



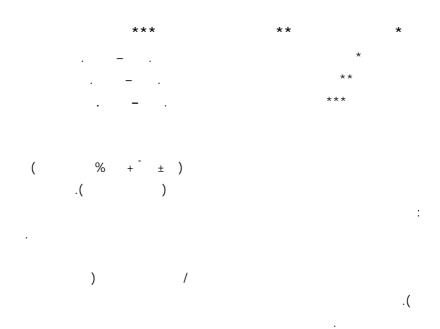
## EFFECT OF ACCELERATED AGING ON VIGOR OF LOCAL MAIZE SEEDS IN TERM OF ELECTRICAL CONDUCTIVITY AND RELATIVE GROWTH RATE (RGR).

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#### ABSTRACT

Maize (*Zea mays* L.) seeds were subjected to accelerated aging treatment for, 3, 7, and 14 days at  $45 \pm 1 \text{ C}^{\circ}$  and 100% relative humidity. These artificially aged seeds were compared to control (Unaged seeds) for evaluation of vigor in seeds. Accelerated aging of Maize seeds up to three days had significant effect on germination percentage & solute leakage. However, further increase in aging period caused an increased in solute leakage and a decrease in germination percentage. Germinability was lost completely at 14 days of accelerated aging. In addition, the reduction in germination percentage was correlated with decrease in seedling length, germination speed index, seed vigor index, seedling fresh & dry weight. Finally, the results revealed that accelerated aging caused depression of maize seeds viability through the above parameters.



## Introduction

The Accelerated aging test (AA) is the second most popular seed vigor test in the United States (1). AA is one of the vigor tests widely used to determine the quality of seed lots. It was initially developed as a test to predict the life span of a number of different species under a range of storage conditions. Accelerated aging is very effective in testing the relative storage potential of seed lots. A seed lot consists of a population of seeds, which may be genetically and physically similar, but vary, in degree of deterioration from relatively non deteriorated to completely dead (100-0%). Deterioration within a lot is on an individual seed basis (2). However, AA is an important procedure for understanding the events that lead to the loss of seed viability. AA damages DNA and mRNA (3), Causes biochemical deterioration of the stored material (4). And reduces the vigor of seedling and early plantlet development shortly after germination (5). AA techniques have great potential for understanding the mechanism of aging and associated deterioration processes of seeds (6). Meanwhile, the process of deterioration under accelerated aging conditions are essentially similar to those under normal conditions, Whereas, the major differences is that the rate of deterioration is much faster, thus, making it possible to be determinate (7; 8; 9). In addition, Seed deterioration can be defined as the loss of quality, viability and vigor either due to aging or effect of adverse environmental factors. The rate of deterioration rapidly increases with increase in either seed moisture content or temperature of storage (10). The principle of this method is based on the artificial acceleration of the deterioration rate of seeds, by exposing them to high temperature and relative humidity levels, which are considered as the most prominent environmental factors with respect to the intensity and velocity of deterioration (6). Consequently, an accelerated aging stress test exposes seeds for short periods (1 to 8 days) to high temperature (40 to  $45C^{\circ}$ ) and high relative humidity (greater than 90%). During the test, seeds absorb moisture from the humid environment along with the high temperature, causing rapid seed aging. High vigor seed lots withstand these stress conditions and deteriorate more slowly than low vigor seed lots. After accelerated aging, high vigor seed lots remained

high in germination, while the germination of low vigor seed lots was reduced (11). The aim of the present research was to investigate the possible effects of accelerated aging upon seeds deterioration (physiological changes) of the local maize seeds.

#### MATERIALS AND METHODS Plant material

Experiments were performed on one Iraqi cultivar (*Zea mays L.*) local variety was used for the study. The seed materials were obtained direct from the field of Babil governorate in the season of (2009-2010) Seeds were surface sterilized using 5% sodium hypochlorite solution for 5 minutes and rinsed thoroughly in distilled water. The seeds were dried at 25°C for 24 hours in the laboratory. as described for pea by (12). Seed material was stored in dark plastic containers at 5C° until use.

## Accelerated aging treatment

Seeds were aged acceleratedly at  $(45 \pm 1C^{\circ})$ and 100% relative humidity up-to 14 days. Seeds were aged in glass desiccators containing distilled water, and spread as a single layer on a metallic net to avoid contact with water. The desiccators were covered and maintained in an incubator at  $45\pm1^{\circ}$ C for 3, 7 and 14 days. Seeds were taken after 3, 7, and14 days of aging treatments. Following the accelerated aging treatment, moisture content was determined and the seeds were air dried at 25°C until their original moisture content (8.3-7.99%) was restored. The seed material was stored at 4°C under the dark until use (12).

## **Moisture content**

Carried out in an oven at  $105\pm3^{\circ}$ C/72h, using three samples of 4.0 g of seeds, for each lot. Results were expressed as mean percentages for each lot (fresh weight basis) (13).

## **Germination test**

Five replicates, each of 20 seeds, were germinated in 9 cm diameter Petri dishes on Whatman NO.1 filter paper. Just enough distilled water (2.5 ml) to moisten the filter paper was provided initially. Moisture level was checked daily and topped-up as necessary. Percentage radicle emergence and seed germination speed was recorded at 25°C after every 24 h time interval. Time for initial signs of radical emergence and maximum emergence was recorded up to 7 days (14).

### Solute leachate test

Twenty five seeds were weighed and placed in 100 ml beaker containing 30 ml of distilled water. Beaker were covered and left undisturbed for overnight. The Elute was collected and the final volume was made to 50 mL with distilled water (15). The conductivity measurements were expressed in ( $\mu$ s\cm\25 seed).

#### The germination speed index (GSI)

The germination speed index (GSI) was calculated as described in the Association of Official Seed Analysts (16) by following formula:

GSI =

No.of germinated seed Days of first count + ... + ... + No.of germinated seed Days of final count

#### Seedling vigor index (SVI)

Seedling vigor index (SVI) was calculated following modified formula of Abdul-Baki and Anderson (17):

 $SVI = [seedling length (cm) \times germination percentage]/100$ 

# Growth analysis: Relative Growth Rate (RGR)

Seedlings of maize cultivar were transplanted into plastic trays filled with clean sawdust .Water was topped after 7 days of planting, seedlings were harvested from trays. Root and shoot were separated, fresh and dry weights were determined, and shoot: root lengths were calculated (14).

#### Statistical analysis:

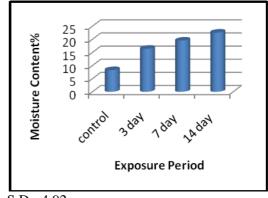
Data were subjected to an analysis of variance, a completely randomized split plot design and LSD (least significant difference) values were calculated at  $P \leq 0.05$ .

## Results

#### Moisture content:

A significant increased was observed in moisture content after aging for (3, 7, and 14 day) compared to control. Under accelerated aging moisture content increased from 8.29% (in control) to 16.4, 19.53 and 22.56 in (3, 7 and 14 day of aging, respectively). As shown in (Figure 1).

(Figure 1) Effect of A.A. conditions on moisture content (%) for maize seeds

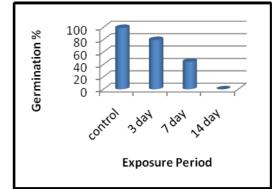


#### L.S.D=4.92

#### Standard germination test:

Accelerated aging had a significant effect on germination. During the first three days of aging, the seeds become unviable and there was significant reduction in the germination percentage. Further increase in aging period caused a suppressive effect on germination percentage at 7 days. Practically no normal seedlings developed at 7 or more days of aging. There was a complete loss of germination at 14 days of accelerated aging. Unaged seeds average germination exhibited of 99% (Figure 2).

(Figure 2) Effect of A.A conditions on germination percentage (%) for maize seeds.

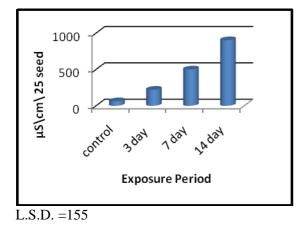


## L.S.D. = 9.84

#### **Electrolyte Leakage:**

Solute leakage in terms of permeability perturbation (measured as electrical conductivity) was increased with accelerated aging period. There was a linear increase in electrolyte leakage with the period of aging (Figure 3).

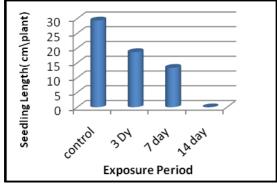
(Figure 3) Effect of A.A conditions on membrane permeability in term of electrical conductivity ( $\mu$ s\cm\25 seed) for maize seeds.



#### Seedling length:

Accelerated aging significantly inhibited seedling growth (Figure 4). Aging up to three, seven days and control produced statistically different seedling length. No seedlings were produced by seeds of 14 days of accelerated aging.

(Figure 4) Effect of A.A. conditions on maize Seedling length (cm\ plant) (7 day old).

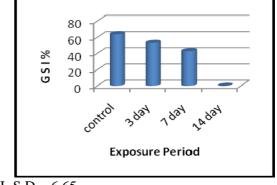


L.S.D. =4.13

#### Germination speed index (GSI):

Germination speed is a direct measure of seed vigor. It may be defined as "number of germinated seeds per unit day". Accelerated aging also decreased the germination speed of seed material. The fastest germination speed was observed in control (63.8) compared to the lowest (0.0) at 14 days of aging treatment (Figure. 5). For example the germination speed of control was maximum (63.8) followed by 3, 7 and 14days of aging (52.9, 42.7 & 0.0, respectively).

(Figure 5) Effect of A.A conditions on germination speed index (GSI %) for maize seeds.

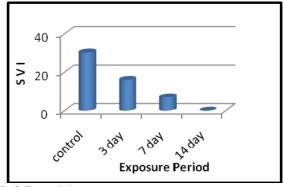


## L.S.D = 6.65

### Seedling vigor index (SVI):

Besides the decrease in germination percentage and seedling length the seedling vigor index also showed a decline pattern during accelerated aging (Figure 6). Vigor index decreased from (30.07 in control) to (16.06, 7.02 and 0.0) in (3, 7 and 14) day of accelerated aging treatment, respectively.

(Figure 6) Effect of A.A conditions on seedling vigor index for maize seeds.

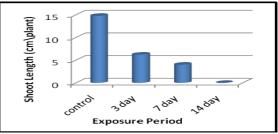


L.S.D. = 5.4

#### **Growth analysis (Relative Growth Rate):**

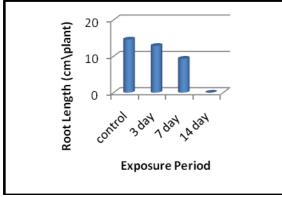
Shoot and root lengths also elicited a significant decline compared to control seeds (Figure 7 and 8) after accelerated aging treatment.

(Figure 7) Effect of A.A. conditions on shoot length (cm\plant) of seven days old maize seedling.



L.S.D = 3.5

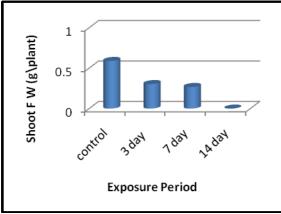
(Figure 8) Effect of A.A. conditions on root length (cm\plant) of seven days old maize seedling.



L.S.D = 3.19

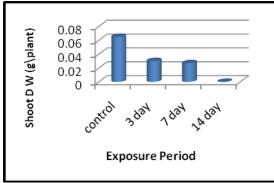
The decrease in the shoot and root lengths of maize seedlings, accelerated with a significant reduction in both fresh and dry weight of both shoot and root(Figure 9 and 10).

(Figure 9) Effect of A.A. conditions on fresh weight of shoot (g\plant) of seven days old maize seedling.



L.S.D = 0.04

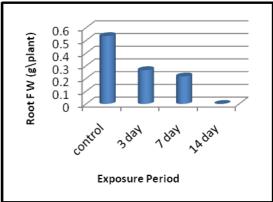
(Figure 10) Effect of A.A. conditions on dry weight of Shoot (g\plant) of seven days old maize seedling.



L.S.D = 0.01

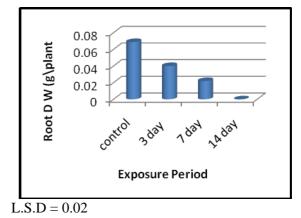
Accelerated aging condition also exhibited a significant effect on fresh and dry weights of seedling root compared to the control (Figure 11 and 12).

(Figure 11) Effect of A.A. conditions on fresh weight of Root (g\plant) of seven days old maize seedling.



L.S.D = 0.03

(Figure 12) Effect of A.A. conditions on dry weight of root (g\plant) of seven days old maize seedling.



#### Discussion

Seed aging decreases the quality of seed and results in agricultural and economic losses. Accelerated aging is one of the most useful tests used for the evaluation of seed vigor and storability. A significant increase was observed in the moisture content after accelerated aging in all periods of aging compared to the control. (Figure 1).Similarly, an increase in moisture content was observed during accelerated aging in chickpea (18) This increase could be explained by an increased in the imbibed water due to the disintegration of cell membranes during aging (19).

Accelerated aging results in progressively loss of seed viability and vigor, seeds of maize exhibited an initial 99% germination which declined till progressively reaches to no germination after 14 day of aging (Figure 2). Accelerated aging also decreased seedling dry and fresh weights, seedling length, seedling vigor index; germination speed index and seedling shoot and root length.(Figures 4, 5, 6, 7, 8, 9, 10, 11, and 12 respectively). Similar results were reported in peanut (20) in cotton seed (21) in chickpea (18) and in Rice (19). The possible reason of this reduction might be the lowering of biochemical activities in seeds. Aging have damaging effect on enzymes that are necessary to convert reserve food in the embryo to usable form and ultimately production of normal seedling (21). Alternatively, the reduction in germination might be due to the degradation of mitochondrial membrane leading to reduction in energy supply necessary for germination (22). The decline in shoot length, root length and seedling vigor index might be attributed to DNA degradation with aging which leads to impaired transcription causing incomplete or faulty enzyme synthesis essential for earlier stages of germination (18).

The decline in seed germination was accompanied with an increase in solute leakage (Figure 2 and 3). There is a strong relationship between lipid peroxidation and electrical conductivity of seeds. It may be stated that rapidly aging had damaging effect on seed membrane and resultantly lipid peroxidation products and electrical conductivity was increased (14). The loss of viability in seeds of onion after aging appeared to be related to the increased membrane destruction (permeability perturbation). This membrane integrity loss may be responsible for the decreased germinability, vigor and ultimately viability (12). The electrical conductivity of carrot seeds increased with the increase in aging time(14). Electrical conductivity of a seed lot is the measure of membrane functions, the results on individual seed conductivity suggest that membrane function is less damaged when the seeds are aged for short time and membranes are likely to be responsible for the slower growth and germination in aged seeds. (12).Finally, we conclude that the foregoing accelerated aging conditions had significant decline in maize seed quality, through the above studied parameters.

## References

- 1. Ferguson-Spears, J.1995. An introduction to seed vigor testing. In: ISTA Seed Vigor Testing Seminar. pp.1-10. (ed. H. A. van de Venter), ISTA, Zurich, Switzerland.
- Delouche, J.C.and C. C. Baskin. 1973. Accelerated aging techniques for predicting the relative storability of seed lots. Seed Sci. & Technol. 1: 427-452.

- 3. Villiers, T.A .**1983**. Ultrastructural changes in seed dormancy and senescence. In: CRC Series in Aging: Senescence in Plants (Thimann KV, ed). pp. 38-66. CRC Press: Boca Raton.
- Murthy, U.M.N; Kumar, P.P. and Sun, W.Q. 2003. Mechanisms of seed aging under different storage conditions for(*Vigna radiata* L.) Wilczek: lipid peroxidation, sugar hydrolysis, Maillard reactions and their relationship to glass state transition. J. Exp. Bot. 54: 1057-1067.
- Bailly, C; Bogatek-Leszczynska, R.; Come, D. and Corbineau, F. 2002. Changes in activities of antioxidant enzymes and lipoxygenase during growth of sunflower seedlings from seeds of different vigor. Seed Sci. Res. 12: 47-55.
- 6. McDonald, M.B. **1999**. Seed deterioration physiology, repair and assessment. Seed Sci. and Technol. **27**: 177-237.
- Aiazzi, M.T; J.A. Aregullo; A. Perez; J. Rienzo and C.A. Guzman. 1996. Deterioration in *Atriplex cordobensis* (Gandoger et sucker) seed: Natural and accelerated aging. Seed Sci. Technol. 25: 147-155.
- 8. Goel, A. and I.S. Sheoran .2003. Lipid Peroxidation and Peroxide Scavenging enzymes in cotton seeds under natural aging. Biol. Plant 46: 429-454.
- Vieira, R.D; A.S. Neto; R.M. deBttencourts and M. Panobianco .2004. Electrical conductance of seed soaking solution and soybean seedling emergence. Sci. Agric. 61: 164-168.
- Ellis, R.H; T.D. Hong & E.H. Roberts
   .1985. Handbook of seed technologies for Gene Banks. Vol. 1 TBPGR ROME, P.P. 518-537.
- 11. ISTA. **2004**. International Rules for Seed Testing Annexes. International Seed Testing Association (ISTA), Zurich, Switzerland.
- Khan, M.M; M.J. Iqbal; M. Abbas and M. Usman .2003. Effect of accelerated aging on viability, vigor and chromosomal damage in pea (*Pisum sativum* L.) seeds. Pakistan J. Agri. Sci., 40: 50–4.
- 13. Woltz, J.M and Tekrony, D.M. **2001**. Accelerated aging test for corn seed. Seed Technology. **v.23**:p. p.21-34.
- 14. Al-Maskri, A; M.M. Khan; O. Al-Manthery and K. Al-Habsi.**2002**. Effect of accelerated aging on lipid peroxidation, leakage and seedling vigor (RGR) in cucumber

(*Cucumis sativus* L.) seeds. Pakistan J.Agri. Sci., **39:** 330–7.

- Simon, E.W and R.M. Rajaharun .1972. Leakage during seed imbibition. J Exp Bot 23: 1076-1085.
- Association of Official Seed Analysts.1983. Seed vigor testing handbook. Contribution 32, Handbook on Seed Testing, AOSA, Lincoln, NE, USA.
- 17. Abdul Baki, A.A. and Anderson, J.D. **1973**. Vigor determinations in soybean seed multiple criteria. Crop Sci. **13:** 630-633.
- Kapoor, N; Arya, A; Siddiqui, Mohd. Asif; kumar, H. and Amir, A. 2010. Seed deterioration in chickpea (*Cicer arietinum* L.) under accelerated aging. Asian j. of plant sci. 9 (3): 158-162.
- Kapoor, N; Arya, A; Siddiqui, Mohd. Asif; kumar, H. and Amir, A. 2011.Physiological & Biochemical Changes During Seed Deterioration in Aged Seeds of Rice (*Oryza* sativa L.). American J. of plant physiology 6(1): 28-35.
- 20. Sung, J. M. and Jeng, T. L. **1994**. Lipid peroxidation and peroxide-scavenging enzymes associated with accelerated aging of peanut seed. Physiologia Plantarum, **91**: 51-55.
- Iqbal, N; A., Shahzad M; Basra & Khalil Ur Rehman .2002. Evaluation of Vigor and Oil Quality in Cottonseed during Accelerated Aging. Int. J. Agri. Biol. 4(3): 318-322.
- Gidrol, X; A. Noubhani; B. Mocquot; A. Fournier & A. Pradet .1998. Effect of Accelerated Aging on Protein Synthesis in Two Legume seeds. Plant Physio. Biochem. 26: 281-288.