

Treatment Induced Germination Improvement in Medicinal Species of *Foeniculum vulgare* Miller and *Cuscuta epithymum*(L.) L

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Abstract

The purpose of this study was to investigate the effects of different treatments on seed germination in two medicinal species (*Foeniculum vulgare* and *Cuscuta epithymum*). Both species are known to have low seed germination. An experiment was performed with 9 treatments and 4 replications in a completely randomized design. Treatments included KNO₃ with concentrations of 0.1, 0.2 and 0.3 percent, immersion in boiling water for five and ten minutes, acetylsalicylic acid 50 and 100 mg L⁻¹ and prechilling for 10 days. Tap water was used as the control. Our findings indicate that 0.1 KNO₃ and 50 mg L⁻¹ acetylsalicylic acid were the most effective treatments for improvement of seed germination percentage in both species.

Keywords: Acetylsalicylic Acid, *Cuscuta epithymum*(L.) L, *Foeniculum vulgare* mill, Germination, KNO₃, Prechiling

1. Introduction

Germination is a critical stage in the life cycle of weeds, medicinal and crop plants, and often controls population dynamics, with major practical implications (Keller and Kollmann, 1999). Plant growth regulators such as GA (gibberellic acid) and IAA (indoleacetic acid) (Hilhorst and Karssen, 1992); chemicals such as KNO₃ (potassium nitrate) (Kevserog̃ lu, 1993; Hartmann *et al.*, 1997) and hot water treatments (Hermansen *et al.*, 1999) have been recommended to break dormancy and enhance germination. The objectives of this study were to determine the effect of acetylsalicylic acid, boiling water and prechiling applied KNO₃ on germination in finding effective methods for improving seed germination characteristics of *Foeniculum vulgare* and *Cuscuta epithymum*. Medicinal plants have been widely used to treat a variety of infectious and non-infectious ailments. According to one estimate, 25% of the commonly used medicines contain compounds isolated from plants. Several plants could offer a rich reserve for drug discovery of infectious diseases (Muhammad *et al.*, 2008). Over 60% of the world's population, 80% in developing countries depends directly on plants for their medical purposes. Medicinal plants have been harvested from the wild since ancient times (Dhillion and Ampornpan, 2000; Dhillion *et al.*, 2002). *Cuscuta epithymum* is a parasitic plant assigned to the Cuscutaceae or Convolvulaceae families, depending the taxonomy. This kind of plants are red-pigmented, because are not photosynthetically active. Its leaves are very little, like flakes. It is a mild laxative. Traditional Western claims for *cuscuta* are that it is a mild diuretic, and that it can be used to treat sciatica and scurvy. Externally, it can be gathered fresh and applied to the skin to treat scrofula derma. They are associated with the liver and kidneys and are used in formulas that help both yin and yang deficiencies, depending on the patient's condition and the other herbs in the formula. *Foeniculum vulgare* has a thick, perennial root-stock, stout stems, 4 to 5 feet or more in height. *F.vulgare* not only improves digestion, but also can reduce bad breath and body odor that originates in the intestines. *F.vulgare* also acts as an excellent digestive aid to relieve abdominal cramps, gas and bloating. *F. vulgare* teas are useful for chronic coughs and act as an expectorant to help clear mucus from the lungs. Oil of *F. vulgare* relieves muscular or rheumatic pains and is warming and soothing in massage oil blends. Women may also benefit from the estrogenic properties of *F.vulgare* (Mozafarian, 1996).

(Keshtkar et al., 2009) Studied the Effect of prechilling and GA3 on seed germination of *Ferula assa-foetida* and *Prangos ferulacea* and reported In the case of *F. assa-foetida* the highest germination percentage (52%) was obtained when the seeds were treated with 250ppm GA-prechilling. For *P. ferulacea* seeds, the highest germination percentage (73%) was found when exposed to 1000ppm GA-prechilling. (Razmjoo et al., 2009) surveyed Breaking seed dormancy of *Prangos uloptera* DC., a medicinal plant of the Iran with Fifteen treatments using sulfuric acid, GA3, ethephon, IBA, ethanol, 2, 4-D, dry heat and chilling and results shown treatments that broke dormancy with the greatest degree of success were GA3 (2500 ppm, 48h) and sulfuric acid (98%, 30 sec) and to a lesser degree IBA (500 ppm, 50 sec) and ethephon (250 ppm, 48h) as well. Hilton (2006) reported that KNO3 treatment in dark situation had every weak influence on seed germination *Avena fatua* while KNO3 with concentrations of 0.2, 0.002 and 0.0002 percent, in light situation induce seed germination. (Nadjafi, et al., 2006) study to species of medical plant *Ferula gumossa* and *Teucrium poliu* concluded that using KNO3, H2-SO4 and gibberelin acid had a significant effect on seed dormancy breaking and germination of two species. (Pérez-Fernández et al., 2006) Seed germination in response to chemicals: effect of nitrogen and pH in the media that *F. vulgare* was on of the species. Their results showed that Nitrogenous compounds increased percent germination (level) and High pH negatively affected the germination rate of seeds from most species, but had no effect on the per cent germination of any of the species.

(Tavili et al., 2009) analyzed the effect of gibberellins acid and KNO3 on germination on *Salsola rigida* and reported that pretreatment with KNO3 0.2% had most influence on seed germination. According to low germination seeds of mentioned species, also considering their medical importance, in this study effect of various treatments on effect seed germination on *F. vulgare* and *C. epithimum* was investigate to find the most effective treatments. *F. vulgare* and *C. epithimum* are valuable medicinal species of rangeland ecosystems and are frequently used in Middle East countries, especially in Iran, for remedy of neurological and women related diseases, so these species are considered to be cultivated in field conditions. Since their seed germination is low therefore it is needed to study and find germination improvement procedures which could help producers to cultivate and harvest big masses of mentioned species with no barrier.

2. Materials and Methods

Seed samples of two medicinal species namely *F. vulgare* and *C. epithimum* were collected from rangelands of Tehran province in 2009. Initial germination test indicated that germination percentage was low in both species. Therefore we decided to test different treatments effects on seed germination of mentioned species. For this purpose, an experiment with a Factorial Randomized Complete Block Design was done. Germination test was conducted by four replications and 9 different treatments. Twenty five seeds for each treatment were put in 10 centimeters Petri dishes. The treatments included 1- pretreatment with KNO3 (0.1, 0.2 and 0.3 percent) for 48 hours 2- add acetylsalicylic acid to the moisture in the petri dish (50 and 100 mg L-1) 3- prechilling (4 degrees centigrade for 10 days) 4- immersion in Boiling water (for Five and Ten minutes) before sowing 5- Control treatment (irrigation with distilled water). To sterilize the seeds Carboxin Tiram was used. Germination percentage was recorded daily during the study period. Rate of germination was estimated using modified Timpson's index of germination velocity (Khan and Ungar, 1984). Mean Germination Time (MGT) was calculated to assess the rate of germination (Ellis and Roberts, 1981).

$$MGT = \frac{\sum D.N}{n}$$

Where N is the number of seeds which in D day grow, n the total number of seeds grown and D is the number of days from the date of germination and the germination rate index was obtained by reversing MGT at the end of this period, final germination percentage was recorded. There are no outliers, normality of data was checked and non-normal data transformed by arc sin to verification of this hypothesis Arc Sin transformation was used for germination percentage before analysis (Khan et al., 2006). Experimental data was analyzed by MSTAT-C program (MSTAT-C, 1990). The difference between the means was compared using Duncan's multiple range tests at %5 level of probability.

3. Results

The results showed that the properties of mean germination time and germination percentage of both species significantly different ($p < 0.05$) under different treatments (Table 1). As table1 interactions of treatment \times species shows germination rate was not significantly affected by interaction of treatment \times species. For this reason, species and treatment affects were survey separately. Germination rate of *C. epithimum* was higher than *F. vulgare* (figure4). Among understand treatments, the highest germination rate belongs to KNO3 (0.1%). As it has been represented in figure 3 mentioned treatment has a significant difference with control.

Germination percentage was significantly affected by different treatments (figure2). Using KNO₃ 0.1, 0.2 and 0.3 percent associated with acetylsalicylic acid 50 and 100 mg L⁻¹ resulted in increasing germination percentage in *F.vulgare* KNO₃ 0.3% affect was not significantly difference. Decrease of germination percentage occurred when seeds of *F.vulgare* were put under treatments of prechiling (for 10 days) and pretreatment with boiling water (for 5 min). Boiling water for 10 minutes of *F.vulgare* had no effect on germination percentage. The results from different treatments on *C.epithymum* seeds revealed that KNO₃ 0.1, 0.2 and 0.3 percent, acetylsalicylic acid 50 and 100 mg L⁻¹, prechiling for 10 days and boiling water for five minutes have had a positive affect on germination percentage of mentioned species while the effect of acetylsalicylic acid 100 mg L⁻¹, prechiling for 10 days and boiling water for 5 minutes was not significantly different. Boiling water for 10 min resulted in decrease of germination percentage in *C.epithymum* seeds.

Mean germination time in *F.vulgare* seeds decreased with using all of the treatment compared to control. Among the treatments, KNO₃ 0.1, 0.2 and 0.3 percent and boiling water 5 min significantly decreased mean germination time. Decrease of mean germination time happened when different treatments were applied except to KNO₃ 0.2% and boiling water. In comparison of mean germination time between control and applied treatments, it was resulted that mean germination times of *C.epithymum* decreased under effect all of the treatments. In separately survey this case, boiling water 10 min and KNO₃ 0.1%, prechiling and acetylsalicylic acid 50 and 100 mg L⁻¹ significantly decreased mean germination time. (figure1).

Figure 3 explains that KNO₃ (0.1%) and boiling water 10minutes had the most and least effect on germination rate of medicinal species respectively. Comparing germination rate of *F.vulgare* and *C.epithymum* (figure4) shows that *C.epithymum* contains higher germination rate (0.16 against 0.12).

4. Discussion and Conclusion

Based on obtained results, it was cleared that suitable treatment for germination properties improvement in two medicinal species were approximately similar. KNO₃ 0.1 and 0.2 percent in addition to acetylsalicylic acid 50 mg L⁻¹ were effective treatments for *F.vulgare* and KNO₃ 0.1, 0.3 percent and associated with acetylsalicylic acid 50 mg L⁻¹ were suitable treatments for *C.epithymum*. In both species, although the were no significant differences between KNO₃ (0.1%) and acetylsalicylic acid 50 mg L⁻¹ in view point of their influence on germination properties but KNO₃ (0.1%) results were better when compared to acetylsalicylic acid 50 mg L⁻¹. This result is like what some other researchers have found (Hilton, 1984) and (Nadjafi et al; 2006). (Pérez-Fernández et al., 2006) showed that Nitrogenous compounds increased percent germination of *F. vulgare* in response to chemicals. *Foeniculum vulgare* showed higher germination percentage compared with *C.epithymum*. This is may be due to soft coat of *F.vulgare* existence of thin and soft coat in *F.vulgare* facilitates imbibitions and gas entrance and stimulates germination. ISTA (International Seed Testing Association) recommended KNO₃ 0.1 and 0.2 percent and for seed germination improvement. In current research it was revealed that KNO₃ (0.1%) suitable level for *F.vulgare* and *C.epithymum* germination improvement. Positive effect of KNO₃ could be due to its role on balancing hormonal portion within seed which in turn results germination inhibitors ratio like A.B.A.(Abscisic Acid), Derkan and Karssen (1993) believed that the reaction of seeds against KNO₃ is related to their sensitivity. Germination decreased in both medicinal species when boiling water (5 and 10min) was used. This might be because of destructive effect of boiling water on embryo. The results showed that boiling water 10min had negative effect compared to 5min. Destructive effect of boiling water depends on two properties: the temperature of water and the time length. In this research the temperature was the same (100° c) but times were different. The effect of acetylsalicylic acid on endogenous phytohormones levels has been mentioned by (Tompsett and Schwabe, 1974). Acetylsalicylic acid treatment could be recommended to enhance plant growth and productivity also to induce plant resistance against biotic and abiotic stresses as well (Raskin et al., 1995).

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Table 1. Variance effect analysis of treatments on measured characteristics of two medicinal species, *F. vulgare* and *C. epithymum*

| Source of Changes | Degrees of freedom | Mean Square of Germination percentage | Mean Square of Mean germination time | Mean Square of Germination rate |
|---------------------|--------------------|---------------------------------------|--------------------------------------|---------------------------------|
| replication | 3 | 144.406 | 1.533 | 0.001 |
| species | 1 | 1180.518** | 0.054** | 0.030** |
| treatment | 8 | 2569.692** | 30.722** | 0.021** |
| species × treatment | 8 | 418.447** | 12.904** | 0.003 ^{ns} |
| Test error | 51 | 46.745 | 2.074 | 0.001 |

*, ** and ns Significant level, refer to %0.1, %0.5 and no significant, respectively.

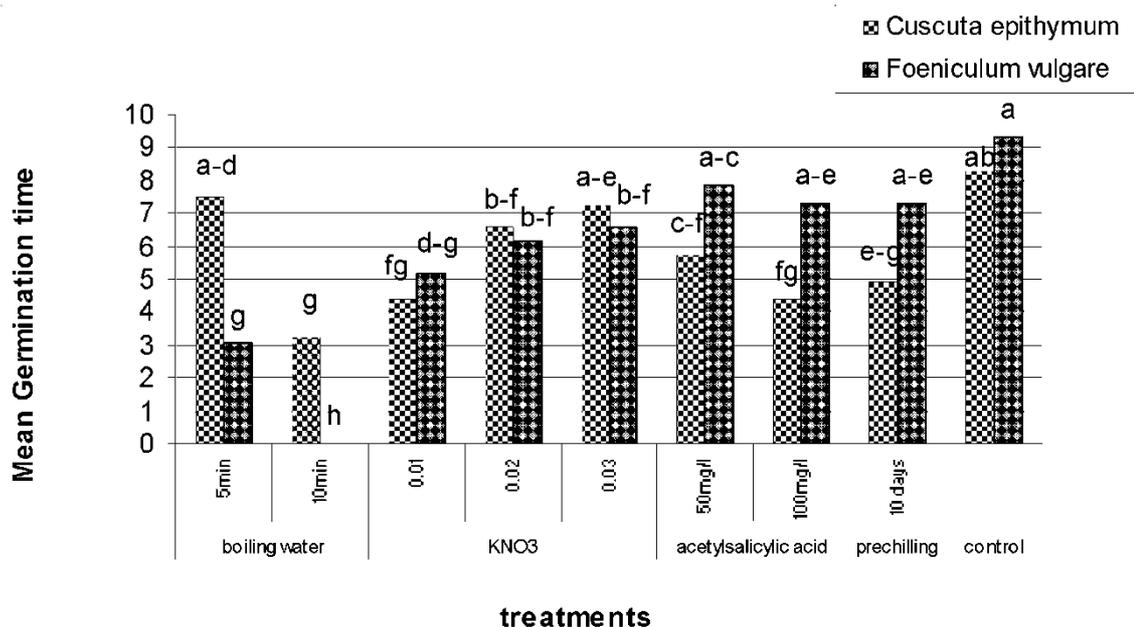


Figure 1. Comparison of mean germination time of *C. epithymum* and *F. vulgare* under effect various treatments

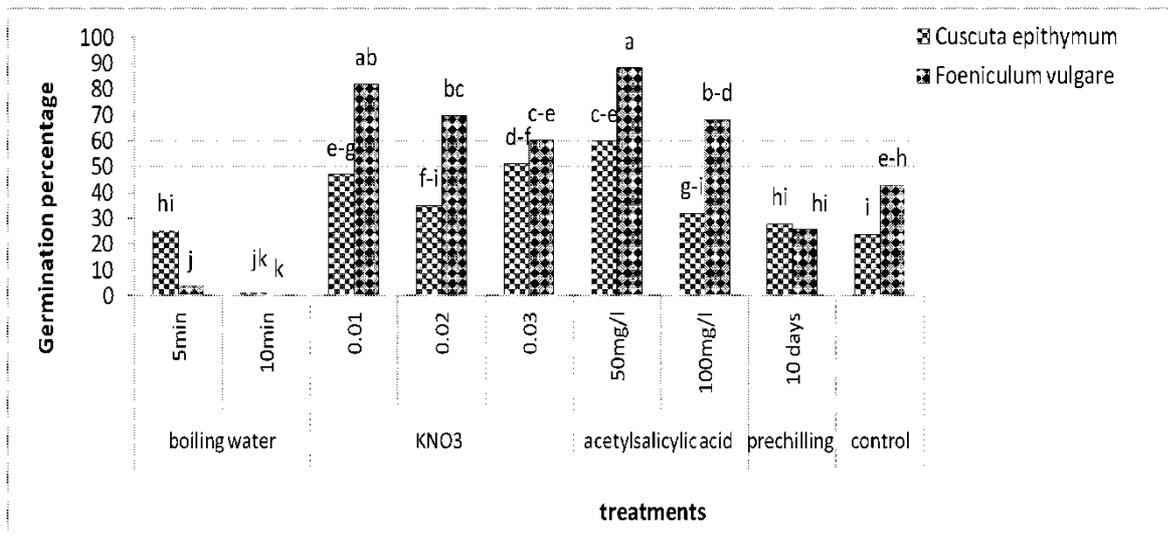


Figure 2. Comparison of average germination percentage of *C. epithymum* and *F. vulgare* under effect various treatments

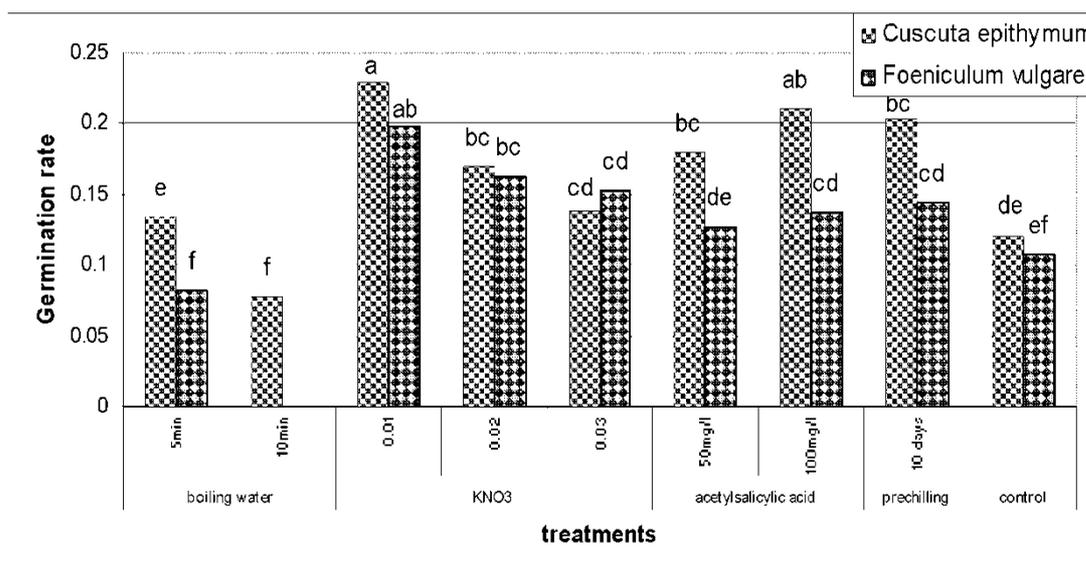


Figure 3. Comparison of germination rate of *C. epithymum* and *F. vulgare* under effect of various treatments

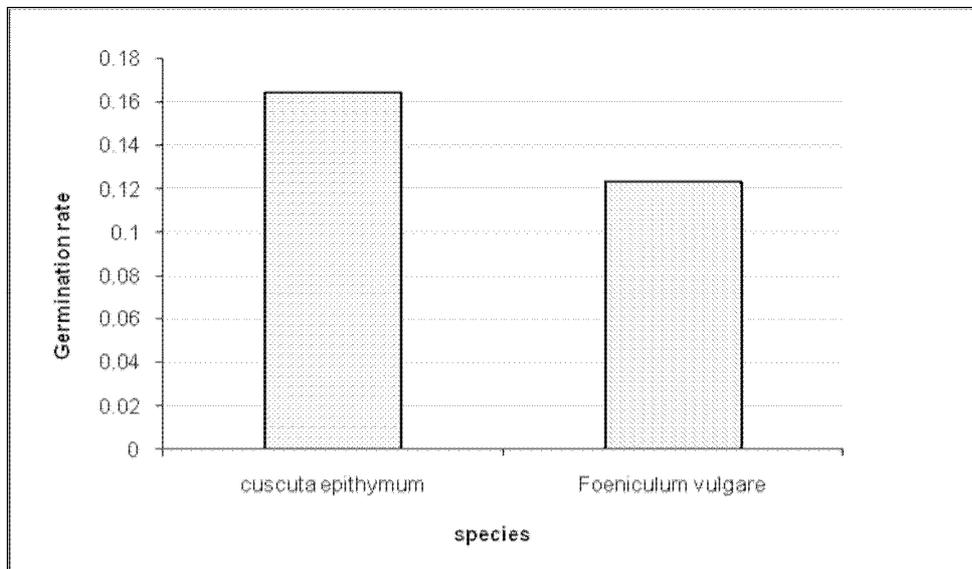


Figure 4. Comparison of average germination rate in two medicinal species *C. epithymum* and *F. vulgare*