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Effect of Different Physicochemical Treatments on Seed Dormancy of Medicinal Herbs (*Portulaca oleracea* L.)

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ABSTRACT

The objective of the present study was an assessment of different treatments on seed dormancy, germination percentage, velocity of germination and seed vigor index on *Portulaca oleracea* L. herb. This experiment was arranged out as completely randomized, based on CRD design with 4 replications. For this purpose seedling characteristics were measured: six different physical and chemical treatments including freezing, wetting and drying, pre-chilling, nitrate potassium and sulfuric acid compared with control sample. The results showed a significant effect of nitrate potassium, wetting and drying, pre-chilling and boiling treatments on seed dormancy ($p<0.01$) and had 52, 49, 40 and 34% germination, respectively. However Freezing (29%) and sulfuric acid (31%) didn't show any significant effect on germination of this plant seeds.

Key words: Dormancy, physical and chemical treatments, germination, *Portulaca oleracea* L.

INTRODUCTION

The family Portulaceae contains about 20 genera and about 250 species in the world (Weston, 1990), which neglected economical application of this herb. *Portulaca oleracea* L. is one of the interesting herbal plants with anti-hypoxic effect (Chen *et al*., 2009), Chinese traditional medicine for thousands of years (Niu and Dan, 2005), asthma curness (Malek *et al*., 2004), reduce inflammation (Wang *et al*., 2007), Ethanol extract induced nerve damage (Wanyin *et al*., 2012), anti-pain, antimicrobial effects and spinal cord Injury (Behravan *et al*., 2011) and Antioxidant properties (Lim and Quah, 2007; Chan *et al*., 2000) were reported by wide reports.

For instance multiple secondary metabolite and active chemical such as different alkaloids (Xiang *et al*., 2005), omega-3 polyunsaturated fatty acids (Stroescu *et al*., 2013) and Furthermore, polysaccharide component (Shen *et al*., 2013), anti-diabetic activity (El-Sayed, 2011), Homoisoflavonoids (Yan *et al*., 2012), Betacyanins (Wanga and Yang, 2010) were reported.

Portulaca oleracea is a suitable source of high crude protein (17.9%), crude fiber (20.3%), fat content (5.6%), magnesium (3.5%) and calcium (1.8%) in chemical profile considerably in livestock feeding and nutrition (Obied *et al*., 2003) for forage purpose.

Portulaca oleracea has red stems 20 to 50 cm in length, yellow flowers and the most literatures has been described both seed propagation and asexual reproduction of this plant with geographical distribution in tropical, sub-tropical and temperate regions.

According to the past literature review; there has been identified some physical and chemical treatment for improvement of seed germination particularly for pasture herbs. However as summary; Oxygen, moisture and light are the three major factors in germination (Berlyn, 1972). Seed dormancy and germination are complex adaptive traits of higher plants that are influenced by a large number of genes and environmental factors (Koornneef *et al*., 2002). Generally, freshly harvested seeds has a more severe dormancy, for example; In freshly harvested achenes, pericopes are permeable and the embryo fully developed, which eliminates the possibility of physical, morphological or morphophysiological dormancy (Zhou *et al*., 2009).

Many species have been reported around the world that have different germination characteristics and dormancy responses (Mandak and Pys, 2005; Brandel, 2004a; Lu *et al*., 2010).

Cooling period and alternative heat and cool influences germination (Barton, 1944; Cullina, 2000). Controlled pre-chilling can improve the germination, however has the negative effect on the dysfunction of germination during exceed of treatment time (Barton, 1944). Chemical treatments such as sulfuric acid (Dirr and Heuser Jr., 1987), nitrate potassium (Uzen and Aydin, 2004) and abscisic acid also enable to eliminate seed dormancy because of rapid germination in some plant species (Del Tredici, 1977).

Knowledge about effect of different physical and chemical treatments on seed dormancy of medicinal *P. oleracea* herbs can help us in understanding of germination and to find the most suitable factors can overcome to the natural barrier of seed dormancy in this plant (*P. oleracea*). As each specific plant has own specific solution for improvement of germination; there is a lack of information about effective physical and chemical treatment on medicinal *P. oleracea* herbs have not been evaluated.

The main objective of the present study was to evaluate effects of different physical and chemical treatments on seed dormancy of medicinal *P. oleracea* herbs.

MATERIALS AND METHODS

Sampling: *Portulaca oleracea* seeds were collected from the Green Garden of Tehran University during spring of 2012.

Laboratory experiment: The 4 physical and 2 chemical treatments including nitrate potassium (KNO_3) (0.1 g L^{-1} for 24 h), sulfuric acid (H_2SO_4) (95%) for 3 min, Freezing (under zero degrees for a week), boiling hot water (95°C per 3 min), wetting and drying (12 h alterative for a week) and pre-chilling (Refrigerators for a week at 5°C) was compared with control group within 4 replicates and 100 numbers of seeds per each treatment. Mentioned treatments were selected based on before studies and also availability.

Measured characteristics: Overall, some characteristics were measured as follows; number of daily germinated seeds, length of seedling (shoot and root).

Size of shoot and root were measured for the total number of germinated seeds (germination percentage) calculates and seed vigor is also specified. Also velocity of germination and germination time were calculated for each treatment.

Germination percentage and velocity of germination were calculated according to following equation:

$$GP = \frac{\sum G}{N} \times 100 \quad (1)$$

GP = Germination percentage
G = No. of germinated seeds in each counting
N = Total number of seeds

$$VG = \sum\left(\frac{n}{t}\right) \quad (2)$$

VG = Velocity of germination (Walker-Simmons and Sesing, 1990)
n = The number of germinated seeds in certain day
t = Day the seeds germinate are counted (Maguire, 1962)

The following formula was used to determine the seed vigor index:

$$VI = \frac{LS \times PG}{100} \quad (3)$$

VI = Vigor index
LS = Length of seedling
PG = Percent of germination

Statistical analysis: After obtaining raw data in the present experiment, data were according to CRD experimental design and ANOVA test and the post hoc test was performed using the Duncan method for grouping treats. using SPSS software (17.0 version).

RESULTS AND DISCUSSION

The following (Fig. 1) shows the number of seeds germinated during the experiment. The first counting of germinated seed was on the third day after cultivation and there weren't germinated seed on the eighth and ninth day after cultivation.

Data were analyzed using SPSS for windows and Microsoft Excel 2007. A one-way Analysis of Variance (ANOVA) was performed on all results and differences between the means were compared using Duncan values ($p<0.01$).

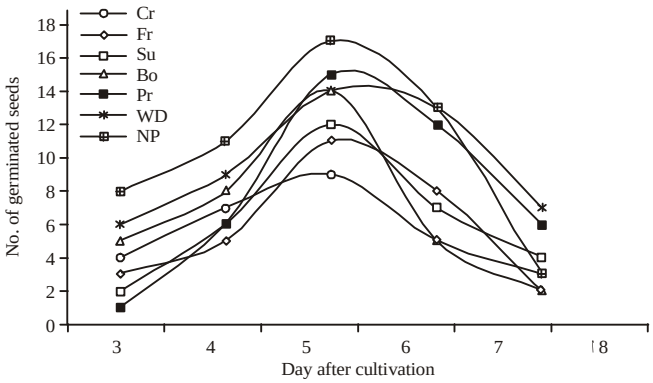

Fig. 1: No. of germinated seeds during germination under various treatments, NP: Nitrate potassium, WD: Wetting and drying, Pr: Prechilling, Bo: Boiling, Su: Sulfuric acid Fr: Freezing and Cr: Control

Table 1: Analysis of variance for seed and velocity of germination

S.O.V	df	SS	MS	Significant	F
Germination					
Treatment	6	2189.71	364.95	0.001**	6.31**
Error	21	1213.25	57.77	-	-
Total	27	3402.96	-	-	-
Velocity of germination					
Treatment	6	177.35	29.56	0.0001**	17.86**
Error	21	34.75	1.65	-	-
Total	27	1290.85	-	-	-

S.O.V: Sources of variation, df: Degrees of freedom, SS: Sum of squares, MS: Mean-squares, **Significant at 1% confidence t at 1% confidence

After the ANOVA analysis and significant result on germination percentage, post hoc test conducted using the Duncan test. Grouping treatments on germination makes it easy to interpret results and similarity the effect of each treatment are better identified.

Velocity of germination (germination rate) is an important factor in germination of seed and makes easier all actions that related to seed breeding and keeping (Table 1). The results of the comparison (grouping velocity of germination under different method by Duncan method) are presented in Fig. 3.

Each of the treatments will be effective on the size of the root and stem of germinated seed and finally on the vigor of the seed. Stability and viability of the seed depending on its vigor completely. Figure 4 shows differences in seed vigor under different treatments.

There are many unique methods to overcome the seed dormancy of different plant seed. Some seeds require several simultaneous treatments to germinate and some else require a specific method only. Double dormancy that has reason needs several methods to remove barriers and germination. For example, secondary dormancy in *Brassica napus* seed, most likely due to changes in gene expression in the final stage of seed formation (Fei *et al*., 2007), needs different methods to break dormancy.

Today, Molecular techniques and especially expression studies, transcriptome and proteome analyses, are novel tools for the analysis of seed dormancy and germination (Koornneef *et al*., 2002) that cause to accelerate and accuracy in testing and are so suitable for tiny seeds. In some cases, physical barriers on the crust of the seed performs as an obstacle to absorb oxygen and piercing the seed coat has a significant effect on seed germination (Duclos *et al*., 2013) but the *P. oleracea* seeds have no special material on their crust except own-crust that cause dormancy of the seed. This can concluded that this plant has physiological seed dormancy. Heat is required for seed embryo growth (Albrecht and McCarthy, 2006), therefore boiling treatment (Fig. 2) had an effect on germinated of this plant seed. Also heat that cause to fluctuations in protein and nitric oxide seed structure could be affect on seed germination (Debska *et al*., 2013). Similar to this research, before studies have shown that the temperature has different effects for the elimination of dormancy of different seeds and high temperature lonely could be breaking the primary and secondary dormancy (Brandel, 2004b). Mohammad and Amusa (2003) research also in order to break seed dormancy of T*amarindus indica* soaking the seeds in hot water to 100°C and there were significantly increased on germination. Soaking the hard seeds of *Parkia bioglobosa* in warm water (70°C) stimulate seed germination compared to control (Aliero, 2004).

Nitrate and light have an effect on seed germination of some plants that usually their seed floated in water (Mollard and Insausti, 2009) and some tiny seed such as *P. oleracea* seeds are sensitive. Nitrate potassium which is one of the nitrate compounds changed all of the germination characteristics of *P. oleracea* seeds (Fig. 1-4). Nitrate potassium treatment performs as a food supplement and a significant increasing root and stem growth. In another study, Shim *et al.* (2008)

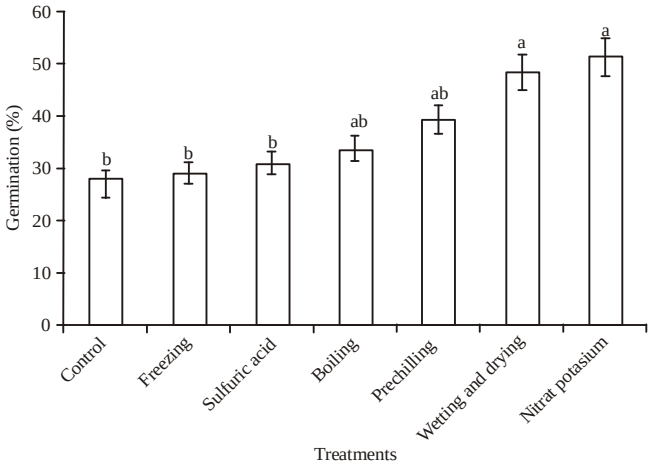

Fig. 2: Comparison and grouping the seed germination of *P. oleracea* under different treatments

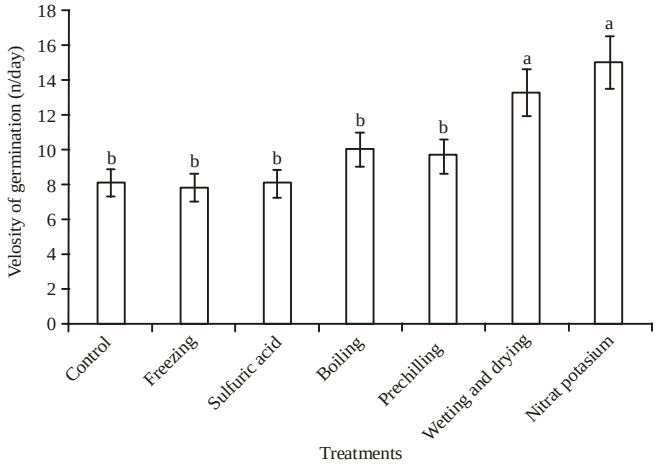

Fig. 3: Comparison and grouping the velocity of germination *P. oleracea* under different treatments

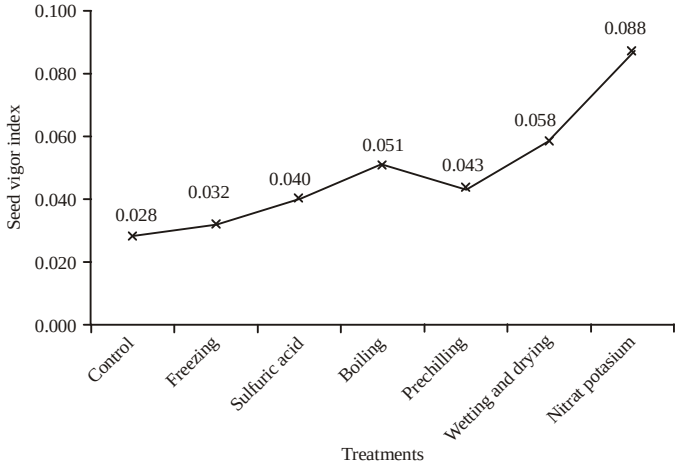

Fig. 4: Seed vigor index changes under different treatments

concluded that priming with 0.2 or 0.5% solution of nitrate potassium for 72 h has a recommended method that can be practically applied for increasing germination of Paspalum under an alternating temperature (25/35°C) condition also in other study nitrate potassium enhanced the characterizes of germination of soybean seed (Ahmadvand *et al*., 2012). The nitrate potassium increase rate of germination of 16 seed per day and shorten the period of germination versus the control sample (9 seed/day) (Fig. 3).

Wetting and drying had the most effect on *P. oleracea* seeds after nitrate potassium. Vincent and Cavers (1978) in a study concluded that some seeds such as *Rumex crispus* seed should be under wetting and drying treat to germinate.

In a study the pre-chilling had a significant impact on *E. angustifolia* seed germination (Macchia *et al*., 2001), also pre-chilling treat eliminate the physiological dormancy (Albrecht and McCarthy, 2006). Prechilling also like boiling treatment increased seed germination and ordered in same group with boiling treatment (Fig. 2). Although, scarification treat could be enhanced germination of *Astragalus fridae* seed dormancy (Arbabian *et al*., 2006) also in other research scarification treatments shell with 90 days chilling, increase germination percentage (Fordham *et al*., 1983), but because the *P. oleracea* seeds are very small, using this treatment would be difficult. In general, natural area, some organization such as fungi are effective on the germination of buried seeds in the soil (Carrillo *et al*., 2009) but for artificial plant propagation, this method maybe is not high efficiency.

Also according to Buriro *et al.* (2011) research, temperature can effect on seed vigor but in his study seed vigor index changed as different treatment. There are maximum and minimum seed vigor index, respectively for the nitrate potassium (0.088) and control sample (0.028) (Fig. 4).

CONCLUSION

The results of this study showed that the *P. oleracea* seed has both physical and physiological dormancy. Treatments such, as nitrate potassium, wetting and drying and boiling water significantly increased the germination percentage and velocity of germination on this plant. But other treatments have no significant effect on the percent of germination and the velocity of germination. Identifying the best way to speed up germination time and also to enhance the germination percentage can remove dormancy on this plant and can be cultured for medicinal and edible usage. Pre-chilling, wetting and drying methods which are natural treatments recommended for break dormancy on this valuable plant.

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