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The Influence of Priming on Viability of Aged Sunflower (*Helianthus annuus* L.) Seeds

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ABSTRACT

This investigation was performed to know the effect of accelerated aging and seed priming on viability of sunflower seeds. Treatments included three periods of accelerated aging (0, 3 and 6 days) under 41°C and 100% humidity and eleven seed priming treatments [Unprimed seeds (control), Distilled water (hydropriming), (25 mM, 50 mM and 75 mM $CaCl_2$), (500 ppm, 1000 ppm and 1500 ppm Salicylic acid [SA]) and (25%, 50% and 75% Yeast)]. A completely randomized design with three replications was used in A factorial experiment. germination percentage (%), mean germination time (MGT), germination index (GI), day germination speed, electrical conductivity (E.C), radical length, seedling dry weight, plumule length, seedling vigor index and seedling growth rate were studied in this investigation. The results showed that, responses of different parameters to aging periods and seed priming treatments were significantly different in all studied traits. With increasing aging duration, there was a reduction in all studied traits except for only mean germination time, day germination speed and electrical conductivity which were increased with increasing aging period. Results also, showed that hydropriming and seed pretreatment with (50 mM $CaCl_2$, 100 ppm Salicylic acid and 50% yeast) were better than other treatments in all traits under this study. Therefore, this investigation recommended with using these treatments to increase viability of sunflower seeds specially after aging stress.

Keywords: Priming, Viability, Sunflower

INTRODUCTION

Seeds of oil crops are very susceptible and sensitive to the different environmental conditions. During the storage period an oxidization will happened easily to the oil inside the seeds and the seed health will deteriorate (Kausar *et al.*, 2009). This deterioration in seeds leads to a great reduction in the viability and vigor (Bailly, 2004 and Kapoor *et al.*, 2011). Accelerated ageing is a way to measure storability of seeds, this way is rapid, cheap, easy and useful for several species (Seiadat *et al.*, 2012 and Kapoor *et al.*, 2011). The viability of seeds can be enhanced by new techniques recently known as seed priming, which improve uniformity and the speed of germination by inducing some physiological changes into target seed using synthetic and natural compounds prior to germination. Seed priming can be carried out using various techniques such as hydropriming (soaking in distilled water), osmo-priming (soaking in several osmotic solutions such as PEG, Kcl, K_2SO_4, potassium salts, etc.,) and plant growth inducers such as Ethephone, IAA and CCC (Chiu, *et al.*, 2002). Many studies have indicated that seed priming causes an increase in the speed uniformity and of germination and seedling emergence in different plant species under suitable conditions (Hadinezhad *et al.*, 2013 and Rehman *et al.*, 2015). Priming also can improve seedling vigor, eliminate seed dormancy, and enhance plant behavior under different environmental conditions, involving different stresses such as salinity and water stress (Hussain *et al.*, 2017 and Tabassum *et al.*, 2017). Various theoretic have been suggested to clean the enhanced germination behavior of primed seeds, including improved efficiency of energy metabolism, embryo growth rates, protein synthesis, genes associated with stress, as well as antioxidant system (Kubala *et al.*, 2015 and Paparella *et al.*, 2015). Priming treatments induce an increase in the expression of antioxidant enzymes (Panuccio *et al.*, 2018), which can enhance superinduces defense mechanisms and minimize the oxidative damage caused by the accretions of reactive oxygen species (ROS). An improvement in the performance of active and aged seeds of many crops using various seed priming treatments has been proved by (Ansari *et al.*, 2013 and Seiadat *et al.*, 2012). This study aimed to know the impact of priming on viability of Sunflower (*Helianthus annuus* L.) seeds after accelerated aging.

MATERIALS AND METHODS

This investigation was carried out under the laboratory condition at the Seed Technology Research Department, Sakha Agriculture Research Station, Egypt during 2020 season. The experiment was carried out as a factorial experiment with completely randomized design with three replicates. This investigation included two factors (aging and priming). For aging treatments, sunflower seeds were put in 41°C and 100% relative humidity for (0, 3 and 6 days) in hermetic aging boxes. Firstly, the aged seeds were treated with (distilled water (hydropriming), (25mM, 50mM and 75 mM $CaCl_2$), (500 ppm, 1000 ppm and 1500 ppm Salicylic acid [SA]) and (25%, 50% and 75% Yeast) in dark for 12 hours as well as one unprimed treatment as control. Then, seeds were washed carefully many times with distilled water and dried on filter paper (Ansari *et al*, 2012). After that standard germination tests were measured at 25°C

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for 8 days according to ISTA (2003). More than 2mm long radical and plumule seed was considered as germinated seed and were counted every day to account the germination rate. Finally, the germination period, germination percentage, germination index, radical length, plumule length, mean germination time were recorded at the end. Also, the seedling dry weight was recorded after putting in the oven 75° C for 48 h.

Germination percentage was measured according to ISTA (2003) using the dropped equation:

$$\textbf{Germination \%} = \frac{\text{Number of normal seedlings}}{\text{Total number of seed tested}} \times 100$$

-**Germination index** was computed as mentioned in the association of official seed analysis (AOSA) 1986 by following equation:

Germination index =

$$\frac{\text{number of germinated seeds}}{\text{days of first count}} + \ldots + \frac{\text{number of germinated seeds}}{\text{days of final count}}$$

-**Seedling growth rate** was calculated using the following formula:

$$\textbf{Seedling growth rate} = \frac{\text{seedling length(cm)}}{\text{number of days}}$$

At 8^{th} day after seed placement, randomized five seedlings of every Petri dish were sampled. Root and length of single seedling was recorded with meter scale. Then the seedlings were completely dried for 48 hour then dry weight of seedlings were recorded using electric balance.

-Mean Germination Time (MGT)

Mean germination time (MGT) was estimated according to Ellis and Roberts (1981), Whereas n refers to the number of germinated seeds in d days and (Σ (nd)) as total germinated seeds:

$$\textbf{MGT} = (\Sigma\,(nd))/(\Sigma n)$$

-Daily germination Speed (DGS)

Daily germination speed was calculated according to Kotowski (1926). A.O.S.A. (1986), in that FGP is the germination percentage, and D refers to days up to attain maximum germination rate:

$$\textbf{MDG=FGP/D}$$

$$\textbf{DGS=1/MDG}$$

-Seedling vigor index

Seedling vigor index was calculated using the following equation**:**

SV1=seedling dry weight (g) × germination (%)

SV2=seedling dry length (cm) × germination (%)

-Electrical Conductivity Test (EC)

250 mL deionized water were used to soak fifty seeds from each treatment and kept at 20°C for 24 h (Ansari *et al*., 2013). A direct reading conductivity meter was used to estimate the electrical conductivity of the seed leachate and expressed in µS.cm-1.

RESULTS AND DISCUSSION

Impact of accelerated aging on viability of sunflower seeds: -

Results obtained from analysis of variances cleared that seed aging significantly affected on germination %, mean germination time, germination index, day germination speed, electrical conductivity, radical length, plumule length, seedling vigor index, seedling growth rate and seedling dry weight (Fig 1, 2).

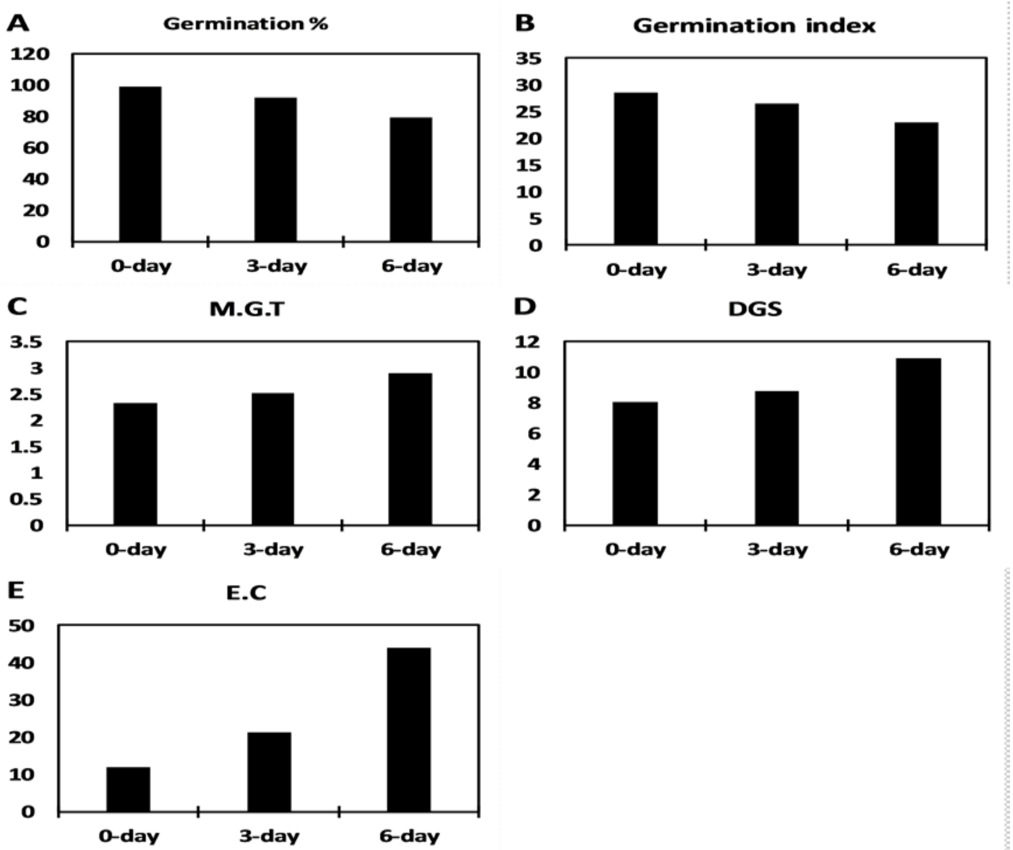

Fig. 1. Impact of accelerated aging on germination %, germination index, Mean germination time (M.G.T), day germination speed (D.G.S) and electrical conductivity test (E.C) of sunflower seeds.

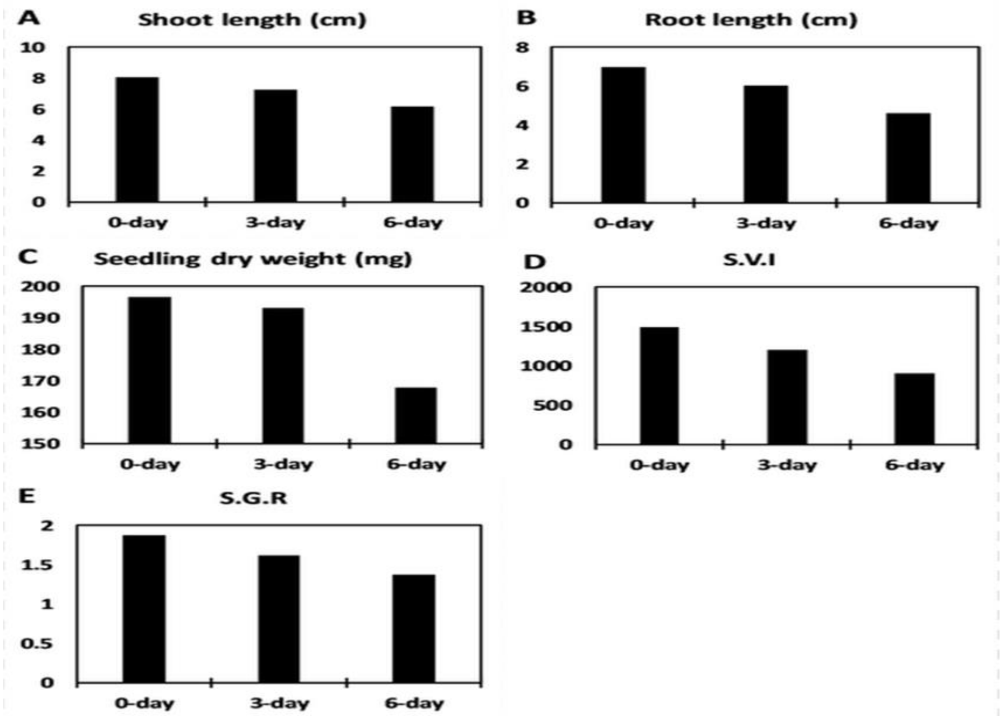

Fig. 2. Impact of accelerated aging on shoot length (cm), root length (cm), seedling dry weight (mg), seedling vigor index (S.V.I) and seedling growth rate (S.G.R) of sunflower seeds.

Germination percentage and germination index of seeds decreased as the aging period progressed (Fig. 1). The highest values of germination % and germination index (99.15 % and 28.62%) were observed under control conditions (0-day aging) and the minimum values (79.33and 22.93) were attained under 6-days aging, respectively. Whereas, increasing period of aging (3 or 6 days) significantly increased mean germination time, day germination speed and electrical conductivity (Fig. 1).The maximum values of these traits (2.9, 10.89 and 44) were recorded by (6-day aging) but the lowest values (2.3, 8.1 and 11.89) were recorded by (0-day aging), respectively. Also, radical length, plumule length, seedling dry weight, seedling growth rate and seedling vigor index were reduced with increasing aging period. Figure 2 shows the effect of seed aging on these traits. Results indicates that the highest values of these traits (8.05, 6.97, 196.72, 1490.56 and 1.88) were observed by (0-day aging) whereas the lowest values (6.15, 4.61, 167.72, 903.59 and 1.38) were observed by (6-days aging), respectively. By increasing seed age period germination% and seedling vigor were decreased and these results are in agree with Ansari and Sharif Zadeh, 2012, Bailly, 2004 and Maryam *et al*., 2019 which have demonstrated negative effects of aging on seed performance, germination % and seedling indices. Also, they concluded that decreasing of radical length, plumule length and seedling dry weight is due to decline in seed vigor. Decreasing germination% in aged seeds may be due to the depression of ά- amylase activity and carbohydrate contents (Bailly *et al*., 2004), or denaturation of proteins (Nautiyal *et al*., 1985). Mohammadi *et al*., (2011) found that aging reduced germination percentage and increased mean time to germinate.

Decreasing in seed viability might be due to the chromosomal aberrations (Akhtar *et al*., 1992) and genetic damage (Abdalla and Roberts, 1968) that occur under storage conditions of seeds. Electrical conductivity increased by increasing seed age and these results are in line with earlier reports of (Seiadat *et al*., 2012). They pointed that cell membrane damage leads to a decrease in the ability of carrier proteins and consequently, caused an increase in electrolyte leakage. Electrical leakage is a sign of permeability of plasma membrane and transition of liquid-crystal phase of membrane to solid-gel state maybe the first phenomenon that affect membrane transporters. After this transition, phospholipid layer of membrane is destroyed by lipid peroxygenase and ROS leading to de-construction of membrane integrity.

Impact of seed priming treatments viability of sunflower seeds:

Results indicated that seed priming treatments had significant impacts on germination %, germination index, mean germination time, day germination speed, electrical conductivity, radical length, plumule length, seedling dry weight, seedling vigor index and seedling growth rate, (Figures 3 and 4).

Germination percentage and germination index were increased in aged seeds with priming. The highest germination percentage (98.2%, 98.6% and 98.6%) and germination index (31.87, 31.99 and 31.86) were achieved by (50 mM CaCl2, 500 ppm SA and 50% Yeast) whereas the lowest germination percentage (76.4%) and germination index (12.1%) were achieved by control (non-primed seeds), respectively. On the other hand, germination mean time, day germination speed and electrical conductivity were decreased by seed priming of aged seeds (Fig.1). The maximum values of germination mean time, day germination speed and electrical conductivity (3.25, 12.72 and 45.56) gained from control (non-primed seeds), respectively. whereas the minimum germination mean time (2.23, 2.22 and 2.20), day germination speed (8.18, 8.15 and 8.17) and electrical conductivity (19.5, 19.7 and 19.4) were gained from (50 mM CaCl2, 500 ppm SA and 50% Yeast), respectively.

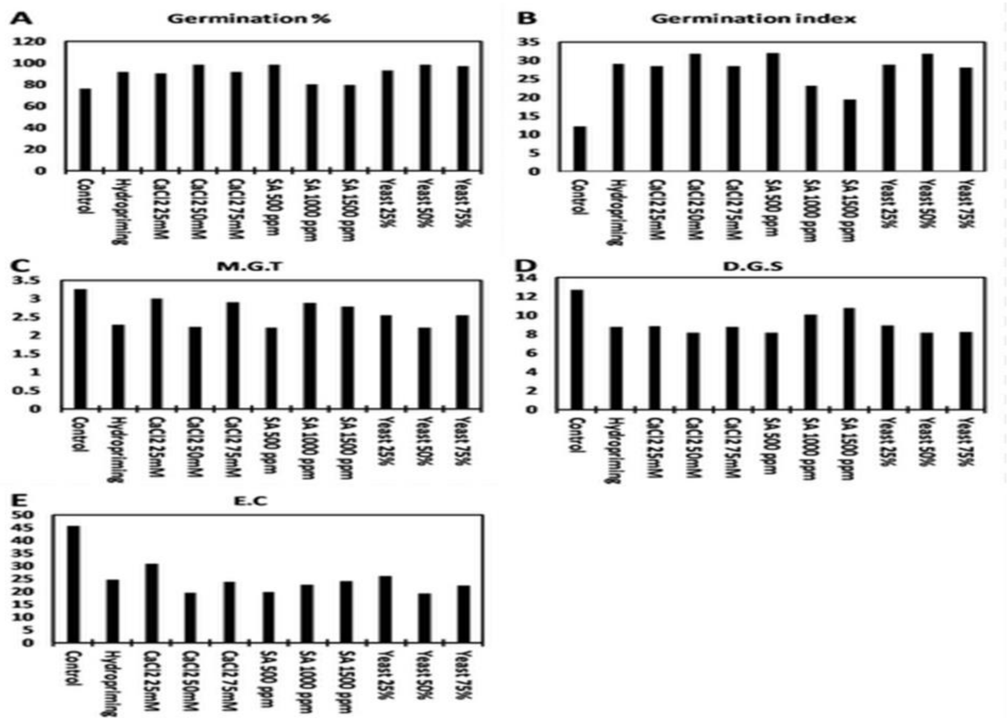

Fig. 3. Impact of seed priming treatments on germination %, germination index, Mean germination time (M.G.T), day germination speed (D.G.S) and electrical conductivity test (E.C) of sunflower seeds.

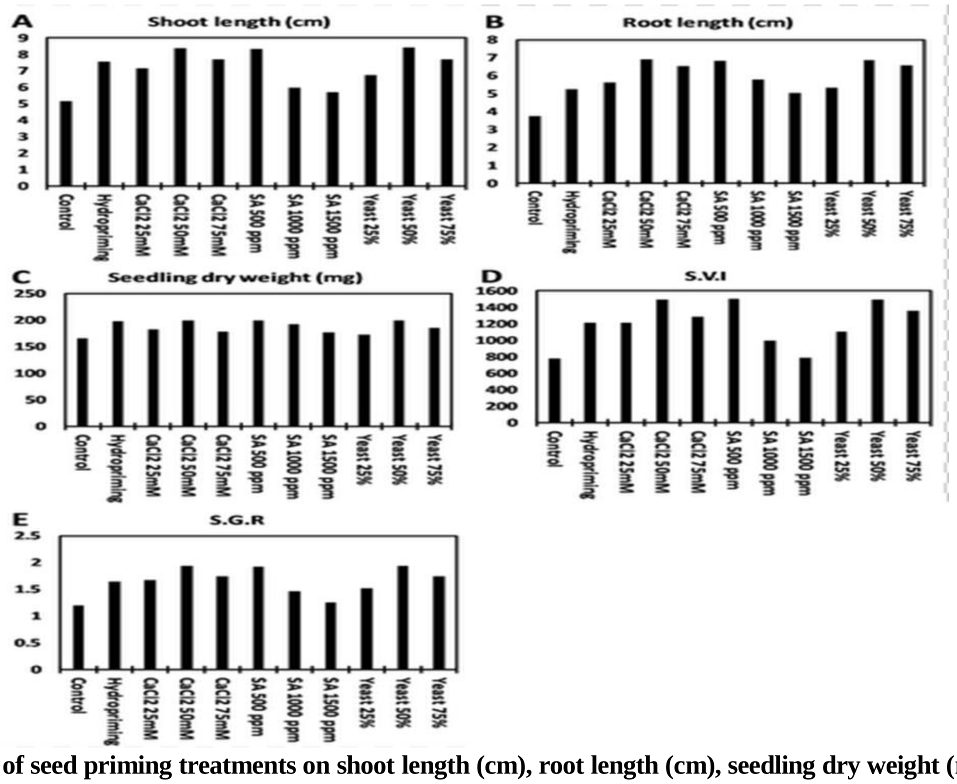

Fig.4. Impact of seed priming treatments on shoot length (cm), root length (cm), seedling dry weight (mg), seedling vigor index (S.V.I) and seedling growth rate (S.G.R) of sunflower seeds.

Also, Seed priming increased radical length, plumule length, seedling vigor index, seedling growth rate and seedling dry weight of aged seeds. The maximum values of shoot length (8.36_cm, 8.36_cm and 8.45_cm), root length (6.89_cm, 6.84_cm and 6.88_cm), seedling dry weight (199.9_mg, 199.8_mg and 199.9_mg), seedling vigor index (1498.5, 1503.8 and 1493.4) and seedling growth rate (1.93, 1.92 and 1.94) were obtained from (50 mM $CaCl_2$, 500 ppm SA and 50% Yeast) and the minimum plumule length, radical length, seedling dry weight, seedling vigor index and seedling growth rate (5.17, 3.77, 165.3, 780.88 and 1.19) were obtained from control (non-primed seeds), respectively.

Priming can enhance viability and seedling vigor. In favor of the findings of Ansari et al., (2013); Hussain et al., (2017) and Seiadat et al., (2012) who reported that seed priming had positive influence on seed viability and seedling vigor traits.

Impact of the interaction between aging and priming on viability of sunflower seeds: -

Data in tables 1 and 2 indicated that there were significant differences due to the interaction between aging and priming in all studied traits (germination %, germination index, mean germination time, day germination speed, electrical conductivity, plumule length, radical length, seedling vigor index, seedling dry weight and seedling growth rate).

The best germination percentage (100%), germination index (32.5) were appeared in non-aged seeds which primed with (water, 50 mM $CaCl_2$, 500 ppm SA and 50% and 75% Yeast), subsequently and the lowest germination percentage (37.3%), germination index (7.6) gained from unprimed but aged seeds for 6-days, subsequently. These results are in agreement with Demir *et al.* (2006).

The most germination mean time, day germination speed and electrical conductivity (3.7, 21.5 and 88.8) were cleared in 6-days aged seeds without any priming. Whereas, the lowest germination mean time (1.5), day germination percentage (8) and electrical conductivity (8.3) were observed in non-aged seeds which primed with (Yeast 50%). These results are supported by Ellis and Roberts (1981).

Data in fig.4 cleared that the best shoot length (10.2cm), root length (8cm), seedling dry weight (217mg), seedling vigor index (1743) and seedling growth rate (2.27) gained of non-aged primed seeds by yeast 50% and the lowest shoot length (3.4), root length (2.1), seedling dry weight (141), seedling vigor index (335) and seedling growth rate (0.73) gained of unprimed but aged seeds for 6-days. Under each level of aging period (0, 3 or 6 days), priming had a positive effect on all studied traits (Fig. 4).

Moreover, our findings exhibited that seed priming improved aged seeds viability and seedling vigor. Priming reduced the aging effect. Among the treatments, (water, 50 mM $CaCl_2$, 500 ppm S.A, 50% and 75%_Yeast) improved germination characteristics and seedling vigor during the aging. In accordance with the previous results, earlier reports (Seiadat *et al*., 2012 and Maryam *et al*., 2019) have proved negative affect of aging on germination. Germination % and germination rate were lost as aging temperature was increased. Ansari and Sharif- zadeh (2012) regarding reported the significant loss of germination and seedling growth occurred when aging period increased. Many researches about different plants show that priming enhances germination parameters (Ansari *et al*., 2013; Khan *et al*., 2019; Roy *et al*., 2019 and Tahjib-Ul-Arif *et al*., 2019).

Table 1. Impact of interaction between aging and priming on germination %, germination index, Mean germination time (M.G.T), day germination speed (D.G.S) and electrical conductivity test (E.C) of sunflower seeds.

Priming	Germ. %			Germ. Index			M.G. T			D.G. S			E.C		
	Aging			Aging			Aging			Aging			Aging		
	0day	3day	6day	0day	3day	6day	0day	3day	6day	0day	3day	6day	0day	3day	6day
Control	98.7	93.3	37.3	25.3	24.23	7.6	2.9	2.9	3.7	8.1	8.6	21.5	15.3	32.5	88.8
Hydro	100	96.0	80.0	31.5	30.53	25.2	2.2	2.2	2.5	8.0	8.3	10.0	11.3	19.4	41.5
$CaCl_2$25mM	98.7	93.3	80.0	31.4	28.67	25.3	2.8	3.0	3.2	8.1	8.6	10.0	13.6	24.6	54.2
$CaCl_2$50mM	100	97.3	96.0	32.5	30.97	25.8	2.1	2.2	2.4	8.0	8.2	8.33	9.6	18.4	41.5
$CaCl_2$75m M	98.7	94.7	81.3	29.2	29.9	26.5	2.7	2.9	3.07	8.1	8.5	9.84	12.8	21.3	39.6
S. A 500 ppm	100	98.7	96.0	32.5	31.86	31.6	1.5	1.7	1.9	8.0	8.1	8.33	8.3	18.2	32.6
S. A 1000 ppm	98.7	76.0	45.0	20.73	14.57	19.9	2.4	2.9	3.4	8.1	9.7	12.5	12.6	19.8	35.9
S. A 1500 ppm	97.3	72.0	60.7	16.43	12.37	11.1	2.1	2.5	3.5	8.2	10.9	13.33	14.4	19.6	38.1
Yeast 255	98.7	93.3	86.7	30.2	29.47	27.2	2.3	2.5	2.8	8.1	9.4	9.23	13.6	21.1	41.2
Yeast 50%	100	100	96.0	32.5	32.07	27.9	2.3	2.4	2.6	8.0	8.0	8.33	8.6	17.6	32.1
Yeast 75%	100	97.3	94.7	32.5	27.77	24.2	2.3	2.5	2.8	8.0	8.2	8.45	10.3	18.4	38.9
L.S.D 0.05	0.99	0.99	0.99	0.71	0.71	0.71	0.11	0.11	0.11	0.25	0.25	0.25	0.86	0.86	0.86

Table 2. Impact of interaction between aging and priming on shoot length (cm), root length (cm), seedling dry weight (mg), seedling vigor index (S.V.I) and seedling growth rate (S.G.R) of sunflower seeds.

Priming	Shoot L.			Root L.			D.W			S.V.I			S.G.R		
	Aging			Aging			Aging			Aging			Aging		
	0day	3day	6day	0day	3day	6day	0day	3day	6day	0day	3day	6day	0day	3day	6day
Control	6.2	5.7	3.4	4.8	4.4	2.1	194	213	144	1088	933	335	1.38	1.25	0.73
Hydro	8.1	7.9	6.6	5.8	5.5	4.5	215	196	188	1393	1324	938	1.74	1.73	1.47
$CaCl_2$25Mm	7.9	7.5	6.1	6.4	6	4.4	199	200	148	1414	1293	940	1.79	1.74	1.47
$CaCl_2$50Mm	9.5	7.5	7.3	7.9	6.9	5.9	215	216	162	1820	1311	1242	2.18	1.69	1.62
$CaCl_2$75m M	8.1	8.1	6.9	7.6	6.5	5.5	181	185	167	1549	1352	967	1.96	1.79	1.49
S. A 0.5	9.7	7.9	7.4	7.7	6.9	5.9	216	219	155	1740	1496	1275	2.18	1.92	1.66
S. A 1	6.7	6.7	5.2	7.4	6.4	3.6	176	176	166	1385	1049	552	1.75	1.56	1.12
S. A 1.5	6	6.1	4.6	6.9	5	3.2	176	188	166	1255	731	355	1.61	1.25	1.08
Yeast 255	7	6.9	6.4	6.2	5.3	4.5	174	160	179	1305	1035	988	1.66	1.49	1.43
Yeast 50%	10.2	8.2	7.1	8	6.7	5.9	217	192	183	1743	1436	1224	2.27	1.79	1.59
Yeast 75%	9.2	7.1	6.8	7.8	6.5	5.3	197	174	182	1700	1253	1119	2.13	1.61	1.48
L.S.D 0.05	0.4	0.4	0.4	0.14	0.14	0.14	2.17	2.17	2.17	63.2	63.2	63.2	0.08	0.08	0.08

CONCLUSION

Loss of seed viability is common problem with aging. our results clearly indicate that seed priming cause an improvement in germination parameters as compared to the non-primed seeds under aging stress. Among the studied treatments, (water, 250 mM $CaCl_2$, 500 ppm S.A, 50% and 75%Yeast) were highly effective to remove damages of aging. Therefore, priming is a useful technique and can be used to enhance aged seeds germination and viability.

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تأثير معاملات التهيئة على حيوية بذور دوار الشمس المتدهورة صناعيا

نجوي إبراهيم شلبي و آلاء محمد المهدي أحمد شاهين
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الملخص

أجريت هذه الدراسة لمعرفة تأثير التدهور الصناعي ومعاملات تهيئة البذور المختلفة على الحيوية في بذور دوار الشمس. تم استخدام ثلاث فترات من التدهور الصناعي (صفر(معاملة المقارنة)، ٣ ، ٦ أيام) تحت ظروف ٤١ درجة ، ١٠٠% رطوبة جوية. وكذلك تم استخدام ١١ معاملات نقع للبذور وهى (بدون نقع (معاملة المقارنة) ، نقع بالماء ، نقع بمحلول كلوريد كالسيوم بتركيز (٢٢٥ ملجم ، ٢٥٠ ملجم ، ٢٧٥ ملجم) ، نقغ بمحلول حمض السالسليك بتركيز (٠,٥ ملجم ، ١ ملجم ، ١,٥ ملجم) ، نقع بمحلول الخميرة بتركيز (٢٥% ،٥٠% ، ٧٥%). وتم تقدير صفات (نسبة الانبات ، دليل الانبات ، متوسط ايام الانبات، سرعة الانبات ، التوصيل الكهربي ، طول الريشة ، طول الجنير ، دليل قوة البادرة ، الوزن الجاف للبادرة ، معدل نمو البادرة). أوضحت النتائج وجود تأثيرات معنوية لكلا من التدهور الصناعي ومعاملات النقع على جميع الصفات المدروسة. زيادة فترة التدهور الصناعي أدت إلي إنخفاض فى جميع الصفات المدروسة فيما عدا متوسط أيام الإنبات والتوصيل الكهربي وسرعة الإنبات. أيضا أوضحت النتائج أن أفضل معاملات النقع هي (النقع بالماء ، محلول كلوريد كالسيوم بتركيز ٢٥٠ ملجم ، محلول حمض السالسليك بتركيز ١ ملجم ، محلول الخميرة بتركيز ٥٠%) وذلك مع جميع الصفات المدروسة. لذلك توصى الدراسة بإستخدام هذه المعاملات كأفضل وسيلة لزيادة الحيوية وقوة البادرة فى بذور دوار الشمس خاصة بعد تعرضها للتدهور الصناعي.