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Effects of Seed Priming on Catalase Activity and Storage Reservoirs of Aged Milk Thistle Seeds (*Silybum marianum* (L.) Gaertn)

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ABSTRACT

In order to study effects of seed priming on storage reservoirs and element contents of aged milk thistle seeds a factorial experiment was conducted based on complete randomized design (CRD) with three replications. Treatments were hydropriming (using distilled water), halopriming (0, 1.5, 3, 4.5 and 6% KNO_3) and accelerated aging (0, 48, 96 and 144 hours under 45 °C and 100% relative humidity). Investigated parameters were germination percentage, mean time germination, seedling length, seedling dry weight, seed reservoirs usage rate (SRUR), seed reservoirs usage efficiency (SRUE), fraction of utilized seed reservoirs (FUSR), catalase activity (CAT), content of sodium, potassium, calcium and sodium to potassium ratio in seedling and cotyledons. The result showed that priming could increase germination percentage, seedling length, and dry weight and reduce mean time of seed germination, while under aging conditions all these traits were decreased except mean time of germination. Priming with concentrations of 3% and 1.5% KNO_3 showed the highest germination percentage, seedling length, dry weight and least mean time germination. Catalase activity deceased at ageing treatment and priming seeds with 3% KNO_3 exhibited the highest value of catalase activity. Aging treatment led to increase of SRUR and in contrast it decreased SRUE and FUSR. The highest SRUR appointed to hydropriming, whereas the highest amount of SRUE and FUSR belonged to 3% KNO_3 treatment. Aging increased Na^+ content and Na^+/K^+ ration while, it deceased K^+ and Ca^{2+} in seedling and cotyledon. In general priming increased germination percent and improved seedling growth, performance of reserves and as well as increased catalase activity and reduced elements leakage from the cells under the aging.

Keywords: Germination; Aged; SRUR; Potassium nitrate; Seedling length

Tohum Yaşlandırma ve Önuygulamanın Meryemana Dikeni (*Silybum marianum* (L.) Tohumlarında Katalaz Aktivitesi ve Depo Rezervlerine Etkisi

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ÖZET

Tohum yaşlandırma ve önuygulamanın meryemana dikeni tohumlarına etkisini belirlemek üzere tesadüf parselleri deneme deseninde ve 3 tekerrürlü bir deneme yürütülmüştür. Önuygulamaları, su önuygulaması (damıtık su) ve tuz önuygulaması (% 0, 1.5, 3, 4.5 ve 6 KNO_3), tohum yaşlandırmayı ise 45 °C ve % 100 oransal neme sahip ortamda bekletme (0, 48, 96 ve 144 saat) oluşturmuştur. Bu çalışmada, çimlenme yüzdesi, ortalama çimlenme süresi, fide boyu, fide kuru ağırlığı, tohum rezervi kullanım oranı (SRUR), tohum rezerv kullanım etkinliği (SRUE), kullanılan tohum rezervi fraksiyonu (FUSR), katalaz aktivitesi (CAT), fide ve kotiledon sodyum, potasyum, kalsiyum ve sodyum içeriği ve sodyumun potasyuma oranı belirlenmiştir. Sonuçlar; tohum önuygulamalarının çimlenme yüzdesini, fide boyunu ve kuru ağırlığını artırıp ortalama çimlenme süresini azaltırken, tohum yaşlandırmanın çimlenme süresi dışında tüm bu özellikleri azalttığını göstermiştir. En kısa ortalama çimlenme süresi ve en yüksek çimlenme oranı, fide boyu ve kuru ağırlık değerlerine % 3 ve % 1.5'lik KNO_3 ile tohum yaşlandırma uygulamalarında ulaşılmıştır. Tohum yaşlandırma uygulamaları katalaz aktivitesini azaltmış ve % 3'lük KNO_3 önuygulamasında ise en yüksek katalaz aktivitesi değerine ulaşılmıştır. Tohum yaşlandırma uygulamaları SRUR değerini artırırken, SRUE ve FSUR değerlerinde düzenli bir azalışa neden olmuştur. En yüksek SRUR değerine su önuygulamasında ulaşılırken en yüksek SRUE ve FSUR değerlerine % 3'lük KNO_3 önuygulamasında ulaşılmıştır. Tohum yaşlandırma, fide ve kotiledonun Na^+ içeriğini ve Na^+/ K^+ oranını artırırken, K^+ ve Ca^{2+} içeriğini azaltmıştır. Genel olarak, önuygulamalar çimlenme yüzdesini artırıp, fide gelişimi, rezerv performansı ve katalaz aktivitesini iyileştirirken, Tohum yalandırma hücrelerden element yıkanmasını azaltmıştır.

Anahtar Kelimeler: Çimlenme; Tohum yaşlandırma; SRUR; Potasyum nitrat; Fide boyu

1. Introduction

Milk thistle (*Silybum marianum* (L.) Gaertn) is cultivated as medicinal plant and also it is known as a weed in some regions (Khan et al 2009). This plant is native to the mediterranean basin and is now widespread throughout the world. Milk thistle is used to treat liver diseases, blood cholesterol and prevention of cancer (Kren & Walterova 2005; Shaker et al 2010). Seeds of milk thistle contain small amounts of flavonoids (taxifolin), fatty acids and other polyphenolic compounds (Ramasamy & Agarwal 2008). Germination and seedling establishment are two important parameters of seed quality. High temperature and humidity are most injurious factors to seed quality (Sveinsdo Ttira et al 2009). Unfavorable conditions and long-term storage can cause seed deterioration, and the aging rate of seeds depend on species (Priestley 1986). Oxidative stress is the result of imbalance condition between production of ROS and antioxidants defensive mechanisms of plants. During aging, free radicals can cause seed cell destruction and finally damage cell vital functions. Any increase in ROS content and specially hydrogen peroxide or OH contents of cell can severely damage cell membrane and injure cell (Bienert et al 2006). Aging could reduce seed reservoirs usage rate while the seed reservoirs use efficiency was not significantly affected by aging (Soltani et al 2008). Priming is an important way for enhancing seed germination. It is the process of partial and monitoring discharge of seeds to begin the biochemical processes and metabolism of sugars and hydrolysis inhibitors during the first and second stages of germination before radicle emergence. Priming enhanced germination percent, germination rate and seedling growth. Priming diminished the effects of seed ageing by reducing malondialdehyde (MDA), a free radicals production and maintenance of antioxidants activities (Basra et al 2003). Plant cell expansion may be limited by at least two factors: low turgor and low cell wall extensibility. Turgor pressure is the driving force for cell expansion and results from the accumulation of osmotically active ions or molecules in vacuoles. Mentianing turgor potential related to cell membrane stability to preventing solutes (such as ions) leakage from the cells. Cell wall extensibility is determined to a great extent by the strength of bindings among various cell wall components such as cellulose

microfibrils, hemicelluloses, and pectins. A reduced growth rate of seedling roots from aged seeds may be related to these factors of cell expansion. The physiological mechanisms that are responsible to control cell expansion have been explained with the acid-growth hypothesis (Sveinsdo Ttira et al 2009). According to this theory, cell expansion is mediated through an acidification of the cell wall, which results in an increase of cell wall elasticity. A higher apoplastic H^+ concentration is assumed to promote exchange of Ca^{2+} from pectinases as well as to activate cell wall-loosening enzymes, such as expansions (Cosgrove 2000). Micro elements play an important role in growth and development of plants (Dewal & Pareek 2004). Metal ions including Fe^{3+}, Zn^{2+}, Mg^{2+}, K^+, Ca^{2+}, and some other micronutrients are cofactor for nearly 100 enzymes which are involved in cell division, nucleic acid metabolism and protein synthesis (Cakmak & Horst 1991). Researches has shown that application of micronutrients reduces the effects of environmental stresses (Wang & Huang 2004). Isamah (2004) showed that sodium, potassium and calcium content of seeds reduced during aging. Calcium could decline plant senescence and increase seed vigour in rice. Calcium might play an important role in reducing O_2 in the germinating stage of naturally or artificially aged rice seeds (Guo 1988). Potassium also plays a key role in osmotic potential and electrical properties of cell cytoplasm, and plasma membrane permeability is influenced by potassium. Moreover, potassium is an important factor for some enzymes (Edgar & Spalding 1999).

This study was conducted to investigate the effects of priming and aging on seed germination, seed storage reservoirs and seedling growth and determine the best treatment for milk thistle seeds.

2. Material and Methods

2.1. Seed materials

Seed of milk thistle (*Silybum marianum* L.) were obtained from the seed and plant improvement institute and to perform accelerated aging treatments, seeds were incubated inside the sealed boxes with 100% relative humidity and afterwards placed at 45 °C for 0, 48, 96 and 144 hours (Gholami Tilebeni & Golpayegani 2011). For seed priming, 150 seeds were placed on two filter papers and 20 ml of osmolite added for seed priming, subsequently and incubated at 20 °C for 12 hours in darkness in 9 cm Petri dishes (Bradford 1985). Treatments included hydropriming and halopriming with KNO_3 at concentrations of 1.5%, 3%, 4.5% and 6%.

2.2. Germination test

To test the germination of milk thistle seeds, 50 seeds were disinfected with 1% sodium hypochlorite for 5 minutes then cultured by sandwiching between two layered filter papers. The counting of germinated seeds was done regularly after every 24 h and the appearance of 2 mm or more of radicle was considered as germination. Germination test was ended after 14 days when the number of germinated seeds was equal in two sequential counting (Sedghi et al 2010). Seedling size was measured on last day of germination test. The seedling dry weight was obtained after putting seedlings in the oven at 75 °C for 48 h.

2.3. Mean time to germination (MTG)

Average seed germination rate was calculated as an index of the germination rate according to Ellis & Roberts (1981), where, (n) shows the number of germinated seeds in days (d) and $\sum$ **(**nd**)** as total germinated seeds.

$$MTG = \sum (nd)/\sum n \quad (1)$$

2.4. Efficiency reservoirs

In order to measure the efficiency reservoirs, seed weight was measured before planting then cultured in petri dish. After 14 days of seedling growth, cotyledon were removed and considered as a seed residual. Dry weight of seedling and cotyledon was calculated using following formulae (Soltani et al 2008).

$$SRUR = ISDW - RSDW \quad (2)$$

$$SRUE = SLDW / SRUR \quad (3)$$

$$FUSR = SRUR / LSDW \quad (4)$$

Where; SRUR, seed reservoirs usage rate; ISDW, residual seed dry weight; RSDW, remaining seed dry weight; SRUE, seed reservoirs using efficiency; SLDW, seedling dry weight; FUSR, fraction of utilized seed reservoirs.

2.5. Determination of catalase activities

The enzyme activity was measured in seedling stage after 14 days of germination. To measure the enzyme activity, 0.2 g of fresh tissue was used. In order to extract protein, 0.2 g of plant fresh tissue was crushed by using liquid nitrogen and then one ml of buffer Tris-HCl (0.05 M, pH= 7.5) was added. Obtained mixture centrifuged for 20 min (13000 rpm and 4 ^{0}C), then supernatant was used for enzyme activity measurements (Sudhakar et al 2001). Catalase activity was assayed according to Karo & Mishra (1976). The 60 µL protein extract was added to tris buffer (50 mM, pH= 7) containing 5 mM H_2O_2 on the ice bath, then the absorbance curve was plotted at a wavelength of 240 nm. Enzyme activity was obtained for OD mg^{-1} protein of fresh tissue. Evaluation of protein carried out by Bradford (1976) method. Briefly, 0.2 g of plant tissue was squashed with 0.6 mL extraction buffer and centrifuged in 11500 rpm for 20 min at 4 °C. The supernatant transferred to the new tubes and centrifuged for 20 minutes at 4000 rpm and the supernatant was obtained. To measure protein amount, 10 µL of supernatant added to 5 mL Bradford solution and 290 µL extraction buffer, then the absorbance rate was read at 595 nm.

2.6. Measuring elements

Sodium and potassium content were determined using Borgan (2006) method. Briefly, 1 g of dried shoot was dry-ashed in an electric furnace at 500 °C for two hours. Then, for each sample, 10 mL of 1 N hydrochloric acid was added and heated to boiling. Finally their volume were made up to 100 mL by using distilled water, and flame spectrometer was used to measure elements concentrations.

2.7. Statistics

An experimental design was arranged in factorial based on a completely randomized design (CRD) with three replicates. The normality test and analysis of variance of data were conducted by the MSTATC and SPSS softwares and comparison of means were done by using the Duncan multiple range test with P value= 0.05. Correlation was determined by Pearson's method (Gomez & Gomez 1984).

3. Results

3.1. Germination percentage and mean time germination

Results of this experiment showed that seed priming significantly increased germination percentage and reduce mean time of germination ($P \leq 0.05$). The highest germination percentage (62.0%) and the lowest mean time of germination (2.01 day) belonged to priming treatments with concentrations of 3% and 1.5% KNO_3, respectively. The least priming impact on germination percentage (56.3%) and mean time of germination (2.39 day) obtained by 1.5% and 6% KNO_3, respectively. Hydropriming caused to increase seed germination from 50.7% to 59.3% and reduced mean time of germination from 3.29 to 2.16 day (Table 1). Aging declined germination percentage and increased mean time of germination ($P \leq 0.05$). During 48 hours of aging, seed germination reduced from 66.2% to 64.2% and mean time of germination from 2.09 to 2.12 days. The maximum reduction of germination percentage observed by 144 hours aging, also the highest postponement of seed germination (mean germination time) appointed to this aging time in compared to control (Table 1).

3.2. Seedling length and dry weight

Seedling growth also was affected significantly by seed priming ($P \leq 0.05$). Results showed seed priming caused higher seedling length and dry weight. The utmost seedling length (7.80 cm) and dry weight (3.92 mg) were observed at seed priming with 3% KNO_3 while the lowest (5.74 cm) was belonged to hydropriming and priming with 4.5% KNO_3 (3.07

Table 1- Effect of seed ageing and priming treatments on GP, MTG, length and weight of seedling, SRUR, SRUE, FUSR and CAT activity in milk thistle

Çizelge 1- Yıllandırma ve tohum önuygulamalarının meryemana dikeninde GP, MTG, fide boyu ve ağırlığıkları, SRUR, SRUE, FSUR ve CAT aktivitesine etkisi

Seed treatment	*GP (%)*	*MTG (day)*	*LS (cm)*	*DWS (mg)*	*SRUR (mg mg^{-1})*	*SRUE (mg mg^{-1})*	*FUSR (mg mg^{-1})*	*CAT OD mg^{-1} protein*
				Priming				
Control	50.7c	3.29a	4.56d	3.13c	0.014a	0.36c	085.3c	5.14b
Hydropriming	59.3ab	2.16bc	5.74c	3.91a	0.011b	0.66b	104.7bc	7.67ab
KNO_3 (1.5%)	56.3b	2.01c	7.63a	3.50b	0.008cd	0.71ab	156.6ab	8.50a
KNO_3 (3%)	62.0a	2.26b	7.80a	3.92a	0.007d	0.99a	197.84a	9.26a
KNO_3 (4.5%)	57.7b	2.08c	6.26b	3.07c	0.009c	0.65b	149.7b	7.35ab
KNO_3 (6%)	58.7a	2.39b	6.56ab	3.18c	0.009c	0.60b	131.4bc	6.87b
$LSD_{0.05}$	8.65	0.29	1.57	0.44	0.00082	0.35	50.3	2.52
				Aged (hours)				
0	66.2a	2.09b	7.27a	3.94a	0.007c	0.93a	191.1a	8.13ab
48	64.2a	2.12b	6.89ab	3.59b	0.007c	0.67b	155.1b	8.88a
96	50.2b	2.51a	6.10ab	3.20c	0.010b	0.73ab	129.8c	7.36b
144	49.1c	2.73a	5.43b	3.07c	0.015a	0.32c	74.7d	5.49b
$LSD_{0.05}$	7.06	0.25	1.36	0.38	0.00071	0.31	43.3	2.06

GP, germination percentage; MTG, mean time germination; CAT, Catalase activity; LS, Length seedling; DWS, Dry Weight seedling; SRUR, seed reservoirs using rate; SRUE, seed reservoirs using efficiency; FUSR, fraction of utilized seed reservoirs

mg), respectively. Hydropriming enhanced seedling length from 4.56 to 5.48 cm and dry weight from 3.13 to 3.91 mg, on the other word lead to enhance seedling length and dry weight by 25.8% and 24.9%, respectively. Aging reduced seedling growth, so seedling length was decreased (25.3%) from 7.27 to 5.43 cm and dry weight (22%) from 3.94 to 3.07 mg in 144 hours aging (Table 1).

3.3. Efficiency reservoirs

Priming also affect significantly SRUR and SRUE ($P \leq 0.05$). According to our results, seed priming lowered SRUR and increased SRUE. Among all priming treatments, the highest (0.011 mg mg^{-1}) and the lowest (0.007 mg mg^{-1}) SRUR belonged to hydropriming and KNO_3 at 3% concentration, respectively. The maximum (0.99 mg mg^{-1}) and the minimum (0.6 mg mg^{-1}) amount of SRUE observed in seed priming with 3% and 6% KNO_3 respectively. Priming with 3% KNO_3 caused to reduce in SRUR by 100% and increasing SRUE by 175%. Aging increased SRUR and decreased SRUE. Aging of 48 hours did not have any changes on SRUR, but it reduced SRUE (27.9%) and aging for 96 and 144 hours declined SRUR by 42% and 144%, respectively (Table 1). FUSR also influenced significantly by priming and aging treatments ($P \leq 0.05$). Priming increased FUSR whereas aging reduced it. The highest (191.1 mg mg^{-1}) FUSR was in 3% KNO_3 with no ageing, while the lowest FUSR (104.7 mg mg^{-1}) observed in hydropriming and in aging the amount of FUSR decreased to 74.7 mg mg^{-1} in aging for 144 hours (Table 1).

3.4. Catalase activities

Catalase activity of milk thistle seedling was affected by seed priming and aging ($P \leq 0.05$). Results showed that priming by 3% KNO_3 exhibited the highest (9.26 OD mg^{-1} protein min^{-1}) and control the lowest (5.14 OD mg^{-1} protein min^{-1}) catalase activity. Aging reduced catalase activity from 8.13 to 5.49 OD mg^{-1} protein min^{-1} (Table 1).

3.5. Elements content

Result showed that seed priming increased Na^+, K^+ and Ca^{2+} in seedlings ($P \leq 0.05$). The highest amount of Na^+ belonged to hydropriming and maximum amount of K^+ (0.41 mg g^{-1}) and Ca^{2+} (0.023 mg g^{-1}) appointed to 6% KNO_3 and hydropriming respectively. The ratio of Na^+/K^+ was changed during seed priming with KNO_3 and showed declining trend ($P \leq 0.05$). Priming with 3% and 1.5% KNO_3 showed the lowest (0.75) and 6% KNO_3 exhibited the highest (0.87) ratios of Na^+/K^+. Aging increased Na^+ content (from 0.25 to 0.34 mg g^{-1}) and Ca^{2+} (from 0.017 to 0.023 mg g^{-1}) while it reduced K^+ content of seedlings (from 0.36 to 0.31 mg g^{-1}). Amount of Ca^{2+} reduced after 96 hours of aging (from 0.023 to 0.018 mg g^{-1}). Aging for 144 hours caused to improve Na^+/K^+ by 58% (Table 2). Also priming prevented significantly cotyledon elements leakage, so the highest and the lowest content of Na^+ appointed to hydropriming and 6% KNO_3, respectively in comparison with control. Maximum K^+ and Ca^{2+} content were in 6% and 1.5% KNO_3 respectively, while minimum amount of these elements observed at hydropriming. Amount of Ca^{2+} increased in seedling after 96 hours of ageing. In severe aging, Na^+/K^+ of cotyledon increased up to 48% (Table 2).

3.6. Correlation coefficient

Results of correlation test showed that germination percentage had a negative correlation with mean time germination and seedling Na^+/K^+, while it has positive correlation with seedling length, dry weight and enzyme activity (catalase). The content of Na^+ in seedlings and cotyledons SRUR had maximum and Na^+/K^+ of cotyledon the minimum correlation with mean time germination. SRUR and SRUE had high correlation with FUSR (r= -0.803 and 0.803) and Ca^{2+} content of cotyledon (r= -0.378 and 0 .311). Catalase enzyme activity positively correlated with germination percentage, seedling dry weight and potassium seedling while it has a negative correlation with mean time germination (Table 3).

4. Discussion

According to the results of this research, seed priming increased germination percentage and reduced mean time of germination, and aging declined germination percentage and increased mean time of germination.

Table 2- Effect priming on Na^+, K^+, Ca^{2+} and Na^+/K^+ in seedling and cotyledon of milk thistle

Çizelge 2- Önuygulamaların meryemana dikeninde kotiledon ve fide Na^+, K^+, Ca^{2+} içeriği ve Na^+/K^+ oranına etkisi

Seed treatment	*Seedling (mg g^{-1})*				*Cotyledon (mg g^{-1})*			
	Na^+	*K^+*	*Ca^{2+}*	*Na^+/K^+*	*Na^+*	*K^+*	*Ca^{2+}*	*Na^+/K^+*
				Priming				
Control	0.24c	0.24c	0.013d	0.94b	0.025bc	0.090a	0.006c	0.17d
Hydropriming	0.52a	0.34c	0.023a	1.51a	0.034a	0.068c	0.009b	1.01a
KNO_3 (1.5%)	0.22d	0.30d	0.015c	0.75c	0.028bc	0.081b	0.011a	0.34b
KNO_3 (3%)	0.25c	0.34c	0.021b	0.75c	0.026bc	0.083b	0.009b	0.34b
KNO_3 (4.5%)	0.24c	0.37b	0.021b	0.65d	0.026bc	0.085b	0.009b	0.39b
KNO_3 (6%)	0.32b	0.41a	0.021b	0.87b	0.024c	0.094a	0.007bc	0.26c
$LSD_{0.05}$	0.013	0.013	0.0047	0.077	0.0038	0.0042	0.0023	0.086
				Aged (hours)				
0	0.25c	0.36a	0.017b	0.73d	0.033a	0.082c	0.010a	0.45b
48	0.30b	0.37a	0.018b	0.81c	0.027b	0.094a	0.010a	0.22d
96	0.31b	0.32b	0.023a	0.95b	0.027b	0.089b	0.006b	0.33c
144	0.34a	0.31b	0.018b	1.16a	0.021c	0.069d	0.009a	0.67a
$LSD_{0.05}$	0.011	0.011	0.0041	0.067	0.0033	0.0036	0.0020	0.074

Table 3- Correlation coefficient in traits effect priming and aged seed

Çizelge 3- Önuygulama ve tohum yaşlandırmasına bağlı olarak incelenen özellikler arası korelasyon katsayıları

	GP	*MTG*	*SRUR*	*SRUE*	*FUSR*	*Length Seedling*	*Weight Seedling*	*CAT*	*K^+ Seedling*	*K^+ Cotyledon*	*Ca^{2+} Seedling*	*Ca^{2+} Cotyledon*	*Na^+ Seedling*	*Na^+ Cotyledon*	*Na^+/K^+ Seedling*	*Na^+/K^+ Cotyledon*
GP	-															
MTG	-.469**	-														
SRUR	-0.197	.490**	-													
SRUE	0.003	-.242*	-.515**	-												
FUSR	0.021	-.315**	-.803**	.803**	-											
Length Seedling	.433**	-.415**	-0.215	0.099	0.177	-										
Weight Seedling	.397**	-.352**	-.257*	0.134	0.23	.441**	-									
CAT	.364**	-.396**	-0.17	-0.022	0.012	0.15	.295*	-								
K^+ Seedling	0.231	-.407**	-0.205	0.043	0.025	0.09	0.102	0.245*	-							
K^+ Cotyledon	0.011	-0.079	-0.033	0.028	0.026	0.001	-0.129	0.093	0.067	-						
Ca^{2+} Seedling	0.077	-0.154	-0.01	0.05	-0.032	-0.031	-0.006	0.069	.431**	.264*	-					
Ca^{2+} Cotyledon	0.161	-.394**	-.378**	.311**	.315**	0.066	0.049	0.002	.246*	.406**	0.184	-				
Na^+ Seedling	-0.138	-0.026	0.108	-0.035	-0.218	-0.194	0.109	-0.064	.283*	-.241*	.376**	0.034	-			
Na^+ Cotyledon	.312**	-0.15	-0.033	-0.066	-0.13	0.231	0.22	0.119	0.128	-0.114	0.222	0.116	0.228	-		
Na^+/K^+ Seedling	-.282*	0.224	0.199	-0.098	-.235*	-.237*	0.011	-0.174	-.282*	-.278*	0.155	-0.12	.816**	0.165	-	
Na^+/K^+ Cotyledon	-0.073	-0.04	-0.044	-0.044	-0.072	0.034	0.107	-0.041	000	-.469**	0.216	-0.106	.566**	.381**	.628**	-

**, correlation is significant at the 0.01 level; *, correlation is significant at the 0.05 level; GP, germination percentage; MTG, mean time germination; CAT, catalase; SRUR, seed reservoirs using rate; SRUE, seed reservoirs using efficiency; FUSR, fraction of utilized seed reservoirs

Lin & Sung (2001) and Hsu et al (2003) reported that seed priming has positive effects on germination of bitter gourd at low temperature and aging conditions (40 °C and 100% relative humidity for 6 days). Seed priming leads to increase germination percentage and decrease dilating of seed germination, whereas seed aging reduced germination percent and seed vigour (Table1).

Seed priming caused to improve germination by reduction activity of lipid peroxidation and enhancement of antioxidants activity. Existence of significantly correlation between catalase activity and germination percentage and mean germination time confirms the impact of antioxidants in improving these traits (Table 3). Seedling growth also was affected by seed priming and aging. Seed priming caused increase and aging caused reduction in seedling growth. Aging by enhancing glucose and seedling respiration affects on proteins and DNA synthase in seedling and reduce mobility of reserves and seedling growth (McDonald 1999; Murthy et al 2003). Seed priming causes to improve in metabolic process that are involved in primary stages of germination and more powerful confirmation with higher uniformity (Basker & Hatton 1987). During seed priming, biochemical and physiological changes are happened and these processes followed by synthesis of macromolecules and enhance seed germination (McDonald 1998), translocation reservoirs, activation and reconstruction of some enzymes, DNA and RNA synthase, ATP production and repairing of damaged membranes (Bray 1995). Reduction of seedling length and dry weight could be due to lower transition of seed reserves such as soluble sugars and protein molecules with low molecular weight from cotyledon to embryonic axis which limiting cell growth and division (Bewley & Black 1994).

Efficiency reservoirs affected by seed priming and aging. Soltani et al (2008) also reported that SRUE would decrease during ageing condition. Reduction of SRUR and FUSR by ageing could be due to decline in gibberellic acid and other hydrolytic enzymes such as α- amylase and β- amylase synthase in the germination process (McDonald 1999). Aging is followed by increasing H_2O_2 and other free radicals activity known as oxidative stress. Accumulation of H^+ in mitochondria inactivates photosynthetic processes and imbalanced conditions between ROS and antioxidants defensive system in plants. These might led to reduce the integrity of proteins and increased sensitivity of protein to protease enzymes and caused to damage cell activity (Kibinza et al 2011). Reduction in replication of genes responsible for catalase activity during ageing might be due to increase in RNA oxidase activity. Priming improves and restores gene expression of catalase and eliminates the impacts of aging on enzyme activity (Kibinza et al 2006). Catalase protects cells from ROS (Shimizu & Kobayashi 1984; Romero-Puertas et al 2002). Catalase accumulates in the cytosol simultaneously with hydrogen peroxide localization during seed priming (Bray et al 1995).

Oxidative stress with production free radicals damages membrane integrity and so membrane stability (Bhattacharjee & Mukherjee 2002). Reduction in content of elements shows the cell membrane injuries during seed aging. Isamah (2004) showed that sodium, potassium and calcium content are reduced during seed aging. The loss of cell membrane stability caused severe damage in exchange of elements between organelles and cell cytoplasm leading to derangement in cell turgor pressure and function. ROS are highly toxic and caused to damage in cell membrane, reduced chlorophyll content and etc. Managing water imbibition to the seeds during priming reduces cell membranes damage. Also osmopriming with different materials can increase the amount of some elements in cells. According to the results of this research, priming with potassium nitrate, potassium increased in the cells (Table 2). Potassium plays a protective role for cells to decrease ROS damages. Potassium increases enzyme activity (antioxidant enzymes) and neutralize negative effects of free radicals by antioxidant enzyme (Cakmak 2002; Hu & Schmidhalter 2005). Correlation positive significant between catalase activity and the amount of potassium in seedling confirming the opposite (Table 3). Results showed that aging decreases potassium content of seedling due to increase activity of free radicals. Priming with KNO_3 also provides

more potassium and help to cell membrane repairing, so hydropriming was less effective than the KNO_3.

5. Conclusions

Seed aging resulted in reduction of germination parentage and SRUE, while increased mean time of germination and as a result of this matter, seedling growth and dry weight were declined. Seed priming by reducing of negative effects of seed ageing, improved germination indices. During ageing, nutrients leakage had been altered and this is due to losing of cell membrane integrity. Seed priming by helping cell membrane to maintain its integrity will play an important role in keeping cell healthy. Priming by 3% KNO_3 was best treatment for seed milk thistle.

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Abbreviation and Symbols	
ROS	reactive oxygen species
MDA	malondialdehyde
SRUR	seed reservoirs using rate
ISDW	residual seed dry weight
RSDW	remaining seed dry weight
SRUE	seed reservoirs using efficiency
SLDW	seedling dry weight
FUSR	fraction of utilized seed reservoirs

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