

Full Length Research Paper

Ash (*Fraxinus excelsior*) seed quality in relation to seed deterioration under accelerated aging conditions

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This study was conducted to evaluate the response of ash (*Fraxinus excelsior*) seeds under accelerated aging test. The accelerated aging test was carried out at three different temperatures: 41, 43 and 45 °C with four duration periods of 48, 72, 96, 144 and a relative humidity of 100%. The two seed lots of *F. excelsior* were subjected to tests of their quality including standard germination, vigor index, seedling growth and seedling dry weight. A completely randomized factorial design with four replications was used. Results indicated that, old seeds were more sensitive to accelerated aging than new seeds. Seeds of both lots had a reduction of viability and vigor after accelerated aging at 41 °C for 96 h, 43 and 45 °C for 72 to 144 h. Germination, vigor index, seedling growth and seedling dry weight showed a greater response to accelerated aging.

Key words: *Fraxinus excelsior*, accelerated aging, germination, seed vigor, seedling growth rates.

INTRODUCTION

Ash (*Fraxinus excelsior*) seeds are one of the most sensitive agronomic seeds where significant deterioration occurs after just one year of storage. Seed quality and longevity of seeds during preservation period mainly depends on two main factors such as biotic like insects, fungi, bacteria, virus, rodents, etc. and abiotic like temperature, relative humidity, moisture content, rainfall, day length and sunshine, etc. Loss of germination capacity is the final manifestation of seed deterioration (Soltani et al., 2009). Seed deterioration can be defined as “deteriorative changes occurring with time that increase the seed’s vulnerability to external challenges and decrease the ability of the seed to survive.” Three general observations can be made about seed deterioration. Firstly, seed deterioration is an undesirable attribute of agriculture. Annual losses of revenue from seed/grain products due to deterioration can be as much as 25% of the harvested crop (Maqsod et al., 2000). An understanding of seed deterioration, therefore, provides a template for improved crop production as well as increasing agricultural profits.

Secondly, the physiology of seed deterioration is a separate event from seed development and/or germination. Thus, the knowledge gained from understanding these events likely does not apply to what occurs during deterioration. Thirdly, seed deterioration is cumulative. As seed aging increases, seed performance is increasingly compromised. Thus, it is important that a fundamental understanding of the process (es) of seed deterioration be gained. This aging is manifested as a reduction in percentage germination, while those seeds that do germinate, produce. During aging, seeds lose their vigor and ultimately viability (Soltani et al., 2009). Losses in seed quality occur during field weathering, harvesting and storage. The losses are exacerbated if seeds are stored at high temperature and/or high relative humidity conditions. Accelerated aging is one of the vigor tests widely used to determine the quality of seed lots (Thant et al., 2010). It is proposed as a prediction test for seed storability and gives an information on the (Nelson et al., 2008) extend of storability of seed as macro, micro and meso-biotic. An accelerated aging stress test exposes seeds for short periods (1 to 8 days) to high temperature (40 to 45 °C) and high relative humidity (greater than 90%). During the test, the seeds absorb moisture from the humid environment along with the high temperature, causing

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Table 1. Results of variance analysis.

Source	d.f	Germination (%)	Moisture content (%)	Vigor index	Shoot length (cm)	Root length (cm)	Seedling dry weight (g)
Temperature (A)	2	6286.948**	222.433**	434.823**	365.241**	410.819**	1.210**
Duration (B)	3	18433.306**	272.343**	1421.667**	938.707**	1040.238**	1.377**
A*B	6	296.795**	3.794**	7.073*	6.863**	9.132**	0.024**
Storage (c)	1	9640.042**	18.463**	181.500**	103.377**	179.580**	0.309**
A*C	2	171.573**	3.645**	33.031**	4.217ns	8.776**	0.083**
B*C	3	1349.181**	0.284ns	14.556*	6.467**	11.606**	0.052**
A*B*C	6	185.670**	0.426ns	0.587ns	1.153ns	1.393ns	0.019**
Error	72	7.194	0.365	3.222	1.528	1.570	0.001

ns, not significant; * significant at 0.05 probability level; **, significant at 0.01 probability level.

rapid seed aging (ISTA, 2004). The changes in germination of seeds after accelerated aging followed a similar trend to the germination of seeds after time in warehouse storage (Siri et al., 2002). Different responses to accelerated aging were found in many crop seeds. The accelerated aging at 45°C for 48 to 72, 72 and 120 h showed potential as a seed vigor test in aubergine, cucumber and melon, respectively (Demir et al., 2004). The purpose of this study was to investigate the responses of storage and seed quality to accelerated aging.

MATERIALS AND METHODS

This study was carried out at the Seed Technology Laboratory and greenhouse, Department of Agronomy, Faculty of Moghan Agriculture, Iran during 2010. The experimental material was the ash (*F. excelsior*) seeds stored in two different conditions. Two different ash seed lots (No 1: collected in autumn 2009 and No 2: one years storage in 2008) were used in this study. Seeds were subjected to tests of standard germination, vigor index and shoot and root length following the AOSA rules for testing seeds (AOSA, 2001; 2002). The experiments were arranged in a factorial completely randomized design with four replications. The test was conducted according to the procedure described by Wongvarodom and Naulkong (2006) and Thant et al. (2010). Accelerated ageing was conducted at three different temperatures (41, 43 and 45°C) and four periods (48, 72, 96 and 144 h) at 100% RH. An aging bottle containing 50 ml of deionized water was used to maintain 100% RH. The accelerated ageing bottles and wire mesh were sterilized with 90% alcohol. Four replications of each lots seed were put in the sterilized wire mesh. The wire mesh was placed in the sterilized aging bottles, which each contained 50 ml of deionized water. The wire mesh in the bottle was held above the water level. The aging bottles were seal-locked and kept in the accelerated ageing chamber at three different temperatures for four periods, as previously mentioned.

Standard germination

One hundred (100) seeds per replication were germinated in between paper (BP) in a 25°C germinator. The germination tests were evaluated after 3 days for 1 month. Numbers of normal seedlings were averaged as the germination percentage (Thant et al., 2010; ISTA, 2004).

Moisture content

Twenty (20) seeds per replication were weighed and dried at 105°C for 24 h. The dried seeds were weighed and moisture content was calculated on a percentage of wet weight bases.

Vigour index

Values were calculated as per adopting the formula vigour (Phyo et al., 2004).

Seedling vigour index = germination% × average seedling length (cm)/100

Shoot and root length

Hundred (100) seeds of each treatment were performed in plastic pots (2 cm depth and with equal distance) containing sand in greenhouse conditions with four replication. The pots were watered on demand. After 3 months of sowing the germination count, ten normal seedlings were measured for their root (tip of root to juncture with shoot) and shoot length (tip of shoot to juncture with root) in centimeter (cm).

Seedling dry weight

The ten seedlings used for the growth measurements were dried under shade for 24 h and then dried in a hot air oven maintained at 75 ± 2°C for 48 h and cooled in desiccator for 30 min, weighed and the mean weight expressed in gram (Navamaniraj et al., 2008).

Statistical analysis

The seed quality were tested for the non-aged seeds, quality of non-aged seeds and that after accelerated aging were compared using a completely randomized design and analyzed using analysis of variance. The statistical significance of means was tested by Duncan's multiple range test (McDonough et al., 2004).

RESULTS

Results of the experiment are shown in Tables 1 to 9 and Figures 1 to 3. Variance analysis has been presented in

Table 1. Results of variance analysis.

Source	d.f	Germination (%)	Moisture content (%)	Vigor index	Shoot length (cm)	Root length (cm)	Seedling dry weight (g)
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Error	72	7.194	0.365	3.222	1.528	1.570	0.001

ns, not significant; * significant at 0.05 probability level; **, significant at 0.01 probability level.

Table 2. Characteristics of seed lots as affected by different temperatures.

Temperature	Germination (%)	Vigor index	Shoot length (cm)	Root length (cm)	Seedling dry weight (g)	Moisture content (%)
41 °C	56.91 ^a	15.41 ^a	13.19 ^a	14.17 ^a	0.648 ^a	17.66 ^c
43 °C	35.09 ^b	9.719 ^b	8.256 ^b	8.970 ^b	0.348 ^b	20.00 ^b
45 °C	30.75 ^c	8.500 ^c	6.731 ^c	7.297 ^c	0.283 ^c	22.92 ^a
LSD	1.774	1.187	0.818	0.829	0.020	0.399

Means followed by the same letter(s) in a column are statistically non-significant at 1% level of probability.

Table 3. Characteristics of seed lots as affected by different durations.

Duration period (h)	Germination (%)	Vigor index	Shoot length (cm)	Root length (cm)	Seedling dry weight (g)	Moisture content (%)
48	70.71 ^a	20.54 ^a	16.82 ^a	17.90 ^a	0.703 ^a	15.75 ^d
72	56.21 ^b	13.79 ^b	11.63 ^b	12.50 ^b	0.506 ^b	19.50 ^c
96	27.50 ^c	7.792 ^c	6.86 ^c	7.64 ^c	0.360 ^c	22.20 ^b
144	9.25 ^d	2.708 ^d	2.26 ^d	2.52 ^d	0.136 ^d	23.32 ^a
LSD	2.04	1.371	0.944	0.957	0.024	0.461

Means followed by the same letter(s) in a column are statistically non-significant at 1% level of probability.

Table 4. Germination percentage of different lots as affected by different temperatures and durations.

Lot	Temperature (°C)	Duration			
		48 (h)	72 (h)	96 (h)	144 (h)
No. 1	41	87.5 ^a	80.25 ^b	60.5 ^d	31.5 ^h
	43	83.25 ^{ab}	72.50 ^c	22.50 ⁱ	0.00 ^k
	45	85.75 ^a	68.50 ^c	19.00 ^j	0.00 ^k
No. 2	41	72.5 ^c	56.50 ^{de}	42.5 ^{fg}	24.00 ⁱ
	43	52.00 ^e	38.00 ^g	12.5 ^j	0.00 ^k
	45	43.25 ^f	21.5 ⁱ	8.00 ^j	0.00 ^k

Means followed by the same letter(s) in a row and column are statistically non-significant at 1% level of probability (LSD= 5.018)

Table 5. Moisture content % of different lots as affected by different temperatures and durations.

Lot	Temperature (°C)	Duration			
		48 (h)	72 (h)	96 (h)	144 (h)
No. 1	41	12.67 ^k	15.67 ⁱ	18.90 ^f	20.07 ^e
	43	15.55 ^{ij}	18.27 ^{fg}	21.50 ^d	23.75 ^b
	45	17.37 ^{gh}	23.10 ^{bc}	24.95 ^a	25.25 ^a
No. 2	41	14.42 ^j	17.90 ^{fg}	20.17 ^e	21.45 ^d
	43	16.30 ^{hi}	18.75 ^f	22.45 ^{cd}	23.45 ^{bc}
	45	18.17 ^{fg}	23.32 ^{bc}	25.25 ^a	25.95 ^a

Means followed by the same letter(s) in a row and column are statistically non-significant at 1% level of probability (LSD = 1.130).

Table 6. Vigor index of different lots as affected by different temperatures and durations.

Lot	Temperature(°C)	Duration			
		48 (h)	72 (h)	96 (h)	144 (h)
No. 1	41	27.5 ^a	19.75 ^{bcd}	14.75 ^{efg}	9.75 ^{ijk}
	43	21.75 ^b	14.00 ^{fgh}	7.00 ^{jkl}	0.00 ^m
	45	19.25 ^{bcd}	12.75 ^{fhgi}	4.50 ^l	0.00 ^m
No. 2	41	20.75 ^{bc}	14.50 ^{efg}	9.75 ^{ijk}	6.5 ^{kl}
	43	17.75 ^{cde}	11.25 ^{ghi}	6.00 ^l	0.00 ^m
	45	16.25 ^{def}	10.50 ^{hij}	4.75 ^l	0.00 ^m

Means followed by the same letter(s) in a row and column are statistically non-significant at 1% level of probability (LSD= 3.358).

Table 7. Shoot length (cm) of different lots as affected by different temperatures and durations.

Lot	Temperature (°C)	Duration			
		48 (h)	72 (h)	96 (h)	144 (h)
No. 1	41	20.90 ^a	17.60 ^b	12.33 ^{def}	7.77 ^{ghi}
	43	18.17 ^b	11.75 ^{ef}	6.42 ^{hij}	0.00 ^l
	45	16.10 ^{bc}	8.92 ^{gh}	5.20 ^{jk}	0.00 ^l
No. 2	41	18.12 ^b	14.15 ^{cde}	8.85 ^{gh}	5.82 ^{ji}
	43	14.42 ^{cd}	10.20 ^{fg}	5.07 ^{jk}	0.00 ^l
	45	13.20 ^{de}	7.12 ^{hij}	3.30 ^k	0.00 ^l

Means followed by the same letter(s) in a row and column are statistically non-significant at 1% level of probability (LSD= 2.313).

Table 1. According to Table 1, the effect of different temperatures, durations and storage were significant on seed germination, moisture content, vigor index, shoot and root length and seedling dry weight ($p < 0.01$). According to Tables of 2 and 3, the highest germination%, vigor index, shoot and root length (cm) and seedling dry weight observed in 41°C and 48 h. The interaction between storage and duration was significant in all traits with the exception of moisture content.

According to Figure 1, the highest and lowest germination was observed in 48 h × seed lot no. 1 (85.5%) and 144 h × seed lot no. 2 (8.00%) respectively. This trend was observed in vigor index, shoot and root lengths and seedling dry weight. The interaction between temperature and duration was significant in all traits. According to Figure 2, the highest and lowest germination observed was 41°C × 48 h (80.00%) and 45°C × 144 h (0%) respectively. This trend was observed in moisture content

Table 8. Root length (cm) of different lots as affected by different temperatures and durations.

Lot	Temperature (°C)	Duration			
		48 (h)	72 (h)	96 (h)	144 (h)
No. 1	41	22.87 ^a	19.12 ^{bc}	13.57 ^e	8.97 ^{ghi}
	43	20.19 ^b	12.84 ^{ef}	7.37 ^{ij}	0.00 ^l
	45	17.12 ^{cd}	9.95 ^{gh}	6.12 ^{jk}	0.00 ^l
No. 2	41	18.45 ^{bc}	14.90 ^{de}	9.27 ^{ghi}	6.17 ^{jk}
	43	15.05 ^{de}	10.65 ^{fg}	5.65 ^{jk}	0.00 ^l
	45	13.72 ^e	7.55 ^{hij}	3.90 ^k	0.00 ^l

Means followed by the same letter(s) in a row and column are statistically non-significant at 1% level of probability (LSD= 2.344).

Table 9. Seedling dry weight (g) of different lots as affected by different temperatures and durations.

Lot	Temperatures (°C)	Duration			
		48 (h)	72 (h)	96 (h)	144 (h)
No. 1	41	0.95 ^a	0.90 ^a	0.72 ^c	0.48 ^e
	43	0.80 ^b	0.42 ^{ef}	0.24 ^j	0.00 ^k
	45	0.72 ^c	0.38 ^{eg}	0.19 ^j	0.00 ^k
No. 2	41	0.74 ^c	0.62 ^d	0.44 ^{ef}	0.34 ^{gh}
	43	0.58 ^d	0.42 ^{ef}	0.32 ^{gh}	0.00 ^k
	45	0.43 ^{ef}	0.30 ^{hi}	0.25 ^{ij}	0.00 ^k

Means followed by the same letter(s) in a row and column are statistically non-significant at 1% level of probability (LSD= 2.344).

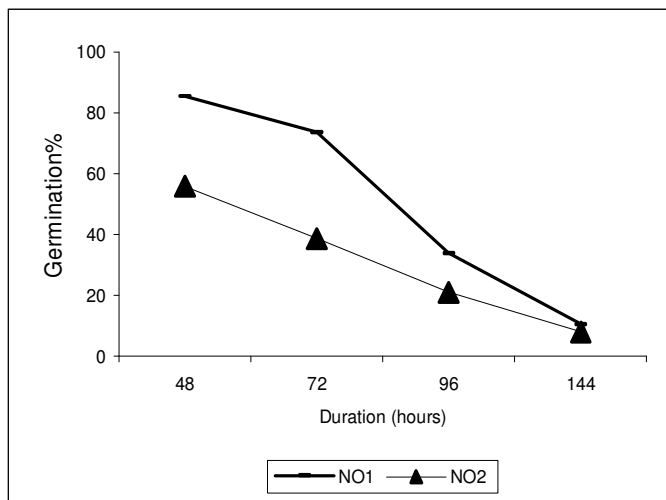
%, vigor index, shoot and root lengths and seedling dry weight. The interaction between temperature and storage was significant in all traits with the exception of root length. According to Figure 3, the highest and lowest germination observed was 41°C × seed lot no. 1 (64.93%) and 45°C × seed lot no. 2 (18.18%) respectively. This trend was observed in moisture content%, vigor index, shoot lengths and seedling dry weight. Interaction effects of storage × temperature × duration were presented in Tables 3 to 9. According to Tables 3 to 9, the optimum range for studied treats were found in seed lot no. 1 × 41°C × 48 h and the lowest ranked for all treats seed lot no. 2 × 45°C × 144 h.

DISCUSSION

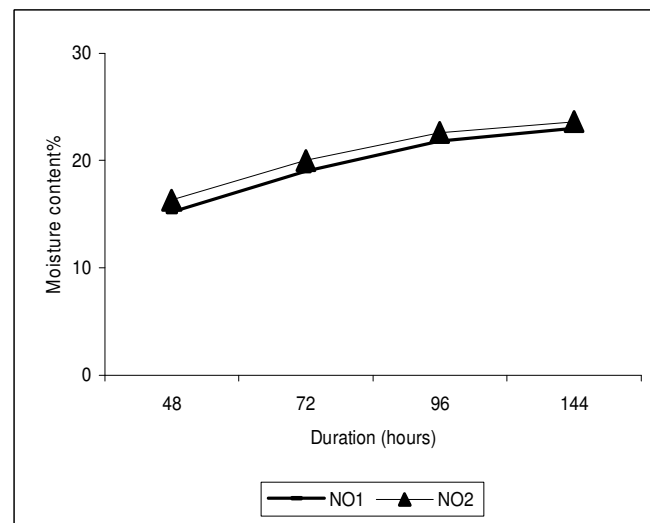
Germination percentages

The results revealed that, accelerated aging at 41°C resulted in the highest average germination percentages (%). However, at 41°C, differences in the mean germination percentages among the seed lots were significant than 43 and 45°C. In terms of aging period, statistically significant differences were found among different aging

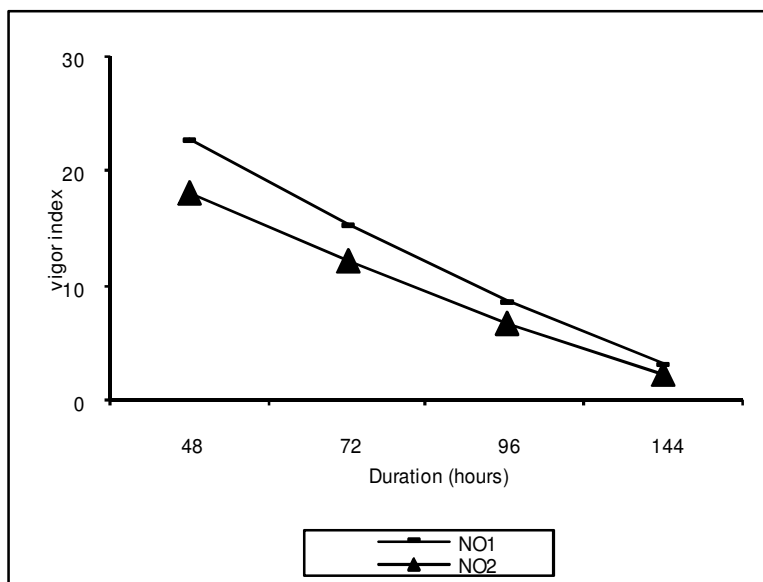
periods for 48 up to 144 h. An accelerated aging time of 48 h gave the highest mean germination percentage (70.71%) and 144 h (9.25%) gave the lowest. The average germination from all seed lots after accelerated aging for 48 h (70.71%) identified the transition point for ash seed quality, after which germination decreased sharply. The germination percentages after aging at 41, 43 or 45°C and 100% RH indicated quadratic responses with the aging period. The germination percentages decreased after aging for 48 h up to 144 h (Table 2). The results showed that, seed lots have difference in germination percentage. The highest mean germination percentage was in seeds no 1. The optimum range for germination percentages is found in seed lot no. 1 × 41°C × 48 h. This may be due to the accelerated aging conditions reducing the effects of internal factors that inhibit the germination process under normal condition. Germination of ash seeds showed a greater response to accelerated aging with a gradual reduction as accelerated aging temperature and duration increased. Such differences in maintenance of seed germination capacity have also been observed by Woltz and Tekrony (2000). This decline is attributed to DNA degradation which leads to impaired transcription causing incomplete or faulty enzyme synthesis essential for earlier stages of germination (Galleschi et al., 2002).



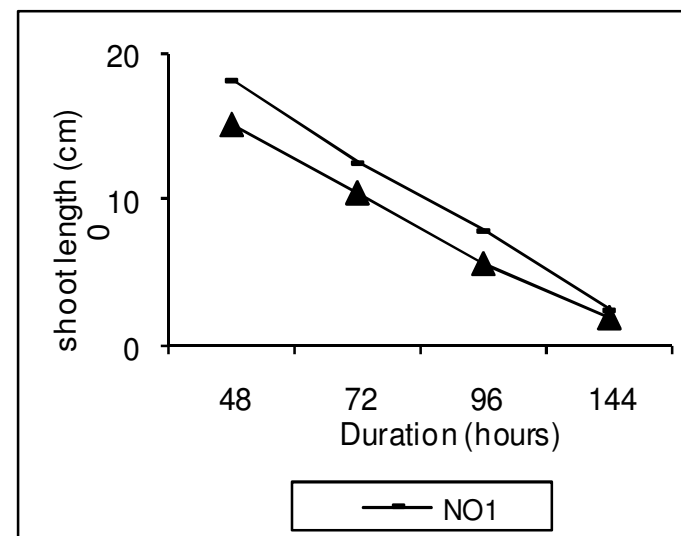
A-1



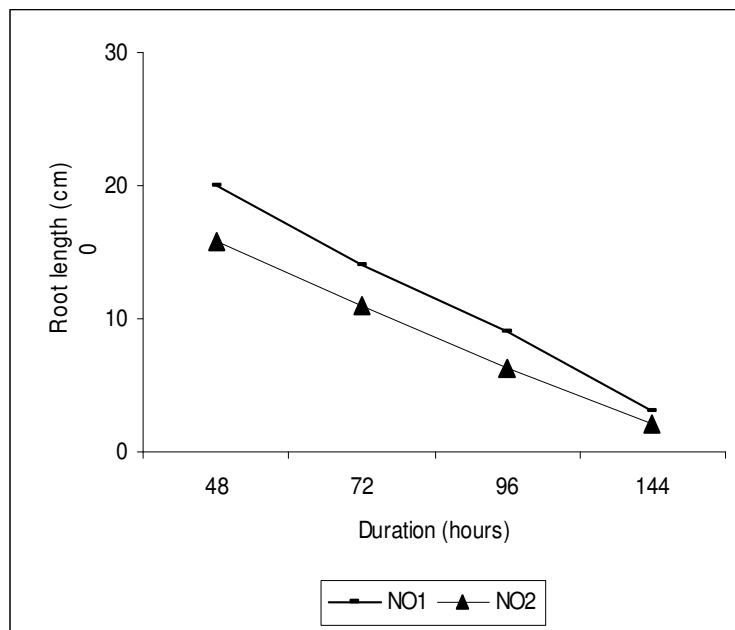
A-2



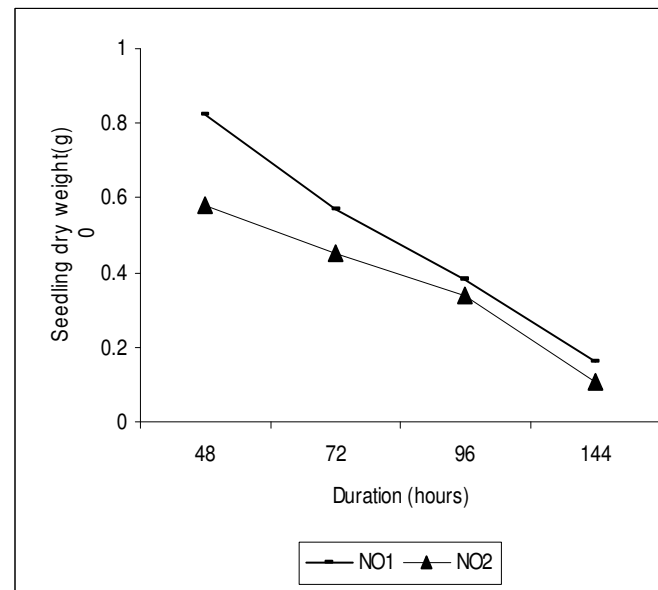
A-3



A-4



A-5



A-6

Figure 1. Interaction of durations and storage on seed germination %, moisture content %, vigor index, shoot and root length (cm) and seedling dry weight.

Malondialdehyde content increased with the degree of seed deterioration, while the activity of free radical scavenging enzymes including, peroxidase, catalase and superoxide dismutase showed inverse relationships with ageing period and direct proportion to reductions in the seed germination (Jatoi et al., 2004).

Moisture content

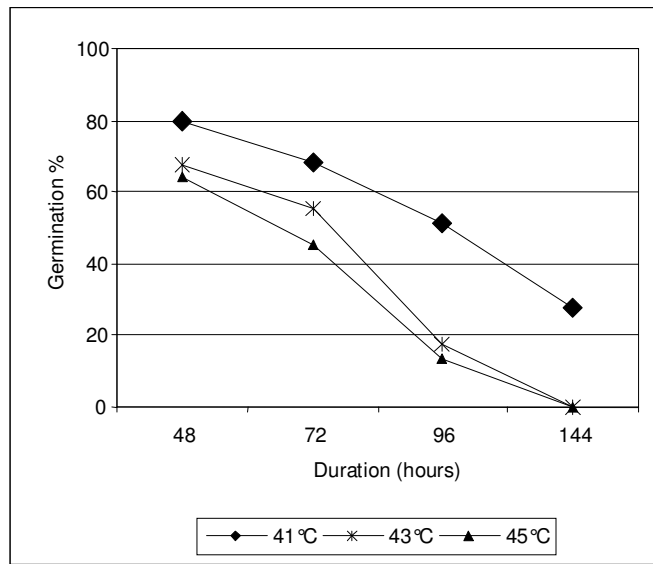
The seed moisture content, after the accelerated aging periods is detected between 12.677 and 25.25% in seeds no. 1 and between 14.42 and

25.95% in seeds no. 2. In this respect, Rodo et al. (2000), working with seeds, detected a marked elevation in moisture content after accelerated aging. On the other hand, one of the most important indicators of uniformity of conditions in accelerated aging is the seed moisture content at the end of the test, since variations.

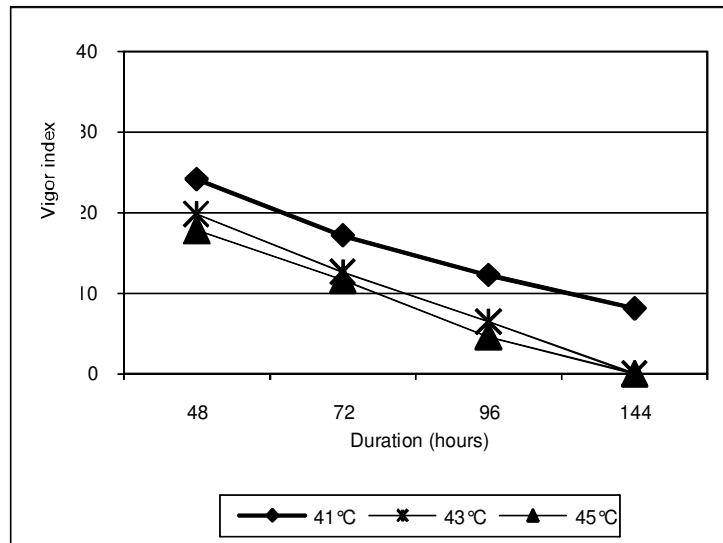
Vigor index

The vigor index of ash seeds was changed distinctly after aging for 41 °C and 48 h. Therefore, the appropriate accelerated condition for ash seed

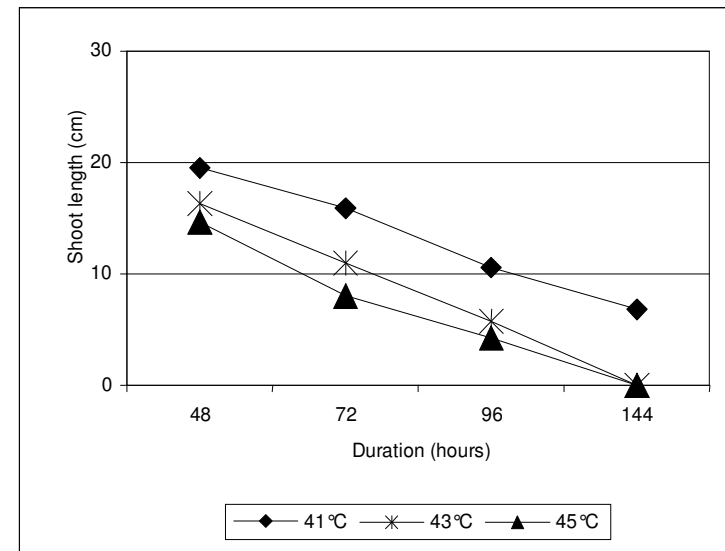
could be aging at 41 °C and 100% RH for 24 h. The results in Table 1 also showed that the vigor index of new seed lots (No. 1) after accelerated aging at 41 °C, with 100% RH were higher than old seed lots (No. 2) in all tested aging periods. Justice and Bass (1979) found that, vigorous seed or new seed lots possessed a greater storage potential than low vigor or older lots and one of the first indications of deterioration was reduced vigor, which was shown by the reduced germination and production. Results from this study are consistent with the suggestion from El-Keblawy (2003) and Basra et al. (2003). The results indicated that, an aging temperature of 41 °C was



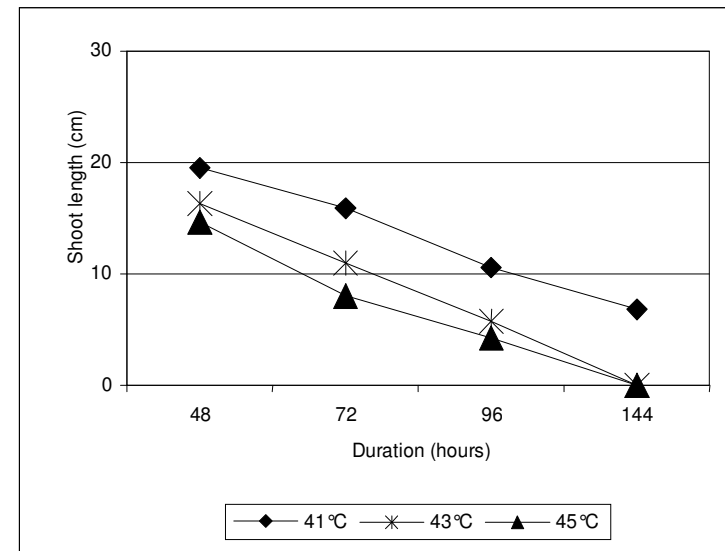
B-1



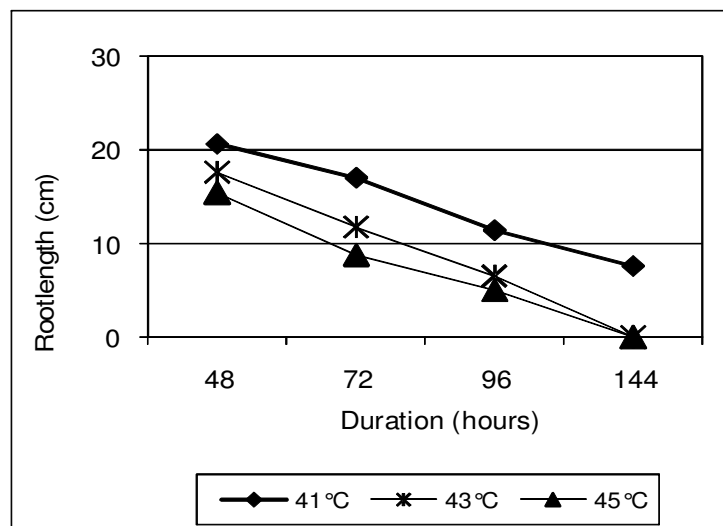
B-2



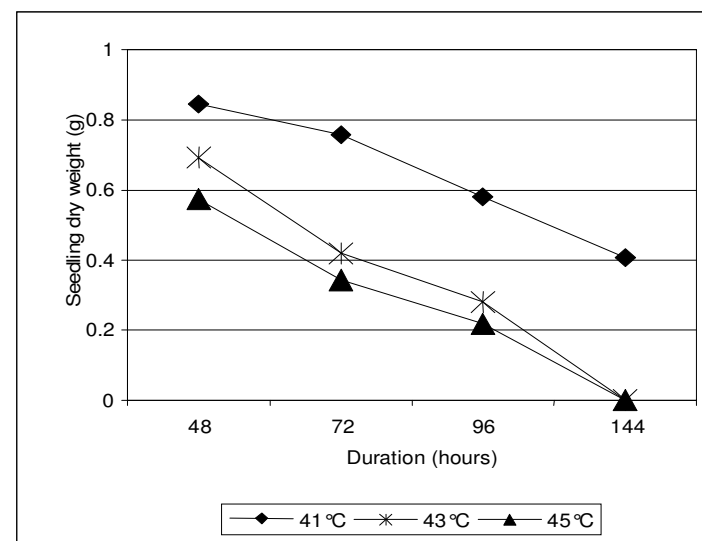
B-3



B-4



B-5



B-6

Figure 2. Interaction of durations and temperatures on seed germination%, moisture content%, vigor index, shoot and root length (cm) and seedling dry weight.

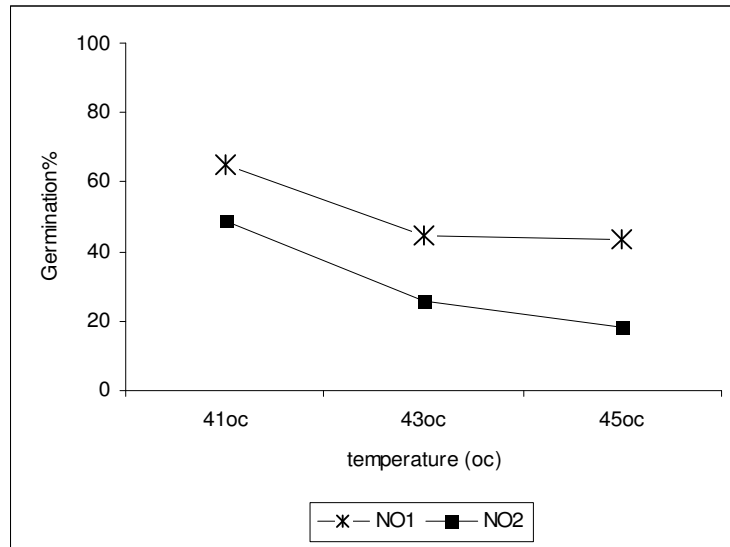
improper for ash seed. Progressive loss of seed viability has also been reported by a number of workers during seed ageing (El-Keblawy, 2003; Jatoi et al., 2004; Sung, 1996). The seedling vigour gradually reduced with accelerated ageing. A number of metabolic processes accompany the loss of seed viability during ageing; hence, use of accelerated ageing in seed biology model experiments has been adequately adopted for determining potential suitability of seeds for long term storage. The increased seed leakage is believed to be associated with aging induced changes in cellular membranes of imbibed seeds. Many biochemical investigations have proven that, lipid peroxidation and fat acidity (free fatty acid percentage) are the major causes of seed deterioration, including cellular membrane

disruption. During aging, peroxidative changes may be the major cause of seed deterioration (Stewart and Bewly, 1980). These results are in agreement with most previous reports (Sung and Jeng, 1994; Jatoi et al., 2004). It is clear that the germination percentage showed a positive and significant correlation with vigor index.

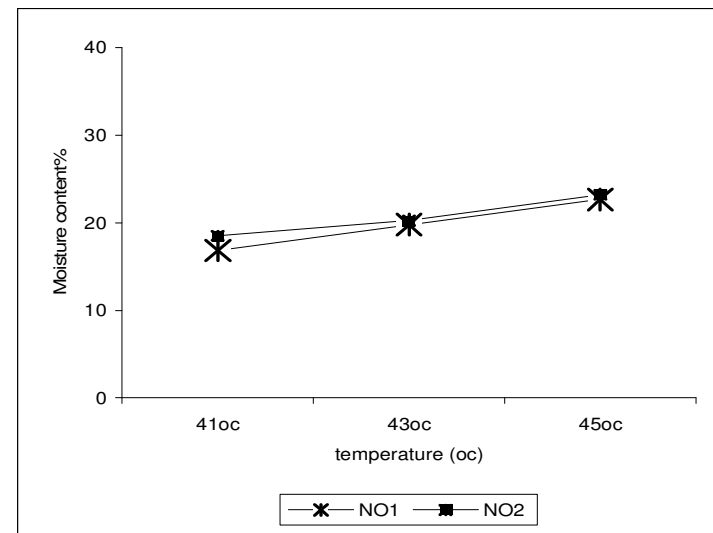
Shoot and root length

The accelerated ageing treatment significantly resulted in reduction of shoot length; root length and seedling dry weight. These were found in both seed lots. Similar results of decrease in seedling length was also reported by Maqsood et al. (2000) in cotton, Roy et al. (1994) in chickpea, Perez and

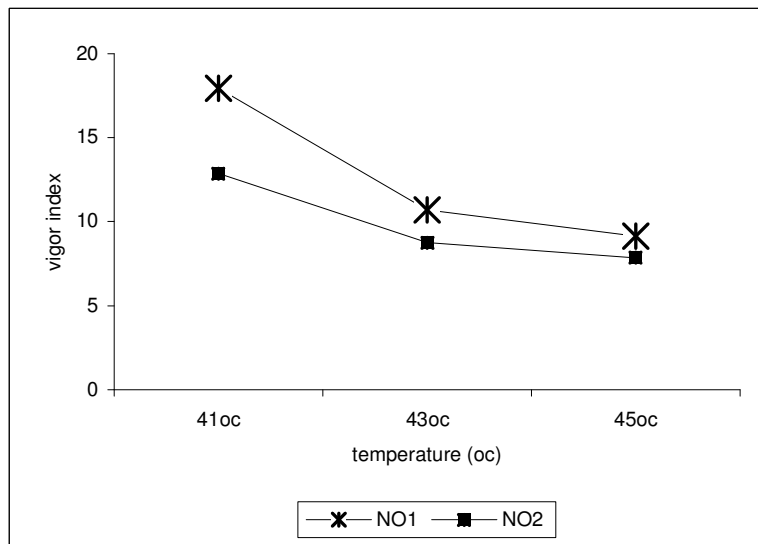
Arguello (1995) in peanuts and Wongvarodom and Naulkong (2006) in bambara groundnut. The seedling growth is the morphogenetic expression of genetic programming. This morphogenetic expression leads to elongation of radical and plumule at more or less defined rate under a particular environmental condition. Under the congenial environmental conditions, the growth of the seedlings takes on a defined temporal pattern, which is the manifestation of the sum, total of activities increasing cell number, cell expansion, fresh and dry weight. Alterations of the environmental condition can modify the growth of the seedlings in different ways affecting various components of the growth (Woltz and TeKrony, 2000; Kanazawa et al., 2000). Reduction of seedling growth as one of the consequences of seed



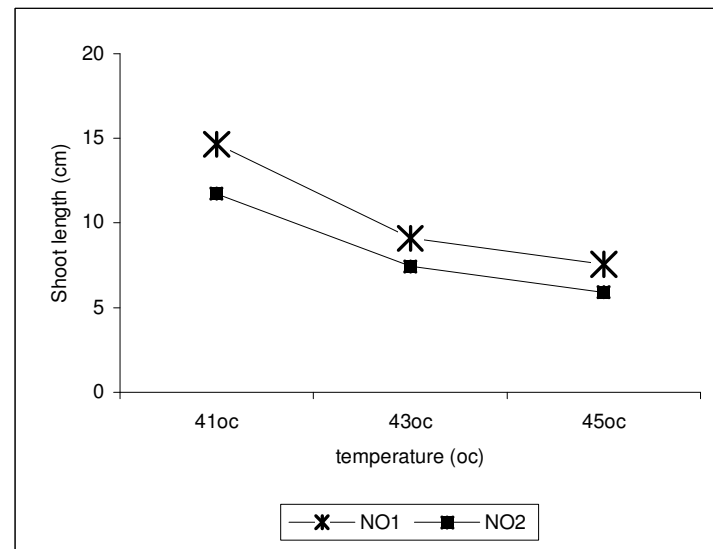
C-1



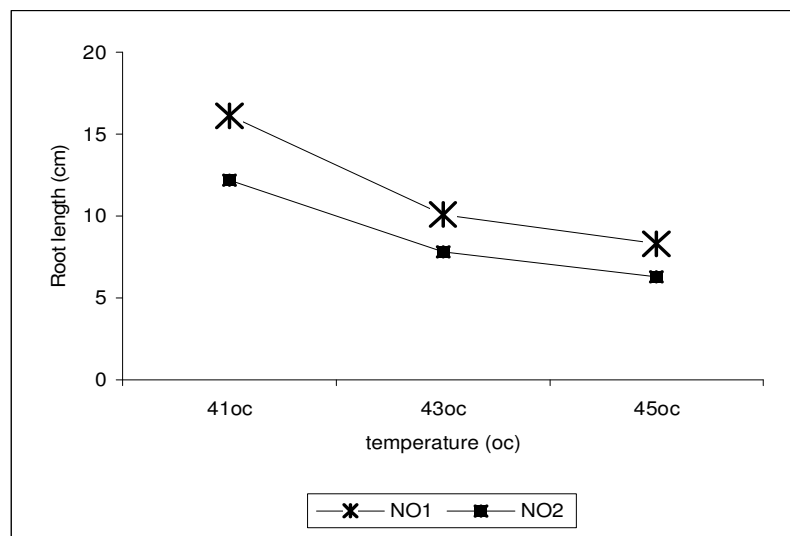
C-2



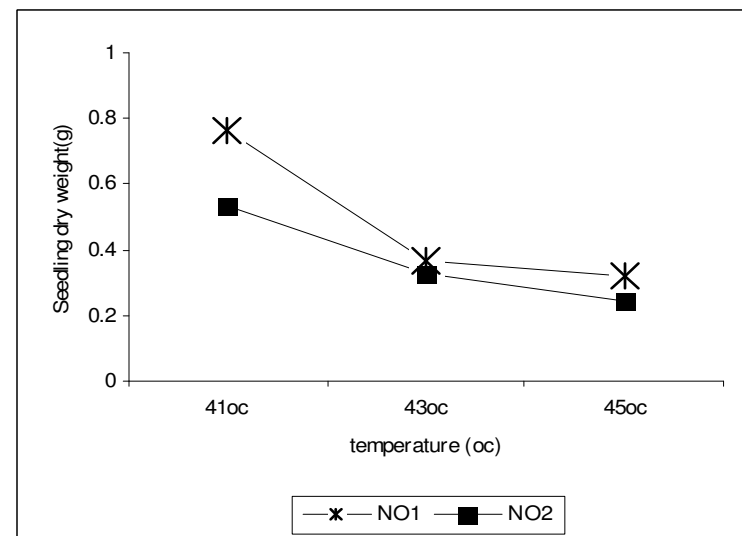
C-3



C-4



C-5



C-6

Figure 3. Interaction of durations and storage on seed germination%, moisture content%, vigor index, shoot and root length (cm) and seedling dry weight.

deterioration in many studies has been considered. Reduce plant growth can result in reduced weed competition, less soil surface shading and reduced soil moisture through evaporation. So weak seedlings that grow less than normal seedlings have facilities such as environmental light, soil moisture and nutrients and less use; more sensitive to environmental conditions are unfavorable difference on plant growth affecting yield of physiological reasons for reduced growth, because the system disorder is caused by plant photosynthesis (Basra et al., 2003). Accelerated aging decreased dry mater production. Results were consistent with those of Eisvand et al. (2010), Sowbhagya and Bhattacharya (2001) and Verma et al. (2003). Sharkey et al. (2001) and Salvucci and Brandner (2004) found that, accele-

rated aging reduced all stage of vegetative and growth of plant organs.

The results of this study revealed that the rate of seed deterioration increased with the increase in storage period and storage temperature. Significant in rate of seed deterioration were observed in seed lots. Seeds no. 2 deteriorated faster than the seeds no. 1 under similar storage condition. These results may be very useful to predict seed longevity during long-term storage. This is a common practice to monitor seed vigor and viability of the stored material through germination and other tests.

Conclusions

It can be concluded that the response of ash

seeds to accelerated aging could differ within storage and seed quality characteristics. The results of the study revealed that the rate of seed deterioration increased with the increase in storage period and storage temperature. Significant in rate of seed deterioration were observed in seed lots. Seeds no. 2 deteriorated faster than the seeds no. 1 under similar storage condition. These results may be very useful to predict seed longevity during long-term storage. This is a common practice to monitor seed vigor and viability of the stored material through germination and other tests.

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