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NUTRITIONAL CHARACTERISTICS AND SEED GERMINATION IMPROVEMENT OF THE FORAGE SHRUB *RETAMA SPHAEROCARPA* (L.) BOISS

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SUMMARY

Retama sphaerocarpa shrubs form populations that can be an important forage resource during the dry season when pasture shortages are common in certain arid and semi-arid Mediterranean basin regions. The leaves of *R. sphaerocarpa* were analyzed for dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents. Leaves were also analyzed for the concentration of macro- (P, K, Ca and Mg) and microelements (Mn, Zn, Fe, and Na). According to the contents of CP, NDF, ADF and ADL in the leaves examined, this species could strike an appropriate balance between available feed ingredients for daily nutritional needs of animals. The contents of Ca, K, Na, P, Fe, Mg, and Zn in *Retama sphaerocarpa* shrubs were found to be high, compared to a number of other forage shrub species. Conversely, the rate of natural regeneration of this shrub in situ was estimated at 2-5%. *R. sphaerocarpa* seeds are affected by seed coat dormancy that prevents seed germination under natural conditions. The seed germination was assessed at a laboratory after the chemical scarification of seeds by concentrated sulphuric acid in the duration range of 0 min and 240 min. The principal component analysis of data related to the germination ability and seedling emergence showed that the best pretreatment was 120 min immersion in sulphuric acid at 25°C (± 2°C), resulting in 86% of the final germination percentage (FGP) and 14.6 cm of the seedling length (SL). According to the results obtained, this species could be considered a ruminant feed of great nutritive value when drought decreases grazing herbaceous biomass yields. These results should encourage farmers and foresters to integrate *R. sphaerocarpa* into their planting programs.

Key words: Fabaceae, Livestock diet, Mediterranean ecosystem, nutrition, *Retama*, rearing, seed coat dormancy

INTRODUCTION

In arid and semi-arid regions, animal feed shortages are a severe problem (Hamadeh et al., 2001). Therefore, the use of local feed resources for animals is necessary and some xerophytic plants with appropriate forage potential offer the opportunity to reduce feed shortages to livestock (Barakat et al., 2013). Native browse species are useful sources of animal feed as these plants remain green during the dry season and provide a better nutritional value than other annual grasses and herbaceous species that wilt readily (Sanon, 2007). Shrubs are key components of these habitats, and the species from the family Fabaceae are among the most important in the Mediterranean climate regions (Pate et al., 1990; Stock & Verboom, 2012).

The genus *Retama* of the family Fabaceae is native to the Mediterranean basin and is distributed in different bioclimatic stages. In Algeria, three species related to this genus have been reported: *Retama sphaerocarpa* (L.) Boiss., *R. raetam* Webb and *R. monosperma* Boiss (Quézel & Santa, 1962). Their ecological interest has been reported in the stabilization of the dunes, the fixing of soils and the reconstitution of the plant cover of semi-arid and

arid ecosystems (Muñoz-Vallés et al., 2013). These species can adapt to xerophytic conditions through the strong regression of the leaf surface and a root system developed to find the water necessary for their persistence (Jiménez-Moreno et al., 2013). Furthermore, they have the property of establishing symbiotic mycorrhizal and rhizobial associations promoting the biofertilization of saline and poor soils (E Castro et al., 2016). These plants also contain products that can be exploited in medicine such as alkaloids and flavonoids (Edziri et al., 2012; El Hamdani et al., 2015). In addition, several studies in the steppe regions have reported that bee's honey contains a good percentage of pollen from species of the genus *Retam* (Manzanares et al., 2017). *Retama sphaerocarpa* is characterized by 6-mm long yellow flowers, a brown calyx, a glabrous corolla and pods are sub-spherical, indehiscent and contain 1 to 2 seeds. *R. sphaerocarpa* is an unarmed shrub of 2 to 3 m height with flexible and alternate branches, with a silver-green color (Cullen & Lewis, 2013). Common in the high steppe plains and in the Northern Sahara, this plant colonizes accumulations of sand and dunes. It can be a significant source of forage for livestock species, particularly during the dry season (Barakat et al., 2013).

The information on the nutritive values of forage species could help range management by selecting suitable grazing sites to sustain animal life without inflicting vegetation damage (Karki et al., 2019). *R. sphaerocarpa* has been the subject of studies relative to its proteins and leaf amino acids, phenolic composition, antioxidant, flavonoid profile, antiglycation properties and cytogenetic characteristics (Zoubir & Meriem, 2012; Touati et al., 2015; Boussahel et al., 2018). However, the improvement of its regeneration by germination and its tolerance to the saline condition has not been studied to date. This is why the lack and rarity of natural sexual regeneration has led to its gradual regression. The lack of data on the germination physiology of *R. sphaerocarpa* seeds, the hardness of their integuments and their impermeability to water and oxygen appears to be comparable to most of the other Fabaceae species (Mehdadi et al., 2017; Kheloufi et al., 2018a; Kheloufi et al., 2019). The positive response of these seeds to special pretreatments is crucial for their better regeneration and effective reforestation programs where the plant cover declines significantly (Kheloufi & Mansouri, 2017). To increase the previously acquired knowledge and contribute to the regeneration by *R. sphaerocarpa* seeds, we have conducted an experiment aimed at breaking its seed coat dormancy. The second objective of this study was to determine, in terms of chemical composition, the nutritional value of the *R. sphaerocarpa* leaves as a forage plant in the semi-arid area of Ras El Aioun (Batna, Algeria).

MATERIAL AND METHODS

Study area and sampling

Batna has two dominant climates, the semi-arid climate and the Mediterranean climate. In 2019, the following climatic conditions were recorded in the region: an average annual temperature of 15.8 °C, an average annual maximum temperature of 23.2 °C, an average annual minimum temperature of 8.0 °C, a total annual precipitation of 170 mm and a total number of 67 rainy days (WCD Tutiempo, 2020).

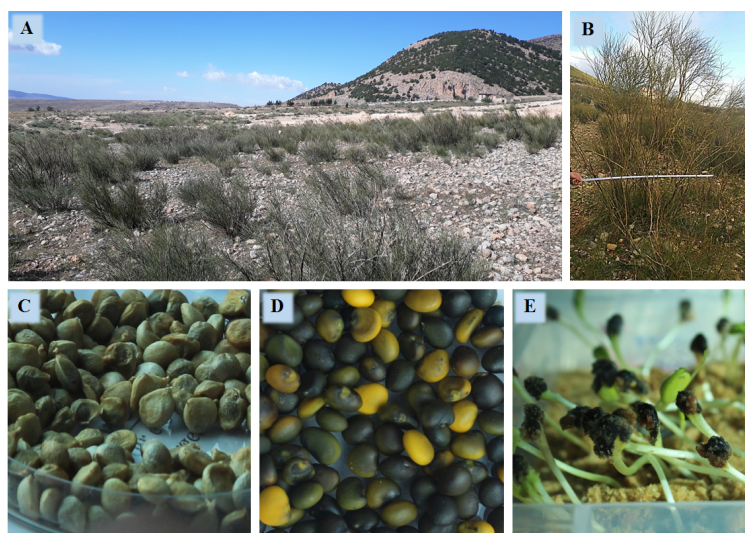


Figure 1. *Retama sphaerocarpa* (L.) Boiss.: (A) Sampling site at Ras El Aioun (Batna, Algeria), (B) Adult shrub, (C) Fruits, (D) Seeds, and (E) Seedlings of 15 days old

The seeds of *R. sphaerocarpa* used in the experiments were obtained from mature pods harvested from 10 different shrubs of approximately the same size (namely a height of 1.60 m and a diameter of 0.9 m) in November 2019, growing at an altitude of 1,175 in a forage area located in the arid region of Ras El Aioun (Batna, Algeria) (Latitude: 35°42' N; Longitude: 5°35' E). The soil in this area is weakly saline ($EC = 1.95 \pm 0.07 \text{ dS.m}^{-1}$) with a pH value of 7.6. The fruit of *R. sphaerocarpa* is a pod of yellow green color, ovoid with 1 to 2 seeds of olive green to yellow color (Fig. 1). After collection, they were transported to the Laboratory of Ecology and Environment, University of Batna 2 (Algeria), where they were selected, described and photographed. The pod sample was obtained by mixing fruits of ten different shrubs. Seeds were manually extracted by opening the pods. A total of 50 seeds were analyzed relative to their size, length, width and thickness, using a digital caliper (Fig. 1). The seeds (length: $5.55 \pm 0.29 \text{ mm}$; width: $4.63 \pm 0.21 \text{ mm}$; thickness: $3.65 \pm 0.31 \text{ mm}$; mean \pm SE; $n = 50$) were then stored in a glass bottle at 4 °C for one month. The thousand-seed weight was 68.6 g.

Nutritional and mineral composition of leaves

The contents of dry matter, ash, and crude protein in *R. sphaerocarpa* leaves were estimated using the AOAC (2000) methods. Neutral and acid detergent fiber contents were determined as described by Van Soest et al. (1991). *R. sphaerocarpa* leaves were analyzed for the concentration of macro- (P, K, Ca and Mg) and microelements (Mn, Zn, Fe, and Na). For the determination of the leaves mineral content, approximately 1.5 g of dried and ground sample was placed in a burning cup and then incinerated in an oven at 550 °C until a constant weight was obtained (for approximately 3 hours). Subsequently, the ash obtained was solubilized with 25 mL of 50% HNO_3 , heated in a water bath for 30 minutes, filtered and the mineral contents were determined with an Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) according to Skujins (1998). The leaves protein content could not be directly determined in actuality, but it was derived from the nitrogen (N) content of the feed sample. On average, all biological proteins produce 16% nitrogen, so the leaves protein content was determined using the 6.25 N percent multiplication (Wu et al., 2014).

Experimental design of seed pretreatment

The seeds analyzed underwent several pretreatments: a chemical treatment which consisted of immersion in 98% sulphuric acid for 30, 60, 90, 120, 150, 180 or 240 minute and subsequent washing in distilled water, or hot water treatment (100°C) for 10 minutes and subsequent washing in fresh distilled water. The seeds were not treated for control purposes. For each treatment, there were four replicates with 50 seeds incubated in a plastic container (15 cm Length \times 8 cm Height \times 10 cm Width) between two layers (1 cm each) of moist sand at 15% (distilled water) and then placed in a culture chamber in total darkness at a culture chamber temperature of 25°C (\pm 2°C) for 15 days. In each procedure, the final germination rate (FGP) was expressed after 15 days as a percentage of the total number of seeds. The seeds were counted as germinated when the radicle growth reached 2 mm. Using a digital caliper, the lengths of approximately 20 seedlings per treatment were also measured.

Statistical analysis

Differences between the treatments after ANOVAs were established by comparing the mean values obtained. Multiple means comparisons were performed using the Duncan's test ($\alpha = 5\%$) and the SAS Version 9.0 program (Statistical Analysis System) (2002).

RESULTS AND DISCUSSION

Nutritional and chemical composition of *R. sphaerocarpa*

The goal of any feeding program is to achieve a balance between available feed ingredients, ensuring that the total nutrient composition of the ration meets the animals' daily nutritional needs. To accomplish such a feat on a daily basis, information on the nutrient content of feed ingredients is of crucial importance. Dry matter is defined as the non-humid portion of an ingredient or diet for feed (Lukuyu et al., 2012). The dry matter of a given feed ingredient or forage contains the necessary nutrients. The chemical composition of the leaves collected from adult plants of *R. sphaerocarpa* is shown in Table 1.

The amount of protein in feeds is also considered a high-quality determinant. Crude protein does not distinguish between N in feed samples from genuine protein or other non-protein nitrogen (NPN) compounds, nor does it distinguish between accessible and unavailable protein (Wu et al., 2014). The amount of the crude protein (CP) was medium ($137 \text{ g.kg}^{-1} \text{ DM}$), Tab. 1. Small domestic ruminants will increasingly resort to natural standing shrubs and ligneous grasses in arid and semi-arid areas, as the only forage options available during the dry season (Spinage,

2012). The CP content of *R. sphaerocarpa* leaves exceeds the minimum level of 7-8% DM needed for optimal rumen function and feed intake in ruminant animals (Van Soest et al., 1991). Throughout several regions of the world, a number of leguminous shrubs and trees were used as basic feed for animals, primarily because of their high protein contents (Soltan et al., 2012).

Table 1. Nutritional and mineral composition of the *R. sphaerocarpa* leaves from the arid region of Ras El Aioun (Batna, Algeria)

Chemical composition of leaves	Values (g.kg ⁻¹ dry matter)
Organic matter	898
Crude protein (CP)	137
Neutral detergent fibre (NDF)	598
Acid detergent fibre (ADF)	432
Acid detergent lignin (ADL)	178
Ca	8.62
Fe	0.34
K	58.7
Mg	3.11
Mn	0.03
Na	2.21
P	2.24
Zn	0.04

According to Harper & McNeill (2015), the detergent feed analysis method is used to describe a forage or feed's fibre or total cell wall material. That portion of a neutral detergent forage or feed sample is called neutral detergent fiber (NDF) containing hemicellulose, cellulose and lignin. When cell wall development increases, the NDF content may increase as it occurs in advancing plant maturity. NDF is a reasonable indicator of fodder quality and plant maturity within a given feed. The NDF content below 40% would be considered good quality for legume forages, whereas over 50% would be considered poor. A second goal will be to have < 35% of acid detergent fibre (ADF) in legumes or grass forages (Makkar et al., 2014). Based on their chemical composition, the leaves of *R. sphaerocarpa* showed high fibre (NDF and ADF) and lignin contents (ADL) with respective values of 598 g.kg⁻¹ DM, 432 g.kg⁻¹ DM and 178 g.kg⁻¹ DM (Tab. 1). The high fiber content of some forage species could be explained in part by the environmental conditions prevailing in the Ras El Aioun region, since low precipitation tends to increase the fraction of the cell wall and decrease the soluble content of the plants (Carberry et al., 2012). Owing to the different proportions and phenological stages of the plants at sampling, our values may somewhat differ from other test results in the literature. In addition, even forages harvested from the same field in the same year can have very different compositions as affected by environmental factors (Kandel et al., 2013). The influence of environmental factors on the forage quality of temperate and tropical grasses has been reviewed by several authors, who have argued how light, temperature, drought and soil nutrients influence the chemical composition and digestibility of forages grown in contrasting areas of the world (Lascano et al., 2001).

Previous studies have found that an adequate intake of elements such as sodium, potassium, magnesium, calcium, manganese, copper, zinc and iodine could minimize individual risk factors, including the cardiovascular disease-related ones (Zamberlin et al., 2012). Major minerals are those needed in quantities exceeding 100 mg per day and represent 1% or less of the body weight (Makkar et al., 2016). Trace minerals are required in smaller amounts. The mineral compositions of *R. sphaerocarpa* leaves are shown in Table 1. The potassium values were found to be high (58.7 g.kg⁻¹ DM) compared to the other minerals determined. The absorption of K usually depends on the form of soil. Potassium is also the main intracellular cation involved in the development of membranes and the electrical excitation of nerve and muscle cells (Sarkar et al., 2015). The daily requirements for adult goat in their diets are 1.8-2.5 g.kg⁻¹ (Medina-Córdova et al., 2014). The potassium content in *R. sphaerocarpa* leaves growing in this semi-arid region exceeded the required levels (Tab. 1).

The Ca, Mg, Na and P contents were 8.62 g.kg⁻¹, 3.11 g.kg⁻¹, 2.21 g.kg⁻¹ and 2.24 g.kg⁻¹, respectively (Tab. 1). Sodium and potassium are essential for many body-regulating systems (Zamberlin et al., 2012). Animal feeding requires a dietary level of 4.3 g.kg⁻¹ calcium (Little, 1982). The leaves from plants that grow in semi-arid regions have ample Ca amounts (Bremner & Kessler, 2012). Calcium is the key component of bones and helps in the growth of teeth (Soetan et al., 2010). The amount of Mg in plants largely depends on the type of soil (Gransee & Führs, 2013). A concentration of magnesium of 2 g.kg⁻¹ in plants is generally considered the minimum dietary concentration for sufficient animal health (Little, 1982). The content of zinc of *R. sphaerocarpa* leaves was 0.04 g.kg⁻¹ (Tab. 1). The plant's physiological activity affects the Zn absorption and the interactions with several elements

such as Fe, Mn and Cu (Siedlecka, 1995). The average tolerable zinc level for cattle is set at 0.5 g.kg^{-1} and for sheep at 0.3 g.kg^{-1} (Jabeen et al., 2010). Goats, on the other hand, need some $0.04\text{-}0.05 \text{ g.kg}^{-1}$ DM of Zn in their diets (Ramirez et al., 2001).

The mean concentration of Mn in the leaves analyzed was 0.03 g.kg^{-1} (Tab. 1). Manganese is the most common divalent cation found intracellularly. It is an integral cofactor for a multitude of enzymatic reactions necessary for energy generation from ATP and for physiological processes, including neuromuscular function and cardiovascular tone maintenance (Rengel, 2000). Our results indicate that the *R. sphaerocarpa* leaves contain a moderate content of iron (0.34 g.kg^{-1}), Tab. 1. Sufficient amounts of Fe in plants are important for both the plant metabolism and the supply of nutrients to animals. The ability of the plant to absorb Fe is variable and is influenced by changing soil and climate conditions, and by plant growth stages and different species genotype properties. On balance, the nutritional requirements of grazing animals are met with the Fe content ranging from about 50 to 100 mg.kg^{-1} (Underwood, 1999).

Breaking seed coat dormancy in *R. sphaerocarpa*

In our study area, the population density of *R. sphaerocarpa* has been estimated at 1,700-2,300 plant/ha. However, the rate of natural regeneration has been estimated at between 2 and 5% by seed germination. In fact, seed propagation is difficult because of the low *in situ* germination rate and long germination time. Moreover, *R. sphaerocarpa* is initially propagated vegetatively by suckers formed from the mother plants. This kind of propagation is very slow and can produce low number of seedlings compared to seed propagation (considering that an adult plant of 1.80 m height and 1 m diameter can produce between 500 and 700 seeds).

According to the results presented in Table 2, the FGP values of 86%, 87%, 88% and 82% were recorded in the seeds immersed in acid for 120 min, 150 min, 180 min and 240 min, respectively. The total length of the seedlings ranged between 14.4 and 14.6 cm during all the treatments. The treatment effect on the FGP and SL values was found to be significant ($P < 0.001$), Tab. 2. The comparison of the mean values obtained at different treatment times shows that the seeds have a dormancy of a physical nature. However, the treatment with sulphuric acid for 120 minutes was found to be the best pretreatment. The immersion of the seeds in hot water for 10 min resulted in only 2% germination. According to Table 2, the seedlings did not exceed an average of 10 cm in length, even with a percentage of 35% FGP in the seeds immersed in acid for 90 min. This can be accounted for by the fact that the seed coat was not abraded enough to allow germination. At the end of the incubation period, there was a germination delay, indicating reduced seedling emergence.

Table 2. Final germination percentage (FGP) and seedlings length (SL) of the *R. sphaerocarpa* seeds exposed to different pretreatments of various durations and after 15 days of incubation

Treatments	FGP (%) (n=4)	SL (cm) (n=20)
0 min SA (Control)	2 ^c	0.02 ^d
30 min SA	4 ^c	1.32 ^c
60 min SA	8 ^c	1.42 ^c
90 min SA	35 ^b	9.47 ^b
120 min SA	86 ^a	14.6 ^a
150 min SA	87 ^a	14.5 ^a
180 min SA	88 ^a	14.6 ^a
240 min SA	82 ^a	14.4 ^a
10 min HW	2 ^c	0.02 ^d
SEM	39.2	6.73
P	< 0.001	< 0.001

Legend: SA - Sulphuric acid; HW - Hot water; the same letters in the column indicate no significance difference (Duncan Multiple Range Test)

Seed dormancy is determined by several factors, including dehydration, oxygen content, extreme temperatures and soil acidity (Ali-Rachedi et al., 2004). Several studies have indicated that wet scarifications (acid or hot water) and dry scarifications (abrasion) applied to seeds with very hard seed coats allowed imbibition and improved respiration in the seed (Kheloufi, 2017; Kheloufi et al., 2018a; Kheloufi et al., 2019). These conditions are important for good production of seedlings. The rest is determined by the internal qualities of the seeds themselves, including metabolism, the content of certain growth regulators and the existence of certain germination inhibitors in the seed

coat, which prevents and delays the imbibition (which is the first step in the germination phase) (Kheloufi et al., 2017).

In the case of seed coat dormancy, germination inhibition occurs due to the action of certain mechanical obstacles that generate a negative osmotic pressure and an imbalance in the seed gas exchanges (Pedrol et al., 2018). This dormancy varies in degree from one species to another (Kheloufi et al., 2018a). However, such inhibition can be minimized by using various pretreatments such as immersion in boiling water, concentrated sulphuric acid and sandpaper abrasion of the seed coat (Kildisheva, 2019). As noted in the results section, the non-scarified *R. sphaerocarpa* seeds, subject to a long incubation period of up to 15 days, exhibited a very low rate of germination, indicating a seed coat inhibitory effect that makes them impermeable (similar to several species of forest legumes). This confirms the results of our previous studies (Kheloufi et al., 2018a; Kheloufi et al., 2019), indicating that the unscarified seeds of leguminous trees do not show imbibition or germination due to the seed coat rigidity. A number of studies on breaking the seed coat dormancy in leguminous species have shown that chemical scarification by concentrated sulphuric acid results in very high germination levels, compared to scarification by boiling water or sandpaper (especially in the instance of a large seed batch of *Retama monosperma* (Bouredja et al., 2011), *Retama raetam* (Mehdadi et al., 2017), *Astragalus armatus* (Kheloufi et al., 2018b) and several *Acacia* species (Kheloufi et al., 2019). The second advantage of sulphuric acid immersion is that it can be reused 2 or 3 times with the same benefits.

CONCLUSION

In vitro and *in situ* measurements are valuable methods for the initial screening of the *R. sphaerocarpa* shrub according to its density, natural regeneration, seed germination and nutritive quality. According to the contents of CP, NDF, ADF and ADL in *R. sphaerocarpa* leaves, this species could be considered a highly nutritious feed for ruminants when drought decreases grazing herbaceous biomass yields. The present study demonstrated the existence of certain major and trace elements in *R. sphaerocarpa*. The mineral contents of Ca, K, Na, P, Fe, Mg and Zn in *R. sphaerocarpa* were found to be high, compared to other forage shrubs. Furthermore, *R. sphaerocarpa* seeds require a specific pretreatment to improve their seed germination. The *R. sphaerocarpa* seed germination rate was improved from 2-5% in nature to 86% after a treatment with sulphuric acid for 120 minutes. The results obtained also indicate that *R. sphaerocarpa* exhibits a physical seed dormancy type. This pretreatment is recommendable to foresters and farmers because it is simple, rapid and can treat big batches of seeds, which would facilitate land restoration in arid and semi-arid zones and increase the nitrogen supply to degraded soils.

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