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## Fixing accelerated ageing test period and evaluation of physical, physiological and biochemical changes during accelerated ageing in groundnut CO 7 pods

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### Abstract

Accelerated ageing test is an efficient vigour test to measure the seed vigour and predict seed storability and their longevity. The laboratory experiment was conducted at Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2017. This study was conducted on groundnut CO 7 pods and the pods were subjected to the accelerated ageing at  $40 \pm 1$  °C and 100% relative humidity for various time intervals (1, 2, 3, 4, 5, 6, 7, 8, 9 & 10 days). The samples were drawn daily from accelerated ageing condition and physical, physiological and biochemical attributes of seeds were evaluated. The result of the study revealed that speed of germination, first count (%), germination (%), root length (cm), shoot length (cm), dry matter production (g seedlings<sup>-10</sup>), vigour index, field emergence (%), viability (%) and dehydrogenase enzyme activity (OD value) decreased and moisture content (%) and electrical conductivity ( $\mu\text{S cm}^{-1} \text{g}^{-1}$ ) increased with increase of accelerated ageing periods. In the present study, initially the pods showed higher germination (84 per cent) and reached Indian Minimum Seed Certification Standards (IMSCS) of 70 per cent germination 4 days after accelerated ageing.

**Keywords:** oil seeds, groundnut, vigour test, accelerated ageing test

### Introduction

Groundnut (*Arachis hypogaea* L.) is one of the most important food and cash crops of our country. It is believed to be a native of Brazil and was introduced in India by sixteenth century. It is a self pollinated, annual herbaceous plant growing up to 50 cm tall with opposite, pinnate leaves having four leaflets and belongs to the Fabaceae family. Rapid seed deterioration during storage is one of the important constraints encountered by groundnut growing farmers. Maintenance of seed quality until sowing is mandatory (Tunwar and Singh, 1988)<sup>[27]</sup> as the irreversible degenerative changes would lead to loss of quality. Hence maintenance of seed quality during storage of seed is essential. More factors affect the seed quality during storage which include genetic, seed maturity, mechanical damage, relative humidity and temperature of the storage environment, seed moisture content and presence of micro flora. The initial seed quality and storage environment are important for increasing the shelf life of seeds. Seed vigor test is being carried out in time to time to check the seed quality and for proper disposal of storage seeds to prevent seed deterioration resulting into poor stand in actual field conditions. Accelerated aging test as a vigour test to predict seed storability or their longevity was developed by Delouche and Baskin, 1973<sup>[6]</sup> and has been used for seed vigor indicator in various crops (Association of Official Seed Analysts, 2002)<sup>[2]</sup>. Delouche and Baskin (1973)<sup>[6]</sup> proposed using the accelerated aging test to predict stand establishment of peanut and suggested that accelerated aging test might have additional utility as a test for predicting seed performance other than storability. Seed ageing can be accelerated during exposure to high temperature and high humidity. In this test, the higher vigor seeds tolerate the high temperature and high humidity and thus retain their capability to produce normal seedlings in the germination test. Low quality seeds deteriorate more rapidly than high quality seeds under these conditions.

Hampton and TeKrony (1995)<sup>[11]</sup> reported that a temperature of 41 °C for 72 hr should be used for the accelerated aging for soybean (*Glycine max* L.) and canola (*Brassica napus* L.).

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Duangpatra and Homdork (1986) [7] stated that for peanut (*Arachis hypogaea* L.) accelerated ageing test with a temperature of 42 °C and 100% relative humidity for 192 hr, while 42 °C and 100% RH for 264 hours was suggested by Promchote and Duangpatra (2002) [24] and Phyo *et al.* (2004) [22]. However, Siri *et al.* (2002) [25] reported that a temperature of 42°C and 100% RH for 264 hours should be used for the accelerated ageing vigor test for peanut. Differences in aging periods for peanut seed from the previous studies may have been attributable to genotypic differences (Phyo *et al.*, 2004) [22]. With this background this study was to investigate the effect of accelerated aging on seed viability and vigour in groundnut CO 7 seeds.

### Materials and Methods

The laboratory experiment was conducted at Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2016 – 2017. The freshly harvested pods of groundnut var. CO 7 collected from Department of oil seeds, Tamil Nadu Agricultural University, Coimbatore. The collected samples were hand sorted and cleaned thoroughly. Pods were subjected to accelerated ageing at 40 ± 1 °C with 100% relative humidity for 1 to 10 days. The samples were drawn daily interval from accelerated ageing condition and shelled. Kernels were tested for different seed quality tests *viz.*, physical, physiological and biochemical tests.

The physical test seed moisture content was determined by low constant temperature method (ISTA, 2010) on three replicates of five gram seeds each held at 103 ± 2 °C temperature for 16 ± 1 hr and the result was expressed in percentage. The physiological tests *viz.*, speed of germination - radicle protrusion was counted daily from third day of sowing until tenth day and it was calculated using the formula by Maguire, 1962. First count - During the germination test, the number of normal seedlings were counted on fifth day and the mean was expressed in percentage. The germination test in quadruplicate using 100 seeds each with 4 replicates of 25 seeds, were carried out in sand medium (ISTA, 2010), in a germination room maintained at a temperature of 25 ± 2 °C and RH 95 ± 2 % with diffuse light (approx. 10 h) during the day. Final count on normal seedling was recorded on 10<sup>th</sup> day observation on germination (%), root length (cm), shoot length (cm), dry matter production (g Seedlings<sup>-10</sup>) after drying at 80 ± 2 °C for 24 h and vigour index (Abdul-Baki and Anderson, 1973) [1] were measured at the end of germination test and also field emergence (%) was observed. The biochemical tests such as electrical conductivity (µS cm<sup>-1</sup> g<sup>-1</sup>), viability (%) (Lakon, 1949) [16], dehydrogenase (Kittock and Law, 1968) [15] and lipid peroxidation (Bernheim *et al.*, 1948) [4] were observed.

The experimental design was completely randomized design (CRD). The data obtained from each of the experiments were subjected to an analysis of variance and treatment differences

tested for significance (P= 0.05) as per the methodology described by Gomez and Gomez (1984) [9]. Wherever necessary, the percent values were transformed to arc-sine values before analysis. The critical differences (CD) were calculated at 5 and 1 per cent probability level.

### Result and Discussion

Accelerated ageing test is a widely used tool to test the seed quality. Seed technologists generally use this technique to predict the storability of seeds as it stimulates conditions conducive for seed deterioration. The process of deterioration of accelerated ageing condition is the same as that of natural ageing but only the rate of deterioration is enormous. In the present study, groundnut CO 7 pods artificially aged at 100 per cent RH and 40 ± 1 °C temperature for various durations from 0 to 10 days to evaluate effect of accelerated aging on seed viability and vigour in groundnut pods.

In the present study, moisture content was increased with increase of storage period under accelerated condition. The results indicated that kernels had low moisture content initially (6.82 per cent) and it was higher in 10 days (8.21 per cent) due to the hydrophilic nature which provided continuous and slow supply of moisture to the pods and increased the moisture. An increase in moisture content increases the respiration rate since it is an energy utilizing process. Due to increased respiration, breakdown of stored food materials takes place that invites the microorganism leading to reduction in seed vigour.

The first count is considered a parameter for assessment of seed vigour. Because in the deterioration process the speed of germination is affected (Nakagawa, 1994) [20]. In the present study, the control seeds showed higher germination (84 per cent) and reached Indian Minimum Seed Certification Standards (IMSCS) of 70 per cent germination, as it is the edge at which the seed lot turns to marginal on 4 days after accelerated ageing and eventually it reached to 38 per cent on 10th day of ageing. Decrease in germination percentage is related to reduction in seed vigour. In the present study, speed of germination, first count, germination percentage, root length, shoot length, dry matter production, vigour index and field emergence was decreased with increase of accelerated ageing periods. Similar results were reported in peanut (Sung & Jeng, 1994) [26], chickpea (Kapoor *et al.*, 2010) [13] and rice (Kapoor *et al.*, 2011) [14]. This reduction might be due to the lowering of the biochemical activities in seeds. The reduction in germination might be due to degradation of mitochondrial membrane leading to reduction in energy supply necessary for germination (Gidrol *et al.*, 1998) [8]. The decline in shoot length, root length and seedling vigor index might be attributed to DNA degradation with ageing which leads to impaired transcription causing incomplete or faulty enzyme synthesis essential for earlier stages of germination (Kapoor *et al.*, 2011) [14].

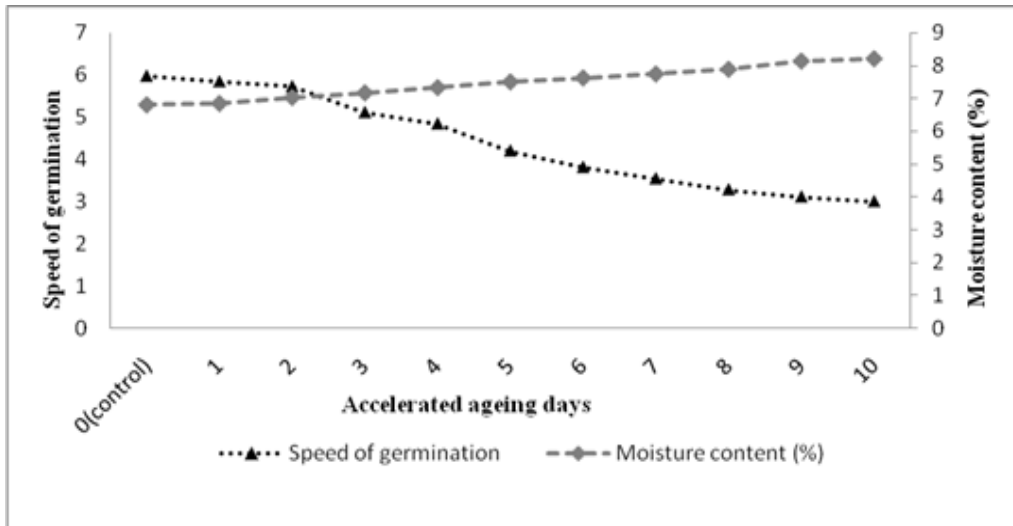


Fig 1: Effect of accelerated ageing on moisture content (%) and speed of germination of Groundnut Co 7 pods

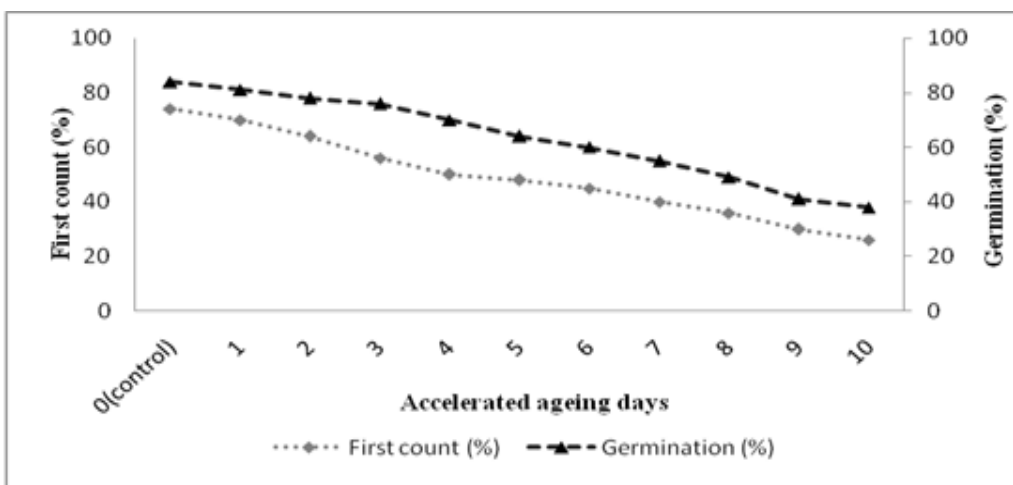


Fig 2: Effect of accelerated ageing on first count (%) and germination (%) of Groundnut Co 7 pods

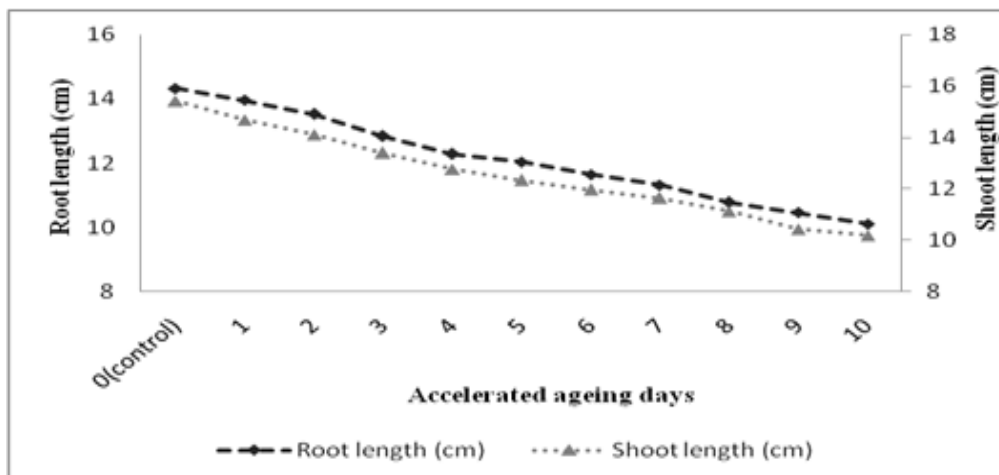


Fig 3: Effect of accelerated ageing on root length (cm) and shoot length (cm) of Groundnut Co 7 pods

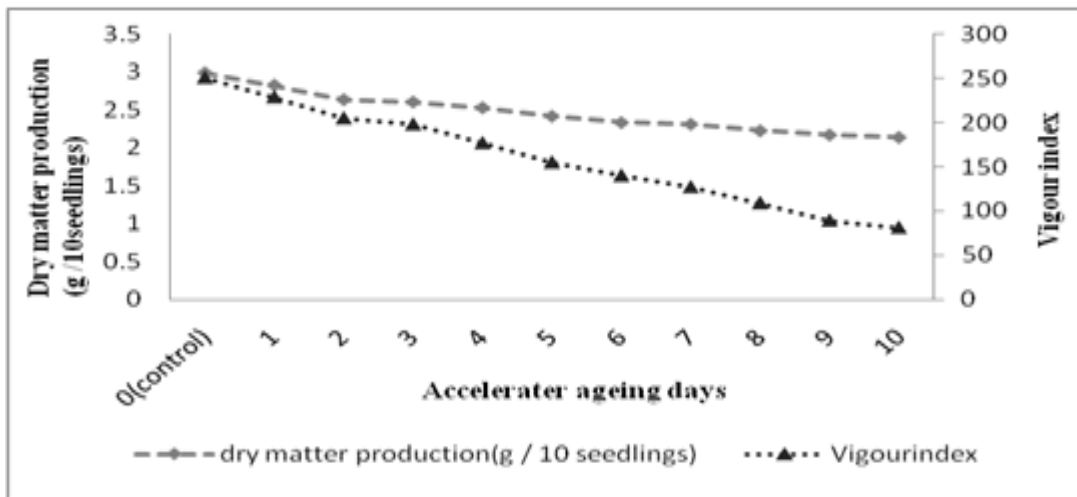


Fig 4: Effect of accelerated ageing on dry matter production (g seedlings<sup>-10</sup>) and vigour index of Groundnut Co 7 pods

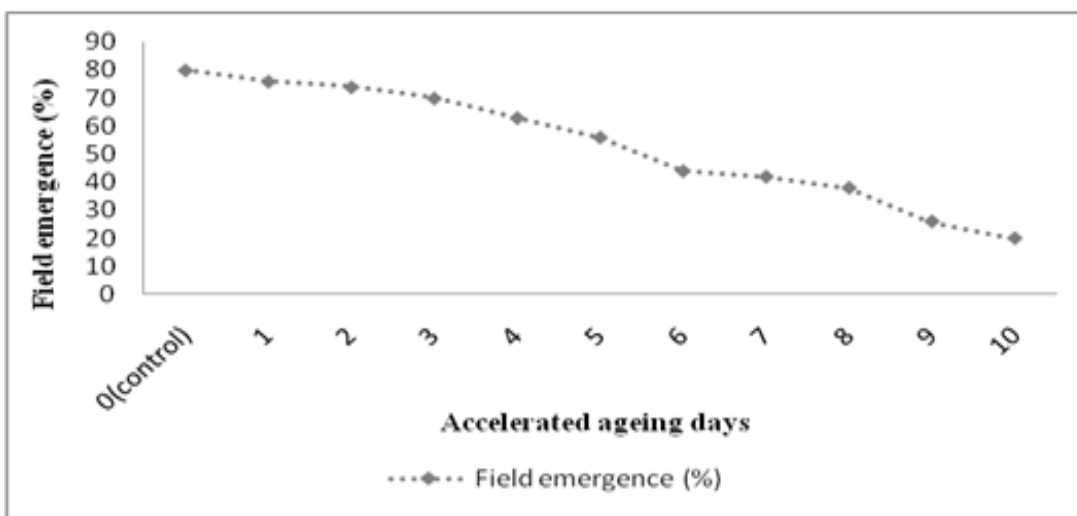


Fig 5: Effect of accelerated ageing on field emergence (%) of Groundnut Co 7 pods

In the present study, the electrical conductivity of seed leachate and lipid peroxidation increased with increase in period of accelerated ageing. Gupta *et al.* (2005) [10] reported that electrical conductivity increased after the seeds were subjected to accelerated ageing because of membrane deterioration and metabolic changes in the seed. Loss of seed vigour and viability is associated with deterioration of membrane properties (Priestley, 1986) [23]. Lipid peroxidation

mediated by free radicals, inactivation of enzymes or decrease in proteins, disintegration of cell membranes and genetic damage (Murthy *et al.*, 2003) [18]. Lipid oxidation products have pronounced effects on other important cellular systems and can damage DNA (Wilson & McDonald, 1986) [29]. So, lipid peroxidation can be considered as the main cause of seed ageing.

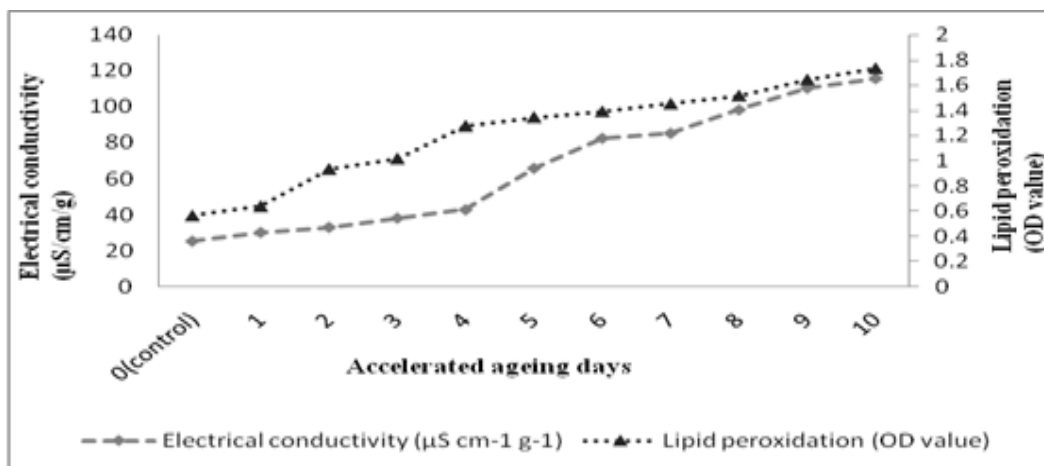


Fig 6: Effect of accelerated ageing on electrical conductivity (µS cm<sup>-1</sup> g<sup>-1</sup>) and lipid peroxidation (OD value) of Groundnut Co 7 pods

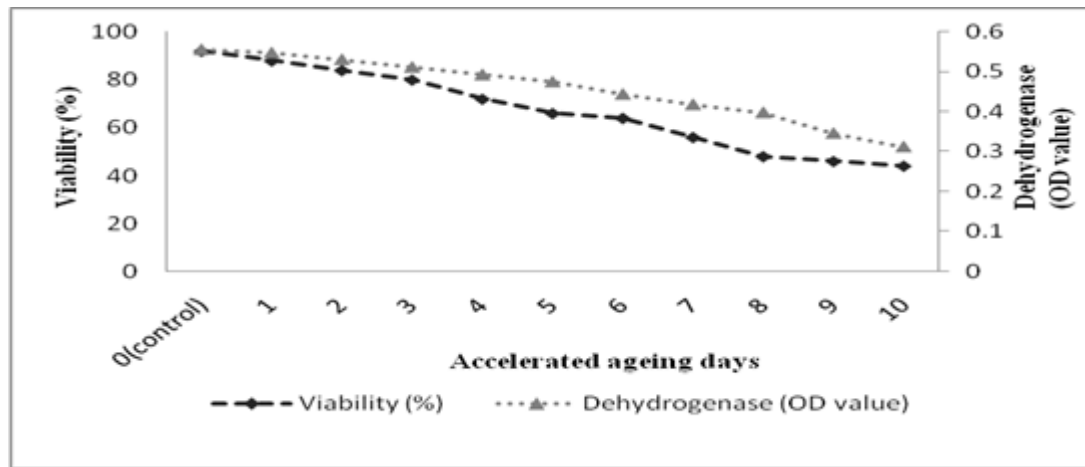


Fig 7: Effect of accelerated ageing on viability (%) and dehydrogenase (OD value) of Groundnut Co 7 pods

In the present investigation, the dehydrogenase enzyme activity was decreased with increasing period of storage under accelerated ageing. Chauhan *et al.*, 2011<sup>[5]</sup> and Pallavi *et al.*, 2003<sup>[21]</sup> noticed declined dehydrogenase enzyme activity due to enzymes undergoing compositional changes by losing or gaining certain functional groups, by oxidation of sulf-hydral groups or by conversion of amino acids within the protein structure. The enzymes may undergo configurational changes such as partial folding or unfolding of ultrastructure, condensation to form polymers and degradation to sub units i.e., absorbance of dehydrogenase enzyme was decreased as the period of storage increased in sunflower. Verma *et al.*, 2003<sup>[28]</sup> observed that the dehydrogenase activity was reduced as the ageing progressed and was found lowest after four year of storage in *Brassica* spp. Mustafa *et al.*, 2010<sup>[19]</sup> found that a high level of correlation was found between the loss of seed viability and the decreases that occurred in dehydrogenase activity in onion.

### Conclusion

In the present study groundnut CO 7 pods were subjected to  $40 \pm 1$  °C with 100% relative humidity for 1 to 10 days. The result showed that the control pods reached Indian Minimum Seed Certification Standards (IMSCS) of 70 per cent germination, as it is the edge at which the seed lot turns to marginal on 4 days after accelerated ageing.

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