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Original Article

Effect of Seed Priming on the Enhancement of Seedling Traits in two Species of *Anthemis* L. Preserved in Medium and Long-term Storage and Accelerated Aged Seeds

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Abstract

The genus *Anthemis* L. (Asteraceae) as medicinal plants are used both for pharmaceutical purposes and in folk medicine. In order to study of seed priming effects on seedling growth of two species of *Anthemis* spp., an factorial experiment based on randomized complete design with three replications was conducted under greenhouse conditions in Research Institute of Forests and Rangelands in 2014-2015.A factorial experiment consisting three factors: 1) two species including *Anthemis tinctoria* L. and *Anthemis triumfettii* (L.) DC. which formed the three levels of factor A, and 2) five conservation methods including: medium-term storage (active cold room 4 °C for 10 years), long- term storage (basic cold room-18 °C for 10 years), regenerated seeds in open air 22 °C for 2 years (Control) and aged seed under accelerated ageing (40 °C,98% of Relative humidity) for 48 and 72h made up the five levels of factor B, and 3) five priming treatments were including: Control (without priming), osmopriming (PEG -0.3Mpa), hormonal priming (Giberlic acid 250 and 500 mg/L), hydropriming (imbibitions with distilled water) were levels of factor C. Data collected for seed emergence percent, root and shoot length, seedling length, vigor index, seedling weight and three proxidase, catalaz and super oxid desmotaz (SOD) enzymatic activities. Result of analysis of variance showed that effects of species, conservation and priming and their interaction were significant for many of seedling traits and enzymatic activities. According to the results, the higher values of seedling emergence, vigor index, seedling length were obtained in *A. tinctoria*. In contrast, peroxidase and SOD enzymatic activity were the higher in *A. triumfettii*. All species had higher seedling growth by using osmopriming. Both osmo and hormonal priming method were effective in recovery of deteriorated seeds. The mean of all traits of three species were higher in base cold room (-18 °C) than active cold room (4 °C) and this a sign effect of low temperature on seed viability. The root length were higher in accelerated ageing test (48h and 72). It was due to positive effect of priming on improvement of deteriorated seed by increasing root length. The more seed emergence characteristics were obtained with effect of osmopriming (PEG 0.3Mpa), and, hormonal priming (Gibberellic acid 250 mg/L). Regarding to result of this research work, It was proved that two priming technique osmopriming using (Poly ethylene glycol) and hormonal priming (Gibberellic acid) were effective method for improvement of aged seed of *Anthemis* spp.

Keywords: Anthemis, Deterioration, Priming, Germination, Seedling growth

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Introduction

Anthemis L. is a genus of aromatic flowering plants in the family Asteraceae [1]. *Anthemis* species are native to the Mediterranean area, southwest Asia and Iran [2]. The species of the *Anthemis* genus are widely used in pharmaceutics, cosmetics and food craft. The flowers of the genus have well-documented uses as disinfectants and healing herbs, the main components being natural flavonoids and essential oils [3].

The *A. tinctoria* species is a biennial plant and used as a popular medical plant. It has a cathartic effect due to its bitter fruit oil. The species grows in rocky slopes in the wild with more sunshine, especially on limestone. This species grows in regions in the north, western north, west and central of Iran [4-2]. *A. triumfetti* is found in Europe, Iran and the Caucasus Mountains. Its flowering and maturity stages are in late spring and late summer, respectively [4].

Despite the impressive medicinal properties and wide usage of *Anthemis* in traditional medicine, it is generally under-utilized. Its plant material is mainly collected from wild in Iran. It is interesting horticultural and medicinal crops in semi-arid areas, due to its adaptation to drought conditions [5]. as well as their medicinal values. Introduction of wild medicinal plants into cultivation represents a great challenge and can result in modifications in the content of bioactive principles [6]. Seed banking is now widely used for the *ex situ* conservation of wild plant species in gene banks. Most of seed banks conserve germplasm of crop species and their wild relatives. Seed banks generally store seeds according to the gene bank standards [7] whoever, there are no specific standards for the conservation of wild plant species that grow in natural habitats. By increase in demand for samples of wild species, it may be necessary to propagated them in larger quantities. Although there are some statistical equations to predict deterioration periods of crop species [8] The are less reports for the determine the best times of regeneration of wild species in seeds bank. [7] recommended monitoring the seeds viability every 5 or 10 years for seeds in medium-or long-term storage, respectively. However, in Iranian natural resource gene bank (Research institute forest and rangeland) there are 45000 accessions that many of them are wild species as range, forage and medicinal plant species, that it is too difficult for monitoring and regeneration all of them. One of the major problems in wild species germplasm is lack of knowledge of how to break dormancy at the arrival time and for regenerating deteriorated seeds after many years. In most cases the seed dormancy is likely to be lost during storage, and the conditions required for germination (in particular, temperature) become less specific [9]. However there are some instances that deteriorated accessions failed to germination using the same treatments and/or conditions that were found to be optimum at the start of storage [9]. One of the methods that is often used as an invigoration treatment to ensure the seed germination is seed priming. This method is useful particularly if the seeds have already aged during storage [10]. On the other hand, insufficient seedling emergence and inappropriate stand establishment are the main constraints in areas receiving less rainfall such as Iran. It is well accepted fact that priming improves germination, reduces seedling emergence time and improves stand [11]. In priming, seeds are soaked in different solutions with high osmotic potential. This prevents the seeds from absorbing in enough water for radicle protrusion, thus suspending the seeds in the lag phase [12]. Seed priming have important role in increasing the yield of different crops in relation to enhance 37, 40, 70, 22, 31, 56, 50 and 20% in wheat, barley, upland rice, maize, sorghum, pearl millet, and chick pea respectively [13]. Seed priming have various techniques for improving the performance of the growth, emergence, and yield of the crop. There are some techniques which are used i.e. hydro-priming, halopriming, osmopriming and hormonal priming [11]. Osmopriming is commercially used technique for improving seed germination and vigor. It involves controlled imbibition of seeds to start the initial events of germination followed by seed drying up to its original weight. Osmopriming has many advantages including rapid and uniform emergence, improved seedling growth and better stand establishment under any environmental and soil conditions [14]. Hydro-priming involves soaking the seeds in water before sowing. In osmopriming, seeds are soaked for a certain period in solutions of sugar, polyethylene glycol (PEG), glycerol, sorbitol, or mannitol followed by air drying before sowing. Hormonal priming is the pre seed treatment with different hormones i.e. salicylic acid, ascorbate, kinetin, etc. which promote the growth and development of the

seedlings [11]. One of main problem in maintenance of seed in gene bank is regeneration of aged seeds that lose their viability over times. For increasing of seed germination traits, it is necessary to apply some seed dormancy breaking and seed priming treatments. Therefore this study was conducted to use the growth regulator substances priming on seeds to increase their germination and seedling growth of wild chamomile seeds that naturally preserved in medium (active store) and long-term storage (base store) and accelerate aged seeds of three species of in two species of *Anthemis tinctoria* and *A. triumfettii* in greenhouse condition.

Material and Methods

Seed of two native accessions of two *Anthemis* species as *A. tinctoria* (14221-Urmia, 18047-Naghdah, 19495-Baneh) *and A. triumfettii* (14170-Urmia, 16724-Mazandaran and 29705-Gilan) were provided from natural resource gene bank, Tehran, Iran. A factorial experiment consisting three factors: 1) two species including *A. tinctoria* and *A. triumfettii* which formed the two levels of factor A, and 2) five conservation methods including: medium-term storage (active cold room 4 °C for 10 years), long- term storage (basic cold room-18 °C for 10 years), regenerated seeds in open air 22 °C for 2 years (Control) and aged seed under accelerated ageing (40 °C,98% of Relative humidity) for 48 and 72h made up the five levels of factor B, and 3) five priming treatments were including: Control (without priming), osmopriming (PEG -0.3Mpa), hormonal priming (Gibberellic acid 250 and 500 mg/L), hydropriming (imbibitions with distilled water) were levels of factor C. The treated seeds of species were sown in 15 cm diameter plastic pots filled with sandy soil. and irrigated with tap water in greenhouse at 22±3°C. The pots were maintained at field capacity. Seedlings growth was complete for 50th days. The emergence characteristics including: emergence percentage, length of rootlet and shootlet, seedling length, vigor index, fresh weight of seedlings were evaluated during 50th days of experiment. The seed emergence percentage was calculated according to total number of emerged seedlings in numbering final day ISTA [15].

After 50th days of start of the experiment, length of rootlet (mm) and shootlet (mm) (that including 5 seedlings per pot on random) was measured according to Lekh and Kairwal [16]. Vigor index was calculated according to Abdulbaki and Anderson (17) that their values obtained from follow equations:

$$Vi = \frac{\%Gr \times MSH}{100}$$

Where:

%G r = final germination percentage

MSH = mean seedling height

VI = Vigor index

Enzyme Activities

Peroxidase:

Peroxidase activity was assayed essentially according to the modified 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 20mM Guaicol solution (0.2% in methanol, freshly prepared). The reaction was initiated with the addition of 0.1mL of0.2 mol/L H2O2. 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.

Catalase:

Catalase activity was estimated by the modified method of Aebi [19]. The reaction mixture contained 0.6 mL of enzyme extract, 0.1 mL of10 mmol/L H2O2 and 2mL of 30 mmol/L potassium phosphate buffer (pH 7.0). The absorbance was read at 240nm immediately after additionof the enzyme extract at an interval of 15 sec for 2 min. The blankwas without enzyme extract.

SOD Super Oxid Desmotaz

The SOD enzyme activity was assayed by the modified method of Giannopolitis and Ries [20]. 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 enzymeextract. SOD activity was assayed by measuring the ability of the enzymeextract to inhibit the photochemical reduction of NBT. Glasstubes containing the mixture was exposed to light (50 µEm–2 s–1).Identical tubes, which were not illuminated, served as blanks. After illuminationfor 10 min, the tubes were covered with black cloth and absorbancewas measured at 560 nm. Log A560 was plotted as a functionof the volume of enzyme extract used in the reaction mixture. Thevolume of enzyme extract, corresponding to 50% inhibition of thephotochemical reaction was obtained from the resultant graph. Oneunit of SOD was defined as the

level of enzyme activity that inhibitedthe photoreduction of NBT to blue formazan by 50% (expressed asunits SOD mg protein–1).

The three populations effects within each species were considered as replications and therefore, the main effects and interactions of population by priming and population by conservation methods were not included in the statistical analysis. Data analysis was carried out using SAS software and the differences between treatment means were tested using Duncan's Multiple Range Test.

Results

Results of the analysis of variance (ANOVA) showed significant differences among species, conservation methods and priming treatments for all of the seed emergence and seedling traits ($p<0.01$) (Table 1).

For enzymatic activities, there were significant differences between species for peroxidase and peroxide dismutase (SOD) ($p<0.01$), between conservation method for SOD (($p<0.05$)), and between priming for catalyze ($p<0.05$) and SOD ($p<0.01$). Interaction between species and conservation was significant for peroxidase ($p<0.01$) and SOD ($p<0.05$). Between species and priming was significant for peroxidase and catalyze ($p<0.01$), and finally, between conservation and priming was significant for SOD ($p<0.01$) (Table 1).

In comparisons between species, the result showed that the higher values of seedling emergence, vigor index, seedling length with average values of 65.85%, 79.06 and 123.04 mm, respectively, were belongs to *A. tinctoria* In contrast, the higher values of peroxidase and SOD enzymatic activity were obtained in *A. triumfettii* (Table 2).

In comparison between conservation method, the highest values for seedling emergence (61.87%), vigor index (72.37 mm), shoot length (49.55mm) were observed in long- term storage (basic cold room). For root length (113.80mm) and seedling length (145.40mm) were obtained in aged seeds in (48 h) and the higher seedling weight (143.71 mg) was obtained in aged seeds in (72 h). For enzymatic activities the higher catalase and peroxidase concentration were obtained in aged seeds in (48h) and the highest SOD activity was obtained in regenerated (control) seeds (Table 3).

In comparisons between priming treatments, the highest seedling emergence (78.67%), vigor index (76.57) were obtained in control (no priming). The highest mean values of root length (93.54 and 93.34 mm), shoot length (42.33 and 41.49 mm), seedling length (135.93 and 134.82 mm) and seedling fresh weight (61.85 and 64.77 mg), respectively, were obtained for seeds treated by GA250 and osmopriming, respectively. For enzymatic activities, the highest peroxidase and catalase activity were observed by GA500 and GA250, respectively (Table 4).

The means of traits of two species at five conservation methods (species by conservation interaction effect is presented in the Table 5. Result showed that in *A. tinctoria* the highest seedling emergence and vigor index were obtained in base cold room followed by active room. For *A. triumfettii* highest seedling emergence and vigor index were obtained in control followed by active cold room

Result showed that the highest mean values of root length, seedling length and seedling weight were obtained with accelerated aged seed (8h and 72h). In both species, the highest and lowest peroxidase and catalase enzymatic activities were obtained in regenerated seeds and aged seed (72h), respectively (Table 5).

The means of species by priming interaction effects are presented in the Table 6. Result showed that in both species the highest values of seedling emergence and vigor index were obtained in regenerated seeds (control). In *A. tinctoria*, the highest values of root length, shoot length, seedling length and seedling fresh weight were obtained in seed priming using GA250mg/L followed by osmopriming. The same trend was observed for *A. triumfettii* In contrast , in both species the lowest root length were obtained in control (no priming).

In both species, the highest catalase activity obtained in seeds treated by GA500 followed by hydropriming. The higher values of peroxidase activity were obtained using osmopriming.

The means of conservation by priming methods interactions are presented in the Fig. 1 and 2. Result showed that the highest seed emergence and vigor index were obtained in regenerated seeds and the old seeds preserved in both base and active cold store without priming, indicating that priming had no impact on increasing of seedling emergence on new seeds. For aged seeds the osmopriming significantly had increased seed emergence percentage and vigor index. However, for seeds preserved in base and active cold store and aged

seeds, the osmopriming had significantly increased root length, shoot length, seedling length and seedling fresh weight than that for control (no priming) (Fig. 2).

For enzymatic activities, the highest Peroxidase activity was obtained in aged seeds using hydropriming, osmopriming and GA500 mg/L. The highest catalyze concentration were observed in regenerated seed (control) using both hydropriming and GA250 mg/L. Similarly, the highest SOD activity was observed using GA250 mg/L and osmopriming in generated (control) seeds (Fig. 2).

Discussion

Result showed that in both species the highest seedling emergence and vigor index were obtained in base cold room followed by active room and regenerated seeds, indicating the effect of low temperature in keeping seed viability for a long time. The seeds preserved in base store with low humidity and low temperature had low metabolic activity and causes it late deterioration. In contest, in active store there was more traffic of staff and open/close the door and also repeated power fluctuations and humidity cause early seed deterioration. Similar to our research [21] showed that by an research which have been done for evaluation and the study of germination potential, rate of emergence and vigour index of the seeds of two species of medicinal plant *Eruca sativa* Mill. and *Anthemis altissima* L.) under cold room and dry storage condition. Their result showed that the seed of *A. altissima* have more germination characteristics in the room temperature compare in active cold room .

In comparisons between priming treatments on seedling traits, result showed that the highest values of root length, shoot length, seedling length and seedling fresh weight seeds treated by osmopriming. In contrast the lowest root length was obtained in control (no priming). Result showed that the highest root length, seedling length and seedling weight were obtained in accelerated aged seed (48h and 72h). The lowest values of these traits were obtained in active cold room. Accelerated aging test is used to evaluation of seeds physiological potential of various species [22]. Alizadeh [23] studied the germination percentage, total rate of germination and vigor of 17 medicinal plants species in following genus: Foeniculum, Saliva, Echium, Hyssopus, Sanguisorba, Nigella, Alyssum, Ziziphora, Anthemis, Melissa, Cuminum, Plantago, Iranian Achillea, Foreign Achillea and Silybum by ageing test and his result showed that germination some those species declined as zero, therefore there were no any planting value for them.

The principle of this method is based on artificial accelerated aging seeds by placing seeds at high temperature and high relative humidity as environmental factors in order to the intensity and speed of aging [24]. In this case, low-quality seeds will deteriorate faster than healthy seeds with higher vigor [25]. The most important changes that happen in deteriorated seeds are oxidation reactions such as the production of free radicals, dehydrogenation of enzymes and proteins, reduction of membrane permeability and increased electrolyte leakage under the influence of free radicals, changing the molecular structure of nucleic acids and reduce enzymes activities [26]. The higher rootlength was obtained in accelerated aging test by application of all of priming treatments except hydropriming. For vigor index, hormonal priming.

This result which agreement with the result of Fallahhossieni *et al*., [27]. Their result showed that The higher root length was obtained in accelerated aging test by application of all of priming treatments except hydropriming.. The positive effect of gibberellic acid may be due to its important role in seedling vegetation and elongation by cell division [28]. However Sajjadi Jaghargh *et al* [29] stated that that seed priming with 125 and 250 mg/L gibberellic acid improved emergence percentage (94%), rate of emergence (18.54 sprout/day) and vigor index (83.29) species of *A. altissima*. [30] stated osmopriming using PEG was to improve germination traits in tomato.

In comparisons between priming treatments on enzymes activity, result showed that the highest peroxidase activity was obtained in aged seeds (48h and 72h) using hydropriming, osmopriming and GA500 mg/L. (Fig. 2). This result was similar with result of [31], they studied effect of seed priming on the enhancement of germination traits of aged seeds of Chamomile (*Matricaria chamomilla* L.) and *Achillea vermicularis* Trin. preserved in medium and long-term storage and their result showed that two priming technique Osmo-priming using (PEG) and hormonal priming (Giberlic acid) were effective method for recovery of aged seed.

Table 1 Analysis of variance and mean of squares of two *Anthemis* L. species seed traits under greenhouse conditions

Source of variation	DF	Seedling emergence %	Vigor index	Root Length mm	Shoot Length mm	Seedling Length mm	Fresh Weight Mg/p	Peroxidase enzymatic activity	Catalyze enzymatic activity	Superoxide dismutase (SOD)
Species	1	38348**	69379**	385.6**	3246.7**	8042.2**	2765**	379.2**	2397.69 ns	14.23**
Conservation	4	19433**	14952**	73847**	5650.1**	50842**	11174**	20.01 ns	3071.55ns	7.80*
Priming	4	16346**	23253**	10981**	2577.2**	21385**	4969.6**	22.71 ns	9016.97*	17.66**
Spec*Conserv	4	4592.2**	8185.9**	805**	253.3**	1487.0**	468.6**	101.55**	1976.05ns	6.86*
Spec * Prim	4	903.0**	2451.3**	895**	220.6**	1016.5**	1235.8**	36.76*	7089.38*	3.74 ns
Conserv *Prim	16	2349**	2950.8**	1743**	359.2**	2361.5**	733.5**	16.76 ns	2535.10 ns	13.48**
Spec*Cons *Prim	16	250.1**	809.1**	469**	127.2**	752.0**	546.0**	24.00 ns	2800.24 ns	4.82 ns
Error	102	165.6	363.12	109.5	52.7	218.4	258.6	10.05	2723.06	2.03
CV%		25.97	31.87	12.60	17.74	11.89	26.37	34.18	27.69	33.02

ns , *, **: non-significant and significant at P= 0.05 and 0.01 levels, respectively.

Table 2 Mean comparison of two *Anthemis* L. species seed traits under greenhouse conditions

Species name	Seedling emergence %	Vigor index	Root Length mm	Shoot Length mm	Seedling Length mm	Fresh Weight mg	Peroxidase enzymatic activity	Catalyze enzymatic activity	Superoxide dismutase (SOD)
A.tinctoria	65.85 a	79.06 a	81.82 a	41.22 a	123.04 a	55.14 a	4.76 b	194.52 a	3.98 b
A.triumfettii	37.59 b	41.17b	78.88 b	37.10 b	116.02 b	56.30 a	9.90 a	189.31a	5.09 a

Means with the same letter are not significantly different (P=0.01) .

Table 3 Mean comparison of five seed deterioration treatments traits under greenhouse conditions

Deterioration	Seedling emergence %	Vigor index	Root Length mm	Shoot Length mm	Seedling Length mm	Fresh Weight mg	Peroxidase enzymatic activity	Catalyze enzymatic activity	Superoxide dismutase (SOD)
Control	49.29 b	60.32 b	85.99 b	40.55 b	126.54 a	57.02b	8.42 b	198.80 a	5.68 a
Aging 48 h	37.47 c	55.22 c	113.80 a	31.64 c	145.40 a	59.18b	9.76 a	192.09 a	4.50 b
Aging 72 h	33.50 c	48.29 d	113.24 a	30.33 c	143.71 a	72.93a	9.26 ab	176.25b	3.87 c
Active store	58.98 a	54.53 c	49.39 c	39.27 b	88.67 b	42.60c	-	-	-
Base store	61.87 a	72.37a	64.34 c	49.55 a	113.89 a	54.55b	-	-	-

Means with the same letter are not significantly different (P=0.01) .

Table 4 Mean comparison of five seed priming treatments traits under greenhouse conditions

Priming Treatments	Seedling emergence %	Vigor index	Root Length mm	Shoot Length mm	Seedling Length mm	Fresh Weight mg	Peroxidase enzymatic activity	Catalyze enzymatic activity	Superoxide dismutase (SOD)
Control	78.67 a	76.57 a	55.81 c	41.40 a	97.20 c	48.02 c	-	-	-
GA250 mg/L	48.82 b	64.00 b	93.54 a	42.33 a	135.93 a	61.85 a	7.99 b	208.00 a	4.63 a
GA500 mg/L	44.36 c	51.27 c	81.28 b	34.23 b	115.52 b	55.48 b	10.41 a	166.67 b	4.80 a
Osmo-0.3MP	51.56 b	69.68 a	93.34 a	41.49 a	134.82 a	64.77 a	9.62 ab	171.22 b	3.44 b
Hydropriming	35.18 d	39.07d	77.77 b	36.37 b	114.17 b	48.49 c	8.55 b	214.04 a	4.78 a

Means with the same letter are not significantly different (P=0.01).

Table 5 Mean comparison of three two *Anthemis* L. species seed traits at five Deteriorate methods under greenhouse conditions

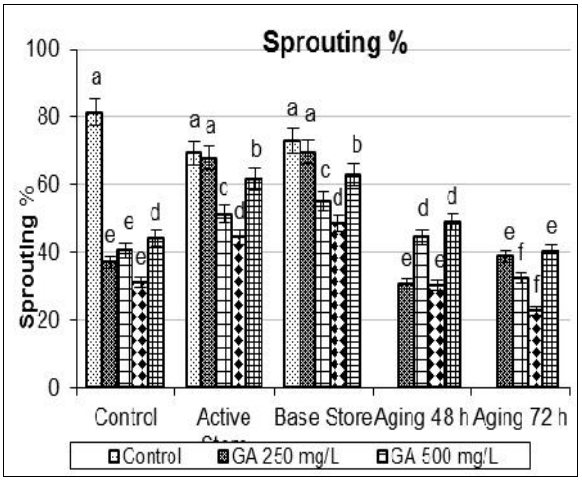
Species name	Superoxide dismutase (SOD)	Catalyze enzymatic activity	Peroxidase enzymatic activity	Fresh Weight mg	Seedling Length mm	Shoot Length mm	Root Length mm	Vigor index	Seedling emergence %	Conservation
A.tinctoria	Control	52.67c	82.27b	65.54c	123.18c	40.91c	3.75c	54.63c	2.10c	194.06a
	Aging 48 h	53.61c	112.82a	79.62b	147.18 a	34.36e	5.59c	57.83c	4.11b	200.98a
	Aging 72 h	41.22e	113.83a	60.07cd	145.95 a	32.13e	4.85c	70.83a	3.83c	188.35ab
	Active store	77.29b	55.06d	79.10b	97.90d	42.84c	-	43.58d	-	-
	Base store	86.93a	67.43c	105.41a	119.94c	52.51a	-	54.98c	-	-
A.tinctoria	Control	45.91d	89.71b	55.10d	129.90c	40.19d	12.82a	59.42b	4.65b	204.16a
	Aging 48 h	21.76g	114.23a	31.27e	142.94 b	28.79f	9.03b	60.66b	5.99a	183.59ab
	Aging 72 h	25.78g	112.65a	36.50e	141.47 b	28.54f	7.46b	75.04a	4.29b	178.11b
	Active store	40.67e	43.73e	32.96e	79.43e	35.70e	-	41.62d	-	-
	Base store	36.80f	61.25c	39.34e	107.84d	46.59b	-	54.11c	-	-

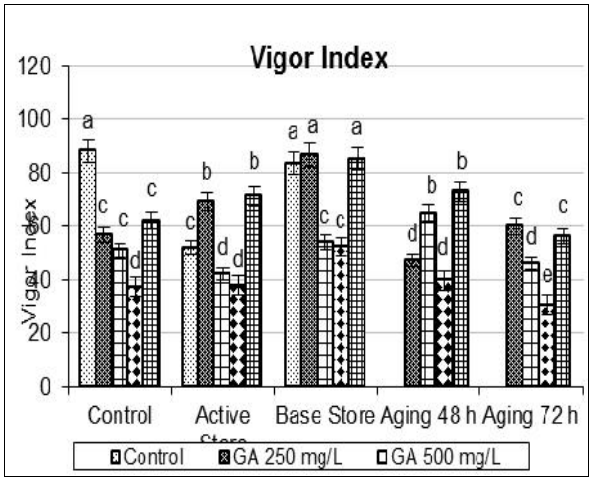
Means with the same letter are not significantly different (P=0.01)

Table 6 Mean comparison of two *Anthemis* L. species seed traits at five priming treatments under greenhouse conditions

Species name	Priming	Seedling Emergence %	Vigor index	Root Length mm	Shoot Length mm	Seedling Length mm	Fresh Weight mg	Peroxidase enzymatic activity	Catalyze enzymatic activity	Superoxide dismutase (SOD)
A.tinctoria	Control	91.19 a	91.27a	57.52c	41.67c	99.19 g	48.96 d			
	GA250mg/L	60.40 c	81.64b	93.01a	46.34a	139.35 a	62.69 a	2.73 c	243.38 a	3.89 b
	GA500mg/L	59.78 c	73.25c	89.45a	36.47e	125.92 d	60.40 b	4.00 c	167.01 b	5.21 a
	Osmoprim	71.73 b	98.16a	92.65a	43.60b	136.24 b	61.69 a	7.66 b	155.78 c	2.42 c
	Hydroprim	46.13 d	50.99e	76.45b	38.05e	114.51e	41.96de	3.87 c	224.58 a	4.45 b
A.triumfettii	Control	66.15 bc	61.87d	54.09c	41.13d	95.22 g	47.07 d			
	GA250mg/L	37.24 e	46.35f	94.06a	38.32e	132.51 c	61.01 a	8.77 b	198.79 ab	5.32 a
	GA500mg/L	28.93 f	29.38g	73.06b	31.96 f	105.02 f	50.75 d	13.38 a	159.94 c	6.03 a
	Osmoprim	31.38 f	41.20f	94.03a	39.38e	133.40 c	67.84 a	8.50 b	170.84 b	3.84 b
	Hydroprim	24.33 g	27.26h	79.34b	34.45 f	113.85 e	55.30 c	8.62 b	240.45 a	5.45 a

Means with the same letter are not significantly different (P=0.01) .





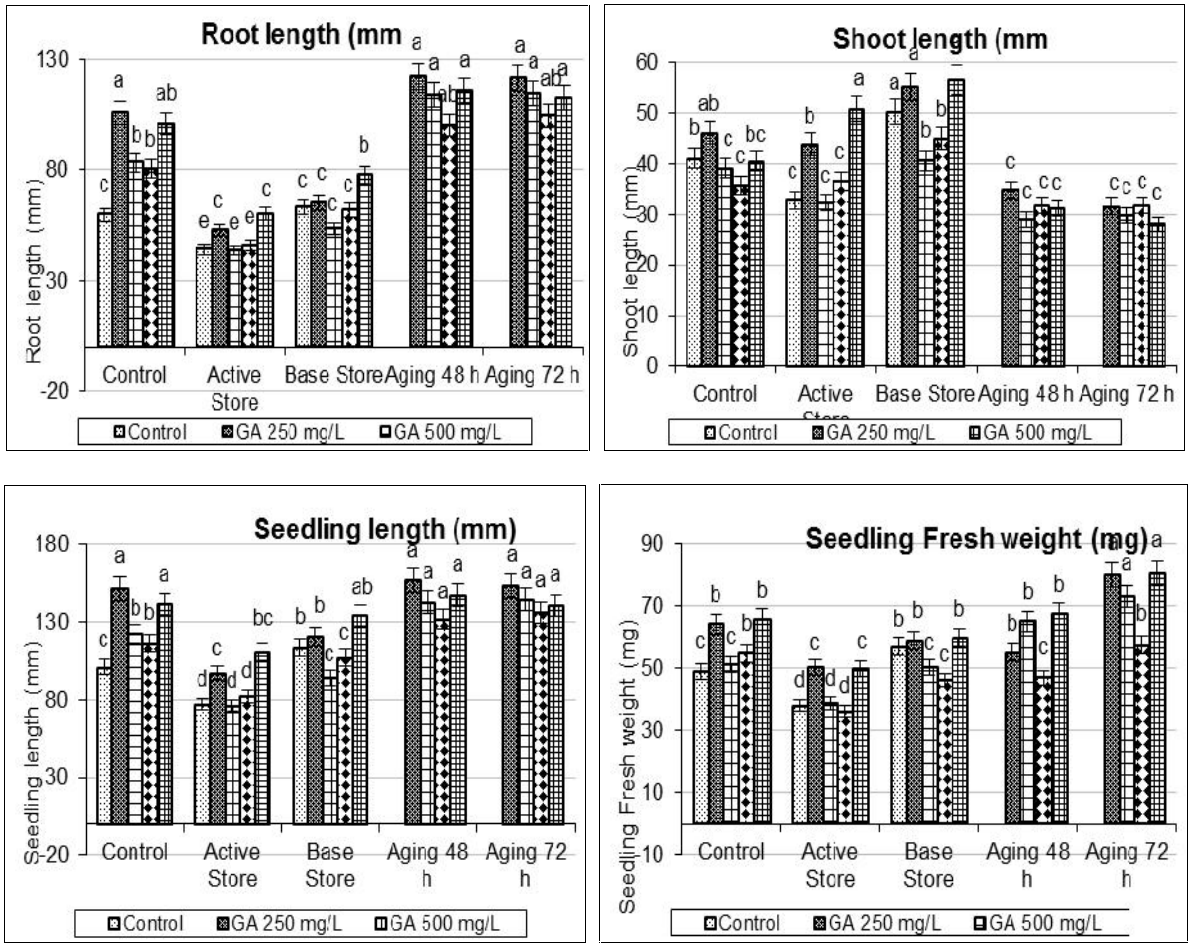
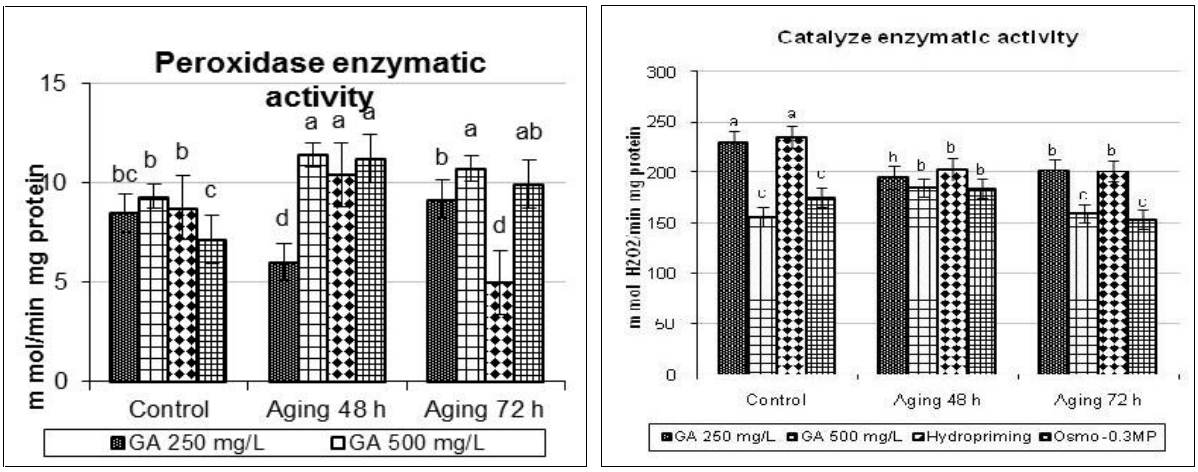


Fig. 1 Priming by deterioration interaction effects for germination percent, root and shoot length seedling length, vigor index and seedling weight two *Anthemis* L. species under greenhouse conditions



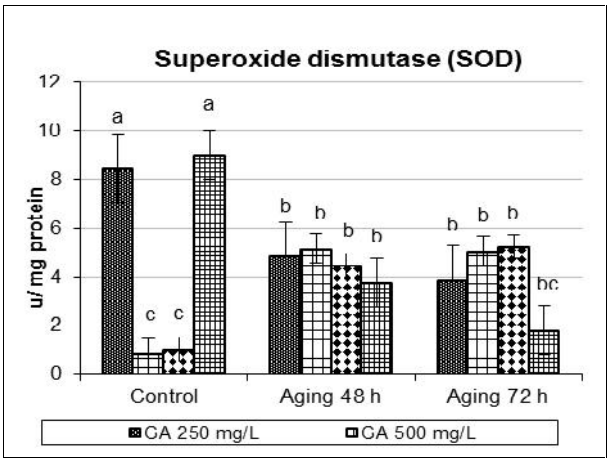


Fig 2. Priming by Deterioration interaction effects for three Proxidase, Catalaz and Super Oxid Desmotaz (SOD) enzymatic activities of two *Anthemis* L. species under greenhouse conditions

The highest catalyze concentration were observed in regenerated seed (control) using both hydropriming and GA250 mg/L. Similarly, the highest SOD activity was observed using GA250 mg/L and osmopriming in generated (control) seeds (Fig. 2).[32] found antioxidation effects of Vitamin C on superoxide dismutase (SOD), and catalase (CAT) activity. [33] reported that priming with 30% PEG for 24 h resulted in increase in the activity of superoxide dismutase (SOD) and peroxidase (POD) and a rapid increase in the respiratory intensity, which were associated with an increase in germination vigor.

Conclusion

Our study showed that priming is useful method to improve quality of deteriorated and old seeds. According to the results the higher values of seedling emergence, vigor index, and seedling length were obtained in *A. tinctoria*. In contrast, peroxidase and SOD enzymatic activity were the high in *A. triumfettii* . Both species had higher seedling growth using osmopriming and hormonal priming. This two priming method were also effective in recovery of deteriorated seeds. The mean of all traits both species were higher in base cold room (-18 °C) than active cold room (4 °C) and this a sign effect of low temperature on seed viability. The root length were higher in accelerated ageing test (48h). It was due to positive effect of priming on improvement of deteriorated seed by increasing root length. The more seed emergence characteristics were obtained with effect of osmopriming (PEG 0.3Mpa), and, hormonal priming (Gibberellic acid 250 mg/L). Regarding to result, of this research work, It was proved that two priming technique Osmopriming using (Poly ethylene glycol) and hormonal priming (Gibberellic acid) were effective method for improvement of aged seed.

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