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Seed Germination and Dormancy Breaking Techniques for *Echinacea purpurea* L.

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ABSTRACT

***Echinacea purpurea* (Asteraceae) is an important medicinal plant known as a disinfectant. Its germination percentage and germination rate is generally low due to dormancy. Dormancy and germination requirements were investigated in this plant. Seeds of *Echinacea purpurea* were subjected to different treatments including various levels of GA3 (100, 200 and 300 ppm), KNO3 (0.5, 1 and 1.5 percentage) and Cold stratification (7, 14, 21 and 28 days). The germination percentage and germination rate significantly increased in all of treatments than control. The highest germination and germination rate were obtained in Cold stratification treatment that induced about 98% germination. The mean germination time also improved in all of treatments than control. Lowest mean germination time also was observed in Cold stratification treatment.**

Key Words: chilling, *Echinacea purpurea*, GA3, KNO3, seed germination, seed dormancy

INTRODUCTION

Echinacea, commonly known as purple coneflower, is a herbaceous perennial plant that is native to North America and widely used for wild flower establishment, perennial gardening, andsome times as a cut flower (Wartidiningsih and Geneve, 1994a). It is also an important medicinal herb that recently gained international popularity in order of its immunostimulatory, antiviral and antibacterial advantages to humans (Li, 1998; Percival, 2000) and Since 1930, it has considered and used as medicinal plant to control Influenza, and reduce upper respiratory infection by human (Fugeh-Berman, A. 2003). Commercial farming of Echinacea is extensively located in United States, Canada and also in Europe, Russia and Australia have well-established cultivation (Wills and Stuart, 1999; Letchamo et al., 2002). *Echinacea purpurea*, one of the main commercial species of purple coneflower, was recently introduced to Iran and was grown well. The germination of *E. purpurea* seeds is generally irregular and poor (Samfield et al., 1990a, 1991b). The low germination percentage of *E. purpurea* is possibly the result of seed dormancy, and chilling stratification improves its germination responses (Wartidiningsih et al., 1994b). In the *Echinacea angustifolia* also Prechilling can remove dormancy only partially (Feghahati and Rees, 1994). Furthermore, there are conflicting results in the literature on the required period of the cold-moist treatment, which can be varied from 2 to 15 weeks (Baskin et al., 1992; Parmenter et al., 1992; Smith-Jochum and Albercht, 1987).

The aim of this research was to determine treatment(s) which are able to stimulate and improve germination factors (Germination percentage, Germination rate and Mean germination time) of *Echinacea purpurea* as an important medicinal plant.

MATERIALS AND METHODS

Seed source: The mature seeds of *Echinacea purpurea* were collected from a field planting in 2010. After collection, immature seeds and those damaged by insects were removed. The seeds were surface sterilized by soaking in 1% sodium hypochlorite (NaOCl) for 5 min and subsequently rinsed thoroughly with sterilized water prior to applying any treatment.

GA3 treatments:
In the first experiment, seeds were soaked in 100, 200, and 300 ppm for 2 hours.

KNO3 treatments:
In second experiment, seeds were soaked in 0.5, 1 and 1.5 percent KNO3 for 2 hours.

Cold stratification treatments:
In third experiment seeds were placed between two layers of paper towel moistened with distilled water inside plastic bags and stored in the dark at 5 0C for 7, 14, 21 and 28 days, respectively. Dishes were sealed with a stripof parafilm to reduce water loss, and darkness was maintained by wrapping the dishes with two layers of aluminum foil.

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All germination experiments were conducted using three replications of 25 seeds per each treatment. Seeds were placed on Wathman No.1 filter paper moistened with 5ml of distilled water in sterilized Petri dishes. Three germination experiments were carried out in completely randomized designs in room temperature (25-30 ^{0}C) and total darkness. Germinated seeds were counted and removed every 24 h for 2 weeks.

A seed was considered germinated when the tip of the radicle had grown free of the seed coat (Wiese and Binning, 1987; Auld et al., 1988). The germination percentage (GP), germination rate (GR) and Mean germination time (MGT) were calculated according to the following formulas (based on Wiese and Binning, 1987 and Scott et al., 1984):

$$1)\ \text{Germination (\%)} = \frac{\text{Number of germination seed}}{\text{Number of viable seeds initiated}} \times 100$$

$$2)\ \text{MGT} = \frac{\sum TiNi}{S}$$

Where Ti is the number of days after beginning of experiment, Ni the number of seeds germinated on day i and S the total number of seeds germinated.

$$3)\ \text{GR} = \sum_{n=1}^{n} \text{(Number germinating since-1)/n}$$

Where, n is the days.

RESULTS AND DISCUSSION

In our experiment, application of GA3 stimulated the germination. All of the concentration of applied GA3 improved germination factors (Germination percentage, Germination rate and Mean germination time) significantly ($P \rangle 0.05$) than control. With increasing GA3 concentration, there were no significant differences ($P \rangle 0.05$) among GA3 concentrations in germination factors expect between 100 and 300 ppm in germination rate (Table1 and Fig.1). Dormant seeds which require chilling, dry storage after ripening and light as a germination stimulator, are often treated with GA3 to overcome their dormancy (Gupta, 2003).

Cold stratification is a standard procedure which have been used to enhance the germination of dormant seeds (ISTA, 1990).in all of exposure days at 5^{0}C (Cold stratification period) there were significant difference ($P \rangle 0.05$) among germination factors than control. The highest germination rate obtained in the 21 days Cold stratification that had significant difference ($P \rangle 0.05$) with other Cold stratification periods and other treatments. The lowest mean germination time also obtained in the 21 days Cold stratification that shown significant difference ($P \rangle 0.05$) with 7 and 28 days Cold stratification and other treatments (Table1 and Fig.1). Wartidiningsih et al., (1994b) and Baskin et al., (1992) reported that prechilling is require in some of Echinacea species (*E. purpurea, E. pallida and E. angustifolia*) to overcome seed dormancy.

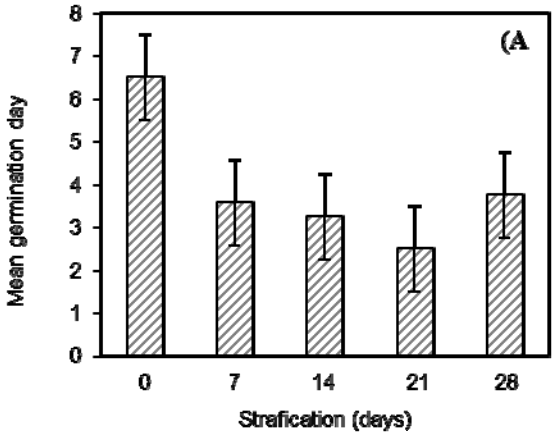
In *E. purpurea* application of KNO3 promoted germination. All of KNO3 levels improved germination factors significantly than control ($P \rangle 0.05$) but among different KNO3 levels there were no significant difference (Table1 and Fig.1). In 10 tasted treatments, Cold stratification treatment (especially 21 days) showed the highest germination percentage and germination rate and the lowest mean germination time than other treatments.

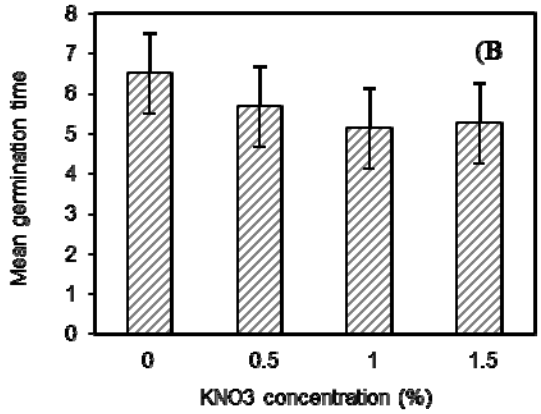
In conclusion, the present work has established some effective methods for breaking seed dormancy and improved germination factors of *Echinacea purpurea* through Cold stratification, application of gibberellin and potassium nitrate.

Table 1. Seed germination and dormancy and applied treatments for *Echinacea purpurea*

Dormancy breaking treatments	Germination percentage	Germination rate
Cold stratification (5 ^{0}C, 7 days)	96.67 a	0.414 bc
Cold stratification (5 ^{0}C, 14 days)	94.17 a	0.455 b
Cold stratification (5 ^{0}C, 21 days)	98.33 a	0.599 a
Cold stratification (5 ^{0}C, 28 days)	96.67 a	0.470 b
KNO3 (0.5%)	88.00 a	0.296 e
KNO3 (1%)	84.00 a	0.303 e
KNO3 (1.5%)	92.00 a	0.333 de
GA3 (100 ppm)	89.00 a	0.393 c
GA3 (200 ppm)	90.00 a	0.373 cd
GA3 (300 ppm)	89.33 a	0.333 de
Control	38.33 b	0.0656 f

Different case letters indicate significant differences (P 〉 0.05, Duncan test) between pretreatments





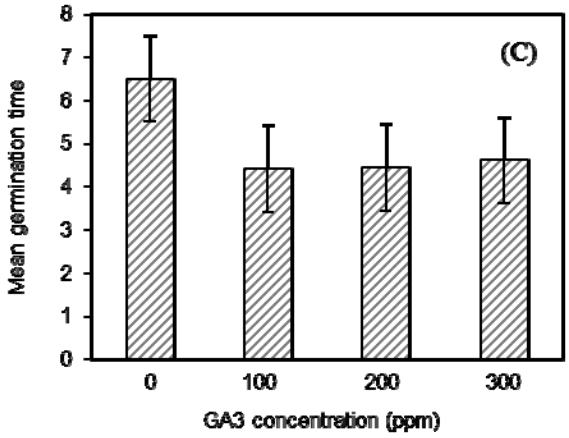


Figure 1. Effect of cold stratification days (A), KNO3 concentrations (B) and GA3 concentrations (C) on Mean germination time of *Echinacea purpurea*. Bars represent the standard errors.

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