Effect of Accelerated Aging Conditions on Viability of Sunflower *(Helianthus annus* L.*)*Seeds

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Abstract :

Sunflower *(Helianthus annus* L.*)*Seeds were subjected to accelerated aging treatment for, 3 and 7 day at 45 ±1 C° and 100% relative humidity. These artificially aged seeds were compared to control (Unaged seeds) for evaluation of seed vigor. Accelerated aging of Sunflower seeds up to three days had significant effect on germination percentage. However, further increase in aging period highly decreased germination percentage . Germinability was lost completely at 7 days of acceleration. In addition, the reduction in germination percentage, there are reduction in seedling length, germination speed index, seed vigor index, seedling fresh & dry weight. These results suggest that, at least within the first 3 days of accelerated aging, there was no direct relationship between lipid peroxidation and deterioration in membrane integrity. Finally, the results revealed that accelerated aging caused depression of sunflower seeds viability through the above parameters**.**

الخلاصة :

عرضت بذور زهرة الشمس إلى ظروف التعمير المعجل (45±1 م° +100% رطوبة نسبية) لمدة 3 و 7يوم وبعدها تمت مقارنة حيوية البذور مع معاملة السيطرة(بذور غير معرضة). وتشير النتائج الى انخفاض معنوي في نسبة الإنبات، حيث كلما زادت مدة التعريض زاد الانخفاض في نسبة الإنبات لحين انعدام الإنبات كلياً في اليوم السابع. إضافة إلى ذلك ،فأن الانخفاض في النسبة المئوية لإنبات البذور قد تلازمت مع انخفاض كل من طول البادرة، معامل سرعة الإنبات، معامل حيوية البذور والوزن الطري/الجاف للبادرات (للمجموع الخضري والجذري).كما اظهرت النتائج عدم وجود تلف في غشاء الخلية بدلالة التوصيلية الكهربائية على الاقل خلال الأيام الثلاث الأولى من ظروف التعمير المعجل. وأخيراً فأن النتائج قد بينت إن ظروف التعمير المعجل أدت إلى انخفاض في حيوية بذور زهرة الشمس من خلال المؤشرات أعلاه.

Introduction

Seeds deteriorate and lose their germinability during periods of prolonged storage. Many theories have been attempted to explain seed aging; external factors such ;as irradiation or fungi attack, and internal factors such as accumulation of toxic compounds, loss of vitamins or hormones, degradation of nucleic acids, proteins or membranes (Priestley 1986). The two most important environmental factors influencing the rate of deteriorative processes in seed aging are the relative humidity of the air, which controls seed moisture content, and the temperature (McDonald, 1999). High temperature, ambient relative humidity, and seed moisture content are the main factors influencing seed storage capability (Abdul-Baki, 1980). However, common interpretations of the Mechanisms of flower seed deterioration Can be Summarized in four points:

Enzyme activities. Most of these studies search for markers of germination such as increases in amylase activity or changes in free radical scavenging enzymes such as superoxide dismutase, catalase, peroxidase and others.

Protein or amino acid content. The consensus is that overall protein content declines while amino acid content increases with seed ageing.

Nucleic acids. The aging leads to decrease DNA synthesis and increased DNA degradation. It is widely believed that degradation of DNA would lead to faulty translation and transcription of enzymes necessary for germination.

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Membrane permeability. Increased membrane permeability associated with increasing seed deterioration has been consistently observed and is the foundation for the success of the conductivity test as a measure of seed quality (McDonald & Kwong, 2005)

Accelerated ageing (A.A) technique is a widely used tool to test the seed quality. This ageing test of seed vigor can give better indications of probable field emergence for vegetable crop seeds than germination and growth tests (Pandey *et al*.,1990). A.A. Initially proposed as a method to evaluate seed storability, this test is rapid, inexpensive, simple and useful for all species (Copeland & McDonald, 2001). AA techniques have great potential for understanding the mechanism of aging and associated deterioration processes of seeds (McDonald, 1999). Meanwhile, the process of deterioration under accelerated ageing conditions are essentially similar to those under normal conditions, however, the major differences is that the rate of deterioration is much faster, thus, making it possible to be determinate (Aiazzi, et al. 1996; Goel and Sheoran, 2003). The aim of the present research was to investigate the possible effects of accelerated ageing upon seeds deterioration (physiological changes) of the local Sunflower seeds.

Materil and methods :

Plant material

Experiments were performed on one Iraqi cultivar (*Helianthus annus* 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 (Khan *et al.*, 2003). Seed material was stored in dark plastic containers at 5C° until use.

Accelerated aging treatment :

Seeds were aged acceleratedly at (45 ±1C°) and 100% relative humidity up-to 7days. Seeds were aged in glass desiccators containing distilled water, seeds 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±1ºC for 3 and 7 days. Seeds were taken after 3 and 7 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 (5.60 - 6.30%) was restored. The seed material was stored at 4°C under the dark until use (Khan *et al.*, 2003).

Moisture content :

Carried out in an oven at 105±3°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) (Woltz & Tekrony, 2001).

Germination test :

Germination test was conducted using between paper (BP) method of germination 50 seed per 5 replication were sown on paper towel. Seeds were placed on the surface of double sheets of towel paper which were moistened with distilled water. The seeds were covered with an another sheet of paper towel. The sheets were rolled and placed vertically in a plastic beaker, covered with a plastic bag at 25Cº in a germinator (ISTA, 1993).

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 (Simon and Rajaharun, 1972). The conductivity measurements were expressed in (µs\cm\25 seed).

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The germination speed index (GI) :

The germination index (GI) was calculated as described in the Association of Official Seed Analysts (AOSA, 1983) by following formula:

$$GI = \frac{\text{No.of germinated seed}}{\text{Days of first count}} + \cdots + \cdots + \frac{\text{No.of germinated seed}}{\text{Days of final count}}$$

Seedling vigor index (SVI)

Seedling vigor index (SVI) was calculated following modified formula of **(Abdul-Baki and Anderson 1973)**:

SVI = [seedling length (cm) × germination percentage]/100

Growth analysis: (Relative Growth Rate)

Seedlings of sunflower cultivar were transplanted into plastic trays filled with clean sawdust .Water was topped after 3 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 (**AL-Maskri, et al, 2002**).

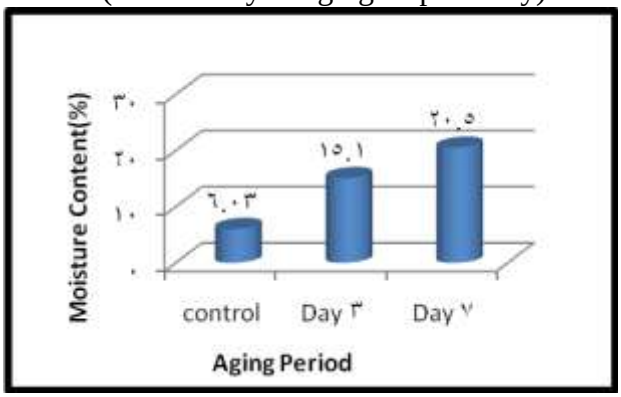
Statistical test:

All treatments were determined by three or more replicates. Data were subjected to an analysis of variance, a completely randomized and LSD (least significant difference) were calculated at $P \leq 0$

Results :

Moisture content:

A significant increase was observed in moisture content after aging for (3 and 7 day) compared to control. Under accelerated aging moisture content increased from 6.03% (in control) to 15.10%, 20.50% in (3 and 7 day of aging respectively). As shown in (Fig1).



Fig(1) Effect of accelerated aging condition on moisture content (%) for sunflower seed
L.S.D= 3.5

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 germination percentage. Further increase in aging period a suppressive effect on germination percentage at 7 days. Practically no normal seedlings developed at 7 days of ageing. There was a complete loss of germination at 7 days of accelerated aging. Unaged seeds exhibited average germination of 75.8% (Fig 2).

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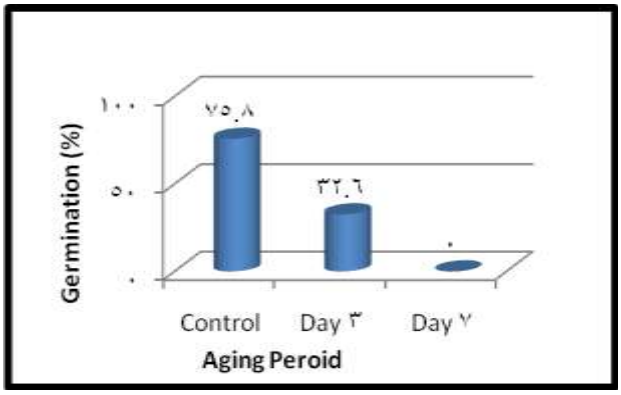

Fig (2) Effect of accelerated aging conditions on germination percentage (%) for sunflower seeds.

L.S.D= 4.6

Electrolyte Leakage:

Solute leakage in terms of permeability perturbation (measured as electrical conductivity). there was no direct relationship between lipid peroxidation and deterioration in membrane integrity in Sunflower Seeds during accelerated aging. A significant increased was not observed between control (Unaged seed) and 3 day Aging Period. And there is no significant effect between 3 day and 7 day accelerated aging. But there is significant effect between Unaged seeds and 7 day accelerated aging (Fig 3).

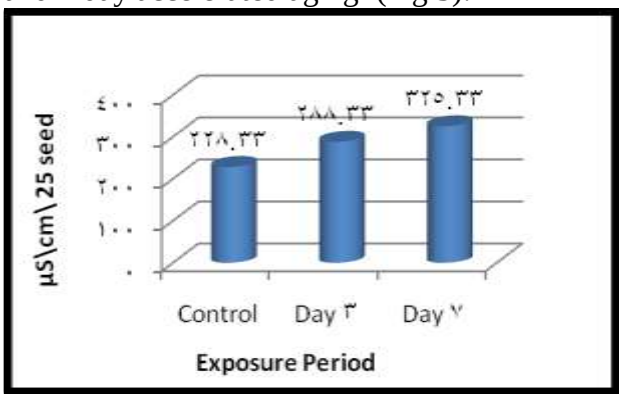

Fig (3) Effect of accelerated aging conditions on membrane permeability in term of electrical conductivity (µs\cm\25 seed) for Sunflower seeds.

L.S.D= 68.8

Seedling length:

Accelerated aging significantly inhibited seedling growth (Fig 4). Ageing up to three, seven days and control produced statistically different seedling length. No seedlings were produced by seeds of 7 days of accelerated aging.

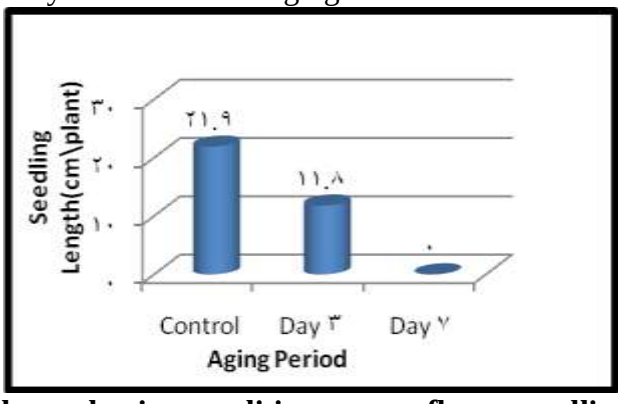

Fig (4) Effect of accelerated aging conditions on sunflower seedling length (cm\plant) 7 day old.L.S.D= 6.4

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Germination speed index (GI):

Germination speed is a direct measure of seed vigor. It may be defined as "number of germinated seeds per unit day". Accelerated ageing also decreased the germination speed of seeds. fastest germination speed was observed in control (45.38) compared to the lowest (0.0) at 7 days of ageing treatment (Fig. 5).

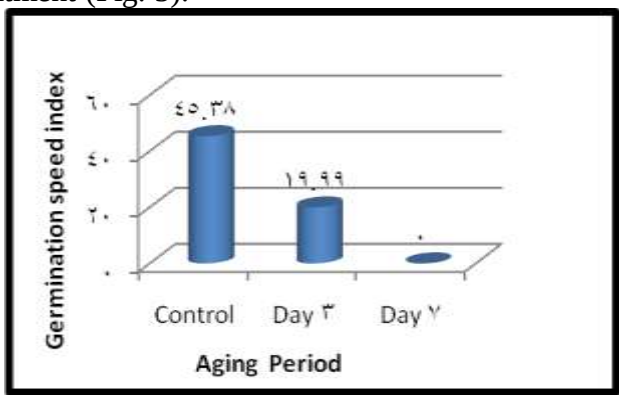

Fig (5) Effect of accelerated aging conditions on germination speed index (GSI) for sunflower seeds.L.S.D=2.15

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 (Fig 6). vigor index decreased from (16.59 in control) to (3.84 and 0.0) in (3 and 7) day of accelerated aging treatment respectively.

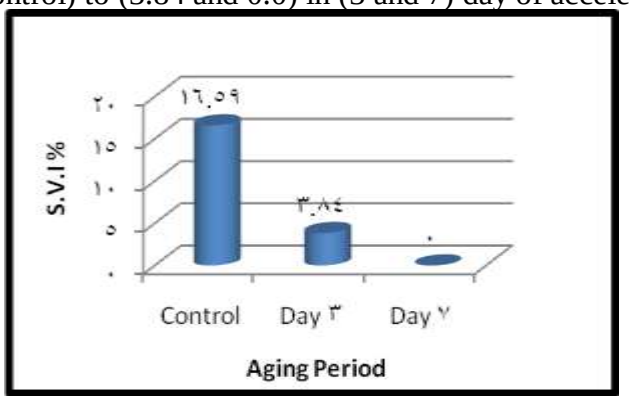

Fig (6) Effect of accelerated aging conditions on seedling vigor index for sunflower seeds.L.S.D=0.58

Growth analysis (Relative Growth Rate):

Shoot and Root length also elicited a significant decline compared to control seeds (Fig 7, 8) after accelerated aging treatment.

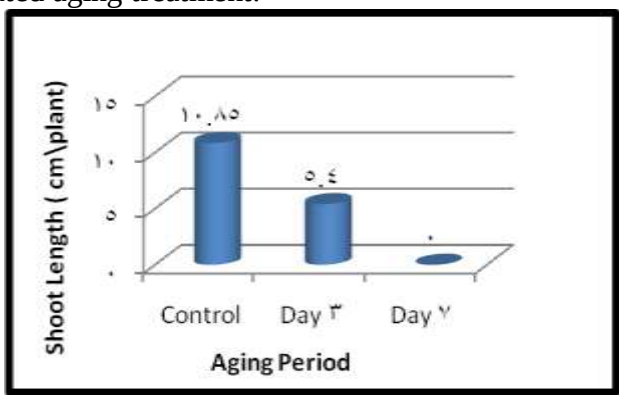

Fig (7) Effect of accelerated aging conditions on shoot length (cm\plant) of 7 days old sunflower seedling.L.S.D= 1.86

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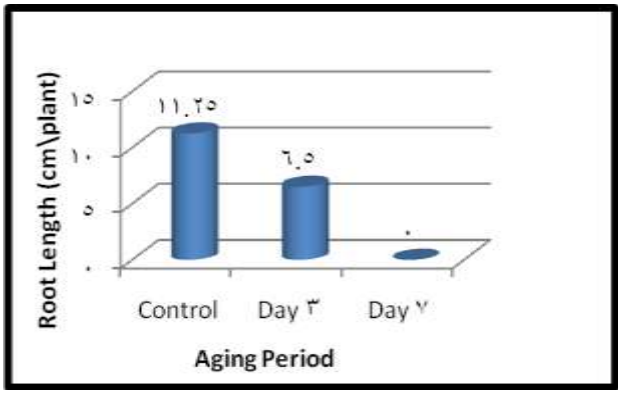

Fig (8) Effect of accelerated aging conditions on root length (cm\plant) of 7 days old sunflower seedling.

L.S.D= 2.4

With the decrease in shoot and root length of Sunflower seedling, Accelerated aging condition showed a decline effects on fresh and dry weight of shoot (Fig 9, 10).

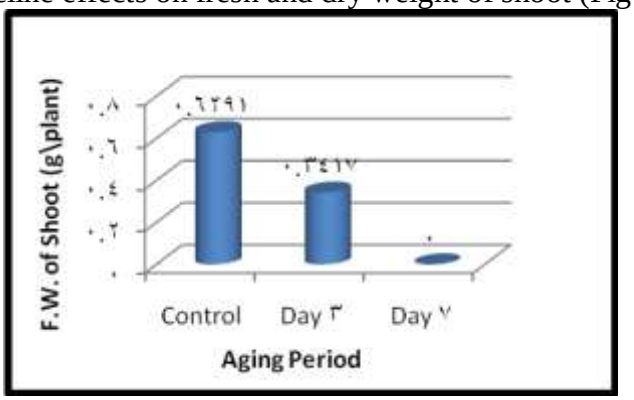

Fig (9) Effect of accelerated aging conditions on fresh weight of shoot (g\plant) of 7 days old sunflower seedling.

L.S.D= 0.10

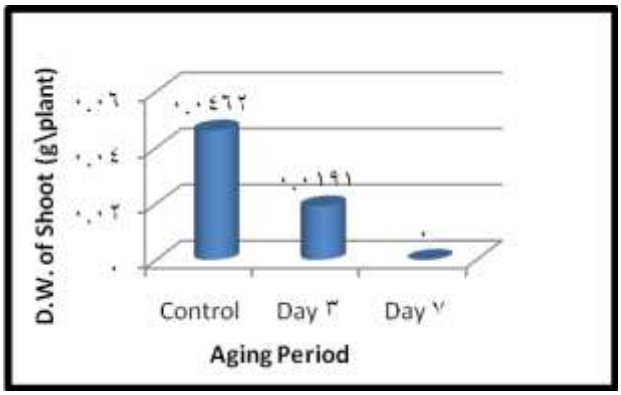

Fig (10) Effect of accelerated aging conditions on dry weight of shoot (g\plant) of 7 days old sunflower seedling.

L.S.D= 0.009

Accelerated aging condition also exhibited a significant effect on fresh and dry weight of seedling root compared to control (Fig 11, 12).

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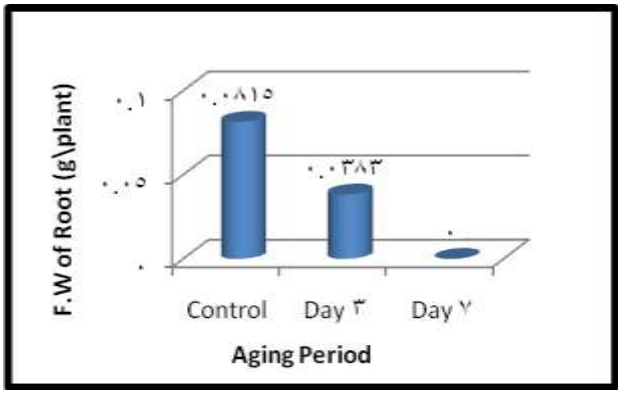

Fig (11) Effect of accelerated aging conditions on fresh weight of root(g\plant) of 7 days old sunflower seedling.

L.S.D= 0.004

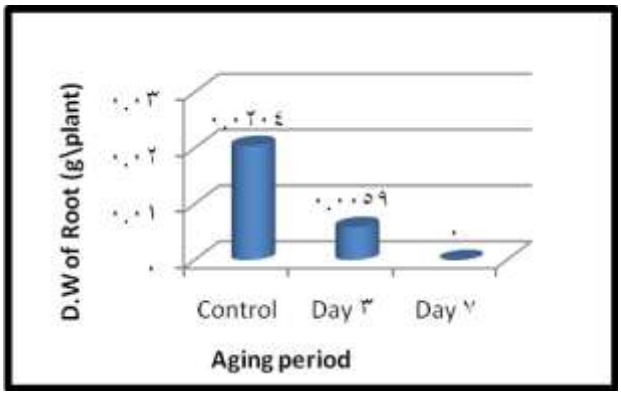

Fig (12) Effect of accelerated aging conditions on dry weight of root(g\plant) of 7 days old sunflower seedling.

L.S.D= 0.003

Discussion

Relative humidity and temperature are the two most important factors determining flower seed deterioration. Relative humidity is important because it directly influences the moisture content of seeds in storage as they come to equilibrium with the amount of gaseous water surrounding them. Temperature is important because it: (i) determines the amount of moisture the air can hold – higher

temperatures holding more water than lower temperatures; and (ii) enhances the rate of deteriorative reactions occurring in seeds as temperature increases (McDonald & Kwong, 2005). These relationships are so important that Harrington (1972) identified the following two rules describing seed deterioration:

Rule 1. Each 1% reduction in seed moisture content doubles the life of the seed.

Rule 2. Each 5°C reduction in seed temperature doubles the life of the seed. A significant increased were observed in moisture content after accelerated aging in all periods of aging compared to control. (Fig1).Similarly, an increase in moisture content was observed during accelerated aging in sunflower seeds (Gidrol, et al. 1989 ; Bailly, et al. 1996). This may be due to the denaturation of seed proteins at high temperature and moisture levels increased (Krishnan, et al. 2004). Or This increased could be explained by increased in imbibed water due to the disorganization of cell membranes during aging (Kapoor, et al. 2011).

Accelerated aging results in progressively loss of seed viability and vigor, seeds of sunflower exhibited an initial 75% germination which declined till progressively reaches to no germination after 7 day of aging (Fig2). Accelerated aging also decreased seedling dry and fresh weights, seedling length, seedling vigor index; germination speed index and seedling shoot and root length.(Fig 4, 5, 6, 7, 8, 9, 10, 11, and 12 respectively). Similar results were reported in peanut (Sung & Jeng, 1994) in cotton seed (Iqbal, et al. 2002) in chickpea

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(Kapoor, 2010) and in Rice (Kapoor, et al. 2011). The possible reason of this reduction might be the lowering of biochemical activities in seeds. Ageing have damaging effect on enzymes that are necessary to convert reserve food in the embryo to usable form and ultimately production of normal seedling (Iqbal, et al. 2002).Alternatively, 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). 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 (Kapoor, 2010). There was no direct relationship between Electrolyte Leakage and deterioration in membrane integrity in Sunflower Seeds during accelerated aging (Fig3). The absence of membrane damage in seeds of sunflower in term of electrical conductivity (Gidrol, et al. 1989). A relationship between leakage and loss of seed viability could not be assumed in sunflower seeds (Bailly, et al. 1996). Our results reveal that, there is no significant effect between control and 3 day Aging Period. And there is no significant effect between 3 day and 7 day accelerated aging. This result could be explained by when sunflower seeds are submitted to accelerated aging for 3 days, the plasma membrane remains undamaged. these results suggest that, at least within the first 3 days of treatment, the lipid reserve in sunflower seeds might act as a detoxifying trap, protecting membranes from excessive damage(Gidrol et al., 1989). Finally, sunflower seed deterioration during accelerated aging is closely related to a decrease in the activities of detoxifying enzymes and to lipid peroxidation (Bailly, et al. 1996). The activities of superoxide dismutase and peroxidase decreased during sunflower seed aging and it was especially pronounced when accelerated aging was applied (Balesevic, et al. 2005).

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