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Original Article

Priming Effect of on the Enhancement of Germination Traits in Aged Seeds of Chamomile (*Matricaria chamomilla* L.) Seeds Preserved in Medium and Long-term Storage

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Abstract

Chamomile (*Matricaria chamomilla* L.) is a widely used medicinal plant possessing several pharmacological effects due to presence of active compounds. In order to study of seed priming effects on seedling growth of chamomile, an experimental design, based on randomized complete design with three replications was conducted under greenhouse conditions in Research Institute of Forests and Rangelands in 2014-2015. Experimental factors were A) three chamomile accessions as code of gene bank 8959 (Brojen), 15123 (Arak) and 23879 (Isfahan), B) five conservation methods including: medium-term storage (active cold room 4 °C for 15 years), long- term storage (basic cold room-18 °C for 15 years), regenerated seeds in open storage 22 °C for 2 years (Control) and aged seed under accelerated ageing (40 °C,98% of relative humidity for 48 and 72h) and C) priming treatments including: without priming /(control), osmopriming (PEG-0.3Mpa), hormonal priming (Gibberllic acid 250 and 500ppm), hydropriming (imbibition with distilled water). Data collected for seed emergence percent, root and shoot length, seedling length, vigor index, seedling weight and three Proxidase, Catalase and Super Oxid Desmotaz (SOD) enzymatic activities. Variance analysis showed significant effects of all factors and their interactions except accession by conservation interaction effects for seedling length ($P<0.01$). Result showed that that accession two accessions 23789 (Esfahan) and15123 (Arak had higher mean values than one other accessions for most of the traits in both medium-term and long- term storage. For aged seeds, the higher root length, seedling length mean values were obtained in 48h accelerate aging test. In comparisons between priming treatments, the higher means of traits were obtained in osmopriming except germination percent. For enzymes activity the trends of accession were not similar but the effect of osmopriming on catalase activity were high and stable in three accessions. The result of priming by conservation interaction showed that the higher seed emergence was obtained in regenerated seeds (control). The higher root length was obtained in accelerated aging test by application of all of priming treatments except hydropriming. For vigor index, hormonal priming (GA250) had higher effect followed by osmopriming. Similarly, for enzymatic activities GA500 had significant impact on proxidas and catalase. It was proved that two priming technique Osmo-priming using (PEG) and hormonal priming (Gibberllic acid) were effective method for recovery of aged seed.

Keywords: Chamomile, *Matricaria,* Deterioration, Priming, Germination, Seedling growth

Introduction

German chamomile plant with the scientific name of *Matricaria recutita* L. and *Matricaria chamomilla* L. Asteraceae family that its flowers

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have medicinal value and are used frequently as anti-inflammatory, antispasmodic and muscle cramps, carminative, Mild sedative and antiseptic therapeutic usage [1]. Chamomile is a perennial plant, 20 to 80 cm in height, which grows naturally in rangelands. The plant has a branched stems, each lead to a flower head with a diameter of 1.5 to 2 cm. The leaves are long and thin with leaflet like notches. The flower heads are constructed by two types of white marginal ligulate florets and non-marginal yellow tubular florets [2]. Wild populations of chamomile are diploid. Diploid and artificially generated tetraploid varieties are cultivated, the tetraploid varieties having larger flower heads and a higher 1000-seed weight [3].

Its distributed and cultivated in the temperate regions of Europe, Asia, and America, as well as in northern and southern Africa is an important medicinal plant in Iran that traditionally have been used for the treatment of various diseases [4]. The medicinal and pharmacological effects of chamomile are mainly connected with its essential oil for its antispasmodic, antimicrobial and disincentive properties Chamomile essential oil is widely used in food, cosmetics and pharmaceutical industries [5]. Chamomile flowers contain 0.3 to 1.5% oil which is mostly composed of the bulk bisabolol sesquiterpens, bisabolol oxide, farnesene, spathulenol and chamazulene. The European chamomile includes Bisabolol rather than Bisabolol oxides [6].

Despite the impressive medicinal properties and wide usage of chamomile in traditional medicine, it is generally under-utilized. Plant material is mainly collected from wild, and thus its gene pools are shaped by environmental factors. It is interesting horticultural and medicinal crops in semi-arid areas, due to its adaptation to drought conditions as well as their high essential oil production. Introduction of wild medicinal plants into cultivation represents a great challenge and can result in modifications in the content of bioactive principles [7].

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 genebank standards [8] 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 [9]. They are less reports for the determine the best method of regeneration of wild species in seeds bank. FAO [8] 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 [10]. 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 [10]. 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 [11]. In 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 [12].

Priming technique is one of the most important methods for seed enhancement. In this method, the seed would be imbibed with water firstly and then those imbibed seeds dried in air room temperature. This process induced to increasing of germination percentage and rate of germination, improvement of seedling establishment and seedling vigor in different environmental condition [13,14].

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 [13]. 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 [15].

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 [16]. Osmopriming is commercially used technique for improving seed germination and vigour. 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 [17].

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 [12]. Soaking seed with an appropriate level of plant growth hormones has positive effect on germination and the performance of different plant species in normal and stress conditions [18,19].

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 Chamomile (*Matricaria chamomilla* L.) in greenhouse condition.

Material and Methods

Seed of three native accessions of Chamomile as codes of 8959 (Brojen), 15123 (Arak) and 23879 (Isfahan) were provided from natural resource gene bank, Tehran, Iran.

A factorial experiment consisting three factors: 1) three accessions including 8959, 15123 and 23879 which formed the three levels of factor A, and 2) five conservation methods including: medium-term storage (active cold room 4 °C for 15 years), long-term storage (basic cold room-18 °C for 15 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 (Gibberllic acid 250 and 500ppm), hydropriming (imbibitions with distilled water) were levels of factor C.

Seeds of various treatments 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. Data collected for number of emerged seedlings, shoot length, root length, seedling length seedling fresh weight and seed vigor index. The rate of emergence and seed vigor were recorded according [20] and [21], meanwhile, the Proxidase, Catalaz and Super Oxid Desmotaz (SOD) enzymatic activities were measured [22]. Data analysis was carried out using SAS software and the differences between treatment means were tested using Duncan's Multiple Range Test.

Results and Discussion

Results of the analysis of variance (ANOVA) showed significant differences among different levels of accession (except root length), conservation methods and priming treatments for all of the traits. There were significant differences between accession by conservation interaction for all of traits except seedling length and seedling weight. Similarly, were significant differences between accession by priming and conservation by priming for all of traits (Table 1).

In comparisons between accession the higher seedling emergence, vigor index, root and shoot length were obtained in two accessions 23789 (Esfahan) and15123 (Arak) in at five conservation methods with priming technique. It was expected since these two accessions were newer than other one accession.

The means of traits of three accessions at five conservation methods are presented in the Table 2. Result showed that the highest root length, shoot length, seedling length and seedling fresh weight was obtained in long-term storage (base cold room). The same trend was observed for accessions with smaller values in medium- term storage (active store) (Table 2) indicating the effect of low temperature in keeping seed viability. The lower germination characteristics including: germination percentage (21, 20.67), vigor index (31.23, 30) of the accession of 8959-Brojen observed in aged seeds with Ageing 48 h and 72h (Table 2). The higher germination characteristics, shoot length and vigor index obtained in base and active cold room for both accessions of 15123 (Arak) and 23879 (Isfahan) (Table 2).

The seeds preserved in base store with low humidity and 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. Meanwhile, in chamomile, the base temperature of germination is 6 °C [23] so; it is likely to start primary metabolites activities the seeds in the active store. Similar to our research Rincker [24] showed that during the 20 years of storing 37 accessions of alfalfa seeds at (-15 °C) with a relative humidity of 60%, the trend of germination decreases were low from 91 to 81%, whereas, In open storage conditions during 10 years Priestley [25] reported the half of the

Seeds lost their viability. Alizadeh [26] studied the germination percentage, total speed of germination and vigor of 17 medicinal plants species by ageing test and his result showed that germination some those species declined as zero, therefore there were no any planting value for them.

In comparisons between priming treatments on seedling traits, result showed that the in active store gibberellic acid (GA 250) and osmopriming increased shoot length, seedling length, seedling fresh weight and vigor index in comparison to control (Table 3 and Fig. 1). Both hormonal priming (GA 250) and osmopriming (PEG 0.3MP) induced more root length (95.10, 96) and fresh weight (85.61, 85.87) for the accession of 8959-Brojen. Also shoot length, seedling length, seedling fresh weight and vigor index of both accession 15123 (Arak) and 23879 (Isfahan) in increased with hormonal priming (GA 250) and osmopriming (PEG 0.3MP) (Table 2). In contrast, hydropriming had no more effects on the improvement of traits for these three accessions. Priming of the Iranian Angelica seed with poly ethylene glycol lead to improve of germination of percentage, speed of germination and seedling vigor (27).

In accelerate aging for 48 and 72 hours, gibberellic acid and osmopriming had significant impact on vigor index and all of priming treatments had increased root length, seedling length and seedling weight that for control (Fig. 1).

Accelerated aging test is used to evaluation of seeds physiological potential of various species [28]. 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 [29]. In this case, low-quality seeds will deteriorate faster than healthy seeds with higher vigor [30]. 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 [31].

Similar to our study, Amooaghaie [32] showed that both osmopriming and hydropriming improved alfalfa seedling germination and growth than that for control. Farooq *et al*.,[33] in study of the effect seed priming in rice seedling growth found higher effect of priming on root length than shoot length. Similar to our study, gibberellic acid was effective on recovery of deteriorated seeds of rapeseed [34]. Esvand *et al* [35] reported that primed seeds of *Agropyron elongatum* using GA100 ppm led to increasing rate of emergence to 43% and vigor index to 40%. Sajjadi Jaghoroghi *et al* [36] studied Effect of Osmopriming, hydropriming and pre-chilling on seed emergence enhancement and seedling vigor of four medicinal species of Anthemis under greenhouse conditions, their result showed that primed seeds with gibberellic acid and potassium nitrate (osmopriming) improved species of Anthemis germination potential and seedlings growth.

The positive effect of gibberellic acid may be due to its important role in seedling vegetation and elongation by cell division [37]. El-Araby and Hegazi [38] stated osmopriming using PEG was to improve germination traits in tomato.

In comparisons between priming treatments on enzymes activity (Table 3 and Fig. 1), result showed that GA 500 followed by osmopriming had increased Catalase enzyme activity in deteriorated seeds of accession 15123 (Arak in (48 h) than other treatments. Similarly, GA 250 had increased peroxidase enzyme activity and Catalase enzyme activity in deteriorated seeds of 23879 (Isfahan) in (72 h) than other treatments. Osmopriming using PEG (0.3MP) had increased Superoxide Desmotas (SOD) activities in deteriorated seeds (72 h) for the23879(Isfahan . In similar results Amooaghaie [32] showed that priming increased peroxidase, catalase and superoxide dismutase activity in two varieties of alfalfa seedlings under saline stress conditions. The activity of antioxidant enzymes including:Acsorbat proxidase and Glutation reductase decreased in deteriorated of soybean seed (39).

Azooz [40], reported priming of salicylic acid increased antioxidant enzymes in *faba bean* seedling. Burguieres, *et al* [41] found antioxidation effects of Vitamin C on superoxide dismutase (SOD), and catalase (CAT) activity.

Yang *et al* [42] in soybean found drought stress priming decreased seedling membrane permeability and increased SOD and peroxidase (POD) activities. Jie *et al*. [43] 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.

Table 1 Analysis of variance and mean of squares of *Matricaria chamomilla* L. seed traits under greenhouse conditions

Source of variation	DF	Germination %	Vigor index	Root Length	Shoot Length	Seedling Length	Fresh Weight	Peroxidase enzymatic activity	Catalyze enzymatic activity	Superoxide dismutase (SOD)
Accession	2	1973.8**	4507.4**	72.62^{ns}	2964.4**	4064.6**	673.5*	285.8**	6447**	28.28**
Deterioration	4	13945**	15034**	18683**	722.2**	14651**	3163.1**	347.2**	10519**	15.11**
Priming	4	5073.5**	7723.8**	5251.7**	1418.7**	9659.9**	2226.7**	23.4**	2112**	7.67**
Acc* Deterioration	8	1155.1**	2594.8**	201.6**	64.48**	123. 6 ^{ns}	190.7 ^{ns}	122.9**	4270**	4.81**
Acc* Priming	8	144.8**	515.06**	526.6**	316. 2**	1373.4**	824.7**	132.7**	8455**	19.22**
Deter *Priming	14	939.7**	1657.5**	750.8**	216.3**	1292.4**	730.3**	60.8**	3547**	32.92**
Acc*Deter*Prim	24	201.4**	628.70**	209.2**	80.3**	369.6**	352.8^{ns}	2.9^{ns}	1363*	2.54**
Error	129	45.82	104.14	56.66	16.65	89.86	246.7	2.21	345.5	0.19
CV%		13.66	16.59	8.94	9.02	7.28	22.17	11.11	10.28	11.63

^{ns}, *, **: no significant and significant at P= 5 and 1% respectively.

Table 2 Mean comparison of three accessions of *Matricaria chamomilla* L. seed traits at five conservation methods under greenhouse conditions

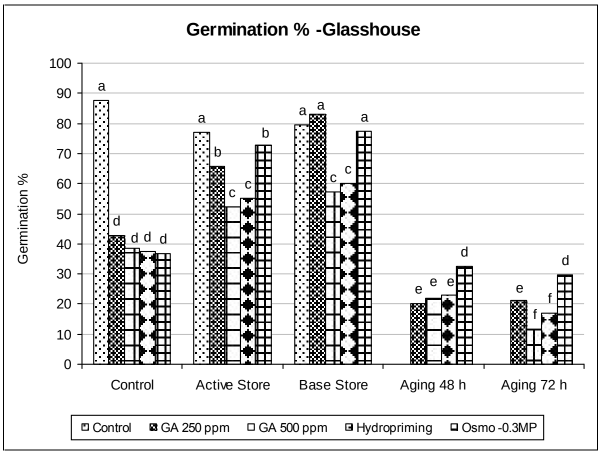
Accession	Deterioration	Germination %	Vigor index	Root Length (mm)	Shoot length (mm)	Seedling length (mm)	Fresh weight (mg)	Peroxidase enzymatic activity (%)	Catalyze enzymatic activity (%)	Superoxide dismutase (SOD) (%)
8959	Control	56.17 c	68.87 b	81.51 b	39.87 b	121.38 b	67.20 c	11.18 a	196.8 a	-
	Aging 48 h	21.00 e	31.23 e	115.22 a	31.66 c	146.87 a	85.55 a	10.12 a	205.8 a	4.38 a
	Aging 72 h	20.67 e	30.00 e	110.43 a	29.69 c	141.48 a	90.30 a	11.73 a	148.3 b	4.33 a
	Active store	44.40 d	43.02 d	55.39 d	36.36 b	94.26 d	63.28 c	-	-	-
	Base store	61.20 c	65.58 b	60.30 c	41.97 b	102.27 c	65.87 c	-	-	-
15123	Control	47.29 d	57.35 c	86.69 b	50.94 a	137.63 b	67.56 c	2.96 b	231.9 a	-
	Aging 48 h	25.33 e	40.73 d	105.33 a	53.33 a	161.70 a	87.50 a	14.48 a	195.3 a	1.54 c
	Aging 72 h	24.67 e	40.54 d	119.06 a	39.89 b	163.27 a	86.33 a	14.73 a	155.6 b	1.29 c
	Active store	70.93 ab	80.36 ab	61.89 c	50.67 a	112.55 c	57.67 d	-	-	-
	Base store	71.87 ab	88.77 a	68.62 bc	53.97 a	122.59 bc	65.27 c	-	-	-
23879	Control	49.73 cd	78.93 b	95.11 b	49.06 a	144.17 b	71.44 b	3.76 b	155.8 b	-
	Aging 48 h	31.56 d	51.28 c	107.83 a	55.72 a	163.55 a	84.39 a	23.97 a	154.3 b	2.89 b
	Aging 72 h	14.67 f	22.95 f	116.42 a	43.74 b	162.46 a	72.68 b	20.18 a	174.3 b	4.55 a
	Active store	78.40 a	89.73 a	61.89 c	50.67 a	112.55 c	57.67 d	-	-	-
	Base store	81.33 a	100.64 a	68.62 bc	53.97 a	122.59 bc	65.27 c	-	-	-

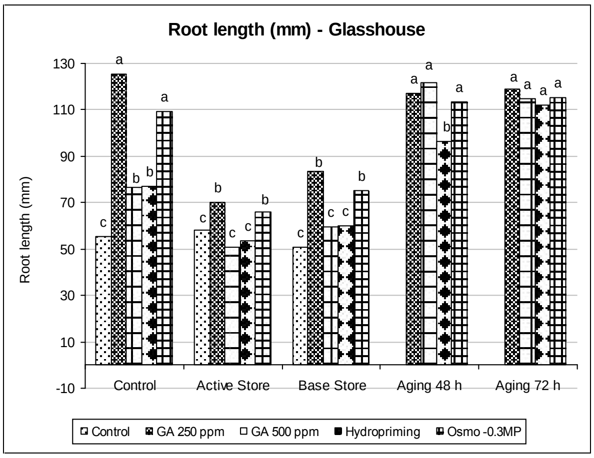
Means with the same letter are not significantly different ($P \leq 0.01$)

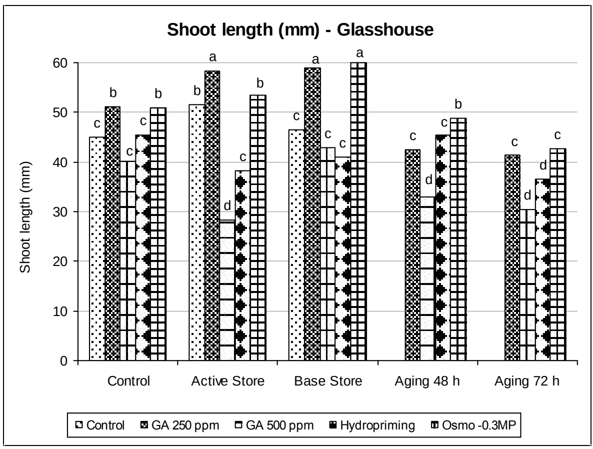
Table 3 Mean comparison of three accessions of *Matricaria chamomilla* L. seed traits at five priming treatments under greenhouse conditions

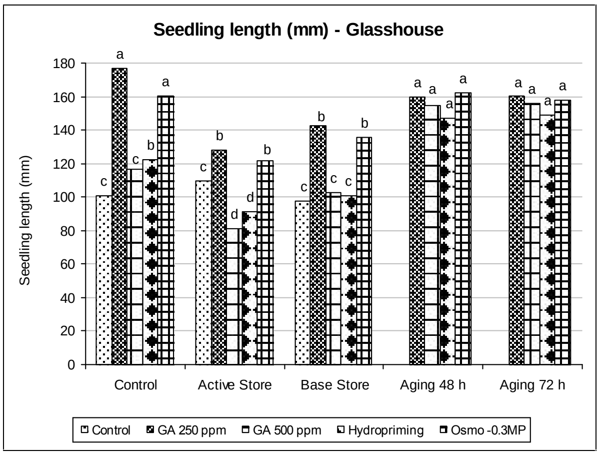
Accession	Priming Treatments	Germination %	Vigor index	Root length (mm)	Shoot length (mm)	Seedling length (mm)	Fresh weight (mg)	Peroxidase enzymatic activity (%)	Catalyze enzymatic activity (%)	Superoxide dismutase (SOD) (%)
8959	Control	74.89 b	84.06 a	60.33 bc	50.22 a	110.56 c	63.56 c	-	-	-
	GA250ppm	39.17 de	53.60 c	95.10 a	41.10 c	136.20 b	85.61 a	10.40 d	155.3 c	6.70 b
	GA500ppm	27.73 f	29.85 d	85.03 ab	29.93 d	117.08 c	70.03 bb	18.08 b	185.1 b	5.30 b
	Hydro priming	32.93 e	28.32 d	68.11 b	27.81 d	95.92 d	61.07 c	11.26 d	225.3 a	4.12 b
	Osmo-0.3MP	46.93 d	61.20 b	96.00 a	38.55	136.02 b	85.87 a	9.10 d	183.7 b	2.49 c
15123	Control	84.44 a	82.60 a	52.14 c	46.33 b	98.48 d	54.22 d	-	-	-
	GA250ppm	54.00 c	81.24 a	101.83 a	55.39 a	157.22 a	81.35 a	15.49 c	170.8 bc	
	GA500ppm	46.00 d	47.17 c	72.82 b	39.40 c	116.55 c	57.86 d	12.46 d	239.2 a	0.58 d
	Hydro priming	41.20 d	52.91 c	88.84 ab	48.98 b	139.04 b	81.14 a	8.51 e	158.3 c	
	Osmo-0.3MP	48.14 d	70.92 b	95.29 a	56.57 a	151.86 a	69.65 c	19.19 b	208.5 ab	2.25 c
23879	Control	91.56 a	89.86 a	52.14 c	46.33 b	98.48	54.22 d	-	-	-
	GA250ppm	52.53 c	79.05 a	103.05 a	55.39 a	158.44 a	77.08 b	21.70 a	231.0 a	3.72 bc
	GA500ppm	44.67 d	49.20 c	78.36 b	36.81 c	118.23 c	56.21 d	15.09 c	110.5 d	-
	Hydro priming	41.60 d	53.77 c	86.21 ab	47.56 b	133.77 b	68.10 c	26.26 a	147.6 c	-
	Osmo-0.3MP	55.33 c	81.36 a	95.09 a	59.16 a	154.26 a	76.76 b	12.62 d	181.2 b	10.74 a

Means in each column with the same letter are not significantly different ($P \leq 0.01$)









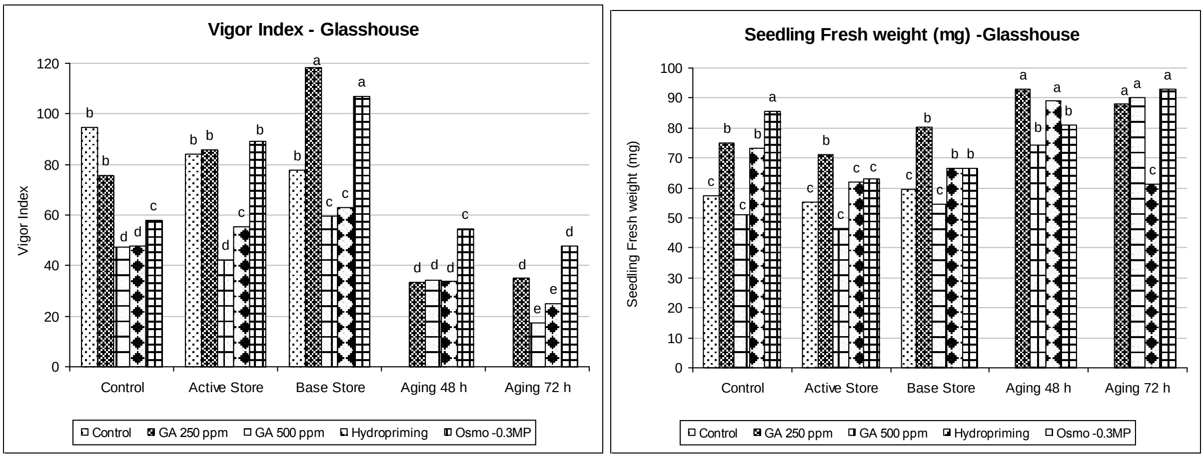


Fig. 1 Priming by conservation interaction effects for germination percent, root and shoot length seedling length, vigor index and seedling weight of *Matricaria chamomilla* under greenhouse conditions

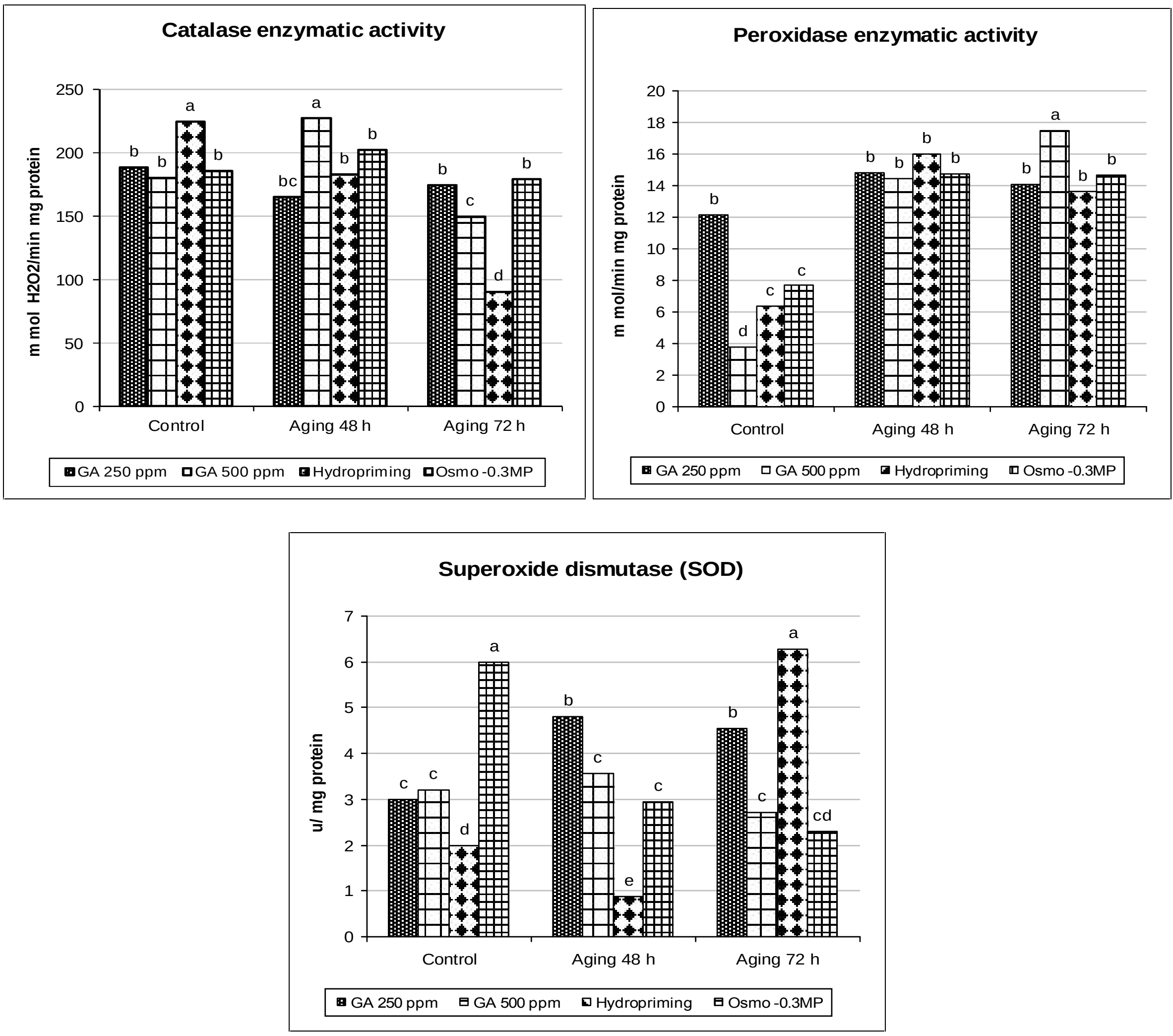


Fig. 2 Priming by conservation interaction effects for three Proxidase, Catalaz and Super Oxid Desmotaz (SOD) enzymatic activities of *Matricaria chamomilla*

Conclusion

Our study showed that priming is useful method to improve quality of deteriorated and old seeds. According to the results two accessions 23879 (Esfahan), 15123 (Arak) have higher mean values than the accession of 8959-Brojen. These two accessions have gotten more values of germination characteristics by effect of osmopriming and hormonal priming but the accession of 8959-Brojen had lower values of germination characteristics with priming techniques. These two priming methods (osmopriming and hormonal priming) were more effective in recovery of deteriorated seeds.

The mean of all traits of three accessions were higher in base cold room (-18 °C) than active cold room (4 °C) and this sign effect of low temperature on seed viability. The root length was 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 (Gibberllic acid 250 ppm). Regarding to result of this research work, it was proved that two priming techniques Osmo-priming using (Poly ethylene glycol) and hormonal priming (Gibberllic acid) were effective method for improvement of aged seed.

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