ANALYZE OF DIFFERENT METHODS OF SCARIFICATION AND BIOCHEMICAL COMPOSITION OF *ACACIA TORTILIS SUBSP RADDIANA* SEEDS

Salima Kebbas[1,2)*], Zahra-Wassila Lekehal[1)], Fatiha Aid[2)]

[1]Département de Biologie, Université Saad Dahleb Blida 1, Algérie

*[2]Laboratoire de Biologie et de Physiologie des Organismes- Equipe de physiologie végétale - Faculté des Sciences Biologiques, Université des Sciences et de la Technologie Houari Boumediene (USTHB), Algérie. *Corresponding author: selmakebbas@yahoo.fr*

Abstract. *Acacia totrtilis subsp raddiana* or more commonly *Acacia raddiana* ; is one of the few leguminous shrub Saharan, which is of great interest to both ecological and economic.The objective of this work is to study the seed of *Acacia raddiana*, focusing both germination and its biochemical composition. Different scarification methods were used to lift the inhibition integumentary seed this taxon and hasten germination. We found that manual scarification has allowed obtaining a germination rate calculated at 85% and a latency of 24 hours. It proves to be the best method, followed by the pure sulfuric acid (98%). Scarification with boiling water gave poor results with a germination rate of 30% and a latency 192 hours (8 days).The study of the biochemical composition of seeds of *Acacia raddiana* reveals that they are very poor in water (2.82%), but rich in dry matter (97.18%), and has an average lipid content (14 085%). *Acacia raddiana* seeds are low secondary metabolites: phenolic compounds (687.8µg /g dry plant material); flavonoids (0.339µg / g dry plant material) and anthocyanins (2.31* 10-5 g /g dry plant material).

Keywords: *Acacia raddiana;* integumentary inhibition; scarification; germination; biochemical composition.

INTRODUCTION

Acacia tortilis subsp *raddiana*; famous tree from Ténéré is native to tropical Africa but thrives in the Sahara, it is particularly strongly present in the edge of the wadi. Formed by trees and shrubs the family of Fabaceae counts more than 1,500 species worldwide including nearly 1,000 in Australia and only 155 species spontaneous in Africa. This species occurs in a wide variety of environmental conditions (Daumont, 1957). *Acacia raddiana* has many uses. Because of his interest in animal feed and occasionally human (food shortages) and other uses in folk medicine, crafts (mortars, dishes, etc.) and the use of its wood, *Acacia raddiana* plays a social role and has an economic importance especially for the first indigenous desert regions (Gast, 1968).This socio-economic interest, gives it a special place to be seen in its use in programs rangeland rehabilitation and afforestation in arid and semi arid areas (Jaouadi et al., 2010).

Overwise, any species regeneration have to pass from the study of the optimal conditions of germination.It is in this context that several studies have been done with the aim of better use and improvement of the regeneration process of this taxon. Measures and restoration techniques to be undertaken are still being defined through long-term studies into the dynamics and development of the species. (Jaouadi et al., 2010).

Our present study was conducted as part of the contribution to the enhancement of *Acacia raddiana* in Algeria particularly in the study of the seed in terms of germination and biochemical composition, since it is the starting point regeneration of any plant species.

MATERIAL AND METHODS

Scarification studies Seeds were scarified according three methods a mechanical one, by using a cuts nail and two chemicals ones using sulfuric acid and boiled water. For this purpose, 20 seeds were used for each test and three replicates were made. After disinfection with hypochlorite the seeds treated by each method were placed on a moisted filter paper on a Petri dishes and placed in an oven at 29°C in the darkness. Germination was scored daily during eight days.

Biochemical composition, After disinfection, Acacia raddiana seeds were dried in an oven at a temperature between 25°C - 28°C, then ground to obtain a fine powder with an electric mill. One sample of this powder was weighed with a precision balance (Pi). This sample is placed in an oven at a temperature of 70°C ± 1°C, 3 hours after, each sample must be weighed. Once the dry weight of the sample is constant (PS), the following equation is established to determine the water content (WC). WC=(Pi-PS/PS)*100 (Audigie et al., 1978). The powder remaining was used to determine, lipids and secondary metabolites content Polyphenols, flavonoids and anthocyanes were determined according to (Singleton and Rossi, 1965). All experiments were repeated three times.

RESULTS AND DISCUSSION

Results regarding the different methods of scarification. Figure 1 shows the percentage of germination of seeds of Acacia raddiana per day for different treatments: manual scarification (nail section) and the chemical scarification H_2SO_4 sulfuric acid and that of boiling water. From the results shown in this figure, the curve of seed germination of Acacia raddiana for each treatment, describes the same pace; with a lag phase of several days, an exponential phase and a stationary phase. Treatment effect was highly significant on the rate and the mean germination time. The results obtained demonstrate the treatment effect at a very important role in seed germination. Our results are close to those reported by of (Cheema and Quadir, 1973) and highlights the need for harsher treatment for *Acacia raddiana seeds* ; this feature is related to the hardness of the seed coat that allows seeds to withstand climate hazards and thereby constitutes insurance for the sustainability of the taxon.

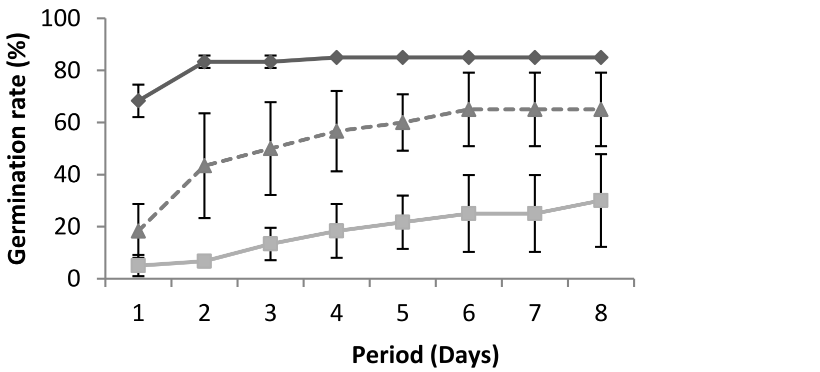

Fig. 1. *Acacia raddiana* kinetic seed germination under different scarification methods (values are means ±SE of three repetitions)

The effectiveness of sulfuric acid (98%) to raise integumentary inhibition was demonstrated by several authors (Behaeghe and Blouard, 1962; Claworthy, 1984; Grouzis, 1987; Vora, 1989) but requires a longer contact (one hour or more) to have high germination rate. Soaking the seeds in boiling water gave low germination rate, this is consistent with the results obtained by Grouzis (2003) - reported that soaking seeds in water, regardless of its duration and temperature, is of no efficacy to lift the integumentary inhibition.

Comparing our results with those of Kebbas et al. (2013) shows that the rate of seed germination of *Acacia raddiana* is slightly lower (81%) in conditions of water and osmotic stress. However, in our case we noticed a significant difference both in rate germination and in average time germination (Table 1). In fact, Manual scarification seems to be very efficient and gives a germination rate of 85% after 03 days. Immersion of the seeds during 30mintes in pure sulfuric acid yielded an average germination rate, which reached 65% after 06 days. boiling water seems ineffective, only 30% of seeds germinated during a period of eight days.Other studies have been done on the germination *Acacia raddiana* seeds using several treatment such as soaking in liquid nitrogen for 2 and 5 days, the germination rate was 45% and 50% respectively according to Alssadawi and Abdelwahad (1977). And even soaking in a rumen fluid collected from animals for 1 and 2 days according to Zohary and Orshansky (1956); gave a germination rate of 15% and 20%; a very low rate compared to the results of the manual scarification which is around 85%.

Experiments by Tran (1979) showed that treatment with dry heat at 120°C, 150°C, 200°C for 02 hours, is not effective on integumentary inhibition and gave a germination rate lower than 10%. Soaking seeds in pure ethanol for 1day, 3 days and even 5 days gave weak germination rate, that was around 20%, 25%, 45% respectively (Chourad, 1975).

Table 1

Average germination rate (mean ±SE (n=3)) of *Acacia raddiana* seeds after three methods of scarifications

	Average germination rate	**Time of germination**	**Statistical similtude(*)**
Manual scarification	85% ± 0	3 days	A
Chemical scarification H_2SO_4	65% ± 14.14	6 days	A
Boilled Water	30% ± 17.80	8 days	B

(*) Diffrent letters indicate significant différences at p < 0,05.

The study of the effect of salt stress found that raising the concentration of sodium chloride NaCl caused a decrease in rate and mean time to germination at high doses. However, this species tolerates salinity to a concentration of 15 g / l, for a germination rate of about 55% [3].

Results regarding to biochemical content of seeds. Water content is very low in *Acacia raddiana* seeds and was estimated to 2.82 ± 0.162% (Table 2). This confirms the hardness of the seed coat in this taxon by the lack of water. However, dry mass was very high and reached 97.18% ± 0.162.Otherwise, lipid content did not exceeding 15% that constitute an average value in seeds of *Acacia raddiana*.

Table 2

Water Content (WC), Dry mass and Lipid Content (mean ±SE (n=3)) of *Acacia raddiana* seeds

Water Content	**Dry Mass content**	**Lipid Content**
2.82% ± 0.162	97.18% ± 0.162	14.085%

Comparing to others leguminous we clearly notice that the water content in the seeds of *Acacia raddiana* is 27 times, 21 times, 25 times lower, respectively, in the seeds of beans, chick peas and lentil. On the other hand, for dry matter; we noticide that Acacia raddiana seeds are very rich and arereached 97%,which allows for better conservation on a much longer duration.

Results regarding the polyphenols content of the seeds. Total polyphenols, total flavonoids and anthocyanins content are expressed respectively as gallic acid quercetin and anthocyandine C-glucoside equivalent, representing an average grade of 687.8 ± 0.052 mg / DM for polyphenols, 0.339 ± 0.046 g/ DM for flavonoids and 2.31 ± 0.07 * 10-5 mg/ DM in *Acacia raddiana* seeds.

Table 3

Total polyphenols, Flavonoids and Anthocyanes content (mean ±SE (n=3)) in *Acacia raddiana* seeds

Polyphenols content in *Acacia raddiana* seeds/g dry mass		
Total polyphenols	Flavonoids	anthocyane
687.8 ± 0.052 μg	0.339 ± 0.046 μg	$2.31*10^{-5}$ ± 0,07

We noticed that polyphenol content in the seeds of *Acacia raddiana* is low compared to the average content of polyphenols consumed per day in humans (approximately 1 g) (Perez-Jimenez et al., 2011), but his virtue against colic sand for animals according to Bellakhdar (1997)] and against the diarrhea (Hernandez-Pacheco et al., 1949), this explain the frequent use of those taxon seeds as anti-spasmodic.In otherwise, those level are very low comparing to the polyphenol content in leaves that are high and have been estimated to 195.45 ± 4.16 mg in rosemary leaves, and 2.06 ± 1.14 mg of flavonoids.

The difference in values of secondary metabolites can be explained by the availability of the polyphenols that may change during development of the plant. This may be due to the harsh weather conditions (high temperature, sun exposure, drought, salinity (Falleh et al., 2008). Indeed, the phenolic content of a plant depends on a number of intrinsic (genetic) and extrinsic (climatic conditions, cultural practices, maturity at harvest and storage conditions) (Falleh et al., 2008; Podsędek, 2007).

Many authors show that contribution to the antioxidant activity of plant extracts is largely due to the presence of some micronutrients such as polyphenols in general and in particular anthocyanins. The polyphenol content in the seeds of *Acacia raddiana* is in the middle of the interval (0.046-4.536mg) found according Mbaiogaou et al. (2013) on voandzou seeds (*Vigna subterranea* (L.) As against the anthocyanin content is low in the range 0.331 mg.

CONCLUSION

Manual scarification stays the better, the simplest and the quickest way to lift the integumentary inhibition in *Acacia raddiana* seeds. Even if other means can be used and are probably very promising as the liquid nitrogene or the microwave method. The low water content in *Acacia raddiana* seeds seems to able them to be store for a long period without alteration and to be used for their nutritional power; because they are fed during the period of scarcity and long periods of drought, and more once the ground seed, and mixed with wheat flour and water, they prepare their porridge. In spite, a very low polyphenol content, *Acacia raddiana* seeds exhibit an importance as medicinal plant particulary used for its antioxydant plant, its anti-inflammatory and antispasmodic activity. In conclusion, this taxon stays a key species in those arids region that deserve more and more interest especially for its regeneration.

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