

The Effect of Seed Priming and Accelerated Aging on Germination and Physiochemical Changes in Milk Thistle (*Silybum marianum*)

Ghasem PARMOON^{1*}, Ali EBADI¹, Soodabeh JAHANBAKHS¹, Mahdi DAVARI²

¹University of Mohaghegh Ardabili, Faculty of Agriculture, Department of Agronomy, Daneshgab Street, Ardabil, Iran; gbasem.parmoon@gmail.com (*corresponding author), ebadi@uma.ac.ir, soodabehjahanbakhs@yahoo.com

²University of Mohaghegh Ardabili, Faculty of Agriculture, Department of Plant Protection, Daneshgab Street, Ardabil, Iran; mdavari@uma.ac.ir

Abstract

Effects of seed priming and aging on some physiological characteristics of Milk thistle was studied in a factorial experiment based on Complete Randomized Design (CRD). Treatments were included hydro priming (using distilled water), halo priming (0, 1.5, 3, 4.5 and 6% KNO₃) and accelerated aging (0, 2, 4 and 6 days under 45°C and 95% humidity) in three replications. Determined parameters were germination characteristics including germination percentage, daily germination speed, mean time of germination, seed vigor index, hypocotile length and hypocotile dry weight. Activity of catalase, peroxidase and polyphenol oxidase were determined at 12 hours after imbibition and seedling stage. According to results of this experiment, germination percentage, seed vigor and seedling growth of seeds were increased under all priming treatments. Improving the catalase and peroxidase activity led to decrease the aging damages. Germination characteristics were improved under both priming treatments at the beginning of germination as well as seedling growth. Polyphenol oxidase activity was increased in the pre-treated seeds but decreased in seedling growth stage. Aging treatments led to reduce the germination percentage, daily germination speed, seed vigor and seedling growth while the germination time was increased. Accelerated aging caused to reduce the germination rate and seedling growth of milk thistle that is probably due to increasing the lipid peroxidation, free radical increment and decreasing the antioxidants activity. The greatest and lowest antioxidants activity, the germination percentage, germination speed and seed vigor were respectively observed under priming using 3% KNO₃ concentration and control seeds.

Keywords: accelerated aging, antioxidant activity, halo priming, milk thistle seeds

Introduction

Plant products, as parts of foods or botanical potions have been used with varying success to cure and prevent diseases throughout some years (Raskin *et al.*, 2002). Traditional herbal medicines have long been a key component of healthcare systems in the developing world (Ma *et al.*, 2005). Most medicinal plants are collected from the wild. Cultivation of these plants is needed to ensure their availability to industry, as well as those people associated with traditional systems of medicine. By bringing herbs into cultivation, traditional and biotechnological plant-breeding techniques can be applied at the genetic level to improve yield and uniformity, and to modify potency. Conventional plant-breeding methods can improve both agronomic and medicinal traits (Canter *et al.*, 2005). Milk thistle (*S. Marianum* L. Gaertn.) is a troublesome weed as well as cultivated as a medicinal plant (Khan *et al.*, 2009).

Milk thistle is native to the Mediterranean basin and is now widespread throughout the world. It is found on roadsides and waste ground, and in cereals crops. Seeds of milk thistle have been used for more than 2000 years to treat liver diseases. The hepatoprotective action of si-

lymarin is mainly through its anti-free radical and anti-carcinogenic roles, antioxidant, anti-lipid peroxidative, antifibrotic, antiinflammatory, immunomodulatory and liver regenerating activity and is subject to enterohepatic circulation (Kren and Walterova, 2005; Shaker *et al.*, 2010). The valuable medicinal compounds of are found in the seeds of plant. The seeds contain the highest amount of silymarin (Engelberth *et al.*, 2008; Sersen *et al.*, 2006) and 25% (w/w) oil (Wallace *et al.*, 2005), but the whole plant is used medicinally. Also, Seeds of milk thistle contain small amounts of flavonoids (taxifolin) and approximately 20-35% fatty acids and other polyphenolic compounds (Ramasamy and Agarwal, 2008). Silymarin is a strong antioxidant that has been proven to promote liver cell regeneration, reduce blood cholesterol and help to prevent cancer (Vaknin *et al.*, 2008). Silymarin is a lipophilic which extracted from the seeds of milk thistle and is composed of three isomer flavonolignans, silybin, silydianin and silychristin. Each flower head produces about 190 seeds, with an average of 6350 seeds per plant (Dodd, 1989). In the soil, seed can remain viable for up to nine years (Sindel, 1991). The seeds show little or no dormancy. Storage of seeds for a long period cause their deterioration

and reduce their survival. Accelerated aging of seeds is different between various plant species and inside one seed clone but it induced by several days of exposure to high temperature and high humidity (Priestley, 1986). Accelerated aging also resulted in increased lipid peroxidation, decreased levels of anti-oxidants and reduced activity of several enzymes involved in scavenging of free radicals and peroxides (Bailly *et al.*, 1998; Chiu *et al.*, 1995; Hsu and Sung, 1997). Thus, reduced anti-oxidative activities, along with other mechanisms, may contribute to the increased susceptibility to deterioration of primed seeds. During aging, free radicals cause damage to cells and cell death finally. Some studies have shown the role of free radicals in aging plants and animals as well as seeds of various plants (Bailly *et al.*, 1996; Kranter *et al.*, 2006). If ROS and hydrogen peroxidase rise a lot more than critical level, can be caused severe damage to the cell membranes due to increased production of OH (Bienert *et al.*, 2006). Oxidative stress is an interaction of the imbalance between ROS production and defense antioxidant against ROS. This result in an oxidative stress is because of Enhancement of combined big molecules with oxygen. In order to inhibition of oxidative damage to tissues, the cells equipped with various enzymes and non-enzymatically for detoxify. The protectant enzymes including Superoxide dismutase that remove oxidizing O_2^- ions through decomposition into hydrogen Peroxidase hydrogen and could be broken and change to oxygen and H_2O by catalase. Other anti-oxidants comprise peroxidase, glutathione and ascorbate (Kibinza *et al.*, 2011).

Priming is an important way for enhancing of seed germination. Priming 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 and reduced germination rate. The priming reduces the effects of aging by reducing Malondialdehyde (MDA) and free radicals production and maintenance antioxidant activities. The radical and seedling growth increase during priming (Basra *et al.*, 2003). The environmental factors effect on seed maturation and agronomic quality of Milk thistle as well as some treatment impact on the enhancement ability of the seeds. The aim of recent study was to evaluate the Milk thistle seed tolerance to wearing and introduce the ways to reduce the effects of aging on.

Materials and methods

Seed materials

In order to perform accelerated aging treatments, seeds were incubated inside the sealed boxes with 90% to 95% relative humidity and afterwards placed at 45°C for 2, 4 and 6 days. 150 seeds were placed inside two filters paper (for hydration inhibition) and prepared osmolites for seed priming were added to them, subsequently incubated at

25°C in darkness for 12 hours. Treatments included hydro priming and halo priming with KNO_3 at concentrations of 1.5%, 3%, 4.5% and 6%.

Germination test

To test the germination of Milk thistle seeds, 25 seeds disinfected with 1% sodium hypochlorite for 5 minutes then placed in petri dishes with two layers of filter paper. Germination rate evaluated each day for 12 days, and seedling size (Hypocotyl) measured. The seedling dry weight (Hypocotyl) was obtained after putting in the oven 75°C for 48 h

Mean time to germination (MTG)

Average seed germination rate was calculated as one index of the germination rate according to Ellis and Roberts (1981), Wherease n shows the number of germinated seeds in d days and ($\Sigma(nd)$) as total germinated seeds:

$$MTG = (\Sigma(nd)) / (\Sigma n)$$

Daily germination Speed (DGS)

Daily germination rate was estimated by of using the Maguire (1962), Daily average germination was calculated according to Hunter *et al.* (1984) in that FGP is the germination percentage, and D show the days up to achieve maximum germination rate:

$$MDG = FGP / D$$

$$DGS = 1 / MDG$$

Seedling vigor index

First and second seed vigor index was calculated using the following equation (Abdul-Baki and Anderson, 1973):

$$SV_1 = \text{seedling dry weight (g)} \times \text{germination (\%)} / 100$$

$$SV_2 = \text{seedling dry length (cm)} \times \text{germination (\%)} / 100$$

Determination of enzyme activities

The enzyme activity was measured twice, 12 hours after the seed imbibition and in the Hypocotyl stage. To measure the enzyme activity of the seeds, 25 seeds were grounded and after that 0.2 g of the obtained powder were used for measuring and for seedlings, 0.2 g of fresh tissue was used. In order to extract protein, 0.2 g of fresh tissue plant was pulverized in a mortar 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 at 13,000 rpm, at 4°C and the supernatant was used for enzyme activity measurements (Sudhakar *et al.*, 2001).

Catalase activity was assayed according to Kar and Mishra (1976). The 60 μ l protein extract was added to Tris buffer (50 mM, pH=7) H_2O_2 5 mM in the ice bath, then the absorbance curve was considered at a wavelength of 240 nm. Enzyme activity was obtained for OD/ mg protein fresh tissue. Peroxidase activity measured as described by Kar and Mishra (1976): 50 μ l protein extract was added to 2.5 ml extraction buffer containing 100 μ M Tris buf-

fer 100 mM and hydrogen peroxide five mM and 10 mM Pirogalol in the ice bath and absorbance changes was read at a wavelength of 425 nm graph. Furthermore, Polyphenol oxidase enzyme activity was measured by Kar and Mishra (1976) method. 100 µl protein extract was solved in 1.5 ml Tris 0.2 M and 0.3 ml Pirogalol 0.02 M and the resulting composition was placed in the bain marie bath at 25°C for five minimums and then the absorbance rate at 420 nm was recorded. The evaluation of protein carried out by Bradford (1976) method. 0.2 g of plant tissue was squashed with 0.6 ml extraction buffer and centrifuged in 11,500 rpm for 20 min at 4°C. The supernatant transferred to in the new tubes and centrifuged for 20 minutes at 4000 rpm and the supernatant was obtained. To measure protein amount, 10 µl obtained to extract was added to 5µl Bradford solution and 290 µl extraction buffer and the absorbance rate was read at 595 nm.

Statistics

An experimental design was arranged in factorial based on a completely randomized design (CRD) with three replications. Means were compared using the LSD method with P value= 0.05. Most of the measured traits were significant at *p* = 0.05.

Results and discussion

Crop seeds live on the environment for several days or years after harvest to start a new growth stage (planting seeds) which causes influence the agronomical quality of seeds. Seed vigor is the main characteristic of crop seed quality. Among the factors affecting seed vigor the maturity stage at harvesting and the seed aging are important (McDonald, 1999), which is influenced by environmental conditions that they placed in. Untreated seeds (control) showed 54% germination percentage which might be due to seeds dormancy. Priming enhances germination of Milk thistle seeds up to 81%. Maximum enhancement belonged to KNO₃ 3% concentrations (from 54% to 81%) while the

minimum exhibited at concentrations of 1.5% from 54% to 60%. (Fig. 1., A). The aging reduced the germination from 54% to 60% (Fig. 1., B).

The seed germination rate was equal to 0.53 days in the controls and it was reduced using priming treatments. KNO₃ 3% and 1.5% concentrations exhibited the highest germination rate equal to 0.34 days (Tab. 1). Aging of seeds increased the daily germination rate (from 0.53 to 0.63). Among all treatments, KNO₃ 3% improved germination during the aging so in the highest aging, daily germination rate reduced from 0.69 to 0.57 days whereas at KNO₃ 4.5% (Tab. 1) it was very lowe (decreased from 0.69 to 0.67 days). Priming increased hypocotyl length and dry weight. Hypocotyl length and dry weight increased during priming with KNO₃ 141% and 90%, respectively. The concentration of KNO₃ at 6% exhibited minimal impact on the Hypocotyl length and dry weight (Tab. 1).

Hypocotyl length reduced during aging (decreased from 2.06 to 1.76 and 1.75 cm at various levels of aging) as well as hypocotyl dry weight (reduced from 1.73 to 1.53 mg). At high levels of aging, KNO₃ 3% exhibited the most positive effect (length increased from 1.75 to 2.96 cm) on the hypocotyl length among other treatments and KNO₃ at 1.5% concentration increased it from 1.53 to 1.80 mg and exhibited the highest influence on the dry weight of hypocotyl (Tab. 1).

Seed vigor is another which affecting seed germination and seedling growth, increase during priming. Among the treatments, the most effective on the first (increased from 0.09 to 0.27) and second (increased from 99.9 to 400.1) vigor belonged to 3% KNO₃ and the lowest was 6% KNO₃. Seed vigor is the first traits would be affected during aging. Both vigor indexes were changed from 0.09 to 0.05 and 99.9 to 71.1 in first and second vigor index, respectively. Priming reduced the aging effect. Among the treatments, KNO₃ 3% exhibited the highest effect on the aging (increase of first vigor from 0.05 to 0.08 and the scnd vigor index from 71.1 to 138.2 at 6 days aging) (Tab. 1). Mean time of seed germination reduced by seed

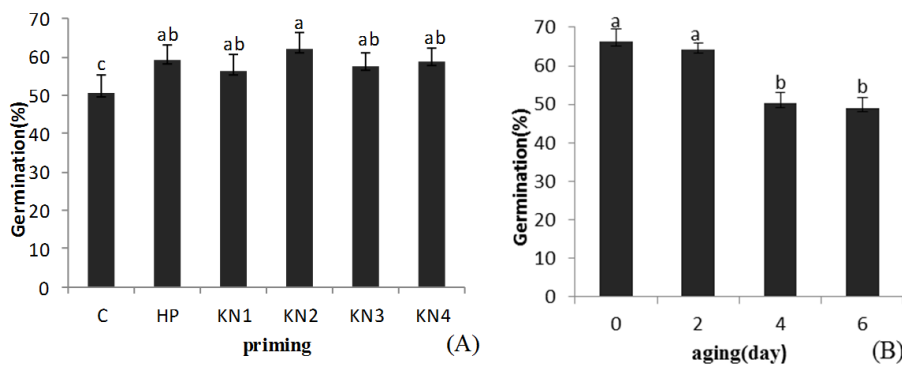


Fig. 1. Effect of seed priming and accelerated aging treatment on germination of milk thistle seeds. A (C: control, HP: Hydro Priming, KN1: Priming with KNO₃ 1.5%, KN2: Priming with KNO₃ 3%, KN₃: Priming with KNO₃ 4.5%, KN4: Priming with KNO₃ 6%) and B (non aging, 2 day aging, 4 day aging and 6 day aging)

Tab. 1. Amounts of DGS, Hypocotyl length, Hypocotyl dry weight, SVI₁ and SVI₂ subjected to different treatments

Seed treatment	Aging (day)	DGS	HYL (cm)	HYDW (mg)	SVI ₁	SVI ₂
Control	0	0.53±0.07	2.06±0.24	1.73±0.07	0.09±0.010	99.9±15.4
	2	0.43±0.01	2.16±0.44	2.13±0.18	0.13±0.010	138.2±28.5
	4	0.63±0.07	1.76±0.26	1.53±0.07	0.06±0.008	76.2±02.7
	6	0.69±0.03	1.75±0.05	1.53±0.24	0.05±0.005	71.1±14.1
Hydro priming	0	0.36±0.01	3.04±0.20	2.43±0.10	0.18±0.007	235.1±12.2
	2	0.45±0.02	3.72±0.27	3.07±0.07	0.18±0.004	227.3±10.8
	4	0.53±0.05	2.10±0.16	2.00±0.12	0.10±0.010	114.6±12.5
	6	0.62±0.04	2.51±0.14	1.67±0.74	0.08±0.030	113.2±17.6
KNO ₃ (1.5%)	0	0.54±0.10	3.62±0.52	2.87±0.13	0.16±0.040	215.5±79.2
	2	0.42±0.02	4.03±0.46	2.47±0.18	0.16±0.010	270.6±39.3
	4	0.47±0.03	3.09±0.89	2.00±0.12	0.10±0.010	178.5±38.4
	6	0.56±0.04	2.82±0.08	1.80±0.12	0.08±0.010	121.1±25.7
KNO ₃ (3%)	0	0.34±0.01	4.97±0.75	3.33±0.13	0.27±0.007	400.1±46.3
	2	0.48±0.03	2.64±0.07	1.87±0.07	0.10±0.008	154.7±9.35
	4	0.50±0.07	2.22±0.40	1.80±0.12	0.09±0.010	151.9±28.5
	6	0.57±0.07	2.96±0.16	1.40±0.12	0.08±0.010	138.2±26.0
KNO ₃ (4.5%)	0	0.44±0.02	4.10±0.67	2.40±0.23	0.15±0.010	261.6±42.6
	2	0.45±0.04	2.87±0.50	1.73±0.07	0.10±0.010	186.6±53.6
	4	0.47±0.05	2.32±0.35	1.67±0.07	0.10±0.010	143.8±18.3
	6	0.67±0.07	2.34±0.13	1.47±0.07	0.06±0.005	96.9±9.40
KNO ₃ (6%)	0	0.47±0.06	2.44±0.23	1.60±0.12	0.09±0.010	153.3±34.6
	2	0.38±0.01	3.05±0.45	1.73±0.18	0.12±0.008	217.8±26.9
	4	0.54±0.05	2.65±0.41	1.53±0.07	0.07±0.010	143.2±29.8
	6	0.59±0.08	2.90±0.42	1.37±0.03	0.07±0.004	132.8±38.0
LSD _{0.05}		0.14	1.16	0.05	0.04	91.94

DGS; Daily germination Speed, HYL; Hypocotyl length, HYDW; Dry weight Hypocotyl, SVI₁ first Seedling vigor index and SVI₂ secondary Seedling vigor index

priming. Potassium nitrate 1.5% had the maximum effect on the mean time of germination (it decreased 37%) and KNO₃ 6% showed the minimum effect (down from 3.29 to 2.39 days). During aging, the mean time of germination was increased (increasing from 2.09 to 2.73) (Tab. 2).

Lin and Sung (2001) and Hsu *et al.* (2003) showed effectiveness of seed priming on the germination in bitter gourd at low temperature and aging conditions. Our results showed that the enhancement of seed germination, and improve establishment candidate factors (including mean germination time, germination rate and seed vigor) are due to the priming. Lipid peroxidation reduction and the antioxidant activity enhancement are the factors that priming affects on germination. Priming can increase the seed survival and improve the stored seeds in the unfavorable conditions. Hsu *et al.* (2003) reported that priming and pre-soaking of bitter gourd seeds enhance the seed germination percentage and reduce its mean time. Also, the aging reduces seed germination percentage and seed vigor and increase the mean time of germination. The priming and soaking in warm water increased Catalase, Ascorbate peroxidase and superoxide dismutase activity and soluble protein content and reduce malondialdehyde levels and total peroxidase and their enhancement over the aging. They believed that priming and soaking the seeds in warm water for a few hours is suitable for long surviving period.

In our study we have found that the rate of the antioxidant activity (Catalase and Peroxidase) reduce both before germination and during hypocotyl growth stage resulting the reduction of seed vigor, whereas the priming with KNO₃ 3% created their highest activity and reduced the effects of aging. Increased activity of this enzyme in the germination initial increases seed germination percentage and speed in which the seedling growth rate improved, too. This finding can be help seedlings to better posture in the field and increased yield. Seed viability declines during aging according to Sung and Jeng (1994) results. Kibinza *et al.* (2011) indicated the reduction of germination percentage during aging while priming with amino acid improves the aged seed. They believe that the decline during aging, resulting in decreased Catalase activities and its improvement during the priming causes seed to be recovered (Pyfrom *et al.*, 1957; Racusen, 1958).

Catalase enzyme activity at germination stage and during the seedling growth was increased by seed priming. Potassium nitrate 3% and 6% showed the highest and lowest activity compared to controls (Tab. 2). Over hypocotyl growth, KNO₃ 1.5% exhibited the greatest (an increase from 1.25 to 2.81 OD/mg⁻¹ protein) whereas KNO₃ 4.5% showed the lowest enzyme activity (reduction from 1.25 to 1.07 OD/mg⁻¹ protein) (Tab. 3). Aging reduced the catalase activity from 8.13 to 5.49 OD/mg⁻¹ protein be-

fore germination and from 1.25 to 0.79 OD/mg⁻¹ protein during the hypocotyl growth (Tab. 2, 3). Results showed that KNO₃ 3% was the compensation better than other treatments (increased from 5.14 to 9.26 OD/mg⁻¹ protein before germination and from 0.79 to 1.71 and 1.29 OD/mg⁻¹ protein in the Hypocotyl stage in 4 and 6 days aging respectively) (Tab. 2, 3).

Peroxidase activity was increased in the primed seeds before germination and during hypocotyl growth. Before germination, peroxidase activity had the maximum activity at KNO₃ 3% (it was increased from 5.94 to 11.45 OD/mg⁻¹ protein) and the lowest activity (7.61 OD/mg⁻¹ protein) obtained by KNO₃ 6%. (Tab. 2). Milk thistle Seed aging led to reduction in peroxidase activity at hypocotyl growth stage, and this effect increases in high levels of aging (reduction of enzyme activity from 3.72 to 1.80 OD/mg⁻¹ protein). Priming treatments reduced aging effect. Among treatments, KNO₃ 3% and hydro-priming exhibited the maximum (18.6 OD/mg⁻¹ protein) and minimum enzyme activity (4.11 OD/mg⁻¹ protein) respectively (Tab. 3).

Catalase and Peroxidase activity was reduced during aging. Reduction of the replication in the genes related to their reduce RNA oxidase activity during aging due to decreases of H₂O₂ leading to increase their activity. Because of the increase in H₂O₂ and free radicals in the stressed cell cytoplasm during oxidative stress, the proton production in the mitochondria increase and leading to inactivation of photosynthetic as well as the non-equilibrium between ROS and antioxidants and also in the reduction of the protein cohesion protein may cause increased protein sensitivity to the protease enzymes, leading to a reduction of seed viability. The priming improves and repairs the gene expression and increases their activity during germination (Berlett and Stadtman, 1997; Kibinza et al., 2006). The antioxidants, especially catalase prevent oxidative ROS activity and then augmentation of enzyme activity increase

prolytic discounting because of cadmium (Shimizu and Kobayashi, 1984; Romero-Pubertas et al., 2002). The aging changes the place of some amino acids and iron-sulfur in structures of some antioxidants resulting ROS activity enhancement. Catalase accumulates in the cytosol during priming simultaneously with hydrogen peroxide localization. Seed protein and nucleic acid synthesis increases and allows different metabolic processes of DNA replication or the synthesis of ethylene (Bray et al., 1995; Fu et al., 1988).

Polyphenol oxidase activity was also influenced by seed priming and aging, so that the activity was increased by seed priming before germination and was reduced over hypocotyl growth. On the other hand, seed aging enhanced polyphenol oxidase activity both before germination and seedling growth. The highest enzyme activity (6.25 OD/mg⁻¹ protein) was observed at 3% KNO₃ and the lowest (4 OD/mg⁻¹ protein) in the control seeds. Also, the enzyme activity increased over aging from 3.80 to 6.24 OD/mg⁻¹ protein (Tab. 2). Enzyme activity at hypocotyl growth stage had the highest rate in the control treatment (2.54 OD/mg⁻¹ protein) and the lowest rate in 3% KNO₃ (1.18 OD/mg⁻¹ protein). Enzyme activity rate increased over the aging so that its activity in control seeds was 2.54 OD/mg⁻¹ protein while enhanced to 3.60 OD/mg⁻¹ protein during the aging (Tab. 3).

Priming could not improve the germination at high levels of aging because irreversible damage repair may not be (Butler et al., 2009). During the programmed cell death (PCD), Catalase rate and sensitivity reduce and indicate that the aging causes PCD and subsequent the reduction of seed's viability. Hydro-priming and priming with ascorbic acid improved germination compared to control. Catalase and peroxidase enzyme activity decreased with increasing of aging severity and Hydropriming with ascorbic acid maintained a high activity of this enzyme

Tab. 2. Amounts of MTG, CAT, POX and PPO of milk thistle seeds subjected to various treatments after 12 hours imbibition

Seed treatment	MTG	CAT (OD/mg ⁻¹ protein)	POX (OD/mg ⁻¹ protein)	PPO (OD/mg-1 protein)
Priming				
Control	3.29±0.15	5.14±0.50	05.94±0.95	04.00±0.75
Hydro priming	2.16±0.14	7.67±0.43	09.07±1.04	04.76±0.63
KNO ₃ (1.5%)	2.01±0.12	8.50±0.92	10.74±1.60	04.97±0.63
KNO ₃ (3%)	2.26±0.11	9.26±1.55	11.45±1.77	06.25±0.71
KNO ₃ (4.5%)	2.08±0.10	7.35±0.64	09.09±1.12	04.99±0.52
KNO ₃ (6%)	2.39±0.16	6.87±0.53	07.61±0.87	05.40±0.45
LSD _{0.05}	0.29	2.52	2.54	1.47
Aging (day)				
0	2.09±0.13	8.13±0.47	10.30±1.44	03.65±0.52
2	2.12±0.12	8.88±0.43	11.63±1.12	04.93±0.50
4	2.51±0.13	7.36±0.96	08.12±0.44	05.43±0.48
6	2.73±0.16	5.49±0.76	05.88±0.56	06.23±0.42
LSD _{0.05}	0.23	2.06	2.89	1.52

MTG; Mean time to germination, CAT; Catalase, POX; Peroxidase and PPO; poly phenol oxidase

Tab. 3. Amounts of CAT, POA and PPO of milk thistle Hypocotyl subjected to various treatments

Priming	Aging (day)	CAT (OD/mg ⁻¹ protein)	POX (OD/mg ⁻¹ protein)	PPO (OD/mg ⁻¹ protein)
Control	0	1.25±0.04	3.72±0.17	2.54±0.22
	2	1.20±0.09	3.40±0.13	2.94±0.22
	4	1.16±0.01	2.67±0.22	3.28±0.29
	6	0.79±0.01	1.80±0.02	3.60±0.01
Hydro priming	0	1.46±0.21	4.11±0.19	2.18±0.21
	2	1.18±0.21	3.52±0.05	2.31±0.36
	4	1.08±0.05	3.10±0.05	2.56±0.29
	6	0.86±0.07	1.98±0.04	2.92±0.22
KNO ₃ (1.5%)	0	2.81±0.18	5.86±0.61	2.44±0.11
	2	1.74±0.01	4.22±0.75	2.63±0.13
	4	1.71±0.11	3.65±0.05	3.01±0.23
	6	1.29±0.25	3.30±0.30	3.32±0.45
KNO ₃ (3%)	0	2.39±0.08	6.18±0.35	1.18±0.01
	2	1.63±0.18	4.36±0.23	1.24±0.04
	4	1.36±0.04	3.91±0.27	1.40±0.03
	6	1.08±0.01	3.72±0.12	1.64±0.04
KNO ₃ (4.5%)	0	1.07±0.03	4.66±0.49	1.28±0.02
	2	1.79±0.04	4.09±0.15	1.34±0.02
	4	1.31±0.14	3.32±0.19	1.51±0.14
	6	0.89±0.04	3.07±0.02	2.06±0.01
KNO ₃ (6%)	0	1.99±0.12	4.36±0.48	1.62±0.02
	2	1.56±0.08	3.87±0.16	1.90±0.06
	4	1.12±0.06	3.13±0.06	2.04±0.07
	6	1.09±0.06	2.73±0.07	2.21±0.04
LSD _{0.05}		0.32	0.81	0.42

compared to control (Goel *et al.*, 2003). Priming and aging are effective on the seed germination rate and pursuant to the seed's vigor. High seed vigor increase the potential of further growth of seedling, and it cause additional dry matter accumulation as well as dry matter. Our results also showed the enhancement of up to germination speed, seed vigor and increase's seedling longitudinal growth and dry weight over priming and reduce them during aging. Seed germination rate and vigor are important indicators of seed quality and influenced seed viability. Rao *et al.* (2006) showed that accelerated aging to reduces the onion seed's vigor. They believe that the decline of seed vigor affected by reducing in antioxidant enzyme activity and increased levels of ROS and malondialdehyde rate during germination and seedling growth. The magnitude of ROS affected by the amount of unsaturated fatty acids and less influenced in the seeds with higher levels of saturated fatty. (Kalpana and Madhava Rao, 1994). Since 50% to 60% milk thistle seed oil are formed from unsaturated fatty acid oleic acid, so it is likely that the seeds produce freer radicals lead to their aging. Perhaps Silymarin found in the milk thistle seed acts as a protective role due to its antioxidant activity against lipid peroxidation and reduces seeds aging (Ghosh *et al.* 2010). The polyphenol oxidase enzyme was significant compared with other enzymes; however, there was no significant relationship to the ag-

ing or priming. Polyphenol oxidase is found in higher plants and its main task catalysis of Quinones of phenol in the presence of oxygen molecules. The enzyme activity also depends upon the plant genotype and growth conditions. Polyphenol oxidase role for the plant, including its effect on Adventitious root forming, root growth organization (Yilmaz *et al.*, 2003) and its role in cell division and primary differentiation and development (Huystee and Cairns, 1982). KNO₃ 3% was the best treatment among of priming methods used in the present study about the most measured characters represents an appropriate level of milk thistle seed priming. It seems that adjustment in elements exchanged of the cell membrane of the seeds in milk thistle is the most important factor that could influence the seed aging improvement.

Conclusions

Potassium nitrate has a positive effect on Antioxidant enzymes activity and is probably highly effective to remove damages of aging.

Acknowledgment

We would like to thank for Dr. Zahri, Mr. Khabiri also Ms. Ali Rezai for his support and encouragement.

References

- Abdul-Baki AA, Anderson JD (1973). Vigor determination in soybean by multiple criteria. *Crop Science* 13:630-633.
- Able AJ, Guest DI, Sutherland MW (2000). Hydrogen peroxide yields during the incompatible interaction of tobacco suspension cells inoculated with *Phytophthora nicotianae*. *Plant Physiol* 124:899-910.
- Bailly C, Benamar A, Corbineau F, Côme D (1996). Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging. *Physiol Plant* 97:104-110.
- Basra SMA, Ahmad N, Khan MM, Iqbal N, Cheema MA (2003). Assessment of cotton seed deterioration during accelerate. *Seed Sci Technol* 31:531-540.
- Berlett BS, Stadtman ER (1997). Protein oxidation in aging, disease, and oxidative stress. *J Biol Chem* 272:20313-20316.
- Bienert GP, Schjoerringa JK, Jahn TP (2006). Membrane transport of hydrogen peroxide. *Biomembranes* 1758 994-1003.
- Bradford MM (1976). A rapid and sensitive for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemis* 72:248-254.
- Bray CM (1995). Biochemical processes during the osmopriming of seeds, 767-789 p. In: Kigel Y, Galili G (Eds.). *Seed Development and Germination*, Marcel Dekker, New York.
- Butler LH, Hay FR, Ellis RH, Smith RD, Murray TB (2009). Priming and re-drying improve the survival of mature seeds of *Digitalis purpurea* during storage. *Ann Bot* 103:1261-1270.
- Canter PH, Thomas H, Ernst E (2005). Bringing medicinal plants into cultivation. opportunities and challenges for biotechnology. *Trends Biotechnol* 23:180-185.
- Ellis RH, Roberts EH (1981). The quantification of aging and survival in orthodox seeds. *Seed Science and Technology* 9:377-409.
- Fu JR, Lu SH, Chen RZ, Zhang BZ, Liu ZS, Cai DY (1988). Osmoconditioning of peanut (*Arachis hypogaea* L.) seed with PEG to improve vigor and some biochemical activities. *Seed Sci Tech* 16:197-212.
- Ghosh A, Ghosh T, Jain S (2010). Silymarin - a review on the pharmacodynamics and bioavailability enhancement approaches. *J Pharm Sci Technol* 2:348-355.
- Goel A, Goel AK, Sheoran IS (2003). Changes in oxidative stress enzymes during artificial ageing in cotton (*Gossypium hirsutum* L.) seeds. *J Plant Physiol* 160:1093-1100.
- Hsu CC, Chen CL, Chen JJ, Sung JM (2003). Accelerated aging-enhanced lipid peroxidation in bitter melon seeds and effects of priming and hot water soaking treatments. *Sci Hortic* 98:201-212.
- Hunter EA, Glasbey CA, Naylor REL (1984). The analysis of data from germination tests. *J Agric Sci, Cambridge* 102:207-213.
- Huystee RB, Cairns WL (1982). Progenitor prospects in the use of peroxidase to study cell development. *Phytochemistry* 21:1843-1847.
- Kalpna R, Madhava Rao KV (1994). Absence of the role of lipid peroxidation during accelerated ageing of seeds of pigeon pea (*Cajanus cajan* L.) *Seed Sci Technol* 22:253-260.
- Kar M, Mishra D (1976). Catalase, peroxidase and polyphenol oxidase activity during rice leaf senescence. *Plant Physiol* 57:315-319.
- Khan MZ, Blackshaw RE, Marwat KB (2009). Biology of milk thistle (*Silybum marianum*) and the management options for growers in north-western Pakistan. *Weed Biol Manage* 9:99-105.
- Kibinza S, Bazin J, Bailly Ch, Farrant JM, Corbineau F, Maarouf-Bouteau HE (2011). Catalase is a key enzyme in seed recovery from ageing during priming. *Plant Science* 181:309-315.
- Kibinza S, Vinel D, Côme D, Bailly C, Corbineau F (2006). Sunflower seed deterioration as related to moisture content during ageing, energy metabolism and active oxygen species scavenging. *Physiol Plant* 128:496-506.
- Kranner I, Birtic S, Anderson KM, Prichard HW (2006). Glutathione half-cell reduction potential: a universal stress marker and modulator of programmed cell death. *Free Radic Biol Med* 40:2155-2165.
- Kren V, Walterova D (2005). Silibin and silymarin. New effects and applications. *Biomed. Pap* 149:29-41.
- Lin JM, Sung JM (2001). Pre-sowing treatments for improving emergence of bitter melon seedlings under optimal and sub-optimal temperatures. *Seed Sci Technol* 29:39-50.
- Ma JKC, Chikwamba R, Sparrow P, Fischer R, Mahoney R, Twyman RM (2005). Plant-derived pharmaceuticals the road forward. *Trends Plant Sci* 10: 580-585.
- Maguire JD (1962). Speed of germination, aid in selection and evaluation for seedling emergence and vigor. *Crop Science* 2:176-177.
- McDonald MB (1999). Seed deterioration: physiology, repair and assessment. *Seed Sci Technol* 27:177-237.
- Priestley DA (1986). *Seed Aging. Implications of Seed Storage and Persistence in the Soil*, Cornell University Press, Ithaca, NY.
- Pyfrom HT, Appelman DW, Heim G (1957). Catalase and chlorophyll depression by 3-amino-1,2,4-triazol. *Plant Physiol* 32:674.
- Racusen D (1958). The metabolism and translocation of 3-amino-1,2,4-triazol in plants. *Arch Biochem Biophys* 74:106.
- Ramasamy K, Agarwal R (2008). Multitargeted therapy of cancer by silymarin. *Cancer Lett* 269:352-362.
- Rao RGS, Singh PM (2006). Mathura Rai Storability of onion seeds and effects of packaging and storage conditions on viability and vigor. *Sci Hortic* 110:1-6.
- Raskin I, Ribnicky DM, Komarnytsky S, Ilic N, Poulev A, Borisjuk N, Brinker A, Moreno DA, Ripoll C, Yakoby N,

- O'Neal JMO, Cornwell T, Pastor I, Fridlender B (2002). Plants and human health in the twenty-first century. Trends Plant Sci 20:522-531.
- Romero-Puertas MC, Palma JM, Gómez M, Del Río LA, Sandalio LM (2002). Cadmium causes the oxidative modification of proteins in pea plants. Plant Cell Environ 25:677-686.
- Shaker E, Mahmoud H, Mnaa S (2010). Silymarin, the antioxidant component and Silybum marianum extracts prevent liver damage. Food Chem Toxicol 48:803-806.
- Shimizu N, Kobayashi K (1984). The reaction of superoxide radical with catalase. Mechanism of the inhibition of catalase by superoxide radical. J Biol Chem 259:4414-4418.
- Sindel BM (1991). A review of the ecology and control of thistles in Australia. Weed Res 31:189-201.
- Sudhakar C, Lakshmi A, Giridara KS (2001). changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. Plant Sci 167:613-619.
- Sung JM, Jeng TL (1994). Lipid peroxidation and peroxide-scavenging enzymes associated with accelerated aging of peanut seed. Physiol Plant 91:51-55.
- Yilmaz H, Taskin T, Otludil B (2003). Polyphenol oxidase activity during rooting in cuttings of grape (*Vitis vinifera* L.) varieties. Turk J Bot 27:495-498.