



EFFECTS OF VARIOUS TREATMENTS ON ECHINACEA PURPUREA L. SEED DORMANCY BREAKING AND GERMINATION

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ABSTRACT : The present study was conducted to evaluate the effect of various treatments including chilling for 2, 5, 9 and 12 weeks, chilling for 12 weeks + %3 H₂SO₄, H₂SO₄ for 2, 5 and 10 min, chilling + GA₃, GA₃ (300 and 600 ppm) as well as %2 KNO₃ to breaking of Echinacea seed dormancy and promote its germination and seedling growth at 2011. This experiment design was completely randomized block with three replications. Analysis of variance results showed that various treatments had significant effect on all studied traits at %1 probability level. Also, mean comparison of treatments indicated that the highest germination percentage, germination rate, plumule and radicle length, seedling dry weight and seed vigour obtained as affected by chilling + GA₃ and GA₃ (600 ppm), while highest value for radicle to plumule ratio observed in seeds that treated by H₂SO₄ for 2 min. In general, each trait showed alternative response to various treatments. Among different treatments chilling + GA₃ and GA₃ (600 ppm) had the best effect on breaking of Echinacea seed dormancy and improving of this plant germination and seedling growth. Therefore, our results showed that dormancy in Echinacea seeds might be physiological.

Keywords: Echinacea, chilling, GA₃, plumule, radicle, seed vigour.

INTRODUCTION

Echinacea (*Echinacea purpurea* L.), is a herbaceous perennial plant that is native to North America and widely used for wild flower establishment, perennial gardening, and sometimes as a cut flower [1].

It is also an important medicinal herb that recently gained international popularity in order of its immune stimulatory, antiviral and antibacterial advantages to humans [2] and Since 1930, it has considered and used as medicinal plant to control Influenza, and reduce upper respiratory infection by human [3].

Different researchers have shown that this plant can be used as enhancer of the immune response, especially of cells such as neutrophils, monocytes and lymphocytes [4].

The seeds of many medicinal plants such as Echinacea species have dormancy. They readily germinate within the native environment, but fail to show good germination under laboratory conditions [5]. The dormancy of dormant seeds must be broken to induce germination. Various methods are used for this, depending on the plant species and type of dormancy. For example, chilling plays an important role in providing the stimulus required to overcome dormancy, increase germination, and produce normal seedlings for *Prunus persica* [6], strawberry tree [7] and *Prunus avium* [8]. Exogenous growth regulator treatments – GA (usually GA₃ and GA₄₊₇) and cytokinins (usually kinetin, benzyladenine) have been shown to break dormancy in many seed species [7].

Researchers noted that, pre-treatments of seed with sulphuric acid and hot water soaking are some factors that can significantly influence germination of *Tamarindus indica* L. Seed germination increased with increasing acid concentration and treatment time [9]. Dewir et al [10], reported that water soaking (24 h) and H₂SO₄ treatments increased the germination percentage of *Sahel* and *Thirax*, respectively. Also, they noted that, prolonged soaking of the seeds in H₂SO₄ possibility imposes injury to the embryo and simply burnt the seeds. Whereas longer soaking duration of 30 min in H₂SO₄ was more effective for *Thirax* seed germination.

However, little investigation have been accomplished on the mechanism of seed dormancy conditions in *Echinacea purpurea*. Therefore, the objective of this research was to evaluate the effects of various treatments on breaking seed dormancy or to enhance *Echinacea purpurea* seed germination and subsequent seedling growth.

MATERIAL AND METHODS

This experiment was conducted in Seed Science Laboratory of Agriculture Faculty, Shahid Chamran University of Ahvaz, Iran during 2011. The experiment design was completely randomized design. There were 13 various treatments including:

- T₁) Control (unsoaked seeds)
- T₂) Chilling for 2 weeks
- T₃) Chilling for 5 weeks
- T₄) Chilling for 9 weeks
- T₅) Chilling for 12 weeks
- T₆) Chilling for 12 weeks + 3% H₂SO₄
- T₇) H₂SO₄ for 2 min
- T₈) H₂SO₄ for 5 min
- T₉) H₂SO₄ for 10 min
- T₁₀) Chilling + GA₃
- T₁₁) GA₃ (300 ppm)
- T₁₂) GA₃ (600 ppm)
- T₁₃) 2% KNO₃

With three replications and each replication represented by 30 seeds.

The Echinacea (*Echinacea purpurea*) seeds were obtained from the Pakan seed company in Isfahan, Iran. All seeds were surface sterilized in 10% sodium hypochlorite (NaOCl) for 3 min. then, these seeds washed with sterile distilled water prior to applying any treatment.

6ml tap water or different treatment added to each petri dish and germination test of Echinacea seeds were performed in petri dishes in laboratory germinator for 14 days at constant 25°C. Data on germinated seed (protrusion of radicle by 2mm) were recorded 24 intervals from the 2th to 14th days after sowing. Radicle and plumule length (cm) and radicle to plumule ratio were recorded on the 14th days by taking five samples at random. Then germination percentage and seed vigor were measured. Dry weight of five seedlings was recorded after drying in hot air oven maintained at 75°C for 48 hr. The dried seedlings were weighted, averaged and expressed in milligrams (mg).

Germination rate [11] and seed vigour index [12] was calculated by the formula:

Germination rate (GR) = Number of germinated seeds / Days of first count + ... + Number of germinated seeds / Days of final count

Seed Vigour Index (SVI) = (Plumule length + Radicle length) × Germination percentage

The data was subjected to the analysis of variance (ANOVA) and were there were significant differences among treatments, means separation was done using the Duncan multiple range test by using MSTAT software at 5% probability level ($p < 0.05$).

RESULT AND DISCUSSION

According to the analysis of variance results that presented in Table 1 and 2, high significant differences among various investigated traits obtained as affected by different treatments at 1% probability level ($p < 0.01$). The results of this experiment showed that the highest Echinacea seed germination percentage and germination rate observed as affected by T₁₂ (GA₃, 600 ppm) and T₁₀ (chilling + GA₃), while the lowest value observed in T₀ or control (Figure 1 and 2). GA₃ has been found to be effective in increasing germination in several species and to break dormancy in dormant seeds. Pre-treatment of blueberry seeds with GA at 100–500 mg/l accelerated germination [13].

It has been reported that germination can be induced by GA in *Vaccinium myrtillus* L. [14], *Vaccinium corymbosum* L. [15] and *Fagus sylvatica* [16] seeds. These results confirm that GA₃ enhances seed germination percentage. GA promotes seed germination and warm plus cold stratification breaks dormancy, thus *Echinacea purpurea* seeds are classified as having physiological dormancy [17]. Recent physiological and molecular studies have shown that physiological dormancy includes an embryo and coat component, and their sum and interaction determine the degree of whole-seed physiological dormancy [18].

For germination to occur in physiologically dormant seeds, the mechanical constraint of the embryo-covering layers must be overcome by the growth potential of the embryo or the mechanical constraint must be reduced, or both. In other hand, seed germination percentage no affected by H₂SO₄ duration. In contrast, Echinacea germination rate decreased by increasing of H₂SO₄ exposure duration from 5 to 10 min. The increase in duration reduced the seed quality due to increased damage to the seed coat [19]. According to the results that obtained from Figure 3, Echinacea plumule length that treated with T₁₀ treatment was significantly higher than other treatments. In contrast, T₇ without significant difference by T₈ and T₉ treatments caused most reduction in this trait. Hejazi et al. [20] announced that application of gibberellic acid can be substitute for cold requirement in dormant seeds. Also, seed germination percentage and germination rate increased by increasing of chilling duration from 2 to 12 weeks. Zare et al [5] showed that, increasing chilling times increased both germination percentage and rate of *Ferula essa foetida*. Also, they noted that, since the method of cold stratification treatment is quite simple and inexpensive compared with GA₃ this can be widely used in practice.

Table 1. Analysis of variance results of Echinacea germination percentage, germination rate as well as plumule and radicle lengths as affected by various treatments.

S.O.V	Mean of square				
	d.f.	GP	GR	P length	R length
R	2	3.477	0.001	0.008	0.555
T	12	224.95 2 **	178.5 85 **	1.649 **	10.86 6 **
Error	24	5.168	2.704	0.012	0.328

** , * and n.s significant at P = 0.01, P= 0.05 and no significant, respectively. d.f. degree of freedom, R: repeat, T: treatments, GP: germination percentage, GR: germination rate, P: plumule, R: radicle,

Table 2. Analysis of variance results of Echinacea germination percentage, germination rate as well as plumule and radicle lengths as affected by various treatments

S.O.V	Mean of square			
	d.f.	R/P	Seedling DW	SVI
R	2	0.624	0.278	0.426
T	12	38.769 **	27.213 **	5.126 **
Error	24	0.51	1.209	0.22

** , * and n.s significant at P = 0.01, P= 0.05 and no significant, respectively. d.f. degree of freedom, R: repeat, T: treatments, P: plumule, R: radicle, DW: dry weight SVI: seed vigour index

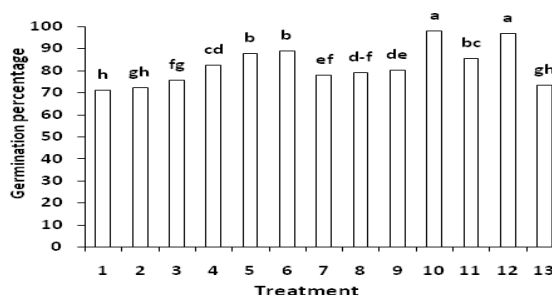


Figure 1. Effect of the various treatments on germination percentage of Echinacea purpurea. Mean values, followed by the same letter were not significantly different (P = 0.05) by Duncan's test.

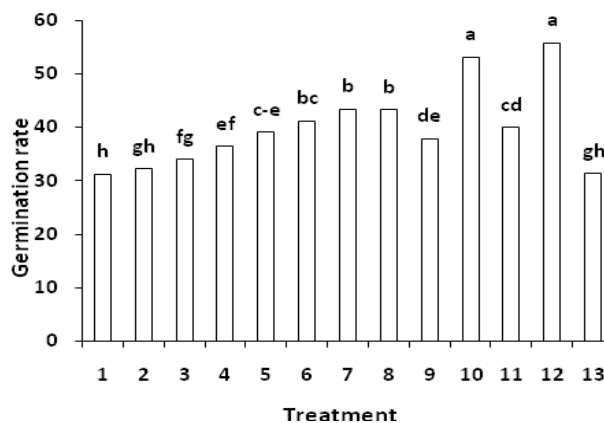


Figure 2. Effect of the various treatments on germination rate of *Echinacea purpurea*. Mean values, followed by the same letter were not significantly different ($P = 0.05$) by Duncan's test

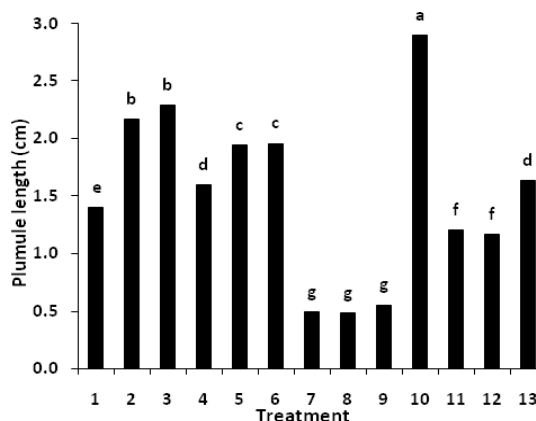


Figure 3. Effect of the various treatments on plumule length of *Echinacea purpurea*. Mean values, followed by the same letter were not significantly different ($P = 0.05$) by Duncan's test.

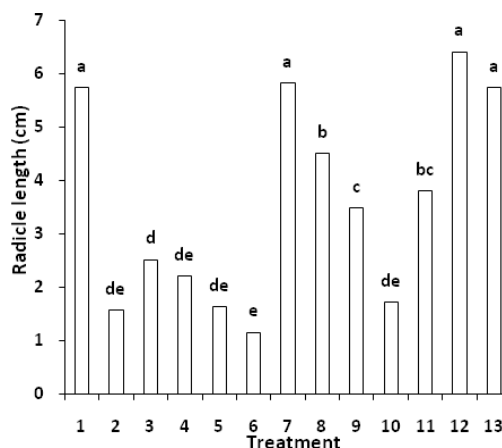


Figure 4. Effect of the various treatments on radicle length of *Echinacea purpurea*. Mean values, followed by the same letter were not significantly different ($P = 0.05$) by Duncan's test

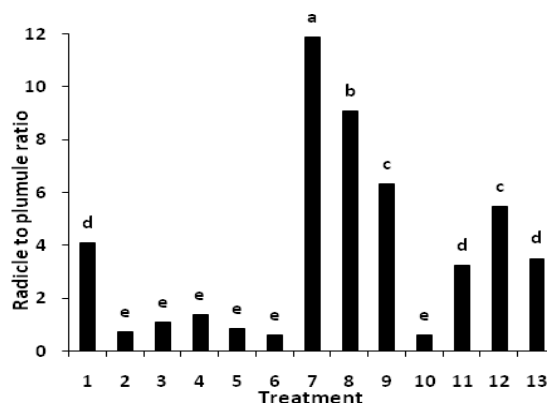


Figure 5. Effect of the various treatments on radicle to plumule ratio of *Echinacea purpurea*. Mean values, followed by the same letter were not significantly different ($P = 0.05$) by Duncan's test.

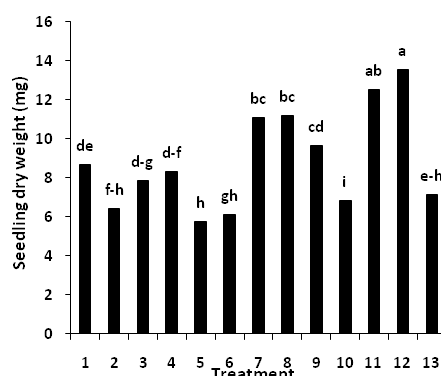


Figure 6. Effect of the various treatments on seedling weight of *Echinacea purpurea*. Mean values, followed by the same letter were not significantly different ($P = 0.05$) by Duncan's test

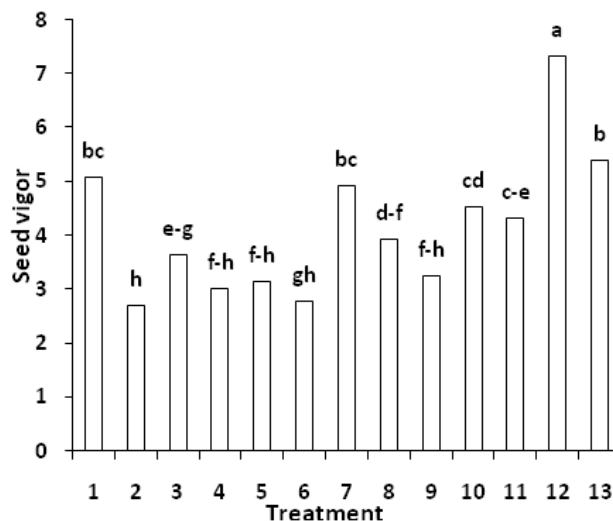


Figure 7. Effect of the various treatments on seed vigor of *Echinacea purpurea*. Mean values, followed by the same letter were not significantly different ($P = 0.05$) by Duncan's test.

The highest radicle length observed at T₁₂ which had no significant difference by T₁, T₇ and T₁₃, and the lowest was observed in T₆ (Figure 4). The mean comparison of treatments that shown in Figure 5, maximum radicle to plumule ratio obtained after T₇ treatment. While the minimum value for this trait observed in T₆ treatment which had no significant differences by treatments from T₂ to T₆ as well as T₁₀. This result obtained due to low plumule length of Echinacea as affected by H₂SO₄ for 2 min. Our results show that the plumule length is more sensitive to H₂SO₄ treatment as compared radical length. The most seedling dry weight obtained as affected by T₁₂, and the lowest was observed after T₅. Of course, no significant differences observed among T₂, T₅, T₆, T₁₀ and T₁₃ (Figure 6). Also, results of Figure 7 revealed that, T₁₂ had the highest positive effects on seed vigour. In contrast, the most effective reduction among all treatments was observed at T₆ treatment (Figure 7).

CONCLUSION AND RECOMMENDATIONS

In conclusion, both chilling and GA₃ applications are effective methods to improve germination in all species tested, indicating the dormancy in these seeds might be physiological. Germination does not seem to be the obstacle for cultivation.

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