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Effect of Priming on Biochemical Regeneration of Chamomile (*Matricaria recutita, Chamaemelum nobile*) Deteriorative Seeds

[1]*Hossein Gholami Tilebeni and* [2]*Manan Sadeghi*

[1]Young Researchers Club, Gorgan Branch, Islamic Azad University, Gorgan, Iran
[2]Young Researchers Club, Bojnourd Branch, Islamic Azad University, Bojnourd, Iran

Abstract: Priming is one of the lipid peroxidation reduce suitable methods in deterioration seeds that might be resulted in increased seed performance (germination and emergence) of deteriorative seeds. Chamomile medical properties is considered to be an antiseptic, antibiotic, disinfectant, bactericidal and vermifuge. The present study was carried out to elucidate the mechanism of seed ageing in two Chamomile species (*Matricaria recutita, Chamaemelum nobile*) (German chamomile and Roman chamomile, respectively). Experiment was factorial conducted based on CRD design with three replicates. Treatments were combinations of 5 levels of seed ageing (0, 24, 48, 72 and 96 hour) and two levels priming (Hydropriming for 2 h and ascorbic acid priming for 12 h). In both cultivars, germinability decreased, whereas membrane deterioration, as assayed by electrical conductivity of the seed leachates, increased progressively with artificial ageing. The decrease in germinability was well correlated with increased accumulation of total peroxide and malondialdehyde content and decreased activities of antioxidant enzymes peroxidase, catalase, ascorbate peroxidase, glutathione reductase and superoxide dismutase. Hydropriming for 2 h and ascorbic acid priming for 12 h partially maintained germination and the activities of various antioxidant enzymes under artificial ageing and the accumulation of peroxide and MDA content was decreased. The results suggested that chamomile seed deterioration during accelerated ageing is closely related to a decrease in activities of various peroxide scavenging enzymes and to lipid peroxidation and priming biochemical improve of deteriorative seeds.

Key words: Chamomile · Accelerated ageing · Priming · Lipid peroxidation · Peroxide-scavenging enzyme
Abbreviations:APX = ascorbate peroxidase · – CAT = catalase · – GR = glutathione reductase · – MDA = malondialdehyde · – POD = peroxidase · – SOD = superoxide dismutase

INTRODUCTION

Chamomile or camomile is a common name for several daisy-like plants. These plants are best known for their ability to be made into a tea which is commonly used to help with sleep and is often served with either honey or lemon. Chrysin a specific flavonoid found in chamomile, has been shown to be anxiolytic in rodents and is believed to be at least partially responsible for chamomile's reputation as a sleep aid. Chamomile is the national flower of Russia. It is known to reduce stress [1]. The flora labelled "chamomile", all of the family Asteraceae, include: 1) *Matricaria recutita* (syn. *M. chamomilla*), German or blue chamomile, commonly used in tea. 2) *Anthemis nobilis* (syn. *Chamaemelum nobile*), Roman chamomile, the "lawn" chamomile. The flowering tops of the chamomile plant are used to make teas, liquid extracts, capsules, or tablets. The herb can also be applied to the skin as a cream or an ointment, or used as a mouth rinse. Chamomile has been widely used in children and adults for thousands of years for a variety of health conditions. The herb is often used for sleeplessness; anxiety; and gastrointestinal conditions such as upset stomach, gas, and diarrhea. It is used topically for skin conditions and for mouth ulcers resulting from cancer treatment [2].

Priming involve a period of controlled hydration of the seeds to a point closed to, but before the emergence of the radicle after which seeds are dried back to their initial moisture content before sowing [3] Successful cultivation of the flower depends on quality of seeds such as viability, vigor and storage potential etc. The deterioration is common biochemical physiological process during prolonged storage of seeds, wherein seed

Corresponding Author: Hossein Gholami Tilebeni, Young Researchers Club, Gorgan Branch, Islamic Azad University, Gorgan, Iran. E-mail: gholamihossein88@gmail.com.

membrane degradation and accumulation of inhibitors occurs, resulting in low field emergence and loss of viability. Seed germination can be enhanced with several treatments such as humidification [4], hydro-priming [5], osmotic priming [6] and alternate hydration-dehydration [7]. Many workers have attempted to increase the seed germination capacity and vigor by using different priming treatments in various field crop and seldomly used to test medicine plant seeds.

Germination ability and subsequent seedling establishment are the most important parameters for seed quality. When stored under conditions of high humidity and temperature, seeds of most crops may be severely damaged and lose vigor [8]. High seed moisture and high temperature further accelerate the seed deterioration. A large number of reactive oxygen species are generated in the seed during ageing which causes lipid peroxidation [9]. This free radical induced non-enzymatic peroxidation, which has the potential to damage membrane, is the major cause of stored seed deterioration. Some protective mechanisms involving free radical and peroxide scavenging enzymes, such as catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR) and superoxide dismutase (SOD) have evolved within the seed [8]. SOD catalyses the disproportionation of two molecules of superoxide into molecular oxygen and H_2O_2 [10]. APX reduces H_2O_2 to water, using ascorbate as an electron donor. Besides, ascorbate and glutathione, two low molecular weight antioxidants are of paramount importance in plants. GR plays a role in the control of endogenous H_2O_2, through an oxidoreduction cycle involving glutathione and ascorbate [11]. CAT and POD are implicated in the removal of H_2O_2. Seed invigoration treatments have been developed to improve seed germination and emergence. Such treatments involve hydration-dehydration, pre-treatment of seeds with diverse chemicals like ascorbic acid, sodium dikegulac, hormones and vitamins prior to accelerated and natural ageing. These treatments have been reported to improve seed vigour and seed storage by scavenging free radicals [12].

Membrane disruption is one of the main reasons of seed deterioration. As a result, seed cells are not able to retain their normal physical condition and functioning. The major causes of membrane disruption are increase in free fatty acid level and free radicals productivity by lipid peroxidation [13]. Protective mechanisms that could scavenge the peroxidatively produced free radicals within the seed to keep these deleterious compounds to a minimum have been reported in soybean and sunflower [10]. This protective mechanism involves several free radical- and peroxide-scavenging enzymes such as catalase, peroxidase and superoxide dismutase and ascorbic acid. In most of the cases in storage seeds have lost their quality when they reach to the farmer but primed seeds may show increased final germination percentage. The priming conditions had a large influence on emergence and seedling vigor in sorghum and argued that germination speed was an important determinant of successful seedling establishment [9].

Chamomile (*Matricaria recutita, Chamaemelum nobile*) is an important medicine plant. However, reports on the biochemical basis of seed deterioration in *chamomile* are very scarce. Hence, in the present study, we investigated seed germinability, and the corresponding activities of peroxidase, catalase, ascorbate peroxidase, glutathione reductase and superoxide dismutase and in *chamomile* seeds of two *chamomile* (*Matricaria recutita, Chamaemelum nobile*) species (German chamomile and Roman chamomile) subjected to accelerated ageing and Effect of priming on biochemical Regeneration chamomile Deteriorative Seeds.

MATERIALS AND METHODS

Chamomile seeds of two chamomile species (*Matricaria recutita, Chamaemelum nobile*) (German chamomile and Roman chamomile,respectively) were procured from the medicine plants research center of Iran . The seeds were delinted using concentrated H_2SO_4 acid at the rate of 100 mL/kg seeds by constantly stirring the slurry for three minutes and then washing with water five to six times to remove the acid (checked with pH paper). After surface sterilization (0.1% $HgCl_2$ for 90 seconds) delinted seeds were divided into three lots. The untreated first lot acted as a control. The second lot was primed for 2 h with water, and the third lot was primed for 12 h with an ascorbic acid solution (100µg/mL), in the dark and adjusted to normal moisture content by drying in lab. Seeds from all sets were aged artificially in an accelerated ageing chamber by incubating the seeds in closed plastic boxes with 100% R.H at 40±1°C for 0, 24, 48, 72 and 96h. All observations were taken in triplicate.

Germination: To determine percentage germination, fifty seeds from each lot were kept on paper towels (pre-soaked with water) and kept in the germination chamber at 24±1°C in the dark. The germination tests were evaluated on the 12th day, and only the seedlings with 2 mm radicle emergence were considered germinated, according to the rules of International Seed Testing Association [14].

Determinations of Malondialdehyde (MDA) and Total Peroxide Contents: For MDA (a product of lipid peroxidation) and total peroxide determinations, three replicates of five seeds, imbibed for 48 h, were hand-homogenized with 4 ml 5% (v/v) trichloroacetic acid at 4 8C using a mortar and pestle and then centrifuged at 14,000 _ g for 20 min. The supernatants were used for MDA [15] and total peroxide [16] determinations.

Determinations of Enzyme Activities: Eighteen h-imbibed seeds from each lot were used for the preparation of enzyme extract. The seeds were decoated, and 200 mg of tissue was ground in a chilled mortar and homogenised with 10 mL of 0.1mol/L phosphate buffer (pH 7.8) containing 0.2 g polyvinylpyrrolidone (PVP), 10 mmol/L β-mercaptoethanol, 10 mmol/L KCl, 1 mmol/L MgCl2 and 1 mmol/L EDTA. The homogenate was centrifuged at 15,000 ×*g* for 15 min at 4°C twice. The resultant supernatant was filtered and used for the enzyme assay. Soluble proteins extracted from seeds, were determined using the method described by Lowry *et al.* [17].

Peroxidase activity was assayed essentially according to the method of Shannon *et al.* [18]. The reaction mixture contained 0.1mL of enzyme extract, 2mL of 0.1mol/L sodium-acetate buffer (pH 4.5) and 0.5 mL of O-dianisidine solution (0.2% in methanol, freshly prepared). The reaction was initiated with the addition of 0.1mL of 0.2 mol/L H_2O_2. The change in absorbance was recorded at 470nm at an interval of 15 sec for 2 min. The enzyme activity was expressed as units (mg protein)$^{-1}$. One unit of POD was defined as 0.1 change in O.D. min^{-1}. Catalase activity was estimated by the method of Aebi [19]. The reaction mixture contained 0.6 mL of enzyme extract, 0.1 mL of 10 mmol/L H_2O_2 and 2mL of 30 mmol/L potassium phosphate buffer (pH 7.0). The absorbance was read at 240nm immediately after addition of the enzyme extract at an interval of 15 sec for 2 min. The blank was without enzyme extract. The enzyme activity was expressed as mmol H_2O_2 decomposed (mg protein)$^{-1}$ min^{-1}.

Ascorbate peroxidase activity was assayed by the method of Nakano and Asada [20]. The reaction mixture contained 0.1mL enzyme extract, 0.2mL of 0.5 mmol/L ascorbic acid in 50 mmol/L potassium phosphate buffer (pH 7.0) and 2 mL of 50 mmol/L potassium phosphate buffer (pH 7.0). The reaction was started by the addition of 0.06 mL of 1mmol/L H_2O_2, and absorbance was recorded at 265 nm every 15 sec. Ascorbate peroxidase activity was calculated using the extinction coefficient of 2.8 mmol/L^{-1} cm^{-1} and expressed as nmol ascorbate oxidised (mg protein)$^{-1}$ min^{-1}.

GR activity was assayed by the method of Goldberg and Spooner [21]. The reaction mixture contained 0.1 mL of enzyme extract, 2.5 mL of 120 mmol/L phosphate buffer (pH 7.2) and 0.1mL of both EDTA (0.015 mmol/L) and oxidised glutathione (0.065 mmol/L). After 5 min 0.05 mL of NADPH (9.6 mmol/L) was added and mixed thoroughly. The reaction was monitored after every 15 sec at 340 nm. The enzyme activity was expressed as µmol NADPH oxidised (mg protein)$^{-1}$min^{-1}. The SOD enzyme activity was assayed by the method of Giannopolitis and Ries [22]. The reaction mixture contained 3mL of 0.1mol/ L phosphate buffer (pH 7.8) containing 1.3 µmol/L riboflavin, 13 mmol/ L methionine, 63 µmol/L nitroblue tetrazolium and 0.1mL of enzyme extract. SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of NBT. Glass tubes containing the mixture were exposed to light (50 µEm^{-2} s^{-1}). Identical tubes, which were not illuminated, served as blanks. After illumination for 10 min, the tubes were covered with black cloth and absorbance was measured at 560 nm. Log A560 was plotted as a function of the volume of enzyme extract used in the reaction mixture. The volume of enzyme extract, corresponding to 50 % inhibition of the photochemical reaction was obtained from the resultant graph. One unit of SOD was defined as the level of enzyme activity that inhibited the photoreduction of NBT to blue formazan by 50% (expressed as units SOD mg protein^{-1}).

Statistical Analysis: For germination, the experiment was arranged as a randomized complete block design with three replicates. Percentage data were arcsine transformed before analysis. All data were subjected to an analysis of variance, and LSD values were calculated at $P \leq 0.05$.

RESULTS

In this study, germination of the seeds decreased progressively with artificial ageing treatment in both species (Fig. 1A, B). Hydration for 2 h and ascorbic acid priming for 12 h improved the germination in comparison to the control. No significant decrease in germination was observed in primed seeds when aged for 24 h, but thereafter, germination was reduced. Seed ageing of 96 h resulted in a significant decrease in germination in both species (Fig. 1 A, B).

Electrical conductivity of the seed leachates increased progressively with the ageing treatment (Fig. 1C, D). Hydropriming and ascorbic acid priming reduced electrical conductivity significantly after seed ageing, as compared to the controls in both species.

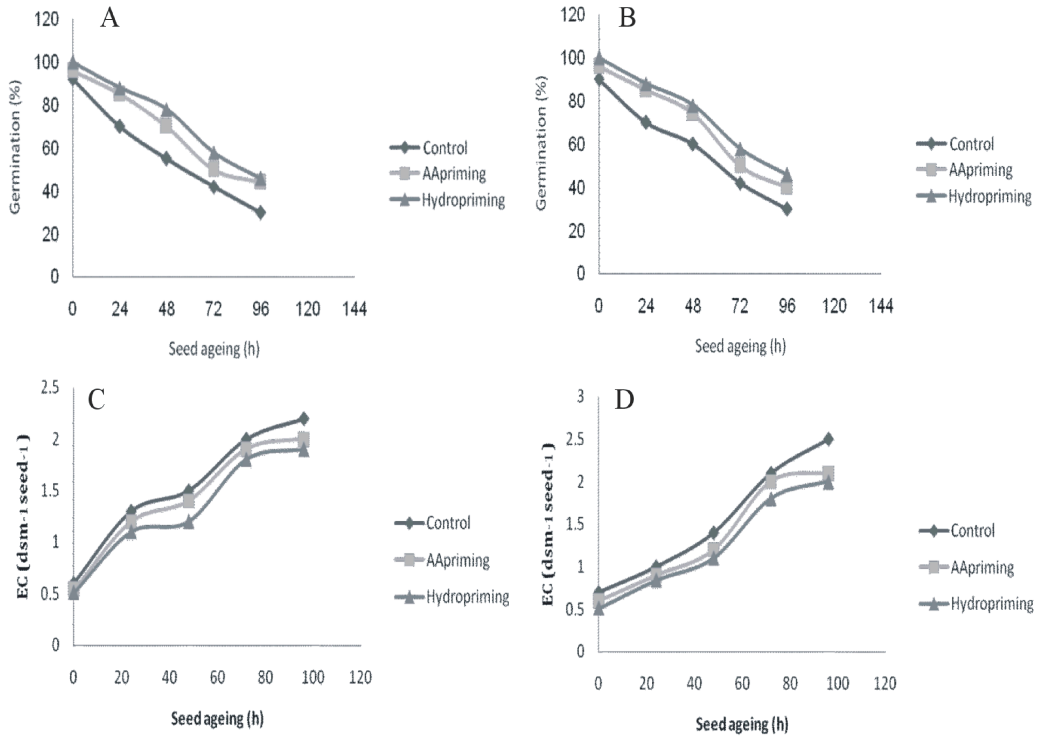

Fig. 1: Changes in germination and electrolyte leakage in seed ageing chamomile seeds of two species *chamomile* German chamomile (A, C) and Roman chamomile (B, D).

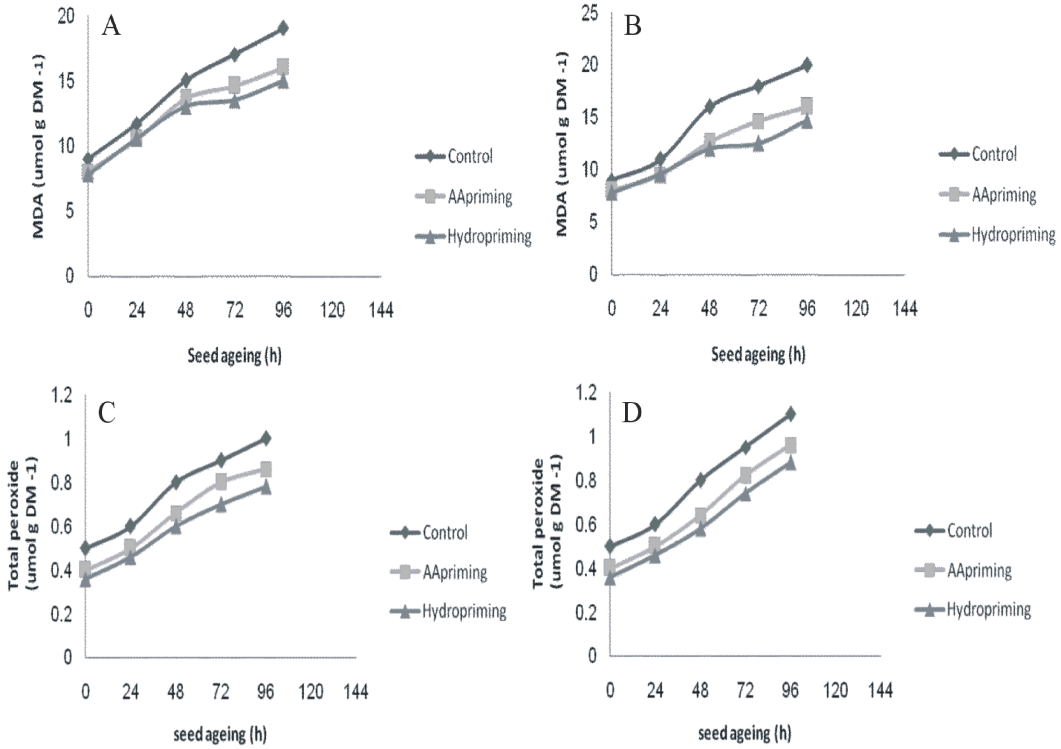

Fig. 2: Changes in malondialdehyde content and total peroxides in seed ageing chamomile seeds of two species *chamomile* German chamomile (A, C) and Roman chamomile (B, D).

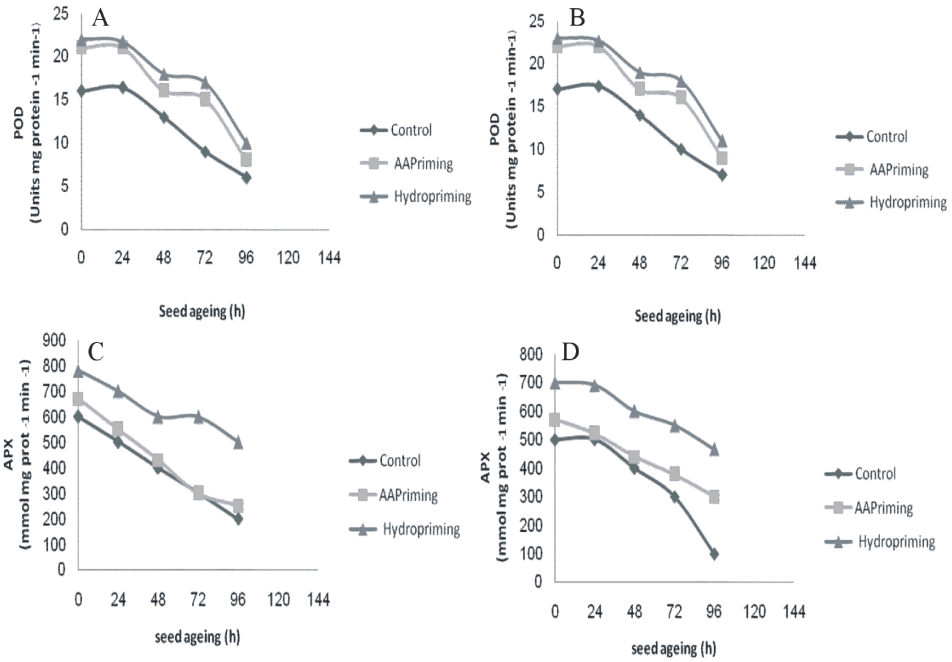

Fig. 3: Changes in activities of peroxidase and ascorbate peroxidase in seed ageing chamomile seeds of two species *chamomile* German chamomile (A, C) and Roman chamomile (B, D).

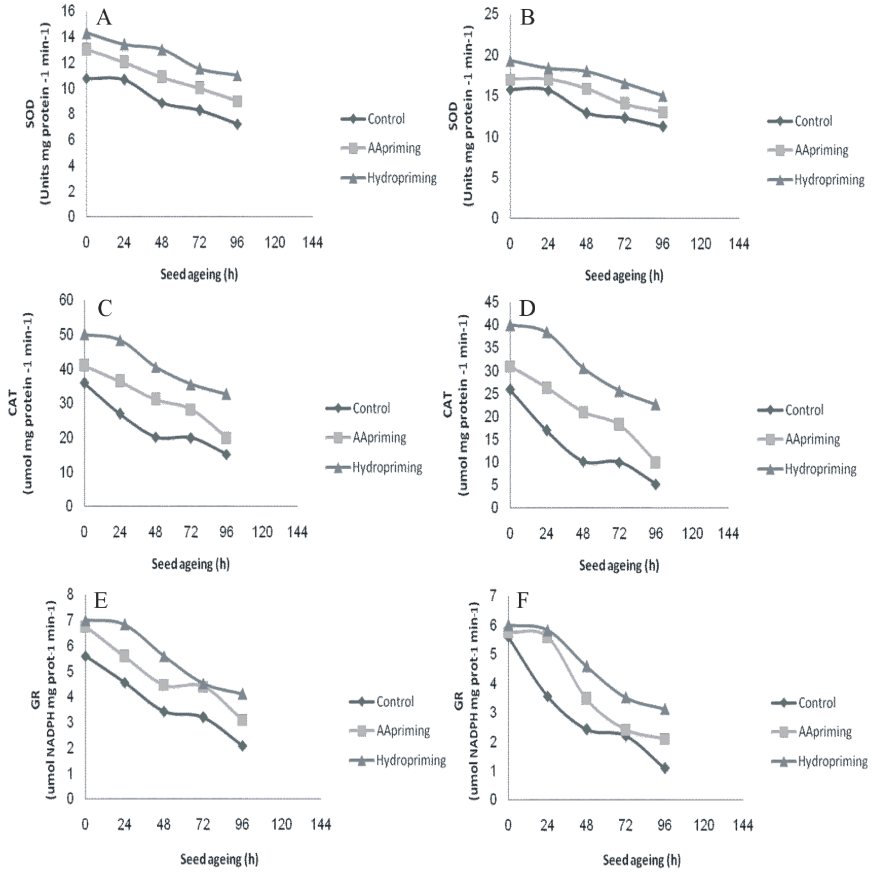

Fig. 4: Changes in activities of superoxide dismutase, catalase and glutathione reductase in seed ageing chamomile seeds of two species *chamomile* German chamomile (A, C, E)and Roman chamomile (B, D, F).

Hydropriming was more effective in keeping the electrical conductivity of seed leachate under check.

Malondialdehyde and total peroxide content increased with seed ageing time from 24 to 96 h (Fig. 2 A, B, C, D). Priming for 2 h with water or 12 h with ascorbic acid lowered the MDA and peroxide content. Seed ageing for 24 h did not show a significant change in MDA or peroxide content, but did increase thereafter.

The activities of various enzymes such as peroxidase (Fig.3A, B), ascorbate peroxidase (Fig. 3C, D), superoxide dismutase (Fig. 4A,B) catalase (Fig. 4C, D), and glutathione reductase (Fig. 4 E, F) decreased progressively with the ageing time. Hydropriming and ascorbic acid priming maintained higher activity of these enzymes in comparison to controls. Activity of various enzymes did not decrease significantly during artificial ageing up to 24 h. However, ageing treatment of 96 h caused a significant decrease in enzyme activities in both species, with a greater decrease in activity in species Roman chamomile.

DISCUSSION

Enhanced lipid peroxidation mediated by free radicals and peroxides is considered to be one of the likely explanations for loss of seed viability during ageing [23]. The purpose of this study was aimed at verifying this statement in chamomile seeds. In this study, germination of the seeds decreased progressively with seed ageing. One possible explanation for the seed deterioration is lipid peroxidation, when membranes are perturbed [24]. Such alterations in the membranes of aged seeds would lead to electrolyte leakage during seed imbibition. In this study the decrease in germinability correlated well with increased electrolyte leakage (Fig. 1C, D), thus reflecting a loss in membrane integrity. This phenomenon may be indicative of an inability to maintain coherent membranes, resulting in losses of germinability [25]. The determination of MDA is a convenient method of quantifying the extent of lipid peroxidation, especially in oil rich seeds having high linoleic and linolenic acid content [26]. In the present study, level of MDA and peroxide content increased with artificial ageing (Fig. 2A, B, C, D). This is clearly reflected in the present study. These results taken in conjunction with reduced germinability (Fig. 1A,B) indicate that increased lipid peroxidation might explain the loss of vigor and viability of chamomile seeds of two species. The increased level of MDA and peroxides in artificially aged seeds indirectly reflects the increased lipid peroxidation [27, 28]. Accelerated ageing of sunflower seeds also resulted in a marked decrease in the germination rate and was associated with an increase in the levels of MDA and conjugated dienes, thus indicating lipid peroxidation [29].

The higher content of MDA and peroxide in aged chamomile seeds might also result from ageing-induced inhibition of free radical and peroxide scavenging activity. Thus, the removal of peroxide from aged seeds might be too slow to prevent accumulation of peroxide [30]. In this study wenoted the decrease in activity of peroxidase (Fig. 3A, B), catalase (Fig. 4 C, D), ascorbate peroxidase (Fig. 3 C, D), glutathione reductase (Fig. 4 E, F) and superoxide dismutase (Fig. 4 A, B) in parallel with decreases in germination, alongside increased levels of MDA and peroxides. Similar decrease in the activity of catalase and peroxidase was reported in artificially aged peanut seeds [31], *Brassica* [32], pea [33] and mungbean [34].

H_2O_2 is partially detoxified by catalase, however H_2O_2 that has diffused into the cytosol is degraded by ascorbate peroxidase [35]. It has been reported that recalcitrant seeds have a high ascorbate peroxidase activity, while orthodox seeds are devoid of ascorbate peroxidase activity [36, 37]. The maintenance of ascorbate peroxidase activity in recalcitrant seeds is very important as they never reach the quiescent phase. In the present study APX activity decreased in the aged seeds (Fig.3C, D).

SOD is considered a key enzyme in the regulation of intracellular concentrations of superoxide radical and peroxides. This removes superoxide and hence decreases the risk of hydroxyl radical formation from superoxide via the metal-catalysed Haber-Weiss-type reaction [38]. In the present study, SOD activity decreased with ageing treatment (Fig. 4 A, B). Similar decreases in SOD activity have been reported in artificially aged peanut seeds [39].

The presence of antioxidants such as, ascorbic acid or glutathione, helps in preventing the lipid peroxidation due to free radical formation [28]. In the present study, activity of GR decreased under artificial ageing treatments (Fig. 4 E, F). This decrease in activity would result in a decreased level of reduced glutathione, a potent free radical scavenger [40]. Hydropriming causes activation of essential germination and repair enzymes, which remain semi-activate following dry back, and are quickly reactivated on imbibitions culminating in more rapid and uniform germination [28]. Hydropriming helps in the repair of membranes, which are disrupted during maturation [41].

Many workers reported improved seed vigour when the seeds were pre-treated with antioxidants prior to accelerated and natural ageing in seeds of various crops

[28]. Reduction in seed deterioration, or improvement in seed vigour by the use of antioxidants, like ascorbic acid, is primarily due to quenching of free radicals, which prevents the peroxidative damage and enhances the activities of peroxide and radical scavenging enzymes [42]. In this study, 2 h hydropriming treatment was more effective in improving the percent germination followed by 12 h priming treatment with ascorbic acid, as compared to untreated control seeds (Fig. 1A, B). Similarly, the priming treatment for 2 h with water or 12 h with ascorbic acid was responsible for lowering the lipid peroxidation, as reflected by the change in MDA content (Fig. 2A,B) as well as peroxide content (Fig. 2C,D) during the artificial ageing treatment. The activities of the various antioxidant enzymes, which decreased during the artificial ageing treatment, were partially restored by 2 h hydration treatment and 12 h ascorbic acid treatment as compared to the untreated controls. The results of priming treatment clearly support the hypothesis that chamomile seed deterioration occurs due to membrane lipid peroxidation caused by the imbalance in the reactive oxygen scavenging system.

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