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# EFFECTS OF STRATIFICATION AND MOISTURIZING TREATMENTS ON BREAKING SEED DORMANCY IN TWO *ECHINACEA* SPECIES

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## ABSTRACT

*Echinacea* (*Echinacea* spp.) has been widely used in folk medicine for centuries and has a high market share and medicinal value, because more than 280 different products are sold economically in Europe. Generally, germination rate of *Echinacea* seeds is very low and poor seedling growth is an ongoing problem compared to other medicinal plants. Different methods were used before sowing that effect seedling quality which have positive effects on yield. In this study, the effects of stratification and moisturizing on the germination of *Echinacea* seeds were investigated. It was determined that stratification + moisturizing treatment at +4 °C with different day intervals (0, 5, 10, 15 days) had a significant ( $p < 0.01$ ) effect on germination rates on seeds of *Echinacea purpurea* L. and *Echinacea angustifolia* L., besides the same effect was observed in epicotyl and hypocotyl lengths. Germination rates and seedling outflows were highest in moist seeds that kept at +4 °C temperature for 15 days. It is clear from the study that stratification and moisturizing methods were efficient in breaking dormancy and also used methods were non-chemical, easily applicable and economically advantage for commercial plantations.

## KEYWORDS:

*Echinacea*, stratification, seed dormancy, seed moisture, germination

## INTRODUCTION

The genus of *Echinacea* belongs to the *Asteraceae* family, consisting of nine species, and called as purple coneflowers, native to North America [1]. In taxonomic classification made by [2] they grouped these plants under 4 species [2-4]. Among these species, *E. purpurea* L. and *E. angustifolia* L. are widely used for medicinal purposes and extensively cultivated in South America, Canada, Europe, Russia, Africa and the Pacific. The emergence of the therapeutic properties of natural products and their uses are as old as human history. Increasing demand

for synthetic products has been caused by the industrial revolution. As a result of this, the development of the organic chemical industry and the emergence and spread of pharmacological treatment gained importance [5].

Today, quarter of the worldwide accepted drugs and 121 effective substances are derived from plants [6]. Twenty-eight (11.1 %) of the 252 drugs that derived from plants were accepted as essential by the World Health Organization (WHO). It is estimated that 60 % of plant-based drugs still present in the market and used as tumor and infection inhibiting agents in clinical trials [7]. Most of these drugs could not be produced synthetically as economically viable. More than 280 different products were procured from *Echinacea purpurea* L. has been sold in Europe. Also; ointments, tinctures, lotions, creams, liquid and dry extracts and toothpastes are the most widely used. In the United States, infusion of fresh and dry roots, powdered roots or encapsulated dry herba is more commonly used internally. It is understood from the market data that the demand for *echinacea* has been increased in recent years. Market sales of plant-based supplements from *Echinacea* ranged between \$ 31 million in 1995 to \$ 39 million in 1998 [8]. *Echinacea* has been traditionally used in folk medicine to treat colds, cough, bronchitis, upper respiratory tract infections and some inflammatory conditions [9]. Leaf and root parts of the plant have been reported to stimulate the immune system and increasing wound healing [10]. Commercial production of *Echinacea* is usually propagated from seeds. It has been reported that the quality and the source of the seed affect the germination of *E. purpurea* L. and *E. angustifolia* L. [11-12]. Extensive studies have been conducted with chemical, environmental and mechanical methods to break dormancy in *Echinacea* spp. [13-14]. In order to increase germination percentage and seedling quality of *E. angustifolia* L.; Ethephon [15], GA<sub>3</sub> [13] and BA-(Benzylamino-purine) [16] could be treated to break dormancy and enhance the seed quality. According to a study was carried out by [17] revealed that stratification and exposing seeds to highly moisture treatments were efficient to break seed dormancy in *Echinacea* compared to stratification treatment was done singly. It was reported that seed moisture content was found as an important factor in breaking seed dormancy in

*Echinacea* [18]. In many studies, it was obvious that stratification, seed moisture content, light and time factors (4-6 weeks) were vital in order to induce germination and enhance germinating rates and promoting seedling growth [11, 13, 17, 19, 20].

In our study, factors that play roles on affecting germinate rates of *Echinacea purpurea* L. and *Echinacea angustifolia* L. plus stratification and moisture treatments methods were investigated to determine optimum germination conditions. Our study aimed to increase the germination rates of *Echinacea* species in order to enhancing propagation rates with a economically favorable method without using any chemical treatment [21].

## MATERIALS AND METHODS

Used methods were applied to break the seed dormancy in order to increase germination rate and ensure early seedling development of the species of *Echinacea purpurea* L. and *Echinacea angustifolia* L. The seeds were obtained from gene bank (USDA) and reproduced in 2019 without chemical treatment. Firstly, the seeds were subjected to two different pre-treatments (stratification + moist and stratification + dry) under controlled conditions in the laboratory, then they were kept at low temperature (+4 °C) for different day intervals (0, 5, 10, 15 days). In line with the principles determined by [22], 40 seeds were placed on each petri dish (100 × 15 mm) containing two Waltman Filter Papers were moisturized in de-ionized water.

The experiment was carried out with two factors using randomized plot design with 3 replications. The petri dishes were placed to the air-conditioning chamber at 25 °C under 16 hours light and 8

hours dark with cold white fluorescent lamps. Germination percentages, epicotyl and hypocotyl lengths were counted and measured when the root length was 2 mm. Obtained results were analyzed using MINTAP statistical analysis program based on ANOVA factor analysis and LSD significance test.

## RESULTS AND DISCUSSION

According to the results of the study, pretreatment of moisturizing and stratification at +4 °C had a significant effect ( $p < 0.01$  level) on germination rates (Figure 1), epicotyl (Figure 2) and hypocotyl lengths of seeds (Figure 3).

It is clear from Figure 1 that the germination rates of *E. purpurea* L. ranged between 63.84-96 %, while germination rates of *E. angustifolia* L. ranged between 54.83-94.28%. Also, *E. purpurea* L. showed more higher germination rates compared to *E. angustifolia* L. These results were in agreement with a study established by [18]. In both genotypes, moisturizing treatment gave the highest germination rate. The highest germination rate was obtained in moist *E. purpurea* L. and *E. angustifolia* L. seeds in 5 days of incubation period at +4 °C respectively (92.54-92.97%) and 10 days of incubation period at +4 °C (96.67-98.20%) respectively. It was found that 11 days of incubation period at +5 °C in a 1mM Ethephon solution in continuous light conditions induced germination in *E. angustifolia* [17]. Our results in dry + stratification treatment was in contradiction with [20]. These researchers reported that *E. purpurea* (L.) germinated best at 0 °C during 1 month of incubation period without moisturizing. Also, [23] found similar results as [20]. In the dry treatment, 15 days of stratification treatment at +4 °C gave the best results

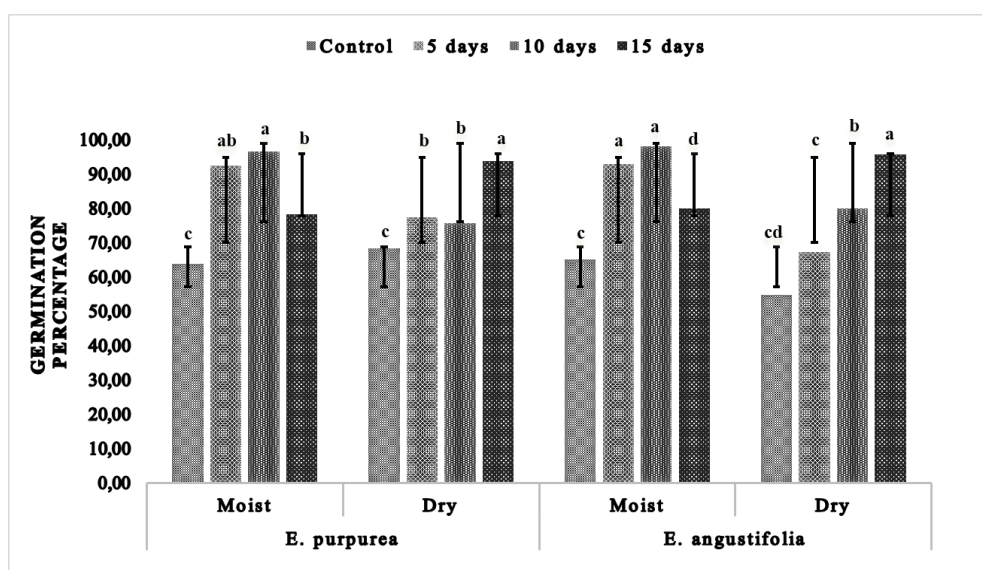


FIGURE 1

Effects of stratification at +4 °C with moist and dry treatments on seed germination percentage of *E. purpurea* and *E. angustifolia*.

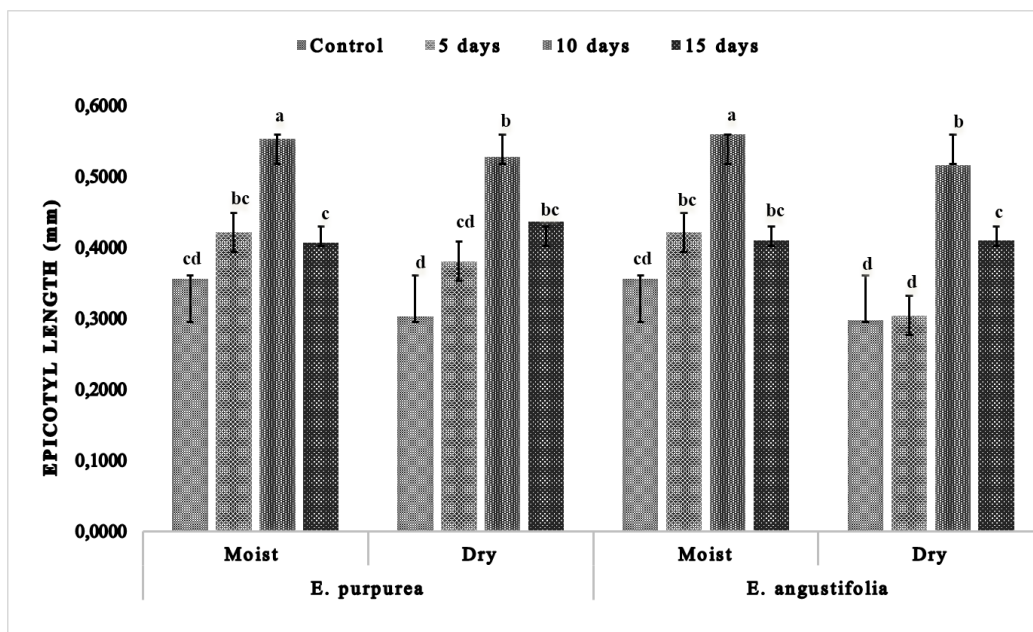


FIGURE 2

Effects of stratification at +4 °C with moist and dry treatments on epicotyl length of *E. purpurea* and *E. angustifolia*.

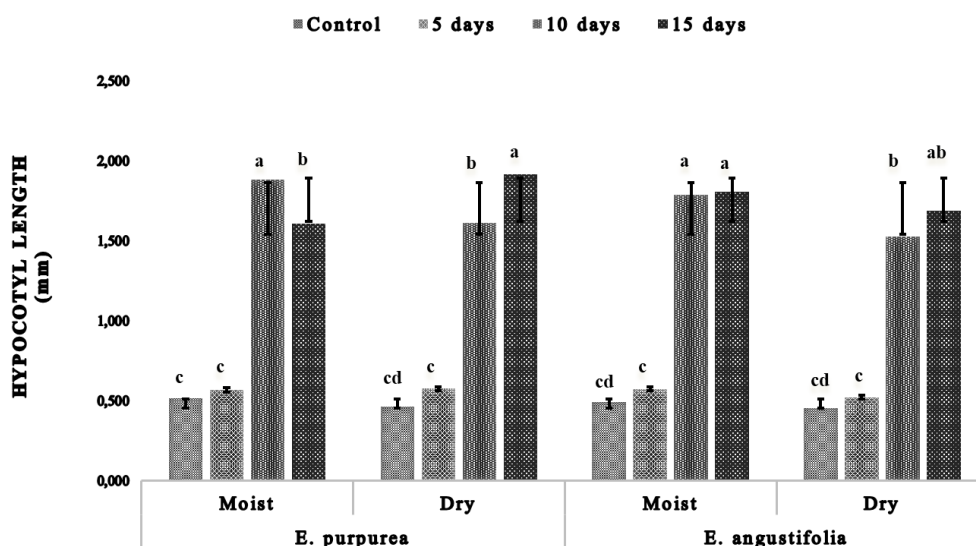


FIGURE 3

Effects of stratification at +4 °C with moist and dry treatments on hypocotyl length of *E. purpurea* and *E. angustifolia*.

(93.84-95.73%). According to the Figure 2 and Figure 3, epicotyl and hypocotyl length in *E. purpurea* L. ranged between 0.302-0.553 mm and 0.463-1.862 mm respectively. Also, epicotyl and hypocotyl length of the *E. angustifolia* L. ranged between 0.297-0.559 mm and 0.453-1.82 mm respectively. The epicotyl and hypocotyl lengths of both species were highest (0.553-0.559 mm and 1.862-1.82) in the seeds that treated with moisturizing and stratification at +4 °C during 10 days. Previous studies had also revealed that combined treatment of stratification and moisturizing were effective in

terms of breaking dormancy of purple cone-flower seeds [11, 13, 17, 19, 20].

## CONCLUSION

The *Echinacea* ssp. has a high market share, commercial and medicinal value. The plant is propagated from seeds as known difficult to germinate. In this study, seed dormancy of *Echinacea purpurea* L. and *Echinacea angustifolia* L. were investigated. It was clear that stratification and moisturizing were

effective to increase germination rates. The best results were obtained in stratification treatment at +4 °C during 15 days combined with moisturizing treatment. This study became prominent as a non-chemical method for germination, provides shorter germination period, economically suitable and easily applicable so advisable for commercial plantations.

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