

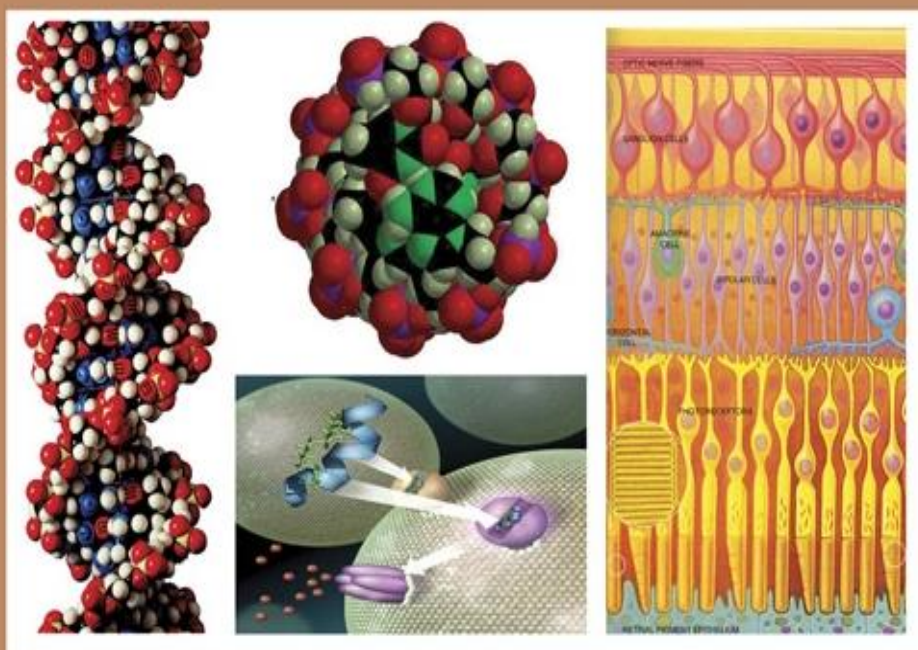


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***Juniperus phoenicea*'s power of responding to ingestion by potential frugivores in the Central Saharan Atlas (semi-arid) - Algeria**

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**ABSTRACT**

Our study goal is to ascertain the effects of *Juniperus phoenicea* seed passage, through potential frugivores' digestive tubes, on their germination behavior in the central Saharan Atlas of Algeria.

The methodology adopted entails comparing germination findings of ingested seeds of *Juniperus phoenicea* by the frugivores, in this case: Ring Ouzels, Wild Boar, Common Genet, Golden Wolf of Africa, red fox, and Shaw's Jird to those of seeds not ingested.

The main findings demonstrate that frugivores' ingestion of *Juniperus phoenicea* seeds does not favorably affect their germination rates. Nonetheless, the seeds ingested by Ring Ouzels provide close results to those of the control, which oscillate by 60%. Also, a notable decrease has been recorded in the batches of the wild boar and the carnivores. The five (05) batches of Shaw's Jird, on the other hand, show a low to no germination rate.

Furthermore, a significant modification of *Juniperus phoenicea* germination pattern is observed after the ingestion of the seeds; not only delaying the latent germination time by a few days but also slowing down the speed of germination. These alterations are unique to each frugivores and such diversity may have a favorable impact on the effectiveness of *Juniperus phoenicea* dispersal.

**INTRODUCTION**

*Juniperus phoenicea* Linnaeus (1753) commonly known as red juniper or Phoenician juniper, is a tiny evergreen shrub or tree with fleshy fruits that resemble berries. The trunk is red to brown in color. *Juniperus phoenicea* is found over the whole Mediterranean Basin, from Jordan and the Sinai Peninsula, along the Red Sea in the east to Portugal on the Atlantic beaches and the Atlas Mountains in the west. Additionally, it occurs in Madeira and the Canary Islands (Farjon 2005). In the Atlas Mountains (Morocco) and the Asir Range (Saudi Arabia), it can grow from sea level up to 2400 m (Benabid 1985; Farjon 2005). In Algeria, the stands of *Juniperus phoenicea* extend over about 227,000 Ha (Hectare), or 10% of the wooded area (Kadik 1987; Louni 1994). This fact gives to the species an evolutionary plasticity and adaptive strategies vis-à-vis to the various ecological constraints (semi-arid and coastal areas).

Thus, it plays an important role especially in the protection of dry soils and areas weakened by desertification and therophytization, however, currently; the species is in a state of intense degradation (Hafsi, 2018).

Red juniper can prosper in arid regions with only 200 mm of rainfall and in temperatures ranging from -12° to 45° C (degrees Celsius). It is unaffected by the kind of soil and is characterized by its excellent wind resistance (Sebastian 1965). According to Djebaili (1984), *J. phoenicea* forms in low materials and forested steppes as a result of forest degradation (Kadik-Achoubi 2005), which occurs frequently and results in very poor regrowth. Despite this, they are highly fascinating since they are the final piece of the forest that may survive in such perilous conditions (Boudy, 1950).

Species from the genus *Juniperus* are mainly dispersed by ornithochory. The female cone has been modified (evolved) to resemble a fleshy fruit which attracts dispersing birds and provides significant nutritional value, with the undigested seeds excreted (more suitable for germination). There are also certain mammals and ants which also swallow the galbulae, digest the fleshy part and return the seeds which will germinate more easily (Hafsi 2018).

The idea that frugivory is the primary method of dispersal for all species of the genus *Juniperus* has been supported by a number of studies (Salomonson 1978; Gaussen et al. 1982; Chavez-Ramirez and Slack 1994; Santos 1999; Garcia et al. 2001; Verdù and Garca-Fayos 2003; Rodrguez-Pérez 2005; Gauquelin 2006; Rumeu et al. 2009; Adams and Thornburg 2010; Rumeu et al. 2011). The frugivores ensure the

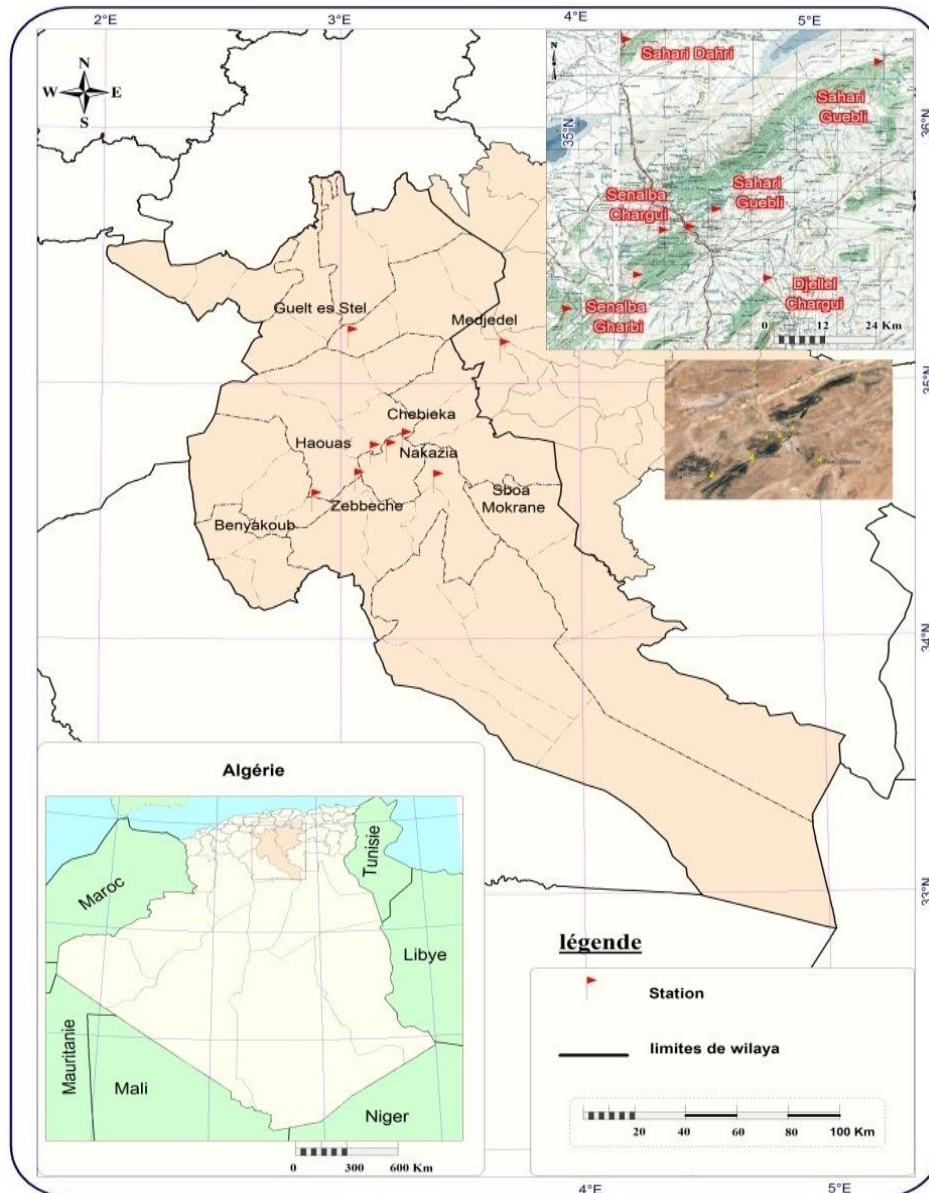
dissemination of these seeds within a radius of a few tens to hundred meters (Genard and Lescourret 1985), which is sufficient to find suitable biotopes for the germination and development of seedlings, away from the mother plant (Genard and Lescourret 1985). The capacity of seeds to germinate past ingestion by frugivores is seminal for the dynamics of certain plant species' populations, and for the evolution of plant-frugivorous interactions (Travest 1998).

The final southern refuge of the Phoenician juniper's range is represented by the mountains of Algeria's Saharan Atlas. This motivated us to conduct a specific study on this plant's genetic resources. The aim of the present study is to measure the ability of *Juniperus phoenicea* seeds to react to ingestion by potential frugivores in the Saharan (semi-arid) region.

## MATERIALS AND METHODS

### 1-Study Area:

The research region is situated in Ouled Nail Mountains, the portion that makes up the central Saharan Atlas (300 km south of Algiers, Wilaya of Djelfa). The five main massifs of Ouled Nail Mountains, Senalba Chergui (1489 m), Senalba Gharbi (1613 m), Sahari Guebli (1484 m), Sahari Dahry (1190 m), and Djebel Djellel Chergui (1478 m), form the limits of this area (Figure 1). The region, as a whole, has a semi-arid Mediterranean bioclimate with a cold winter (Emberger 1971), rainfall is around 310 mm (millimeter) and the average temperatures are between 26 °C (Degree Celsius) in July and 5.4°C in January. The rainfall pattern is A.S.W.S. (autumn, spring, winter, summer). Two classes of soils are noted: Rendzines and brown calcareous soils (Kadik-Achoubi 2005).



**Fig. 1:** The location of the study area (central Saharan Atlas) in Algeria

## 2- Data Collection:

To gather *Juniperus phoenicea* seeds, we surveyed the junipers in our study area between November, December 2020 and January 2021. The samples we collected, including ripe galbuli, depulped seeds, and frugivore droppings, have then been photographed on location and placed in separate bags with labels (origin, said place, GPS point, and other useful descriptions). The data are summarized in Table 1.

Based on droppings' identification on animal behaviour and the place in which they are put and the Droppings' Guide (Gilles

2018).

- The wild boar is a common and very abundant wild species in the study location, making it easy to identify its droppings;
- Shaw's Jird has droppings that are very abundant near its characteristic burrow;
- Common Genet is a stealthy creature that marks its territory with latrines, which correspond to clusters of distinctive feces left in a fixed location, most frequently on rocks (Tessier and Paillat 2001; Mallil 2010).
- The vital range of Ring Ouzels individuals as well as the presence of a white spot, have



been opted for to identify corresponding droppings;

-The red fox and the golden wolf: These two canidés are somewhat few in our study area. Their feces have been identified according to their size and shape and follow the description of Giannakos (1997).

- The process of recovering *Juniperus phoenicea* seeds from the feces of frugivores (Ring Ouzels, wild boar, Shaw's Jird, Common genet, red fox, and golden wolf) involves gently scrambling the samples

between the index finger and thumb to examine their contents. The sorting of such contents has allowed us to identify and select the intact and homogeneous seeds of red juniper.

It is well noted that the amount of *Juniperus phoenicea* seeds in the fecal matter collected is highly important. That is why the galbuli of *Juniperus phoenicea* are a vital food source at this time of year (winter) since resources are less diverse and scarce in our study location.

**Table 1:** Characteristic data summary of batch tested-seeds origins.

No.	Massif	Said Place	Code	Seed Origin	Seed Number	Geographic Coordinations
01	Djellel Chergui	Sboa Mokrane	Control	Manually depulped seeds	60	3.3829°E 34.5699°N
02		Sboa Mokrane	Djellel Bird beaks	Seeds escaped from granivorous birds	60	3.3829°E 34.5699°N
03		Sboa Mokrane	Partial depulped	Partially depulped seeds	60	3.3829°E 34.5699°N
04		Sboa Mokrane	Djellel Ring Ouzels	Ring Ouzels Droppings ( <i>Turdus torquatus</i> Linnaeus, 1758)	10	3.3646°E 34.5549°N
05		Sboa Mokrane	Djellel Wild Boar	Wild Boar Droppings ( <i>Sus scrofa</i> Linnaeus, 1758)	60	3.3866°E 34.5696°N
06		Sboa Mokrane	Djellel Shaw's Jird	Rodent Shaw's Jird Droppings ( <i>Meriones shawii</i> Duvernoy, 1842)	60	3.3609°E 34.5589°N
07		Sboa Mokrane	Djellel Golden wolf	African Golden Wolf Droppings ( <i>Canis anthus</i> F. Cuvier, 1820)	14	3.3981°E 34.5622°N
08	Senalba Chargui	Haouas	Haouas Fox	Red fox Droppings ( <i>Vulpes vulpes</i> Linnaeus, 1758)	60	3.1218°E 34.6829°N
09		Nakazia	Nakazia Genet	Common Genet Droppings ( <i>Genetta genetta</i> Linnaeus, 1758)	60	3.1908°E 34.6914°N
10	Senalba Gharbi	Zebbeche	Zebbeche Ring Ouzels	Ring Ouzels Droppings ( <i>Turdus torquatus</i> Linnaeus, 1758)	60	3.0528°E 34.5563°N
11		Zebbeche	Zebbeche Shaw's Jird	Rodent Shaw's Jird Droppings ( <i>Meriones shawii</i> Duvernoy, 1842)	60	3.0565°E 34.5767°N
12		Benyakoub	Benyakoub Shaw's Jird	Rodent Shaw's Jird Droppings ( <i>Meriones shawii</i> Duvernoy, 1842)	60	2.8782°E 34.4953°N
13	Sahari Guebli	Chebieka	Chebieka Shaw's Jird	Rodent Shaw's Jird Droppings ( <i>Meriones shawii</i> Duvernoy, 1842)	60	3.2555°E 34.7327°N
14		Medjedel	Medjedel Wild Boar	Wild Boar Droppings ( <i>Sus scrofa</i> Linnaeus, 1758)	60	3.6678°E 35.0839°N
15	Sahari Dahri	Guelte es Stel	Guelte es Stel Shaw's Jird	Rodent Shaw's Jird Droppings ( <i>Meriones shawii</i> Duvernoy, 1842)	60	3.0288°E 35.1360°N

### 3- Artificial Germination Tests:

In order to prepare the seeds for artificial germination tests, we first carried out a viability test according to the principle of flotation in distilled water (Willan 1992). Secondly, we disinfected the seeds for 10 minutes by soaking them in sodium hypochlorite (8%). We, then, have concluded the process with three rinses of sterile distilled water (Nedjimi et al. 2014; Moulessehouli et al. 2022).

The 15 batches of *Juniperus phoenicea* seeds that have been selected for germination have been placed in sterile Petri

dishes of 09 cm (centimeter) in diameter, on a double layer of Watman-type filter paper soaked in 5 ml (milliliter) of distilled water, at the rate of 20 seeds per box with 03 replications with the exception of the batches of Djellel Ring Ouzels (10 seeds). The boxes have been placed in an incubator set at a temperature of 20°C, and a photoperiod of 12 h/12 h. The duration of the experiment has been 30 days during which the moistening and counting of the number of germinated seeds have daily been carried out.

One indicator of germination has been the appearance of a 1-mm-long radicle

(El Fels *et al.* 2013; Nedjimi 2013).

The following germination expression parameters have been used:

- Germination rate: It is expressed as the ratio of the number of sprouted seeds to the total number of sown seeds.

- Germination speed: it can be expressed as the median duration of germination (Scott *et al.* 1984) or as the average germination time AGT (the time at which 50% of sprouted seeds are reached) (Come 1970).

$$AGT = \frac{N_1T_1 + N_2T_2 + N_3T_3 + \dots + N_nT_n}{N_1 + N_2 + N_3 + \dots + N_n}$$

AGT: Average Germination Time

N<sub>n</sub>: Number of seeds germinated between time T<sub>n-1</sub> and time T<sub>n</sub>;

T: Number of days past sowing.

#### 4- Statistical Analysis:

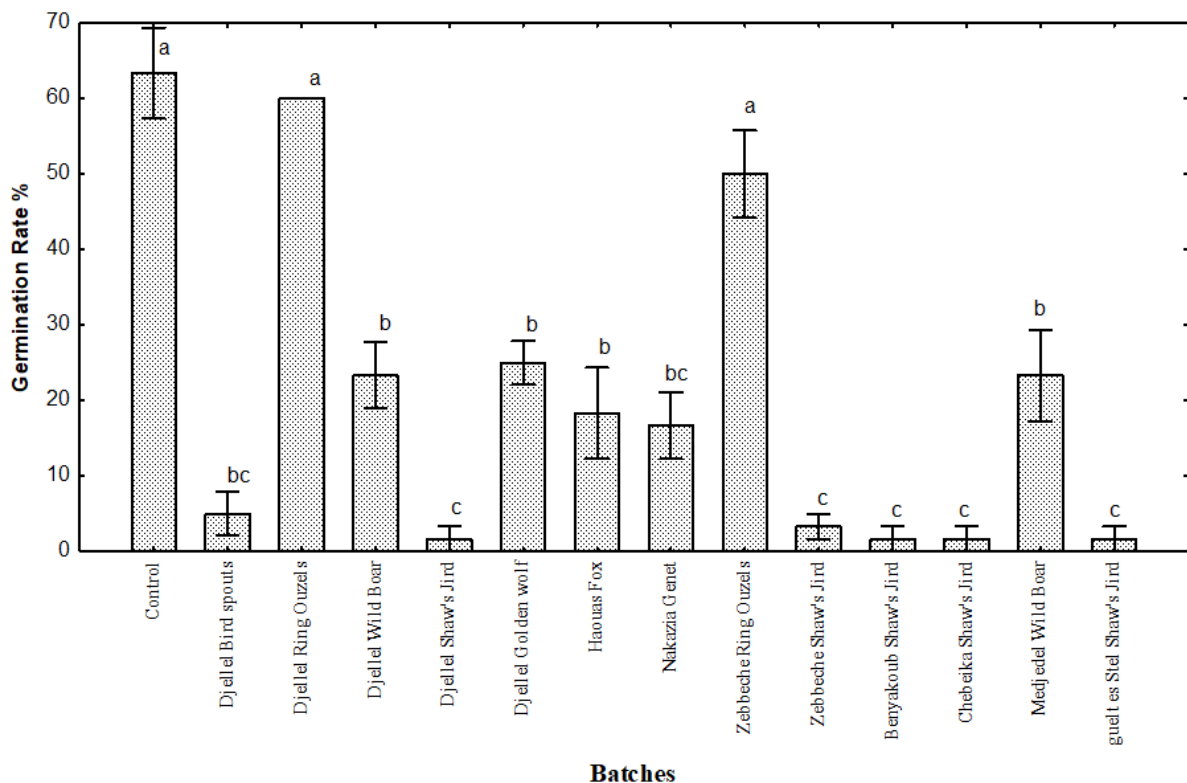
The Fisher test at  $\alpha = 5\%$  has been used to analyze variance with a single factor of variation, and Duncan's test has then been used to determine which groups are homogeneous. Version 8.0 of Statistica is the

statistical software performed. Each average has an assigned letter. Averages that are separated by the same letter are remarkably homogenous.

### RESULTS AND DISCUSSION

*J. phoenicea* germination rates of the various batches experimented on during these studies range widely from 0% to 63.33%.

Statistically, the analysis of variance shows that there is an outstandingly significant effect ( $P < 0.001$ ) of the different batches on the germination rate (Figure 2). In addition, Duncan's test at the 5% threshold makes it possible to classify the batches of seeds (of different origins) into homogeneous groups. According to this test, the batches of control, Djellel Ring Ouzels and Zebbech Ring Ouzels are part of group (a); group (b) corresponds to Djellel wild boar, Zebbech wild boar, Djellel golden wolf, Nakazia genet and Haouas fox, while the five batches of Shaw's Jird and Djellel beak birds are classified in group (c).



**Fig. 2:** Frugivores' ingestion effects on the germination rate of *J. phoenicea* seeds. The bars represent the mean  $\pm$  standard deviation (n=3 replicates). The different letters above the bars indicate a significant difference at  $P < 0.001$  according to Duncan's test.

The partially depulped seed batches have a zero-germination rate. The galbulus is divided into pieces based on how many seeds it contains, but the thick pulp that covers the seeds is left in place; germination inhibitors are present in these pulp tissues (aril). The elimination of this pulp can significantly enhance germination (Izhaki and Safrel 1990; Barnea et al. 1991; Bustamante et al. 1992; Traveset 1998; Samuels and Levey 2005; Pérez-Cadavid et al. 2018). It, also, may reduce the possibility of microbial or fungal infections (Jackson et al. 1988).

With 63.33%, the control batch germination rate is the highest. Such value is comparable to those of Sebastian (1958), Zine El Abidine et al. (1996). Given the limited duration of the experiment (30 days), the germination rate of *J. phoenicea* obtained is notably acceptable for a forest species with abundant fructification rate which in turn confirms the suggestion that red juniper seeds germinate easily (Sebastian 1958). This implies that there is no tegumentary inhibition or embryonic dormancy in this species. On the other hand, Mandin (2010) found out that the stands in the Ardèche gorges (France), had a *Juniperus phoenicea* germination rate of less than 8%.

The fleshy pulp of *Juniperus phoenicea* galbuli, which may contain chemical elements preventing germination, is primarily eliminated by the path of the seeds through the digestive tract of frugivores (Barnea et al. 1990; Traveset 1998; Samuels and Levey 2005; Pérez-Cadavid et al. 2018).

For batches of seeds defecated by frugivores, a better germination rate is marked in batches from Ring Ouzels' droppings, in such cases, batches of Djellel Ring Ouzels and Zebbeche Ring Ouzels are with respectively 60% and 50%. These estimable values could be due to the Ring Ouzels being able to choose the ripest galbuli and; therefore, the mature seeds which have a great germination power. Many studies show that there is a range of species plants whose seeds are not affected by ingestion by *Turdus* ' *Solanum nigrum* (Barnea et al. 1990) of

*Aralia nudicaulis* (Krefting and Roe 1949). Thus, Traveset and Verdú (2002), claim that the intestinal path time of seeds is shorter in birds compared to other non-flying mammals and it has been shown to have a significant positive effect on germination.

Additionally, the germination rate of the seeds defecated by wild boar, golden wolf, red fox, and common genet has been from 17% to 36% on average. Nevertheless, the germination rates for the two kinds of wild boar droppings (Guelt es Stel wild boar and Djellel wild boar) are equal at 23.33%. Nearly one-third of the value of control and *Turdus torquatus* is represented. According to Genard and Lescourret (1985), the disseminating function of the wild boar is minimal because the feces of this animal contain a low abundance of intact seeds. Furthermore, the wild boar's mode of consumption is non-selective since it includes both mature and non-mature galbuli, typically that *Juniperus phoenicea* bears galbuli on the same branches of two generations: first and second years. Consequently, the seeds ingested by wild boar are not all mature. Furthermore, Genard and Lescourret (1985), add that wild boar consume a wide range of plant species diaspores which in response plays a role in the dissemination and structuring of vegetal communities. Besides, the populations of wild boars are rather numerous in the massifs of the Saharan Atlas, allowing us to infer the critical advantage provided by them for *Juniperus phoenicea* spread in our study area.

The seed batches defecated by the golden wolf, red fox, and genet show germination rates of 35.71%, 16.67%, and 18.33%, respectively. These carnivores are occasional frugivores of *Juniperus phoenicea* (Herrera 1989; Willson 1993; Clevenger 1996) that have a high potential to disperse seeds (Herrera 1989; Bustamante et al. 1992). They have the ability to spread seeds away from broodstock due to their extensive vital ranges, high mobility, and prolonged passage times through the digestive tract (Clevenger 1996; Varela and Bucher 2006). As a result,

these mammalian carnivores can deposit seeds in a variety of microhabitats that are more conducive to their establishment (Howe and Miriti 2000; Varela and Bucher 2006). Carnivores represent the weak mesh of disseminators of *Juniperus phoenicea* in view of the number of galbuli consumed. Too, the number of its population is very low compared to other frugivores in the study areas.

The seed batches that escaped from granivorous bird beaks and the five (05) batches of seeds from Shaw's Jird droppings have been responsible for the low germination

rates of 1.67% to 5%. Indeed, granivorous birds spawn the galbuli of *Juniperus phoenicea* and shell the exodus of seeds to reach the endoderm. In this process, there are seeds that accidentally escape. These seeds are depulped by the beaks of granivorous birds. It is appropriate that the number of species of recorded granivorous birds that we have observed frequenting *Juniperus phoenicea* in the study area (Table 2), as well as the number of bare seeds and the number of seeds accidentally released, participate in a significant part in the dissemination of *Juniperus phoenicea*.

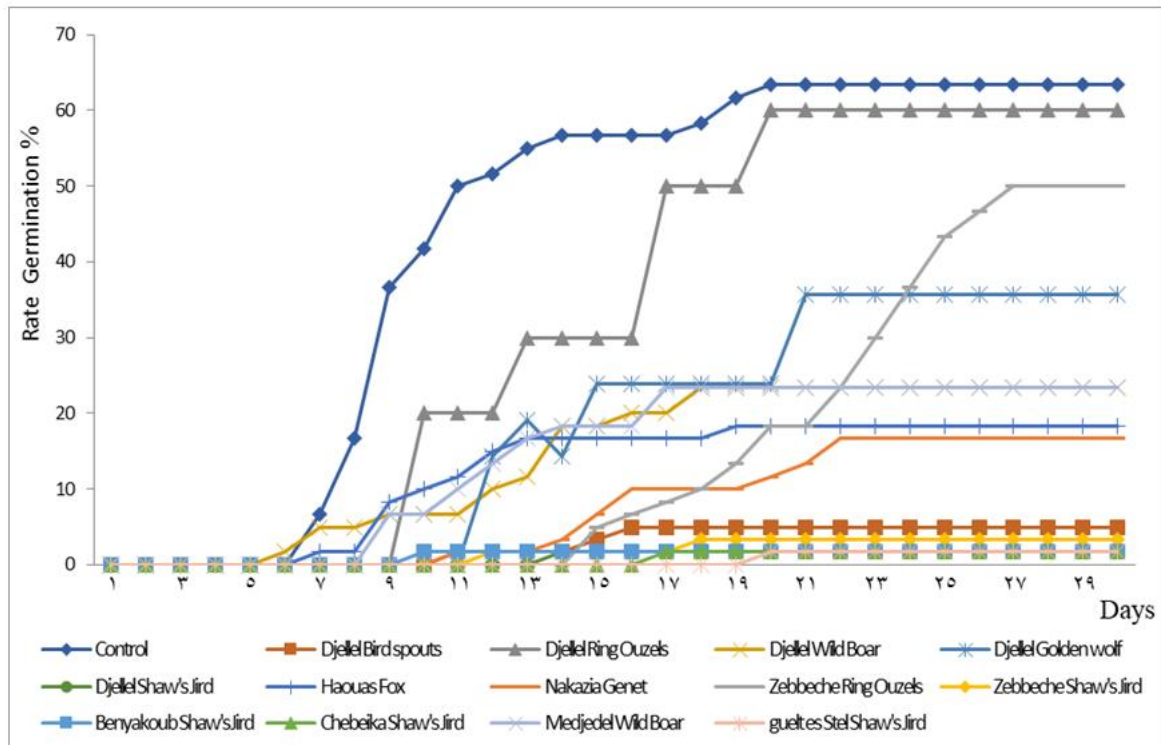
**Table 2:** Main granivorous birds observed frequenting *Juniperus phoenicea* in the study area

French Name	Scientific Name	Common Name
<b>Bruant fou</b>	<i>Emberiza cia</i> Linnaeus, 1766	<i>Rock Bunting</i>
<b>Pinson d'arbres</b>	<i>Fringilla coelebs</i> Linnaeus, 1758.	<i>Common Chaffinch</i>
<b>Linotte Mélodieuse</b>	<i>Linaria cannabina</i> Linnaeus, 1758.	<i>Common Linnet</i>
<b>Verdier d'europe</b>	<i>Chloris chloris</i> Linnaeus, 1758.	<i>European Greenfinch</i>

Despite the fact that a number of authors (Salomonson 1978; Chavez-Ramirez and Slack 1993, Garcia 2001; Horncastle *et al.* 2004; Dimitri *et al.* 2017) claim that rodents are pure predators of *Juniperus phoenicea* seeds, the intact morphological appearance of *Juniperus phoenicea* seeds recovered from the fecal matter of Shaw's Jird (02 to 03 seeds per excreta) has prompted us to test their germination power. The low germination rates of batches recovered from *Meriones shawii*'s droppings, not exceeding 5% in the case of "Guelt es Stel Shaw's Jird" batch, could be due to the passage of *Juniperus phoenicea* seeds in the digestive tract of Shaw's Jird having destructive effects on the intrinsic characteristics of the seeds.

For the present case, these rodents heed the edges of *Juniperus phoenicea* matorrals and feed on its galbuli in times of scarcity (the winter season). Consequently, the obvious overgrowth of this species in the highland region has been confirmed by Le Berre (1990), Adamou-Djerbaoui (2010), Soutou *et al.* (2012) and Adamou-Djerbaoui *et al.* (2013). Several authors (Adamou-Djerbaoui *et al.* 2013) report that *Meriones shawii* is one of the most wonderful crop pests. An individual can store a large amount of fruit in his burrow. Some of the buried seeds are forgotten and germinate in sufficient numbers to ensure effective regeneration (Charles-Dominique 1995).





**Fig. 3:** Evolution of the germination rate of ingested and non-ingested seeds of *Juniperus phoenicea*.

As a function of time (Figure 3), the evolution of the cumulative germination rate of 15 batches of different origins of *Juniperus phoenicea* seeds made it possible to distinguish that the curve relative to the control is dominant. The 14 left batches are gradually approaching the null in that the very last ones are of Shaw's Jird and those partially depulped.

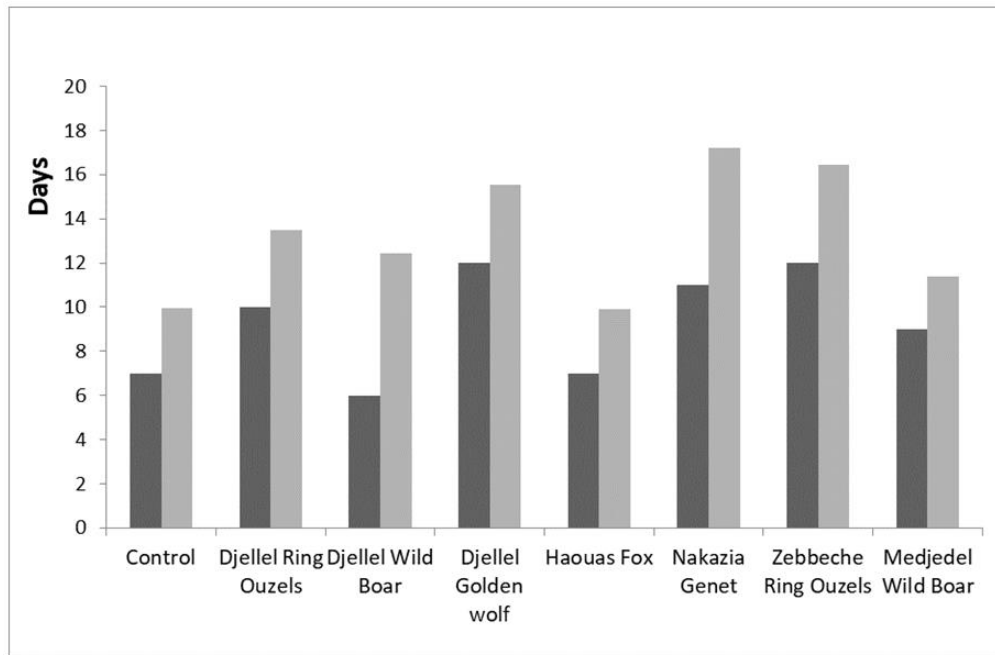
According to the origin of the seeds that have been recovered, the latent lifespan T1 is divided into intervals ranging from five days to seventeen days after the sowing date. Early germination can be observed in the batches of beak birds (5 days), Djellel wild boar (6 days), control (7 days), and Haouas Fox (7 days). Yet, late germination has been spotted in the batches of Djellel Shaw's Jird (14 days), Zebbech Shaw's Jird (12 days), Chebeika Shaw's Jird (17 days), Mejedel wild boar (09 days), Guelt es Stel Shaw's Jird (11 days), Benyakoub Shaw's Jird (10 days), , Djellel Ring Ouzels (10 days), Zebbech Ring Ouzels (12 days), Djellel golden wolf (12

days) et Nakazia Genet (11 days).

For this reason, we have deduced that after passing through the digestive tracts of the Ring Ouzels, Shaw's Jird, Golden Wolf and Common Genet, the latent lifespan of *Juniperus phoenicea* seeds is significantly extended, compared to that of the control.

The exponential germination time is prominently fast and short for the control (5 days), followed by the two batches of the Ring Ouzels, slow for the batches of carnivores and indeterminate for the ones where germination is greatly low (case of Shaw's Jird).

The germination rate stabilizes on the 26th day in all batches placed in germination. However, it is impossible to determine the commonly used germination parameters T1 and AGT due to the low germination rates of 1.67% to 5% observed in the batches obtained from Shaw's Jird's droppings. In this case, each contains one to three germinated seeds.



**Fig. 4:** Effects of ingestion by different frugivores on average germination rate (AGT) and latent lifespan T1 of *Juniperus phoenicea* seeds.

The average germination rate (AGT) of various batches of frugivore-ingested seeds (Fig. 4) is significantly higher than the AGT of the control with the limitation where the germination rate is significant. The AGT ranges from 9.97 days to 17.2 days with the control being faster (10 days) than the seeds passing through frugivores' digestive tubes, Nakazia Genet (17 days). This reduction of germination speed of seeds ingested by frugivores is probably due to, according to (Traveset 1998), a process of ingestion that can slow down germination, which signifies a delay in germination rates (The mechanism for which is unknown).

When compared to non-ingested seeds, we have observed a difference in the germination kinetics of frugivore-defecated seeds. This difference is reflected in the lengthening of the latent lifespan (T1) and the average germination time (AGT), which in turn modifies the seed germination pattern of *Juniperus phoenicea*. According to Verdù and Traveset (2004), the passage of seeds of many plant species through the digestive tract of frugivores affects the speed of germination. This germination pattern is particularly advantageous in Mediterranean habitats, where the risk of seed mortality must be

extended over time. It is more adapted to climatic conditions, mainly unpredictable rain (Izhaki and Safriel 1990; Barnea *et al.* 1991; Izhaki *et al.* 1995; traveset 1998).

As the berries of the genus *Juniperus* L. species are very rich in resin and essential oils, they provide for the birds the necessary energy for spring migration. However, in fleshy fruit plants, frugivores play an important role in the efficiency of seed dispersal over available microclimates (Schupp 1993; Adams 1998; Rumeu *et al.* 2009; Hafsi *et al.*, 2017). Birds are the primary vector of seed dispersal for many plant species, contributing to their migration over long distances (Whittaker and Fernández-Palacios 2007). Moreover, for species of the genus *Juniperus* L., Grant (1980) reported the effect of good seed dispersal by ornithochory on the variability of *Juniperus* populations in contrast to those of *Cupressus*. In Algeria, the study of Milla *et al.* (2013) attested the trophic behavior of birds on the diversity of fleshy fruits from the Algiers Sahel including *Juniperus phoenicea* subsp. *turbinata*, according to color, volume and number of seeds.

### Conclusion

The frugivores studied are keenly

involved in removing the fleshy pulp (depulping) from the seeds of *Juniperus phoenicea* as the main barrier to their germination. Certainly, the frugivores, the object of our studies, do not positively affect the germination rate of *Juniperus phoenicea* but the specific diversity of the consumers of its galbula as a nutritional source, their modes of exploitation and dissemination contribute to the heterogeneity of the germination characteristics of the seeds which can; thus, diversify the habitats on which the new seedlings settle and conquer the new territories more adequate to global changes.

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