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Field : Ecology and Environnement

Option : Plant Ecology and Environnement

### **Theme**

Effect of the chemical nature of the substrate on the  
parameters of the germination and growth of *Astragalus*  
*armatus*.

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## **Dedication**

*I dedicate this work to*

*To my dear father, who enlightened and encouraged me  
all through my studies;*

*To my dear mother, who is the light of my life and who has waited patiently for  
the fruit of her good upbringing;*

*May God give them good health and a long life.*

*To my lovely grandmother; with my wishes for a long life.*

*To my dear brothers: TAHER and IBRAHIM*

*To my sisters: AFFAF and NASSIMA*

*To my nephews: DJAWAD, EYAD and IMRAN*

*Thank you for your prayers and continued support. May the spirit of love reign  
among us all the time.*

*My big family.*

*To my cousins and the whole ALLALI and GOUDJAL family,*

*To my precious friend: Elie*

*To all the people who helped me from near and far.*

*To all of you, from the bottom of my heart, I dedicate this work to you.*

*Hadjer*

## **Dedication**

*I dedicate this work*

*To my parents who supported me throughout my studies.*

*To my brothers .*

*To my sisters.*

*To my whole family.*

*To all my friends*

*To all my promotion.*

***Abdelrahmane***

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## الملخص: تأثير الطبيعة الكيميائية للركيزة على معاملات إنبات ونمو *Astragalus armatus*.

تهدف الدراسة المقدمة في هذه المذكرة إلى تحديد الوسط الذي تنبت فيه بذور *Astragalus armatus* بشكل أفضل. لهذا الغرض، ولمدة 18 يوماً، تابعنا معدل الإنبات؛ ومتوسط مدة الإنبات؛ ومعدل الإنبات اليومي؛ وطول الجذور وطول السيقان؛ معدل النمو ومؤشر القوة لبذور *Astragalus armatus*، المزروعة في أربعة وسائط (المياه المقطرة،  $\text{CaSO}_4$ ،  $\text{CaCO}_3$ ، و  $\text{NaCl}$ )، بتركيزات مختلفة ( $T_0 = 0\%$ ؛  $C_1 = 5\%$ ؛  $C_2 = 10\%$  و  $C_3 = 15\%$ ). أظهرت النتائج اختلافات كبيرة لجميع المعلومات التي تم قياسها ( $P < 0.05$ )، ونلاحظ معدل إنبات بنسبة 100% في ( $\text{CaSO}_4 5\%$ )؛ 75% في ( $\text{CaCO}_3 5\%$ )؛ 42% في ( $\text{NaCl} 5\%$ )، طول السيقان 45 مم في ( $\text{CaSO}_4 5\%$ )؛ 40 مم في ( $\text{CaCO}_3 5\%$ )؛ وطول الجذر 4 مم في ( $T_0$ ). لوحظ أعلى مؤشر قوة 0.5 في ( $\text{CaSO}_4 5\%$ )؛ تم تمييز أقل معدل نمو للجذور ( $< 0.001$  سم/يوم) في  $\text{NaCl}$  ويلاحظ الحد الأقصى لمعدلات النمو ضعيف ( $< 0.25$  سم/يوم) في (الماء المقطر 0% و  $\text{CaSO}_4 5\%$ ). أظهر تحليل المكونات الرئيسية أن إنبات بذور *Astragalus armatus* أفضل في وسط الجبس  $\text{CaSO}_4 5\%$  بالإضافة إلى نموه؛ ولكن في  $\text{NaCl}$  يكون النبات عرضة للمخاطر.

**الكلمات المفتاحية:** نمو، *Astragalus armatus*، الجبس، الحجر الجيري، كلوريد الصوديوم، الإنبات.

## Résumé: Effet de la nature chimique du substrat sur les paramètres de la germination et de la croissance d'*Astragalus armatus*.

L'étude présentée dans ce mémoire vise à déterminer le milieu dans lequel les graines de *Astragalus armatus* germent le mieux. Pour cela durant 18 jours, nous avons suivi le taux de germination ; la durée médiane de germination ; la moyenne journalière de germination ; la longueur des racines et celle des glumelles; la vitesse de croissance et l'indice de vigueur pour des graines de la plante, semées dans quatre milieux (Eau distillée,  $\text{CaSO}_4$ ,  $\text{CaCO}_3$  et  $\text{NaCl}$ ), à concentrations différentes ( $T_0 = 0\%$  ;  $C_1 = 5\%$  ;  $C_2 = 10\%$  et  $C_3 = 15\%$ ). Les résultats ont montré des différences significatives pour l'ensemble des paramètres mesurés ( $P < 0,05$ ), nous observons un taux de germination de 100% dans ( $\text{CaSO}_4 5\%$ ) ; 75% dans ( $\text{CaCO}_3 5\%$ ) ; 42% dans ( $\text{NaCl} 5\%$ ), la longueur des glumelles 45 mm dans ( $\text{CaSO}_4 5\%$ ) ; 40 mm dans ( $\text{CaCO}_3 5\%$ ) ; et longueur des radicules 4 mm dans ( $T_0$ ). L'indice de vigueur le plus élevé 0,5 est observé dans ( $\text{CaSO}_4 5\%$ ) ; la plus faible vitesse de croissance des racines ( $< 0,001$  cm/jour) est marqué dans le  $\text{NaCl}$  et les vitesses maximales de croissance des glumelles ( $> 0,25$  cm/jour) sont observées dans (l'eau distillée 0% et  $\text{CaSO}_4 5\%$ ). L'Analyse en Composante Principale a démontré que la germination des graines d'*Astragalus armatus*, est meilleure dans le milieu gypseux  $\text{CaSO}_4 5\%$  aussi bien que sa croissance ; mais dans  $\text{NaCl}$  la plante est sujette à des risques.

**Clés-Mots :** *Astragalus armatus*, germination, croissance,  $\text{NaCl}$ ,  $\text{CaSO}_4$ ,  $\text{CaCO}_3$ .

## Abstract: Effect of the chemical nature of the substrate on the parameters of the germination and growth of *Astragalus armatus*.

The study presented in this master's report, aims to determine the substratum in which the seeds of *Astragalus armatus* germinate best. During 18 days, we monitored the germination rate; the median germination duration; the daily germination average; root length and stem length; the growth rate and the vigor index for seeds of the plant, sown in four substratum (distilled water,  $\text{CaSO}_4$ ,  $\text{CaCO}_3$  and  $\text{NaCl}$ ), at different concentrations ( $T_0 = 0\%$  ;  $C_1 = 5\%$  ;  $C_2 = 10\%$  and  $C_3 = 15\%$ ).

The results showed significant differences for the measured parameters ( $P < 0.05$ ). We observe a germination rate of 100% in ( $\text{CaSO}_4 5\%$ ) ; 75% in ( $\text{CaCO}_3 5\%$ ) ; 42% in ( $\text{NaCl} 5\%$ ). Stems length were 45 mm in ( $\text{CaSO}_4 5\%$ ) ; 40 mm in ( $\text{CaCO}_3 5\%$ ). The rootlets length were 4 mm in ( $T_0$ ). The highest vigor index 0.5 is observed in ( $\text{CaSO}_4 5\%$ ) ; the slowest root growth speed ( $< 0.001$  cm/day) is marked in  $\text{NaCl}$  and the maximum stems growth speeds ( $> 0.25$  cm/day) are observed in (0% distilled water and  $\text{CaSO}_4 5\%$ ).

Principal Component Analysis demonstrated that germination of the seeds of *Astragalus armatus* is better in the gypsum substratum  $\text{CaSO}_4 5\%$ , where its growth showed also good results, then  $\text{CaCO}_3 5\%$ . However in  $\text{NaCl}$  the plant faces risks.

**Keywords:** *Astragalus armatus*, germination, growth,  $\text{CaCO}_3$ ,  $\text{NaCl}$ ,  $\text{CaSO}_4$ .

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## List of abbreviations

**CaSO<sub>4</sub>**: Calcium sulphate.

**CaCO<sub>3</sub>**: Calcium carbonate.

**NaCl**: Sodium chloride.

**GR**: Germination rate.

**DMG**: Median germination time.

**MDG**: Mean daily germination.

**LS**: Stems length.

**LR**: Rootlet length.

**SG**: Speed growth.

**VI**: Vigor index.

**ANOVA**: Analysis of variance.

**A C P**: Principal component analysis.

**P**: Probability.

**%**: percentage.

## Introduction

Seed germination is an important and fragile stage in the plant life cycle that determines the establishment of seedlings and plant growth. Seed germination is regulated by the interaction of environmental conditions and physiological preparation (Steckel et al., 2004). Each plant has a specific set of environmental requirements required for germination (Baskin and Baskin, 1998). The success of natural reproduction depends primarily on the seed's response to external environmental influences. Furthermore, germination success may reflect plant population size, distribution and abundance (Rojas-Arechiga et al., 1998; Ramirez-Padilla and Valverde, 2005). Several environmental factors such as temperature, soil chemistry, light and soil moisture simultaneously affect germination (El-Keblawy and Al-Rawai, 2006). Soil nature is the most prevalent factor limiting plant distribution and productivity (Qin et al, 2010). Edaphic tolerance during germination is critical for the establishment of plant cultivation in dry land soils (Khan and Gulzar, 2003). For example an increase in salinity induces decrease in the percentage of germinated seeds (Meot-Durosand Magné, 2008).

According to (Mallem , 2018), *Astragalus armatus*, a chamaephyte with low pastoral value, has shown a remarkable proliferation in sandy areas. It is a species that appears particularly in cultivated areas and overgrazed areas (Chaieb (1997). explains this proliferation by the root architecture of this plant, favoring the absorption of water and also to its high germination power. The growth of *Astragalus armatus* could be a sign of soil degradation in the steppe ecosystem, as it is linked to desertification. (Hirche and al., 2010).

The floristic modifications of pastoral ecosystems in arid and desert regions, under the effect of animal pressure and water deficit, affect primarily the palatable grasses. The maintenance of the biological productivity of the environment remains, under such conditions, linked to the appearance of a new type of vegetation that is not very productive but in balance with a new environment (Nedjraoui and Bédrani 2008).

According to (**Chaieb, 1997**), *Astragalus armatus* is considered to be a species marking degradation; however it can contribute to the process of restoring the ecological balance in the degraded environments. The ability of *Astragalus armatus* to trap sand and build a wind veil and its ability to fix atmospheric nitrogen can contribute to improving the fertility of degraded soils (**Mallem, 2018**).

In this context, our work of memory was undertaken and whose objective is to determine the chemical nature of the lands which would be most favorable to the germination of seeds of *Astragalus armatus*. To achieve this objective a germination test of the seeds of the plant is carried out in three substratums: Limestone, gypsum and saline and with varying concentrations.

The dissertation is structured in three chapters:

- In the first chapter we present a bibliographical review regarding *Astragalus armatus*;
- In the second chapter are presented the material and methods used to achieve our objective;
- The third chapter exposes the results obtained and their discussion; and we will end with a conclusion and perspectives.

# **Chapter I: Bibliography synthesis**

# 1 General information about *Astragalus armatus*

## 1.1 History:

The word *Astragalus* is of Greek origin; it means one of the bones of the Tibiotarsian articulation. This denomination comes from the similarity of the sound of the dried grains of the plant with that of the bone, when they fall on a solid surface. (**James and al, 1981**).

This genus of legumes includes some two thousand species of annuals, perennials and shrubs found throughout much of the temperate zone of the Northern Hemisphere (**Burnie et al., 2006**). *Astragalus* species grow from fall to spring and remain green during winter when grass is not available (**Colegate et al., 1985**).

According to **Chaib (1997)**, *Astragalus armatus* is considered as a species marking degradation, it can contribute, in partial ways, to the process of restoring the ecological balance in these degraded environments.

## 1.2 General information on Fabaceae :

The species chosen for our work *Astragalus armatus* is a species belonging to the family *Fabaceae*. The *Fabaceae* are one of the most important families among the dicotyledons (**Bonnier, 1905**). It is a group represented by more than 20,000 cosmopolitan species from cold to tropical regions (**Gepts et al., 2005; Cronk et al., 2006**). The APG III (2009) phylogenetic classification divides this group into 3 subfamilies: Papilionoideae, Caesalpinioideae and Mimosoideae.

The subfamily Papilionoideae is monophyletic, cosmopolitan, and includes nearly 11,300 species in 440 genera (**Labat, 1996**), most of which have root nodules (**Raven et al., 2000**). Their flowers are irregular and the corolla is in the form of a "butterfly" (**Baillon, 1867**) with an upper petal (standard) located outside two lateral petals (wings) and two fused lower petals (carina) enclosing the style and fused stamens. The fruit is a single carpel pod with two opposite suture zones that, in spontaneous species, open at maturity to expel the seeds (**Caratini, 1984; Polhill, 1994**).

### 1.3 Geographical distribution:

The genus *Astragalus*. Is the most abundant of the Fabaceae family with about 2500 species and with more than 250 taxonomic sections in the world (Yuan et al, 2012) and (Fogg, 2004). This genus is widely distributed throughout the temperate and arid regions of the world and mainly localized in Asia (1500 species), North America (500 species) and South America (150 species) and Europe (120 species) (Sahli et al, 2013), in the countries of the Mediterranean basin 500 species have been described of which about 50 are in North Africa (Crotti et al, 2014), Mediterranean or Arab. They are represented by more than 50 species delimited in several sections and of which 15 are found in the Sahara of Algeria. In the flora of North Africa, 10 species of *Astragalus* are endemic to Algeria, Morocco and Tunisia (Anastas and Warner, 1998) and (Bahn, 2011).

In Algeria, the genus *Astragalus* is distributed in the pre-Saharan zone and associated with desertification in arid areas due to overgrazing (Braun et al, 1990) and (Yuan et al, 2012) However, the center of origin and biodiversity of the genus *Astragalus* is Eurasia, particularly the mountainous parts of Southwest and South-Central Asia (Choi, 2007) and (Van Gompel and Schuster, 1987).

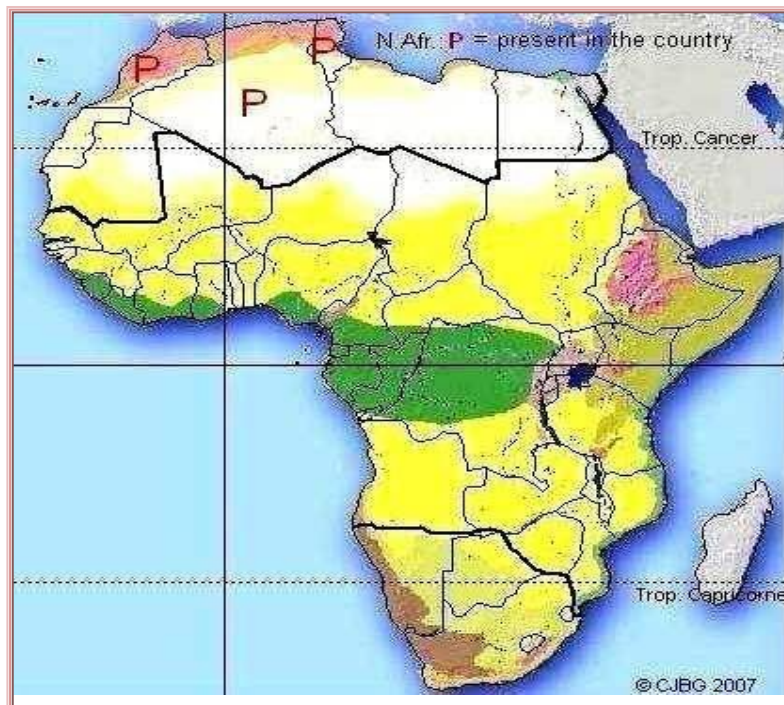


Figure 01: Geographical distribution of the species *Astragalus armatus*

Willd (Labeled,2016).

## 1.4 Classification

The species *Astragalus armatus sp. numidicus* (Coss.et Dur.) Maire is a fabaceae that follows the classification presented in table 01.

**Table 01:** Place of *A. aramatus* in the systematic (APGIII classification (2009)).

|                    |  |
|--------------------|--|
| <b>Reign:</b>      | Plant( <i>plantae</i> )                                    |
| <b>Class:</b>      | Broadleafplants  |
| <b>Subclass:</b>   | Dialypetal   |
| <b>Order:</b>      | Fabales  |
| <b>Family:</b>     | Fabaceae   |
| <b>Sub-family:</b> | Papilionaceae  |
| <b>Tribe:</b>      | Galegee  |
| <b>Genre:</b>      | <i>Astragalus</i>  |
| <b>Species:</b>    | <i>Armatus</i>   |
| <b>Subspecies:</b> | <i>Astragalusarmatussp .numidicus</i><br>(Coss.andDur.) M. |

The species *Astragalus armatus* growing in North Africa (fig.1).It is an endemic species, foundin Algeria, Morocco and Tunisia(Labed, 2016).

### 1.4.1 The genus *Astragalus*:

*Astragalus armatus* genus has three synonyms; *Acacia armata* (Willd.) Batt, *Anthyllis tragacanthoides* (Desf.) Pomelet *Anthyllis traga canthoides* Desf. (Greuter et al 1989). This genus is present in North Africa. The genus *Astragalus*, part of the family Fabaceae, is a collection of more than 3,000 species (Scherson et al., 2008) with a high rate of diversification, speciation, and endemism (Sanderson and Wojciechowski, 1996) and whose probable origin is Eurasia, regions between the mountains of southwestern Asia and the Himalayan plateau (Podlech, 1986; Lock and Simpson, 1991). Two centers of diversification that are Eurasia and North America have been identified by Wojciechowski

**and al.** The genus *Astragalus*, the largest in the family Fabaceae, includes about 2500 species with more than 250 taxonomic sections worldwide (**Yuan et al., 2012**).

### 1.4.2 Botanical description

*Astragalus armatus* is a champhyte, very twiggy, growing up to 1 meter in height. This species is very thorny; the rachis of the leaves becomes a sharp thorn after the fall of the leaflets. The petioles become hard and sharp. The small leaflets are very deciduous; scaly and hairless branches. The flowering takes place at the end of the winter and continues until the end of the spring. One underlines in this species the importance of the reproduction: An adult individual can form until 1500 flowers. (**Sahara Nature**)



**Figure 02:** Species of *Astragalus armatus* Willd (original 2022).

### 1.4.3 Mode of adaptation:

The perennial plants adapt to the climate and to the soil by the reduction of the number of leaves, their size in spine or kind of scales; the thickening by an epidermis cuticle of the stomata. To fight against the warming, the fatty plants or Cactaceae reserve an important

quantity of water at the level of the leaves, stems and roots (Quezel, 1978; Ozenda, 1983). To absorb the maximum amount of water, the superficial roots extend over a wide area horizontally to collect the lowest rainfall on the sand, while the very long, vertical roots sink to reach deep layers. In some species, these roots have a clumped sleeve of sand that prevents evaporation (Benchelah et al., 2011).

*Astragalus armatus* is a plant with pinnate compound leaves and whose leaflets fall off and the rachis becomes spiny: in this case, it is not a seasonal but a permanent change. In addition, in summer, we observe a stop of the vegetation with a total fall of the leaflets. These two modes allow the plant to reduce the leaf surface and thus to decrease the losses of water by transpiration. This taxon presents some eco-physiological particularities which favor rapid proliferation on the steppe:

- Good germinate abilities in natural environment, in particular by comparison with *Rhanteriumsuaevolens*.
- Power of its rooting able to develop the weak hydrous reserves of the ground,
- And importance of the phytomass produced.

However, although *Astragalus armatus* is considered a species marking degradation, it can contribute, even if only partially, to the process of restoring the ecological balance in these degraded environments. The trapping of sand and the reconstitution of the aeolian veil by the very developed tufts lead to an improvement of the water balance of the soil and favour the germination of species that were previously rarefied. Finally, its ability to fix atmospheric nitrogen contributes to improving soil fertility (Chaieb, 1997).

### 1.4.4 The genus *Astragalus* in Algeria:

The genus *Astragalus* is quite well represented in Algeria (Quézel et Santa, 1962). Indeed, the Algerian flora includes 40 species of *Astragalus* (45 taxa counting the subspecies). These species are divided into annual and perennial species which colonize several types of habitat and belong to different ranges.

## 1.5 Toxicity of the genus *Astragalus*

The genus *Astragalus* includes both toxic and non-toxic species.

### 1.5.1 Non-toxic *Astragalus*

Most *Astragalus* are non-toxic. Some are used as grazing species, such as *Astragalus nuttalianus* and *Astragalus cicer* found respectively in the southwest and western United

States are highly valued by livestock (**Sutharsan, 2010**). The same applies to the species *Astragalus gyzen sis* Bunge. And *Astraglus gombo* Bunge .Located in the northern Algerian Sahara (**Vejdelek and Protiv, 1990**).

### 1.5.2 Poisonous Astragalus

The toxic species of this genus are classified according to the nature of the dominant toxin and the type of intoxication they cause, and are divided into three categories (Barberoand al, 2012):

- Selenium-accumulatin astragalus (**Ablinger,2013**).
- *Astragalus* synthesiz esnitrate products (**Nagasakaetal,1989**).
- *Astragalus* containing indolizidic alkaloids (**Kutyrevand Kappe,1997**).

## 1.6 Interest of Astragalus

### 1.6.1 Medicinal interest of the plant:

The species of the genus *Astragalus* are used in traditional medicine all over the world as medicinal herbs against stomach ulcer, cough, chronic bronchitis, hypertension, gynecological disorders, diabetes and venomous scorpion stings (**Bellakhdar, J. 1997**).

### 1.6.2 Economic importance of the plant:

According to (**Moussaoui et al, 2011**), the studies confirm that by physiological characteristics the *Astragalus armatus* it is presented as a good primary material for the manufacture of papers.

### 1.6.3 Ecological interest:

According to (**Mallem et al, 2017**), the studies able to demonstrate the conjugated action of two factors; the silting up and the agriculture (ploughing and overgrazing) on the floristic diversity of the fragile steppe environments, in spite of Despite the low diversity of palatable species, the proliferation and adaptation of species rejected by sheep and species related to the presence of sand, the environment has improved from the point of view of sand fixation and wind erosion control .

## **CHAPTER II: MATERIALS AND METHODS**

## 1 Plant material

Seeds of *Astragalus armatus* (photo 01), were collected during April 2022 in the rangelands of Ksar El Hiran at Laghouat province.



**Photo 01: Representation of the seeds of *Astragalus armatus* (original April 2022).**

## 2 Experimental design for seeds germination

Germination tests were carried out under different concentrations of four substratums: sodium chloride (NaCl), calcium carbonate (CaCO<sub>3</sub>), and calcium sulfate (CaSO<sub>4</sub>) and distilled water, to determine the suitable substratum for the germination of *Astragalus armatus* seeds. The *Astragalus* seeds were first disinfected in ethanol solution (60%), for 10 minutes and then washed three times with distilled water and placed in petri dishes lined with filter paper at about 10 seeds per dish. In the first case, we added distilled water which

## Materials and methods

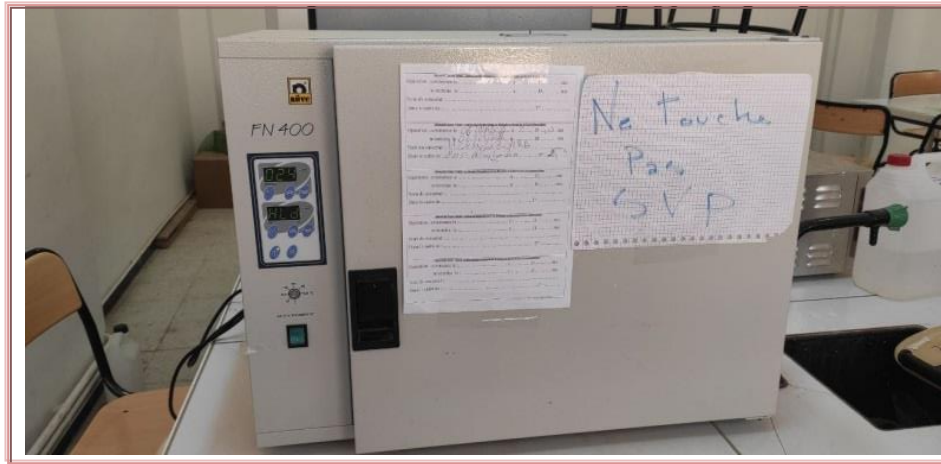
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constitutes the control substratum recorded (T0). In the other cases, we added CaCO<sub>3</sub> solutions at 5mg/100ml, 10 mg/100ml, and 15 mg/100ml; CaSO<sub>4</sub> solutions at 5 mg/100ml, 10 mg/100ml, and 15 mg/100ml; and NaCl solutions at 5 mg/100ml, 10 mg/100ml, and 15 mg/100ml, with three (3) replicates for each substratum.

However, we have four substratum (distilled water, CaSO<sub>4</sub>, CaCO<sub>3</sub>, and NaCl) with three treatments C1 (5%), C2 (10%), and C3 (15%) for substratum (CaSO<sub>4</sub>, CaCO<sub>3</sub> and NaCl). The Petri dishes are placed in an incubator that is set to 25°C and also has a 12 hour dark / 12 hour light cycle. Seed germination is noted when the rootlet, which is at least 2 mm long, emerges from the seed.



**Photo 02 : Representation of the experimental design for the germination seeds of *Astragalus armatus* (original April 2022).**



**Photo 03 : Incubator machine**

### **3 Germination monitoring and measured parameters**

The following parameters were measured throughout this work:

#### **3.1 The kinetics of germination**

To better understand the physiological significance of the germination and growth behavior of *Astragalus armatus*. We measured the germination rate, the lengths of the rootlets and the lengths of the stems during the 18 days of the test.

### 3.2 Germination rate (GR)

Rate of germination is expressed as the ratio of the number of germinated seeds by the total number of seeds:

$$\text{GR} = \text{number of germinated seeds} / \text{total number of seeds} \quad (\text{Radford, 1967})$$

### 3.3 Median germination time (MGT)

The median germination time, allows us to express the germination energy that is responsible for the depletion of the seed's reserves (Scott and Jones, 1984). It can also be expressed as the average time of germination (T50) (the time required to reach 50% of germinated seeds) (Côme, 1970).

$$\text{MG (T50)} = \text{T1} + (0.5 - \text{G1} / \text{G2} - \text{G1}) \times (\text{T2} - \text{T1})$$

Where:

- G1 = cumulative percentage of germinated seed with a value closer to 50% or less in value.
- G2= Cumulative percentage of germinated seed that is closer to 50% by greater value.
- T1: the number of days corresponding to the cumulative percentage of germinated seed with a value of more than 50 % for each lower value.
- T2: the number of days corresponding to the cumulative percentage of germinated seed with a value closer to 50 % per higher value.

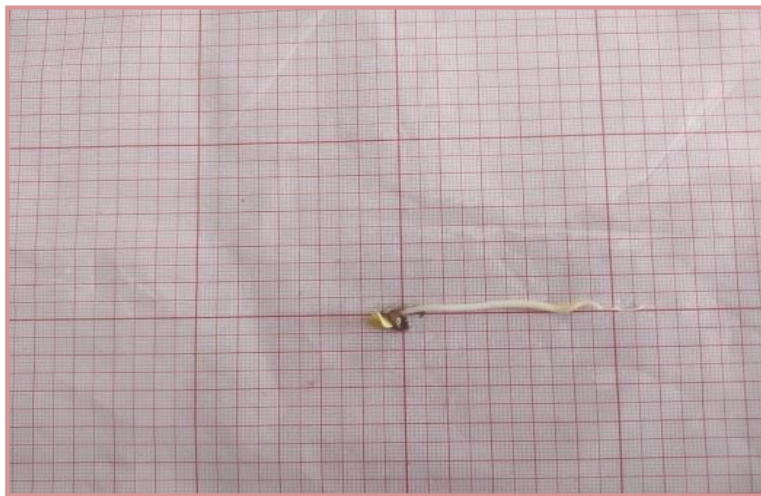
### 3.4 Mean Daily Germination (MDG)

The MDG is represented by the ratio of final germination percent to final germination days. (Osborne and Mercer, 1993).

$$\text{MDG} = \text{Final germination percentage} / \text{Number of days to final germination.}$$

### 4 Rootlet length and Stems length

The length of the primary rootlet and the length of the stems were measured by projection on the millimeter paper to evaluate the growth of the plant in relation to the preferred substratum (Radford, 1967).



**Photo 04: Measure of rootlet (original, 2022)**

### 5 Developing speed (D S)

The developing speed expresses the ratio of the length of the stems or rootlets at the end of the trial divided by the final growth time (Delamarre et al, 2014)

$$\mathbf{D\ S = LR\ or\ LS\ (cm)/final\ growth\ time\ (day)}$$

### 6 Vigor Index (V I)

This is a critical indicator for determining which plant substrates are more resistant (Radford, 1967).

$$VI = (LR + LS) \times GR$$

Where:

VI: Vigor Index

RL: Rootlet length

SL: Stems length

GR: Germination rate

### 7 Determination of proline (mmol/g MF):

At the end of the trial, this parameter is measured, in order to evaluate the physiological behavior of the *Astragalus* seedlings relating to the substrate in which their seeds germinated. The accumulation of proline is one of the adaptive strategies triggered by the plant in the face of environmental constraints (**Belkhodja and Benkabilia, 2000**). Many studies report that proline is accumulated in the plant when it is in unfavorable conditions for its development and growth (**Sivaramakrishnan et al., 1988**).

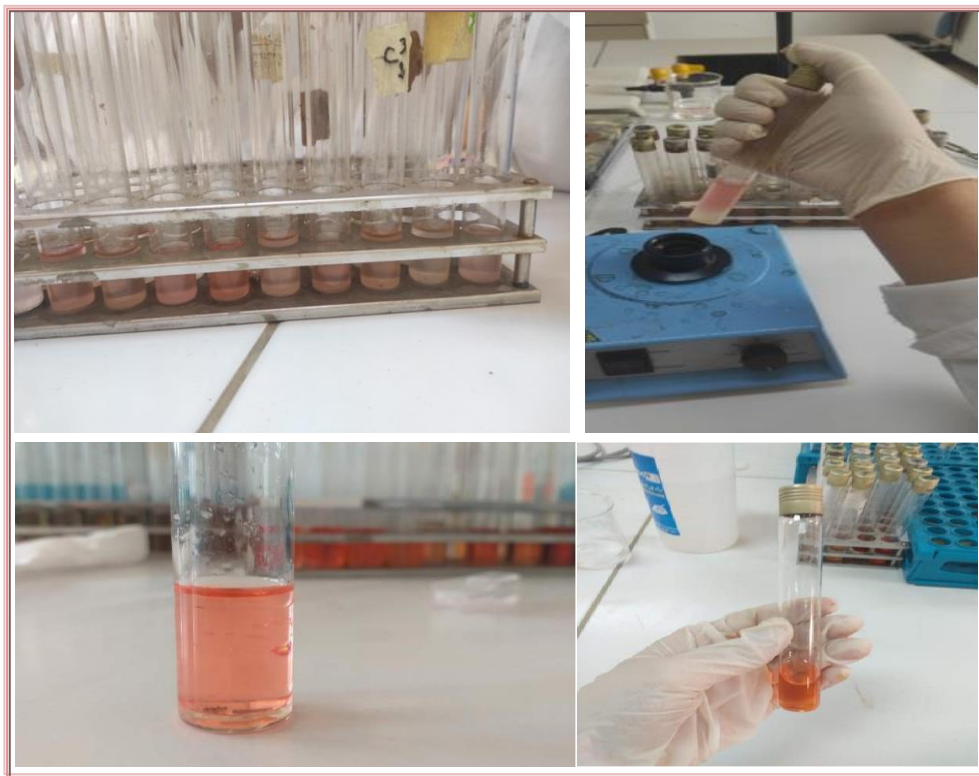
Proline, also known as pyrrolidine 2-carboxylic acid, is one of the twenty main aminoacids that make up proteins. Proline is easily oxidized by ninhydrin or tricetohydrindene, and the protocol for detecting proline in leaf samples is based on this reaction (**El Jaafari, 1993**). **Trolls and Lindsey's (1955)** method was simplified and developed by (**Rasio and al. (1987)**).

It entails placing 100 mg of fresh material in test tubes containing 2 ml of 40% methanol. For 60 minutes, the whole is heated in a water bath at 85°C. (During heating, the tubes are covered with aluminum foil to prevent the alcohol from volatilizing.) After cooling,

## Materials and methods

1 ml of extract and 1 ml of acetic acid ( $\text{CH}_3\text{COOH}$ ): 25 mg of ninhydrin ( $\text{C}_6\text{H}_6\text{O}_4$ ) are added; 1 ml of mixture containing: 120 ml of distilled water; 300 ml of acetic acid; and 80 ml of orthophosphoric acid ( $\text{H}_3\text{PO}_4$ ,  $d=1.7$ ).

After boiling for 30 minutes at  $100^\circ\text{C}$ , the solution turns red. After cooling, 5 ml of toluene is added to the solution, which is stirred, and two phases separate (an upper phase with red color contains proline and a transparent lower phase without proline). After the lower phase has been removed, the upper phase is recovered and dehydrated by adding 5mg of anhydrous Sodium Sulfate  $\text{Na}_2\text{SO}_4$  (to eliminate the water it contains).



**Photo 03: Proline determination (Original plate, 2022).**

The optical density (**OD**) is measured with a spectrophotometer (type 20D) at 528nm. The values obtained are converted into proline content using a standard curve previously established from a series of known proline concentration solutions. This curve is used to calculate the proline content of plant leaves.

### **8 Statistical analysis**

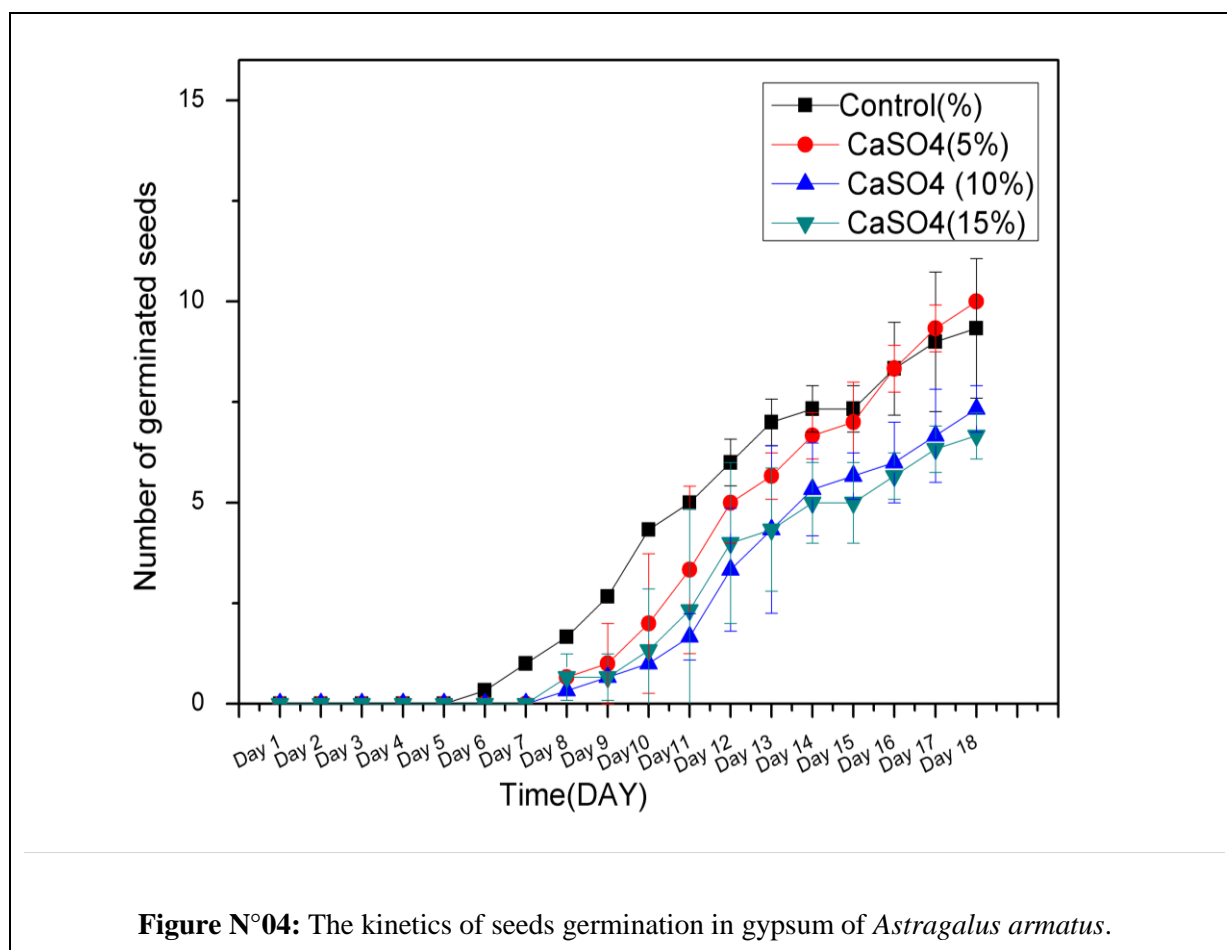
For the whole test, an analysis of variance is performed by Tukey's test at 5% using Minitab 2018, the means are compared according to the method of Newman and Keuls, each mean is assigned a letter, the means followed by the same letter are not significantly different. Also at the end of the test, a principal component analysis (PCA) is performed in XI-Stat.

## **CHAPTER III: RESULTS AND DISCUSSION**

## 1 The kinetics of germination

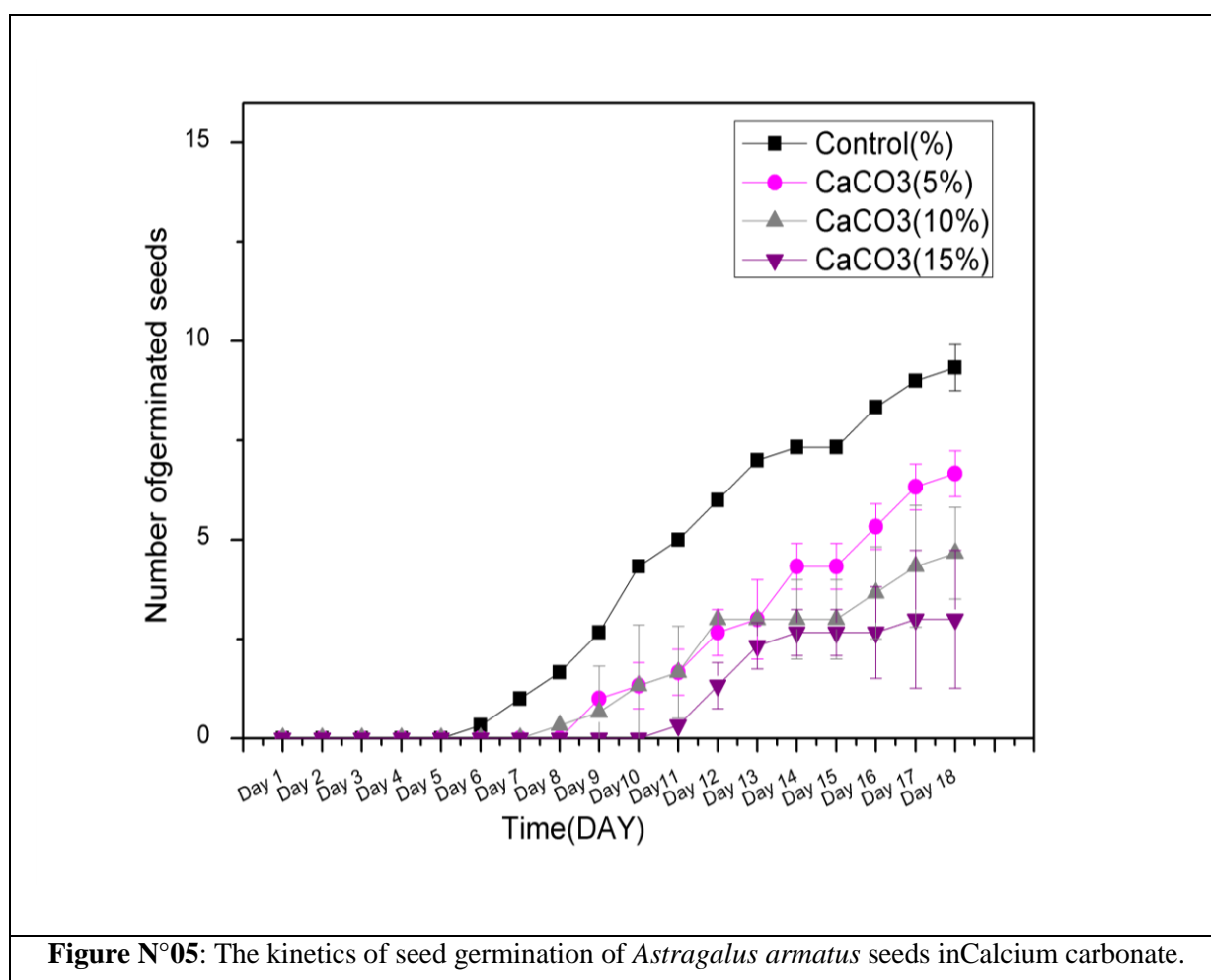
### 1.1 Germination kinetic in Calcium Sulfate substrate

The figure (04), shows the kinetics of seed germination in gypsum ( $\text{CaSO}_4$ ) at various concentrations. Germination begins on the sixth day in the control substratum (0 %) at a rate of 10%. A seed has germinated in the other concentration since the eighth day. On the 18th day, the maximum number of seeds (10) germinated in  $\text{CaSO}_4$  (5%).



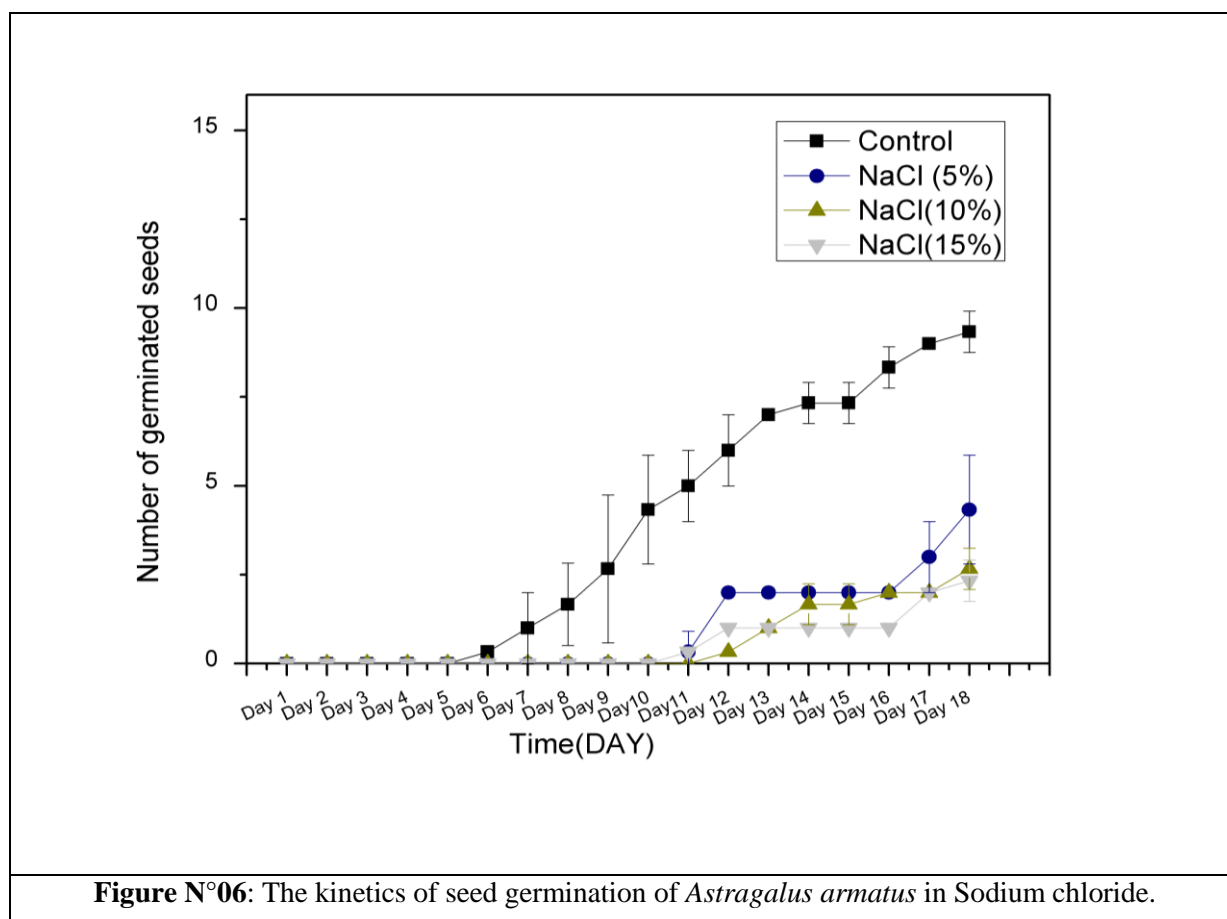
## 1.2 Germination kinetic in Calcium Carbonate substrate

Figure (05), shows the germination kinetics of *Astragalus armatus* seeds in calcium carbonate at various concentrations (5%, 10% and 15%). The germination begins on the 6th day in the control substrate (0%) with a rate of 10 %, in the other concentration of CaCO<sub>3</sub> (5%, 10% and 15), the germination starts on the 9th day. The maximum number of seeds (07) germinated on the 18th day, in CaCO<sub>3</sub> (5%).



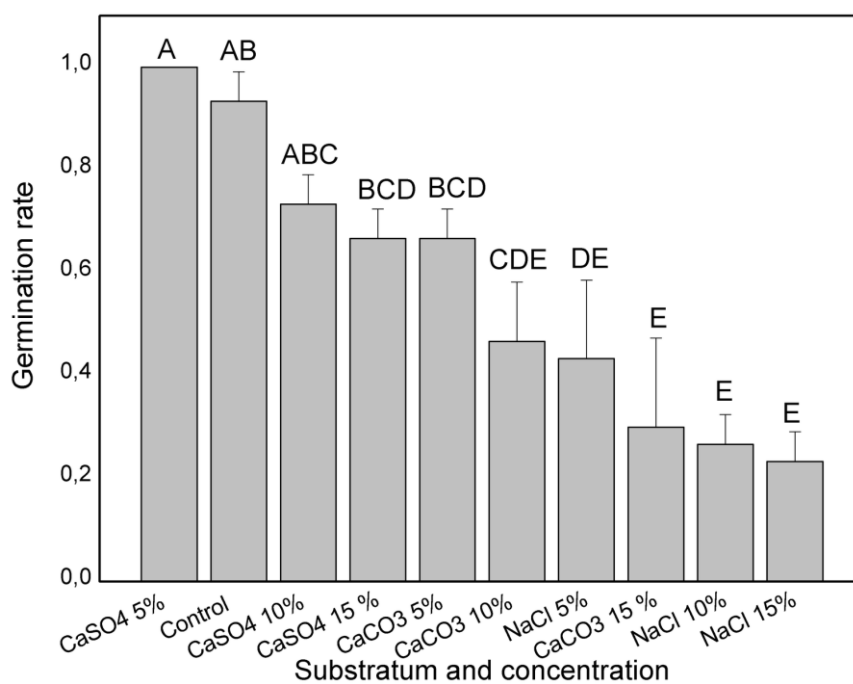
### 1.3 Germination kinetic in Sodium Chloride substrate

In sodium chloride at various concentrations (5%, 10% and 15%) the kinetics of *Astragalus armatus* seeds germination is shown in Figure (06). Germination begins on the sixth day in the control substrate (0 %) with a rate of 10%, on the 12th day in each of the other concentrations of NaCl one seed (10%) germinated. On the 18th day, the greatest number of seeds (4) germinated in the substrate NaCl (5%).



## 2 Germination rate

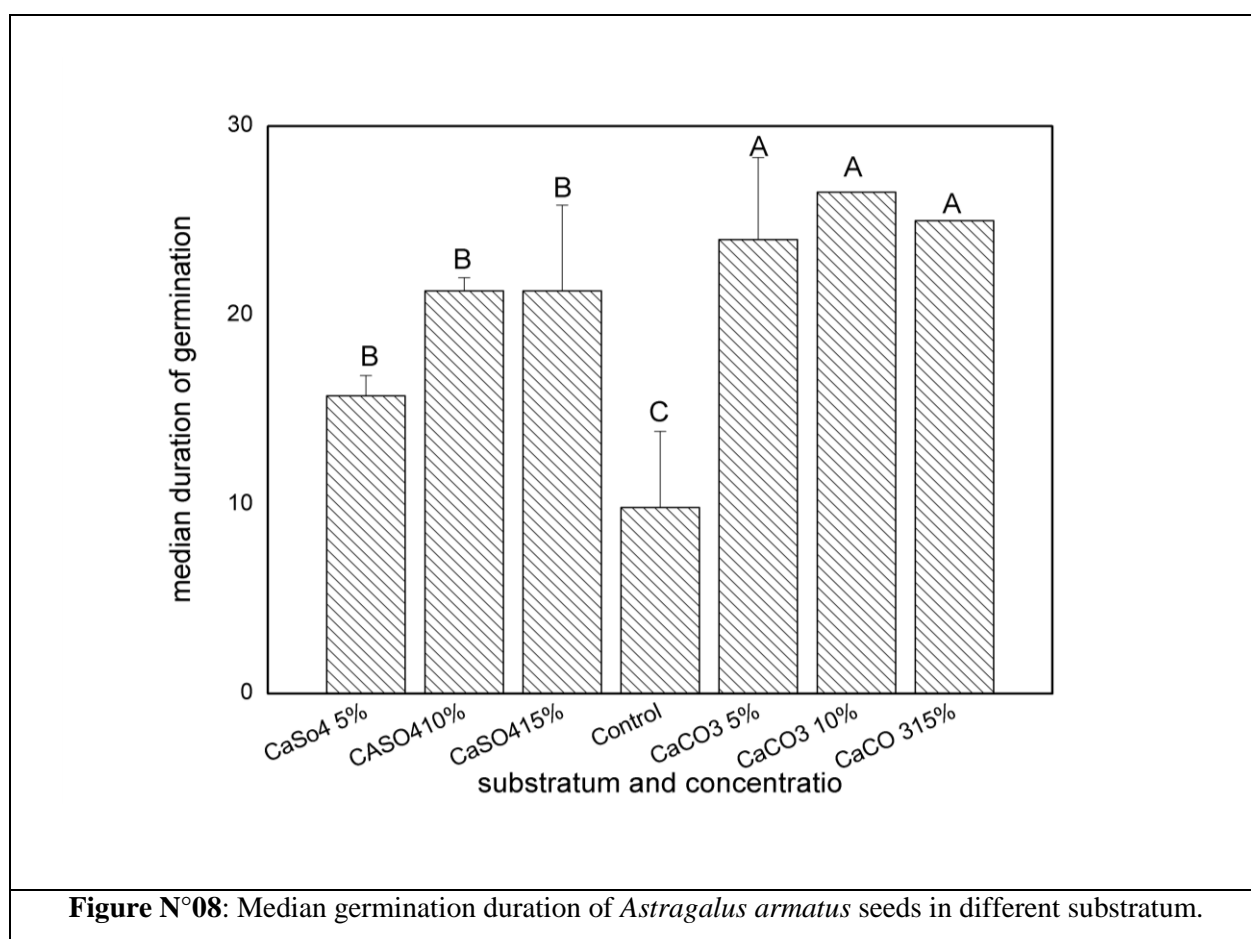
Figure (07), shows that the germination rate of *Astragalus armatus* seeds in various substrates is maximum (100%) in CaSO<sub>4</sub> (5%), followed by that of control (93%). The lowest germination rate (25%) is observed in the substrates of NaCl (10%), CaCO<sub>3</sub> (15%), and NaCl (15%). ANOVA reveals statistically significant differences ( $P < 0,05$ ), resulting in seven statistical groups.



**Figure N°07:** Germination rate of *Astragalus armatus* seeds in different substratum.

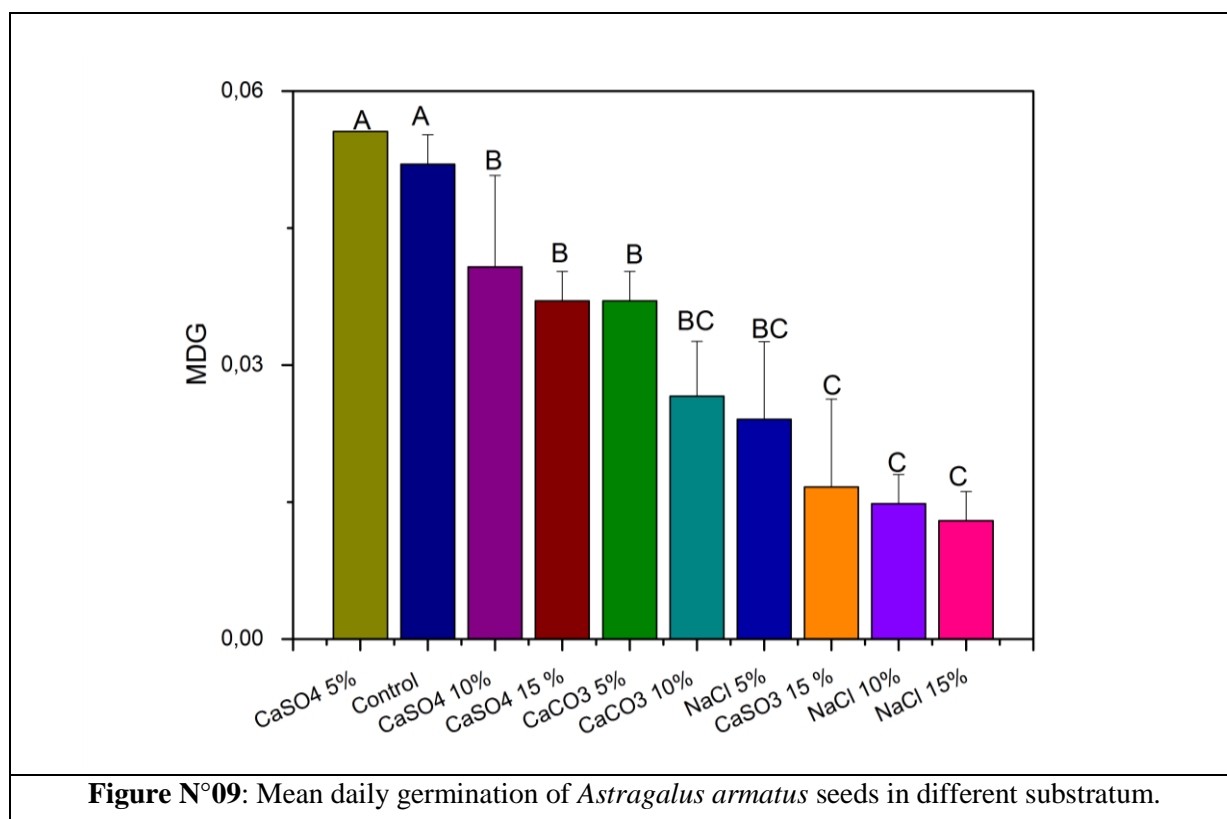
## 2.1 The Median germination duration (MGD)

Figure (08), presents the variation in median germination duration of *Astragalus armatus* seeds different concentrations of CaCO<sub>3</sub> and CaSO<sub>4</sub>. In the CaSO<sub>4</sub> substratum, the increase in the concentration generates a slight increase in MGD. In CaCO<sub>3</sub>, MGD is highest around 25. The lowest value of MGD (9) is observed for the control. ANOVA reveals a significant difference ( $P < 0.05$ ) forming three statistical groups.



## 2.2 Mean Daily Germination (MDG)

Figure (09), shows that the mean daily germination is highest around 0.06 for the CaSO<sub>4</sub> (5%) and the control (0%). MDG is about 0.04 in CaCO<sub>3</sub> (5%), CaSO<sub>4</sub> (10%) and CaSO<sub>4</sub> (15%), and also NaCl substrates. In the salt substrate and in CaCO<sub>3</sub> (5%), the MDG values are lower than 0.03. The ANOVA test carried out on the 18<sup>th</sup> day reveals significant difference for MDG of *Astragalus armatus* seeds germinating at different concentrations (5%, 10% and 15%) of CaCO<sub>3</sub>, CaSO<sub>4</sub> and NaCl thus forming 4 statistical groups.



### 3 Stems length and rootlets length of *Astragalus armatus* seedlings

#### 3.1 Stems length

Figure(10), represents the variations in stems lengths of *Astragalus armatus* germinating in different substrate and at various concentrations. The stems lengths are greater than 4.5 cm in CaSO<sub>4</sub> 5% and the control. The lengths of *Astragalus armatus* stems are between 0.9 and 2.2 cm in the substrates CaCO<sub>3</sub> (5%, 10%, and 15%); CaSO<sub>4</sub> (10%, 15%). ANOVA test revealed significant difference ( $P < 0.05$ ) forming 4 statistical groups of stems length.

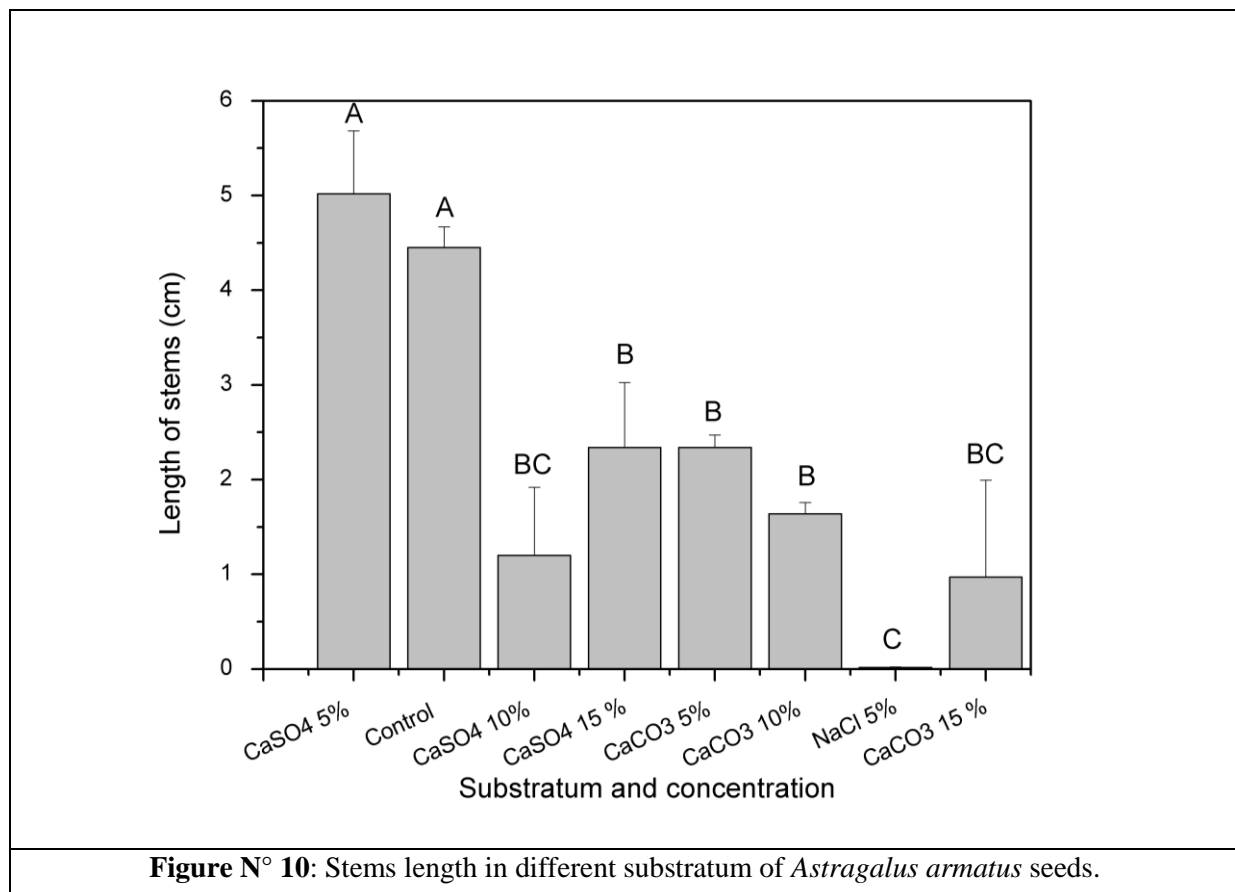
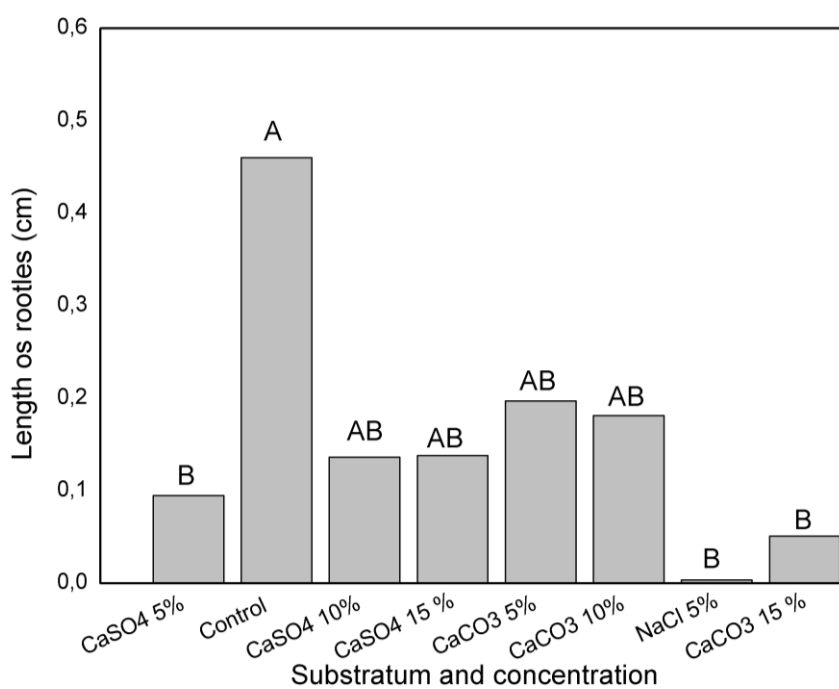


Figure N° 10: Stems length in different substratum of *Astragalus armatus* seeds.

### 3.2 Rootlets length

Rootlets length of *Astragalus armatus* germinating in CaSO<sub>4</sub> and CaCO<sub>3</sub> at various concentrations are shown in Figure (11). The longest rootlet higher than 4 cm is observed for the control substratum (0%); in the other substrates, the length of the rootlets is less than 0.2 cm. ANOVA test showed significant differences ( $P < 0.05$ ) 3 statistical groups for the rootlets length.

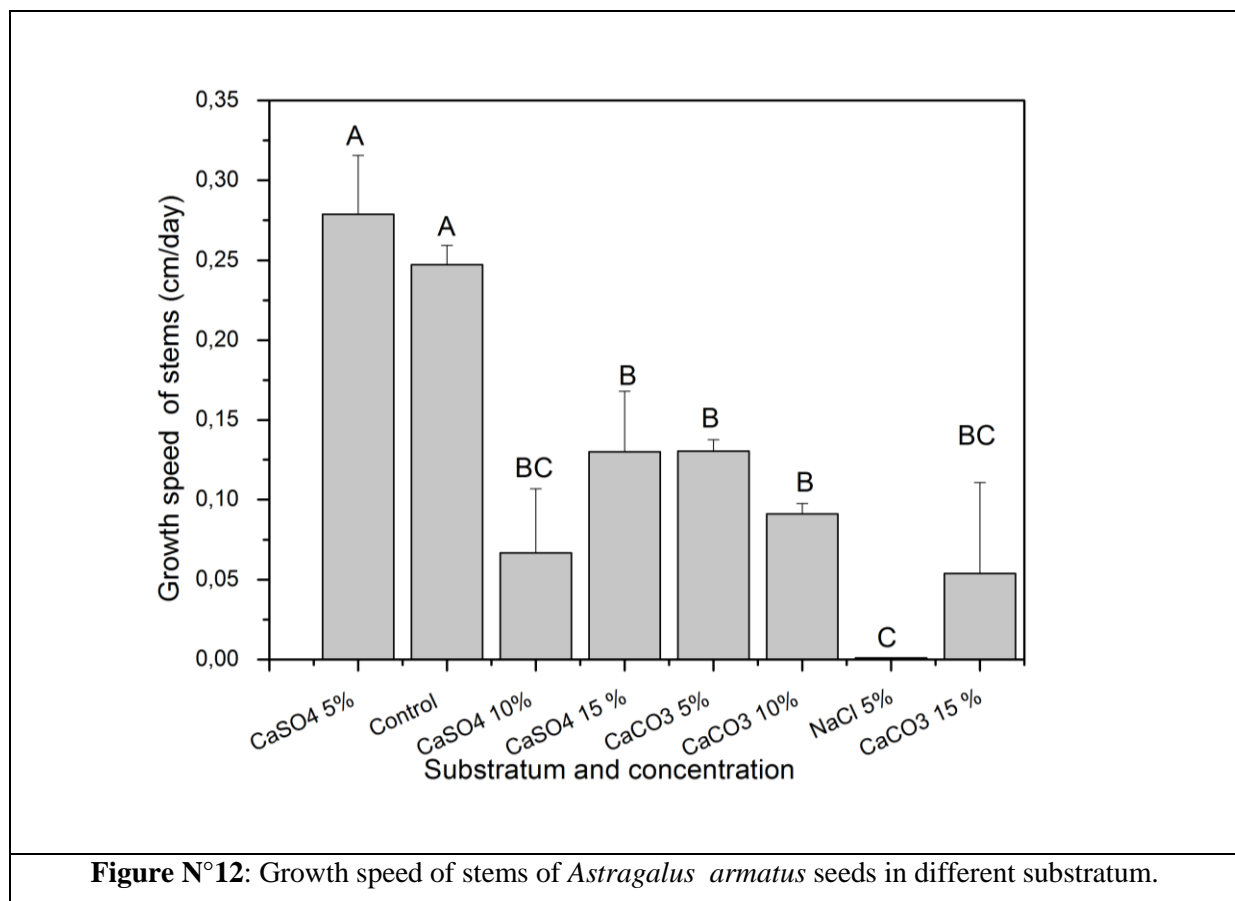


**Figure N°11:** Rootlets length of *Astragalus armatus* seeds in different substratum.

## 4 Growth speeds

### 4.1 Growth speed of stems

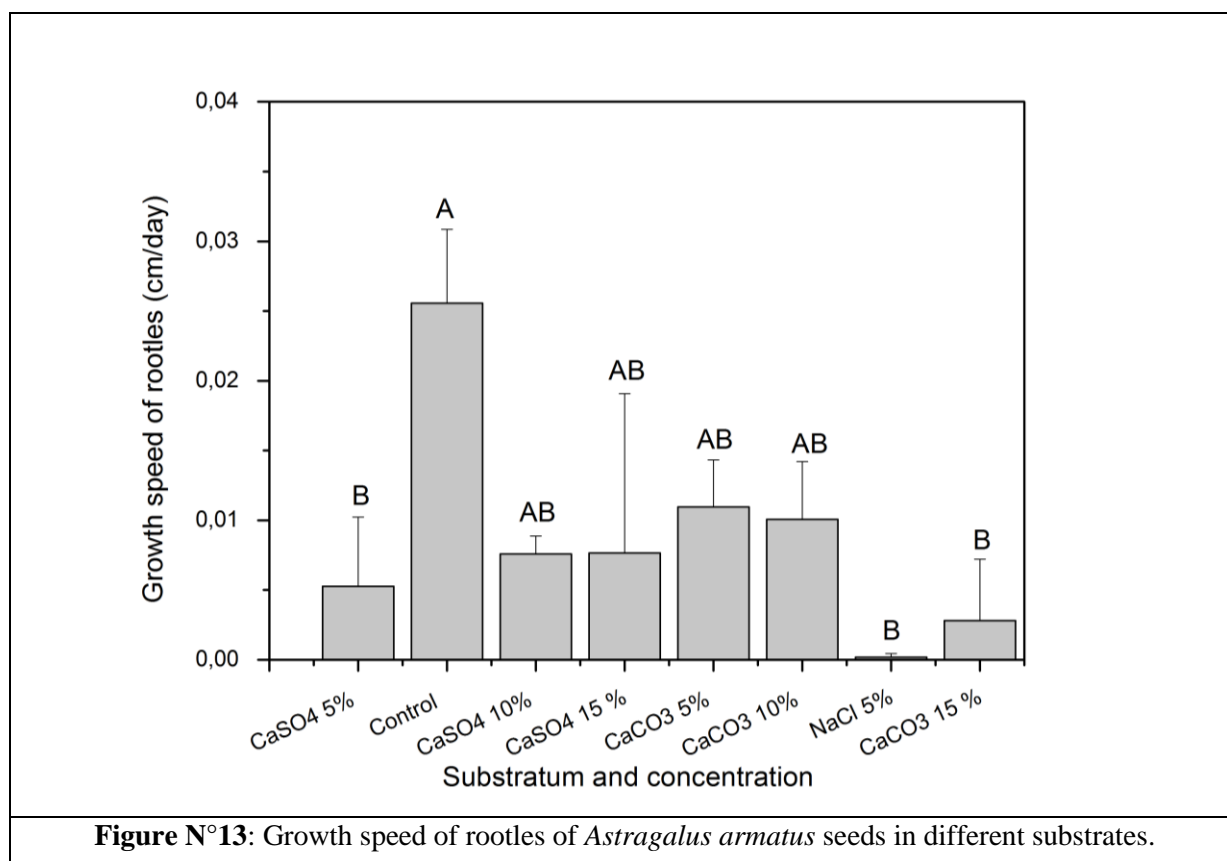
Figure (12), presents the growth speed of stems of *Astragalus armatus* germinating in different substrates and at various concentrations. The fastest shoot growth is observed for the control and also in CaSO<sub>4</sub> (5%), followed by those in the substrates (CaSO<sub>4</sub> (15%) and CaCO<sub>3</sub> (5% and 10%) in which the growth speed of *Astragalus armatus* stems is between 0.09 and 0.12 (cm/day). The lowest growth speed of *Astragalus armatus* 0.05 (cm/day) is registered in the substrate of CaCO<sub>3</sub> 15%. ANOVA test revealed significant difference forming 4 statistical groups of growth speed of *Astragalus armatus* stems.



**Figure N°12:** Growth speed of stems of *Astragalus armatus* seeds in different substratum.

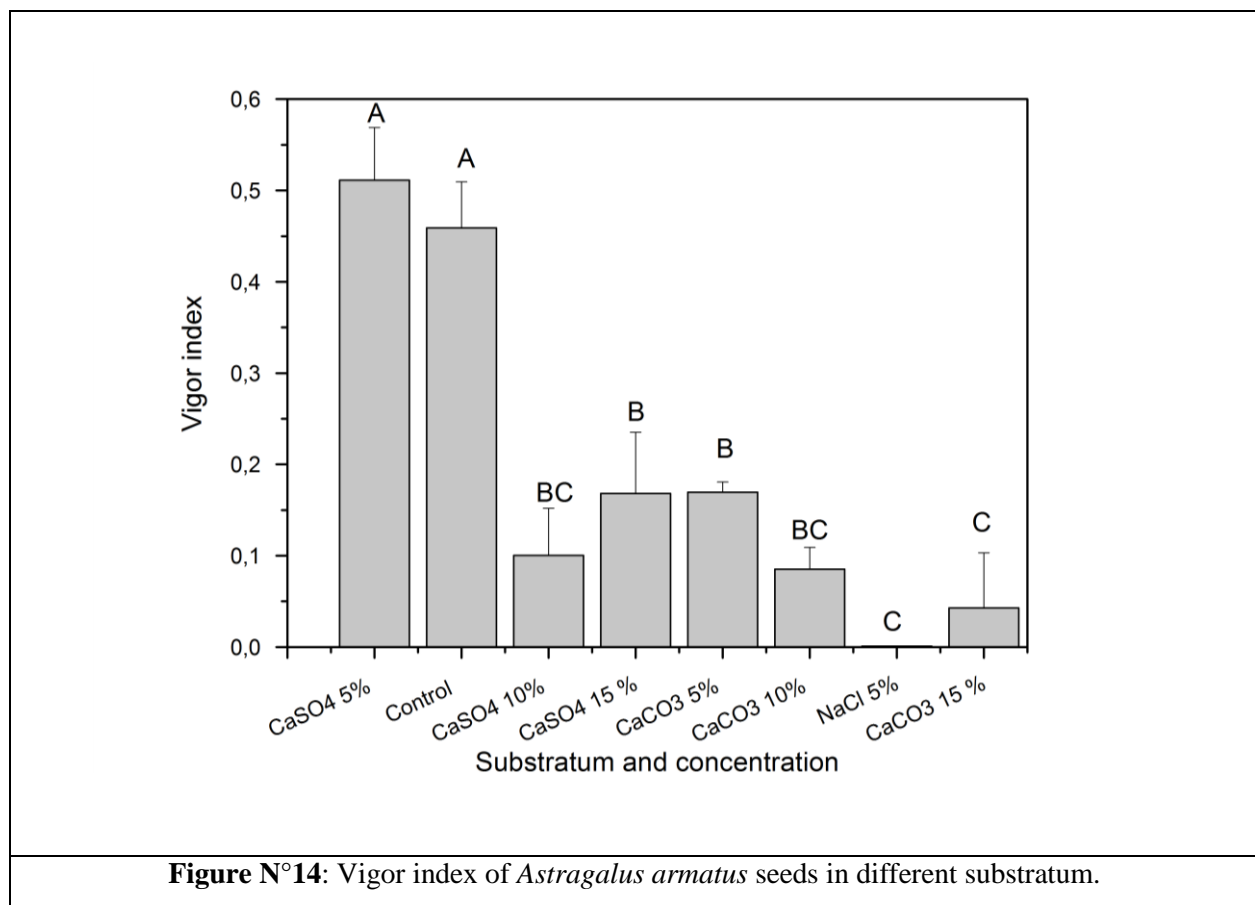
## 4.2 Growth speed of rootles

The growth speed of rootlets length *Astragalus armatus* seedlings germinating in CaSO<sub>4</sub> and CaCO<sub>3</sub> at various concentrations are shown in Figure (13). The slowest speed (0.01 cm/day) is observed in NaCl (5%). In the other CaCO<sub>3</sub> and CaSO<sub>4</sub> substrates for all concentrations, the growth rates of *Astragalus* rootlets are relatively higher, between 0.001 and 0.0012(cm/day). It is in the control (0%) that the rootlets of *Astragalus* develop rapidly 0.025 (cm/day). ANOVA test reveals significant differences ( $P < 0.001$ ) forming three statistical groups.



## 5 Vigor Index

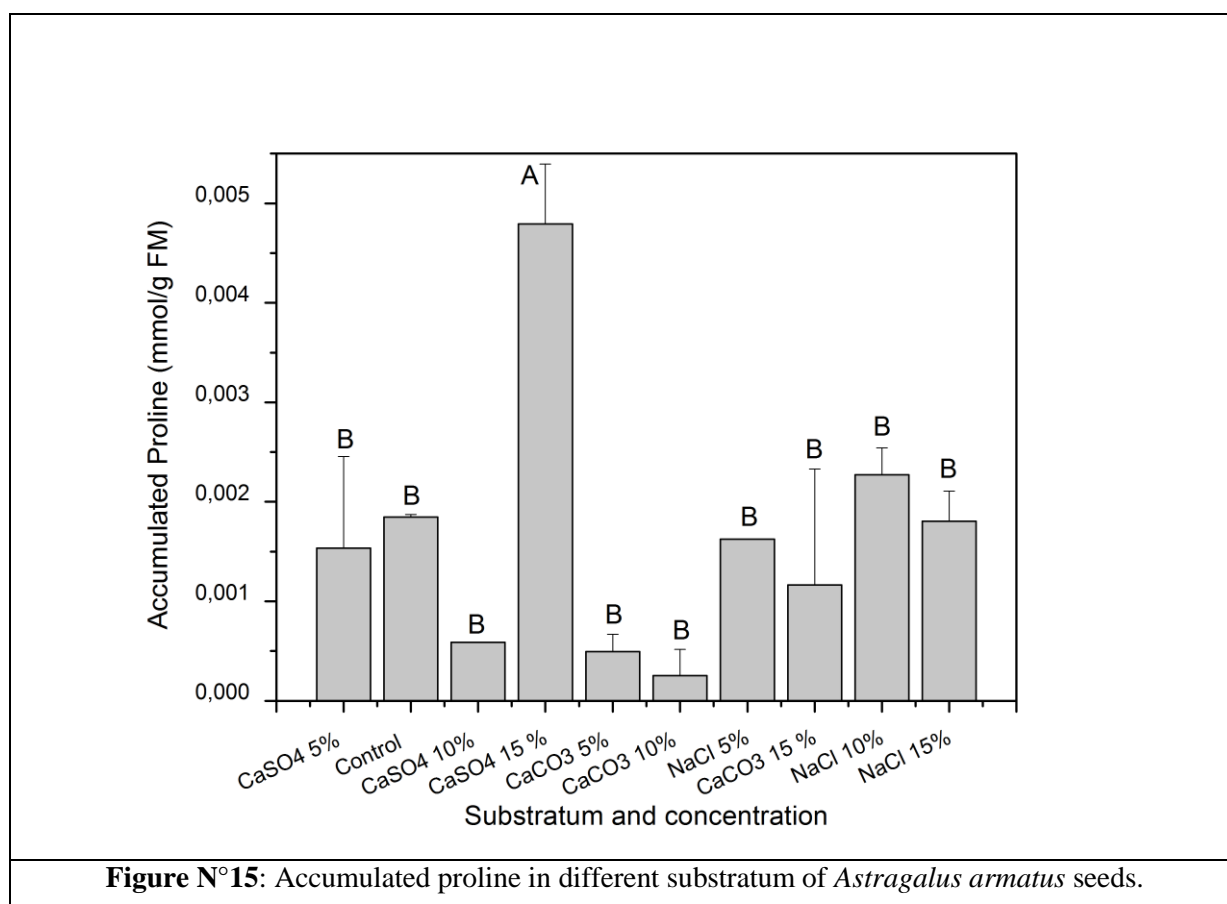
Figure (14), shows that *Astragalus armatus* is most resistant in the substratum CaSO<sub>4</sub> (5%) and in the control substrate, *Astragalus armatus* seedlings recorded vigor indices greater than 0.45. In the other substrates where *Astragalus armatus* has germinated, the seedling vigor index is less than 0.2. The ANOVA test reveals significant difference ( $P < 0.001$ ) thus forming each one of the four statistical groups.



**Figure N°14:** Vigor index of *Astragalus armatus* seeds in different substratum.

## 6 Accumulated proline(mmol/g FM)

Figure(15), represents the accumulated proline in the fresh stems of *Astragalus armatus* germinating in different substrates at various concentrations. The highest accumulated proline 0.0045 (mmol/g FM) is observed in CaSO<sub>4</sub> (15%), lower values and ranging between 0.0005 and 0.002 (mmol/g FM) are registered for the others substratum. ANOVA test reveals a significant difference ( $P < 0,05$ ), forming 2 statistical groups.



## 7 Principal component analysis

The principal component analysis (PCA) visualizing (Figure 18), the correlations between the treatments and the measured parameters. Through (figure18), we observe that the CaSO<sub>4</sub> 5% treatment is the most related to the measured parameters. This is presented by 100% with the germination rate and 92% with the length of stems; 92% with the growth speed of stems; and 99% with the vigor index. In CaCO<sub>3</sub> 5%, 91% of the rootlets are longer; 91% of the rootlets are growing faster. Then we observe that the CaSO<sub>4</sub> (10%, 15%) and CaCO<sub>3</sub> (10%, 15%) substrates seem to have similar behavior. These treatments are moderately correlated to the measured parameters compared to the control, which is placed below these two substrates. While for the treatments with NaCl, we observed no correlation with the measured parameters.

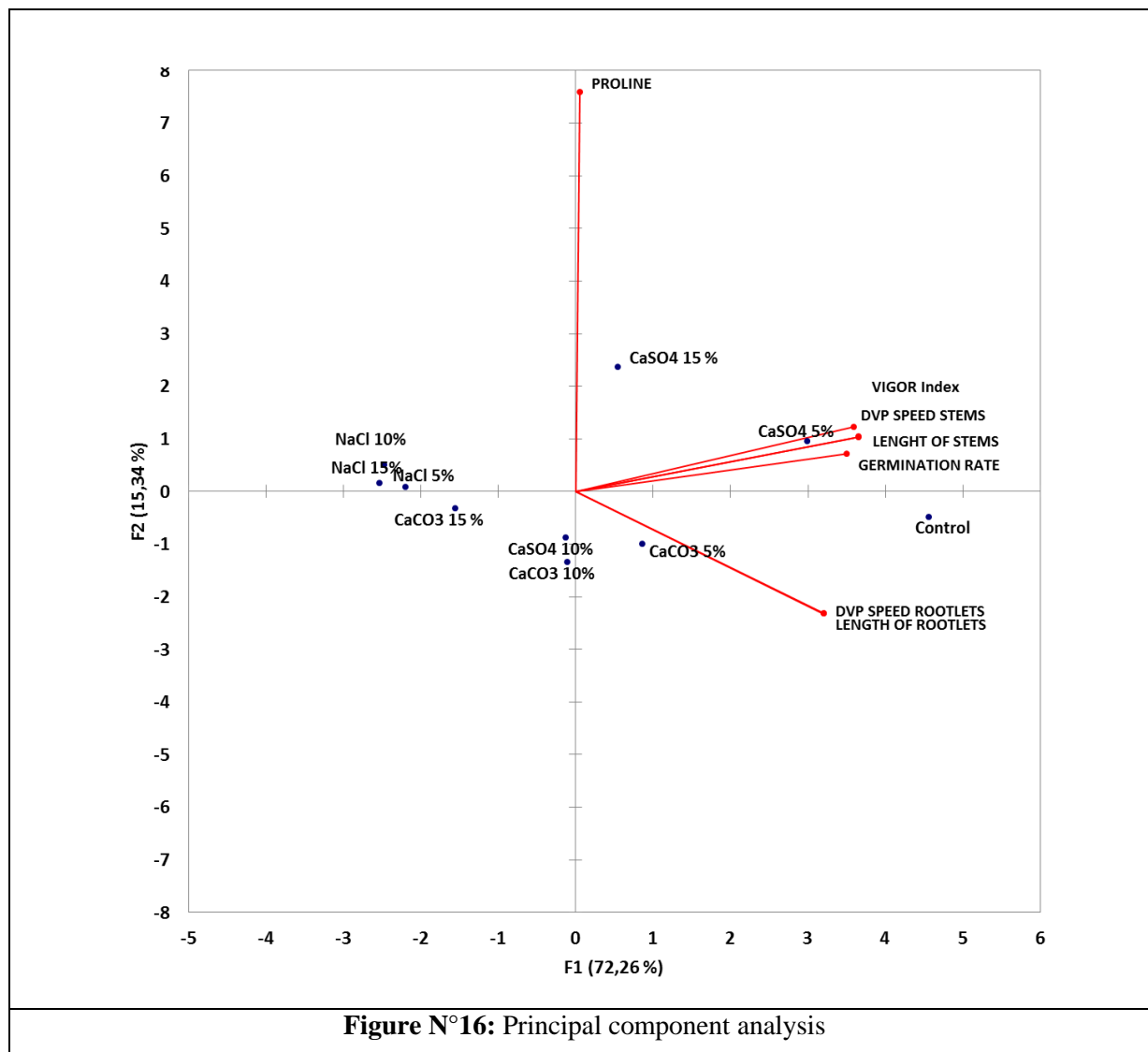


Figure N°16: Principal component analysis

## **Chapter Discussion**

When the germination of *Astragalus armatus* seeds was examined (PCA), it was discovered that they had distinct germination behavior in the used treatments. Calcium sulfate (CaSO<sub>4</sub>) had a positive impact on the germination rate and daily mean, as well as stem length, stem development speed, germination kinetics, etc. Seeds grew best in (CaSO<sub>4</sub>) compared to other treatments, according to resistance testing. *Astragalus armatus* is well suited to the gypsum substrate. However, some researchers have shown that gypsum can help acupuncture needles develop faster. Ca<sup>2+</sup> is the most abundant cation in alkaline soils. As a result, the proportion of germination impacted by Ca<sup>2+</sup> salts is essential.

It restricts the production of stems and roots. Increasing the calcium concentration in the gypsum substrate had no effect on the development of rootlets and stems in this study. The data obtained with the sharp tip in this experiment show that excess Ca<sup>2+</sup> has no effect on sharp tip growth, implying that calcium plays a significant role in plant germination and growth. As a result, it can be hypothesized that this calcium acts as a mineral fertilizer, improving plant germination and growth. Excess limestone has been found to disrupt *Astragalus armatus* root length and growth speed when treated with CaCO<sub>3</sub>.

According to (Ozenda, 1962), *Astragalus armatus* is a plant that grows in soils that are generally calcareous or calcareous and well-drained.

The effect of excess limestone on acupuncture germination and growth is not mentioned in any published research. This situation makes understanding and interpreting our results difficult. A lack of calcium during germination and emergence, according to (Wortmann et al, 2009), leads to reduced root growth. Concerning the effects of excess Ca<sup>2+</sup> in gypsum substrate, it is worth noting that the increase in calcium carbonate causes an early drop in calcium uptake during germination, consequently delaying plant seed germination, but it does not cause dormancy or kill the seeds. Germination and emergence of seedlings under salt stress conditions indicate genetic potential for salt tolerance at this stage of plant development.

Laboratory studies have revealed that NaCl has varying effects on the germination rate and emergence of *Astragalus armatus*. NaCl has similar effects on the growth of many plant species, according to research. Ungar (1996), Belkhodja (2004) pointed out that given the importance of the germination stage in following developmental phases, the reaction of plants to salt is extremely varied depending on whether we are interested in the germination stage or

another stage. In any plant species, especially in arid regions (Lachiheb et al., 2004), this becomes a vital component for the successful growth of plants in saline conditions.

The time it takes for *Astraglus armatus* seeds to trigger mechanisms that regulate osmotic pressure is the cause of delayed germination (Bliss R, 1986). According to Asloum (1990), plant growth is significantly reduced as NaCl concentration increases. The majority of authors believe that the decrease in growth is primarily due to osmotic pressure. For Atmanerochdi and al. (2005), plant growth was reduced under salinity conditions due to the uptake of abnormal salt concentrations rather than reduced water uptake. According to Asloum (1990), the degree of ion uptake by plants from salt solutions varies, resulting in ion accumulation and a decrease in essential nutrient uptake.

The accumulation of Na<sup>+</sup> ions in plants reduces the uptake of essential cations like Ca<sup>2+</sup>. Na<sup>+</sup> and Ca<sup>2+</sup> are competing for the same apolipoprotein binding site. When essential ions like Na<sup>+</sup>, Ca<sup>2+</sup>, or SO<sub>4</sub><sup>2-</sup> become scarce, nutrient imbalances can cause stunted growth in the presence of salt. Other important molecules required for plant growth are inhibited by the presence of Cl<sup>-</sup>.

Based on our findings results *Astraglus armatus* germination and development, we can say that *Astraglus armatus* does not grow in saline soil.

## **Conclusion**

Our work has been undertaken to test the most favorable substrate for seed germination of *Astragalus armatus* our results show that:

- ✓ The monitoring of the germination kinetics revealed that it can be of variable duration depending on the chemical nature of the substrate ;
- ✓ The germination rate is very low in saline substratum;
- ✓ The maximum germination rate is observed in the lowest concentration 5%, of the gypsum substrate;
- ✓ The average germination duration and average daily germination rate, were the best in gypsum substratum for all concentrations combined;
- ✓ The length of stems and rootlets and their growth rate decreased in saline substrate and it is more pronounced in that of gypsum.
- ✓ The plant is strongest in gypsum substratum, but shows very weak resistance in limestone and salt substratums.

From this study, we can suggest that *Astragalus armatus* seeds show good germination in gypsum substratum, this has been confirmed by PCA.

These results open up many interesting research perspectives in physiology and ecology, it would be really interesting:

- Run other concentrations of these solutions to see the effect of excess concentrations on germination;
- Repeat these experiments to test the effect of other environments on the germination of *Astragalus armatus* seeds;
- Complement these results by measuring protein, sugar, and chlorophyll to better understand the impact of the environment on plant metabolism;
- Test other measurements, such as temperature and pH, to determine which of these *Astragalus armatus* seeds will germinate.

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