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# Effect of salicylic acid on antioxidant enzyms of accelerated aging seeds of milk thistle (*Silybum marianum*)

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## Abstract:

**Effects of accelerated aging and seed priming treatments were studied on milk thistle seeds during germination process. An experiment was performed in a factorial based on Completely Randomized Design (CRD). Treatments were Salicylic acid concentrations (0, 500, 1000, 1500 and 2000 mg. $L^{-1}$) and accelerated aging for (0, 48, 96 and 144 hours under 45 °C and 95% humidity) with three replications. Our results showed that salicylic acid dramatically enhanced seed germination, seedling vigor and primary seedling growth. Catalase, polyphenol oxidase and peroxidase activity were increased by seed priming. Reduction in germination parameters were observed by increase in accelerated aging durations. The highest antioxidant activity, germination percentage and daily germination rate and seed vigour were observed at primed seeds with 1000 mg. $L^{-1}$ Salycilic acid on non-aged seeds. Salicylic acid treatment with a concentration of 1000 mg. $L^{-1}$had the greatest impact on healing harmful effects aging.**

**Keywords: Accelerated aging, Catalase, Milk thistle, Peroxidase, Germination, Seed Priming.**

## Introduction:

Milk thistle (*Silybum marianum*) is cultivated as a medicinal plant and also known as nexus weed (Khan *et al*., 2009). This plant is native to the mediterranean basin and is now widespread throughout the world. Seeds of milk thistle have been used for more than 2000 years to treat liver diseases, blood cholesterol, and containment of cancer (Shaker *et al*., 2010; Kren and Walterova, 2005). Seeds of milk thistle contain small amounts of flavonoids (taxifolin) and approximately 20-35% fatty acids and other polyphenolic compounds (Ramasamy and Agarwal, 2008). Seeds of Sylimarin have no dormancy or little dormancy, and their viability up to nine years in soil condition (Sindel, 1991).

Long-term storage or adverse environmental factors cause seeds to deteriorate and loss in seed viability, vigor, and overall seed quality due to aging. Seed moisture content and storage temperature are the most critical factors in seed deterioration. Percentage and rate germination in aged seeds are much lower than healthy seeds. Deteriorated seed often produces uneven stands and fewer plants per unit area than fresh seed (Ellis *et al*., 1985).

Kapoor *et al* (2010) reported that seed quality is affected by accelerated ageing treatment using 45 °C and 100% humidity for 24, 48, and 72 hours. Moisture content of aged seeds was increased and all physiological parameters such as germination percentage, seedling root and shoot length, and vigor index, seed protein and sugar content were deceased (Abbas *et al*., 2011). Under ageing conditions, free reactives damage the seed cells and finally lead to cell death. These components are very important in senescence process of animals and plants (Kranner *et al*., 2006; Bailly *et al*., 1996). If the amounts of reactive oxygen species (ROS) and hydrogen peroxide ($H_2O_2$) is higher than critical level, it increases the OH reactive and also severe damages to cell and its membrane (Bienert *et al*., 2006). Oxidative stress is occurred due to imbalance between ROS production and antioxidant defense system (Kibinza *et al*., 2011). Priming (controlled hydration) by reducing germination meantime and improvement of germination rate causes to better seedling establishment under different environmental stress (Nawaz *et al*., 2012). Hydro priming, osmo priming, matrix priming, hormone priming and halo priming are used for seed pre-treatment (Ghassemi-Golezani *et al*., 2010; Hu *et al*., 2006). Priming causes to biochemical, cellular and molecular changes such as DNA and protein synthesis of seed and also increases their enzymes activity to overcome ageing process. Seed priming significantly increases activity of catalase, peroxidase, superoxide dismutase, sugar content and malondialdehyde (MDA) under different environmental stresses (Hu *et al*., 2006).

The aim of this study was to investigate the effect of seed hormonal priming using salicylic acid on germination and seedling growth parameters and activity of antioxidant enzymes of milk thistle seeds to reduce the effects of ageing and seed deterioration.

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## Material and methods:

In order to perform accelerated aging treatments, seeds were incubated inside the sealed boxes with 90% to 95% relative humidity and then placed at 45 °C for 48, 96 and 144 hours. Aged and Non-aged seed were soaked at (500, 1000, 1500 and 2000 mg. $L^{-1}$) salicylic acid solutions for 24 hours. Moisture content of the seeds was 14% and germination 60%. For seed priming 150 seeds were placed in two filters paper (for Hydration inhibition) and prepared solutions for treatments were added then incubated at 25°C in darkness for 24 hours. Then seeds were removed from solutions and surface dried at room temperature and prepared for standard germination test.

In order to performe standard germination test, 25 seeds were sterilized using 1% sodium hypochlorite for 5 minutes, and then seeds were placed in petri dishes with two layers of filter papers. Germinated seed were counted everyday till day $12^{th}$, then root and shoot length was measured. The seedling dry weights (hypocotyl) were measured after drying in the oven at 75 °C for 48 h.

Mean time to germination calculated using the following equation (Ellis and Roberts, 1981):

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

$\sum(nd)$ where n is number of germinated seeds in d days and $\sum(nd)$ is total germinated seeds till the end of experiment.

Daily germination Speed was calculated according to to Hunter *et al.* (1984) in that FGP is the germination percentage, and D shows the days to maximum germination rate:

$$DGS = D / FGP$$

First and second seed vigor indices were calculated using the follow equation (Abdul-Baki and Anderson, 1973):

$SV_1$= seedling dry weight (g) × germination (%)

$SV_2$= seedling length (cm) × germination (%)

Membrane permeability was determined based on the electrolyte leakage. Fifty seeds of each treatment were weighed then putted in 250 ml distilled water under 25 °C for 24 hours. Membrane permeability index was determined using the following equation and Ec meter model Mi170 (ISTA, 2003):

$$EC_T = \frac{EC_2 - EC_1}{m}$$

$EC^-_1$= Electrical conductivity of sample at starting time of experiment

$EC^-_2$= Electrical conductivity of sample after 24 hrs

M: weight of 50 seeds

Enzymes activity was measured at two times, 12 hours after the seed imbibition and at seedling stage (on day $14^{th}$ after starting germination test because at this time root and shoot are grown enough for furthure investigations). Seeds were grinded and after that, 0.2 g of the obtained powder was used for measurments, but for seedlings, 0.2 g of fresh tissue was used. In order to extraction protein, 0.2 g of fresh tissue was pulverized in a mortar using liquid nitrogen and then 1 ml of Tris HCl buffer (0.05 M, pH= 7.5) was added. Obtained mixture was centrifuged for 20 min. at 13000 rpm, at 4 °C and the supernatant was collected for enzyme activity measuring (Sudhakar *et al*., 2001). Catalase activity was determined according to Kar and Mishra (1976). 60 µl of protein extract was added to Tris buffer (50 mM, pH=7) and Hydrogen Peroxide($H_2O_2$) 5 mM in the ice bath, then the absorbance curve was considered at 240 nm using spectrophotometer (model UV2100, Company Unike, USA). Enzyme activity was obtained for OD/ mg protein fresh tissue. Peroxidase activity was measured as described by Karo and Mishra (1976); 50 µl protein extract was added to 2.5 ml of extraction buffer containing 100 µM Tris buffer 100 mM, $H_2O_2$ 5 mM and 10 mM Pirogalol in the ice bath then absorbance changes was determined at 425 nm spectrophotometer. Polyphenol oxidase activity was measured according to Kar and Mishra (1976). 100 µl protein extract was solved in 1.5 ml Tris 0.2 M and 0.3 ml pirogalol 0.02 M and the resulted composition was placed in the bain marie bath at 25 °C for five minutes, then the absorbance rate at 420 nm was recorded. Evaluation of protein content was carried out using Bradford (1976) method. 0.2 g of fress tissue was squashed with 0.6 ml extraction buffer and centrifuged in 11500 rpm for 20 minutes at 4 °C. The supernatant transferred to new tubes and was centrifuged for 20 minutes at 4000 rpm and the supernatant was collected. To measure the protein content, 10 µl of obtained extract was added to 5µl Bradford solution and 290 µl extraction buffer and the absorbance rate was read at 595 nm.

Means were compared using the LSD test and Data were analysed using SAS, ver 9.2 and charts were created using Microsoft Excel 2010.

## Results:

Results of experiment showed that germination percentage of treated seeds was significantly increased by priming seeds with salicylic acid (Table 1). The highest germinstion perecentage was observed at 1000 mg. $L^{-1}$ Salicylic acid (63.3%), while 500 mg. $L^{-1}$ Salicylic acids had the least effect. Germination percentage was reduced from 66.9% to 58%, 4.48% and 16.8% after 48, 96 and 144 hours (Table 2). Seed priming reduced daily germination rate and mean time to germination. Minimum daily germination rate (0.47) and mean time to germination (1.7) were observed at 1000 and 500 mg. $L^{-1}$ Salicylic acid treatments, respectively. Unlike seed priming, accelerated ageing caused increased daily germination rate (0.44 to 0.57 day) and mean time to germination (1.8 to 2.4 day) (Table 2).

Seedling length was affected by salicylic acid treatments (Table 3). Application of 1000 mg. $L^{-1}$ Salicylic acid exhibited the highest seedling length. Ageing reduced seedling growth. After 48 hrs. of ageing treatment seedling length was reduced by (3.56%) while, using seed priming concentrations increased



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**Table1. Analyss variance of germination characteristics of Milk thistle under different priming and aging conditions.**

| S.OV. | df | Ms | | | |
|---|---|---|---|---|---|
| | | GR% | MTG | DGS | EC |
| Salicylic | 4 | 1.33* | 0.508** | 0.010* | 5336.8** |
| Aging | 3 | 3.89** | 0.135** | 0.022* | 162.2** |
| A × S | 12 | 0.688$^{ns}$ | 0.019$^{ns}$ | 0.0065$^{ns}$ | 1723.5** |
| Erorr | 40 | 0.754 | 0.022 | 0.0053 | 0.524 |
| Cv% | - | 11.51 | 10.36 | 10.39 | 6.5 |

**GR; Germination Percentage, MTG; Mean Time to Germination, DGS; Daily Germination Rate. Ns: nosignifican, ** and *: significant in level 1 and 5%.**

**Table 2. Germination characteristics of milk thistle under different priming and aging conditions (data are means of three replications ±standard error)**

| Seed treatments | Germination characteristics | | |
|---|---|---|---|
| | GR% | MTG(day) | DGS(day) |
| Salicylic (mg. $L^{-1}$) | | | |
| 0 (Control) | 50.6±4.4$^{b}$ | 3.2±0.15$^{a}$ | 0.57±0.03$^{a}$ |
| 500 | 57.0±4.3$^{ab}$ | 1.7±0.15$^{b}$ | 0.49±0.03$^{ab}$ |
| 1000 | 63.3±4.5$^{a}$ | 2.0±0.14$^{b}$ | 0.47±0.03$^{b}$ |
| 1500 | 60.6±2.6$^{ab}$ | 1.9±0.16$^{b}$ | 0.47±0.02$^{b}$ |
| 2000 | 57.3±3.5$^{ab}$ | 1.8±0.13$^{b}$ | 0.51±0.03$^{ab}$ |
| Aging(hours) | | | |
| 0 | 66.9±3.4$^{a}$ | 1.8±0.16$^{b}$ | 0.44±0.02$^{c}$ |
| 48 | 58.9±1.9$^{ab}$ | 1.9±0.17$^{b}$ | 0.48±0.01$^{bc}$ |
| 96 | 56.2±3.5$^{ab}$ | 2.3±0.18$^{a}$ | 0.53±0.03$^{ab}$ |
| 144 | 49.0±3.9$^{c}$ | 2.4±0.23$^{a}$ | 0.57±0.03$^{a}$ |

**GR; Germination Percentage, MTG; Mean Time to Germination, DGS; Daily Germination Rate. Distinct letters differ significantly according to the LSD test (p≤0.05) among the treatments.**

**Table 3. Analyss variance of vigor indices of Milk thistle under different priming and aging conditions.**

| S.OV. | df | Ms | | | | |
|---|---|---|---|---|---|---|
| | | RL | HL | HDW | $SVI_1$ | $SVI_2$ |
| Salicylic | 4 | 0.402$^{**}$ | 0.124$^{**}$ | 0.0064$^{**}$ | 0.007$^{**}$ | 26.66$^{**}$ |
| Aging | 3 | 0.053$^{*}$ | 0.197$^{**}$ | 0.017$^{**}$ | 0.020$^{**}$ | 17.33$^{**}$ |
| A × S | 12 | 0.123$^{*}$ | 0.0042$^{ns}$ | 0.0009$^{ns}$ | 0.0018$^{ns}$ | 3.14$^{ns}$ |
| Erorr | 40 | 0.045 | 0.016 | 0.0009 | 0.0014 | 3.27 |
| Cv% | - | 11.78 | 7.95 | 2.81 | 12.54 | 14.81 |

**RL; Radical length, HL; Hypocotyl length HDW; Hypocotyl weight, $SVI_1$; First Seedling vigor index, $SVI_2$; Secondary Seedling vigor index, Ns: nosignifican, ** and *: significant in level 1and 5%.**

**Table 4. vigor indices of Milk thistle under different priming and aging conditions (data are means of three replications ±standard error).**

| Seed treatment | vigor index | | | |
|---|---|---|---|---|
| | HL.(cm) | HDW.(mg) | $SVI_1$ | $SVI_2$ |
| Salicylic (mg. $L^{-1}$) | | | | |
| 0 (Control) | 1.93±0.13$^{b}$ | 1.3±0.09$^{c}$ | 0.07±0.009$^{b}$ | 96.4±11.0$^{b}$ |
| 500 | 2.87±0.15$^{a}$ | 1.5±0.07$^{b}$ | 0.08±0.009$^{ab}$ | 166.8±17.3$^{a}$ |
| 1000 | 2.95±0.10$^{a}$ | 1.6±0.05$^{ab}$ | 0.10±0.010$^{a}$ | 186.3±14.6$^{a}$ |
| 1500 | 2.75±0.18$^{a}$ | 1.7±0.06$^{a}$ | 0.10±0.006$^{a}$ | 167.4±14.2$^{a}$ |
| 2000 | 2.73±0.14$^{a}$ | 1.6±0.07$^{ab}$ | 0.09±0.008$^{a}$ | 157.2±13.0$^{a}$ |
| Aging(hours) | | | | |
| 0 | 3.01±0.17$^{a}$ | 1.7±0.06$^{a}$ | 0.11±0.008$^{a}$ | 185.9±15.8$^{a}$ |
| 48 | 2.90±0.13$^{a}$ | 1.7±0.04$^{a}$ | 0.10±0.004$^{a}$ | 164.6±10.4$^{ab}$ |
| 96 | 2.45±0.12$^{b}$ | 1.4±0.05$^{b}$ | 0.08±0.007$^{b}$ | 140.5±11.9$^{bc}$ |
| 144 | 2.22±0.13$^{b}$ | 1.3±0.05$^{c}$ | 0.06±0.006$^{c}$ | 128.3±16.1$^{c}$ |

**HL; Hypocotyl length HDW; Hypocotyl weight, $SVI_1$; First Seedling vigor index, $SVI_2$; Secondary Seedling vigor index. Distinct letters differ significantly according to the LSD test (p≤0.05) among the treatments.**

seedling length and dry weight compared to the control. The highest dry weight was produced at concentration of 1500 mg. $L^{-1}$ (30.76%), and the lowest was produced at 500 mg. $L^{-1}$ (15.3%) (Table 4).

Seed vigor also affected by seed priming. Treating seeds with 1000 and 1500 mg. $L^{-1}$ Salicylic acid



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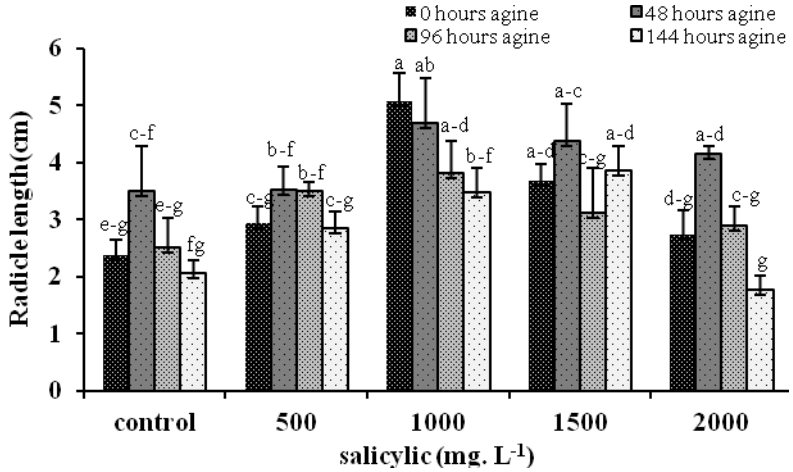


**Figure 1. Effects of seed priming and accelerated aging on root length of milk thistle seeds. Distinct letters differ significantly according to the LSD test (p≤0.05) among the treatment**

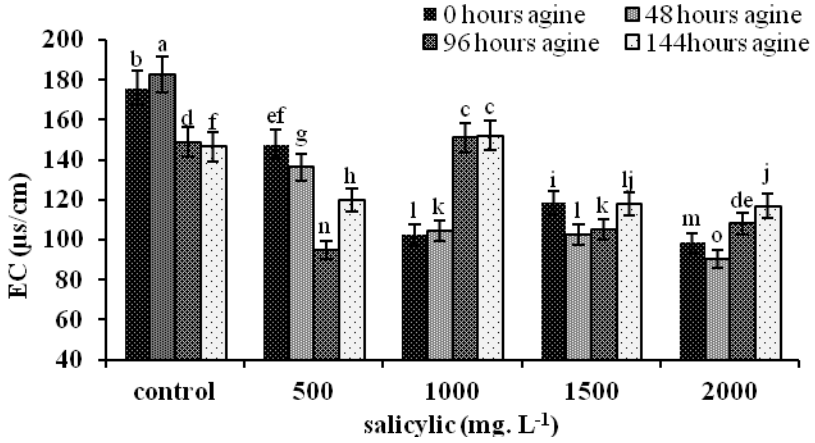


**Figure 2. Effects of seed priming and accelerated aging on cell membrane permeability of milk thistle seeds. Distinct letters differ significantly according to the LSD test (p≤0.05) among the treatment.**

exhibited the highest values for the first and secondary vigour index up to 42.8% for the first vigor index, and 93.5% and 73.6% for second vigour compared to the control. Ageing reduced first and secondary vigor index, for instance, ageing for 48 hours led to reduction of the first and the second vigor index from 0.11 to 0.06 185.9 to 128.3 respectively. Ageing durations for 96 and 144 hours reduced the first vigor index by 20% and 40% and secondary vigor 14.6% and 22%, respectively (Table 4).

Root growth was influenced by seed priming and ageing treatments (Table 3). The highest root length was obtained at 1000 mg. $L^{-1}$Salicylic acid in no aged seeds, that 112% increase in root growth compare to the control. Pattern of root length variations didn't show a clear trend. Fourty eight hours of ageing increased root length while longer durations of seed ageing produced lower root length (Figure 1).

Membrane permeability was affected by seed priming and ageing treatments (Table 1). Membrane permeability increased at higher durations of ageing treatments. Seed priming saved membrane permeability. However, priming increased leakage from aged seeds surprisingly, and the lowest amount of leakage was in seed priming with concentration 1500 mg. $L^{-1}$ (Figure 2).

Enzyme activity also was affected by seed priming (Table 5). The highest catalase activity was observed at 1500 mg. $L^{-1}$salicylic acid (7.04 OD/ mg protein/$min^{-1}$) while the lowest activity was observed at 500 mg. $L^{-1}$ (5.28 OD/mg protein/ $min^{-1}$) at seedling stage, 1000 mg. $L^{-1}$ Salicylic acid, catalase activity was reached to (2.9 OD/ mg protein $min^{-1}$) and at 2000 mg. $L^{-1}$ it reached to to (2.36 OD/ mg protein/ $min^{-1}$). Catalase activity was reduced by ageing duration, it was decreased from 7.07 to 6.74 OD/ mg protein/$min^{-1}$ at germination stage while in seedling stage, it reduced from 3.02 to 2.35 OD/ mg protein/$min^{-1}$. Increase in ageing duration for 96 and 144 h, reached the catalase activity to 5.81 and 4.35 OD/ mg protein/$min^{-1}$, respectively at germination stage, while at seedling stage, it was 2.05 and 1.67 OD/mg protein/$min^{-1}$ for 96 and 144 hours ageing treatment respectively (Table 6). Peroxidase activity was influenced by application of seed priming under ageing conditions. The highest peroxidase activity was recorded for concentration of 1000 mg. $L^{-1}$salicylic acid at both seedling and germination stages. For germination and



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**Table 5. Analyss variance CAT, POA and PPO of milk thistle under different priming and aging conditions.**

| S.OV. | df | Ms | | | | | |
|---|---|---|---|---|---|---|---|
| | | Seed | | | Hypocotyl | | |
| | | CAT | POX | PPO | CAT | POX | PPO |
| Salicylic | 4 | 0.28$^{*}$ | 0.80$^{*}$ | 0.324$^{*}$ | 0.75$^{**}$ | 5.37$^{**}$ | 0.615$^{**}$ |
| Aging | 3 | 0.97$^{**}$ | 0.87$^{*}$ | 0.216$^{*}$ | 0.45$^{**}$ | 1.58$^{**}$ | 0.336$^{**}$ |
| A × S | 12 | 0.014$^{ns}$ | 0.053$^{ns}$ | 0.122$^{ns}$ | 0.011$^{ns}$ | 0.023$^{ns}$ | 0.008$^{ns}$ |
| Erorr | 40 | 0.107 | 0.27 | 1.10 | 0.037 | 0.038 | 0.012 |
| Cv% | - | 13.57 | 19.29 | 3.77 | 13.14 | 6.43 | 5.60 |

**CAT; Catalase, POX; Peroxidase and PPO; polyphenol oxidase. Ns: nosignifican, ** and *: significant in level 1and 5%.**

**Table 6. CAT, POA and PPO of Milk thistle under different priming and aging conditions (data are means of three replications ±standard error)**

| Seed treatment | CAT OD/mg protein min$^{-1}$ | | POX OD/mg protein min$^{-1}$ | | PPO OD/mg protein min$^{-1}$ | |
|---|---|---|---|---|---|---|
| | Seed | Hypocotyl | Seed | Hypocotyl | Seed | Hypocotyl |
| Salicylic ( mg. L$^{-1}$) | | | | | | |
| 0 (Control) | 5.14±0.50$^{b}$ | 1.10±0.06$^{b}$ | 5.94±0.95$^{b}$ | 03.55±0.24$^{c}$ | 6.48±0.94$^{c}$ | 3.09±0.15$^{c}$ |
| 500 | 5.28±0.28$^{b}$ | 2.40±0.33$^{a}$ | 6.98±0.62$^{ab}$ | 11.51±0.79$^{ab}$ | 10.59±1.14$^{ab}$ | 4.86±0.23$^{a}$ |
| 1000 | 6.37±0.54$^{ab}$ | 2.90±0.23$^{a}$ | 9.61±0.89$^{a}$ | 12.03±0.74$^{a}$ | 11.95±1.86$^{a}$ | 3.33±0.20$^{c}$ |
| 1500 | 7.04±0.73$^{a}$ | 2.59±0.19$^{a}$ | 8.28±0.82$^{ab}$ | 11.34±0.63$^{ab}$ | 10.90±0.97$^{ab}$ | 4.25±0.23$^{b}$ |
| 2000 | 6.11±0.54$^{b}$ | 2.36±0.21$^{a}$ | 7.19±0.88$^{ab}$ | 10.76±0.63$^{b}$ | 7.92±0.98$^{bc}$ | 5.14±0.16$^{a}$ |
| Aging(hours) | | | | | | |
| 0 | 7.07±0.47$^{a}$ | 3.02±0.34$^{a}$ | 8.68±0.70$^{a}$ | 11.12±0.93$^{a}$ | 7.23±0.62$^{b}$ | 3.38±0.23$^{d}$ |
| 48 | 6.74±0.49$^{a}$ | 2.35±0.20$^{b}$ | 8.75±1.02$^{a}$ | 11.38±1.06$^{a}$ | 8.46±1.16$^{b}$ | 3.98±0.24$^{c}$ |
| 96 | 5.81±0.41$^{b}$ | 2.05±0.14$^{bc}$ | 7.10±0.71$^{ab}$ | 09.81±0.95$^{b}$ | 10.01±1.47$^{ab}$ | 4.41±0.26$^{b}$ |
| 144 | 4.35±0.32$^{b}$ | 1.67±0.13$^{c}$ | 5.88±0.46$^{b}$ | 07.04±0.63$^{c}$ | 12.57±0.96$^{a}$ | 4.75±0.24$^{a}$ |

**CAT; Catalase, POX; Peroxidase and PPO; polyphenol oxidase Distinct letters differ significantly according to the LSD test (p≤0.05) among the treatments.**

seedling growth stages its activity was changed from 5.94 to 9.61 OD/mg protein/min$^{-1}$ and from 3.55 to 12.03 OD/ mg protein/min$^{-1}$ repectively. The lowest enzyme activity was recorded under treatment of 500 mg. L$^{-1}$of germination (6.98 OD/ mg protein/min$^{-1}$) and 2000 mg. L$^{-1}$on seedling stage (10.76 OD/mg protein/min$^{-1}$). Seed ageing could cause reduction of peroxidase activity in 96 and 144 hours of ageing treatment from 8.68 to 7.10 and 5.88 OD/mg protein/min$^{-1}$ and in seedling stage from 11.12 to 9.81 and 7.04 OD/ mg protein/min$^{-1}$ (Table 6). Polyphenol oxidase activity were changed prior to seed priming at both germination and seedling growth stages. The highest activity of this enzyme in germination phase (11.95 OD/mg protein/min$^{-1}$) was at concentration of 1000 mg. L$^{-1}$and the lowest activity (7.92 OD/mg protein/min$^{-1}$) was at concentration of 2000 mg. L$^{-1}$. The highest enzyme activity of polyphenol oxidase in seedling phase was at concentration of 2000 mg. L$^{-1}$ which changed from 3.09 to 5.14 OD/mg protein/min-1 Ageing led to drastical increase in polyphenol oxidase activity. Ageing treatment for 48 hours at germination phase showed activity of 7.23 to 8.46 OD/mg protein/min$^{-1}$and at seedling stage from 3.38 to 3.398 OD/mg protein/min$^{-1}$. The highest enzyme activity was recorded at 144 hours ageing in germination (12.57 OD/mg protein/min$^{-1}$) and seedling stage (4.75 OD/ mg protein/min$^{-1}$) (Table 6).

## Discussion:

Results of this study showed that aging decreased seed germination characteristics (germination percentage, mean time to germination and daily germination rate). Reduction of these characteristics could be attributed to an increase seed in reservoirs leakage (Figure 2). Aging causes reduction in selective permeability and destruction of cell membrane which leads to an increases in leakage of reserves from seed. External leakage and membrane damage impair cell development and seed imbibition and thus reduces seed germination. These results are confirmed by Goel *et al* (2003) findings on oxidative stress changes during the period of cotton seeds. They declared that during seed deterioration process germination rate is decreased and leakage rate rises. Reduction in germination indices during aging could also be related to decrease of antioxidant enzymes activity duration (Table 6). As shown in table 3 reduction in germination percentage and antioxidant enzymes activity during seed aging is consistent with the results of Hsu *et al* (2003). Priming of seeds with salicylic acid decreased the detrimental effects of aging and increased germination indices. Aging had several effects on seeds such as reduction in antioxidant enzymes activity and increases in electrolyte leakage from membranes. Hydration control of seeds during seed priming prevents from further destruction of damaged cell membrane and leakage of stored materials. Achieving favorable results by applying higher concentration of salicylic acid (1000 mg. L$^{-1}$) may be due to production of osmotic solutions with low osmotic potential caused hydration control of seed and improvement turgidity of cell membrane. Goel *et al* (2003) showed hydro priming and priming with ascorbic acid led to reduced leakage, improved



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antioxidant enzymes activities, and germination of cotton seeds during oxidative stress. Vigor index (hypocotyl length, hypocotyl weight, first seedling vigor index and secondary seedling vigor index) decreased during aging (Table 4). Reduction of these characteristic can be attributed to decrease in germination rate in early germination process that finally led to reduction in seedling growth (Table 2).

Seeds with high germination potential could germinate rapidly and seedling growth development and production phases could be hastened and finally greater powerful seedling production. Aging with disrupting the cell membrane activity inhibits from swelling and essential turgor pressure for cell growth and division of cells can prevent development. Aged seeds had more leakage than none-aged seeds, therefore lower amounts of stored material was holding up and appropriate environment provided for the development of fungal disease which has a considerable effects on seed vigor and seedling growth (Figure 2). Reduced activity of antioxidant enzymes in early stages of germination, affects on activity of these enzymes in the seedling stage also the impact of their activities reduces (Table 6). Reduced antioxidant enzymes activity in seedlings reduces the seedling vigor under unfavorable environmental factors that lead to reduced seedling growth. Soltani *et al* (2008) showed that during aging process the rate of reserves efficiency is decreased. This may be due to reduced levels of hydrolytic enzymes such as amylase and hydrolase activities that is related to lower levels of the gibberellic acid. So this is responsible for reduction in seedling growth and vigor. This result is confirmed by Cholami tilebeni and Golpayegani (2011) findings. Salicylic acid can influences on gibberellin hormone levels and increases levels of it and in this way affect the performance of reserves and improved seedling growth. The role of salicylic acid on the germination and membrane permeability and growth rate has been reported by other investigators (Barkosky and Einhellig 1993; khan *et al*., 2003). The activities of antioxidant enzymes (catalase, peroxidase and polyphenol oxidase) decreased during aging; this reduction was observed during hampering of germination and seedling growth stage observed (Table 6). The other researchers also demonstrated that the addition of enzymes and other antioxidant enzymes such as superoxide dismutase, glutathione reductase and ascorbate peroxidase also decreased during aging (Hu *et al*., 2006; Hsu *et al*., 2003; Goel *et al*., 2003). Reduction in antioxidant enzymes activity could be due to reduction in transcription of genes related to antioxidant enzyme activity. Aging increases Oxygen free radicals and hydrogen peroxides and the factors that increase the activity of RNA oxidase, and discount factor reduces transcription of antioxidant enzyme activity (Berlett and Stadtman 1997; Kibinza *et al*., 2006). Fujikura and karssen (1995) suggested that during aging molecular change in DNA and protein structure of the seed occurs, which in turn, can reduce the expression of genes related to the antioxidant enzymes activity. Priming increased the antioxidant enzymes activity in the early stages of germination and seedling growth (Table 6). Reduction of free radicals and hydrogen oxidation during priming can be considered a major cause for improvement in antioxidant enzyme activity (Kang *et al*., 2003; Kibinza *et al*., 2011). Among the different concentrations of salicylic acid on antioxidant enzyme activity changes were different. Catalase activity of seeds in 1500 mg. $L^{-1}$ concentration was maximum, while enzyme activity in seedlings at all concentrations showed no significant difference. Peroxidase and polyphenol oxidase activities in the most active concentrations were observed in 1000 mg. $L^{-1}$ that most of germination characteristics, seedling growth and seed vigor of the concentration obtained. Different results may be due to the seed treatments which were applied over a period of time after the change is attributed to the plant tissue. However, in most of the measured traits 1000 mg. $L^{-1}$ concentration of salicylic acid was most effective and reduced the effects of aging. These effects can be related to the hormonal and chemical properties of matter that requires further study.

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