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EFFECT OF TEMPERATURE REGIMES ON SEED GERMINATION
ASAFOETIDA (*FERULA ASSAFOETIDA* L.)

ABSTRACT

Asafoetida is a medicinal plant belonging to the *Apiaceae* family. Gum obtained from the lower part of the stem and roots of this plant has many industrial and pharmaceutical applications. This plant is subject to extinction in its natural habitats due to over-utilization. Understanding the biology of seed germination can help to restore such degradation by implementing agricultural development programs. The present study is an attempt to determine the germination responses to two temperature regimes (constant and fluctuating) during the course of the study. The experiment was planned based on a factorial-completely randomized design with two factors (constant and fluctuating temperature regimes) at 3 levels each (15, 20 and 25°C) with 4 replications. The results showed that the characteristics of germination in asafoetida were significantly improved under the fluctuating temperature as compared with the constant regime. It showed a mean germination time of 1.88 days for the fluctuating regime, while it was 4.88 days for the constant regime. The same results were found on germination rates in favor of fluctuating (0.62 per day) in comparison with constant regime (0.33 per day). Under the fluctuating regime, the lowest level of imposed temperature (daily application of 10 and 20 degree during the first and the second 12 hours, respectively) was the best for seed germination in this experiment.

Key words: asafoetida, constant temperature, fluctuating temperature, medicinal plants, seed biology

INTRODUCTION

Asafoetida (*Ferula assa foetida* L.) is a medicinal plant belonging to *Apiaceae* family. It's a perennial plant native to steppe region of Iran which

includes most parts of Khorasan, Yazd, Kerman, Sistan and Baluchestan and Esfahan provinces (Pirmoradi, 2002). If the root of this plant is scarified by a sharp object a valuable gum exuded. The gum forms a good income for a large number of rural residences. It has a disagreeable smell caused by sulfur compounds (Omidbaigi and Pirmoradi, 2006). The essential oil of Asafoetida (Terpene, Pinene and a sesquiterpene and sulfur compounds) forms up to 65 percent of the gum content. About 14 different compounds such as episamarcandin, umbelliperenin and coniferol have been found in asafoetida resin_ (Nassar *et al.*, 1995). The essential oil has pharmaceutical usage as well as large consumption in food industry. Asafoetida has several medicinal properties including antispasmodic, digestive, expectorant, laxative, sedative, nervine, analgesic (Eighner and Scholz, 1998., Bremness, 1994).

Germination is a critical stage in the life cycle of plants which greatly affected by environmental and physiological factors such as humidity, light, temperature and dormancy (Alvarado and Bradford, 2002). Temperature as the most effective factor controls the metabolic rate and the subsequent growth in all organisms (Oliver and Annandale, 1998). It plays the main role on germination characteristics such as germination onset, the rate and the percentage in which whether or not lead to plant establishment (Jami Al-Ahmadi and Kafi, 2007).

The sensitivity to temperature regimes (constant or fluctuating) varies in different species. When the condition for seedling growth becomes favorable, seed germination can greatly affect the survival of the species (Baskin and Baskin, 1998). Responses to temperature fluctuation in some cases may be influenced by the low active form of phytochrome (Probert, 2000). In contrary, there are species that fluctuating temperature can greatly stimulate germination even in darkness. Vazquez-Yanes (1974) reported that the seeds of *Ochroma lagopus* never germinate at a constant temperature but when are exposed to fluctuating temperatures, the germinating initiate even in darkness. In a laboratory work, Thompson and Grime (1983) found that 46 out of 112 species were germinated using fluctuating temperatures.

Unfortunately there is no relevant information regarding asafoetida in literature. Over-utilization of asafoetida in natural ecosystems has put the plant survival at a critical risk therefore due to lack of knowledge there is a need to investigate the biology and the seed germination responses to climatic factors mainly temperature. The authors are hopeful that the outcomes can help to a better understanding of the current issues on propagation and plant establishment in natural ecosystems where the species is at brink of extinction. This laboratory research project aims to study the effects of selected degrees of temperature under the different regimes on biology and seed germination of asafoetida in South Khorasan province of Iran.

MATERIALS AND METHODS

This study was conducted in 2011 in laboratory of seed technology, Faculty of Agriculture of Birjand University. The seeds of asafoetida were collected from Rashid Kooch in Jandagh region, 1800 meters above sea level in center of Iran. After the seeds' collection they were incubated at 20°C for a while and then were pretreated by Benzyl Amino Porine 0/25 Mg per liter at 5°C (Otroshy *et al.*, 2009) for 28 days to break dormancy. The seeds were then sterilized by 5% Sodium Hypochlorite solution for 2 minutes and washed three times by sterilized distilled water. The study design was a factorial completely randomized with two factors (constant and fluctuating temperature regimes) each at three levels of 15, 20 and 25°C in 4 replications. In constant regime, the applied temperatures remained constant during the experiment while in fluctuating, two temperatures (10 and 20°C, 15 and 25°C and 20 and 30°C) were applied daily (12 hours each) as representation of 15, 20 and 25 degree Celsius, respectively.

The experiment includes Petri dish with 10 centimeters in diameter containing 20 seeds. After placing two layers of Whatman filter papers in Petri dishes, 5 ml of distilled water was added. Germinated seeds were counted daily and continued until the cumulative value of the germinated seeds reached to a constant level. In this experiment root emergence (visible with naked eye) in the Petri dishes was regarded as seed germination (Bradel and Jense, 2005; Adam *et al.*, 2007). Germination percentage and rate was calculated at each temperature. By counting the germinated seeds for each day, mean germination time (MGT) was calculated (Flores and Briones, 2001; Bradel and Jensen, 2005). Here we calculated MGT using the following method of Matthews and Khajeh Hosseini, 2006:

$$MGT = \frac{\sum n \times t}{\sum n}$$

where, n= number of seed germinated per day and t= number of day

Before analysis, the percentage data were converted to $\text{Arcsin} \sqrt{x/100}$ Analysis of variance and mean comparison test was performed by SAS version 8.2 software. Mean comparison test were made by FLSD test.

RESULTS

The results on temperature regimes showed that the fluctuating temperature had significant effect on both germination rate and germination time ($p < 0.01$) but not on germination percentage. The temperatures applied at three levels on the other hand only had significant effect on seed germination percentage ($P < 0.01$) with no effect on germination rate and mean ger-

mination time. The interaction between the regimes and the temperatures on mean germination time was significant ($P < 0.01$) while it had no effect on the others (Table 1).

Table 1
Mean square values of seed germination percentage and rate and mean germination time of *asafetida*

| Variables | DF | Germination rate [1/day] | Germination percentage [%] | Mean germination time [day] |
|----------------------|----|--------------------------|----------------------------|-----------------------------|
| Regime | 1 | 0.562** | 551.04 ^{ns} | 30.27** |
| Temperature | 2 | 0.063 ^{ns} | 2101** | 9.58** |
| Regime × Temperature | 2 | 0.064 ^{ns} | 182.29 ^{ns} | 21.93** |
| Error | 18 | 0.044 | 136.46 | 1.46 |

^{ns} and ** indicate non-significant and significant ($\alpha \leq 0.01$) respectively

The temperature regimes showed great effects on both germination rate (Fig 1) and germination time (Fig 2) but had no effect on germination percentage. The fluctuating temperature regime increased germination rate significantly as compared with the constant regime. The results showed that the germination rate increased high up to 0.622 (1/day) on the fluctuating regime while it only reached to 0.332 (1/day) under the constant regime. With such a great effect on germination rate under the fluctuating temperature regime it is most likely to have a much better seedling establishment of *asafetida* than the constant regime.

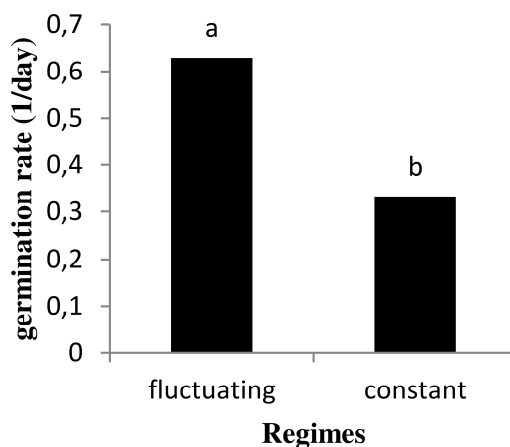


Fig. 1: Effect of the temperature regimes on germination rate

In Fig. 2 the mean germination time in fluctuating temperature regime (1.87 days) was significantly lower than the time at the constant regime (4.12 days). In other words, the time to have at least 50% of the seeds germinated under fluctuating regime is lower than the constant regime.

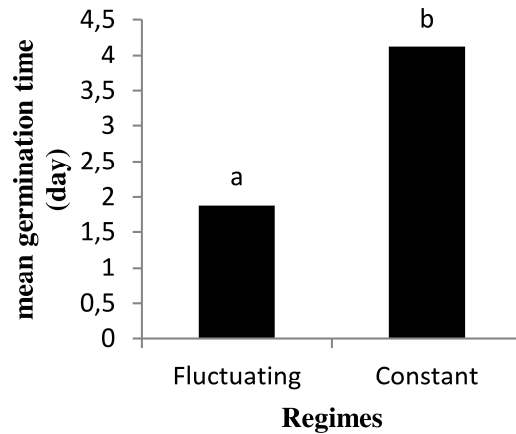


Fig. 2: Effect of the temperature regimes on mean germination time

The overall results shown in the figure above illustrate a big difference between the temperature regimes in favor of the constant one. Details on such a significant variation between the regimes is addressed in Fig 3 where germination time decreased largely from 6.58 to 2.16 days by increasing temperature from 15 to 20°C under constant temperature regime. Further increase in temperature to 25°C on the other hand had some increase in the germination time but did not reached to a level to make a difference to that it was at 20°C. In fluctuating temperatures there was found no significant differences between the mean germination times observed under the three temperature categories.

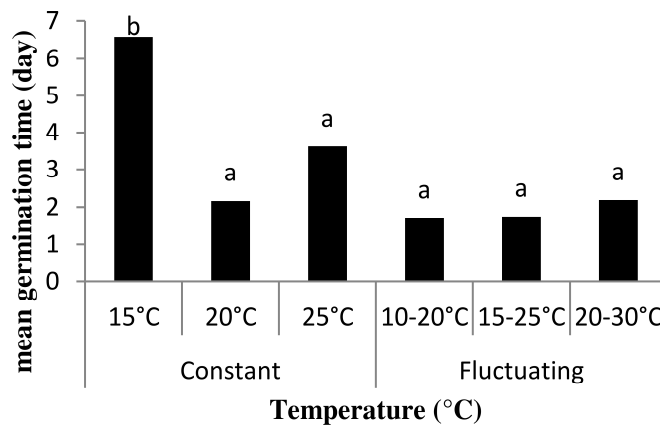


Fig. 3: Interaction effect of temperature and regimes on mean germination time

Based on the results obtained above, the lowest germination time (1/69 days) was occurred in the fluctuating temperature regime (10-20°C) while in the constant regime the mean germination went up to 6.58 days (the

maximum mean germination time). This finding highlights the significance of the temperature regimes on reducing the germination time.

As reported earlier, the temperature regimes had no effect on the seed germination percentage however a significant variation on germination percentage in relation to the temperature levels has been observed.

Such variations demonstrate an adverse relationship between the temperatures and germination percentages (Fig 4). The Maximum germination percentage occurred at the mean temperature of 15 degree (48.75 percent-ages) and decreased with the increase in temperature to 20 and 25°C respectively ($P < 0.01$).

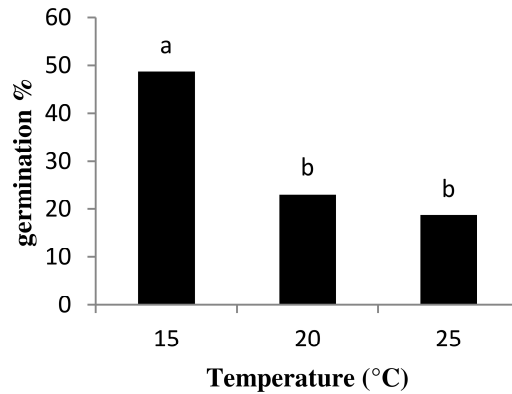


Fig. 4: Effects of the selected temperatures on germination percentage

The same as the germination time, germination percentage also has shown significant responses to the temperatures ($P < 0.05$). As the Fig 5 illustrates, the highest mean germination time (4.14 days) was observed at 15°C while it dropped to 1.95 at 20°C and then rose up to 2.91 when the temperature increased to 25°C.

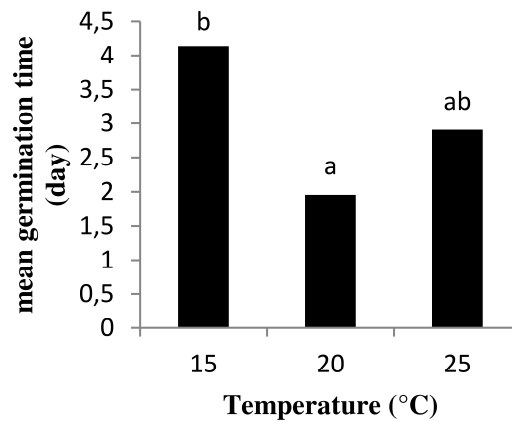


Fig. 5: Effect of temperature on mean germination time

Low germination time means higher germination rate, therefore it can play a key role in seedling emergence and establishment. The graph depicts that the temperature of 20 degree could be regarded as an optimum thermal threshold necessary to achieve the lowest germination time in asafoetida.

DISCUSSION

The results of this study showed that the temperature regimes have great effect on germination rate of asafoetida. Generally, the germination rate is a sensitive index to temperature that can affect germination (Schimpf *et al.*, 1977). In this study, the fluctuated temperature led to significant changes in germination rate as well as germination time compared with the constant regime where a constant temperature is applied during the experiment. The significance of such an effect is almost double in favor of fluctuating regime for germination rate and half for the germination time over the constant regime.

Although the findings of this study is new and no relevant report could be found in literature however there are instances on some other plant species such as *Chenopodium album*, *Panicum maximum* (Murdoch *et al.*, 1989) and *Orbanch* spp (Kebriab and Murdoch, 1999b) in which imposing more than one temperature during the experiment return higher germination rate. According to Evers (1991), rapid germination can increase the likelihood of early radicle departure from seed facilitating water usage and better seedling establishment.

The germination percentage in this study was observed to be highest averaged at 15 °C and showed an inverse relation with temperature when it rose up to 20 and 25. The result confirm the outcomes of a previous work (Zangoie *et al.*, 2012) in which the 15 °C is the favorable temperature for Asafoetida in relation to seed germination percentage. In our data there seems to be an optimum degree of temperature recognizable in which above that the germination percentage is greatly reduced which is in line with the outcomes of Tabrizi *et al.* (2007). The reduction of germination percentage is attributed to the structural changes in some proteins essential for germination (Copeland and McDonald, 1995) and seed deterioration (Hardgree, 2006a) under higher temperatures.

Comparing the temperature regimes, the fluctuating temperature leads to a significant reduction in mean germination time of asafoetida that is more evident in lower temperatures. From the other hand, it was observed that germination times did not respond differently to the fluctuated temperatures (10-20, 15-25 and 20-30). The results suggest that only fluctuating temperature itself is effective in reducing the germination time of asafoetida not the amounts of the temperatures applied. Nevertheless the great outcome of the fluctuating regime in reducing germination time provides the seeds of Asa-

foetida with a faster growth leading to a better seedling emergence and establishment. This is more important in regeneration of species in wild habitats (Chen and Maun, 1998) where the plants aggressively compete for water, nutrient and energy to death.

As was mentioned earlier, the fluctuating temperatures generally are more effective in stimulating seed germination (Baskin and Baskin, 1998) and this the one is precisely provided by the Mother Nature to the species in natural ecosystems. Plant adaption to the natural fluctuation of temperature in wild is regarded as a key to the species survival (Borges and Rena, 1993., Copland and McDonald, 1995).

CONCLUSION

The findings of this study showed that the thermal regime of fluctuating using temperatures averaged at 15°C returns the best germination condition for asafoetida seeds. To overcome seed dormancy and better growth and seedling establishment of asafoetida in natural environment, the results produced a guideline to select appropriate seeding time using local weather data. It suggests the days with average minimum and maximum temperatures of 10 and 20 respectively after the freezing peaks in winter.

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