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Effect of Acceleratedly Aged Groundnut (*Arachis hypogaea* L.) Seeds on Physiological and Biochemical Properties

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ABSTRACT

Background: Accelerated ageing causes seeds to deteriorate that affect the seed quality of groundnut seeds. Seed deterioration will cause metabolic and chemical changes *viz.,* lipid peroxidation and autoxidation, leading to loss of membrane integrity and enzyme activities. These changes are associated with reduced germination and vigour.

Methods: The groundnut seeds cv. VRI 8 was subjected for accelerated ageing at 40°C with 100% RH upto 11 days and evaluated the physiological and biochemical changes in groundnut seeds associated with accelerated ageing.

Results: The germination and seed vigour of accelerated aged groundnut seeds exhibited declining trend which was well correlated with antioxidant enzyme activities *viz.,* catalase, peroxidase, superoxide dismutase, ascorbate peroxidase and glutathione reductase activities whereas negatively correlated with electrical conductivity, electrolyte leakage and proline content. The decline in physiological and biochemical activity in accelerated aged seeds was associated with impairment in metabolic process caused by membrane damage during ageing and the inability of the seeds to repair and compensate the accumulated damage.

Key words: Accelerated ageing, Antioxidant activity, Electrolyte leakage, Germination, Groundnut, Membrane stability index, Seed vigour.

INTRODUCTION

Groundnut is an annual allotetraploid crop, native to South America and belongs to Fabaceae family. India is the second-largest producer of groundnut in the world. In 2021-2022, India has exported 514,163.87 MT of groundnut (APEDA, 2021). It is the second most important cultivated legume crop and the fourth largest edible oilseed crop in the world (Shilman *et al*., 2011) as it has high oleic acid (MUFA) that provides lower rate of oxidation and less pungent flavor in storage (Mozingo *et al.,* 2004).

The groundnut kernel consists of total carbohydrate (9.89%-23.62%), crude lipids (32.7%-53.9%) and crude protein (25.9%-32.4%) (Musa, 2011; Wang *et al.,* 2016). In addition, it is also rich in vitamins (*e.g*., vitamin E), minerals (*e.g*., copper, manganese, iron and phosphorus), phytosterols, phenolics and antioxidants (Arya *et al.,* 2016).

Fatty acid profiling of groundnut depicts 42% monounsaturated fatty acids and 37% polyunsaturated fatty acids which include 45% oleic, 35% linoleic, 10% palmitic, 3% stearic and 2% behenic, with the other 5% comprising small amounts of linolenic, arachidic eicoseonic, lignoleic and gadoleic acids (Ahmed and Young, 1982).

These fatty acids are the major energy source for the germinating seedlings next to carbohydrates, especially in the early developmental stages. But these fatty acids and oil content are modified by undergoing various chemical changes and reacts with other constituents producing undesirable compounds which is detrimental to seed quality during storage (Fennamo, 1996).

Seed deterioration occurs during storage is associated with various metabolic and chemical changes leading to physical and physiological alterations including loss of membrane integrity and cell compartmentalization (McDonald, 1999). Also, this seed deterioration affects the unsaturated fatty acids resulting in increased cell permeability (Simon, 1974).

Lipid peroxidation and auto-oxidation are involved in seed ageing in groundnut (Sung and Jeng, 1994) and many studies also revealed that accelerated ageing is related with decrease in antioxidant enzymes, total lipids, phospholipids and unsaturated fatty acid content (Kalpana and Madhav Rao, 1994).

In the present study, it has been investigated whether accelerated ageing is related to (1) Membrane damage through measuring electrical conductivity, electrolyte leakage and membrane stability index (2) Changes in total oil content (3) Changes in antioxidant levels through measuring various antioxidant enzymes *viz.,* catalase, peroxidase, superoxide dismutase, ascorbate peroxidase, glutathione reductase and proline content.

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MATERIALS AND METHODS

Seed material

Freshly harvested seeds of groundnut cv. VRI 8 were procured from the Regional Research Station, Vridhachalam. The procured seeds were finely dried, cleaned and graded for further use of conducting the experiments. The experiments were carried out in Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore in the year 2022-2023. The seeds were subjected to accelerated ageing by placing the seeds in controlled environment (temperature-40°C and relative humidity - approximately 100%) for 11 days to create a lot with various germination percentage and vigour.

Germination, vigour index and dehydrogenase activity

Germination test was investigated in roll towel method with 4 replication of 100 seeds each under controlled condition of 25±2°C and 95±2% of temperature and RH respectively (ISTA, 2015). On the day of final count (10th day), seedlings were evaluated as normal seedlings, abnormal seedlings, hard seeds and dead seeds as per ISTA. Based on the number of normal seedlings, the germination % was calculated by using the following formula:

$$\text{Germination (\%)} = \frac{\text{Number of normal seedings}}{\text{Total number of seeds sown}} \times 100$$

Vigour index was calculated as per the formula given by Abdul Baki and Anderson (1973) and the mean values were expressed in whole number.

$$\text{Vigour index} = \text{Germination (\%)} \times \text{Seedling length (cm)}$$

The dehydrogenase activity in seeds were assessed by following the procedure given by Kittock and Law (1968).

Electrical conductivity, Electrolyte leakage and Membrane stability index

The electrical conductivity of the seed leachate was measured using electrical conductivity meter and was indicated in dSm^{-1} (Presley, 1958). The electrolyte leakage and membrane stability index were determined by measuring electrical conductivity at 25°C (C1) and after incubating at 121°C for 20 mins (C2) (Kocheva *et al.,* 2005). It is measured by adopting the following formula:

$$\text{Electrolyte leakage (\%)} = \frac{C1}{C2} \times 100$$

$$\text{Membrane stability index (\%)} = 1-(C1/C2) \times 100$$

Oil content

The oil was extracted from the seeds by cold press technique. The cold pressed extraction of groundnut oil was accomplished using a twin-screw press at temperatures below 60°C (Wang *et al.,* 2016). The oil content was determined by using the following formula.

$$\text{Oil content (\%)} = \frac{\text{Weight of oil extracted (g)}}{\text{Weight of sample (g)}} \times 100$$

Antioxidant enzymes activity

Spectrophotometric method was followed for determining all the antioxidant enzymes. The procedures were followed for catalase activity as per Aebi (1984), peroxidase (Malik and Singh, 1980), superoxide dismutase (Beauchamp and Fridovich, 1971), ascorbate peroxidase (Nakano and Asada, 1981), glutathione reductase (Elavarthi and Martin, 2010) and proline content (Bates *et al.,* 1973).

Statistical analysis

The data recorded were statistically analyzed using completely randomized design and wherever necessary the per cent values were transformed to angular (Arc-sine) values before the analysis and the critical difference (CD) was computed at 5 per cent probability using SPSS software (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The results revealed that the physiological parameters of the accelerated aged seeds was found to decrease significantly when compared to control. Reduction in germination percentage to the tune of 76% and a significant reduction in vigour index (87%) were registered when the seeds were subjected to 11 days of accelerated ageing when compared to control (Fig 1). The results are in accordance with Das *et al.* (2018), where the sunflower registered a similar decrease in germination and seedling length when it is kept for storage. As seed deterioration increased, seed germination and vigour index progressively decreased indicating a biochemical manifestation *viz.,* cellular, metabolic and chemical alterations including chromosome aberrations and DNA damage, RNA impairment and enzyme denaturation (Kapoor *et al.,* 2010). According to Bewley *et al.* (2013), after 48 hrs of ageing, the seeds cannot tolerate severe biochemical changes and cannot repair the damage resulting in reduction in seed germination.

The electrical conductivity of the seed leachate significantly increased with ageing. It increased considerably in 11 days accelerated aged seeds to reach 0.489 dSm^{-1} from 0.156 dSm^{-1} in control seeds. Similar increase was recorded in electrolyte leakage from the aged seeds *i.e.*, 15% increased electrolyte leakage in solution was observed when the seeds were acceleratedly aged for 11 days (Table 1). Similar increase in electrical conductivity along with increased leakage of nutrients in medium were recorded in sweet pepper (Kaewnaree *et al.,* 2011). Whereas, decrease in the membrane stability index of the aged seeds was determined as compared to control seeds. This continuous efflux of solute leakage in medium during water uptake is in accordance with the result in sunflower seeds by Corbineau *et al.* (2002).

Electrolyte leakage from seed is correlated with cell membrane damage and its repair during ageing (Khan *et al.,* 2005). Free radicals produced during accelerated ageing will be detrimental to the cell membrane resulting in ion leakage in response to imbibition which includes substance like both organic ions *viz.,* sugars, enzymes, amino acids, protein, nucleosides and inorganic ions. These electrolyte efflux indicate the cell rupture and increased membrane permeability. Therefore, seed deterioration can be determined by the amount of seed solute leaked in solution.

When the seeds were subjected to accelerated ageing, the germination, vigour index and membrane stability of the seeds were decreased and the electrical conductivity and electrolyte leakage were also increased indicating that germination, vigour index, membrane stability is negatively correlated to electrical conductivity and electrolyte leakage (Table 2). This result is confimed with Mandizvo and Odindo (2019) who observed a similar loss of germinating ability of bambara groundnut landrace after ageing for 48 hrs which is well correlated with increased electrical conductivity and electrolyte leakage and in soybean seeds (Vijay *et al.,* 2010).

When the accelerated ageing increased, decrease in the dehydrogenase activity of the seeds were recorded. Significant decrease in the oil content from the day one of accelerated ageing was found as compared to control. As the ageing increased, the oil content is further decreased *i.e*., 9.2% reduction was observed on 11th day of ageing than control seeds (Table 1). The results observed are in confirmity with Suresh *et al.,* (2019) who showed a similar significant reduction in total oil content in accelerated aged

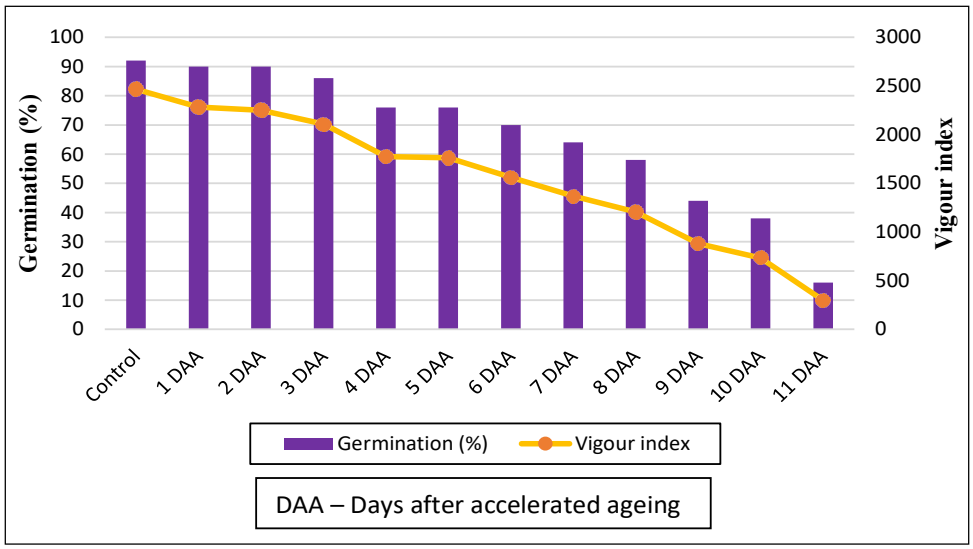


Fig 1: Effect of accelerated ageing on physiological parameters in groundnut seeds.

Table 1: Changes in biochemical parameters due to accelerated ageing in groundnut seeds.

Storage period (Days)	Electrical conductivity of seed leachate (dSm^{-1})	Electrolyte leakage (%)	Membrane stability index (%)	Dehydrogenase activity (OD value)	Oil content (%)
Control	0.156	45.4 (42.36)	54.5 (47.58)	2.850	49.7 (44.83)
1 DAA	0.183	47.3 (43.45)	52.7 (46.55)	2.743	48.9 (44.37)
2 DAA	0.189	47.5 (43.57)	52.6 (46.49)	2.685	43.8 (41.44)
3 DAA	0.204	47.5 (43.57)	52.5 (46.43)	2.651	42.4 (40.63)
4 DAA	0.222	49.0 (44.43)	50.9 (45.52)	2.641	44.5 (41.84)
5 DAA	0.283	54.2 (47.41)	45.6 (42.48)	2.620	42.4 (40.63)
6 DAA	0.315	56.4 (48.68)	43.7 (41.38)	2.607	42.8 (40.86)
7 DAA	0.356	62.6 (52.30)	41.9 (40.34)	2.575	42.3 (40.57)
8 DAA	0.400	63.1 (52.59)	37.5 (37.76)	2.547	42.3 (40.57)
9 DAA	0.413	59.8 (50.65)	36.9 (37.41)	2.496	41.6 (40.16)
10 DAA	0.432	62.4 (52.18)	43.1 (41.03)	2.425	42.0 (40.40)
11 DAA	0.489	60.4 (51.00)	39.6 (39.00)	2.381	40.5 (39.52)
Mean	0.304	54.6 (47.64)	46.0 (42.71)	2.602	43.6 (41.32)
SEd	0.004	0.908	0.637	0.045	0.41
CD (P=0.05)	0.008	1.873	1.314	0.092	0.85

(Figures in parentheses indicate arc sine value).

Jatropha seeds. Increased relative humidity and temperature during accelerated ageing resulted in increased moisture content in seeds through attaining equilibrium resulting in lipid peroxidation and autooxidation (Suriyong, 2007). These will produce free radicals that would act upon the lipids thereby reducing the oil content (Sushma, 2014). As the lipids are the major energy source next to sucrose, these lipids are utilized as energy source during aging might be another reason for reduced oil content in accelerated aged seeds (Ghasemnezhad and Honermeier, 2009; Lakshmi *et al*., 2021).

The accelerated ageing decreased the antioxidant activity of the seeds. There was a significant reduction in all the enzymatic activities when the seeds were subjected to 11 days of accelerated ageing under 40°C and 100% RH *i.e*., 50% in catalase activity, 41% in peroxidase activity, 61% in superoxide dismutase activity, 35% in ascorbate peroxidase activity and 78% in glutothione reductase activity whereas 25% increased proline content was recorded in 11 days accelerated aged seeds (Table 3 and 4). Increase in proline content indicates that seed activates the defense system to tolerate the alterations occurred during ageing but the antioxidant system decreases as ageing increases depicting the loss of seed capacity to tolerate the damage caused by ageing *i.e*., free radical damage to mitochondria and DNA damage. Reduction in enzymatic activities, an indication of seed deterioration during ageing includes reduction in both the synthesis and activity due to oxidative stress (Mittler, 2002; Kumari *et al*., 2021). This loss of enzyme activity *viz.,* catalase, peroxidase,

Table 2: Changes in biochemical parameters (enzymatic activities) due to accelerated ageing in groundnut seeds.

Storage period (Days)	Catalase activity (µM H_2O_2 reduced min^{-1} mg^{-1} protein)	Peroxidase activity (U mg^{-1} protein min^{-1})	Superoxide dismutase activity (mg $protein^{-1}$)
Control	1.89	1.27	0.88
1 DAA	1.71	1.05	0.81
2 DAA	1.66	1.01	0.75
3 DAA	1.59	0.95	0.69
4 DAA	1.47	0.89	0.67
5 DAA	1.43	0.76	0.61
6 DAA	1.34	0.73	0.59
7 DAA	1.22	0.71	0.53
8 DAA	1.16	0.67	0.47
9 DAA	1.11	0.64	0.44
10 DAA	1.06	0.57	0.39
11 DAA	0.94	0.52	0.34
Mean	1.38	0.81	0.60
SEd	0.019	0.012	0.011
CD (P=0.05)	0.039	0.024	0.024

Table 3: Changes in biochemical parameters (enzymatic activities) due to accelerated ageing in groundnut seeds.

Storage period (Days)	Ascorbate peroxidase activity (mM min^{-1} mg^{-1} protein)	Glutothione reductase activity (µM reduced glutathione formed min^{-1} mg^{-1} protein)	Proline content (µM/ gram of FW)
Control	2.15	1.98	23.4
1 DAA	2.11	1.67	24.3
2 DAA	2.07	1.13	24.9
3 DAA	2.05	1.07	24.3
4 DAA	1.98	1.18	23.2
5 DAA	1.99	1.11	25.1
6 DAA	1.94	1.03	24.9
7 DAA	1.86	0.78	26.2
8 DAA	1.82	0.77	27.8
9 DAA	1.77	0.54	28.9
10 DAA	1.54	0.49	28.8
11 DAA	1.39	0.43	29.2
Mean	1.89	1.02	25.92
SEd	0.03	0.02	0.31
CD (P=0.05)	0.06	0.04	0.64

Table 4: Correlation between physiological parameters and biochemical parameters.

	Germination (%)	Vigour index I	Electrical conductivity of seed leachate (dSm^{-1})	Electrolyte leakage (%)	Membrane stability Index	Oil content (%)
Germination (%)	1					
Vigour index I	0.989	1				
Electrical conductivity of seed leachate (dSm^{-1})	-0.960	-0.982	1			
Electrolyte leakage (%)	-0.814	-0.874	0.938	1		
Membrane stability index	0.820	0.875	-0.933	-0.944	1	
Oil content (%)	0.691	0.754	-0.758	-0.717	0.729	1

glutothione reductase is in accordance with Silva *et al.* (2018) in *Jatropha* seeds stored at different maturity stages and sunflower seeds (Bailly *et al.,* 1996).

CONCLUSION

The present study in groundnut cv. VRI 8 revealed that when the seeds are subjected to accelerated ageing for 11 days, the seed deterioration started. It was evaluated by observing a decline in germination percentage, seed vigour and the antioxidant defense system associated with increased electrical conductivity and electrolyte leakage resulting in loss of membrane integrity. These changes might be due to high temperature and relative humidity during accelerated ageing that increased the seed moisture which further triggered the biochemical changes which are irreversible, leading to a loss of vigour and viability.

Conflict of interest

All authors declared that there is no conflict of interest.

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