Comparison the Effect of Different Treatments for Breaking Seed Dormancy of *Citrullus colocynthis*

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Abstract

Citrullus colocynthis is one of the major medic plants and it has many medical values. This plant adopts in desert regions and specifically in sand dunes. Forasmuch as seeds of this plant germinate not easily, so in this research tried to study about effect of different treatments for breaking seed dormancy, germination stimulus and growth of *Citrullus colocynthis* seeds. The treatments were include sulfuric acid 98% in 20 and 40 minutes time intervals, potassium nitrate 0.2% within 72 hours, hot water in 90 centigrade degree during 10 minutes and scratching by sand paper. For comparing this treatments and normal germination is used distilled water for control. Experiments were performed in a completely randomized design with four repetitions and six treatments. Results of variance analysis and mean comparison showed that there are significant statistical differences (0.01 levels) between treatments for percentage and velocity of germination, length of the Root and Shoot. The maximum percentage and velocity of germination and length of the Shoot obtain in scratching by sand paper treatment and results of other treatments were in lower level than this one. According to got results determined that scratching is the most suitable method for dominance on seed dormancy of *Citrullus colocynthis* species.

Keywords: Seed dormancy, Germination, Scratching, *Citrullus colocynthis*

1. Introduction

Citrullus colocynthis (bitter apple) is a desert plant of the Cucurbitaceous family, naturally adapted to arid environments. Yield potential should be estimating under desert conditions in order to evaluate the plant's economic future as a crop suitable in an arid environment (Schafferman et al, 1998). The fruit is globular, smooth, with a hard but thin rind, something like a gourd. It is filled with a soft, white pulp, in which are imbedded numerous seed. This pulp is the article used in medicine (Lloyd, 1898).

According to human approach to medical plants and increasing cultivation area of these plants, are required to resolve the production blockage of medical plants. For germination, the dominated seeds (Brandel, 2004), it is necessary to apply some treatments for elimination the blockage of germination (Baskin, 1998). It is possible to cancel the mechanical dormancy with deleting seed frill, abrasion of seed crust, mechanical tools and using acids like sulfuric or nitrate acid (Black and Bewley, 2000; Kapland, 1996). Aliero (2004) studies effects of different treatments of sulfuric acid with 98%, 90%, 70% and 50% concentrations in 1, 3 and 5 minutes time interval and hot water treatment during 1, 2, 3, 4 and 5 seconds on germination of *Parkia biglobosa* 's seeds. He reached the conclusion that seeds by infusion in sulfuric acid 98% for 3 minutes in compared to other treatments, showed

highest level of germination percentage (50%), while sulfuric acid treatment 90% for 3 and 5 minutes lead to respectively 28 and zero germination percentage.

Sxitus et al. (2003) worked on breaking dormancy of *Ulex europaeus*'s seeds, which have hard crust they concluded that sulfuric acid and sand paper treatments cause an increase in germination of its seeds. In the another research Rehman et al. (1999) for dominance on seed dormancy of *Acacia salicina* which have hard crust, report that sulfuric acid 98% originate germination of this seeds and increasing time of connection with this acid solution causes increasing number of buds. They notify that this seed treatment with sulfuric acid 98% during 10 minutes have no effect on breaking seed dormancy but seed infusion for 30 minutes in mentioned acid show significant germination increasing. Rana and Nuatiyal (1989) also conducted similar experiment on the seeds of *Acacia farniesiana*; they observe using sulfuric acid causes increasing in germination, but with increasing time of seed connection with acid originate injury to embryo structure that produce more abnormal seedling. Research of Uzen and Aydin (2004) signify positive effect of scratching on breaking dormancy and germination stimulus on some seeds of *Medicago* genus. Mechanical scratching of *Ulex europaoeus*'s seeds with sand paper causes germination increasing in this seed.

Proliferation or reproduction and increased of *Citrullus colocynthis* is via seed, but with hard crust, impervious and dormancy period of this plant seeds, germination is very difficult. So, production of this plant faces with many problems. With respect to medicinal importance of *Citrullus colocynthis* (bitter apple) plant, in this study have trying to determine and introduce the best treatment for breaking dormancy, germination stimulus, increasing percentage and velocity of germination, and consequently, increasing in seedling establishment.

2. Material and Methods

Seeds of *Citrullus colocynthis* use in this study. Seeds were collected from arid regions of southeast of Iran, Sistan. Seeds cleaned and prepared. The study conducts in seed laboratory of Natural Resources faculty, university of Tehran. Seeds were disinfected using hypo sodium chloride for 5 minutes, then washed with distilled water and prepared for different experiments.

To overcome the dormancy imposed by the hard seed coat and embryo and to achieve rapid, uniform and high germination rates, a completely randomized design with six treatments and two replications conducted in this study. Seeds of *Citrullus colocynthis* were subjected to chemical scarification, as well as different concentrations and soaking times of 98% sulphuric acid. Treatments consist of T_1: control (distilled water), T_2: scratching with sand paper, T_3: pretreatment with H_2SO_4 (98%) for 20 and 40 minutes, T_4: pretreatment with KNO_3 (0.2%) for 72 hours, and T_5: soaking in hot water ($90^{\circ C}$) for 10 minutes.

The first and last counting of germinated seeds do in 3rd and 30th day of the experiment. After the day third, counting perform daily. Germination and early growth properties include percentage of germination, rate of germination, Root and Shoot length. Germination percentage (Camberato and Mccarty, 1999) and germination rate (Walker and Sesing, 1990) were calculated using equation 1 and 2.

Germination percentage

$$GP = \frac{\sum G}{N} \times 100$$

GP: germination percentage, G: number of germinated seeds in each counting, N: number of seeds

germination speed

$$GR = \sum_{i=1}^{n} \frac{S_i}{D_i}$$

Where S_i is the number of germinated seed at each counting, D_i is the number of day until n counting and n is the number of counting.

The obtained data was analyzed using analysis of variance (ANOVA). Means were compared at the 5% level of significance using Duncan's multiple range test with statistical software MSTAT-C version 2.00.

3. Results

As it has showing in table 1, there is significant difference ($P \leq 0.01$) among stimulator treatments in terms of their effects on germination percentage, germination rate, Root and Shoot length.

3.1 Scratching of Seed crust with sand paper

A germination percentage of 61% recorded with abrasion usage. This amount of germination was the highest one and significantly differed from the others (Fig. 1). In addition, figs 2 and 4 dedicates that germination rate and Shoot length resulted from scarification are largest one among different treatments while in Shoot length no significant difference is considered between scarification and boiling water.

3.2 Sulfuric Acid

Sulfuric acid usage as a stimulator resulted in four and 6 times greater germination percentage compared to distilled water (control) in 20 and 40 minutes treatments respectively. Fig 1 reveals that there is significant difference between germination percentage of sulfuric acid and the other three treatments so that germination percentage of sulfuric acid lies in fourth position when comparing with scarification, potassium nitrate and hot water. In addition, similar status could be considering for germination rate of treatments (Fig. 2). Root and Shoot Length impress under sulfuric acid as increasing time of seed contact with acid cause decreasing in seedling growth (Fig. 3 and 4).

3.3 Potassium Nitrate

Seed placing for 72 hours in potassium nitrate (KNO_3) cause significant increasing than control treatment in germination of *Citrullus colocynthis* seeds. Amount of germinated seeds under this treatment was 50% (Fig. 1) that showed the most germination percentage, velocity, Root and Shoot length after sand paper treatment (Figs. 2, 3 and 4).

3.4 Hot Water

When seeds placed in hot water $90^{\circ C}$ for 10 minutes originate significant increasing than control treatment in germination. Amount of germinated seeds under this treatment is 40 percent (Fig. 1) that after sand paper and potassium nitrate have maximum germination percentage and velocity and Shoot length (Fig. 2 and 4). This treatment shows the most Root length among the others (Fig. 3).

4. Discussion

Many seeds fail to germinate after processing and placement in favorable growing conditions - such seeds say to be dormant. In some dormant seeds, morphological changes must take place before germination can start. For others, parts of the seed must undergo physiological changes before germination can occur. Under natural conditions, necessary changes take place gradually under varying combinations of aeration, moisture, temperature, and light. By duplicating key conditions of the natural environment in the laboratory or nursery, dormant seeds can induce to germinate with a reasonable length of time. Seed dormancy is a phenomenon that distributes seed germination in time interval, and has vital role in seed plants permanence (Gu et al., 2003). This process in seeds has special importance and is noticeable in different viewpoints like amount, velocity and uniformity. Nikoleve (1977) divide seed dormancy to two section: endogenous (inborn) and exogenous. One of the methods for plant reproduction is seed, but Orphanos (1998) says that seed crust impenetrability is because of one-sclreedi layer cells (Roleston, 1987). Breaking of these cells coating or mechanical pressure can cause water infiltration and then germination (Brant et al., 1971; Egley, 1993). Therefore, Roleston (1987) express that in this seeds often use pre-germination treatments for crust penetrably and breaking dormant. Seed soaking in hot water or scratching with chemical or mechanical methods are pre-germination treatments in seeds with hard crust or shell.

Therefore, *Citrullus colocynthis* seeds germinate under effective treatments on seed crust like sand, H_2So_4, KNo_3 and hot water, and remove seed dormancy problem. We can say that this plant seeds dormancy presumably intercommunicate physical factors. As it result in this research is that mechanical resistance of seed crust against seed emergence cause *Citrullus colocynthis* seeds dormancy. When scratching treatment uses, seed crust being thin (sand paper) and create crack in seed crust (sulfuric acid and hot water). Sulfuric acid is able to solve the shell and breach the skin inhibited the germination process greatly reduce. Concentrated sulfuric acid with the destruction of seed coating and Asklrydy cells to allow penetration of water to dewatering process and seed dormancy cause by impenetrability water from skin remove (Levitt, 1974; Nikoleve, 1977). The investigations surrounding the effect of sulfuric acid on seed germination is corroborated this subject that seed germination rate depends on acid concentration and contact time with acid and seed shell. In this way, mechanical resistance against the bud emergence reduces. Positive effect of acid concentration on increasing germination velocity reports by Sparg et al. (2006). Successful seed germination of *Citrullus colocynthis* under crust scratching treatments confirming mechanical resistance of shell against sprouts for emergence. Like this condition may observe in mention plants: *Parkia biglobosa* (Aliero, 2004), *Tamarindus indica* (Mohammad and Amusa, 2003), *Ulex europaeus* (Sxitus et al., 2003), *Medicago* (Uzen and Aydin, 2004), *Ferula gummos* and *Teucrium polium* (Nadjafi et al., 2006).

With more study in this field and usage suitable treatments, may solve seed germination problem of this valuable medical plant. Generally, Results show that scratching is more important in breaking seed dormancy of *Citrullus colocynthis* plant compared to other treatments. However, this aspect can be cheaper and low risk, probable harm

to embryo compared to chemical material and especially acid has important, and its application is more advisable.

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Table 1. Variance analysis for studied properties of *Citrullus colocynthis* affected by various treatment

S.O.V	ss	df	ms	f
Germination percentage	8780.8	5	1756.1	198.8**
Germination speed	5.53	5	1.1	276.3**
Root length	48.84	5	9.76	23.1**
Shoot length	37.97	5	7.59	31.71**

Significant difference between treatments at 1% levels**

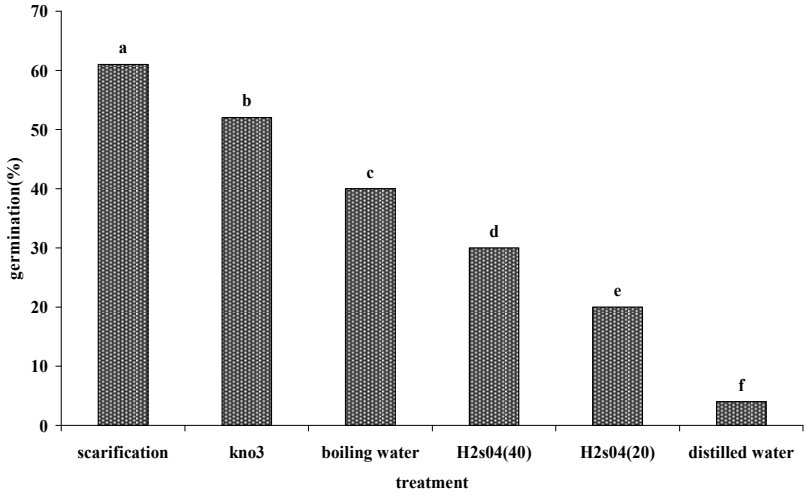

Figure 1. Comparing affects of various treatment on germination percentage of *Citrullus colocynthis*

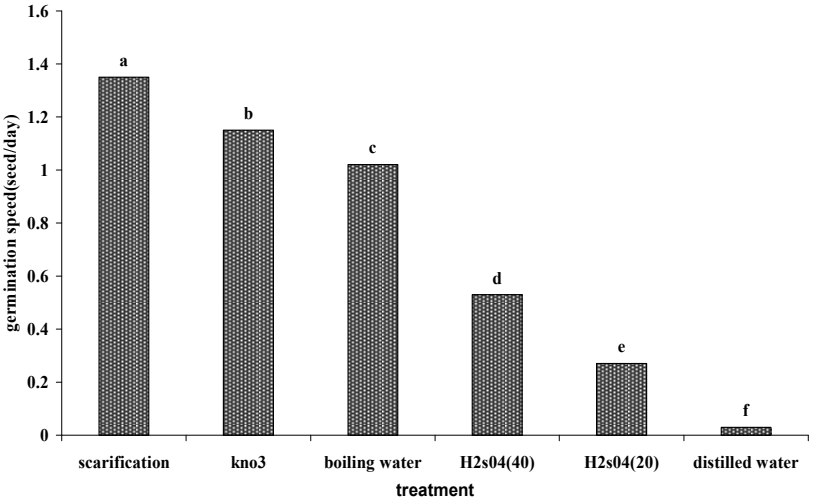

Figure 2. Comparing affects of various treatment on germination speed of *Citrullus colocynthis*

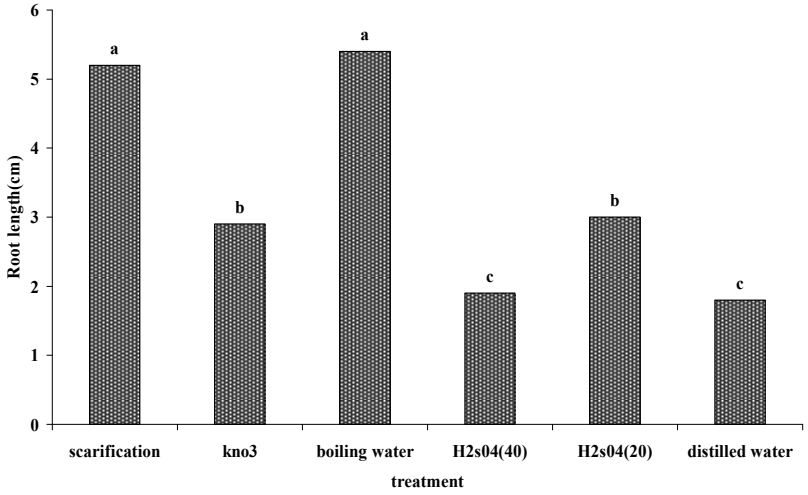

Figure 3. comparing affects of various treatments on Root length of Citrullus colocynthis

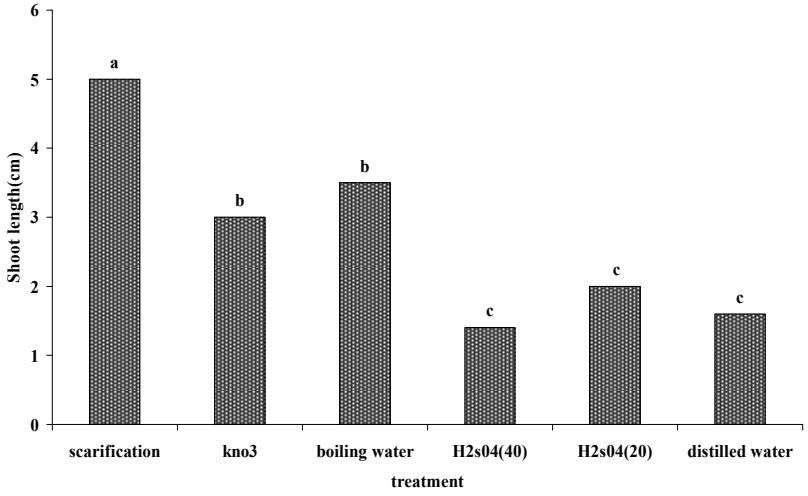

Figure 4. Comparing affects of various treatment on Shoot length of *Citrullus colocynthis*