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Research Article

SOME ECOLOGICAL FACTORS AFFECTING SEED GERMINATION IN WILD FIG (FICUS JOHANNIS BOISS.; MORACEAE)

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ABSTRACT
Wild fig (Ficus johannis) grows in the arid regions of Iran and has an important role in preventing soil erosion and feeding some animals. In this work the ecological factors of temperature, light, culturing substrate and fungal infections affecting seed germination of wild fig was investigated. Fresh and dried fig fruits were collected from Isfahan province, Iran. Three groups of seeds were used: washed in running water, unwashed and contaminated to fungi. Four ranges of temperatures, each in tow status (darkness/light; darkness) were also performed for the washed seeds. In each treatment 100 seeds in four replicates were used and the germinated seeds were counted weekly until the fourth week. The percent of and the time required for 50% of seed germination (T50) was calculated. Results showed that the wild fig seeds were positively photoblastic with lesser germination percentage at the darkness in comparison to the darkness/light condition. Maximum germination, 94 ± 4.32 %, was achieved at the alternating darkness/light at 30/10 °C in the end of fourth week. The seeds did not germinate at the temperatures below 10 °C. The percentage germination was 10 ± 2.31% and 2 % for unwashed and fungal contaminant seeds, respectively. Also the germination amount of fig seeds cultivated in the habitat soil was 45 % while it was 98 % in the sand as cultivation substrate.

Keywords: *Ecological Factors; Ficus Johannis; Seed Germination; Wild Fig*

INTRODUCTION
Wild fig (*Ficus johannis* Boiss.) is a member of the mulberry family (Moraceae) and grows naturally in the arid and mountainous regions of Iran (Browicz, 1982), Afghanistan, Pakistan, Oman and United Arabic Emirates (Jongbloed *et al.*, 2003). *F. johannis* is essentially resistant to drought and salinity and hence, it can be used to prevent soil erosion, establishment a sustainable landscape and restoration of degraded vegetation (Shanahan *et al.*, 2001). Furthermore, it plays an important role in the nutrition of some animals, especially some mammals and birds (Lisci and Pacini, 1994). So, it is important to be aware of its seed germination.

Some plant taxonomists believe that the two species of *F. johannis* and *F. carica* are very close relatives and *F. carica* is a hybrid that its probable parents could be *F. carica* subsp *rupestris* and *F. johannis* (Browicz, 1982). In the nature, these two species are reproduced through seeds and cuttings (Wondye, 2011; Caliskan *et al.*, 2012). A comprehensive study on the ecology of seed germination in *F. carica* was available (Lisci and Pacini, 1994) while the seed germination of *F. johannis* had not been reviewed so far. Therefore, the present work was performed to investigate the ecological factors such as temperature, light, cultivation substrates and fungal contaminants affecting seed germination in the wild fig (*F. johannis*).

MATERIALS AND METHODS
Fresh and dried fruits (syconia) of *Ficus johannis* subsp. *johannis* were collected from a habitat in the southwest of the Isfahan city, at position of 32°, 53.591' N and 51°, 55.809' E, with the elevation of 1842-1862 meters above sea level. Three groups of seeds were examined: washed in running water for 2 hours; unwashed with the around materials; and contaminated with fungi. Four ranges of temperatures (0/10; 10/20; 10/30; 20/30 °C), each in tow status (alternate periods of darkness/light; absolute darkness) were applied (adapted from Lisci and Pacini, 1994). In each treatment 100 seeds were placed on moist filter paper in Petri dishes at a distance of 5 mm from each other (Vazquez-Yanes *et al.*, 1996) and irrigated with distilled water every 24 hours. Germinated seeds were counted weekly until the fourth week. The

percentage of and the time required for 50% of seed germination (T50) was calculated (Shah *et al.*, 2002; Coolbear *et al.*, 1984). Four replicates in a randomized block design (Montgomery, 2008) were performed and the mean ± SD of them were calculated. By using analysis of variance (ANOVA) and Tukey's method for multiple comparisons (Montgomery, 2008) the significant differences and comparison between treatments and replicates were determined. All statistical analysis and drawing of diagrams were performed using Minitab 16.

After determining the optimum conditions of temperature and light, the rate of seed germination on the unwashed seeds and contaminated seeds were studied. The seeds of infected fig fruits were examined using a stereomicroscope to confirm the existence of fungal mycelia around them. Such seeds considered as fungal infected and used for germination test.

To investigate the effect of soil, a number of washed seeds were planted in two sets of polyethylene pots; one set filled with the soil of natural habitat and the second filled with sand. In each pot 25 seeds were planted in 0.5 cm depth. The pots were placed in the greenhouse at optimum conditions of temperature and light and irrigated with distilled water every 24 hours. The seedlings emerged from the soil surface were counted as germinated seeds.

RESULTS AND DISCUSSION

The highest percentage of seed germination in the wild fig (*Ficus johannis*) was 94% ± 4.32% that occurred at a period of 16 hours darkness with 10 °C and 8 hours light with 30 °C (Table 1 and Figure 1). In this status the time required to reach 50 % of germination was 11.5 days (T50 = 11.5). At the same temperature, but in the darkness, the amount of germination in the end of fourth week reached to 78.5 % that was approximately 15.5% lower in compared to the alternate periods of darkness/light (Figure 3). Under these conditions, 50 % of the seeds germinated after 19.8 days (T50=19.8). Seed germination did not occur at the temperatures ranging zero to 10 °C (Table 1). In the all treatments at temperatures above 10 °C, seed germination occurred in darkness, but much less than the speed of it in the alternate periods of light/dark. The results of the present study are consistent with the results for edible fig (*F. carica*) reported by Lisci and Pacini (1994), except for less germination percentage in the wild fig at the all treatments. The amount and rate of seed germination in seed plants is under influence of both internal factors (Genetics) and environmental factors such as humidity, temperature, light and substrate (Baskin and Baskin, 1998; Milberg *et al.*, 2000; Urgesa, 2011). Temperature is an important environmental factor affecting the seed germination and each species requires its own optimum temperature (Bewley, 1997). The role of temperature is more related to the seed internal changes, kinetic of enzymes and the biochemical nature of the seed cells (Vazquez-Yanes *et al.*, 1996).

Light is another environmental factor affecting seed germination in a number of angiosperms. Accordingly, some fig species have photoblastic seeds (Titus *et al.*, 1990). Vazquez-Yanes *et al.*, (1996) for example, reported that the seeds of three tropical fig species (*Ficus insipida*, *F. syoponensis*, *F. petensis*) with no germination in the darkness were positively photoblastic. Our findings revealed lower percent of seed germination at darkness for the wild fig (Table 1 and Figure 1). It may be attributed to the seed size and temperature ranges, because according to Milberg *et al.*, (2000) the dependence of seed germination to the light decreases with increasing in the seed size. Also, according to Baskin and Baskin (1998) and Chen *et al.*, (2013) suitable temperature can replace the need to light for germination of some positively photoblastic seeds. Direct observations showed fungal contaminants in most of the fig fruits fallen on the ground. Presence of fungal hyphae surrounding the fig seeds through microscopic examination confirmed this subject (Figure 2). At the optimum temperature and light, germination percent in the contaminated seeds was maximum 2 % at the end of the third week (Figure 3). Competition between fig seeds and fungal hyphae for more oxygen uptake, enzymatic modifications after hyphae penetration into the fig seeds that causes seedling death, are the probable explanations for impaired germination in the contaminant seeds (Debnath *et al.*, 2012). However, in some cases has been reported that the seed germination and seedling establishment have been increased due to mycorrhizal fungi (Harper and Lynch, 1981; Debnath *et al.*, 2012).

Germination percentage in the unwashed fig seeds (inserted into the fleshy pulps) at the optimal temperature and light was respectively 6 % and 10 % at the end of third and fourth weeks (Figure 3). Osmotic pressure caused by the fleshy tissues and nutrients surrounding the fig seeds prevent water absorption and consequently delay or prevent the seed germination (Lisci and Pacini, 1994; Chen *et al.*, 2013). Indeed washing eliminates these barriers.

In the nature, fig fruits are eaten by some species of mammals and birds. This process causes accelerating the seed germination because of elimination of substances surrounding the fig seeds after passing through the digestive system of these animals (Shanahan *et al.*, 2001; Wondye, 2011). Hence, these animals have an important role in distribution of the fig trees. Rain and running water also helps in this process (Lomascoloa *et al.,* 2010). At the optimum conditions of temperature and light, germination amount of fig seeds cultivated in the habitat soils was approximately 45% at the end of fourth week (Figure 3). While at the same conditions the amounts of germination for the seeds cultivated in sand and distilled water were respectively 98% and 94% (Figure 3). Indeed, the fig seeds germinated faster and with a higher amount in the sand substrate. Urgessa (2011) by investigating the role of soil factors on seed germination of some fig species concluded that some components of the natural soils may have negative effects on the seed germination; hence sand is the best substrate for these species. This conclusion is compatible with our results. Different fig species usually produce a plentiful fruits (syconia) with numerous seeds inside each of them (Figueirredo and Perin, 1995; Garcia *et al.*, 2005). However newly emerged seedlings are normaly rare in beneath and around of the wild figs. There are some explanations for this. First, according to Wondye (2011) a lot of seeds are not fertile. Second, the fungal contaminations reduce or prevent seed germination (Harper and Lynch, 1981; Debnath *et al.*, 2012). Microscopic observations on the wild fig (syconia) revealed that the ratio of empty to the intact seeds was relatively high due to the presence of fig wasp larvae that feed on the embryo and nutrients inside them. Wondye (2011) reported that in *F. vasta* the seed embryo is eaten by the larvae of a tiny wasp that lives inside the seeds. Specific symbiosis between a kind of wasp and the wild fig has been specified (Ghahari and van Noort, 2011). Therefore, the empty seeds in the wild fig may be attributed to these wasps. Furthermore, field observations indicated that the wild fig seeds are eaten by birds, wild wasps and ants. Also, according to Hadas (2004) and Urgessa (2011) a lot of fig seeds could not germinate due to their incompatibility with the soils of the habitat, or their growth is ceased before establishment.

Conclusion

1-Maximum seed germination of the wild fig (Ficus johannis) occurs at a period of 16 hours darkness with 10 °C and 8 hours light with 30 °C.

2-The wild fig seeds are positively photoblastic with lesser germination percentage at the darkness in comparison to the darkness/light conditions.

3-Seed germination in the wild fig is inhibited by its surrounding materials as well as through the fungal contaminations.

4-Seed germination in wild fig may be influenced by the substrates. some components of the natural habitat soils may have negative effects on the seed germination.

5-The optimal conditions of light and temperature to achieve the maximum seed germination in the wild fig (*F. johannis*) are relatively similar to those reported for the edible fig (*F. carica*); It is assumed that the two species are close relatives, so should be reduced to a single species and the latter becomes a synonymous. For this, more researches is required.

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