on the germination and seed vigour. Similar results, i.e., priming induced improvement in seed germination have been reported earlier [8,25-28]. However, our data indicates that 12-17% improvement occurs in primed seed compared to aged seeds; such improvement only limited up to 50% germination level of ageing (Table 1). No improvement occurs below 50% germination level of ageing. Seed vigour is a complex physiological feature that ensures rapid

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