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Pre-germination treatment and temperatures conditions on *Echinacea purpurea* seed germination

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Abstract: *Echinacea purpurea* (L) Moench, is a medicinal and herbaceous plant from Asteraceae family, and has an important role on economics of many communities. Germination is one of the main steps on understanding this species. Then, the aim of this study was to evaluate the effect of temperature conditions and precooling treatment on *E. purpurea* seed germination. This study was conducted in a completely randomized design, with four repetitions and tree treatments, constituted by three germination temperatures (20, 30 and 20-30°C) and two conditions, which is, breaking dormancy by precooling (5°C) for seven days and the control sample without precooling. Gerbox was used for germination test. The germination was determined by primary root protrusion. As a result, the germinations were satisfactory on three temperatures at the two groups. The highest germination potential was observed in alternating temperature 20-30°C (97.00 ± 0.93%). Better percentage of germination was observed in precooling treatment (97.75 ± 0.62%) and in the treatment without precooling (95.58 ±0.54%). Temperature directly impacts the coefficient of velocity of germination (CVG), with no statistical difference in the group subjected to precooling for temperatures of 20-30°C and in constant temperature of 30°C. However, the alternating temperature gets better CVG for the group without precooling treatment. Temperature of 20-30°C get better result (2.92 ± 0.13) for mean germination time (MGT),. *E. purpurea* presents fast germination lasting less than five days.

Keywords: Asteraceae; Medicinal Plant; Temperature; Precooling.

Tratamento pré-germinativo e diferentes condições de temperatura na germinação de sementes de *Echinacea purpúrea*

Resumo: *Echinacea purpurea* (L) Moench, é uma planta medicinal herbácea pertencente à família Asteraceae, com alto potencial medicinal e econômico em muitas comunidades. A germinação é um dos primeiros passos visando o cultivo de uma espécie. O objetivo desse trabalho foi avaliar o efeito da temperatura e do pré-resfriamento sobre a germinação de sementes de *E. purpurea*. Esse estudo foi realizado em um delineamento inteiramente casualizado, com quatro repetições e três tratamentos, constituídos por três temperaturas de germinação (20, 30 e 20-30°C) e dois tratamentos, os quais, a quebra da dormência por pré-resfriamento (5°C) por sete dias e a amostra controle sem pré-resfriamento. Para o teste de germinação foram usados caixas Gerbox. A germinação foi determinada pela protrusão da raiz primária. Como resultados, as germinações foram satisfatórias para as três temperaturas nos dois grupos. O maior potencial de germinação foi observado na temperatura alternada de 20-30°C (97.00 ± 0.93%). Melhores percentuais de germinação foram observados no tratamento de pré-resfriamento (97.75 ± 0.62%) e no grupo sem pré-tratamento (95.58 ±0.54%). A temperatura diretamente impactou o índice de velocidade da germinação (CVG), com nenhuma diferença estatística no grupo submetido ao pré-resfriamento para a temperatura de 20-30°C e em temperatura constante de 30°C. No entanto, nos grupos sem tratamento de pré-germinação, a temperatura alternada obteve melhores resultados em CVG. Para o tempo médio de germinação (MGT), a temperatura de 20-30°C obteve melhores resultados (2.92 ± 0.13). *E. purpurea* apresenta germinação rápida em tempo médio menor que 5 dias.

Palavras-chave: Asteraceae; Plantas medicinais; Temperatura; Pré-resfriamento.

Introduction

Echinacea purpurea (L) Moench is a species from Asteraceae family, native from Western United States prairies. This species has been used for many years with medicinal purpose, being one of the most widely herbal medicines used in many countries (BERGERON et al., 2002; DAS, 2009). There are nine species of *Echinacea* and the more commons are *Echinacea angustifolia, E. purpurea* e *E. palida* (THOMSEN et al., 2012; ROMERO e CASTELLA, 2012). Echinacea is an herbaceous plant that presents erect and robust stalk, and its height can reach up to one meter. Their leaves are elongated, slightly elliptical and have entire margins. The root coloration is grayish brown with some white spots (DAS, 2009). It is popularly well-known as cone flower or purpurea. Complete life cycle in twelve months at tropical conditions (LOAIZA et al., 2004). It is an excellent ornamental plant due to the beauty and durability of its flowering that happens from July to August.

Medicinal plants are used all around the world for many purposes, especially in Europe. However, a major problem faced on their utilization is the obtaining, which generally is performed by extractivism. This could bring to wrong identification and several problems to the species (SARTI, 2008).

Thus, germination studies are very important on species perpetuation and one of the main topics on agricultural production. Germination process will happen when several conditions were favorable to the germination, growth and development of the plant (FERREIRA et al., 2001). Germination is determined by essential structures emergence, such as root system, shoot system and terminal buds, making possible embryo to transform in a normal plant.

Propagation depends on several factors such as seed quality. Each species requires different temperature, moisture and luminosity conditions, which lead to demand specific studies in order to express maximum productive potential (CARVALHO e NAKAGAWA, 2012). Environmental factors that more common affect germination processes are the intensity and quantity of light, temperature and alternations, gas concentration and water (FERREIRA et al., 2001). Temperature impacts on velocity reactions and water absorption is essential to germination process form the embryo (VELTEN e GARCIA, 2005). It is recommended pre-germination treatment of seeds with cooling to break dormancy for some species of Asteraceae family, (MC DONOUGH, 1974; BRASIL, 2009).

Even with the advancement of technology in modern medicine, natural products are still used in large scale and play an important role on global health. However, the difficulty of multiplication can influence the use of some species; therefore, it is essential many agronomic studies, especially on seed germination. Seed germination studies have been performed often for many years, yet it is necessary specific studies of some important species like *Echinacea purpurea* to the determination of ideal conditions for germination and factors that affect the germination

Therefore, the aim of this study was to evaluate the effect of temperature and precooling treatment on seed germination of *E. purpurea*, contributing to the understanding of this important medicinal plant.

Material and methods

This research was conducted at Plant Physiology and Systematic Botanic laboratory of Agricultural Sciences Institute from Federal University of Minas Gerais. The inflorescences, used as experimental material, were collected with physiological maturity established. Seeds from twenty inflorescences were extracted, and were processed to remove floral debris and other plant parts. By visual selection, seeds with best conditions to express full germination potential were selected. The seeds were previously disinfected with 1% hypochlorite for one minute, and then washed in sterile distilled water.

Freshly harvested seeds were used in order to obtain better results. Also, in this study was considered that the storage did not influence light requirements and temperatures during the germination process (FERREIRA et al., 2001).

Gerbox previously sterilized were used for germination test, with wet germination paper that was autoclaved. The volume of sterile distilled water was equivalent to 2.5 times the mass of dry germination paper. Fifty seeds over the germination paper were arranged in each Gerbox. The first group with 1200 seeds was subjected to the temperature of 5°C for seven days, in germination equipment (BOD), with a photoperiod of 12 hours to evaluate if seed had dormancy. The second groups, also with 1200 seeds, were prepared according previous methodology (without precooling treatment).

Treatments were arranged in three temperature systems, which are 20°C, 30°C and alternating temperature, 20-30°C, with a photoperiod of 12 hours, in germination equipment (BOD). Daily evaluations were performed, and observations were completely finished after 21 days.

Some germination parameters were calculated: Final germination percentage: G = (n / a) x 100, being n = number of germinated seeds a = total number of seeds; mean germination time: $t = \sum(n_i t_i)/\sum n_i$, which ni = Number of germination seeds on the day and ti =Incubation time in days (Harrington, 1972); Coefficient of Velocity of Germination: CVG (N1/1 + N2/2 + N3/3 +... + Nn/n) which is N1, N2, N3,..., Nn are numbers not accumulated of germinations seed from first day until last day after experiment beginning (MAGUIRE, 1962).

Experiment was conducted on completely randomized design, with four repetitions to each treatment. General Linear Models (GLM) were constructed for data analysis, using statistical software (R DEVELOPMENT CORE TEAM, 2014). To analysis of seed germination percentage due to the different temperatures, treatment to break dormancy (seed cooling at 5°C for a week), and also the interactions between explanatory, were used the error quasibinomial distribution. To analyze of CVG and mean germination time, the same explanatory variables were fitted models with Gaussian error distribution. Contrast analysis with support of car pack was done for comparing the means of the explanatory variables with more than two levels. Significant amounts of p lower than 0.05 were considered.

Coefficient of velocity of germination and mean germination time was calculated in the end of experiments. Data obtained by germination test were statically analyzed in R® software.

Results and discussion

Germination process occurred in all treatments. The germination percentage is shown in Table 1 for the evaluated variables. Both germinated seeds percentage and the CVG were influenced by precooling treatment (Table 1), which higher germination percentages were observed in precooling treatment (97.75 ± 0.62 %) and in without precooling treatment (95.58 ± 0.54%).

The germination of the seed presents satisfactory performance in the three temperatures on the two groups. The highest germinated potential was observed on alternating temperature 20-30°C (97.00 ± 0.93%). Germination percentage was also positively affected by precooling treatment (Table 1).

Temperature variation can benefit the seeds germination of several species of the Asteraceae family. There are reports that seeds from others medicinal species of this family have better germinations at 20°C, which allows settle after the winter and have strong permanent on site (FERREIRA et al., 2001). Many species express highest germination potential when are subjected to daily alternating temperature, which can be related to seed dormancy, although alternating temperatures can accelerate the germination of non-dormant seeds (Kissmann et al., 2008). Velten and Garcia (2005) observed that this temperature range promote germination from others species of Asteraceae family, such as *Eremanthus glomerulatus* e *E. Elaeagnus*. *Stenachaenium campestre* also presents higher germinability to this temperature range.

Germination percentage of this seeds tends to be higher when temperature conditions are similar to the same temperatures. This can correspond to the period of the year where environmental conditions are favorable to emergence and establishment of the seedling (VELTEN e GARCIA, 2005). Thus, the optimum temperature range for seed germination is affected by physiologic adaptability of species in cultivation or origin place. Therefore, optimum temperature range has strong relation with climate and biome.

Considering the high germination percentage of the precooling treatment, an association with the climatic temperature conditions of plant origin place can be performed. According to Tang et al. (2008) *Chenopodium album* seeds subjected to low temperatures present germination capacity due the synthesis of gibberellin, since, gibberellin biosynthesis is regulated by light and cold temperatures.

Plants from *Asteraceae* family frequently present seed with physiologic dormancy as germination process impediment. There are performances of some hormones ruling seed germination process. Abscisic acid can affect dormancy and gibberellin can stimulate and complete germination. Also, ethylene can act on breaking seed dormancy. Besides, precooling treatment can also promote breaking seed dormancy (WOOD et al., 2013). During precooling treatment, the induction of ethylene hormone release can be occurred, but necessary levels of ethylene for breaking dormancy are very high. Nonetheless, ethylene can reduce abscisic acid effect and improve gibberellin performance.

CVG was affected by temperatures. The alternating temperature 20-30°C and constant temperature of 30°C do not differ statistically and present higher amounts of CVG 28.92±1.10 e 27.83±2.17, respectively, in the group subjected to precooling treatment (Figure 1). TO second group, not subjected to this treatment, the temperature that presented higher CVG was alternating temperature 20-30°C (21.12±0.91). The smaller CVG occurred on temperature of 20°C both to the group subjected to precooling treatment (16.93±0.78) and without this treatment (13.01±0.35) (Figure 1). Temperatures of 20°C, with precooling treatment, and 30°C without precooling treatment do not differ statically in relation to CVG (Figure 1).

Table 1. Results from statically analyses using General Linear Models (GLM), between germination seed percentage, CVG e MGT (response variables) and different temperatures on presence and absence on pre-germination treatment (explanatory variables).

Response variables	**Explanatory variables**	**DF**	**F**	***p*-value**
Germination seed percentage	Temperature	2	0,2256	0,79899
	Precooling	1	5,5968	0,02246
CVG	Temperature	2	40,7567	$1,453 \times 10^{-10}$
	Precooling	1	68,1705	$2,460 \times 10^{-10}$
	Temp: Precooling	2	5,4043	0,008155
MGT	Temperature	2	19,531	$8,494 \times 10^{-7}$
	Precooling	1	19,691	$6,023 \times 10^{-5}$

DF: degrees of freedom, CVG: coefficient of velocity of germination, TMG: mean germination time.

The presence or absences of light and different temperatures are environmental factors that most commonly impact germination. These, together with water, especially in soil microsites, regulate germination (FERREIRA et al., 2001). It was obtained higher germinability and fewer MGT (3.19 ± 0.16) with variations in temperature (20-30°C). Some seeds react to the temperature alternate, because have enzymatic mechanisms which act all different climate and temperature conditions. This may be related to ecological adaptations of the species to the environment (OLIVEIRA et al., 2011).

Temperature has huge influence on seed germination, since this parameter is considered regulatory of germination speed. Optimum temperature range provides higher germination percentage, while extreme temperature (below or above optimum) can cause germination loses or even embryo death (CARVALHO e NAKAGAWA, 2000). Temperatures Bellow optimum temperature range can cause reduction of enzymes linked to respiration and cellular metabolism (TAIZ and ZEIGER, 2009).

Optimum germination temperature occurred when germination takes place with high efficiency, that is, high germination percentage together with fast seed germination, shortening exposure time of seeds to adverse conditions (SCREMIN-DIAS et al., 2006).

1

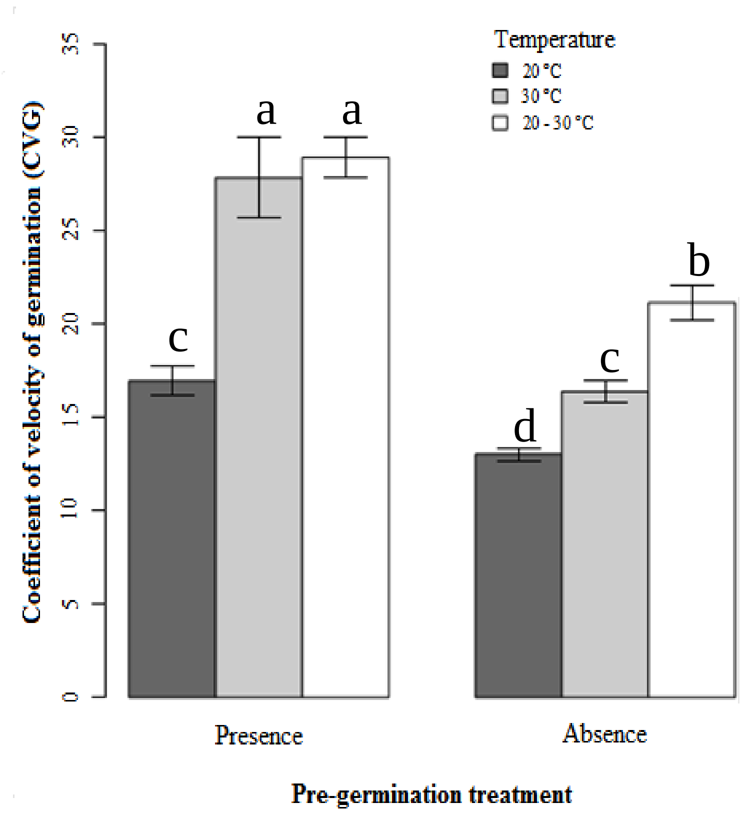

Figure 1. Mean coefficient of velocity of germination (± standard error) due to different temperatures and presence/absence of pre-germination treatment.

Alternating temperature also promoted lower mean germination time (2.92± 0.13), and constant temperature of 20°C got higher MGT (4.22 ± 0.19). Seeds submitted to precooling present fewer mean germination time (3.20 ± 0.17) and with treatment present higher MGT (Figure 2). Mean germination time can be used as evaluation index of the speed of occupation for some species (FERREIRA et al., 2001). When germination was fast, it is possible to the species establish in the environment taking advantage of favorable conditions. However, rapid environmental change conditions can cause loss of all germinated seedlings and even loss of individuals. According to Ferreira et al. (2001), about the mean germination time, seeds can be classified as fast (mean time <5 days); intermediate (mean time> 5 <10 days) and slow (mean time> 10 days). According to the results obtained in this study, *E. purpurea* seeds are fast (Figure 2).

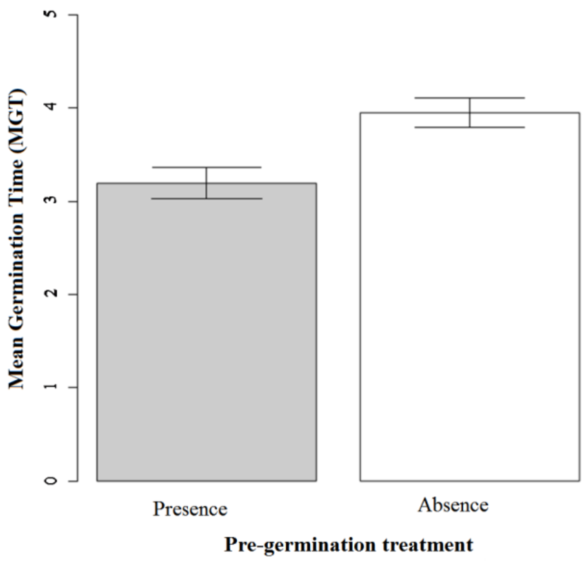

Figure 2. Mean germination time (mean ± standard error) due to presence/absence of pre-germination treatment.

Seeds that respond to alternating temperatures can maybe have enzymatic mechanisms that function in different climatic and temperature conditions. This response is probably related to the ecological adaptations of species to the environment (OLIVEIRA et al., 2011).

Results indicated that variables that more benefit CVG were precooling treatment interacting with temperatures. This treatment was crucial to high value of CVG. According to Bufalo et al. (2012) it is likely that this treatment altered hormonal balance between promoters and inhibitors on the germination process, increasing the limit temperature of Echinacea seed germination and suggesting that precooling at 5°C required a longer time to reduce the internal levels of germination inhibitors. Brasil (2009) reported that treatment benefited CVG due to breaking dormancy of Echinacea seeds.

Conclusions

Different temperatures and precooling treatment impacted seed germination.

Better temperature range for *E. purpureae* germination was alternating temperature 20-30°C. Temperature was crucial to CVG and to MGT and influence germination percentage. According to MGT, *E. purpurea* presents fast seeds. However, temperature does not impact on germination percentage.

Precooling treatment affected positively percentage of germinated seeds. Also, precooling treatment affected positively CVG especially at temperature range of 20-30 °C. Precooling treatment influenced positively MGT, especially at temperature range of 20-30 °C.

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