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THEME

Optimisation de la production de la sesbania par l'utilisatin de souches rhizobiennes adaptes aux sols saharien

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THEME

Optimization of sesbania production using rhizobial strains adapted to Saharan soils

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Dedication

we hereby dedicate this thesis to our esteemed families, whose unwavering support and encouragement have been instrumental in the successful completion of this work. We extend our deepest gratitude to our parents for their steadfast belief in our potentials and for providing the essential foundation upon which our academic endeavors were built.

List of Abbreviations

- M.C: media culture
- T.S: thermal stress
- S.S : salinity stress
- YEM: yeast extract mannitol
- Dpi: days post inoculation
- DO: density optic
- E.C : electrical conductivity
- B.T : biochar and peat

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Introduction

Sesbania is an economically important leguminous plant, especially in regions with marginal or degraded soils, where it plays multiple roles in sustainable agriculture. Its ability to fix atmospheric nitrogen through symbiotic relationships with rhizobial bacteria enhances soil fertility, reduces dependence on synthetic nitrogen fertilizers, and lowers input costs while promoting environmentally friendly farming practices. Beyond soil enrichment, *Sesbania* is recognized for its versatility in agroecosystems—commonly used as green manure, forage for livestock, and a biomass source for fuel and industrial applications.

In arid and semi-arid regions, particularly in **Saharan environments** marked by water scarcity, high temperatures, and salinity, Sesbania cultivation offers promising solutions. It improves **soil structure**, mitigates **desertification**, and contributes to both **food and fodder security**. Given the growing global emphasis on sustainable agriculture and the urgent need to combat **land degradation**, Sesbania provides a viable path to improving **the productivity of marginal lands** while supporting **rural livelihoods and local economies**.

However, the successful establishment and performance of Sesbania in these harsh environments depend heavily on **the efficiency and resilience of its rhizobial partners**. The selection and optimization of rhizobial strains capable of tolerating **Saharan stress conditions**—including **high salinity**, **drought**, and **thermal extremes**—is critical for maximizing biological nitrogen fixation and plant growth (Zahran, 1999; Hungria & Vargas, 2000). While some rhizobial strains may perform similarly under standard conditions, studies indicate **significant variation in their stress tolerance**, which can directly affect nodulation efficiency and Sesbania's biomass production in challenging climates (**Laranjo et al., 2014**).

The objective of this study is to evaluate the performance of various rhizobial strains under controlled stress conditions that mimic the challenges of Saharan soils. By subjecting these strains to different abiotic stress factors, this research seeks to identify the most resilient and effective strains for promoting optimal *Sesbania* growth. Ultimately, the findings will contribute to improving the cultivation of *Sesbania* in arid environments, with significant implications for sustainable agriculture and local economic development.

Chapter I: BIBLIOGRAPHICAL REVIEW

I.1. INTRODUCTION TO THE GENUS SESBANIA AND SESBANIA ACULEATA

The genus *Sesbania* is part of the legume family **Fabaceae**, subfamily **Faboideae**, and comprises approximately 60–70 species globally. These species are predominantly distributed in tropical and subtropical zones, where they play vital roles in agroecology and soil improvement due to their fast growth and nitrogen-fixing capabilities. Among these species, ***Sesbania aculeata*** (syn. *Sesbania bispinosa*) is of particular agronomic importance because of its adaptability, biomass productivity, and soil-enhancing properties. It has become a subject of growing interest in sustainable agriculture and environmental stress management systems.

I.1. Presentation of *Sesbania aculeata*

I.1.1. Geographic Distribution and Ecological Adaptation

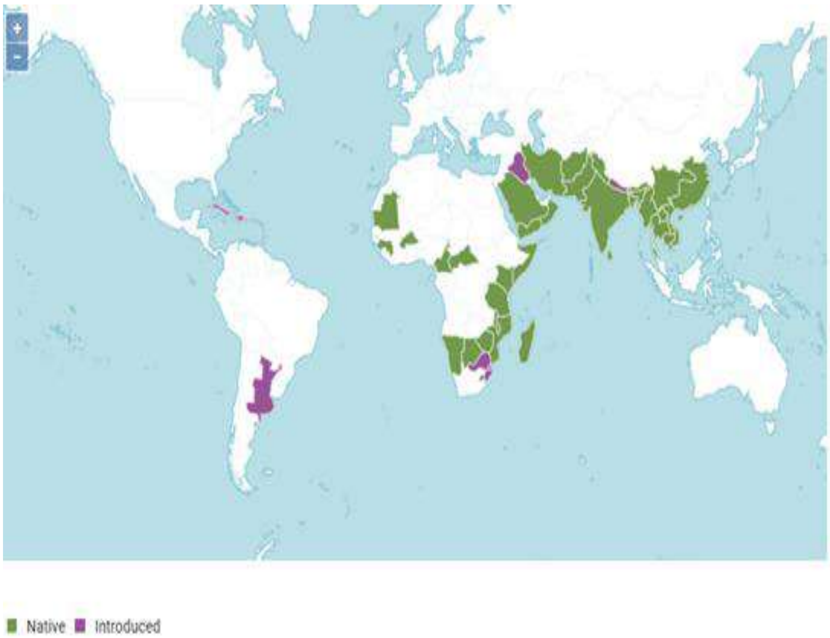

Figure 1 geographic distribution of sesbania aculeata

Originally native to South and Southeast Asia, Sesbania aculeata has been successfully introduced into various regions of Africa, including Algeria, as well as parts of the Middle East and South America. It thrives in tropical and subtropical environments and is particularly well adapted to arid and semi-arid zones (Larkem et al., 2017). Its tolerance to salinity, waterlogging, and poor soil fertility makes it a suitable species for marginal lands and for use in climate-resilient farming systems. Recent Algerian studies have confirmed its potential under Saharan saline conditions, with plants maintaining growth and physiological activity even at elevated salinity levels (Mokrane, 2021)

. I.1.2. Biology and Life Cycle

Sesbania has been characterized as a perennial woody shrub, but an annual at high latitudes (Duke, 1981). Munz (1973) terms it an annual in California. In U.S. agriculture, it is treated mainly as a summer annual legume (McLeod, 1982), and it grows rapidly during a 60- to 90-day summer growing period (Miller et *al*., 1989). *Sesbania bispinosa* is self-pollinating and requires no isolation for pure seed production. Flowers are mainly pollinated by bees, and ripe pods shatter to release the seeds.

I.1.3. Botanic Description

Sesbania aculeata is an annual herbaceous legume that grows rapidly and can reach a height of 1.5 to 3 meters. The stem is typically erect, ribbed, and may bear paired spines at the nodes, which is a distinguishing feature. Its leaves are pinnately compound, with 20–40 pairs of small linear to oblong leaflets. The plant produces yellow, papilionaceous flowers arranged in axillary racemes. Flowering is followed by the formation of long, slender pods that contain 20–30 seeds (Satnami & Yadava, 2012). Root systems are well developed, forming numerous nodules in association with symbiotic nitrogen-fixing bacteria, which contribute to soil fertility and reduce the need for synthetic nitrogen fertilizers.

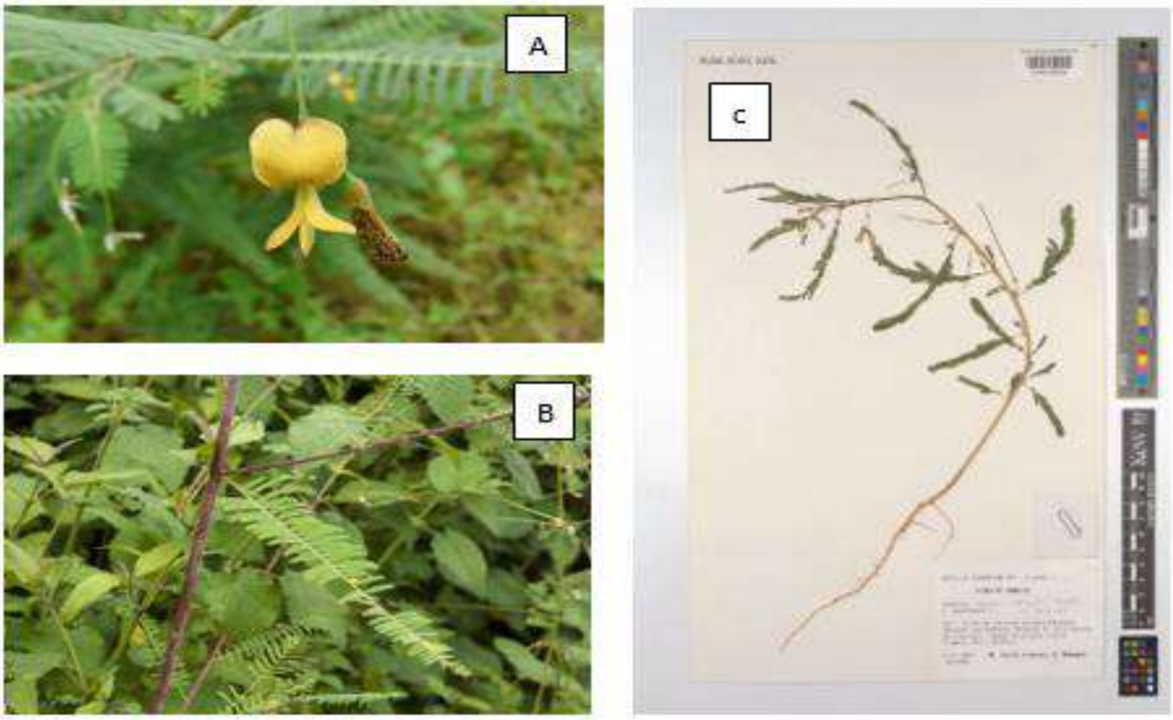

Figure 2 (A) = sesbania aculeata flower , (B)= sesbania aculeata stem and foliage , (c)= sesbania aculeata stem& foliage and roots

I.1.4. Systematics and Classification

Table 1classification of Sesbania aculeata

Taxonomic Rank	**Classification**
Kingdom	Plantae
Phylum (Division)	Tracheophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Genus	*Sesbania*
Species	*Sesbania aculeata*

Sesbania is a genus within the legume family **Fabaceae**, known for its ability to form symbiotic associations with **nitrogen-fixing bacteria**, particularly rhizobia. Species within this genus are valued for multiple agricultural and ecological uses, including **forage**, **fuelwood**, **soil fertility restoration**, and **traditional medicinal applications** (PROTA, 2016).

An estimated **50 to 60 species of *Sesbania*** have been described, primarily from **tropical and subtropical regions**, with a large number native to **Africa** (Onim & Dzowela, 2016). The name *Sesbania* is believed to be derived from the **Arabic name 'Siesaban'**, reflecting the plant's historical and regional significance.

Taxonomic synonyms for *Sesbania bispinosa* include numerous historical or misapplied names, such as *Coronilla aculeata*, *Emerus aculeatus*, *Sesban aculeatus*, *Sesbania aegyptiaca*, and *Sesbania bispinosa Steud.*. Valid synonyms also include *Sesbania bispinosa (Jacq.) Steud. ex Fawc. & Rendle*, depending on the regional flora or taxonomic authority (APNI, 2016; The Plant List, 2016).

I.1.5. Plant Ecology

Sesbania species are well adapted to wetlands, marshy areas, and poorly drained soils, often thriving in heavy clay soils with minimal land preparation. Under waterlogged conditions, the stem develops a spongy aerenchymatous parenchyma, enabling gas exchange and maintaining root respiration in anaerobic environments (Dreyfus & Dommergues, 1981; PROTA, 2016). The plant is commonly found at low to medium elevations, especially along streambanks, open wetlands, and rice paddies, where it often behaves as a volunteer or weedy species.

Sesbania also colonizes disturbed sandy soils, margins of waterways, and marshlands, indicating its ecological plasticity. It is distributed across a wide range of climatic zones, from subtropical moist and tropical dry forests to tropical moist forests (Onim & Dzowela, 2016). A unique physiological trait of Sesbania is nyctinasty—a movement behavior in which its leaves follow the sun during the day and fold at night, likely aiding in temperature regulation and reducing nighttime water loss (Kumar & Ponnuswami, 2015)

I.1.6. Uses of the Plant

Sesbania bispinosa (syn. *Sesbania aculeata*) is a multipurpose legume with a wide range of agricultural, industrial, and medicinal applications. Agriculturally, it is widely cultivated as **green manure** and **fodder**, contributing to soil fertility through **biological nitrogen fixation** and enhancing livestock productivity due to its high-protein foliage (Kumar et al., 2004; Singh et al., 2001). The plant also serves as **natural firewood** and is traditionally used for **intercropping** and **soil improvement** in rice-based cropping systems (Ahlawat et al., 2007).

Industrially, the **fibers of *S. bispinosa*** are comparable to those of birch trees, making them suitable for manufacturing **rope, sackcloth, sailcloth, and paper pulp** (Rai et al., 2012). In certain regions, its seeds have been used as **famine food**, and the plant's **natural gum** has demonstrated value as a **thickening and emulsifying agent** in food processing and other applications (PROTA, 2016).

From a medicinal standpoint, *S. bispinosa* has a long history of use in **traditional medicine**. Its **leaves and flowers** are used in **poultices** for treating **inflammation**, **skin infections**, and **tumors**, while seed preparations have been applied topically for **ringworm** and internally for **intestinal worms** (Prajapati et al., 2003). These therapeutic applications are largely attributed to its **astringent, antimicrobial, and anti-inflammatory** properties, making it a valuable resource in ethnomedicine across South Asia and Africa (Kirtikar & Basu, 1987).

I.1.7. Economic Value

Sesbania bispinosa fibres, known as ‘Dundee fibre’, have been used to construct ropes, fish nets, sackcloth and sailcloth (Orwa, 2009; PROTA, 2016). The fibres are regarded as very durable in water-related activities. Its fibres are also a source of paper fibre (Flore de La Réunion, 2016). Its seeds are a potential source of galactomannan gum, which is used as a thickening agent (Orwa et al., 2009; Useful Tropical Plants, 2016).The seed also contains about 53% protein, which can be used for the preparation of medium for culturing antibiotic-producing fungi such as Penicillium (Prasad, 1993)

.1.8. Cultivation and Agronomic Importance

Sesbania aculeata is widely cultivated as a **green manure crop**, particularly in **rice-based cropping systems**, where it is incorporated into the soil before planting to enhance organic matter and nitrogen content (Boparai et al., 1992). It is also grown as a **forage crop**, **fodder**, and for **biomass production**. The species is sown directly in the field under rainfed or irrigated conditions and grows well in **loamy to clay soils**, though it tolerates a range of soil types including saline and alkaline conditions (Singh & Gangaiah, 2001). In regions like the Algerian Sahara, *S. aculeata* has shown remarkable performance even in **saline soils**, maintaining shoot and root development while contributing to soil reclamation (Mokrane, 2021).

Additionally, its potential use extends to **aquaculture feed**, **bioremediation**, and **pharmaceutical applications**, as several studies have identified **bioactive flavonoids** with antioxidant and antimicrobial activity (Satnami & Yadava, 2012).

I.1.9. Pests and Diseases

Sesbania is a hardy leguminous plant that generally requires **minimal agronomic care** during its growth cycle. Although a variety of **insect pests** have been reported to infest the plant, the resulting damage is usually **minor and rarely economically significant** (ICAR, 2015; Kumar & Rathi, 2005). One of the notable pests is the **caterpillar of Azygophleps scalaris**, which bores into the **stems and roots**, consuming the **pith tissue** while leaving the epidermis intact. Affected plants become structurally weak and often collapse. The most effective management strategy involves **uprooting and destroying the infested plants** to prevent further spread (Singh et al., 1992).

Another common pest is the **leaf webber Striglina scitaria**, whose larvae **twist the terminal leaflets** and reside within them, feeding internally. A **spray of 0.1% BHC (Benzene Hexachloride)** or **Dieldrin** has historically been recommended for control, although these chemicals are now largely restricted or banned due to environmental concerns (CABI, 2020). Additional insect pests include **caterpillars of Thyposidra successaria and Amsacta moorei**, which also damage leaves and young stems but can be controlled with BHC or **DDT**, though more environmentally friendly alternatives are now advised (Kalaisekar et al., 2017).

Sesbania is also susceptible to **pod and seed predation** by various **beetles and bugs**, while **improperly dried seeds** are vulnerable to **fungal infection and weevil damage** during storage. **Cladosporium spp.**, a saprophytic fungus, may appear on aerial plant parts, but typically causes **only superficial infection** without major yield loss (Meena et al., 2018).

I.1.10. Environmental Impact

Sesbania is a good soil improver: fallen leaves, leftover stalks and roots add organic matter to the soil. The roots improve soil permeablility. It is useful for alkaline and saline soils remediation (Orwa et al., 2009; Qadir et al., 2002; NAS, 1980). Prickly sesban is a N-fixing legume and is often used as green manure in rice fields where it yields up to 12 t/ha (Arunin et al., 1987). Ploughing in foliage 60-70 days after planting and just before rice being planted out improves rice yield as much as an application of 80 kg - 150 kg N/ha (Orwa et al., 2009; Arunin et al., 1987).

II. SYMBIOTIC NITROGEN FIXATION

II.1. General Concepts :

II.1.1. Definition

Nitrogen fixation is the biochemical process through which atmospheric nitrogen gas (N_2)—which is inert and unavailable to most organisms—is converted into ammonia (NH_3), a biologically usable form that plants can assimilate for growth and development (Udvardi & Poole, 2013).

II.1.2. Physical Nitrogen Fixation

Of the total nitrogen fixed by natural processes, approximately **10% is contributed by non-biological (abiotic) mechanisms**, particularly through **atmospheric phenomena** such as **lightning, thunderstorms**, and **atmospheric pollution** (Moyes et al., 2016). During **lightning storms**, the intense heat and energy facilitate the combination of **gaseous nitrogen (N_2)** and **oxygen (O_2)** to form **nitric oxide (NO)**:

$$N_2 + O_2 \rightarrow 2NO \quad \text{(during lightning and thunderstorm)}$$

Subsequently, nitric oxide undergoes further oxidation to form **nitrogen dioxide (NO_2)**:

$$2NO + O_2 \rightarrow 2NO_2 \quad \text{(in the presence of atmospheric oxygen)}$$

These nitrogen oxides then dissolve in rainwater, reacting with water to form **nitrous acid (HNO_2)** and **nitric acid (HNO_3)**:

$$2NO_2 + H_2O \rightarrow HNO_2 + HNO_3$$

Once deposited on the soil surface with rain, these acids react with **alkaline soil minerals** (e.g., calcium and potassium salts) to produce **water-soluble nitrates (NO_3^-)** and **nitrites (NO_2^-)**:

$$HNO_3 + \text{Ca/K salts} \rightarrow \text{Ca/K nitrates}$$

These water-soluble forms of nitrogen are then **readily absorbed by plant roots**, contributing to the nitrogen nutrition of plants in ecosystems not supported by biological nitrogen fixation.

II.1.3Biological nitrogen fixation

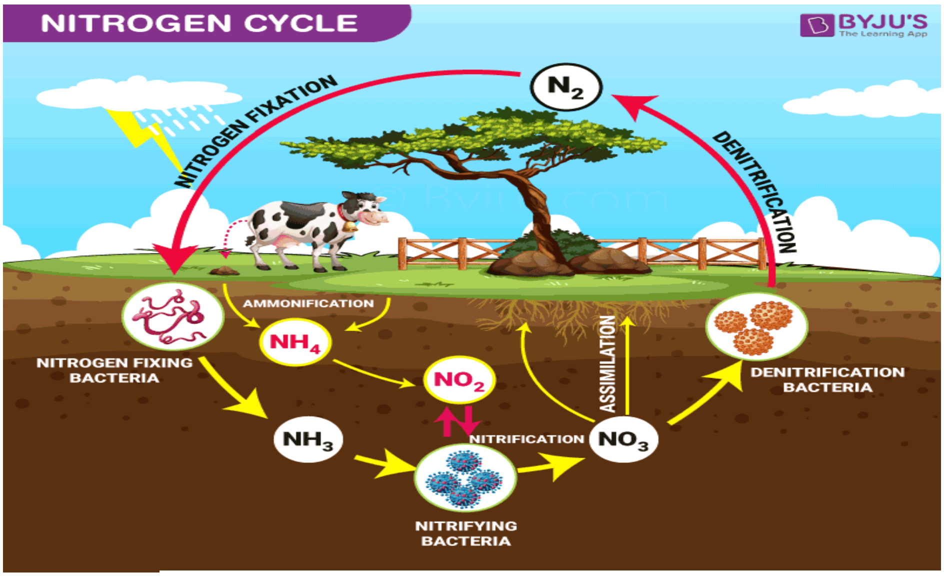

Figure 3 nitrogen fixation cycle

The **conversion of atmospheric nitrogen (N_2)** into plant-usable inorganic or organic forms by living organisms is known as **biological nitrogen fixation (BNF)**. This natural process plays a fundamental role in maintaining soil fertility and reducing the need for chemical nitrogen inputs in agriculture (Herridge et al., 2008). BNF is primarily performed by two major groups of microorganisms: those that exist independently in the soil (**asymbiotic or free-living**) and those that engage in **symbiotic associations** with plants, particularly legumes.

In addition to these, a third category, known as **associative symbiotic nitrogen fixation**, has been recognized. This involves microorganisms that form loose but effective associations with the **roots of cereals and grasses**, such as sugarcane and maize, without forming specialized nodules (James, 2000; Baldani et al., 2014).

II.1.4 Asymbiotic Nitrogen Fixation:

Several types of **free-living bacteria and cyanobacteria** are capable of fixing atmospheric nitrogen into usable forms:

- **Aerobic nitrogen-fixing bacteria**, such as *Azotobacter*, *Beijerinckia*, and *Derxia*, function in oxygen-rich environments.
- **Anaerobic nitrogen-fixing bacteria**, like *Clostridium pasteurianum* and *Bacillus polymyxa*, operate under oxygen-free conditions.
- **Photoautotrophic nitrogen-fixing bacteria**, such as *Chlorobium*, *Rhodopseudomonas*, and *Rhodospirillum*, use light energy and fix nitrogen in aquatic or flooded soils.
- **Chemosynthetic nitrogen-fixing bacteria**, like *Desulfovibrio*, utilize chemical energy from inorganic compounds for nitrogen fixation.
- **Nitrogen-fixing cyanobacteria**, particularly heterocyst-forming types like *Nostoc*, *Anabaena*, *Trichodesmium*, *Calothrix*, and *Cylindrospermum*, contribute significantly to nitrogen fixation in aquatic and agricultural systems. For example, *Aulosira fertilissima* is active in rice paddies, while *Cylindrospermum* is known to fix nitrogen in sugarcane and maize fields (Roger & Ladha, 1992; Rai et al., 2000).

These free-living organisms represent a crucial component of nitrogen cycling, particularly in **non-legume cropping systems** and **low-input farming environments**.

II.1.5 Symbiotic biological nitrogen fixation

Sesbania aculeata forms a mutualistic association with nitrogen-fixing bacteria—primarily **Rhizobium** and **Sinorhizobium/Sinorhizobium saheli**—that colonize root nodules and occasionally stem nodules. These symbionts convert atmospheric N_2 into ammonia via the nitrogenase enzyme, supplying the plant with essential nitrogen and significantly enhancing soil fertility (Akter Najmun et al., 2016).

II.2. Benefits of Nitrogen-Fixing Legumes

Global data indicate that, on average, the **symbiotic relationship between legumes and rhizobia** is responsible for fixing approximately **30–40 kg of nitrogen (N)** per tonne of shoot **dry matter** produced, considering the total plant biomass (both shoots and nodulated roots) (Peoples et al., 2009). Therefore, factors that directly influence **legume growth**—such as **water and nutrient availability**, **pest and disease pressure**—become the primary determinants of total **N_2 fixation** in cropping systems.

In addition, agronomic practices that **reduce the presence or effectiveness of rhizobia** in the soil—such as **lack of inoculation**, **poor-quality inoculants**, or **excessive nitrogen fertilization**—can limit BNF. Practices like **frequent tillage**, **long fallows**, or **intercropping legumes with cereals** (which compete for available nitrogen) can also inhibit symbiotic efficiency (Giller, 2001; Herridge et al., 2008).

Although a significant proportion of the nitrogen fixed by legumes is removed at **harvest**, particularly in the form of **protein-rich seeds**, the **inclusion of legumes in crop rotations** generally enhances the productivity of subsequent crops. This improvement is not solely due to residual soil nitrogen, but also linked to **non-nitrogen benefits**, such as changes in **soil biological activity**. Recent findings suggest that **molecular hydrogen (H_2)**—a by-product of nitrogenase activity in nodules—may influence **soil microbial communities** and contribute to improved crop responses in legume-based rotations (Dong et al., 2003; Peoples et al., 2009).

III -Rhizobial Strains and Stress Factors

III.1. Diversity and Functionality of Rhizobia

III.1.1. Phylogenetic Classification of Rhizobia:

Rhizobia are a paraphyletic group within the classes Alphaproteobacteria and Betaproteobacteria. They are primarily categorized into several genera, including *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Azorhizobium*, and *Sinorhizobium* (also known as *Ensifer*). Phylogenetic analyses, particularly those based on 16S rRNA gene sequences, have been instrumental in delineating these genera and understanding their evolutionary relationships.

Rhizobia are very diverse bacteria that have been identified in distant phylogenetically branches, interspersed with non rhizobial bacterium species (Remigi, Zhu, Young, & Masson-Boivin, 2016). So far, up to 18 different rhizobium genera and hundreds of species have been described among α- and β-proteobacteria, thus now referred as α- and β-rhizobia. The rhizobium genetic diversity is reflected by the diversity of molecular mechanisms these bacteria use during the symbiotic process. Comparative genomic studies of rhizobium symbiotic genes indeed do not support a unique shared genetic strategy for symbiosis in rhizobia (Amadou et *al.*, 2008, Tian et *al.*, 2012). Instead various functions, whose phylogenetic distribution ranges from ubiquitous to lineage- or even strain-specific, have been recruited for symbiosis.

III.1.2 Morphological Characteristics:

Rhizobia, or root nodule bacteria, are medium-sized rod-shaped cells, measuring 0.5 to 0.9 µm in width and 1.2 to 3.0 µm in length. They do not form endospores, are Gram-negative, and are motile by means of a single polar flagellum or two to six peritrichous flagella. Uneven Gram staining is frequently observed in Rhizobium depending on the age of the culture. Cells from young cultures and nodule-derived bacteroids typically show uniform Gram staining, whereas older and elongated cells often exhibit a banded appearance with unstained zones. These unstained zones have been identified as large granules of the polymeric acid poly-β-hydroxybutyrate (PHBA). PHBA appears refringent under phase-contrast microscopy (Hoben, 2012).

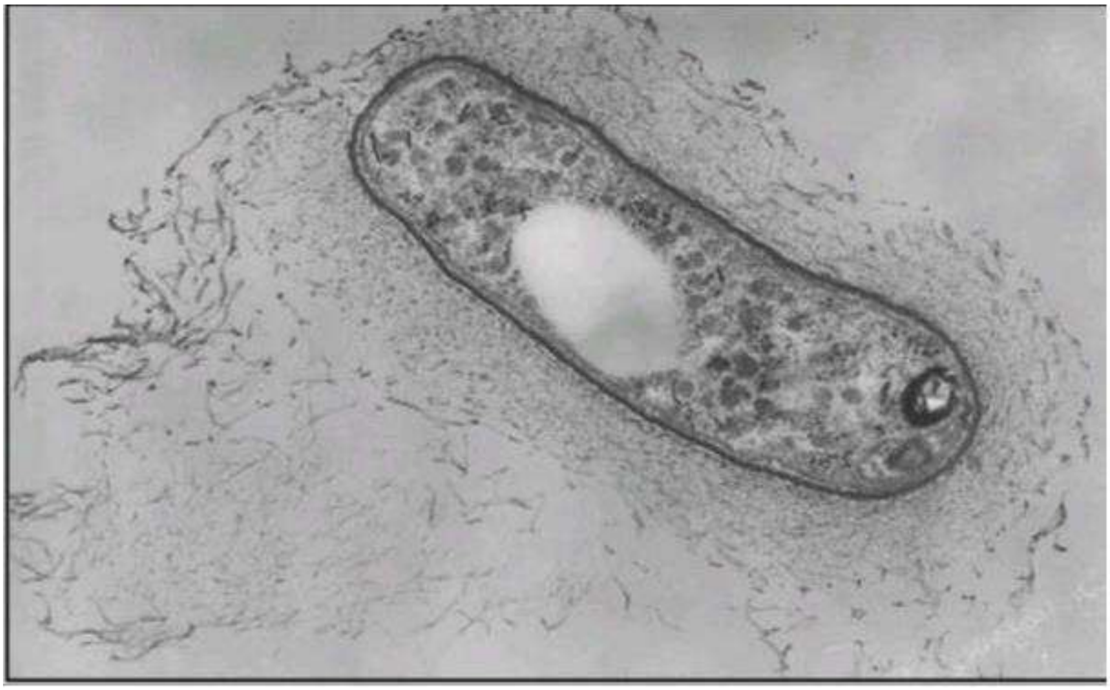

Figure 4 microscopical view of rhizobium trifolii by(Vincent,1982).

III.1.3 Symbiotic specificity of rhizobial strains

The specificity between rhizobia and legume hosts is controlled by molecular signaling, particularly flavonoid–Nod factor interactions. This specificity determines the efficiency and success of the symbiosis and varies widely among both bacterial strains and host plants (Oldroyd & Downie, 2008; Gage, 2004).

III.1.4 Molecular Mechanisms Underlying Symbiotic Specificity

The establishment of a successful symbiosis between legumes and rhizobia involves several key molecular interactions:

Flavonoids and isoflavonoids are components of the plant root exudates. Rhizobia express a transcriptional factor called NodD, which is activated by the specific flavonoid secreted by the compatible legume (Broughton *et al*., 2000;

Chen *et al*., 2005; Peck *et al*., 2006). Different rhizobial species respond to different flavonoids. Often, rhizobia contain multiple *nodD* copies that encode different NodD isoforms (Ferguson *et al*., 2020). These isoforms may perform divergent roles, may be involved in distinct stages during symbiotic infection, or may be required under different environmental conditions, and have been described to enhance nodulation competitiveness or to extend symbiotic host range through perception of different plant signalling molecules (Demont *et al*., 1994; del Cerro *et al*., 2015, 2017, 2020; Kelly *et al*., 2018; Acosta-Jurado *et al*., 2019; Ferguson *et al*., 2020).

Once activated, NodD induces the expression of bacterial genes involved in the synthesis of the so-called Nod factors (Broughton *et al*., 2000). Nod factors are lipochito-oligosaccharides with different substituents that constitute a characteristic molecule which will be recognized by specific receptors (LysM receptor-like kinases) on the root of the compatible legume (D'Haeze and Holsters, 2002; Madsen *et al*., 2003; Radutoiu *et al*., 2003; Smit *et al*., 2007).

Once the legume recognizes the specific Nod factors, a cascade of signals is initiated in the plant tissue, promoting the physiological, morphological and molecular changes that characterize the nodulation process. The molecular signal cascade induced in the plant by the specific Nod factor recognition has been extensively revised (Ferguson *et al*., 2010; Oldroyd *et al*., 2011; Liu *et al*., 2018; Tang and Capela, 2020).

III.1.5Genetic Determinants of Host Range in Rhizobia

The host range of rhizobia is largely determined by their symbiotic plasmids, which encode key nodulation (nod) and nitrogen-fixation (nif) genes. Differences in these genetic factors explain why some rhizobial strains nodulate specific legumes more effectively than others (Perret et al., 2000; Masson-Boivin et al., 2009).

- **Nod Gene Clusters**: The presence and structural organization of **nodulation (nod) gene clusters** in rhizobia are critical determinants of **host specificity**. The conserved **nodABC** genes encode enzymes essential for synthesizing the **Nod factor backbone**, a signaling molecule required for initiating symbiosis. Additional accessory nod genes—such as **nodX**, **nodZ**, or **nodH**—modify the Nod factor, thereby influencing the range of compatible legume hosts (Perret et al., 2000; Masson-Boivin et al., 2009).

- **Horizontal Gene Transfer**: Rhizobia can expand their **symbiotic capabilities** through **horizontal gene transfer (HGT)**. This includes acquiring symbiotic

plasmids, **genomic islands**, or **integrative conjugative elements** that carry key **nodulation (nod)** and **nitrogen fixation (nif/fix)** genes. HGT can lead to significant changes in host compatibility, allowing previously incompatible rhizobial strains to nodulate new legume species (Sullivan & Ronson, 1998; Marchetti et al., 2010).

- **Secreted Effector Proteins**: Some rhizobia employ **type III secretion systems (T3SS)** to deliver **effector proteins** directly into host cells. These effectors can suppress **plant innate immune responses** or reprogram signaling pathways to facilitate infection and **nodule formation**. The diversity and specificity of these effectors among rhizobial strains contribute to **differential symbiotic outcomes** across legume species (Marie et al., 2001; Okazaki et al., 2013).

III.1.6Characteristics of Effective Strains

Effective rhizobial strains exhibit several key characteristics that enhance their symbiotic relationship with leguminous plants, contributing to improved plant growth and soil fertility. One of the most critical traits is **high nitrogen fixation efficiency**, which enables these bacteria to convert atmospheric nitrogen (N_2) into plant-usable forms such as ammonium, thereby reducing the need for chemical nitrogen fertilizers (Hungria & Vargas, 2000). Additionally, effective strains demonstrate strong **competitiveness**, meaning they can outcompete indigenous soil rhizobia to colonize legume roots and form nodules, which ensures effective nitrogen fixation even in soils with established microbial populations (Sessitsch et al., 2002).

Adaptability to **abiotic stress** is another essential characteristic. Some rhizobial strains can endure extreme conditions such as **high salinity**, **drought**, and **acidic soils**, while still maintaining their symbiotic effectiveness (Zahran, 1999). The **soil pH**, in particular, plays a pivotal role in influencing nodulation and nitrogen fixation, as it affects the availability of nutrients and microbial activity in the rhizosphere (Giller, 2001). Moreover, certain rhizobia produce **phytohormones**, such as **indole-3-acetic acid (IAA)**, which enhance root elongation and development, thereby facilitating improved plant–microbe interaction and nutrient uptake (Liu et al., 2016). These multifaceted traits make efficient rhizobial strains valuable allies in sustainable agriculture, where they improve crop productivity and soil health while minimizing the reliance on synthetic inputs..

III.1.7 Indigenous vs. Commercial Strains

In agricultural practices, the choice between **indigenous** and **commercial rhizobial strains** for legume inoculation significantly affects crop productivity and soil health. Indigenous rhizobia, which naturally inhabit local soils, are often better adapted to specific environmental conditions such as **soil pH**, **temperature fluctuations**, and **moisture availability** (Thies et al., 1991; Giller, 2001). This environmental adaptability allows for more effective symbiotic relationships with host plants, enhancing **biological nitrogen fixation** and promoting robust plant growth, especially under marginal conditions (Howieson & Ballard, 2004).

The decision between indigenous and commercial strains should consider **local edaphic conditions**, the **physiological demands** of the cultivated crop, and the intended **agricultural outcomes**. To improve inoculation efficiency and nitrogen fixation, several strategies are recommended

- Selecting **highly competitive and efficient strains** through genomic and phenotypic screening (Sessitsch et al., 2002).
- Co-inoculating with **plant growth-promoting rhizobacteria (PGPR)** to improve establishment and nutrient uptake (Vessey, 2003).
- **Optimizing soil parameters**, such as pH and micronutrient levels, to support symbiosis (Giller, 2001).
- Developing or selecting **stress-tolerant strains** capable of nodulation and nitrogen fixation under abiotic stress (Zahran, 1999).

In some cases, **native populations of rhizobia** may be insufficient in number or lack the necessary symbiotic traits, requiring supplementation with **well-adapted commercial inoculants** (Herridge et al., 2008). Consequently, a **comprehensive understanding of the native soil microbiota** and agroclimatic context is essential for designing optimal inoculation strategies. Tailoring the approach to **leverage the strengths of local strains**—possibly in combination with commercial formulations—offers a sustainable pathway to maximizing legume productivity and biological nitrogen fixation.

III.2. Interaction of Rhizobia and Sesbania

The symbiotic relationship between **rhizobia** and *Sesbania* plays a crucial role in **biological nitrogen fixation (BNF)**, significantly contributing to **soil fertility** and **plant productivity** in tropical and subtropical agroecosystems. *Sesbania*, a fast-growing leguminous genus, establishes a symbiotic association with specific **rhizobial strains**, leading to the formation of **nodules** on either roots or stems. Inside these nodules, rhizobia reduce atmospheric nitrogen (N_2) into ammonia (NH_3), a plant-available form of nitrogen, thereby benefiting both the host plant and the surrounding soil ecosystem (Beck et al., 1993; Dakora & Keya, 1997).

This symbiotic interaction is influenced by numerous factors, including **soil characteristics**, **environmental stressors**, and the **genetic compatibility** between the host and microbial symbiont. Some species, such as *Sesbania rostrata*, are particularly remarkable for their ability to develop **both root and stem nodules**, enabling enhanced nitrogen fixation even under **flooded or poorly aerated conditions** (Dreyfus & Dommergues, 1981). The presence and expression of key **nodulation (nod)** and **nitrogen fixation (nif)** genes in rhizobia are essential for a successful interaction, ensuring optimal nutrient exchange and nodule functionality (Perret et al., 2000; Masson-Boivin et al., 2009).

III.3. Nodulation Process of *Sesbania aculeata*

III.3.1. Root Nodule Formation

Nodulation is a key biological process in leguminous plants, enabling them to form a symbiotic association with nitrogen-fixing bacteria known as rhizobia. This process plays a fundamental role in sustainable agriculture by enhancing soil fertility through biological nitrogen fixation. Sesbania aculeata, a fast-growing legume widely cultivated in arid and semi-arid regions, is particularly known for its ability to establish effective nodulation under diverse environmental conditions, including saline and nutrient-poor soils (Zahran, 1999; Lupwayi & Haque, 1999). Experimental studies have shown that S. aculeata forms both root and, under some conditions, stem nodules, making it an ideal green manure crop for restoring soil fertility and supporting crop rotations in degraded landscapes (Beck et al., 1993; Hungria & Vargas, 2000).

In *Sesbania aculeata*, nodulation occurs primarily in the roots, where compatible rhizobial strains initiate infection by recognizing flavonoid signals released by the host plant. This leads to the formation of infection threads and cortical cell divisions that culminate in nodule development (Gage, 2004; Oldroyd & Downie, 2008). The nodules house nitrogen-fixing bacteria that convert atmospheric nitrogen into ammonia, which the plant can assimilate for growth. Although root nodulation is most common in *S. aculeata*, under certain conditions, stem nodules may also form, especially when exposed to flooding or waterlogged environments (Beck et al., 1993; Zahran, 1999). This dual nodulation capacity enhances its effectiveness as a green manure crop in diverse agroecological zones.

III.3.2. Development of Nodule

Immediately following the release of rhizobia into the cytoplasm of host cortical cells, the plant undergoes **hyperplasia**, a rapid and localized increase in cell division in the nodule primordium. Within these cortical cells, rhizobia differentiate into **bacteroids**, enlarged and functionally specialized forms capable of nitrogen fixation (Oldroyd & Downie, 2008; Udvardi & Poole, 2013). This cellular reprogramming results in the formation of a **tumor-like nodule structure**, densely packed with bacteroid-containing cells. As part of the symbiotic adaptation, many of the host cells in the nodule exhibit **endoreduplication**, a process where the chromosome number increases without mitotic division, resulting in **polyploidy** (Cebolla et al., 1999; Suzaki et al., 2014). This phenomenon is observed across both **diploid and polyploid legumes**, and is believed to enhance the metabolic capacity of infected cells to support the high energy demand of nitrogen fixation..

III.3.3 Root Nodule process :

When soil nitrogen levels are low, leguminous plants initiate root nodule development to establish symbiosis with nitrogen-fixing rhizobia. This interaction is tightly regulated by bidirectional **cell signaling** between the host plant and rhizobia (Oldroyd & Downie, 2008; Gage, 2004). The process of nodulation involves the following key stages:

1. The roots of *Sesbania aculeata* secrete **flavonoids** into the rhizosphere, which act as chemoattractants to rhizobia, promoting their accumulation near the root hairs (Hassan & Mathesius, 2012).
2. In response, rhizobia produce **nodulation factors (Nod factors)**, which are lipochitooligosaccharide signals that cause **root hair curling**, a critical early event in infection (Spaink, 2000).

3. Nod factors also trigger various physiological changes in the host, such as **membrane depolarization**, **cytoskeletal reorganization**, and **calcium spiking**, all of which support nodule organogenesis (Oldroyd & Downie, 2006).
4. Nod factor receptors located on the **plasma membrane** of root hairs recognize these signals, initiating the formation of **infection threads**, which serve as conduits for rhizobial entry. Rhizobia can also occasionally enter through cracks or wounds in the epidermis (Gage, 2004).
5. The infection thread penetrates deeper into the root tissue, delivering the rhizobia into **epidermal and cortical cells**.
6. Within cortical cells, rhizobia are surrounded by plant-derived membranes, forming a structure known as a **symbiosome**, where they are compartmentalized and protected (Jones et al., 2007).
7. Rhizobia stimulate **nodule formation** through signaling cascades involving calcium-calmodulin-dependent kinases and **cytokinin**, a hormone that induces cortical cell division (Murray et al., 2007; Tirichine et al., 2007).
8. Inside the nodules, the bacteria differentiate into **nitrogen-fixing bacteroids**, while the plant develops **vascular tissues** to facilitate nutrient exchange between the two partners (Udvardi & Poole, 2013).

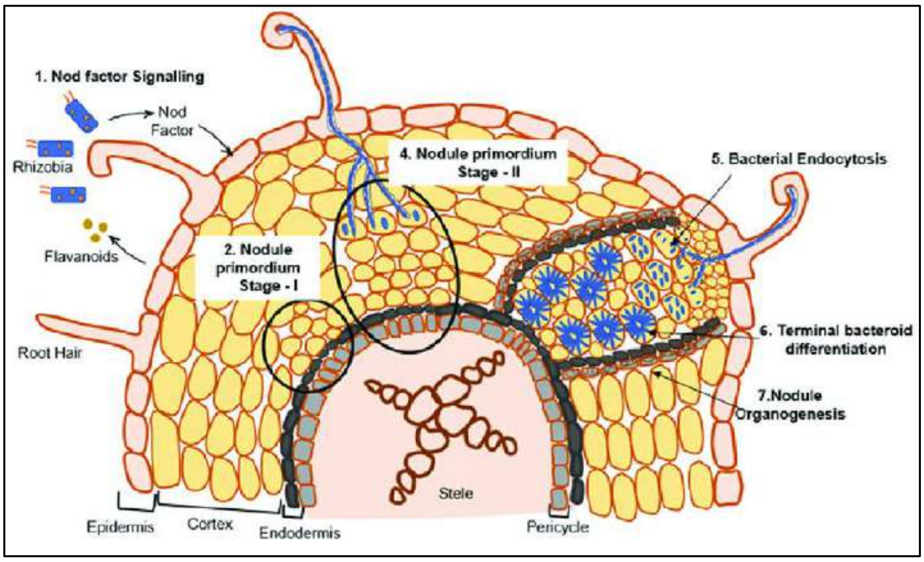

Figure 5 infection thread foration

III.3.4 Structure of root nodule

The formation of root nodules is initiated by the action of **rhizobial signaling molecules**—notably Nod factors—and is supported by plant hormone-mediated responses, particularly **cytokinesis regulated by cytokinin and auxin pathways** (Murray et al., 2007; Suzaki et al., 2014). In mature nodules, the central **bacteroid zone** is the site of active nitrogen fixation and is surrounded by multiple layers of plant-derived **cortical cells**. Within this zone, rhizobia differentiate into **bacteroids**, which are housed either individually or in groups within **symbiosomes**—plant-derived vesicles enclosed by **peribacteroid membranes** (Udvardi & Poole, 2013).

Effective nodules are typically **large and pink** in color due to the presence of **leghaemoglobin**, an oxygen-binding molecule that facilitates efficient respiration for nitrogen fixation while maintaining low free oxygen levels to protect nitrogenase (Ott et al., 2005). As nodules **senesce**, they degrade and release **stationary-phase rhizobia** back into the soil, where they can potentially reinfect host roots or persist in the rhizosphere until conditions become favorable again (Puppo et al., 2005).

III.3.5 Function of the nodule bacteroids

According to isotopic labeling and metabolic studies, **bacteroids**—the differentiated forms of rhizobia inside legume nodules—serve as the primary sites of **biological nitrogen fixation** (Kouchi & Yoneyama, 1984; Udvardi & Poole, 2013). Unlike free-living rhizobia, bacteroids lose the ability to catabolize sugars efficiently and instead focus on producing **ammonium ions (NH_4^+)**, which are subsequently assimilated by host plant cells via the enzyme **glutamine synthetase** into organic nitrogen compounds (Vance, 2001).

This interaction exemplifies a true **mutualistic symbiosis**, where both partners contribute distinct but complementary metabolic functions. A key player in this process is **leghaemoglobin**, a red oxygen-binding hemoprotein that is structurally similar to animal hemoglobin. It is localized in the cytoplasm between bacteroids and the **peribacteroid membrane**, providing a **low-oxygen microenvironment** essential for protecting the oxygen-sensitive **nitrogenase** enzyme (Ott et al., 2005; Appleby, 1984).

Leghaemoglobin consists of a **haeme group** synthesized by the bacterial partner and a **globin peptide** encoded by the plant's nuclear genome, reflecting the integrated biosynthetic cooperation between the two symbionts (Jacobsen-Lyon et al., 1995). The pigment not only enhances nitrogen fixation efficiency by buffering free oxygen but also stands as a **distinct molecular hallmark** of a successful rhizobium–legume symbiosis. In this context, **effectiveness** refers to the plant-microbe association's capacity to fix and assimilate atmospheric nitrogen under functional nodule conditions (Herridge et al., 2008).

IV. IMPACT OF ENVIRONMENTAL STRESS ON NODULATION AND RHIZOBIA

Nodulation is a **highly sensitive and regulated process**, and its success is significantly influenced by a range of **abiotic and biotic stress factors**. Environmental conditions such as **drought**, **salinity**, **extreme temperatures**, and **nutrient deficiencies** can severely impair the initiation, development, and functionality of root nodules (Zahran, 1999; Hungria & Vargas, 2000). Biotic stresses, including **pathogen attacks** or **competition with native soil microbes**, can also disrupt the delicate balance required for successful **rhizobium–legume symbiosis** (Sessitsch et al., 2002).

Understanding how these stressors affect nodulation is critical for enhancing the **resilience and productivity** of legume-based cropping systems, particularly in **arid, semi-arid**, and **marginal soils** where such stresses are prevalent. Developing or selecting rhizobial strains with improved **stress tolerance** and maintaining appropriate soil health can significantly improve **symbiotic nitrogen fixation** and, by extension, **crop yield and soil fertility** in challenging environments (Laranjo et al., 2014; Liu et al., 2011).

IV.1. Definition of Stress

Environmental stress refers to any external abiotic or biotic factor that adversely affects an organism's **growth**, **development**, **reproduction**, or **survival**. In plants, such stresses can disrupt **physiological** and **biochemical processes**, resulting in reduced productivity and posing significant challenges to **adaptation and resilience** (Mittler, 2006).

According to Levitt (1980), environmental stress is classified into two main categories:

IV.2. Abiotic Stress

Abiotic stress is any environmental factor that imposes a negative effect on plant growth and productivity by altering cellular homeostasis, leading to physiological and metabolic disruptions." (Zhu, 2016, Annual Review of Plant Biology)

Caused by non-living environmental factors such as:

- **Drought stress**: Water deficiency
- **Salinity stress**: High salt concentrations
- **Temperature stress**: Extreme heat or cold
- **Heavy metal stress**: Toxic metal accumulation
- **Nutrient deficiency**: Lack of essential nutrients

IV.3Biotic Stress : is the damage occur to plants Resulting from living organisms such as:

- **Pathogens and pests**: Bacterial, fungal, or viral infections
- **Competition with other soil microbes**: Presence of non-beneficial microbes

IV.3 How does the environmental stressors affect nodulation ?

Environmental stressors, particularly **drought**, exert a substantial influence on the process of **nodulation** in leguminous plants, compromising both the initiation and functionality of root nodules. Drought stress has been widely reported to reduce **nodule number**, **biomass**, and **nitrogenase activity**, ultimately leading to decreased biological nitrogen fixation (Sinclair et al., 2007; Zahran, 1999). In a study investigating the effects of water availability on nodulation, plants subjected to **moderate and severe drought** showed significant reductions in both **nodule quantity and dry weight**, resulting in lower nitrogen fixation efficiency (Gil-Quintana et al., 2013).

In addition to drought, environmental stressors such as **salinity** and **extreme temperatures** also adversely affect the **legume–rhizobia symbiosis**, disrupting nodulation and reducing the activity of the **nitrogenase enzyme** responsible for nitrogen fixation (Laranjo et al., 2014). These stress-induced limitations on BNF can significantly impair **plant growth**, **biomass accumulation**, and **crop yield**, particularly in arid and semi-arid regions where legumes play a key role in sustainable agriculture (Hungria & Vargas, 2000).

Chapter II: Materials and methods

II- Materials and methods

II-1 Objective

The primary objective of this study is to evaluate the efficiency of different rhizobial strains in promoting **Sesbania** growth under controlled conditions. Specifically, this study aims to:

1. 1-Characterization of rhizobia strains associated to *Sesbania aculeata*
2. **Compare the performance of different rhizobial strains** to identify the most efficient symbiotic associations.
3. **Examine the direct influence of rhizobial inoculation** on Sesbania root architecture and biomass accumulation, and nutrient uptake efficiency in a controlled, microbe-free environment.

II -2 Material

II-2-1 Plant Material:

In this study, *Sesbania aculeata* seeds from the 2023 production were used as the plant material. **Biological material** (Root nodules) were collected from three different stations, including the ITDAS and Chott stations (Ouargla) , with two nodules sampled from each plant at each location. The collected nodules were surface disinfected and sterilized to ensure aseptic conditions for the isolation and purification of rhizobial strains, which were subsequently evaluated for their effectiveness under arid environmental conditions.

photo 1 sesbania seeds

II-3 Methods

II-.3.1 Chemical Analysis of Electrical Conductivity (EC) and pH

To determine the electrical conductivity (EC) and pH of the sand, representative samples were first sieved to remove residual debris and impurities.A 20 g portion of the sieved sand was then placed into a small bottle, followed by the addition of 200 mL of distilled water. The mixture was subjected to rotary agitation for 30 minutes to ensure thorough homogenization. As discribed by Aubert, G. (1957)

After agitation, the mixture was filtered to separate the sand from the filtrate. The pH of the filtrate was then measured using a pH meter, while the electrical conductivity (EC) was determined using a conductivity meter.

photo 3 sand filtration

photo 2 rotation agitator

II-3-2 Isolation of Rhizobial strains from *Sesbania aculeata*

The selection of rhizobial strains was carried out through **isolation (Somasegaran & Hoben, 1994),** cultivation, and characterization using a selective culture medium. The process involved the following steps:

1. Preparation of YEM Culture Medium

The **Yeast Extract Mannitol (YEM)** medium was prepared to support the growth of rhizobial strains. The medium contained:

Table 2 m.c yem composition

ingredients	Importance	Quantity
Mannitol	primary carbon source.	10g
Yeast extract	provides essential growth factors, vitamins, and nitrogen compounds	0.5g
K_2HPO_4	serves as a buffering agent	0.5g
$MgSO_4 \cdot 7H_2O$	supplies magnesium for enzymatic activities	0.2 g
NaCl	maintains osmotic balance	0.1 g
Agar	solidifying agent	15g

Figure 6 media culture preparation steps

After weighing the exact amount of each component and mixed into a 500 ml of distillated water on a heated magnetic stirrer complete with distillated water until reaching 1L then Cover the mouth of the Erlenmeyer flask using an aluminum paper then let it boil for 15 minutes with heating and stirring . The medium was poured into a sterilized vial in a sterile environment then destinated to autoclaving at 121°C for 20 minutes for the purpose of sterilization , then poured into sterile Petri dishes and allowed to solidify under aseptic conditions.

2-Nodules pretreatment

Nodules were pretreated as described by Somasegaran and Hoben (1994).

Initial Cleaning

- Nodules were washed with sterile distilled water to remove adhering soil and debris.

b) Surface Sterilization

- Immersion in 70% ethanol for a short period in a aseptic condition in a microbiological laminar flow .
- Treatment with diluted bleach solution to eliminate external contaminants.
- Multiple rinses with sterile distilled water to remove disinfectant residues.

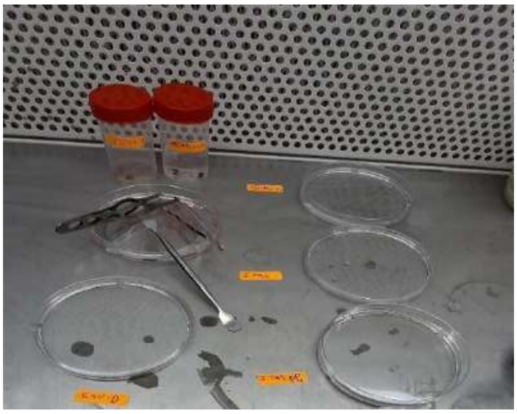

photo 4 nodules pretreatment

3. Streaking Technique for Isolation

To ensure the successful isolation of rhizobial strains, the streak plate method was employed. This technique allows for the separation of individual bacterial colonies from a mixed sample. The procedure involved the following steps :

Figure 6 isolation of bacteria using quadrant streaking method

a) Inoculum Preparation

- The surface-sterilized nodules were crushed in sterile distilled water to release the bacteria.
- A sterile loop was used to collect a small amount of the nodule suspension for streaking.

b) Quadrant Streaking Method

- A sterile inoculating loop was dipped into the bacterial suspension and used to streak the first quadrant of the YEM agar plate in a zigzag pattern.

- The loop was then sterilized by flaming in a Bunsen burner and allowed to cool.
- A small portion of the first streak was dragged into the second quadrant using the sterilized loop, creating a dilution effect.
- This process was repeated for the third and fourth quadrants, ensuring a gradual reduction in bacterial density.

c) **Incubation**

- The inoculated plates were **incubated at 28°C for 3–5 days** under controlled conditions.

Rhizobial colonies were identified based on **morphological characteristics** (white, circular, mucus-producing, and slightly raised).

4- Purification and Storage

Selected colonies were subcultured onto fresh YEM plates using the same streaking technique to ensure purity. Once pure strains were obtained, they were transferred to YEM agar slants for short-term storage at 4°C.

II- Eco-physiological characteristics test of the isolates

To evaluate the efficiency and resilience of the isolated rhizobial strains, different abiotic stress factors were applied, including drought stress, salinity stress, and thermal stress. Each stress condition was imposed using controlled laboratory techniques to simulate extreme environmental conditions typical of arid regions (Somasegaran & Hoben, 1994).

1. pH stress test

To evaluate the impact of pH stress on Rhizobium bacteria associated with Sesbania plants, different pH levels were tested:

- The bacteria were grown in YEM medium adjusted to pH levels 4, 5, 6, 7, 8, and 9.
- The pH of the medium was modified using HCl for acidic conditions and NaOH for alkaline conditions.
- Bacterial response was monitored by observing growth, changes in colony appearance, and overall adaptation at each pH level

2. Salt stress test

Salinity stress was imposed by supplementing the growth medium with sodium chloride (NaCl) at different concentrations:

- YEM agar was prepared with increasing NaCl levels (0.5 g/250ml, 1g/250ml, 1.5g/250ml, 2g/250ml.).
- Strains were inoculated and incubated to determine their ability to grow under saline conditions..

3. Thermal stress test

To evaluate the impact of temperature fluctuations on rhizobial strains, different incubation temperatures were tested:

- Strains were grown in YEM agar at low (0°C, 15°C), medium (28°C), and high temperatures (45°C).
- The survival and adaptation of the strains were analyzed by measuring growth rates, colony morphology changes, and biochemical responses.

II-3-4 **Physiological characteristics test of the isolates**

To investigate the influence of different sugars, starch, and amino acids on the growth of rhizobial strains by supplementing both liquid and solid yeast extract mannitol (YEM) media, and to evaluate bacterial growth quantitatively (optical density) and qualitatively (colony morphology) (Somasegaran & Hoben, 1994).

1. Preparation of Culture Media

1.1. Components Used

- **Carbon sources** (added at 1% w/v):
    - Glucose
    - Fructose
    - Maltose
    - Sucrose (saccharose)
    - Lactose
    - Starch (amidon)
- **Amino acids / Nitrogen sources** (added at 0.1% w/v):
    - Glutamine
    - Proline
    - Arginine
    - Cysteine

  - Asparagine

1.2. Media Types

Two types of media were prepared:

- **Liquid YEM medium**, used for spectrophotometric measurement of rhizobial growth.
- **Solid YEM medium**, used for observing colony morphology and growth characteristics on agar plates.

Agar (1.5% w/v) was added to the media intended for solid culture plates.

II-3-5- Inoculation of Media

II-3-5-1. Preparation of Rhizobial Inoculum

- Actively growing rhizobial strains were cultured in standard YEM broth.
- The optical density (OD600) of each culture was measured using a spectrophotometer and adjusted to approximately 0.5 to standardize the bacterial concentration across all treatments.

II-3-5.2. Liquid Media Inoculation

- In each treatment flask (containing 50 mL of supplemented YEM broth), 1 mL of the standardized rhizobial inoculum was added under sterile conditions.
- Flasks were incubated at 28°C with shaking at 120 rpm for 5 days.

II-3-5.3. Solid Media Inoculation

- Solidified agar plates containing each supplement were inoculated by spreading a small volume (approximately 50–100 µL) of the rhizobial suspension onto the surface using a sterile micropipette or loop.
- Plates were incubated at 28°C for 5 days under static conditions.

II-3-5 4 Evaluation of Rhizobial Growth

1. In Liquid Media (Quantitative Analysis)

After 5 days of incubation, bacterial growth was measured by reading the optical density at 600 nm (OD600) using a UV-visible spectrophotometer.

- Results were recorded and compared to the control (YEM without any added carbon or nitrogen source) to determine the effect of each supplement on rhizobial growth.

2. On Solid Media (Qualitative Analysis)

- Plates were observed for differences in colony color, shape, edge characteristics, and overall density.
- Any noticeable enhancement or inhibition in growth compared to the control was documented.

II-3-.6 Antibiotic Stress Tolerance Assay of Rhizobial Strains

The tolerance of rhizobial strains against various antibiotics was assessed using the disc diffusion method, as described by **Bauer et *al*., 1966**. In this method, 1 mL of each rhizobial culture (previously grown in yeast extract mannitol [YEM] broth) was applied aseptically onto sterile YEM agar plates using a micropipette. Prior to application, the optical density (OD) of each culture was measured using a spectrophotometer to standardize the inoculum. The cultures were allowed to stand on the plates for 15 minutes to ensure uniform distribution, after which the excess liquid culture was carefully removed under sterile conditions.

Antibiotic-impregnated discs containing: **erythromycin, clindamycin, amoxicillin, chloramphenicol, netilmicin, penicillin, and vancomycin**. Were then placed on the surface of the inoculated YEM agar. The plates were incubated at 28°C for 48 hours, after which zones of inhibition were measured to evaluate the sensitivity of the rhizobial strains to the antibiotics.

II-3-7 Experimental authentication

II-3-7 -1 Sand pretreatment

It was then subjected to drying at a high temperature (230**C°** for two hours continuously) ensuring the elimination of bacteria and other microorganisms. The treated sand was subsequently stored under aseptic conditions until further use in the experiment. The sand pretreatment was assessed using the standard soil sterilization protocol, as described by Somasegaran & Hoben (1994) ,The sand used in this study underwent a pretreatment process to ensure sterility and eliminate any potential microbial contaminants. First, it was thoroughly washed with distilled water to remove dust, debris, and soluble impurities.

II-3-7 -2 inoculum production (liquid culture)

To prepare a viable rhizobial inoculum, a liquid culture was established using the Yeast Extract Mannitol Broth (YEMB) medium under sterile conditions.

1. **Dissolution of Components**
    - The required components were weighed and dissolved in 500 mL of distilled water then complete volume up to 1 L in Erlenmeyer flask.
    - The solution was stirred using a magnetic stirrer to ensure complete dissolution.
2. **pH Adjustment:**
    - The pH of the medium was adjusted to 6.8–7.0 using 1N hydrochloride acid to optimize rhizobial growth.
    - The pH was confirmed using a pH meter before autoclaving.

Picture 7 sterilsation of sand

Picture 8 sand washing

3. **Sterilization:**
    - The medium was dispensed into 250 mL Erlenmeyer flasks, each containing 100 mL of YEM broth.
    - The flasks were covered with cotton plugs or aluminum foil and sterilized in an autoclave at 121°C for 15 minutes at 15 psi.
    - After sterilization, the flasks were left to cool at room temperature under aseptic conditions inside a laminar flow hood.

Inoculation of Rhizobial Strains

- A single colony of the purified rhizobial strain was picked from a **YEM agar plate** using a sterile loop.
- The colony was transferred into a **250 mL Erlenmeyer flask** containing **100 mL of sterile YEM broth**.
- The culture was incubated at **28°C** under **continuous shaking (120–150 rpm)** for **48–72 hours** to ensure proper aeration and bacterial multiplication.

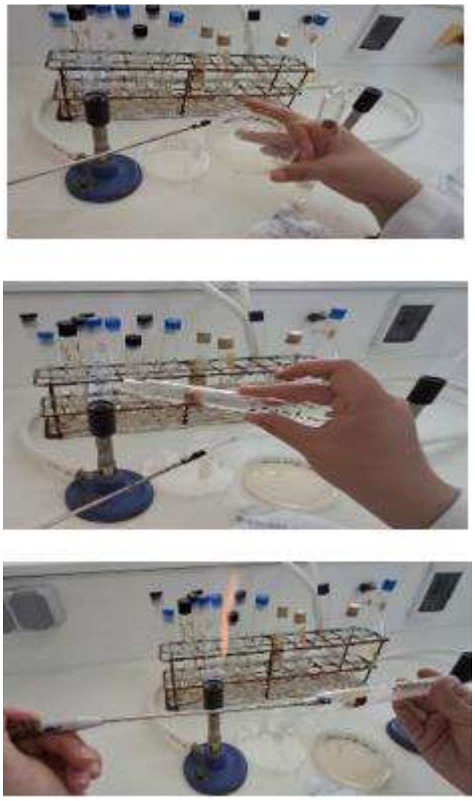

Figure 9 liquid inoculum preparation

II-3-7 -3 Preparation of culture substrate (Leaonard jars)

Leonard jar system is a specialized apparatus designed for the aseptic cultivation of plant roots, commonly utilized in studies involving legume-rhizobium symbiosis. It consists of two sterilized vessels stacked vertically and connected by a wick, allowing nutrient solutions to be drawn from the lower vessel into the growth medium in the upper vessel through capillary action. This setup provides a controlled environment to evaluate the effectiveness of rhizobial strains in nitrogen fixation.

1. Components of the Leonard Jar System

Each Leonard jar system consists of two main parts:

a) **Upper Jar (Growing Chamber):**
   - A sterilized plastic or glass container (in this case we used the first half of a perforated sterillised plastic bottle) filled with a sterile growth substrate (sand).
   - This section houses the Sesbania aculeata seedlings after inoculation.

b) **Lower Jar (Nutrient Solution Reservoir):**

- A larger glass container or bottle (thr second half of the platic bottle) filled with nitrogen-free nutrient solution to support plant growth.
- The lower chamber prevents external contamination and allows controlled nutrient supply.

photo 5 Leonard jar preparation

c) Sterile compress :

- Sterile compress is inserted between the two containers, acting as a capillary system to transport moisture and nutrients from the lower chamber to the roots in the upper jar (Somasegaran & Hoben, 1994).

2.Assembly of Leonard Jars

A. Sterilization of Components

- The materials were autoclaved at 121°C for 20 minutes before assembly.

b) **Placement of Wick:**

- A sterile compress was inserted through the perforation in the upper jar, extending into the lower jar to ensure continuous moisture supply.

c) **Filling the upper chamber:**

- The sterilized sand was evenly distributed in the upper jar, ensuring proper aeration and root penetration.

d) **Addition of Nutrient Solution:**

- The lower jar was filled with a nitrogen-free nutrient solution, essential for evaluating biological nitrogen fixation (BNF) by the inoculated rhizobia.
- Composition of nutrient solution:

Table 3 macro element composition of nutrient solution

Macro-element	
CaCl2	0.10g
MgSo4 7H2O	0.12g
KH2PO4	0.10g
Na2HPO4 2H2O	0.15g
steel citrate	0.005g

Table 4 micro-element composition of nutrient solution

Micro-elements	
H_3BO_4	2.86g
$MnSO_4\ 4H_2O$	2.03g
$ZnSO_4\ 5H_2O$	0.22g
$CuSO_4\ 5H_2O$	0.08g
$Na_2\ MO_4\ 2H_2O$	0.14g

2. Planting and Inoculation

Sesbania aculeata seeds were carefully transferred into the Leonard jars containing the sterilized sand substrate. Following transplantation, a sterile syringe was used to apply the four liquid rhizobial inoculums directly near the root zone. This ensured effective bacterial-root interaction, promoting successful colonization and symbiotic establishment while maintaining aseptic conditions (Somasegaran & Hoben, 1994).

photo 6 seeds preparation

photo 7 Leonard jars for liquid inoculation

3. Preparation of Solid Inoculum for Rhizobia

A solid inoculum refers to a form of microbial culture where the bacterial strain is embedded in a solid carrier instead of a liquid medium. In the context of rhizobial inoculation, solid inoculants are commonly prepared using sterile peat, vermiculite, perlite, or biochar as carriers to ensure longer shelf life and better adherence to seeds or soil.

photo 8 solid inoculums ingredients

photo 1 greenhouse of Kasdi Merbah University - Ouargla

- **Carrier Preparation:**
    - The chosen carrier material (sterile peat and biochar) was prepared by thoroughly mixing it with *Sesbania aculeata* seeds.
    - To enhance adhesion, a small amount of distilled water was added, ensuring the peat mixture evenly coated and sticked to the seeds.
- **Seed Inoculation and Planting:**
    - The inoculated seeds were planted directly into Leonard jars, where they were well covered with a layer of sterile sand to provide stability and promote root development.
- **Bacterial Culture Integration:**
    - the liquid rhizobial inoculum was applied directly on top of the planted seeds using a sterile syringe to ensure immediate contact between the bacteria and the root zone.
- **Storage & Application:**
    - The freshly inoculated substrate was applied without incubation, facilitating early root colonization and symbiotic establishment under aseptic conditions in a closed greenhouse.
    - This method ensured that the rhizobia were introduced into the growth environment without delays, maximizing their effectiveness in plant symbiosis.

VI.6.1 Nodulation and Growth Measurements

To evaluate the symbiotic efficiency of rhizobial strains and their impact on the growth of *Sesbania aculeata*, both nodulation and vegetative growth parameters were systematically measured. Data collection was carried out at regular intervals, typically 30 days post-inoculation (dpi), under controlled environmental conditions.

Nodulation Assessment:

Nodulation was quantified by carefully uprooting plants and gently washing the root systems under running tap water to remove adhered soil. The total number of nodules per plant was manually counted. In addition to counting, nodules were categorized based on their position (main root vs. lateral roots) and size (small, medium, or large), and the color of the nodules was recorded to distinguish between active (pink/red, indicating leghemoglobin presence) and inactive nodules (white or greenish). These qualitative observations help infer nodule functionality.

Growth Measurements:

Vegetative growth was evaluated using several morphological parameters:

- **Plant Height (cm):** Measured from the soil surface to the apical meristem using a measuring scale.
- **Stem Diameter (mm):** Measured at 1 cm above the soil surface using a digital caliper.
- **Leaf Number:** Counted manually on each plant.
- **Dry Biomass:** After harvesting, shoot and root systems were separated and oven-dried at 70°C for 72 hours until constant weight was achieved. Dry weight was measured using an analytical balance.
- **Root-to-Shoot Ratio:** Calculated to assess biomass allocation patterns between the aerial and subterranean parts of the plant.

Chapter III: Results and discussions

results of sand's ph & CE parameters:

The results obtained from these analyses are presented as follows.

Table 5 results of sand's ph & CE parameters

Replication	R0	R1	R2	R43	Average
PH	6.07	6.25	6.43	6.50	6.31
Temperature	23.8	24.0	23.7	23.5	/
CE(ms/cm)	2.33	2.34	2.36	2.34	2.34

1. Results of liquid inoculum preparation :

The image displays strains(ITAS1&ITAS2 and CHOTT1&CHOTT2) test tubes containing a turbid liquid, indicating successful growth of rhizobial strains in the liquid inoculum preparation. The turbidity reflects good biomass density, suggesting the culture has likely reached a high bacterial cell concentration, probably in the late logarithmic or early stationary growth phase. Importantly, there are no visible signs of contamination—such as distinct layers, unusual coloration, or clumping—which confirms the culture's apparent purity. Additionally, the tubes are well sealed with aluminum foil, indicating the use of proper aseptic techniques and possibly supporting anaerobic or microaerophilic conditions, depending on the strain's requirements.

Figure 7 liquid inoculum results

Results of pure strains :

The image presents the final outcome of the bacterial purification process, showcasing agar plates containing the rhizobial strains CHOTT1, CHOTT2, ITAS1, and ITAS2. The presence of well-isolated colonies with uniform morphology—consistent in size, shape, and opacity—strongly suggests successful purification and the absence of contamination. Particularly for CHOTT1 and CHOTT2, the clear streaking patterns have led to distinct, well-spaced colonies, indicating that the streaking technique and dilution were effectively applied. While ITAS1 and ITAS2 exhibit slightly fainter or fewer colonies, their growth still confirms viability and purity. The use of replicated plates labeled as "copie 1" and "copie 2" further supports the reliability of the purification process. Overall, these results confirm that all four strains have been successfully purified and are ready for downstream analyses.

Figure 11 pure isolated strains

Results of parameters effects on strains growth :

Visual observation :

The purified rhizobial strains demonstrated clear differences in tolerance across temperature, salinity, and pH conditions. **Temperature** had a notable impact: while all strains grew well at 15 °C to 35 °C, only the CHOTT strains maintained growth at 45 °C, indicating greater thermotolerance compared to ITAS strains. In terms of **salinity**, all strains showed robust growth across NaCl concentrations ranging from 0.5 g to 2 g, suggesting a strong salt tolerance. Regarding **pH**, the strains failed to grow under highly acidic conditions (pH 4–5), but resumed strong growth from pH 6 to 9, with optimal performance around neutral to slightly alkaline levels. These results suggest that the strains are well adapted to moderate salinity and pH, while CHOTT strains also possess better heat resistance, which could reflect adaptation to harsher environmental conditions.

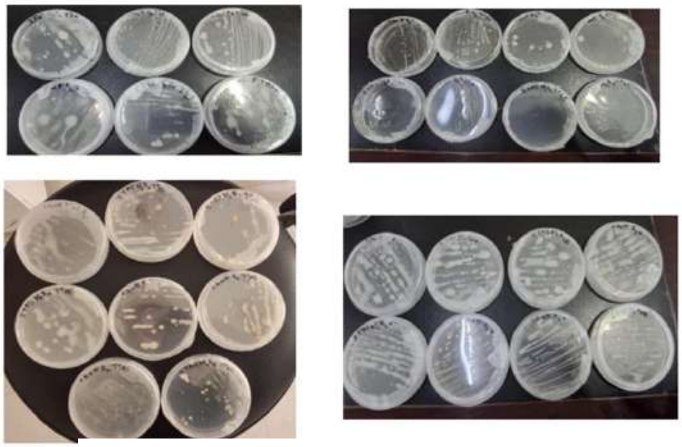

Figure 12 ph. s.r

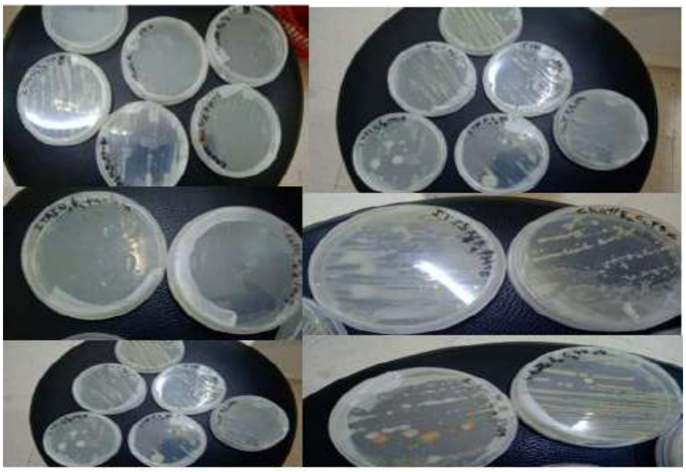

Figure 13 T r.s

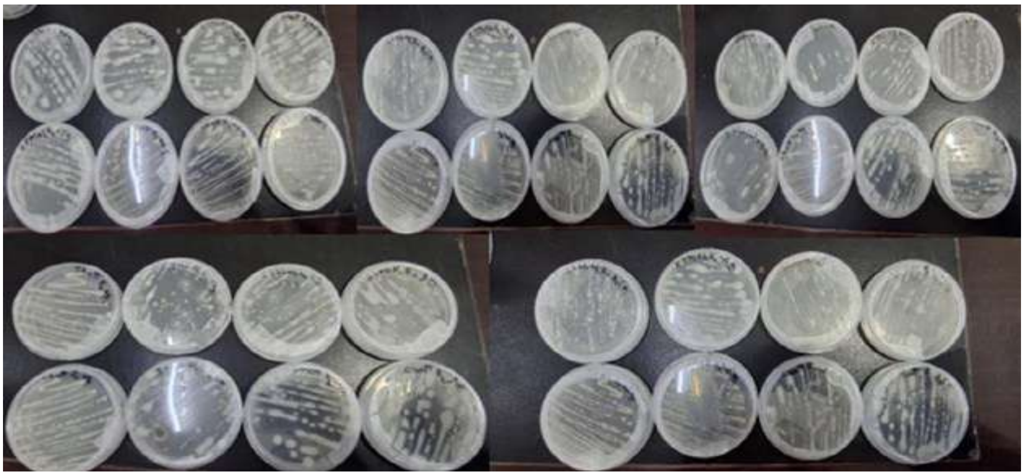

Figure 14 salinity S.r

R SOFTWARE barplots discussion :

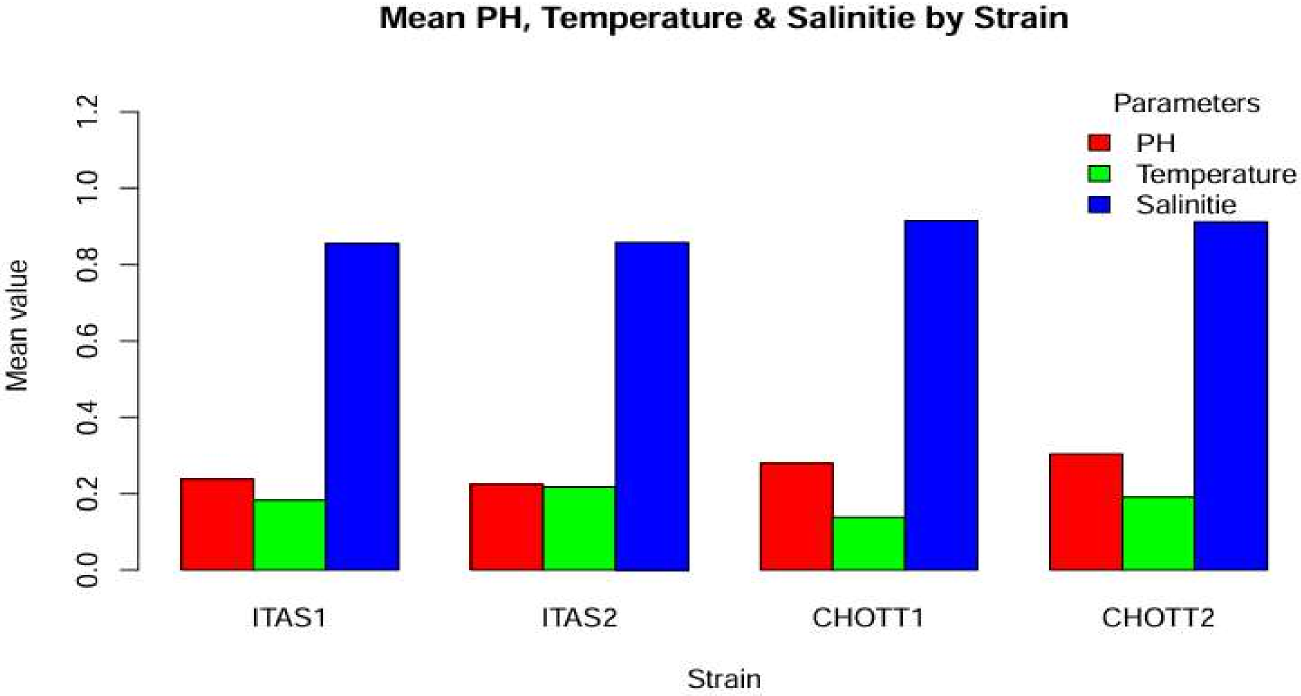

Figure 15 bar plots of mean PH, TEMPERATURE & SALINITY BY STRAINS

The bar plots above shows the 3 parameters effects on chott1&chot2 and Itas1&itas2 growth

CHAPTER III : RESULTS AND DISSCUSIONS

The ANOVA results show that **pH** (p = 0.569) and **temperature** (p = 0.351) did **not have a significant effect** on the growth of the tested rhizobial strains.

The **Tukey HSD test** also confirmed the absence of significant differences between strains under varying pH and temperature conditions, with all adjusted *p-values* exceeding 0.05.

These results suggest that the strains may be **tolerant or adapted** to the tested conditions. Further research could explore more extreme stress levels to better assess their sensitivity.

Results& discussion of sugar levels on strains growth :

Visual observation :

The visual assessment of rhizobial strains Chott1, Chott2, ITAS1, and ITAS2 grown on agar plates supplemented with different sugars (glucose, fructose, maltose, sucrose, and lactose) reveals notable differences in their growth responses. Glucose and sucrose supported the most robust growth, particularly for Chott1 and Chott2, as indicated by dense, well-defined streak patterns on the corresponding plates. This suggests these sugars are efficiently utilized carbon sources for these strains. Moderate growth was observed on maltose and fructose plates, especially for ITAS1 and ITAS2, indicating that these sugars are metabolized to a lesser extent. In contrast, lactose resulted in minimal to no visible growth across all strains, implying limited or no ability to utilize lactose, likely due to the absence or low expression of lactose-metabolizing enzymes.

Figure 16 results of different sugar application on strains

Excel histograms discussion:

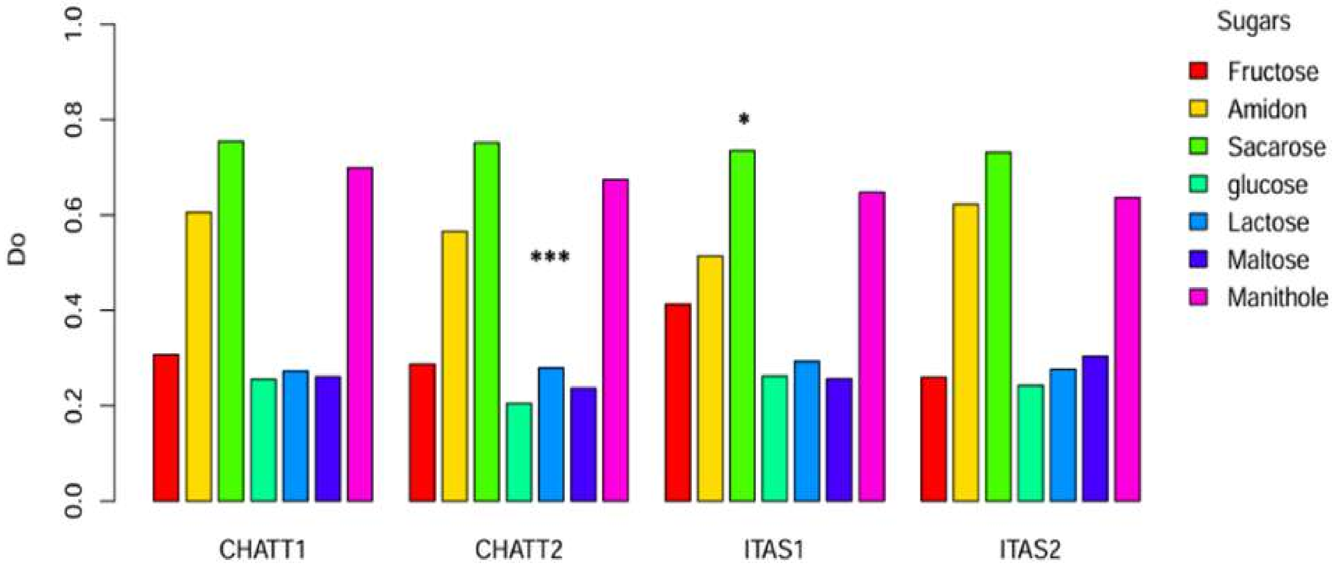

Figure 17 Boxplots of sugar effect on strains

The histogram above quantitatively illustrates the average growth of rhizobial strains ITAS1, ITAS2, CHOTT1, and CHOTT2 in response to various carbon sources, complementing the visual observations from the agar plates. Among the tested sugars, sucrose (saccharose) and starch (amidon) promoted the highest growth across all strains, particularly in CHOTT1, CHOTT2, and ITAS2, confirming their effectiveness as primary carbon sources. Moderate growth was observed with fructose , suggesting these sugars are metabolizable but less efficient than sucrose or starch. In contrast, glucose and maltose resulted in lower average growth, especially in CHOTT strains, which slightly contrasts the initial visual assessment that suggested stronger glucose-induced growth. This may indicate differences in early versus sustained growth or the influence of sugar concentration. Lactose consistently showed the lowest growth values across all strains, aligning with the minimal visual colony development and confirming its limited utility for rhizobial metabolism,

R. software bagplots discussion :

The figure above represent different sugar effects on chott1&chott2 and Itas1&Itas2 growth

The ANOVA results ($p = 0.0256$) indicate that sugar utilization significantly differs among the tested rhizobial strains. The Tukey HSD test confirms this difference, revealing that CHOTT2 and ITAS1 strains utilize sugars at significantly different levels compared to CHOTT1, with adjusted p-values below 0.05. These differences suggest that specific strains may have varying affinities or metabolic efficiencies for the tested sugars. Notably, CHOTT1 appears to utilize sugars more effectively overall, while CHOTT2 and ITAS1 show reduced sugar assimilation. These findings could reflect inherent metabolic specialization among strains or adaptation to different environmental sugar sources.

CHAPTER III : RESULTS AND DISSCUSIONS

Results of Amino acids effects on strains growth :

Visual observation :

The petri dishes show variable bacterial growth among the four rhizobial strains—**ITAS 1**, **ITAS 2**, **CHOTT N1**, and **CHOTT N2**—in response to different amino acids. **Arginine** supports visible bacterial growth in **all four strains**, with clearly defined streaks present on each corresponding plate, indicating a broadly favorable effect across strains. **Proline** also supports bacterial growth in all four strains, as evidenced by streaks or colonies on each plate. **Cysteine** promotes growth specifically in **ITAS 1**, **ITAS 2**, and **CHOTT N1**, where clear bacterial colonies are observed, while no visible growth appears for **CHOTT N2**. **Glycine** is associated with visible, though generally weak, bacterial growth in **all four strains**; the colonies are faint but consistently present. **Aspartic acid** supports very limited growth, with only **faint streaks** observed in a few strains, suggesting a marginal effect. Overall, **ITAS 1** and **ITAS 2** show stronger and more consistent growth across most amino acid plates, whereas **CHOTT N2** tends to show reduced growth or absence of colonies, particularly in response to cysteine and aspartic acid. These visual differences reflect varying degrees of metabolic compatibility with the tested amino acids among the rhizobial strains.

Figure 19 results of different amino acids applications effects on strains

R . Software barplots discussion

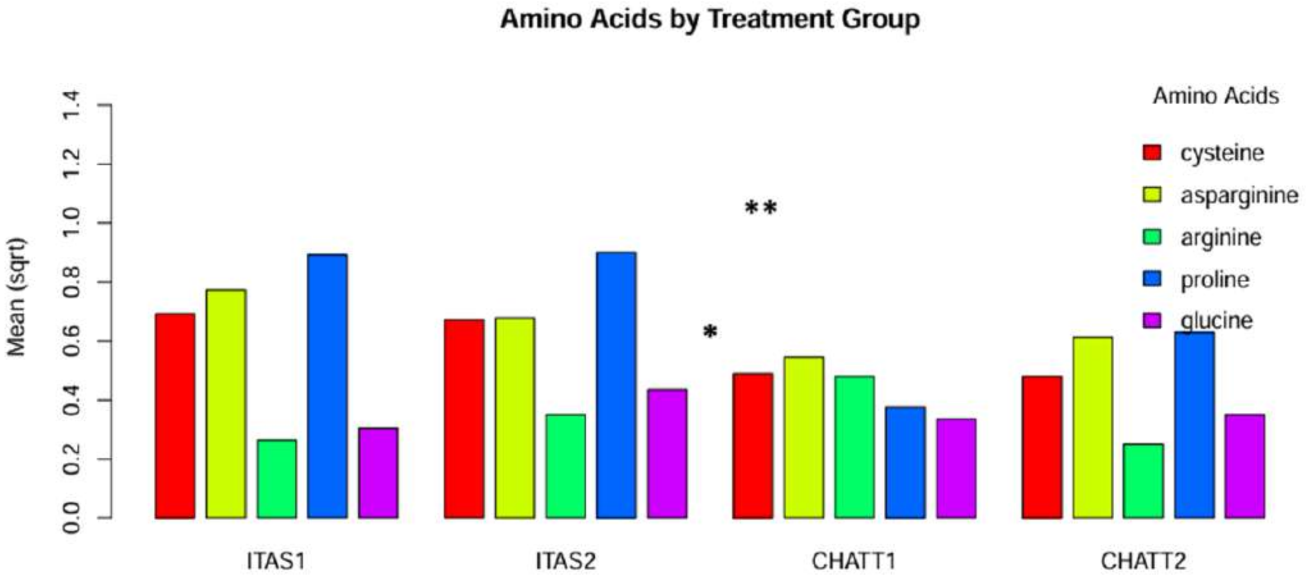

Figure 20 mean barplots of results of different amino acids applications effects on strains

The figure above represent barplots of results of diffrenet amino acids applications effects on strains

The ANOVA results show that, in most cases, the tested amino acids did **not have a statistically significant effect** on the growth of the rhizobial strains, as their associated p-values exceeded the conventional significance threshold of 0.05.

Tukey HSD post-hoc comparisons confirmed the absence of significant pairwise differences for several amino acids, with most adjusted p-values greater than 0.05. An exception was observed for **arginine**, where significant differences were found between specific strain pairs, suggesting a potential strain-dependent response to this amino acid.

Overall, these findings indicate that the bacterial strains may exhibit a degree of **tolerance or metabolic flexibility** toward the tested amino acid profiles. This could reflect their ability to utilize a broad range of nitrogen sources without strong preferential growth responses.

Results of antibiotics responses by strains :

Visual observation :

The figure below shows a series of Petri dishes used for antimicrobial activity assays, likely involving rhizobial strains such as ITAS1 & ITAS2 and chott 1 & chott2 grown on YEM medium. Small paper discs placed on the agar surface suggest the application of different treatments or antimicrobial agents. Clear zones of inhibition are visible around several discs, indicating effective suppression of bacterial growth in those areas. The degree of inhibition varies across the plates, with some discs producing strong, distinct halos while others show little to no effect, suggesting differential sensitivity or resistance among the tested bacteria. The presence of uniform bacterial lawns and clearly defined zones supports the reliability of the assay, although the inclusion of labeled controls and replicates would enhance data accuracy.

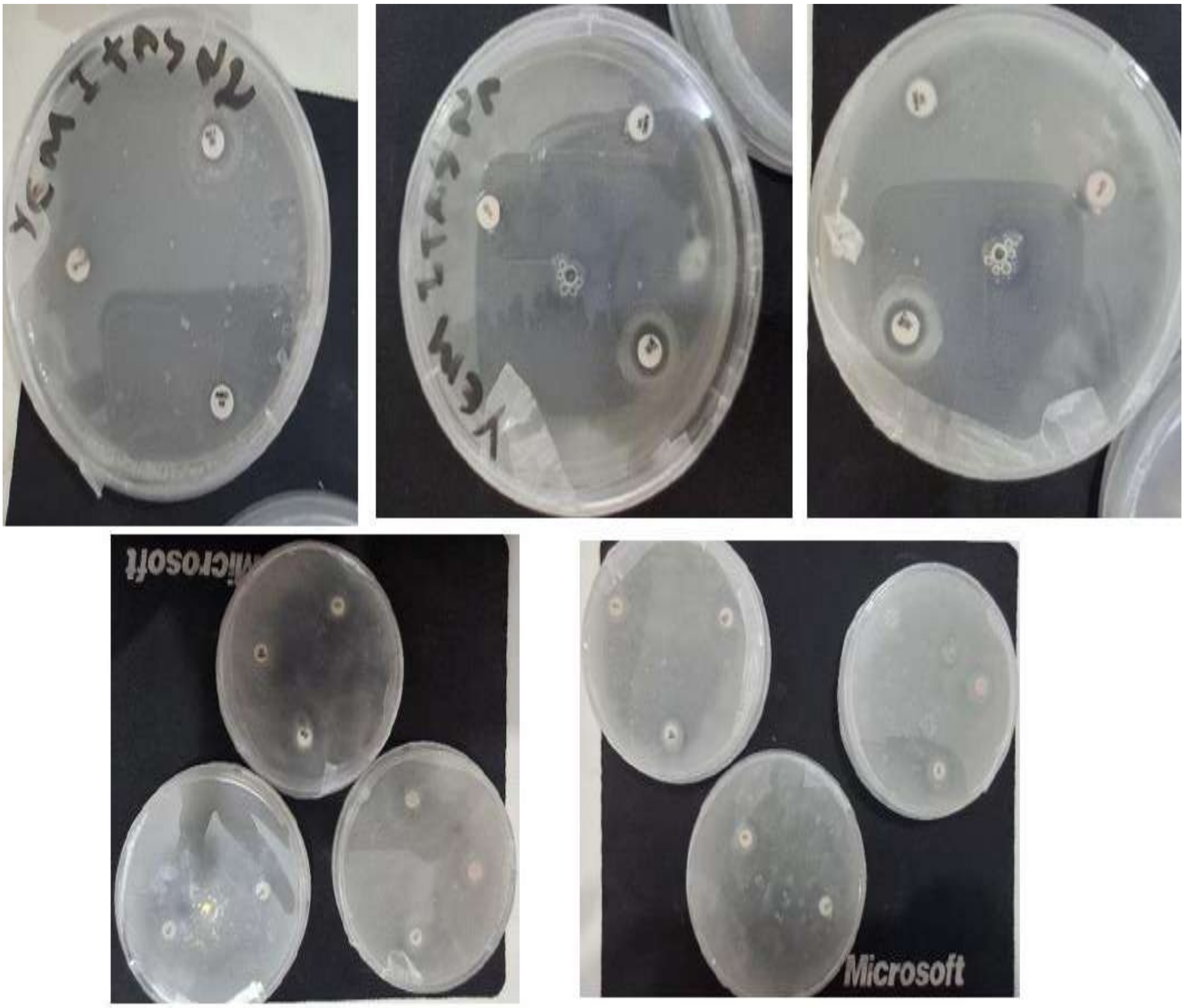

Figure 21 antibiotics responses by strains

Excel histograms discussion :

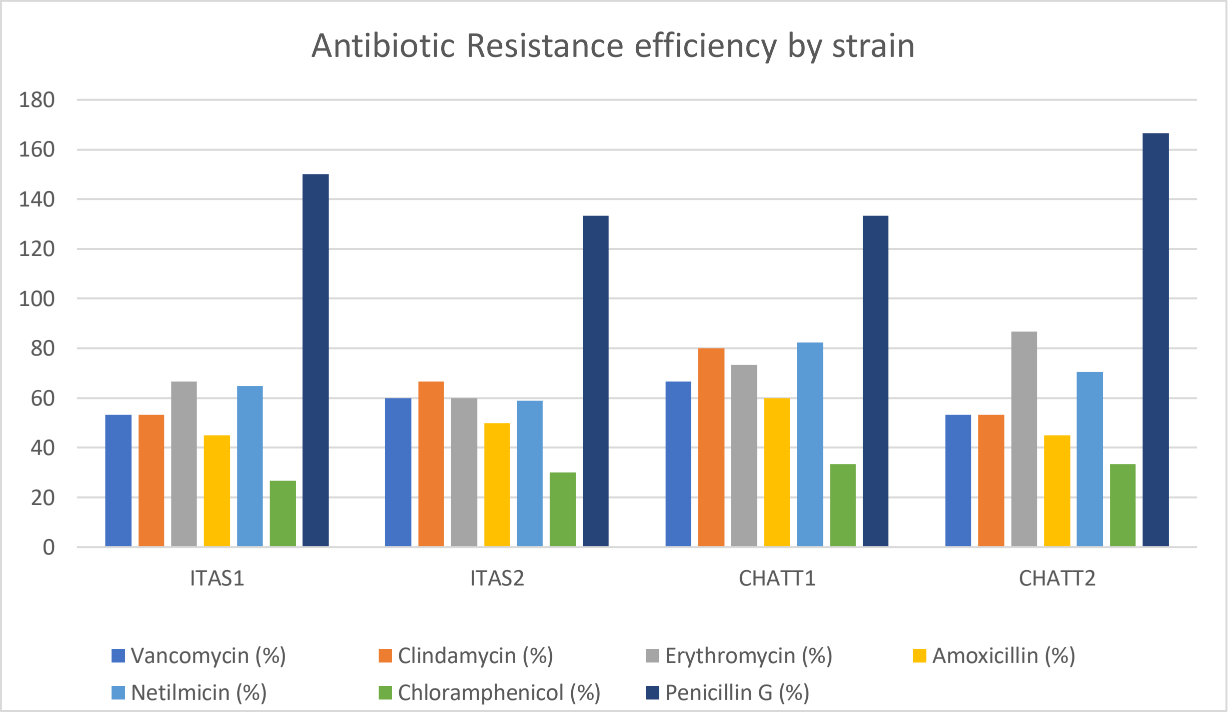

Figure 22 histograms of antibiotics response by strain

The figure above represent histograms of antibiotics response by strains

Based purely on the relative heights of the bar clusters for each strain, we can assign performance groups A–D to highlight their overall antibiotic responsiveness. CHOTT1 clearly stands out with the tallest bars—driven by NET30 (1.4), AMC30 (1.2) and E15 (1.1)—and so it belongs to Group A. CHOTT2, with similarly high but slightly lower peaks (NET30 = 1.2, E15 = 1.3) and consistently strong responses across other antibiotics, falls into Group B. ITAS2 occupies Group C, as its bars remain in the midrange (0.8–1.0) without extreme highs or lows, reflecting moderate susceptibility. Finally, ITAS1 shows the shortest bars overall (0.8–1.1) and thus is placed in Group D. This grouping scheme provides a quick visual summary: CHOTT1 is the most responsive strain, followed by CHOTT2, then ITAS2, with ITAS1 exhibiting the lowest antibiotic response among the four.

The bar chart shows that Penicillin G is the most effective antibiotic across all four bacterial strains (ITAS1, ITAS2, CHATT1, CHATT2), with the highest inhibition levels, especially in CHOTT2 and ITAS1. In contrast, Chloramphenicol consistently exhibits the lowest inhibition in all strains. Erythromycin and Netilmicin show moderate and fairly stable effectiveness across the strains, while Vancomycin, Clindamycin, and Amoxicillin display variable inhibition levels depending on the strain. Overall, Penicillin G stands out as the most potent antibiotic, while Chloramphenicol is the least effective.

Results of Nodulation and Growth Measurements:

CHAPTER III : RESULTS AND DISSCUSIONS

Visual observation :

Figure 23 liquid inoculum results

Figure 24 solid inoculum results

CHAPTER III : RESULTS AND DISSCUSIONS

Numeric results and discussion :

Table 6 data of nodulation

Treatment	Nodule Size	Nodule Color	Nodule Position
Itas N1	Small	Light Brownish	Lateral / Main Root
Itas N2	Large	Brownish	Main Root
Chott N1	Small	Brownish	Lateral Roots
Chott N2	Small	brownish beige	lateral roots
B.T.ITAS.N1	Small	dark Brown	lateral roots
B.T.ITAS.N2	Small	dark brown	Main Root
B.T.chott.N1	Large	light brown	Main Root
B.T.chott.N2	medium	brown	main root
Control B (B.T. + N source)	**Control B** (B.T. + N source)	**Control B** (B.T. + N source)	**Control B** (B.T. + N source)
Control A (B.T., no N)	**Control A** (B.T., no N)	**Control A** (B.T., no N)	**Control A** (B.T., no N)

The data presented in the tables highlight the differential effects of rhizobial strains and inoculation carriers (B.T: solid inoculum forms such as peat and biochar) on plant growth and nodulation efficiency compared to control .

The comparison between inoculated plants and control treatments reveals clear differences in nodulation efficiency and symbiotic performance. Control A, which consisted of seeds coated with biochar and peat but received no external nitrogen, showed poor development and either an absence of nodules or the formation of small, pale nodules, indicating limited symbiotic activity. Control B, treated with biochar, peat, and a chemical nitrogen source, displayed healthy vegetative growth but showed very few or no nodules. This is expected, as the presence of readily available nitrogen typically inhibits nodule formation by suppressing the plant's symbiotic signaling.

In contrast, all inoculated treatments demonstrated the formation of functional nodules, with notable variation depending on the strain and the presence of the solid support. The ITAS and CHOTT strains alone formed small to medium-sized nodules, with CHOTT N2 and ITAS N2 performing slightly better than their counterparts. However, the addition of biochar and peat (B.T.) significantly improved nodule development in both strains, particularly in B.T. CHOTT N1 and N2 treatments, where nodules were larger, darker in color, and located primarily on the main root—signs of effective nitrogen fixation. Among all treatments, B.T. CHOTT N1 produced the most pronounced results, with large, light brown nodules on the main root, indicating active symbiosis and optimal conditions for bacterial function.

Overall, the inoculated plants, especially those treated with the CHOTT strains in combination with the solid support, outperformed both control groups. These results confirm the positive role of rhizobial inoculation and the biochar–peat carrier in enhancing biological nitrogen fixation, which can potentially reduce or replace the need for synthetic nitrogen fertilizers, especially in early plant development stages.

Histograms of plant growth numeric data :

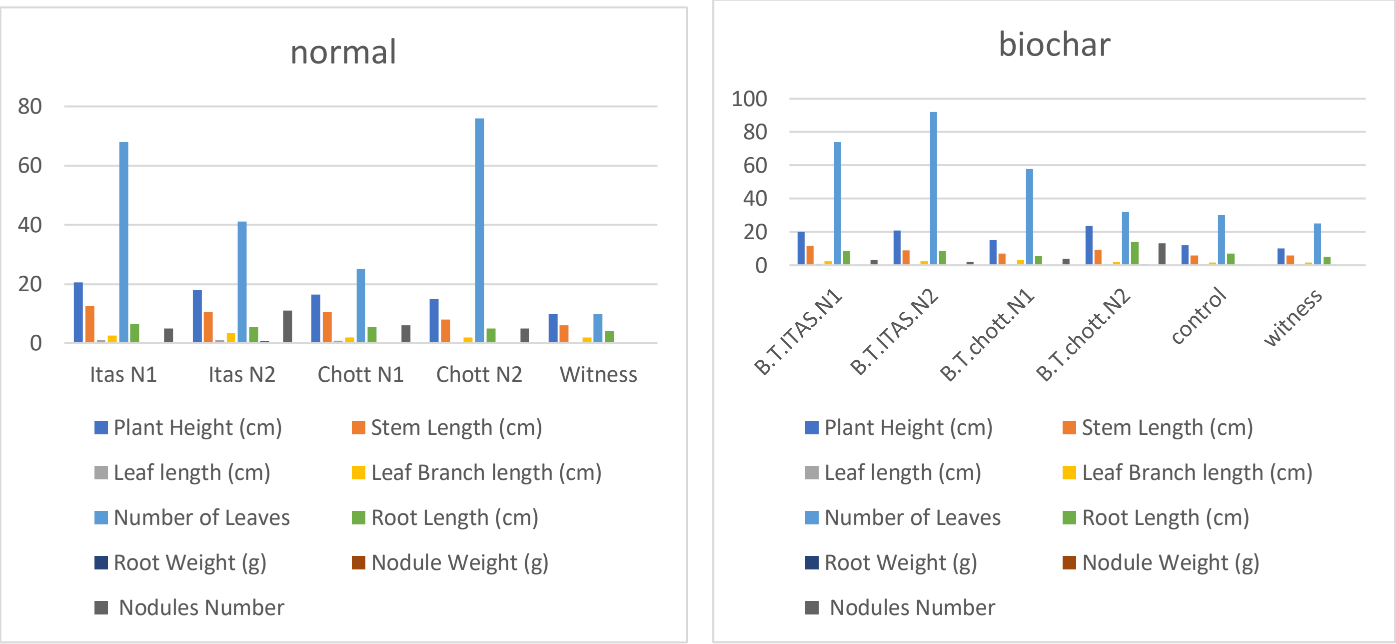

Figure 25 histograms of strains effects on plants growth

The figure above represent the effects of strains on sesbania aculeata plants developments compaired to control with no source of azote and to control with source of azote also to controle seeds covered with peat and biochar , another naked seeds control

strains effects on plants growth

The bar graph shows that Sesbania plants grown differently to various microbial strains. Chott N2 resulted in the highest number of leaves and strong shoot growth, making it the most effective for above-ground development. Itas N1 also promoted good plant height and leaf traits, while Itas N2 led in root length, root weight, and nodule formation, indicating its strength in below-ground growth and symbiosis. Both Chott strains supported moderate nodulation and growth, whereas the control group (Witness) showed the weakest performance across all parameters, confirming that microbial inoculation significantly enhances Sesbania growth

strains effects on plants growth (biochar and peat)

The bar graph shows that Sesbania plants grown with the addition of biochar and peat responded very positively to microbial inoculation, with notable differences among strains. The B.T.Itas.N2 strain produced the highest number of leaves—nearly 90—and also led in plant height, root weight, and nodule development, making it the most effective overall. B.T.Itas.N1 and B.T.Chott.N1 also showed strong results, especially in leaf number and root traits. B.T.Chott.N2 performed moderately, while the control and witness groups had significantly lower growth across all parameters, particularly in leaf number, root development, and nodulation. This demonstrates that combining biochar, peat, and effective microbial strains greatly enhances Sesbania growth and root symbiosis

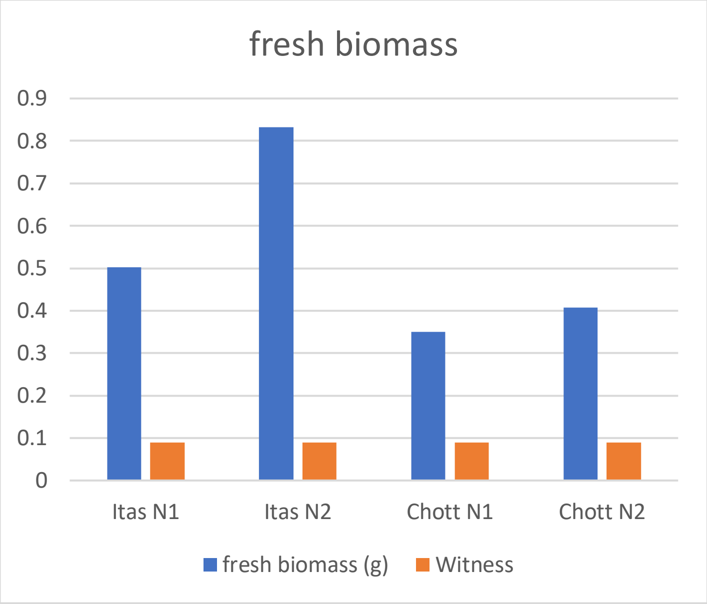

Figure 26 histograms of fresh plants biomass naked seeds

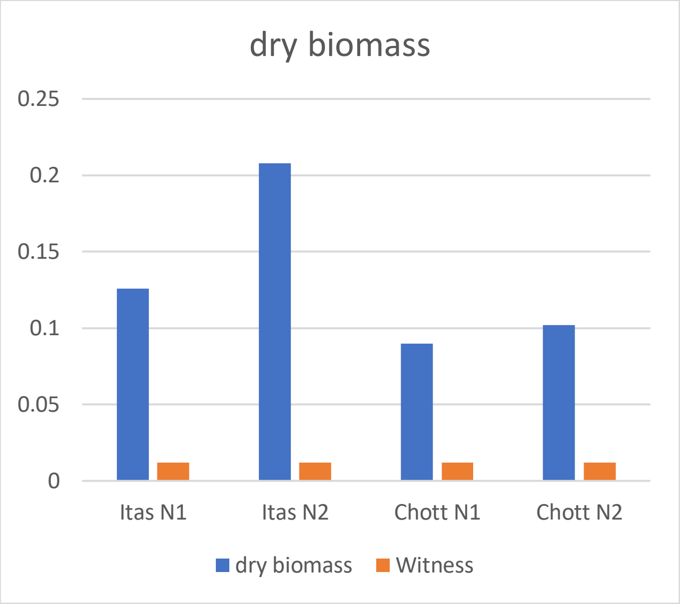

Figure 8 histograms of dry plants biomass naked seeds

The figures above represent histograms of fresh and dry biomass of naked seeds results of the four strains effects

In both dry and fresh biomass measurements, the treated plants (blue bars) produced significantly higher biomass than the control plants (red bars). Among the treatments, Itas N2 resulted in the highest biomass for both fresh and dry weight, indicating its superior effectiveness. Chott N1 and Chott N2 also showed moderate increases in biomass, though still substantially higher than the control. The control plants consistently exhibited very low biomass, demonstrating that without the addition of bacteria and minerals, even with peat and biochar, Sesbania plants grew very poorly

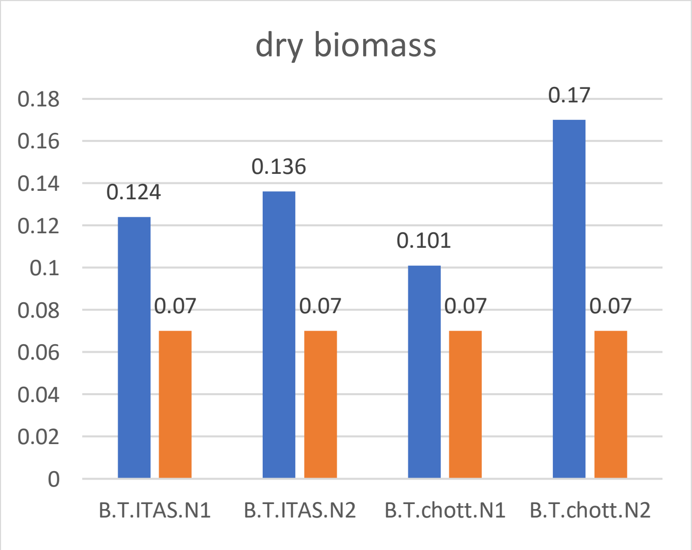

Figure 29 histograms of dry biomass of covered seeds

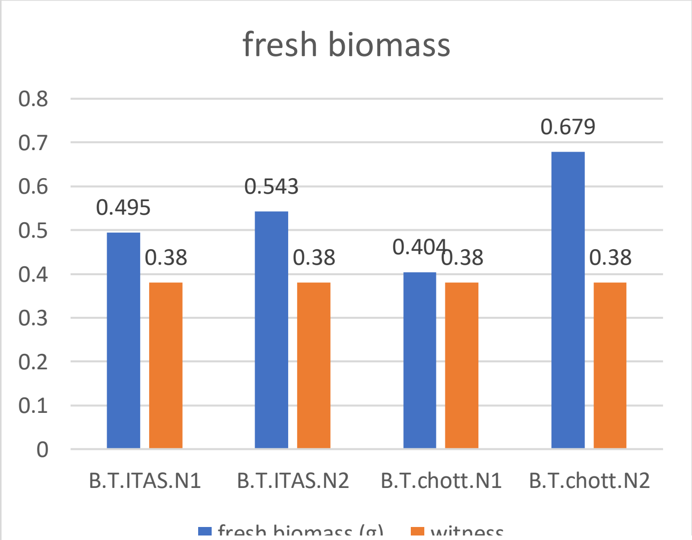

Figure 28 hisotgrams of fresh biomass of covered seeds

The figures above represents the dry biomass and fresh biomass result of strains effects compared to control with and without source of azote

In both dry and fresh biomass results, all treatments involving biochar and peat (blue bars) outperformed the control (red bars), indicating that these amendments significantly enhanced plant growth. For dry biomass, the B.T.chott.N2 treatment produced the highest values, suggesting it was the most effective among all tested options. Similarly, in fresh biomass, the same treatment (B.T.chott.N2) again showed the greatest increase, confirming its consistent effectiveness in promoting biomass accumulation

Final discussion :

Building on classic studies of arid-zone rhizobia—and recognizing that ***Sesbania aculeata*** also forms natural symbioses with partners such as *Azorhizobium doebereinerae* (first isolated from Sesbania roots in Florida, USA), *Neorhizobium huautlense* sp. nov. (described from Mexican semi-arid soils), and *Rhizobium* sp. SIN-1 (recovered from Sesbania nodules in India)—our CHOTT isolates behaved like microbial marathoners, not merely surviving but actively proliferating at 45 °C—conditions under which many temperate strains falter—likely thanks to robust upregulation of heat-shock proteins (e.g., GroEL/GroES chaperonins) and adaptive shifts in membrane fatty acid saturation that maintain fluidity and prevent thermal denaturation. When presented with a panel of carbon sources, CHOTT1, CHOTT2, and ITAS2 exhibited a clear, statistically significant (ANOVA $p = 0.0256$) preference for sucrose and starch; this suggests these strains secrete higher levels of extracellular polysaccharide-degrading enzymes (e.g., amylases and sucrases) and channel the resultant monosaccharides into more efficient glycolytic and oxidative phosphorylation pathways, thereby maximizing ATP yield to support nodule invasion and bacteroid differentiation. Our amino acid assays—which revealed broad growth across glycine, cysteine, and aspartate but a distinctive, strain-specific responsiveness to arginine—point toward differential expression of arginine decarboxylase and associated polyamine biosynthetic pathways, mechanisms known to confer osmoprotection and reactive-oxygen-species scavenging under nutrient stress. Finally, by delivering CHOTT1 within a finely milled biochar–peat matrix, we created a high-surface-area microhabitat that both retains moisture and buffers rhizosphere pH fluctuations, facilitating sustained bacterial viability, enhanced colonization of root hairs, and elevated nitrogenase activity—findings that mirror the soil-conditioning benefits of biochar documented in field and greenhouse trials. Collectively, these mechanistic insights not only validate our experimental framework but also chart a clear path for harnessing thermotolerant, metabolically versatile rhizobia—packaged in optimized carriers—to bolster Sesbania aculeata productivity in hot, nutrient-poor soils

CONCLUSION

Conclusion

This study demonstrates the potential of native rhizobial strains, particularly those isolated from arid Algerian soils, to serve as effective bioinoculants for the sustainable cultivation of ***Sesbania aculeata*** under stress-prone environments. The CHOTT and ITAS strains, especially CHOTT1 and ITAS2, exhibited remarkable resilience to high temperatures (up to 45 °C), salinity, and variable pH conditions, reflecting an ecological adaptation shaped by their native habitats. These findings are further supported by the strains' metabolic flexibility, with statistically significant preferences for sucrose and starch as carbon sources, and distinctive amino acid utilization patterns, notably the arginine responsiveness of CHOTT1—suggesting specialized nitrogen assimilation strategies under nutrient-limited conditions.

The integration of solid inoculation carriers, particularly biochar–peat formulations, further enhanced symbiotic efficiency by supporting higher microbial viability, stimulating root development, and improving nodulation outcomes. The superior performance of CHOTT1 when delivered through this matrix aligns with broader research on biochar's role in improving soil structure, moisture retention, and microbial colonization.

Importantly, these results align with and extend previous international findings on Sesbania symbioses, such as those involving Azorhizobium doebereinerae (USA), Neorhizobium huautlense sp. nov. (Mexico), and Rhizobium sp. SIN-1 (India), reinforcing the concept that local environmental pressures shape symbiont competitiveness and functionality. The ability of CHOTT1 and ITAS strains to combine abiotic stress tolerance with effective symbiosis positions them as strong candidates for inoculant development in regions challenged by climate change and soil degradation.

This study demonstrates that *Sesbania* can be cultivated in arid and semi-arid regions, and its production may be improved by employing rhizobial strains adapted to Saharan soils.

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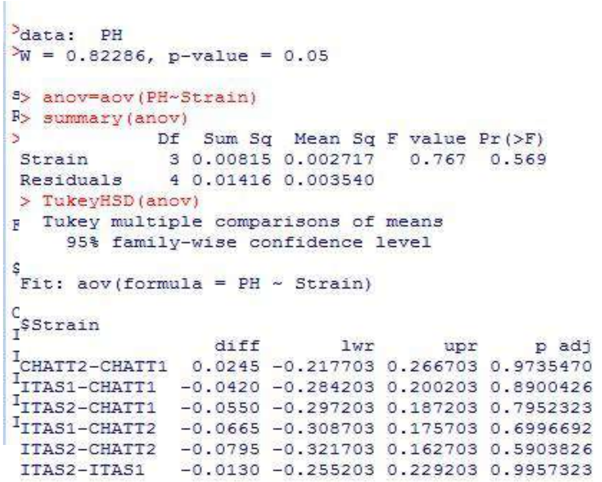
- Sesbania bispinosa var. bispinosa | Plants of the World Online | Kew Science

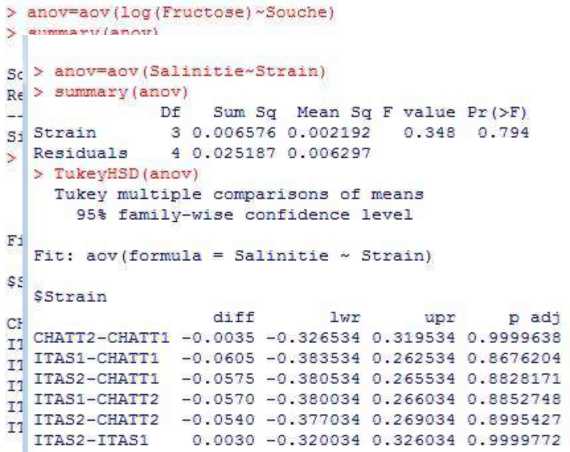
ANNEXES

ANNEXES

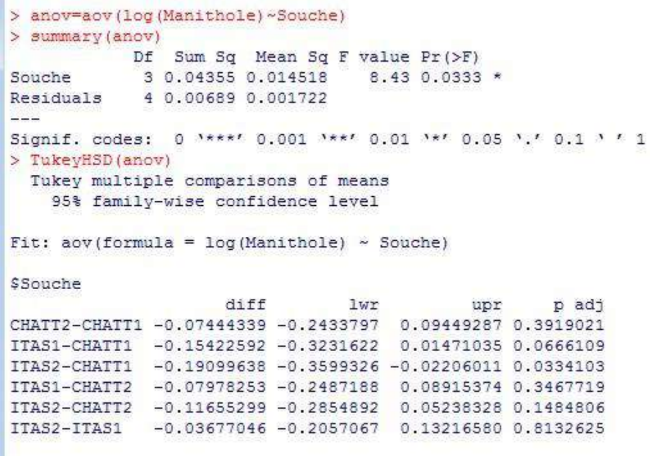
- Anova and tukey hsd test using R softwar :



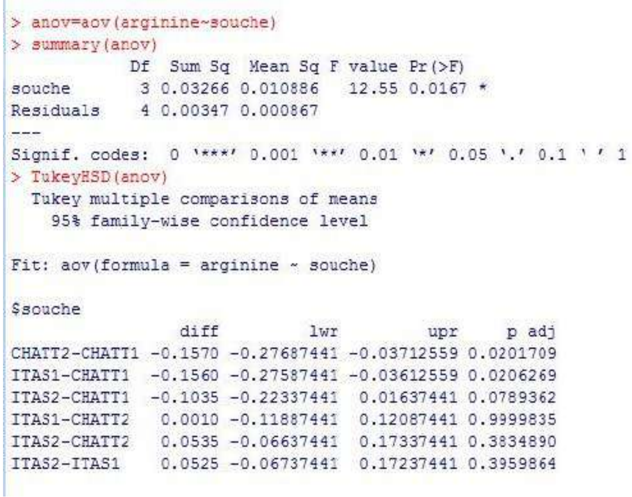
```
>data:   PH
>W = 0.82286, p-value = 0.05

> anov=aov(PH~Strain)
> summary(anov)
              Df  Sum Sq  Mean Sq F value Pr(>F)
 Strain        3 0.00815 0.002717   0.767  0.569
 Residuals     4 0.01416 0.003540
 > TukeyHSD(anov)
   Tukey multiple comparisons of means
     95% family-wise confidence level

 Fit: aov(formula = PH ~ Strain)

 $Strain
                  diff        lwr      upr     p adj
 CHATT2-CHATT1  0.0245 -0.217703 0.266703 0.9735470
 ITAS1-CHATT1  -0.0420 -0.284203 0.200203 0.8900426
 ITAS2-CHATT1  -0.0550 -0.297203 0.187203 0.7952323
 ITAS1-CHATT2  -0.0665 -0.308703 0.175703 0.6996692
 ITAS2-CHATT2  -0.0795 -0.321703 0.162703 0.5903826
 ITAS2-ITAS1   -0.0130 -0.255203 0.229203 0.9957323
```



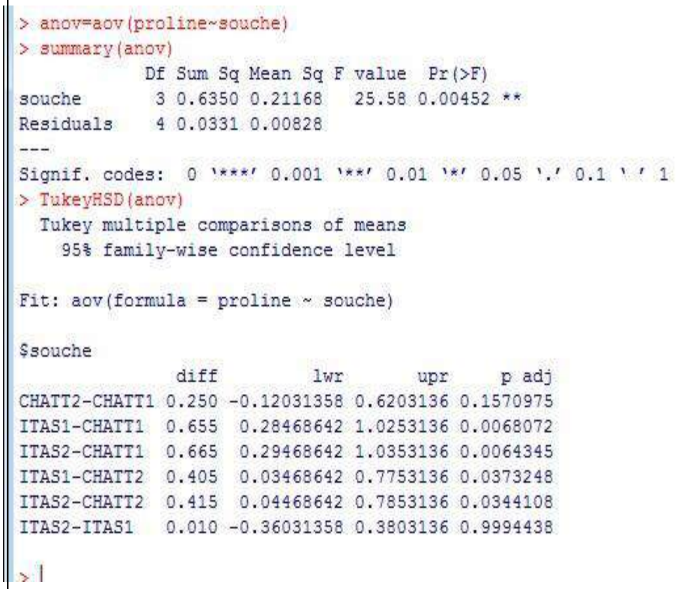
```
> anov=aov(log(Fructose)~Souche)
> summary(anov)

> anov=aov(Salinitie~Strain)
> summary(anov)
             Df   Sum Sq  Mean Sq F value Pr(>F)
Strain        3 0.006576 0.002192   0.348  0.794
Residuals     4 0.025187 0.006297
> TukeyHSD(anov)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = Salinitie ~ Strain)

$Strain
                 diff       lwr      upr     p adj
CHATT2-CHATT1 -0.0035 -0.326534 0.319534 0.9999638
ITAS1-CHATT1  -0.0605 -0.383534 0.262534 0.8676204
ITAS2-CHATT1  -0.0575 -0.380534 0.265534 0.8828171
ITAS1-CHATT2  -0.0570 -0.380034 0.266034 0.8852748
ITAS2-CHATT2  -0.0540 -0.377034 0.269034 0.8995427
ITAS2-ITAS1    0.0030 -0.320034 0.326034 0.9999772
```



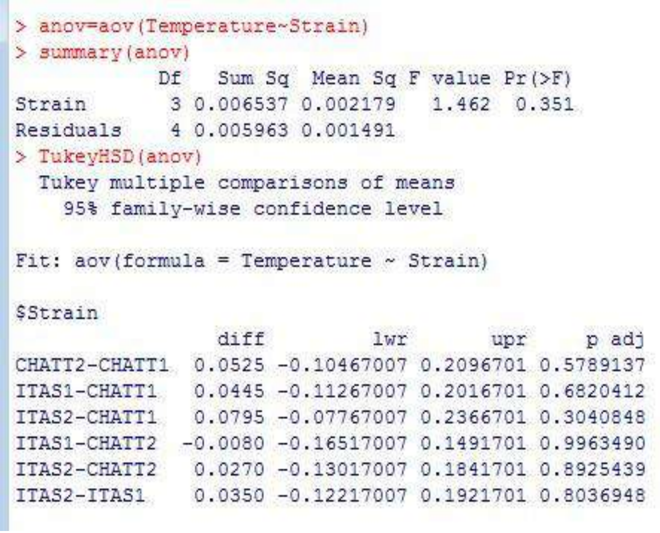
```
> anov=aov(log(Manithole)~Souche)
> summary(anov)
            Df  Sum Sq  Mean Sq F value Pr(>F)
Souche       3 0.04355 0.014518    8.43 0.0333 *
Residuals    4 0.00689 0.001722
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> TukeyHSD(anov)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = log(Manithole) ~ Souche)

$Souche
                    diff        lwr         upr     p adj
CHATT2-CHATT1 -0.07444339 -0.2433797  0.09449287 0.3919021
ITAS1-CHATT1  -0.15422592 -0.3231622  0.01471035 0.0666109
ITAS2-CHATT1  -0.19099638 -0.3599326 -0.02206011 0.0334103
ITAS1-CHATT2  -0.07978253 -0.2487188  0.08915374 0.3467719
ITAS2-CHATT2  -0.11655299 -0.2854892  0.05238328 0.1484806
ITAS2-ITAS1   -0.03677046 -0.2057067  0.13216580 0.8132625
```



```
> anov=aov(arginine~souche)
> summary(anov)
            Df  Sum Sq  Mean Sq F value Pr(>F)
souche       3 0.03266 0.010886   12.55 0.0167 *
Residuals    4 0.00347 0.000867
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> TukeyHSD(anov)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = arginine ~ souche)

$souche
                diff         lwr         upr     p adj
CHATT2-CHATT1 -0.1570 -0.27687441 -0.03712559 0.0201709
ITAS1-CHATT1  -0.1560 -0.27587441 -0.03612559 0.0206269
ITAS2-CHATT1  -0.1035 -0.22337441  0.01637441 0.0789362
ITAS1-CHATT2   0.0010 -0.11887441  0.12087441 0.9999835
ITAS2-CHATT2   0.0535 -0.06637441  0.17337441 0.3834890
ITAS2-ITAS1    0.0525 -0.06737441  0.17237441 0.3959864
```

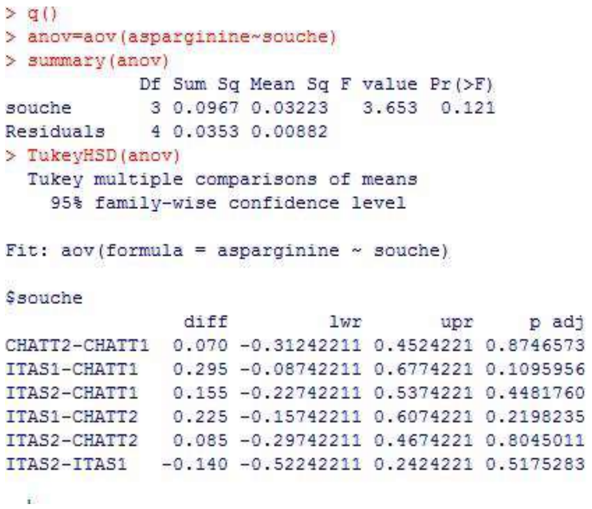


```
> anov=aov(proline~souche)
> summary(anov)
            Df Sum Sq Mean Sq F value  Pr(>F)
souche       3 0.6350 0.21168   25.58 0.00452 **
Residuals    4 0.0331 0.00828
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> TukeyHSD(anov)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = proline ~ souche)

$souche
               diff         lwr       upr     p adj
CHATT2-CHATT1 0.250 -0.12031358 0.6203136 0.1570975
ITAS1-CHATT1  0.655  0.28468642 1.0253136 0.0068072
ITAS2-CHATT1  0.665  0.29468642 1.0353136 0.0064345
ITAS1-CHATT2  0.405  0.03468642 0.7753136 0.0373248
ITAS2-CHATT2  0.415  0.04468642 0.7853136 0.0344108
ITAS2-ITAS1   0.010 -0.36031358 0.3803136 0.9994438

> |
```

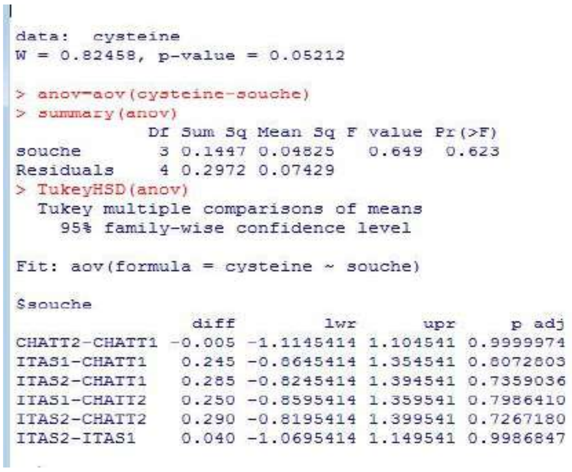


```
> anov=aov(Temperature~Strain)
> summary(anov)
            Df   Sum Sq  Mean Sq F value Pr(>F)
Strain       3 0.006537 0.002179   1.462  0.351
Residuals    4 0.005963 0.001491
> TukeyHSD(anov)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = Temperature ~ Strain)

$Strain
                 diff         lwr       upr     p adj
CHATT2-CHATT1  0.0525 -0.10467007 0.2096701 0.5789137
ITAS1-CHATT1   0.0445 -0.11267007 0.2016701 0.6820412
ITAS2-CHATT1   0.0795 -0.07767007 0.2366701 0.3040848
ITAS1-CHATT2  -0.0080 -0.16517007 0.1491701 0.9963490
ITAS2-CHATT2   0.0270 -0.13017007 0.1841701 0.8925439
ITAS2-ITAS1    0.0350 -0.12217007 0.1921701 0.8036948
```

ANNEXES



```
> q()
> anov=aov(asparginine~souche)
> summary(anov)
            Df Sum Sq Mean Sq F value Pr(>F)
souche       3 0.0967 0.03223   3.653  0.121
Residuals    4 0.0353 0.00882
> TukeyHSD(anov)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = asparginine ~ souche)

$souche
                diff         lwr       upr     p adj
CHATT2-CHATT1  0.070 -0.31242211 0.4524221 0.8746573
ITAS1-CHATT1   0.295 -0.08742211 0.6774221 0.1095956
ITAS2-CHATT1   0.155 -0.22742211 0.5374221 0.4481760
ITAS1-CHATT2   0.225 -0.15742211 0.6074221 0.2198235
ITAS2-CHATT2   0.085 -0.29742211 0.4674221 0.8045011
ITAS2-ITAS1   -0.140 -0.52242211 0.2424221 0.5175283
```



```
data:  cysteine
W = 0.82458, p-value = 0.05212

> anov=aov(cysteine~souche)
> summary(anov)
            Df Sum Sq Mean Sq F value Pr(>F)
souche       3 0.1447 0.04825   0.649  0.623
Residuals    4 0.2972 0.07429
> TukeyHSD(anov)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = cysteine ~ souche)

$souche
                diff        lwr      upr     p adj
CHATT2-CHATT1 -0.005 -1.1145414 1.104541 0.9999974
ITAS1-CHATT1   0.245 -0.8645414 1.354541 0.8072803
ITAS2-CHATT1   0.285 -0.8245414 1.394541 0.7359036
ITAS1-CHATT2   0.250 -0.8595414 1.359541 0.7986410
ITAS2-CHATT2   0.290 -0.8195414 1.399541 0.7267180
ITAS2-ITAS1    0.040 -1.0695414 1.149541 0.9986847
```

- Excel data of growth measurments :

Treatment	**fresh biomass (g)**	Witness
Itas N1	0.503	0.09
Itas N2	0.833	0.09
Chott N1	0.35	0.09
Chott N2	0.408	0.09

Treatment	**fresh biomass (g)**	witness
B.T.ITAS.N1	0.495	0.38
B.T.ITAS.N2	0.543	0.38
B.T.chott.N1	0.404	0.38
B.T.chott.N2	0.679	0.38

Treatment	**dry biomass**	Witness
Itas N1	0.126	0.012
Itas N2	0.208	0.012
Chott N1	0.09	0.012
Chott N2	0.102	0.012

Treatment	**dry biomass**	witness
B.T.ITAS.N1	0.124	0.07
B.T.ITAS.N2	0.136	0.07
B.T.chott.N1	0.101	0.07
B.T.chott.N2	0.17	0.07

Treatment	**Nodules Number**
Itas N1	5
Itas N2	11
Chott N1	6
Chott N2	5

Treatment	**Nodules Number**
B.T.ITAS.N1	3
B.T.ITAS.N2	2
B.T.chott.N1	4
B.T.chott.N2	13

ANNEXES

- Data table of carbon sources :

Amidon	Sacarose	glucose	Lactose	Maltose	Manithole
0.25	0.55	0.072	0.082	0.064	0.43
0.28	0.53	0.065	0.089	0.068	0.41
0.26	0.53	0.068	0.096	0.087	0.39
0.54	0.54	0.051	0.059	0.098	0.42
0.22	0.56	0.085	0.068	0.058	0.5
0.55	0.58	0.048	0.08	0.079	0.48
0.34	0.56	0.044	0.088	0.056	0.44
0.3	0.57	0.04	0.068	0.056	0.47

- Data table of antibioics inhibition zones in cm :

Strain	VA	CM	E15	AMC30	NET30	C30	P10
ITAS1	0.8	0.8	1	0.9	1.1	0.8	0.9
ITAS2	0.9	1	0.9	1	1	0.9	0.8
CHATT1	1	1.2	1.1	1.2	1.4	1	0.8
CHATT2	0.8	0.8	1.3	0.9	1.2	1	1

- Data table of arameters effects on strains growth :

strain	**PH**	**Temperature**	**Salinitie**
ITAS1	0,247	0,15	0,886
ITAS1	0,228	0,216	0,823
ITAS2	0,247	0,219	0,943
ITAS2	0,202	0,217	0,772
CHOTT1	0,321	0,108	0,862
CHOTT1	0,238	0,169	0,968
CHOTT2	0,373	0,222	0,95
CHOTT2	0,235	0,16	0,873

- Data table of amino acids effects on strains growth :

Results	cysteine	asparginine	arginine	proline	glucine
ITAS1	0,59	0,68	0,06	0,68	0,34
ITAS1	0,38	0,52	0,08	0,92	0,27
ITAS2	0,16	0,42	0,09	0,82	0,29
ITAS2	0,89	0,5	0,16	0,8	0,58
CHATT1	0,25	0,4	0,23	0,1	0,26
CHATT1	0,23	0,21	0,23	0,19	0,41
CHATT2	0,3	0,35	0,09	0,4	0,29
CHATT2	0,17	0,4	0,04	0,39	0,41

ANNEXES

- Antibiotics inhibition percentage formula :

$$\text{Inhibition percentage} = \left(\frac{\text{Inhibition zone in mm}}{D} \right) \times 100$$

- **Committee of the Antibiogram of the French Society for Microbiology. (2024).** ***CASFM/EUCAST v1.0: Antibiogram guidelines (June 2024)*****. French Society for Microbiology.**:

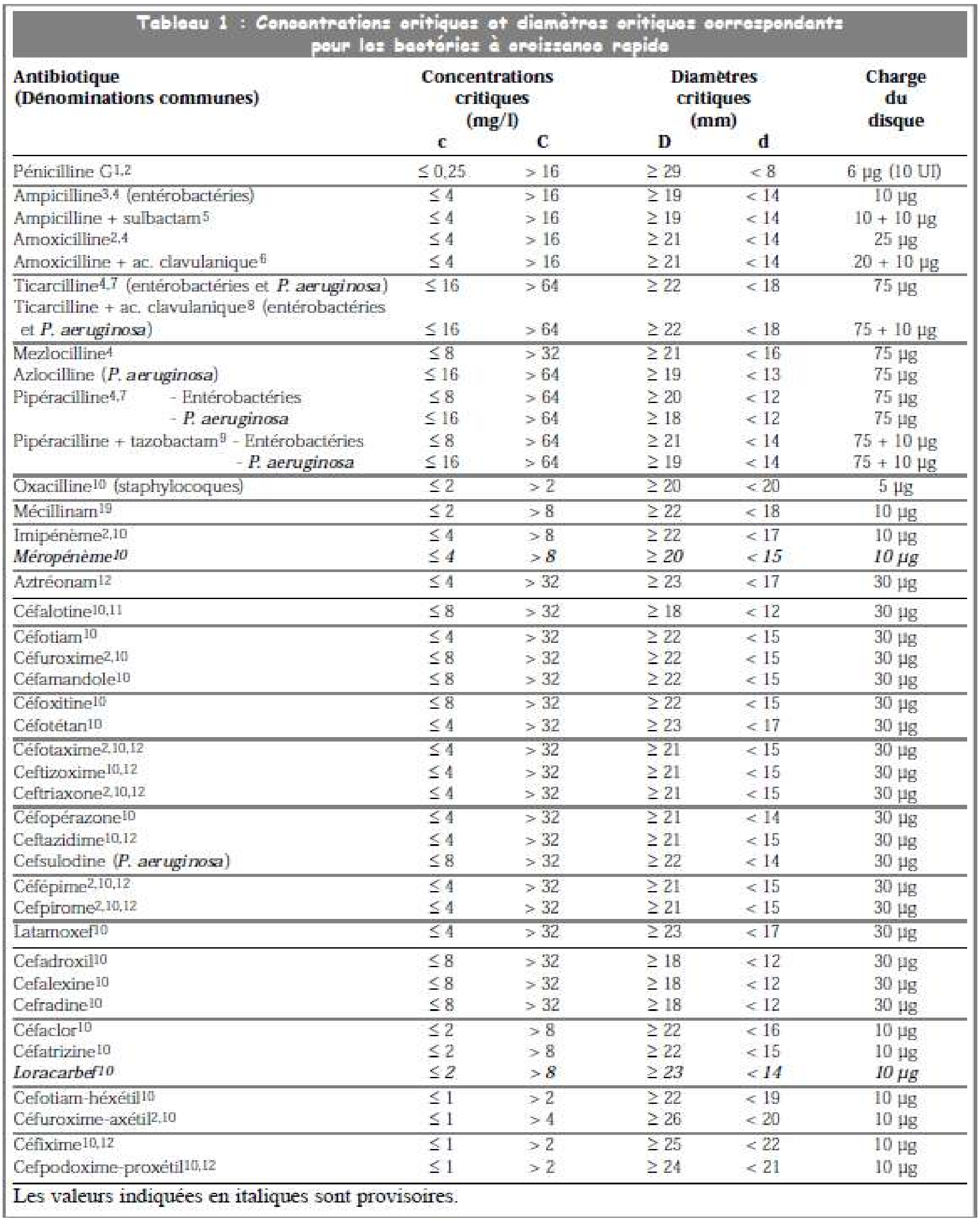


Tableau 1 : Concentrations critiques et diamètres critiques correspondants pour les bactéries à croissance rapide

Antibiotique (Dénominations communes)	Concentrations critiques (mg/l)		Diamètres critiques (mm)		Charge du disque
	c	C	D	d	
Pénicilline G[1,2]	≤ 0,25	> 16	≥ 29	< 8	6 µg (10 UI)
Ampicilline[3,4] (entérobactéries)	≤ 4	> 16	≥ 19	< 14	10 µg
Ampicilline + sulbactam[5]	≤ 4	> 16	≥ 19	< 14	10 + 10 µg
Amoxicilline[2,4]	≤ 4	> 16	≥ 21	< 14	25 µg
Amoxicilline + ac. clavulanique[6]	≤ 4	> 16	≥ 21	< 14	20 + 10 µg
Ticarcilline[4,7] (entérobactéries et *P. aeruginosa*)	≤ 16	> 64	≥ 22	< 18	75 µg
Ticarcilline + ac. clavulanique[8] (entérobactéries et *P. aeruginosa*)	≤ 16	> 64	≥ 22	< 18	75 + 10 µg
Mezlocilline[4]	≤ 8	> 32	≥ 21	< 16	75 µg
Azlocilline (*P. aeruginosa*)	≤ 16	> 64	≥ 19	< 13	75 µg
Pipéracilline[4,7] - Entérobactéries	≤ 8	> 64	≥ 20	< 12	75 µg
- *P. aeruginosa*	≤ 16	> 64	≥ 18	< 12	75 µg
Pipéracilline + tazobactam[9] - Entérobactéries	≤ 8	> 64	≥ 21	< 14	75 + 10 µg
- *P. aeruginosa*	≤ 16	> 64	≥ 19	< 14	75 + 10 µg
Oxacilline[10] (staphylocoques)	≤ 2	> 2	≥ 20	< 20	5 µg
Mécillinam[19]	≤ 2	> 8	≥ 22	< 18	10 µg
Imipénème[2,10]	≤ 4	> 8	≥ 22	< 17	10 µg
Méropénème[10]	*≤ 4*	*> 8*	*≥ 20*	*< 15*	*10 µg*
Aztréonam[12]	≤ 4	> 32	≥ 23	< 17	30 µg
Céfalotine[10,11]	≤ 8	> 32	≥ 18	< 12	30 µg
Céfotiam[10]	≤ 4	> 32	≥ 22	< 15	30 µg
Céfuroxime[2,10]	≤ 8	> 32	≥ 22	< 15	30 µg
Céfamandole[10]	≤ 8	> 32	≥ 22	< 15	30 µg
Céfoxitine[10]	≤ 8	> 32	≥ 22	< 15	30 µg
Céfotétan[10]	≤ 4	> 32	≥ 23	< 17	30 µg
Céfotaxime[2,10,12]	≤ 4	> 32	≥ 21	< 15	30 µg
Ceftizoxime[10,12]	≤ 4	> 32	≥ 21	< 15	30 µg
Ceftriaxone[2,10,12]	≤ 4	> 32	≥ 21	< 15	30 µg
Céfopérazone[10]	≤ 4	> 32	≥ 21	< 14	30 µg
Ceftazidime[10,12]	≤ 4	> 32	≥ 21	< 15	30 µg
Cefsulodine (*P. aeruginosa*)	≤ 8	> 32	≥ 22	< 14	30 µg
Céfépime[2,10,12]	≤ 4	> 32	≥ 21	< 15	30 µg
Cefpirome[2,10,12]	≤ 4	> 32	≥ 21	< 15	30 µg
Latamoxef[10]	≤ 4	> 32	≥ 23	< 17	30 µg
Cefadroxil[10]	≤ 8	> 32	≥ 18	< 12	30 µg
Cefalexine[10]	≤ 8	> 32	≥ 18	< 12	30 µg
Cefradine[10]	≤ 8	> 32	≥ 18	< 12	30 µg
Céfaclor[10]	≤ 2	> 8	≥ 22	< 16	10 µg
Céfatrizine[10]	≤ 2	> 8	≥ 22	< 15	10 µg
Loracarbef[10]	*≤ 2*	*> 8*	*≥ 23*	*< 14*	*10 µg*
Cefotiam-héxétil[10]	≤ 1	> 2	≥ 22	< 19	10 µg
Céfuroxime-axétil[2,10]	≤ 1	> 4	≥ 26	< 20	10 µg
Céfixime[10,12]	≤ 1	> 2	≥ 25	< 22	10 µg
Cefpodoxime-proxétil[10,12]	≤ 1	> 2	≥ 24	< 21	10 µg

Les valeurs indiquées en italiques sont provisoires.

ANNEXES

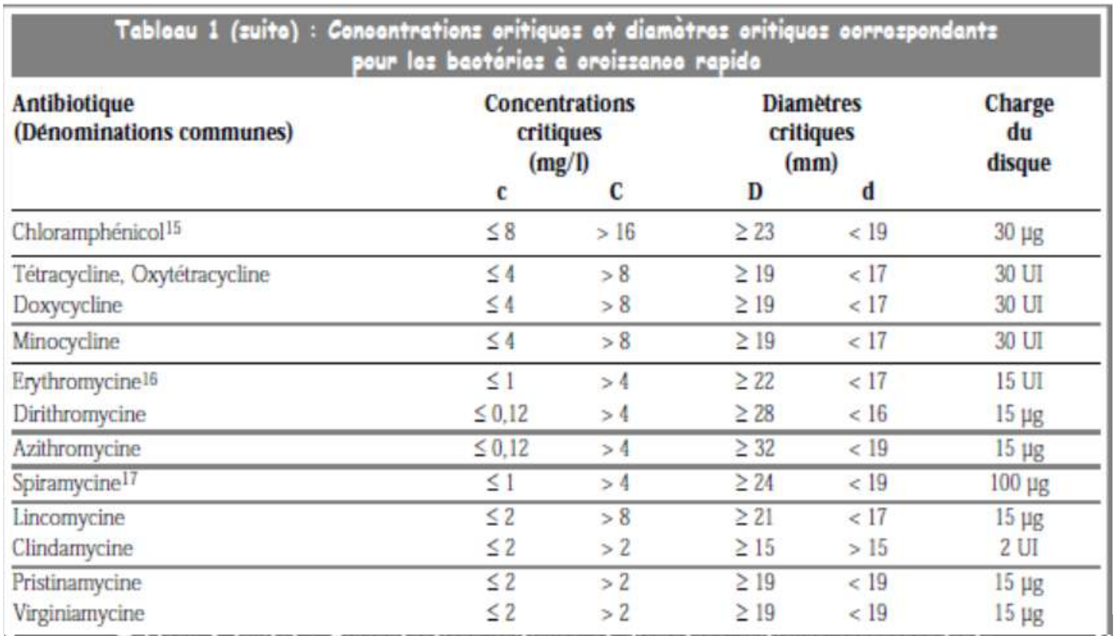


Tableau 1 (suite) : Concentrations critiques et diamètres critiques correspondants pour les bactéries à croissance rapide

Antibiotique (Dénominations communes)	Concentrations critiques (mg/l)		Diamètres critiques (mm)		Charge du disque
	c	C	D	d	
Chloramphénicol[15]	≤ 8	> 16	≥ 23	< 19	30 µg
Tétracycline, Oxytétracycline	≤ 4	> 8	≥ 19	< 17	30 UI
Doxycycline	≤ 4	> 8	≥ 19	< 17	30 UI
Minocycline	≤ 4	> 8	≥ 19	< 17	30 UI
Erythromycine[16]	≤ 1	> 4	≥ 22	< 17	15 UI
Dirithromycine	≤ 0,12	> 4	≥ 28	< 16	15 µg
Azithromycine	≤ 0,12	> 4	≥ 32	< 19	15 µg
Spiramycine[17]	≤ 1	> 4	≥ 24	< 19	100 µg
Lincomycine	≤ 2	> 8	≥ 21	< 17	15 µg
Clindamycine	≤ 2	> 2	≥ 15	> 15	2 UI
Pristinamycine	≤ 2	> 2	≥ 19	< 19	15 µg
Virginiamycine	≤ 2	> 2	≥ 19	< 19	15 µg

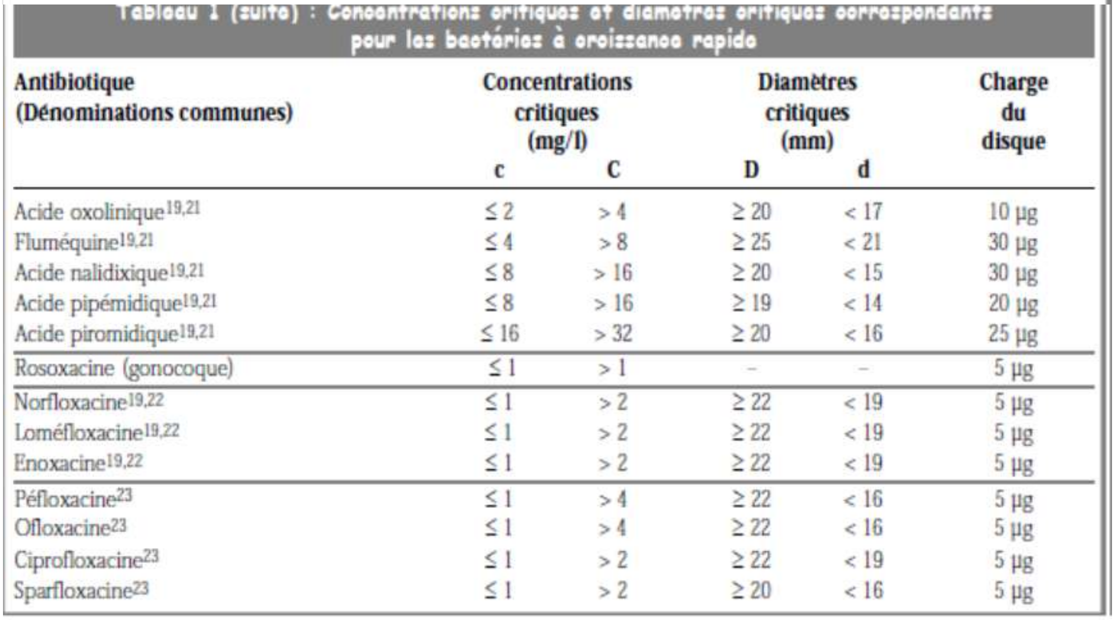


Tableau 1 (suite) : Concentrations critiques et diamètres critiques correspondants pour les bactéries à croissance rapide

Antibiotique (Dénominations communes)	Concentrations critiques (mg/l)		Diamètres critiques (mm)		Charge du disque
	c	C	D	d	
Acide oxolinique[19,21]	≤ 2	> 4	≥ 20	< 17	10 µg
Fluméquine[19,21]	≤ 4	> 8	≥ 25	< 21	30 µg
Acide nalidixique[19,21]	≤ 8	> 16	≥ 20	< 15	30 µg
Acide pipémidique[19,21]	≤ 8	> 16	≥ 19	< 14	20 µg
Acide piromidique[19,21]	≤ 16	> 32	≥ 20	< 16	25 µg
Rosoxacine (gonocoque)	≤ 1	> 1	–	–	5 µg
Norfloxacine[19,22]	≤ 1	> 2	≥ 22	< 19	5 µg
Loméfloxacine[19,22]	≤ 1	> 2	≥ 22	< 19	5 µg
Enoxacine[19,22]	≤ 1	> 2	≥ 22	< 19	5 µg
Péfloxacine[23]	≤ 1	> 4	≥ 22	< 16	5 µg
Ofloxacine[23]	≤ 1	> 4	≥ 22	< 16	5 µg
Ciprofloxacine[23]	≤ 1	> 2	≥ 22	< 19	5 µg
Sparfloxacine[23]	≤ 1	> 2	≥ 20	< 16	5 µg

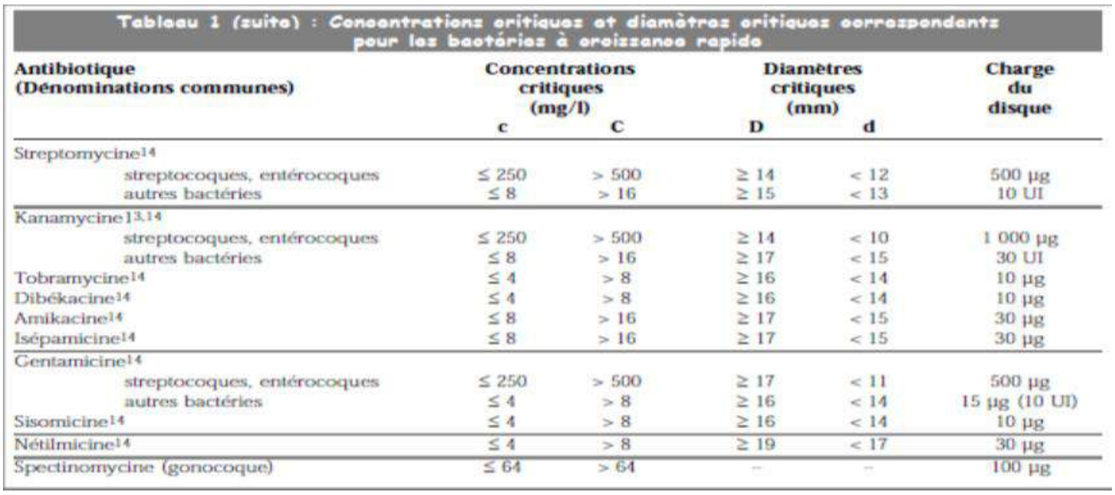


Tableau 1 (suite) : Concentrations critiques et diamètres critiques correspondants pour les bactéries à croissance rapide

Antibiotique (Dénominations communes)	Concentrations critiques (mg/l)		Diamètres critiques (mm)		Charge du disque
	c	C	D	d	
Streptomycine[14]					
streptocoques, entérocoques	≤ 250	> 500	≥ 14	< 12	500 µg
autres bactéries	≤ 8	> 16	≥ 15	< 13	10 UI
Kanamycine[13,14]					
streptocoques, entérocoques	≤ 250	> 500	≥ 14	< 10	1 000 µg
autres bactéries	≤ 8	> 16	≥ 17	< 15	30 UI
Tobramycine[14]	≤ 4	> 8	≥ 16	< 14	10 µg
Dibékacine[14]	≤ 4	> 8	≥ 16	< 14	10 µg
Amikacine[14]	≤ 8	> 16	≥ 17	< 15	30 µg
Isépamicine[14]	≤ 8	> 16	≥ 17	< 15	30 µg
Gentamicine[14]					
streptocoques, entérocoques	≤ 250	> 500	≥ 17	< 11	500 µg
autres bactéries	≤ 4	> 8	≥ 16	< 14	15 µg (10 UI)
Sisomicine[14]	≤ 4	> 8	≥ 16	< 14	10 µg
Nétilmicine[14]	≤ 4	> 8	≥ 19	< 17	30 µg
Spectinomycine (gonocoque)	≤ 64	> 64	–	–	100 µg

ABSTRACT

ABSTRACt

Title: Optimization of *Sesbania* production using rhizobial strains adapted to Saharan soils

ABSTRACT

Rhizobial strains were isolated from root nodules of *Sesbania aculeata* plants grown in Algerian desert soils, using material from the 2023 production season (ITAS and Chott stations). Their tolerance to abiotic stresses (temperature, salinity, and pH) was tested in vitro. Chott strains showed greater high-temperature tolerance, while all strains tolerated salinity similarly and grew best at pH 6–9.Symbiotic performance was assessed under field-relevant stress (2.34 ds·m^{-1} salinity, pH 6.31) using Leonard jars with solid and liquid inoculants. Solid inoculants, especially CHATT N1, promoted higher nodulation and plant biomass. Nitrogen metabolism profiling revealed that ITAS strains preferred proline and asparagine, while Chott N1 utilized arginine more effectively. Antibiotic sensitivity tests showed Chott strains were more sensitive, while ITAS strains exhibited greater resistance. These results highlight the potential of native rhizobia as stress-tolerant bio-inoculants to enhance *Sesbania aculeata* cultivation in arid environments.

Key words : **Rhizobia, abiotic stress, nitrogen metabolism, symbiotic efficiency, bio-inoculants, *Sesbania aculeata*.**

TITRE: Optimisation de la production de la s*esbania* par l'utilisatin de ouches rhizobiennes adaptees aux sols saharien

RESUME :

Des souches rhizobiennes isolées de *Sesbania aculeata* cultivée dans les zones désertiques d'Algérie (stations ITAS et Chott, campagne 2023) ont montré une bonne tolérance au pH (6–9) et à la salinité (jusqu'à 400 mM), avec une meilleure tolérance thermique chez les souches Chott (croissance à 45 °C). En pots de Leonard, l'inoculation solide, notamment avec CHATT N1, a induit la plus forte nodulation et biomasse. Métaboliquement, les souches ITAS préféraient la proline et l'asparagine, tandis que CHATT N1 utilisait mieux l'arginine. Les souches Chott étaient plus sensibles aux antibiotiques, contrairement aux souches ITAS plus résistantes. Ces résultats confirment le potentiel des rhizobiums autochtones pour améliorer la culture de *Sesbania aculeata* en zones arides.

Mots clés : Rhizobium, stress abiotiques, métabolisme de l'azote, efficacité symbiotique, bio-inoculants, (*Sesbania aculeata*).

. العنوان : تحسين إنتاج باستخدام سلالات الريزوبيا المتكيفة*سيسبانيا اكيلياتا*.(*Sesbania aculeata* مع التربة الصحراوية

ملخص:

تم عزل سلالات من تمّ عزل سلالات بكتيرية عقدية من جذور نبات السيسبانيا المزروع في التربة الصحراوية الجزائرية، باستخدام بذور وعُقد من إنتاج موسم سنة ٢٠٢٣، من محطّتي إيتاس وشط. وقد تم تقييم قدرة هذه السلالات على تحمّل الظروف البيئية غير الملائمة مثل درجات الحرارة المرتفعة والملوحة وتغيّر درجة الحموضة في ظروف مخبرية. أظهرت سلالات الشط تحمّلاً أفضل للحرارة المرتفعة، بينما أبدت جميع السلالات قدرة متقاربة على تحمّل الملوحة، وسُجّل أفضل نمو لها في درجات حموضة معتدلة تتراوح بين ٦ و٩. تم اختبار الفعالية التكافلية لهذه السلالات في أواني معقّمة باستخدام وسط غذائي، من خلال نوعين من التلقيح: أحدهما صلب يحتوي على خليط من الخث والفحم النباتي والسلالات والبذور، والآخر سائل يحتوي على السلالات والبذور فقط. أظهر التلقيح الصلب، خاصة مع السلالة شط ١، نتائج متميزة في عدد العُقد الجذرية ووزنها الجاف، مع زيادة ملحوظة في نمو الجذور والسيقان مقارنة بالنباتات غير المُلقّحة. كما بيّنت التحاليل الحيوية وجود اختلاف في قدرة السلالات على استهلاك مصادر النيتروجين؛ حيث فضّلت سلالات إيتاس البرولين والأسباراجين، بينما أظهرت سلالة شط ١ قدرة أكبر على استهلاك الأرجنين. أما في اختبارات مقاومة المضادات الحيوية، فقد اتّضح أن سلالات الشط أكثر حساسية، في حين أبدت سلالات إيتاس مقاومة أعلى.

تؤكد هذه النتائج أن السلالات البكتيرية المحلية تملك قدرة عالية على تحمّل الإجهادات البيئية، وتُظهر فعالية في التلقيح الحيوي، مما يجعلها خياراً واعداً لتحسين زراعة السيسبانيا في المناطق الصحراوية.

الكلمات المفتاحية: السيسبانيا، البكتيريا العقدية، التربة الصحراوية، الإجهادات البيئية، التكافل الحيوي، التلقيح الحيوي.