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TITLE

Development of a NanoBio Herbicide (Phytotoxic Plant Extract) for controlling *Setaria verticillata*

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In the name of Allah, the Most Gracious, the Most Merciful.

"All praise is due to Allah, by whose grace good deeds are accomplished."

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الإهداء

لكل من خانته العزائم ..

لكل من حركت الرياح سفينته دون رغبته فوقف ينظر دون فعل شيء ..

لكل من رأى نهاية عمله قبل أن يبدأ فاتخذ من خدود الحيطان مخبأ و انتكس ..

لك مني هذه الرسالة: ما كان الله ليقذف بها لك لو لم تكن انت لها.

ختام عملنا هدية لكل من ساهم و قاسم بعمل، بنصيحة، بكلمة فبدعاء

لكم أهدي عملي وجهدي وتعبي بارك الله لكم وفيكم

أدامكم الله لي ولي وحدي

لك أمي التي لم تنجبني " طاطا" جزيل شكري وامتناني وعرفاني ما كان شكري ليوفي ربع حقك فاعذريني. جعلك الله منارة علم وركيزة دين ورزقك من كل طيب يا طيبة الأخلاق يا طيبة الأعراق. لأخوالي "لحمادي، العيد" لكم مني جزيل شكري وامتناني فقد كنتم مصدر الإلهام ومنبع الأفكار.

لجميع خالاتي ولك أمي مباركة و أمي سعيدة أخط بأناملي حروف شكري فشكرا وشكرا ورحم الله من أنجبكن.

أشكرك " عمي لخضر " لما كان لك من أثر على هذا العمل فأنت موردي وناصحي يوم تعثرت بي الكلمات فشكرا لك وبارك الله فيك.

ختام الكلام مسك وأي مسك كوالديّ المحبين الشاهدين على ما بذلته في هذا العمل؛ فشكرا لكما على ما بذلتماه طيلة حياتي من جهد وصبر ووقت شكرا.

ابنتكم المحبة دوما

أسماء

الإهداء

أهدي هذا العمل إلى كل من مدّ يد العون بكلمة أو ساهم بنصيحة وتوجيه في إنجاز هذا البحث لكم مني كل الشكر والامتنان.

فاطمة

Abstract

Modern agricultural systems in the Souf Valley region face increasing challenges due to the spread of weeds, primarily *Setaria verticillata*, which possesses a remarkable physiological ability to compete with strategic crops and deplete water resources. Despite the historical reliance on manufactured chemical herbicides, field data and farmer surveys have demonstrated their declining effectiveness. The weed exhibits severe cellular resistance to common commercial herbicides (such as Select), particularly in advanced phenological stages (flowering and seed formation), in addition to the environmental risks resulting from their toxic accumulation in permeable desert soils.

This study aims to develop a sustainable bio-herbicide alternative by synthesizing a complete plant extract combining *Mentha piperite*, *Rosmarinus officinalis* , and *Artemisia herb-alba* . Chemical analysis revealed a strong synergistic effect of flavonoids, alkaloids, and surfactants in necrosing and killing the weed. To enhance stability and adhesion, the active mixture was loaded onto cellulose nanocrystals (CNCs). The developed bio-nano-herbicide demonstrated superior and sustained field control efficacy with reduced active ingredient dosages. Biological tests have also confirmed the complete safety of the compound and its lack of negative impact on the soil microbiome (bacteria and fungi), thus providing a smart applied model that integrates nanotechnology with agricultural sustainability in arid regions.

Kay words: Oued souf, *Setaria verticillata,* NanoBio Herbicide, Plant Extract,Phytotoxicity

Résumé

Les systèmes agricoles modernes de la région de la vallée du Sudf sont confrontés à des défis croissants en raison de la prolifération des adventices, principalement *Setaria verticillata*, qui possède une remarquable capacité physiologique à concurrencer les cultures stratégiques et à épuiser les ressources en eau. Malgré le recours historique aux herbicides chimiques de synthèse, les données de terrain et les enquêtes auprès des agriculteurs ont démontré leur efficacité décroissante. Cette adventice présente une forte résistance cellulaire aux herbicides commerciaux courants (tels que Select), en particulier aux stades phénologiques avancés (floraison et formation des graines), sans compter les risques environnementaux liés à leur accumulation toxique dans les sols désertiques perméables.

Cette étude vise à développer une alternative bioherbicide durable en synthétisant un extrait végétal complet combinant *Mentha piperita, Rosmarinus officinalis* et *Artemisia herb-alba.* L'analyse chimique a révélé un fort effet synergique des flavonoïdes, des alcaloïdes et des tensioactifs dans la nécrose et la destruction de l'adventice. Pour améliorer la stabilité et l'adhérence, le mélange actif a été chargé sur des nanocristaux de cellulose (CNC). Le bio-nano-herbicide développé a démontré une efficacité supérieure et durable sur le terrain, avec des doses réduites de matière active. Des tests biologiques ont également confirmé l'innocuité totale du composé et son absence d'impact négatif sur le microbiome du sol (bactéries et champignons), offrant ainsi un modèle d'application intelligent qui intègre la nanotechnologie à une agriculture durable dans les régions arides.

Mots-clés : Oued souf, *Setaria verticillata*, NanoBio Herbicide, Extrait de plante, Phytotoxicité

ملخص

تواجه النظم الزراعية الحديثة في منطقة وادي سوف تحديات متزايدة نتيجة انتشار الأعشاب الضارة، ولا سيما نبات (*Setaria verticillata*)، الذي يتمتع بقدرة فسيولوجية ملحوظة على منافسة المحاصيل الاستراتيجية واستنزاف موارد المياه. وعلى الرغم من الاعتماد التاريخي على مبيدات الأعشاب الكيميائية المصنعة، فقد أظهرت البيانات الميدانية واستطلاعات رأي المزارعين انخفاض فعاليتها. يُظهر هذا النبات مقاومة خلوية شديدة لمبيدات الأعشاب التجارية الشائعة (مثل سيليكت)، خاصةً في المراحل الفينولوجية المتقدمة (الإزهار وتكوين البذور)، بالإضافة إلى المخاطر البيئية الناجمة عن تراكمها السام في التربة الصحراوية النفاذة.

تهدف هذه الدراسة إلى تطوير بديل حيوي مستدام لمبيدات الأعشاب من خلال تصنيع مستخلص نباتي كامل يجمع بين النعناع وإكليل الجبل والشيح. وكشف التحليل الكيميائي عن تأثير تآزري قوي للفلافونويدات والقلويدات والمواد الفعالة سطحياً في إتلاف وقتل هذا النبات. ولتعزيز الاستقرار والالتصاق، تم تحميل المزيج الفعال على بلورات نانوية من السليلوز. أظهر مبيد الأعشاب النانوي الحيوي المُطوَّر فعالية فائقة ومستدامة في مكافحة الأعشاب الضارة في الحقول مع تقليل جرعات المادة الفعالة. كما أكدت الاختبارات البيولوجية سلامة المركب تمامًا وعدم وجود أي تأثير سلبي له على الميكروبيوم الموجود في التربة (البكتيريا والفطريات)، مما يوفر نموذجًا تطبيقيًا ذكيًا يدمج تقنية النانو مع الاستدامة الزراعية في المناطق القاحلة.

الكلمات المفتاحية: وادي سوف ,*Setaria verticillata* ,مبيد حيوي نانوي ,مستخلص نباتي , السمية النباتية.

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Introduction

The agricultural sector in Algeria is a strategic pillar for achieving food security, diversifying the national economy, and promoting sustainable agricultural development. In recent years, the Algerian government has implemented several programs and initiatives to enhance agricultural production, particularly in desert regions. These regions possess unique natural and economic characteristics that offer significant opportunities for agricultural expansion and economic growth.

Among these regions, the Souf Valley stands out as one of the most successful examples of agricultural development in Algeria. Desert agriculture in the Souf Valley has experienced remarkable progress over the past decades, leading to a significant expansion of cultivated land and greater crop diversification. This development has been driven by several factors, including abundant groundwater resources and the ability of local farmers to adapt to the harsh environmental conditions that characterize the region **(ADJLANE S. et al., 2023)**.

In this context, the Souf Valley has witnessed the emergence of new agricultural activities following its integration into agricultural development programs launched in 1983. These initiatives have contributed substantially to increasing the production of several strategic crops, including potatoes, wheat, barley, and tomatoes, allowing the region to play an important role in meeting the food needs of the Algerian population. Consequently, the Souf Valley has become one of the country's leading agricultural production areas, achieving remarkable crop yields during recent agricultural seasons **(Mubaraki I., 2015)**.

However, despite these remarkable achievements, the expansion of agricultural activities has also generated several challenges. Among the most serious constraints is the widespread infestation of weeds, which represents one of the main biological factors responsible for reducing crop productivity, increasing water consumption, and degrading soil quality. Among these weeds, ***Setaria verticillata*** is considered one of the most problematic species. This annual grass is characterized by its rapid spread and its strong invasive capacity in cultivated fields. Moreover, it exhibits a remarkable physiological ability to compete with economic crops for water, nutrients, and light, making its management a major challenge that requires effective and sustainable control strategies to prevent its continuous mechanical and biological dissemination. These findings are supported by the study of **Artur A. and Isabel M. C. (2024)**.

To control this invasive weed, farmers frequently rely on chemical herbicides. However, the extensive use of these products has raised serious concerns regarding human health and environmental safety. Several scientific studies have demonstrated that chemical herbicides may contribute to respiratory disorders, chronic diseases, and significant environmental damage affecting soil organisms, beneficial plants, and wildlife **(Rad, S. et al., 2022; Parven, A. et al., 2025)**. Despite these documented risks, the use of chemical herbicides continues to increase in the Souf Valley in an attempt to control harmful weeds, including *Setaria verticillata*, thereby aggravating existing environmental and health problems.

Therefore, there is an urgent need to develop sustainable and environmentally friendly alternatives capable of reducing dependence on chemical herbicides while maintaining effective weed control. In this context, bioherbicides based on natural plant extracts and nanotechnology represent a promising approach because of their potential effectiveness and lower environmental impact.

Accordingly, the aim of this study is to develop and evaluate an effective bio-nanoherbicide for controlling *Setaria verticillata* in the Oued Souf region while reducing the environmental pollution associated with conventional chemical herbicides. More specifically, this study seeks to answer the following research questions:

- How effective is the bio-nanoherbicide formulated from the plant extracts of *Mentha piperita*, *Rosmarinus officinalis*, and *Artemisia herba-alba* in controlling and eliminating *Setaria verticillata*?
- What are the possibilities of using this bio-nanoherbicide as a safe, sustainable, and environmentally friendly alternative to conventional chemical herbicides?

To address these questions, this thesis is divided into two complementary sections. The theoretical section reviews the fundamental concepts related to the studied plants, weeds, nanotechnology, and bioherbicides. The applied section presents the experimental procedures, the protocols used to prepare the bio-nanoherbicide, and the results obtained from evaluating its effectiveness against *Setaria verticillata*.

Theoretical Part

ChapterI:

Weed of *Setaria verticillata*

1.Definition of weed:

Although there is no universally accepted definition, a weed is generally defined from a human perspective as a plant that is out of place. This means that a plant considered a weed in one location may not be considered one in another. **(Rana, S. S. 2020)** Other official views and definitions include:

Human Preference: Historically, weeds have been defined as plants growing in undesirable places. A. E. Georgia (1916) defined them more precisely as plants growing in places where other plants are desired. **(Rana, S. S. 2020)**

Interference: The Weed Science Society of America (WSSA) defines a weed as any plant that is undesirable or interferes with human activities or well-being. Similarly, the European Weed Research Society defines it as any vegetation cover, excluding fungi, that interferes with people's goals or needs. **(Rana, S. S. 2020)**

Ecological Role: From an ecological perspective, weeds are considered secondary succession pioneers or opportunistic species that grow after human-induced disturbances in habitats. They are also defined as plants that have evolved to interfere with crops and human activities**. (Rana, S. S. 2020) (Vallotton, A. (n.d.). 2005)**

Biological characteristics: Some scientists define weeds by their behavior, noting their exceptional tenacity, harmful nature, and high capacity to occupy areas disturbed by human activity**. (Vallotton, A. (n.d.). 2005)**

Agricultural impact: In agriculture, they are often referred to as invasive plants (plantes adventices), species that grow spontaneously in human-modified environments and compete with cultivated crops for vital resources such as water, nutrients, and light **.(Hinana, I et *al* 2017. Achour, L. 2005).**

Philosophical perspective: Ralph Waldo Emerson described the weed as "a plant whose virtues have yet to be discovered."**(Rana, S. S. 2020)**

2. *Setaria verticillata*:

2.1 Definition *Setaria verticillata*, commonly known as bristly foxtail, is a C4 grass species considered a serious weed in at least 11 countries across Europe, Asia, Africa, South America, and the Pacific (Figure 1) **(Kazemi, F. 2023)**.

It originates from Europe and possibly Africa and has spread widely due to its sticky seeds that facilitate dispersal and its rapid growth rate, enabling it to dominate crops and grasslands; it also adapts rapidly to local conditions and has developed resistance to some herbicides **(Parker, 2022)**. Morphologically, *S. verticillata* is distinguished by the presence of bristles in its inflorescence (Figure 2) and shows significant phenotypic variation useful for taxonomic differentiation from related species **(Palakurthi et *al*., 2022).** Genetically, it is a tetraploid species containing both A and B genomes, which differentiates it from related Setaria species such as *S. italica* and *S. viridis* that have only the A genome **(Benabdelmouna, et *al*, 2001)**; this genomic composition clarifies its taxonomic position within the genus.

Additionally, aqueous extracts of S. verticillata inhibit seed germination percentage and seedling growth in wheat varieties at higher concentrations **(Pyar, et *al*, 2019)**, and the species has been identified as a host for phytoplasma pathogens in India **(Mall, et *al* 2020).**

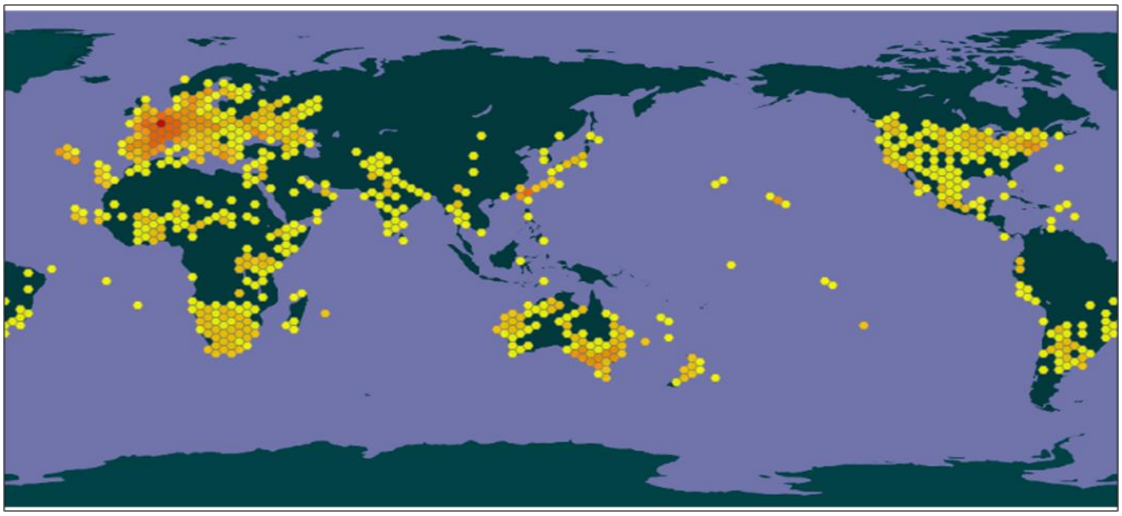

Figure 1: Global geographical distribution of *Setaria verticillata* (GBIF Secretariat, 2023)

Figure 2***:*** Inflorescence of *Setaria verticillata* (Hyde, M.et *al.*, 2026)

2.2 Botanical classification

Setaria verticillata (Figure 3), belongs to the genus Setaria within the family Poaceae, subfamily Panicoideae, and tribe Paniceae. It is characterized by unique phytolith signatures and epidermal patterns that aid in its taxonomic identification, including a novel "Λ" (Lambda) type of undulated ornamentation on leaf surfaces **(Bhat, M. A. et *al*., 2018).** Its botanical classification places it firmly within the foxtail group of grasses, distinguished by specific morphological and biochemical traits of phytoliths found in various plant parts **(Bhat, M. A.et *al*., 2018).** Although detailed molecular phylogenetic studies focus more broadly on Setaria species, *S. verticillata*'s distinct morphological features support its clear taxonomic status within the genus **(Bhat, M. A.et *al*. 2018, Quaedvlieg, W.et *al.*, 2013).**

Figure 3: *Setaria verticillata (L)* P Beauv (Pierre coulot, 2022).

2.3 Structural Characteristics

The physical structure of Setaria verticillata is designed for both resilience and effective seed dispersal (Figure 4):

- **Stems and Roots**: The plant features fibrous roots and erect stems (culms) that typically range from 0.3 to 1.2 meters tall. These stems often branch more than those of related foxtail species and are "geniculately ascending," meaning they are bent like a knee at the lower nodes**. (NZPCN.2024).**
- **Leaves:** The leaf blades are broadly linear, 5–30 cm long, and 4–16 mm wide. They are described as flaccid and scabrous (rough) on both surfaces. The leaf sheaths have ciliate hairs on their margins, a feature used to distinguish them from tropical relatives) **(Franke, A. C, et *al*. 2009, NZPCN.2024).**
- **Panicles and Spikelets:** The inflorescence is a dense, spiciform panicle between 2 and 15 cm long. It is characterized by spaced clusters of spikelets, particularly at the base. The spikelets themselves are ellipsoid and approximately 1.5–2.5 mm long. **(Franke, A. C, et *al*., 2009 . Kamran, I.,2015)**
-

- **Barbed Bristles**: A defining structural feature is the presence of one to three bristles at the base of each spikelet. These bristles are retrorsely barbed (hooked), which makes the seed heads feel rough and causes them to stick tenaciously to surfaces like "Velcro **". (NZPCN.2024).**

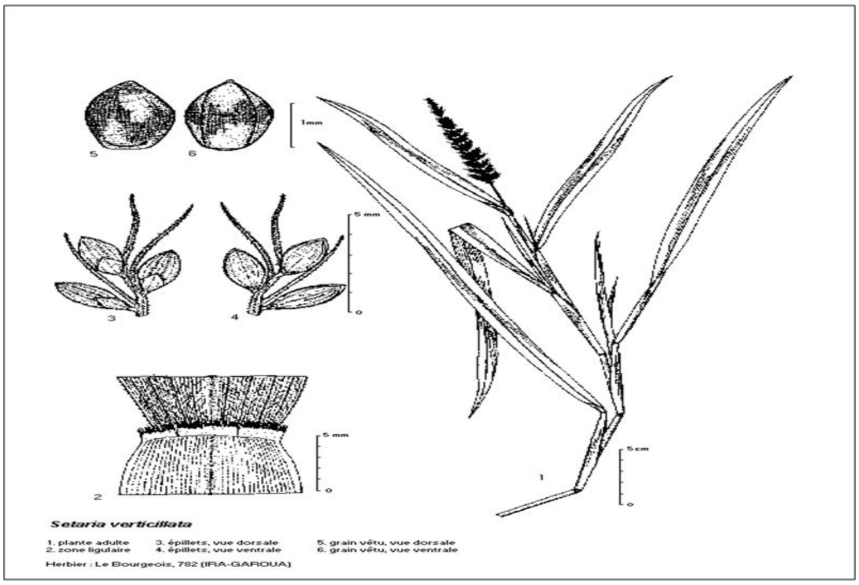

Figure 4: Anatomy of the plant *Setaria verticillate* (SETVE, 2016)

2.4 Germination

Setaria verticillata seeds germinate optimally at temperatures around 30.5 °C under light/dark conditions, with maximum seedling emergence occurring when seeds are sown on the soil surface; emergence declines sharply with increasing sowing depth, reaching zero at 6 cm depth 1. Seed dormancy is induced during embryogenesis, producing long-lived heterogeneous seed banks in the soil; seedling emergence timing is regulated by environmental signals such as soil oxygen, moisture, and temperature, which communicate through a complex soil-seed system to stimulate embryo respiration and growth **(Dekker, J., 2003) ., Dekker, J. 2013).** After germination, the plant develops tillering shoots whose number and stature adjust plastically to local conditions, optimizing stem length and branching patterns to maximize growth and reproduction**(Dekker, J., 2003) .** Stem length can vary depending on environmental factors and competition, with phenotypic plasticity allowing adaptation in plant architecture including leaf development to suit local habitats **(Dekker, J., 2003) .** Seed production occurs after vegetative growth and flowering; however, salt stress above 75 mM NaCl significantly reduces dry matter accumulation in roots and shoots as well as seed viability, indicating sensitivity during both vegetative and reproductive phases **(Ben Ahmed et *al.*, 2008).** The species spreads effectively through sticky seed heads that facilitate dispersal across disturbed sites, enabling rapid colonization and persistence in diverse environments **(Dekker, J., 2003) .**

2.5 Mechanisms of Spread

Setaria verticillata spreads primarily through its highly effective seed dispersal mechanisms:

- **Animal and Human Transport:** Because the retrorse barbs on the bristles stick to fur, feathers, and clothing, seeds are often carried great distances by humans and animals **(Ben Ahmed.et *al* 2008)**.
- **Adherence:** The "sticky" nature of the panicles causes seed heads to often become entangled with one another or with other vegetation, facilitating movement through physical contact **(ISSG, 2010**).
- **Seed Rain:** The plant produces a heterogeneous seed rain (1 to 12,000 seeds per plant), creating long-lived seed pools in the soil that ensure survival over many seasons **(Franke, A. C, et *al*. 2009).**

2.6 Evolution

The evolution of Setaria verticillata is marked by its ability to rapidly adapt to local environments through genetic mechanisms and structural advancements, though it has also developed significant resistance to certain classes of chemical herbicides.

2.6.1 Evolutionary Origin and Genetic Structure

- **Polyploidization:** Setaria verticillata is hypothesized to be the evolutionary product of chromosome doubling (polyploidization) of Setaria viridis (green foxtail) **(Franke, A. C, et al. 2009)**
- **Genetic Diversity:** The species is characterized by unusually low genetic diversity within a single population but very high diversity between different populations. This is primarily attributed to its high level of self-pollination, combined with genetic drift and founder effects **(Franke, A. C, et al. 2009).**
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- **Evolutionary Hierarchy:** In the evolutionary hierarchy of the Setaria genus, S. verticillata occupies an intermediate position. It possesses "advanced" characteristics, such as cross-shaped silica bodies, while retaining "primitive" traits like dome-shaped subsidiary cells in its leaf epidermis**.(Kamran, I.,2015)**
- **Adaptive Plasticity:** A key driver of its evolutionary success is its phenotypic plasticity, which allows the plant to adapt its growth habits (such as height and leaf length) and seed germination timing in response to environmental stresses like shade, drought, or competition **(Kamran, I., 2015)**.

2.6.2 Evolution of Herbicide Resistance

Setaria verticillata has evolved resistance to several chemical herbicides, particularly those used in large-scale agriculture:

- **Photosystem II Inhibitors (Group C 1/5):** The species has developed widespread resistance to this category of herbicides. Specifically, resistance to atrazine has been documented in Spain, and resistance to triazine has been observed in the United States **(Kamran, I., 2015)**.
- **Mechanisms of Survival:** Its resistance is often linked to its adaptable seed emergence patterns. By spreading out seedling emergence over a long period, the plant can "escape" early-season herbicide applications, ensuring that some individuals survive to reproduce even after chemical treatment **(Franke, A. C, et *al*., 2009)**.
- **Resilience to Control:** Beyond chemical resistance, the herb is evolutionarily resilient to mechanical damage and drought, further complicating management efforts in agricultural settings like corn crops, where it can cause significant damage **(Franke, A. C, et al. 2009)**.

While related species like Setaria viridis have developed resistance to a broader range of chemicals—including trifluralin, fenoxaprop-p-ethyl, and sethoxydim—current records for *S. verticillata* focus primarily on its robust resistance to triazines and other Photosystem II inhibitors.

2.6.3 Ecological Escape and Germination Patterns

A critical "defense" mechanism for *S. verticillata* is its ability to bypass chemical treatments through its germination behavior:

- **Variable Seed Dormancy:** Seeds produced by a single plant do not all germinate at the same time. This heterogeneous seed pool ensures that seedling emergence is spread out over a long period **(ISSG, 2010, Franke, A. C, et al. 2009)**.

- **Timing of Emergence:** Because germination requirements are highly variable, the plant can "escape" early-season herbicide applications by delaying its emergence until after the chemical's active period has passed **(ISSG, 2010, Franke, A. C, et *al*., 2009)**.

2.6.4 Genetic and Adaptive Strategies

- **Rapid Local Adaptation:** The species is known for its ability to adapt to local conditions rapidly. This trait allows populations to evolve defenses specific to the herbicide regimes used in particular agricultural regions **(Ben Ahmed.et *al.*, 2008).**
- **Genetic Diversity**: While genetic diversity within a single population is low, there is huge genetic diversity between different populations. This broad genetic pool across the species increases the likelihood that some populations will possess or develop mutations that confer resistance to new chemical threats **(Franke, A. C, et *al*., 2009).**
- **General Resilience**: Beyond chemical resistance, the herb is evolutionarily resilient to mechanical damage and drought, which further ensures its survival in managed agricultural environments where herbicides are used **(Franke, A. C, et *al*., 2009).**

2.7 Agronomic Impacts

Setaria verticillata is considered one of the most problematic weed groups interfering with world agriculture.

- **Competition and Yield Loss:** It is a major invasive weed that flourishes in agricultural fields, grasslands, and arable land. It exhibits high **phenotypic plasticity**, allowing it to adapt its growth and reproduction based on available nutrients, light, and water, which enables it to outcompete desirable plants. Its presence is known to cause significant **damage to corn (maize) crops**.
- **Herbicide Resistance:** A major challenge in managing *S. verticillata* is its development of **resistance to Photosystem II inhibitors**, specifically atrazine and other C 1/5 herbicides. Resistance has been documented in both Spain and the United States.
- **Pathogen Reservoir:** It serves as an alternative host for numerous agricultural pests and diseases, which complicates crop protection:

    - **Viruses:** It is a reservoir for **wheat spot mosaic**, **barley stripe mosaic**, **wheat streak mosaic**, and **maize rough dwarf** viruses.

  - **Fungal and Bacterial Diseases:** It is a host for **yellow dwarf diseases in rice** and ergot (*Claviceps fusiformis*).
  - **Insects and Nematodes:** It hosts the thrips *Anaphothrips swezeyi* and various nematodes, including **Meloidogyne** sp. and **Pratylenchus pratensis**.
- **Interference with Operations:** Like many weeds, it can interfere with cultural operations and mechanical harvesting, leading to increased labor and equipment costs.

2.8 Ecological Impacts

The ecological threat of *S. verticillata* stems from its invasive nature and its ability to dominate disturbed environments.

- **Displacement of Native Species:** Its aggressive growth and adaptability allow it to **displace native grasses**, thereby reducing local biodiversity.
- **Invasive Dispersal:** The plant's seed heads have bristles with retrorse barbs that cause them to **stick to humans and animals like 'Velcro'**. This mechanism allows seeds to be carried over great distances, facilitating rapid spread into new territories.
- **Persistence in the Soil:** It forms long-lived, heterogeneous seed pools. Seeds can remain viable in the soil for an extended period, with a maximum recorded **longevity of 39 years**.
- **Environmental Resilience:** The seeds are highly resilient to temperature extremes, surviving **cold as low as -50°C** and **heat from burning up to 100°C** for short durations. Although it is very sensitive to salinity—which can inhibit its growth and grain production—it remains selective for potassium, allowing it to survive in various soil types.

2.9 Natural bioherbicides for *Setaria verticillata* (bristly foxtail)

Research has identified several plant-derived essential oils and extracts that can act as natural herbicides against *Setaria verticillata* or closely related foxtail species. Most work is at laboratory or greenhouse scale but shows strong inhibition of germination and early growth (Table 1).

- **Direct bioherbicides against *Setaria verticillata***

  - **Ferulago campestris essential oil**
    - Essential oil rich in myrcene, α-pinene and γ-terpinene showed **selective antigerminative activity** on *S. verticillata* seeds, strongly inhibiting germination

and radicle elongation while not affecting common bean (*Phaseolus vulgaris*) seeds **(Ascrizzi et *al.*, 2021).**

- Applied as a seed-coating (in chitosan) it combined weed suppression with bean seed protection from an insect pest **(Ascrizzi et *al.*, 2021).**

- **Ficus carica (fig) leaf aqueous extract**
    - Strong, dose-dependent inhibition of *S. verticillata* germination (≈46% reduction) and root growth (≈89% reduction); foliar sprays reduced shoot and root growth and leaf number by up to **100%** in *S. verticillata* **(Mergby et *al.*, 2025).**
    - Only slight, non-significant effects on tested crops, indicating promising selectivity **(Mergby et *al.*, 2025).**

Table 1: Natural agents tested against foxtail weeds.

Bioherbicide source	Target foxtail species	Main effect at tested doses	Citations
Artemisia argyi water extract	***Setaria viridis***	**Inhibited seed germination and seedling growth**	**(Li et *al.*, 2021)**
Asteraceae essential oils	***Setaria viridis***	**100% germination inhibition with some oils**	**(Benvenuti et *al.*, 2017)**
Sinapis alba seed meal extract	***Setaria viridis***	**Bioherbicidal activity pre- and post-emergence**	**(Dolianitis et *al.*, 2025)**
Pseudomonas fluorescens BRG100	***Setaria viridis* (green foxtail)**	**Bacterial bioherbicide targeting green foxtail**	**(Raza et *al.*, 2025)**

Broader context and mode of action

- Essential oil components like terpinen-4-ol, lavandulol and linalyl acetate completely inhibited germination and root growth of bristly foxtail (*S. verticillata*) in vitro at 80–320 nL/cm³, suggesting lavender-type oils as ingredient sources **(Koiou et *al.*, 2020).**
-

- Plant extracts and allelochemicals typically disrupt membranes, cell division, nutrient uptake, pigments, and hormone balance in weeds **(Hasan et *al*., 2021, Radhakrishnan et *al*., 2018, Roberts et *al*., 2022).**

Chapter II:

Medical plants

3. Artemisia herb-alba:

3.1 Definition

Artemisia herba-alba L. (white wormwood, "Chih" in Arabic, "armoise blanche" in French), resilient Shrub, also known as desert wormwood, is a resilient, perennial dwarf shrub belonging to the Asteraceae family. It is described as a typical species of the steppe and Saharan landscape and is very popular with sheep and goats). **(Baranová, B.et *al*,2025)**

It is native to the Mediterranean-steppe zone of Algeria and occurs widely in semi-arid and arid bioclimates, from the eastern Batna region through Djelfa, M'Sila, Boussaâda, Biskra, and the northeastern Tébessa area **(Saida M, et *al*. 2024)**. The plant thrives on calcareous, low-moisture soils and tolerates temperature ranges of 19–24 °C during its flowering stage **(Stambouli F et *al*.2022.)**

Phytochemically, the aerial parts are rich in polyphenols (≈45 mg GAE g^{-1} dry weight) and flavonoids such as catechic acid, rutin, caffeic acid and quercetin, especially in methanolic extracts (flavonoid content up to 1.966 µg eq Qu mg^{-1} extract) **(Bouatrous Y., Mechaala S. 2020, Ghareeb H.S. 2023).** Essential-oil yields vary between 0.3 % and 1.6 % of dry material, with a characteristic composition dominated by camphor 51% usually and 1,8-cineole, as well as davanone, thujone and other oxygenated monoterpenoids **(Goudjil M., 2025, Chenoui H., Cherif H. 2020)**

These chemical profiles underlie its traditional and modern uses: antimicrobial and antioxidant agents, bio conservatives for raw milk, bio-pesticides against plant pathogens and mollusks, and potential therapeutic applications for diabetes, leishmaniasis and inflammatory disorders **(Adewumi O.A et *al*. 2020, Wahnou H.,et *al* .2024, Mohammed M.J.,2022)**

3.2 The botanical and scientific classification of Artemisia herba-alba

. Kingdom (Règne): Plantae **(Chenoui, H., & Cherif, H. 2019)**

. Division (Embranchement): Spermaphytes (Phanérogames) **(Chenoui, H., & Cherif, H. 2019, Sahel, R., & Abidi, Y. 2023)**

. Sub-division (Sous-embranchement): Angiospermes**(Chenoui, H., & Cherif, H. 2019, Sahel, R., & Abidi, Y. 2023)**

. Class (Classe): Dicotyledones (Magnoliopsida)

. Sub-class (Sous-classe): Asteridae

. Order (Ordre): Asterales (also referred to as Synantherales in some classifications)

. Family (Famille): Asteraceae (formerly known as Composées/Compositae)

. Tribe (Tribu): Anthemideae

. Sub-tribe (Sous-tribu): Artemisiinae

. Genus (Genre): Artemisia

. Species (Espèce): Artemisia herba-alba Asso**(Chenoui, H., & Cherif, H. 2019, Sahel, R., & Abidi, Y. 2023)**

3.3 Botanical and Morphological Description

- Is a dwarf, silver-green shrub of the Asteraceae family, typically 20–40 cm tall, with dense root systems that can access soil moisture down to ~50 cm depth (Figure 5 and 6**) (Saida M, et *al*. 2024)** -
- Leaves: Its leaves are small, white and woolly with a silvery appearance**(Chenoui, H., & Cherif, H. 2019)**
- Flowers: The flowers are grouped in clusters, featuring tiny ovoid flower heads (capitules) 1.5 to 3 mm in diameter. **(Chenoui, H., & Cherif, H. 2019)**
- Sensory Traits: The plant presents a characteristic thymol-like odor and a bitter taste, and its essential oil is a viscous, yellowish liquid with a strong odor. **(Bouatrous, Y., & Mechaala, S. 2020)**.

Figure 5: Artemisia herb-alba in the stage of flowering

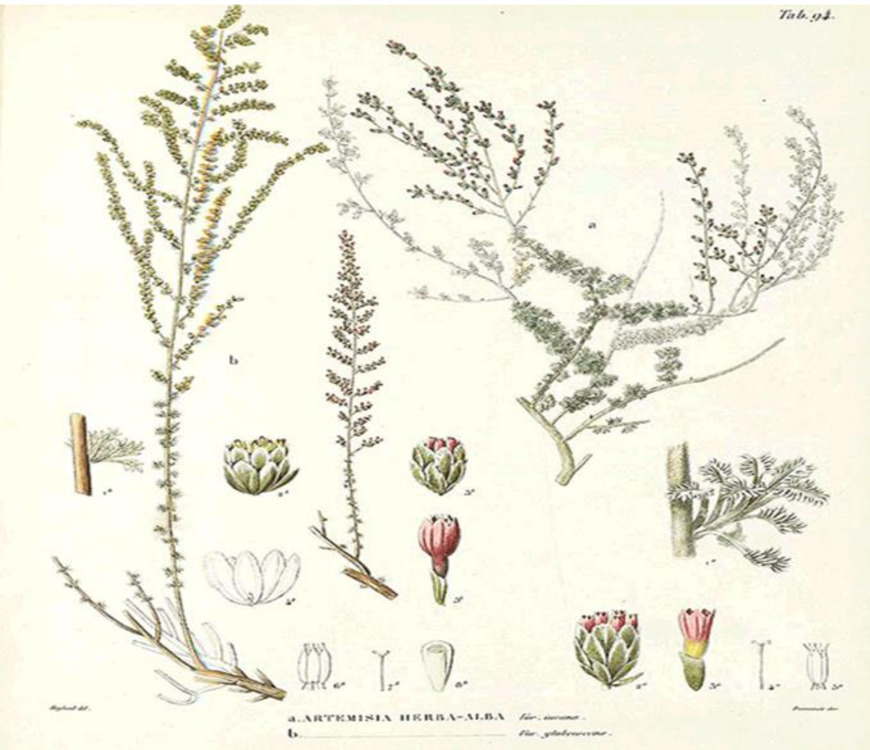

Figure6: Anatomy of the plant Artemisia herb-alba **(Bouafia Abderrhmane, 2022)**

3.4 Chemical Characteristics and Chemotypes

- **General Chemotype:** Algerian Artemesia herba-alba EOs corresponds to the camphor/ thujones/ chrysanthenone chemotype **(Medjeldi, S. et *al*,2024)**.

. Regional Variations:

-Djelfa: The main compounds are Davanone (42.8%), Camphor (15.96%) and Thujone (%9.63) **(Goudjil, M. B et *al* .2016).**

-Tébessa: The oil profile is led by camphor (51.14%), followed by 1,8 Cineole (19.30%), camphene (6.90%) and chrysanthenone**. (Goudjil, M. B et *al* .2016)**.(%5.68).

-Bordj Bou Arréridj: The oil is composed mainly of chrysanthenone (54.5%), Camphor (15.9%), 1,8-cinéol (5.7%) and β-Thujone.(%5.5) .**(Chenoui, H., & Cherif, H. 2019)**

Phenolic Profile: The plant is rich in flavonoids, with chlorogenic acid identified as the most abundant compound in some samples**.(Mahboub, N.et *al* ,2024)**

The general properties of liquid extracts (aqueous, ethanolic, and methanolic) of Artemisia herba-alba (Chih) are characterized by their diverse phytochemical composition and significant biological activities:

3.4. 1. Physical and Organoleptic Properties

- **Yields:** Extraction yields vary by solvent. The ethanolic extract typically provides the highest yield at approximately 15.64%.
- **Appearance:** The ethanolic extract is dark greenish, whereas the aqueous extract is light brownish.

- **Solvent Power:** Ethanol is noted as having a power to penetrate the plant matrix (0.121 mL/g) or water (0.032 mL/g). **(Chenoui, H., & Cherif, H. 2019)**

3.4.2. Phytochemical Composition

- **Secondary Metabolites:** The extracts are rich in polyphenols, flavonoids, alkaloids, saponins, tannins, and quinones (Figure7, 8, 9 and 10) (**Stambouli, F.et *al*.2022).**

- **Key Phenolic Compounds:** HPLC analysis identifies chlorogenic acid as the most abundant compound. Other identified compounds include rutin, vanillin, gallic acid, caffeic acid, vanillic acid, coumaric acid, catechic acid, and quercetin**.. (Mahboub, N.et *al* ,2024)**
- **Fatty Acids:** Identification of the antibacterial fraction in aqueous extracts revealed the presence of palmitic acid and oleic acid. .(**Stambouli, F.et *al.*2022)**

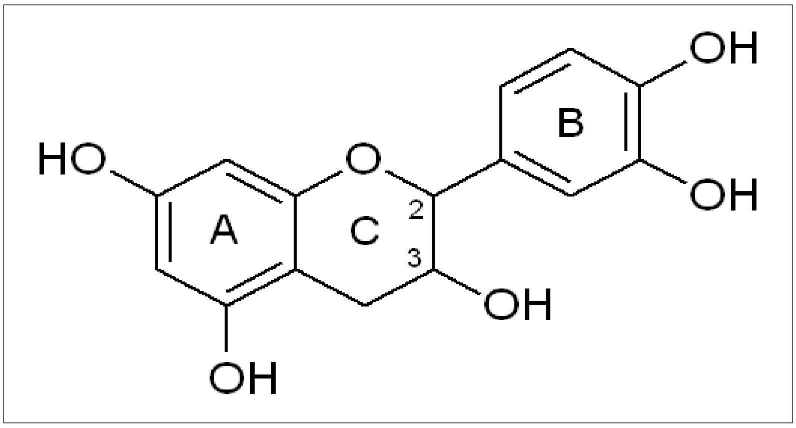

Figure7: Flavonoids

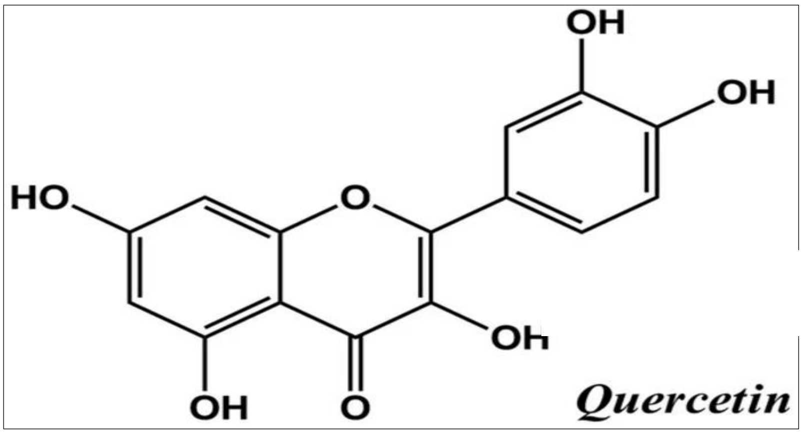

Figure8: Phenol

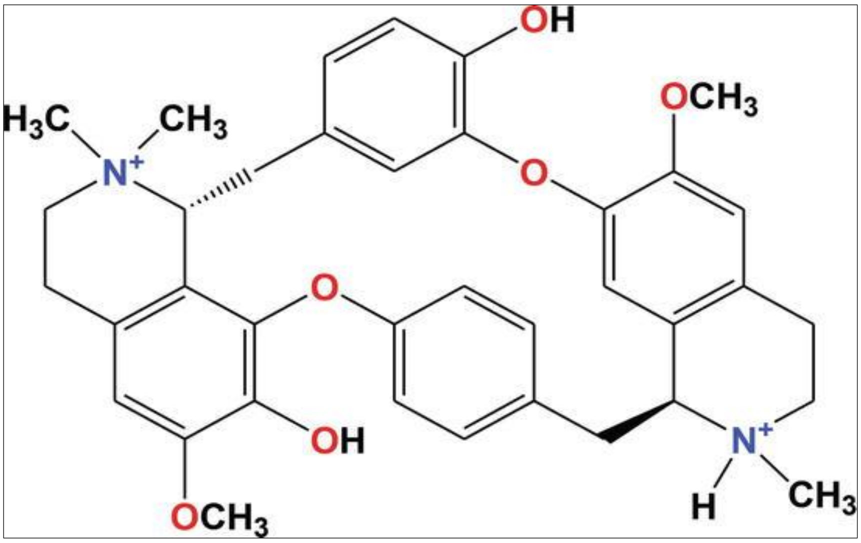

Figure9: Alcaloide

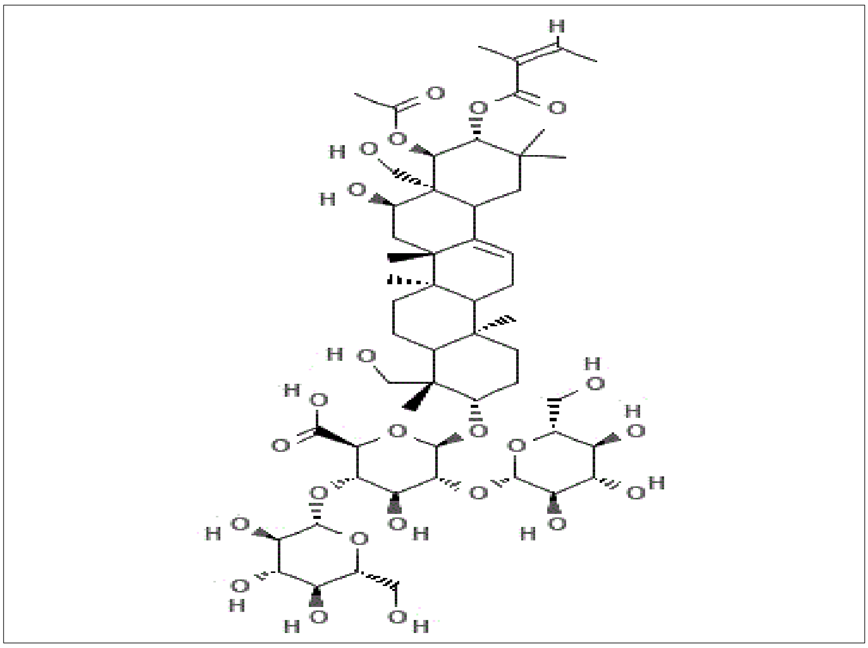

Figure10: Saponin

3.5 Agricultural and Pest Control Properties

- **Natural Bio-Herbicide (Allelopathy):** Shoot aqueous extracts at concentrations of 5% to 20% can completely inhibit the germination and significantly suppress the growth of various weeds (e.g., *Panicum turgidum*, *Portulaca oleracea*, *Melilotus*). **(Al Harbi, N. A. 2016)**
- **Odor and Taste:** It possesses a strong, pungent, and pleasant aromatic odor, often compared to thymol. The taste is characterized as excessively bitter. **(Al Harbi, N. A. 2016)**

Wormwood (*Artemisia herba-alba*), commonly known in Algeria as "Chih," acts as a powerful natural herbicide primarily through a phenomenon called allelopathy. This process involves the plant releasing secondary metabolites—such as phenolic compounds, terpenoids, and alkaloids—into the environment, which can negatively impact the development of neighboring harmful plants.

3.5.1 Total Inhibition of Seed Germination

- **Cereal Weeds:** Aqueous extracts of *Artemisia herba-alba* at concentrations of 10% and 20% have been shown to cause a total absence of germination in various weeds that infest Algerian cereal crops **(Sahel, R., & Abidi, Y. 2023).**

- **Specific Weed Sensitivity:** The extract is highly effective against common agricultural weeds such as ***Galium tricorne*** (rough corn bedstraw), *Melilotus* (sweet clover), and ***Centaurea*** **(Al Harbi, N. A. 2016).**
- **Dose-Dependency:** Research indicates that at a 5% concentration, shoot aqueous extracts can completely inhibit the germination of seeds from harmful plants like ***Panicum turgidum*** and ***Portulaca oleracea*** **(Al Harbi, N. A. 2016).**

3.5.2 Selective Action and Environmental Safety

- **Selective Potency:** Interestingly, while wormwood is highly toxic to weeds, it often shows a lesser inhibitory effect on crop species like wheat and barley, suggesting it could be used as a selective bio-herbicide. **(Chenoui, H., & Cherif, H. 2019)**

- **Replacement for Synthetic Chemicals:** Algerian researchers propose these natural extracts as a "green" alternative to synthetic herbicides like 2,4-D, which are increasingly regulated due to environmental pollution and weed resistance. **(Hacini, N.et *al*,2022)**
- **Soil Safety:** Preliminary ecotoxicity tests indicate that concentrations effective against weeds are generally safe for beneficial soil organisms like earthworms (*Eisenia foetida*). **(Hacini, N.et *al*,2022**

4. Rosemary (*Rosmarinus officinalis)*

In Algeria, the genus **Rosmarinus** (Lamiaceae family) is represented by aromatic shrubs that are deeply integrated into the country's traditional medicine, landscape, and emerging bio-industrial sectors **(Gadoum et al.2025).**

4.1 Taxonomy and Species in Algeria

The genus *Rosmarinus* L. includes three primary species native to the Mediterranean: *R. officinalis* L., *R. eriocalyx* Jord. & Fourr. (also known by the synonym *R. tournefortii* de Noé), and *R. tomentosus*.

- **Rosmarinus officinalis:** The most widely distributed species, an evergreen shrub known globally as "rosemary".
- **Rosmarinus eriocalyx:** A lesser-known aromatic shrub that predominantly flourishes in the hilly soils and pastures of Algeria. Based on recent DNA evidence, it is sometimes classified under the name *Salvia jordanii*. **(Gadoum et al.2025)**
- **Local Names:** In Algeria, rosemary is commonly known by several vernacular names, including Klil or Iklil Aljabal (Arabic), and Lazir, Ouzbir, or Touzala (Berber/Targui). **(Bousbia et *al*,2021)**

4.2 Botanical Description

- **Appearance:** Rosemary is a hardy, evergreen perennial bush that typically reaches heights of 0.5 to 2 meters. It features a deep taproot and woody, tortuous stems (Figure11and 12).
- **Leaves:** The plant is characterized by rigid, coriaceous, needle-like leaves that are dark green and shiny on top, and whitish/hairy underneath. These leaves contain the highest quality of essential oils.
- **Flowers:** Grouped in small axillary clusters, the flowers are purplish-white, blue, or lilac, featuring a two-lipped corolla and protruding stamens. In Algeria, flowers can bloom year-round **(Mamen et *al,* 2024).**

Figure11: Rosmarinus officinalis

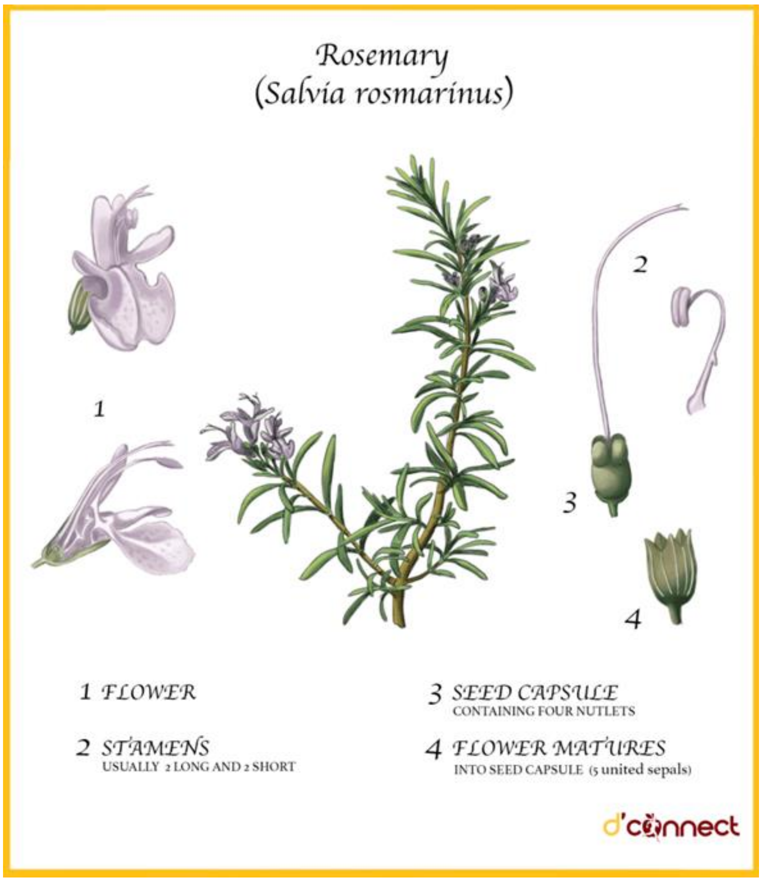

Figure12: Anatomy of *Rosmarinus officinalis* (McKechnie, C. 2022)

4.3 Geographical Distribution

Rosemary thrives in the Mediterranean climate of Algeria, particularly in warm, moderately dry areas with limestone soils.

- **North-West:** *R. eriocalyx* is found in unanthropised mountainous regions such as the Tissemsilt Province (Larjam) and Sidi Bel Abbes. **(Gadoum et al. 2025)**
- **East and Central:** *R. officinalis* is common in Khenchela (Khirane region) **(Mamen et al.2024).** El Tarf (Haddada forest) , and the Ain Defla province (Djelida and Bordj el Amir Khaled). **(Mouissi et al.2022)**
- **South and Sahara:** It is also cultivated or found spontaneously in Saharan regions like El Oued and Adrar. **(Hacini et *al*.2022)**

4.4 Chemical Composition and Diversity

The therapeutic and industrial value of Algerian *Rosmarinus* is derived from its complex chemical profile, which varies significantly by region (chemotypes) and season.

- **Essential Oils (EO):** The yield typically ranges from 0.3% to 2.5%. The major volatile compounds found in Algerian samples include camphor, 1,8-cineole (eucalyptol), alpha-pinene, borneol, and camphene **(Badache et al. 2026)**.
- **Phenolic Compounds:** Rosemary is rich in antioxidants, specifically polyphenols, flavonoids, and tannins. Key bioactive markers include carnosic acid, carnosol, and rosmarinic acid. **(Gadoum et al. 2025)**
- **Chemotypes:** Studies in Algeria have identified distinct profiles, such as a camphor-dominant type in Djelida and a 1,8-cineole-rich type in Bordj el Amir Khaled .**(Badache et al. 2026)**

4.4.1 Types and Preparation of Liquid Extracts

Aqueous Extracts: Produced by soaking dried or fresh plant material in distilled water, these extracts primarily capture water-soluble compounds like certain phenols and tannins.

Methanolic/Ethanolic Extracts: These are prepared by soaking plant material in organic solvents, often yielding higher concentrations of polyphenols and flavonoids compared to water-based methods **(Senanayake, S. P. J. N, 2018)-.**

4.4.2 Chemical Composition

The biological effectiveness of rosemary liquid extracts is driven by their high concentration of secondary metabolites:

Phenolic Diterpenes: Carnosic acid and carnosol are the most potent antioxidants, accounting for over 90% of the extract's antioxidant activity. **(Satyal, P.et *al*,2017)**

Phenolic Acids and Flavonoids: These include rosmarinic acid, caffeic acid, apigenin, and luteolin, which contribute to anti-inflammatory and antimicrobial effects. **(Satyal, P.et *al*,2017)**

Volatile Monoterpenes: Dominant in the essential oil fraction, the primary constituents are typically 1,8-cineole (eucalyptol), camphor, and alpha-pinene**. (Ben Kaab, S., et *al*.2019)**

4.4.3 Biological and Pharmacological Properties

Antioxidant Activity: Rosemary extracts are powerful free radical scavengers that inhibit lipid oxidation and combat oxidative stress. Their efficacy is often comparable to synthetic antioxidants like BHT and BHA. **(Badache et *al*. 2026)**

Antimicrobial Efficacy: Liquid extracts exhibit broad-spectrum activity against foodborne pathogens. They are particularly effective against Gram-positive bacteria (e.g., Staphylococcus aureus) but also inhibit Gram-negative strains (e.g., Escherichia coli and Klebsiella pneumoniae) . **(Mamen, N.et *al*,2024)**

Antifungal Properties: The extracts significantly inhibit the growth and spore germination of fungi such as Candida albicans, Fusarium oxysporum, and Penicillium italicum. **(Ben Kaab, S., et al.2019)**

- Anti-inflammatory Action: Studies show these extracts can inhibit protein denaturation and stabilize cell membranes with IC_{50} values comparable to aspirin. **(Badache et al. 2026)**
- Bio-herbicidal Potential: Rosemary liquid preparations can completely inhibit seed germination and slow seedling growth in various weeds (e.g., wild mustard and wild oat), serving as an eco-friendly alternative to synthetic pesticides.**(Gadoum, A., et *al*. 2025)**

5. Peppermint (*Mentha piperita*)

5.1 Definition

Peppermint (*Mentha piperita*) is a perennial aromatic herb native to Europe (Figure 13), but it is now cultivated in the northern United States, Canada, Asia, North Africa, and many other parts of the world. It is of considerable economic importance.

Furthermore, peppermint is one of the most widely consumed single-ingredient herbal teas. Its extracts and essential oil possess diverse biological activities, and peppermint (*Mentha piperita L.*) is considered one of the best potential sources of bioactive compounds for the food, cosmetic, and pharmaceutical industries. Peppermint, a hybrid species, is the result of crossing water mint (*Mentha aquatica L.*) and spearmint (*Mentha spicata L.*). Other mint species are also cultivated worldwide for the production of essential oils.(Hudz et *al*,2023) .

-grows in cool, moist, humus-rich soils up to an altitude of 1,800 m .It is a common plant in all temperate regions of the world. It is a perennial plant with a long, creeping, spreading, hairy rhizome. The stem, 50 to 80 centimeters tall, is erect or ascending and divides into opposite branches. Its leaves are 4 to 10 cm long, oval, opposite, with short petioles, lanceolate, acute, and toothed. They are a beautiful green and take on reddish hues in the sun and coppery red in the shade. They are covered with large, rounded secretory hairs in which the fragrant volatile substances accumulate. The flowers, purplish, form very short, ovoid spikes at the tips of the branches. The fruit, divided into four parts, is surrounded by a persistent calyx. Its odor is strong, its flavor pungent and refreshing (Figure 14) **(ABADLIA & CHEBBOUR, 2014)**

- In Algeria: The plant grows wild in humid regions, but it is more commonly found cultivated (Benhalima, 2015)

Figure 13: Mentha piperita

Figure 14: ***Mentha piperita***

5.2 Chemical composition of Extracts of Oils compositions

The essential oils of *M. piperita* represent 1 to 3% of the mass of the dry matter, which contains numerous compounds such as flavonoids (in particular polysubstituted flavones common in Lamiaceae) (Figure 15), triterpenes, carotenoids, but the major constituent is always menthol (30 to 70%), as well as menthone (content between 15 and 76%), menthyl acetate (2 to 5%, even up to 23%), 1,8-cineole (3 to 8%), menthofide (0 to 7%), neomenthol (2.5% to 5%), and pulegone (0.5 to 1.5%). These major constituents are accompanied by trans-sabinene hydrate, piperitenone, α- and β-pinenes, and veridiflorol, as well as other chemical constituents in very small quantities. (Benhalima, 2015)

5.2.1 The hydroalcoholic compositions

The hydroalcoholic and other extracts of *M. piperita* contain flavonoids from different groups (epicatechin; rutin; quercetin; naringenin; naringenin-7-0-glucoside; kaem-ferol; hesperidin (3',5,7-trihydroxy-4'-methoxyflavanone 7-rutinoside); luteolin (3',4',5,7-tetrahydroxyflavone); luteolin-7-0-glucoside; eriocitrin ((S)-3',4',5,7-tetrahydroxyflavanone-7-[6-O-(x-L-rhamnopyranosyl)-β-D-glucopyranosid], apigenin, etc.) and phenolic acids, including caffeic on the solvent chosen for extraction **(Hudz et *al., 2023*)**

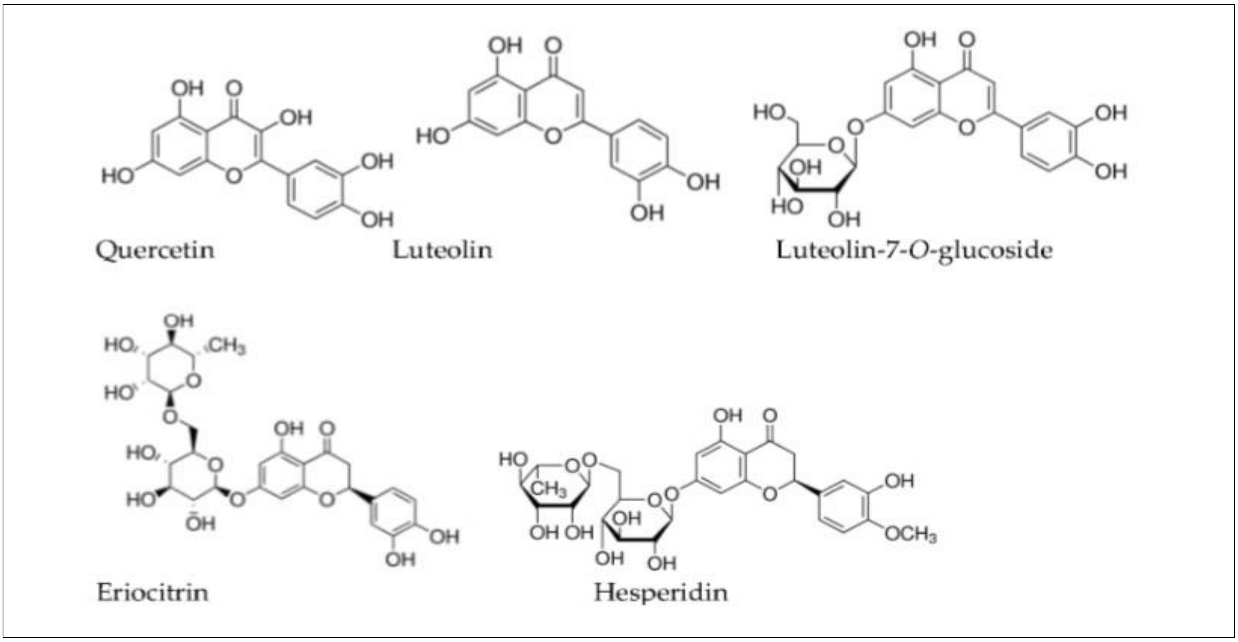

Figure 15.The chemical structure of main flavonoids of *M.piparita* **(Hudz and *al., 2023*)**

5.2.2 Allelopathic activity

Controlling invasive weeds remains a major challenge for the agricultural sector, causing environmental, economic, and agricultural obstacles that result in crop losses exceeding US$100 billion annually worldwide.

This situation highlights the urgent need for innovative, effective, and environmentally sustainable herbicides, with a focus on natural materials as potential alternatives.

The results point to the promising potential of a natural lactone known as menthalactone, extracted from mint.

Its phytotoxicity was evaluated against several weed species. Menthalactone demonstrated superior efficacy against brown millet, and its toxic properties were assessed in detail, showing significant inhibition of A. stolonifera seed germination. The susceptibility of two common weed species—millet (*Echinochloa crus-galli (L.) P. Beauv.*) and crabgrass (*Digitaria sanguinalis (L.) Scop.*)—to menthalactone was also evaluated. A concentration of 1000 µmol of menthalactone completely inhibited the germination of both weed species, while a concentration of 330 µmol inhibited germination by less than 50%. Post-germination application of 1% menthalactone showed no significant inhibitory effect on millet or crabgrass **(Sultani and *al*., 2024)**

The release of growth-inhibiting chemicals into the soil from mint residues (roots, stems, and leaves) negatively impacts photosynthesis and transpiration, leading to reduced plant growth and productivity. Furthermore, phenolic compounds, such as ellagic acid, hesperidin, cinnabenic acid, and trans-ferric acid, present in aqueous extracts of mint, may be responsible for reduced germination and growth by inducing oxidative stress. The weed-inhibiting ability of mint has also been investigated using its essential oils. In this context, significant toxic effects of peppermint essential oil against several weed species have been observed. **(Karkans, 2019)**

Chapter III: Nanotechnology

6. Entrance in the world of nanotechnology

Nanotechnology is the science and engineering of the very small, typically structures 1–100 nanometers in size, with special properties and wide applications.

It has grown into a major, multidisciplinary field touching physics, chemistry, biology, engineering, medicine, electronics, and more. It focuses on designing and using materials and devices whose behavior changes when things get extremely small.

It deals with structures, devices, and systems whose properties arise from controlling matter at 1–100 nm. **(Bayda et *al*., 2019, Hulla et *al*., 2015).** A key idea is that new phenomena and properties appear at this scale (for example in electrical, optical, or mechanical behavior), which can be used in technology **(Rozra, 2026, Rafique et *al*., 2020).**

6.1 Nanoscience vs. nanotechnology

Nanoscience studies materials and phenomena at atomic/molecular scales; nanotechnology is using that knowledge to observe, manipulate, and manufacture matter at the nanoscale. **(Bayda et *al*., 2019, Madkour, 2019)**

Nanotechnology now influences almost every field of science, including materials, electronics, biology, and computer science (Table 4) **(Bayda et *al*., 2019).** It is considered a general-purpose, “megatrend” technology, with large public and private investment and rapidly growing revenues from nano-enabled products. **(Wolf, 2021).** Important application areas include medicine and nanomedicine, catalysis, energy, water treatment, electronics, and environmental protection. **(Bayda et *al*., 2019, Nasrollahzadeh et *al*., 2004, Rozra, 2026)**

Table4: Basic manufacturing approaches in nanotechnology

Concept	Description	Citations
Top-down	Start from larger pieces of material and miniaturize them.	**(Bayda et *al*., 2019)**
Bottom-up	Build structures atom-by-atom or molecule-by-molecule, often via self-assembly.	**(Bayda et *al*., 2019, Patel et *al*., 2021)**

6.2 Nanomaterials: Main Types of "Nano"

Nanomaterials are materials where at least one dimension is between 1–100 nanometers, giving them special properties compared with bulk materials. Different "types of nano" are usually organized by dimensions **(**0D–3D) and by composition (carbon, metals, polymers, etc.).

- **By Dimension (0D–3D)**

Many reviews group nanomaterials into four dimensional classes:

- **0D (zero-dimensional)** – all three dimensions are nanoscale; examples: quantum dots, fullerenes, nanoparticles **(Joudeh & Linke, 2022)**.
- **1D** – one dimension is large, two are nanoscale; examples: nanotubes, nanorods, nanowires, nanofibers **(Joudeh & Linke, 2022).**
- **2D** – two dimensions large, one nanoscale; examples: nanosheets, nanofilms, nanolayers, thin coatings **(Joudeh & Linke, 2022).**
- **3D** – collections/assemblies of nano-building blocks (bulk nanomaterials, porous structures, nanowire arrays) **(Joudeh & Linke, 2022)**.

- **Material Type**s

- **Carbon-based** – fullerenes, carbon nanotubes, graphene, carbon nanofibers, carbon black. **(Ijaz et *al*., 2020).**
- Inorganic (metal / metal oxide / ceramic) – metal nanoparticles (e.g., Au, Ag), metal oxides (e.g., ZnO, TiO_2), other ceramics. **(Ijaz et al., 2020)**
- **Organic / polymeric / lipid-based** – polymer nanoparticles, liposomes, other soft organic nanosystems. **(Al-Harbi & Abd-Elrahman, 2024).**
- **Composite / hybrid** – core–shell particles, nanocomposites combining several materials **(Barhoum et *al*., 2022).**

6.3 Nanocellulose

- Nanocellulose is a **nano-structured form of cellulose** with remarkable mechanical strength, biocompatibility, and wide industrial and biomedical uses. Nanocellulose refers to cellulose broken down to the nanoscale (≤100 nm in at least one dimension), typically as cellulose nanocrystals, nanofibrils, or bacterial/microbial nanocellulose. It combines cellulose's renewability and biodegradability with nanomaterial properties like high surface area and tunable chemistry, making it a key "green" material for advanced applications **(Randhawa et *al*., 2022, Patil et *al*., 2021).**

6.3.1 Types of Nanocellulose

- **Cellulose nanocrystals (CNCs)**: rod/needle-like, highly crystalline particles obtained mainly by acid hydrolysis of plant cellulose or other biomass**. (Randhawa et *al*., 2022, Patil et *al*., 2021).**
- Cellulose nanofibrils (CNFs / NFC / MFC)**:** longer, flexible fibrils containing both crystalline and amorphous regions, produced by mechanical and/or chemical pretreatment of cellulose. **(Randhawa et *al*., 2022, Patil et *al.*, 2021)**
- Bacterial nanocellulose (BNC / BC): nanofibers biosynthesized by certain bacteria, forming very pure cellulose hydrogels with high porosity and crystallinity. **(Klemm et *al.*, 2018, De Amorim et *al*., 2020)**

6.3.2 Sources of Nanocellulose

Nanocellulose is obtained from plants, bacteria, algae and some animals (tunicates), and can also be produced from agricultural/industrial biowaste, supporting sustainability. **(Randhawa et *al*., 2022, Bačáková et *al*., 2019, Ansari et *al*., 2024)**

- **High mechanical strength and stiffness, low density**. **(Hansini et *al*., 2025, Sharma et *al.*, 2019)**. Large surface area and many surfaces hydroxyl groups, enabling extensive chemical functionalization. **(Hansini et *al*., 2025, Trache et *al*., 2020).** Biocompatible, biodegradable, generally low toxicity, attractive for biomedical uses. **(Patil et *al*., 2021; Lin & Dufresne, 2014)**. Good optical and barrier properties, useful in transparent films and packaging. **(Sharma et *al*., 2019, Trache et *al*., 2020)**

6.3.3 Preparation and Functionalization (High Level)

Common routes include mechanical fibrillation, acid hydrolysis, oxidation (e.g., TEMPO), enzymatic treatments, or bacterial biosynthesis, often preceded by pretreatments. **(Noremylia et *al*., 2022; Qi et *al*., 2023)** Surface chemistries such as esterification, silylation, amidation, phosphorylation, carboxymethylation tune hydrophilicity, charge, and interaction with polymers or drugs**. (Noremylia et *al*., 2022, Tahir et *al*., 2022)**

6.3.4 Representative application domains of nanocellulose

Table 5: Representative application domains of nanocellulose

Area	Example uses	Citations
Biomedical	Drug delivery, wound dressings, tissue engineering scaffolds, implants	**(Patil et *al*., 2021, Nicu et *al*., 2021, Lin & Dufresne, 2014)**
Composites & plastics	Reinforcement in polymer nanocomposites, lightweight structures, armor, blades	**(Trache et *al*., 2020; Tahir et *al*., 2022)**
Packaging & paper	Transparent, strong, biodegradable films and coatings	**(Sharma et *al*., 2019; Tahir et *al*., 2022)**
Water & environment	Membranes, water purification, adsorption	**(Heise et *al*., 2020; Patel et *al*., 2019)**

Table 6: Main ways nanomaterials are typed and classified

Criterion	Examples of Types	Citation
Origin	Natural, incidental, engineered, bioinspired nanomaterials	**(Harish et *al*., 2022)**
Crystal structure	Crystalline, polycrystalline, amorphous; single-phase vs multi-phase	**(Barhoum et *al*., 2022)**
Morphology	Full particles, hollow particles, nanosheets, core–shell, complex architectures	**(Sannino, 2021)**

6.3.5 Nanoformulation Principles for Bioherbicides

The principle of mixing a **bioherbicide** with **nanoparticles** is to use the nanoparticle as a carrier or formulation aid that protects the active natural agent, improves its dispersion or solubility, helps it stick to leaves or move through soil and plant tissues, and then releases it in a controlled way at the weed target **(La Iacona et *al*., 2025, Campos et *al*., 2023, Kremer, 2019).** This approach is mainly used because many bioherbicides are unstable, poorly water-soluble, short-lived, or weak under field conditions, and nanoformulation is intended to overcome those limits while reducing dose and off-target exposure **(Pérez-De-Luque, 2023, Scavo et *al*., 2025, Campos et *al*., 2023).**

Table7: Core Principles

Principle	Definition
Encapsulation	The bioactive can be enclosed inside a nanocapsule, trapped in a polymer matrix, adsorbed on a surface, or attached to a nanoparticle core **(Melanie et *al*., 2022)**
Protection	Nanocarriers shield sensitive natural compounds from light, temperature, humidity, microbes, and other degradation routes **(La Iacona et *al*., 2025, Khan et *al*., 2024)**
Controlled Release	The active ingredient is released gradually or in response to triggers, extending action time and avoiding a rapid burst loss **(La Iacona et *al*., 2025; Zargar et *al*., 2023, Pontes et *al*., 2025)**
Better Delivery	Small particles improve wettability, foliar adhesion, dispersion, tissue penetration, and sometimes vascular transport **(Zargar et *al*., 2023, Yang et *al*., 2024, Bombo et *al*., 2019)**
Safer Use	Effective weed control can sometimes be achieved with lower doses and less toxicity to non-target organisms **(Pérez-De-Luque, 2023, Maruyama et *al*., 2016, An et *al*., 2023)**

Nanocellulose is a biobased carrier that can give bioherbicides controlled, sustained release, better stability, and lower toxicity compared with many synthetic carriers.

Nanocellulose is widely studied as a carrier for drugs and other bioactive molecules because it is renewable, biodegradable, tunable, and can form many structures (particles, films, hydrogels) that control how an active compound is released. These same principles can be applied to bioherbicides, even though most current work is in medicine, packaging, and crop protection more broadly.

6.3.6 Key Carrier Properties of Nanocellulose

Biocompatible, low toxicity, “green”: Nanocellulose has low cytotoxicity, is biodegradable, and is viewed as a sustainable alternative to synthetic carriers**. (De Carvalho et *al*., 2024; Channab et *al*., 2024)** High surface area and tunable chemistry give good loading of bioactive molecules and control over release (Table8) **(Huo et *al*., 2022, Sheikhi et *al*., 2018)** Multiple forms (cellulose nanocrystals, nanofibrils, bacterial nanocellulose) allow design of particles, films, hydrogels, and aerogels for different delivery routes. **(Huo et *al*., 2022, Patil et *al*., 2021)**

Table8: How nanocellulose structures modulate active release

Function	Evidence (non-herbicide models)	Relevance to bioherbicides
Sustained / controlled release	CNC-CTAB systems gave ~50% curcumin release over 8 h, versus faster release of free compound. **(Casanova et *al*., 2023)**	Similar slow release could extend field persistence of bioherbicides.
pH- or environment-responsive release	CNC–alginate microspheres showed pH-dependent release suited to stomach/intestine conditions. **(Batta-Mpouma et *al*., 2025)**	Environment-responsive systems could trigger more release in rhizosphere or leaf surface conditions.
Hydrogel-based delivery	Nanocellulose-based hydrogels provide sustained drug release from minutes to days. **(Liu et *al*., 2021)**	Analogous hydrogels could deliver bioherbicides slowly in soil or on seeds.

6.3.7 Evidence from Agriculture and Crop-Protection Contexts

A moisture-responsive hydrogel with nanocellulose carrying the synthetic herbicide atrazine achieved >91% entrapment and sustained release over 40 h, versus complete dissolution of pure atrazine in 3 h, with weed control efficiency up to 95%. **(Prasanna et *al*., 2025).** Reviews on nanocellulose in agriculture highlight its role in slow/controlled release and crop protection**,** e.g., seed coatings and long-lasting protection systems **(Channab et *al*., 2024).**Cellulose-based nanocarriers have been used to encapsulate fungicides and other agrochemicals, improving activity and stability, illustrating feasibility for bio-based actives too. **(Sánchez-Hernández et *al*., 2024)**

- **Nanocellulose as a carrier**

Nanocellulose carriers can, in principle, help bioherbicides that are unstable, poorly soluble, or quickly washed away by:

- Improving loading and protection of natural herbicidal compounds. **(De Carvalho et *al*., 2024).** Providing sustained and environment-responsive release, reducing dosing frequency and off-target impacts. **(Kupnik et *al.*, 2020).**Offering a biodegradable, low-toxicity matrix fitting sustainable weed-management goals. **(Lunardi et *al.*, 2021)**

Research shows nanocellulose (CNC, CNF, BNC) is an effective, tunable carrier for many bioactive molecules, giving high loading, sustained and stimuli-responsive release, and generally low toxicity. Direct studies with bioherbicides are limited, but work with herbicides and other agro-bioactives indicates nanocellulose can act as a "green" delivery platform that could stabilize bioherbicides, prolong their action, and improve selectivity in agricultural systems.

6.3.8 Cellulose vs. Nanocellulose: Structure, Types, and Uses

Cellulose is the basic structural polymer in plant cell walls, while nanocellulose is cellulose broken down to the nanoscale, gaining new properties like very high strength, surface area, and reactivity. Research compares how conventional cellulose and its nano-forms are produced, structured, and applied in advanced materials and biomedicine.

6.3.8.1 Structure and abundance of cellulose:

Cellulose is the most abundant natural biopolymer, a linear chain of β-1,4-linked glucose units forming strong microfibrils in plant cell walls. **(Hansini et *al*., 2025, Sharma et *al*., 2019).** It is fibrous, water-insoluble, and a key raw material in wood and fiber industries **(De Amorim et *al*., 2020)**.

6.3.8.2 Definition and main types of nanocellulose

Nanocellulose is cellulose with at least one dimension in the nanometer range, typically obtained by breaking down bulk cellulose. **(Randhawa et *al*., 2022, Klemm et *al*., 2018)** Common categories:

- **Cellulose nanocrystals (CNC/NCC)** – rod-like, highly crystalline particles, usually from acid hydrolysis of plant cellulose. **(Cheng et *al*., 2022; Patil et *al*., 2021)**
- **Cellulose nanofibrils (CNF/NFC)** – long, flexible fibrils with crystalline and amorphous regions, made by mechanical/chemical fibrillation. **(Randhawa et *al*., 2022)**

- **Bacterial (microbial) nanocellulose (BNC/BC)** – nanofibers biosynthesized by bacteria such as Gluconacetobacter, forming very pure, porous hydrogels. **(De Amorim et *al.*, 2020; Klemm et *al.*, 2018)**

6.3.9 Transformation of Cellulose into Nanocellulose

Plant cellulose's hierarchical, semicrystalline structure allows extraction of nanofibers and nanocrystals by top-down mechanical and chemical de-structuring (e.g., refining, homogenization, acid hydrolysis). **(De Amorim et *al*., 2020, Li et *al*., 2022).** Bacterial cellulose is made bottom-up, directly as nanoscale fibrils secreted by bacteria**. (De Amorim et *al*., 2020)**

6.3.10 Key advantages over bulk cellulose

Nanocellulose keeps cellulose's renewability and biodegradability but adds:

Very high mechanical strength and stiffness, low density**. (Trache et *al*., 2020, Xue et *al.*, 2017).** Large surface area and many reactive hydroxyl groups for functionalization. **(Trache et *al*., 2020).** Non-toxicity, biocompatibility, and biodegradability, attractive for biomedical and packaging uses **(Sharma et *al*., 2019, Xue et *al*., 2017)**

Table9: Example application domains

Domain	Example roles	Evidence
Composites & plastics	Reinforcing lightweight, strong polymer composites	**(Sharma et *al*., 2019, Trache et *al*., 2020)**
Food packaging	Barrier films, coatings with strength and low permeability	**(Li et *al*., 2022)**
Biomedicine	Drug delivery, wound dressings, tissue engineering scaffolds	**(Xue et *al.*, 2017, Patil et *al.*, 2021, Bačáková et *al*., 2019)**
Biomedicine	Drug delivery, wound dressings, tissue engineering scaffolds	**(Xue et *al.*, 2017, Patil et *al.*, 2021, Bačáková et *al*., 2019)**

Cellulose is the abundant, fibrous glucose-based polymer forming plant cell walls. Nanocellulose is cellulose engineered to the nanoscale—most often as CNC, CNF, or bacterial nanocellulose—gaining exceptional strength, surface area, and functionalization capability while remaining renewable and biodegradable. These nanoscale forms enable advanced applications in composites, green packaging, and biomedicine that are not possible with bulk cellulose alone **(**Table9).

6.3.11 Plant sources

Plant sources of nanocellulose are extremely diverse, ranging from wood pulp to many agricultural residues and non-wood plants.

Nanocellulose (nanofibers and nanocrystals) is obtained wherever there is lignocellulosic biomass rich in cellulose. Research has moved from traditional wood to many non-wood crops, residues, and even aquatic weeds to improve sustainability and use waste streams.

6.3.11.1. Wood and Forestry Biomass

Wood pulp and other wood-based biomass still dominate nanocellulose production (about **57%** of 3358 cellulose nanofiber papers, 2004–2018). **(Pennells et *al.*, 2019).** Forestry biomass and wood (e.g., pine, poplar) are key sources of cellulose nanocrystals (CNCs). **(Qureshi et *al*., 2024)**

6.3.11.2. Non-wood Crops and Natural Fibres

Non-wood plant fibers used include banana rachis, sisal, kapok, pineapple leaf, coir and other lignocellulosic fibers. (**Deepa et *al.*, 2015).** Bast and leaf fibers such as flax, hemp, jute, sisal, abaca are highlighted as non-woody sources. **(Owonubi et *al*., 2021)**

6.3.11.3. Agricultural Residues and Food-Processing Wastes

Table 10: Examples of agricultural residues used for nanocellulose

Residue type	Examples mentioned	Citation
Straws & stalks	Rice straw, wheat straw, maize stalk, elephant grass	**(Nargotra et *al*., 2023, Das et *al*., 2023, Owonubi et *al*., 2021)**
Bagasse & husks	Sugarcane bagasse, cassava bagasse, peanut shuck, coconut husk/coir, rice husk	**(Boufi, 2017, Qureshi et *al*., 2024, Owonubi et *al*., 2021)**
Leaves & peels	Pineapple leaves/crown, banana leaves/rachis, jackfruit peel, tea leaf, banana plant waste, eggplant plant residue, tomato plant residue	**(Nargotra et *al*., 2023, Owonubi et *al*., 2021, Bahloul et *al*., 2021, Kassab et *al*., 2020)**
Other agro-wastes	Corn cobs, coconut husks, palm fruit bunch, shells (walnut, groundnut, pistachio, sago), soy hull, white coir	**(Nargotra et *al*., 2023, Owonubi et *al*., 2021)**

6.3.11. 4. Other Plant and Aquatic Biomass

Energy crops and lignocellulosic agro-industrial crops generally are seen as promising nanocellulose sources. **(Pires et *al*., 2019)** Aquatic weed Eichhornia crassipes (water hyacinth/Nile rose) has been used to produce CNCs. **(Hemida et *al*., 2023)**

6.3.11.5. Wood vs Non-wood: Trends

From 2004–2018, **30%** of cellulose nanofiber papers used non-wood biomass, with 100 unique biomass sources identified, especially agricultural residues and industrial wastes. **(Pennells et *al*., 2019)** Reviews emphasize non-woody grasses, seed-hair fibers (e.g., cotton), bast, leaf, fruit and legume residues as ideal for low-energy nanocellulose production due to lower lignin and higher hemicellulose contents. **(Das et *al*., 2023)**

Nanocellulose can be extracted from a very wide range of plant materials. Traditional wood pulps are still the main industrial source, but research strongly targets non-wood biomass—especially agricultural residues (straws, bagasse, husks, leaves, peels) and other underused plant wastes—to improve sustainability, reduce waste, and lower production costs.

6.3.12 Agricultural applications

Nanocellulose has many agricultural applications in fertilizers, soil, crop protection, and postharvest use .Nanocellulose is being used as a biodegradable, tunable material in agriculture to improve fertilizer efficiency, protect crops, retain water, and create smart packaging and sensors. Most work is still at lab or pilot scale, but reviews show rapidly expanding applications across the whole crop value chain.

- **Fertilizers, Soil Health, and Water Management**

Nanocellulose is used in slow/controlled-release fertilizers, improving nutrient use efficiency and reducing environmental impact. **(Channab et *al*., 2024, Lakhani et *al*., 2025). As a** matrix or coating in slow-release nitrogen fertilizers, nanocellulose's hydrophilicity, hydroxyl groups, and easy surface modification help delay N release, enhance soil microbes, and maintain moisture. **(Sartika et *al.*, 2025).** Nanocellulose-hydrogel hybrids act as soil conditioners with high water retention and fertilizer carriers, supporting precision agriculture. **(Lakhani et *al*., 2025).** CMC–nanocellulose hydrogels encapsulating NPK show slow nutrient release in water and soil and improved cucumber growth. **(Bauli et *al*., 2021)**

- **Crop Protection and Pesticide / Bioactive Delivery**

Nanocellulose is used as a nanocarrier for pesticides and fertilizers, enabling pH-responsive, controlled delivery and better leaf adhesion. **(Ning et *al.*, 2023; Ahmed et *al*., 2024).** Nanocellulose-containing suspensions significantly improve wettability, adhesion, and rainfastness of agrochemicals on leaves, enhancing topical delivery**. (Jiang et *al.*, 2023)** Cellulose-based coatings and carriers can combine pesticide and nutrient functions, increasing insect control while supplying nitrogen. **(Zhao et *al*., 2021)** Lignocellulosic nanocarriers (including cellulose-based) are highlighted as eco-friendly nanopesticide platforms for controlled release. **(Lima et *al*., 2021)**

- **Seed Coatings, Seed/Grain Protection, and Postharvest Uses**

Nanocellulose-based **seed coatings** can provide long-lasting crop protection and improve germination and stress tolerance**. (Channab et *al*., 2024, Ahmed et *al*., 2024).** A cellulose-based photothermal coating eradicates seed-borne fungi and bacteria under NIR light, preventing diseases and enabling long-term grain storage. **(Ji et *al*., 2023).** In postharvest horticulture, nanocellulose is used in coatings and packaging films to extend shelf life and in SERS sensors to detect pesticide residues on fruits and vegetables. **(Alvarado & Bulfa, 2025)**

- **Environmental Protection and Circular Bioeconomy**

  - Functionalized nanocellulose hydrogels act as adsorbents, sensors, and photocatalyst supports for soil and water pollution prevention and remediation.
  - Using agricultural wastes to produce nanocellulose supports circular bioeconomy and reduces reliance on fossil resources. **(Mateo et *al*., 2021; Ahmed et *al*., 2024)**

Across fertilizers, soil conditioners, crop protection, seed/grain treatments, and postharvest systems, nanocellulose functions mainly as a biodegradable carrier, coating, or hydrogel matrix that controls water and agrochemical release, improves adhesion and protection, and helps remediate pollution. Reviews emphasize strong potential for more sustainable, precise agriculture, but also note challenges of production cost, scalability, and the need for more field-scale and safety studies.

Chapter IV:

Bio-herbicide

7. Bioherbicide

7.1 Definition

The most traditional definition describes a bioherbicide as a biological control agent applied in a way similar to chemical herbicides to suppress weeds **(Auld & Morin, 1995)**. Many reviews specify the active ingredient as a living microorganism, usually fungi, bacteria, or sometimes viruses, formulated with ancillary ingredients and applied inundatively **(Aneja, 2024; Auld & Morin, 1995).** Other recent reviews use a broader definition that includes natural products such as microbial phytotoxins, plant extracts, and other phytotoxic metabolites from biological sources **(Hasan et *al*., 2021, Kubiak et *al*., 2022)**.

7.2 Main Types

The table 2 summarizes the main categories of **bioherbicides**, classified according to their biological sources and active agents. It presents examples of microbial, plant-derived, and natural-product herbicides as sustainable alternatives for weed management.

Table 2: Major bioherbicide categories described across the literature.

Type	Compounds	Examples
Microbial bioherbicides	Live or killed microbes used to kill or manage weeds	Fungi, bacteria, viruses (Duke et al., 2022)
Mycoherbicides	Fungi-based microbial bioherbicides	*Colletotrichum, Alternaria, Phoma* **(Aneja, 2024, Golijan-Pantović et *al*., 2023)**
Bacterial bioherbicides	Bacteria or their phytotoxic metabolites used against weeds	*Xanthomonas campestris, Pseudomonas fluorescens* **(Aneja et *al*., 2017, Ocán-Torres et *al*., 2024)**
Plant-derived bioherbicides	Plant extracts, allelochemicals, and essential oils with herbicidal activity	Allelochemicals, essential oils **(Rou & Raul, 2025, Raza et *al*., 2025)**
Biochemical / natural-product herbicides	Purified or semi-purified natural phytotoxins or microbial metabolites	Natural phytotoxins, cell-free metabolites **(Aneja, 2024, Ocán-Torres et *al*., 2024)**

In short, a bioherbicide is a biologically derived weed-control product, and its main types are microbial**,** plant-derived, and biochemical/natural-metabolite products, with mycoherbicides as the dominant microbial subtype.

Advantages and Limitations of Bioherbicides

Bioherbicides offer selective, lower-persistence weed control with less environmental contamination than synthetic herbicides, but their field performance and commercialization remain the main barriers. Across the literature, the strongest recurring limitations are inconsistent efficacy under field conditions, short persistence or shelf life, formulation and storage problems, and costly or slow regulatory and market uptake **(Roberts et *al*., 2022, Harding & Raizada, 2015, Triolet et *al*., 2020).**

Advantages and Limitations of Bioherbicides

Table 3: Strongest evidence on bioherbicide benefits and constraints

Evidence Strength	Claim
Evidence strength: Strong (9/10)	Bioherbicides are generally **more environmentally friendly** than synthetic herbicides because they biodegrade faster, have shorter environmental persistence, and reduce soil and water contamination risk **(Islam et *al*., 2024, Petraki et *al*., 2024, Kubiak et *al*., 2022).**
Evidence strength: Strong (9/10)	Bioherbicides often show **high target specificity**, which helps protect crops and non-target plants, but that same specificity can leave mixed-weed infestations insufficiently controlled **(Islam et *al*., 2024, Duke, 2023, Petraki et *al*., 2024).**
Evidence strength: Strong (8/10)	The main practical limitation is **variable field efficacy** driven by humidity, temperature, formulation, and application constraints, often making bioherbicides slower or less reliable than synthetic herbicides **(Islam et *al*., 2024, Petraki et *al*., 2024, Harding & Raizada, 2015).**

Bioherbicides are consistently positioned as useful in **integrated weed management**, especially where herbicide resistance and environmental restrictions make synthetic options less attractive **(Islam et *al*., 2024, Roberts et *al*., 2022, Harding & Raizada, 2015).** They also tend to have multiple natural modes of action and, so far, no evolved resistance has been reported for microbial bioherbicides in the reviewed literature **(Duke, 2023, Kubiak et *al*., 2022).**

Their limits are equally consistent. Many products act more slowly, require repeated application, or fail when environmental conditions are not **favorable (Islam et *al*., 2024, Petraki et *al*., 2024, Aneja, 2024).** Formulation is a central bottleneck because living agents must remain viable during storage and after application, while natural compounds can be unstable under light, heat, humidity, and microbial degradation **(Roberts et *al*., 2022, Campos et *al*., 2023, Pérez-De-Luque, 2023).**

The most striking gap is the disconnect between many experimental candidates and few commercial successes. Reviews repeatedly attribute this to production hurdles, ecological fitness, duration of effect, formulation difficulties, and expensive or slow registration rather than to lack of candidate organisms alone **(Triolet et *al*., 2020, Roberts et *al*., 2022).**

Safety is another underdeveloped area. Although bioherbicides are usually less toxic overall, some natural toxins are harmful to mammals, and this complicates development and registration **(Hasan et *al*., 2021,Petraki et *al*., 2024, Cai & Gu, 2016).** Newer work suggests encapsulation, nanotechnology, cell-free metabolites, and precision application could improve stability, targeting, and cost-effectiveness, but these solutions are still emerging rather than established field standards **(Ocán-Torres et *al*., 2024, Campos et *al*., 2023, Hewitt et *al*., 2023).**

In sum, the main advantages of bioherbicides are environmental compatibility, selectivity, and value against resistant weeds, while the main limitations are inconsistent field efficacy.

8. Plant-Extract Bioherbicide

8.1 Definition

A plant extract bioherbicide is a weed-control product made from extracts of plant parts such as leaves, roots, stems, or seeds that contain phytotoxic compounds capable of suppressing weed germination, growth, or metabolism. In the broader bioherbicide literature, plant extracts are one major type alongside microbial agents; the active substances are usually allelochemicals or essential-oil constituents such as phenolics, flavonoids, terpenoids, and related secondary metabolites **(Islam et *al*., 2024, Hasan et *al*., 2021, Rou & Raul, 2025).**

Plant extracts suppress weeds by damaging membranes and interfering with DNA, mitosis, amylase activity, nutrient uptake, photosynthetic pigments, and growth hormones **(Radhakrishnan et *al*., 2018, Ammar et *al*., 2023, Hasan et *al.*, 2021).** They also often increase oxidative stress and reactive oxygen species, which further slows weed establishment and seedling growth **(Radhakrishnan et *al*., 2018; Ammar et *al*., 2023).**

Examples repeatedly cited as plant-based bioherbicides include **sorghum water extracts**, **Juglans nigra**, **Ocimum basilicum**, **Eucalyptus**, and aromatic-crop essential oils and aqueous extracts **(Roberts et *al*., 2022, Islam et *al*., 2024, Poveda et *al.*, 2026).**

8.2 Main Types

- **Aqueous extracts** use water-soluble phytotoxins from plant tissues and are widely studied for practical, low-residue formulations **(Susilo & Pujiwati, 2026, Marques et *al*., 2023).**
- **Essential-oil bioherbicides** use volatile plant compounds, especially terpenes and terpenoids, as active ingredients **(Abd-ElGawad et *al*., 2020, Špirović-Trifunović & Tojić, 2022).**
- **Allelochemical-based products** use purified or semi-purified natural compounds released by plants that inhibit neighboring plants **(Roberts et *al.*, 2022, Habibi, 2024).**

A plant extract bioherbicide, then, is best defined as a plant-derived weed-control product whose extracted natural compounds directly inhibit weed germination or growth, most often through allelochemicals or essential-oil constituents.

Practical Part

Chapter I: Materials and Methods

1. Study Area

1.1 Geographical location and climate

El Oued, also known as the the Oued Souf region, is a significant Saharan province located in south-eastern Algeria, approximately 600 km northeast of the capital, Algiers **(Med Agri Food Resilience, 2023).** It is bordered by Tunisia to the east, and by the Algerian states of Tébessa, Khenchela, Biskra, El Meghaier, Touggourt and Ouargla. The province is divided into two main regions: the **Oued Souf** in the west and the border region with Tunisia in the east.

- **Terrain:** A major portion of the region (three-quarters of the Souf’s surface) is occupied by the Grand Erg Oriental, which consists of a vast succession of sand dunes.
- **Climate:** As a Saharan region, it faces a harsh climate where summer temperatures often exceed 40°C, and annual rainfall is extremely low, generally less than 100 mm **(Hacini, N et *al.*, 2022)**

1.2 Demographics and Agriculture

- **Population:** El Oued is one of the most densely populated Saharan Wilayas in Algeria, with more than 900,000 inhabitants as of 2020. It has a very young population, with 39% of residents under the age of 14 **(Med Agri Food Resilience, 2023)**.
- **Agriculture:** This is the backbone of the region, employing 42% of the active workforce. The area sits atop massive groundwater resources estimated at 4.9 billion cubic meters, which supports its booming agricultural sector **(Hacini, N et *al* ., 2022)**.

The Oued Souf region in El Oued is a booming agricultural center characterized by a diverse range of crops, supported by both the traditional Ghout system and modern irrigation techniques. The crops can be categorized as follows:

1.2.1 Date Palm Cultivation (The Strategic Mainstay)

The date palm (*Phoenix dactylifera L.*) is the region's most important crop, with an estimated 279,565 trees cultivated in the Ghouts alone as of 2022.

- **Key Cultivars:**
    - **Deglet Nour:** Known as the "date of light," it is the most exported and appreciated variety, accounting for 62% of Algeria's national production.
    - **Ghars:** A hardy variety used for soft dates, often processed into pastes or "Rob" (date jam).
- **Other Varieties:** Mech Degla, Degla Beidha (white dates), Takarmoust, Timdjouhart, Tantboucht, and Tinissine.

1.2.2 Market Gardening and Protected Crops

El Oued has become a leading producer of vegetables in Algeria, utilizing both open fields and greenhouses.

- **Potatoes:** A flagship crop introduced in the 1990s; the region now produces 24% of Algeria's total potato output.
- **Tomatoes:** Both field and greenhouse tomatoes are prominent, contributing 16% of national production. Cherry tomatoes are also grown in some Ghouts.
- **Peppers and Chillies:** Includes local landraces known as "Felfel Arbi" or "Laarbi".
- **Other Vegetables:** Green beans, peas, eggplants, onions, zucchinis, pumpkins, and cucumbers.

1.2.3 Fruit Trees (The Second Layer)

In the traditional Ghout system, fruit trees are often planted underneath the canopy of date palms.

- **Pomegranates:** Found in most fruit-bearing Ghouts; varieties include "Hlou" (sweet) and "Hamad" (sour).
- **Figs:** Five local cultivars are known: Kharfi, Ngaoussi, Bou3ank, Tinedli, and Bakor.
- **Others:** Table grapes, apples, pears, and lemons.

1.2.4 Industrial, Field, and Fodder Crops

- **Industrial Crops:** Primarily tobacco (snuff), henna, and various spices, covering approximately 1,127 hectares.
- **Field Crops:** Cereal crops (wheat and barley) and forage crops for livestock.
- **Fodder:** Fodder cabbage is grown in some Ghouts for goats and sheep, though the cultivation of alfalfa has declined.

1.2.5 Medicinal and Aromatic Plants

El Oued is home to a diverse array of spontaneous (wild) flora, including over 73 species of medicinal plants belonging to 37 botanical families. The local population remains deeply attached to traditional medicine, frequently using these local plants to treat digestive and respiratory ailments (**Khechekhouche, A et *al,* 2020).**

- **Common Families:** Asteraceae and Lamiaceae (9 species each).
- **Examples:** *Artemisia herba alba* (Chih), *Mentha spicata* (Nânâa), *Rosmarinus officinalis* (Klil), and *Peganum Harmala* (Harmel).

1.2.6 Traditional and Legacy Crops

- **Purslane:** One of the oldest crops grown in the Ghouts, traditionally used in couscous.
- **Legacy Crops:** Historically, the region saw more cultivation of carrots, turnips, broad beans, and lettuce, though many farmers have stopped growing these in the traditional Ghouts in recent years.

2. Survey on the Impact of *Setaria verticillata* in Agriculture

2.1 Objective of the Survey

The aim of this survey is to identify the problems and damages caused by the weed *Setaria verticillata*, as well as the common methods used to get rid of it, and the methods and costs required for the removal process.

2.2 Study Population

The study community consists of farmers, agricultural specialists, and those interested in ecological agriculture.

The sample consists of 63 individuals, 85 % of whom are farmers and the rest are specialists in agriculture and agroecology. Participants were chosen on the basis of the following criteria:

- Suitability and eligibility: The participant should possess experience or direct characteristics related to the research topic.
- The sample should accurately reflect the population, including age, gender, and geographic distribution.
- The target group should be easily accessible and communicable to ensure a high response rate.

2.3 Methodology

- **Type of Survey (questionnaire, interview)**

To meet the core objectives of this study, a structured, sequential questionnaire was carefully developed. Rather than just a random list of questions, the survey follow a thoughtful, logical progression, designed to naturally chosen to keep participants engaged, maintain clarity, and ensure that their answers build up cohesively-ultimately leading to genuine, precise data that serves the ultimate goal of our research (Appendix 1) .

- **Structure of the Questionnaire**

a. Title and introduction

Title: A survey about the plant *Setaria verticillata* commonly known as "Ellafa" or "Sboul lfar" in Oued Souf region.

Introduction: The aim of this study is to estimate the extent of the damage caused by this weed and how farmers deal with it. We can confirm that this questionnaire will be used for scientific research purposes only.

b. Demographic data

This includes background information for participants such as age, educational level, specialization, and years of experience.

c. Core sections

The questions are arranged in order of complexity, from the easiest and most superficial to the most profound, in order to maintain a logical flow of answers and the participant's focus.

d. Types of questions

Open questions**.**

e. Conclusion

Thank you for your time and awareness.

2.4 Statistical Analysis of Data

To analyze the collected data, statistical methods will be applied using statistical software such as Excel. The analysis will be carried out in 2 main phases:

- Descriptive statistics; including frequencies and percentages, will be used to summarize the respondents' profile and their answers to the 14 questions.
- Inferential statistics were implemented, beginning with Cronbach's Alpha to verify the internal consistency and reliability of the questionnaire

Cronbach's Alpha

$$\alpha = \frac{k}{k-1}\left(1 - \frac{\sum \delta_i^2}{\delta_i^2}\right)$$

$$0.8 \approx 0.775 = \frac{19}{19-1}\left(1 - \frac{2.523}{9.5}\right)$$

3. Plant Material

- **Cotton (Gossypium hirsutum)**

Cotton (*Gossypium hirsutum*) was selected as the primary source for cellulose extraction due to its exceptionally high cellulose content, which typically ranges between 90% and 95% in its purified form, making it one of the richest and most accessible natural sources of cellulose. Furthermore, cotton fibers are widely recognized for their purity, availability, and ease of processing, which makes them particularly suitable for laboratory extraction procedures.

The cotton samples (Figure 16) used in this study were collected from agricultural farms located in the Gamar region, El Oued Province, during the flowering and fruiting stages. This region is well known for its favorable environmental conditions for cotton cultivation. The use of locally sourced plant material ensured the freshness and quality of the samples while contributing to the valorization of local agricultural resources.

Figure16: *Gossypium hirsutum*

- ***Artemisia herba-alba, Rosmarinus officinalis, Mentha piperita***

The use of Artemisia herba-alba, Rosmarinus officinalis, and Mentha piperita was selected because of their high allelopathic potential, attributed to the presence of numerous bioactive compounds capable of exerting phytotoxic effects on competing plant species. These substances can interfere with various physiological processes involved in seed germination and plant growth. Furthermore, the use of plant-derived extracts is part of an approach aimed at developing more sustainable and environmentally friendly weed management strategies. Therefore, A. herba-alba, R. officinalis, and M. piperita can represent promising natural sources of bioherbicides for the control of Setaria verticillata.

The Artemisia herb-alba was brought from the provinces of M'Sila and Djelfa (Figure17), located in the High Plateaus region. The harvest took place in the middle of January when the plant was found in the flowering stage.

Figure17: Artemisia herb alba

Mentha piperita and Rosmarinus officinalis (Figure18) were harvested from the Gumar region of El Oued province in January, when the rosemary was in bloom.

Figure18: Rosmarinus -A- and mentha piperita -B-

The cotton tree branches were picked from the Qamar region of El Oued province while in flowering and bearing fruit.

4. Preparation of Nanocellulose

4.1 Cellulose Extraction

The stems of Gossypium hirustum were oven-dried (80°C for 2 hours), then finely chopped and ground into a fine powder.

. The waxes were removed from the plant powder by sequential extraction using n-hexane (4 hours), then ethyl acetate (4 hours), then methanol (4 hours), and finally distilled water (4 hours), using a Soxhlet apparatus to ensure the gradual removal of the raw wax material.

. The resulting material was then oven-dried at 65°C for up to 4 hours.

. The dried, extractable material underwent autoclaving, where the sample was immersed in a 5 w/v aqueous sodium hydroxide (NaOH) solution and heated at 120°C under 2 atm for 45 minutes. After autoclaving, the material was filtered and washed with distilled water until a neutral pH was reached. It was then bleached to remove any remaining hemicellulose and lignin. After bleaching, the material was filtered and washed again until a neutral pH was reached, and then dried to obtain pure cellulose (drying: 60–70°C for 4–12 hours, cooled in a desiccator, stored in an airtight container with silica gel desiccant). The bleaching process consists of two main steps:

A. The cellulose material is treated with 2% hydrogen peroxide (H_2O_2) and 0.2% TAED. Tetraacetylethylenediamin) at pH 12, stirred at 50°C for 12 hours. After treatment, the material is filtered and washed with distilled water until neutral, then dried.

B. The dried material from the first step is treated with acetic acid (80%) and nitric acid (65%) at 120°C for 30 minutes. It is then filtered and washed with distilled water and ethanol until neutral pH is reached, and finally dried to obtain pure cellulose.

. The pure cellulose is dried at 65°C in a drying oven and then weighed to determine the extraction yield.

4.2 Preparation of Nanocellulose

. The pure cellulose is subjected to acid hydrolysis using sulfuric acid (64% H_2SO_4) with continuous stirring at 25°C for 60 minutes. This treatment allows the cellulose structure to be broken down into nanoparticles through the selective hydrolysis of amorphous regions. At the end of the reaction, it is stopped by adding cold deionized water to dilute the acid and prevent further decomposition.

. The resulting suspensions are then washed several times with distilled water using high-speed centrifugation. After each centrifugation cycle, the filtrate is discarded and replaced with fresh water. This process is repeated until the pH reaches neutral, ensuring the removal of any residual acid.

. Finally, the nanocellulose suspension is kept under continuous magnetic agitation or gentle mechanical stirring to improve particle dispersion, and then stored at a low temperature (freezer) for subsequent analysis (Fifure 19).

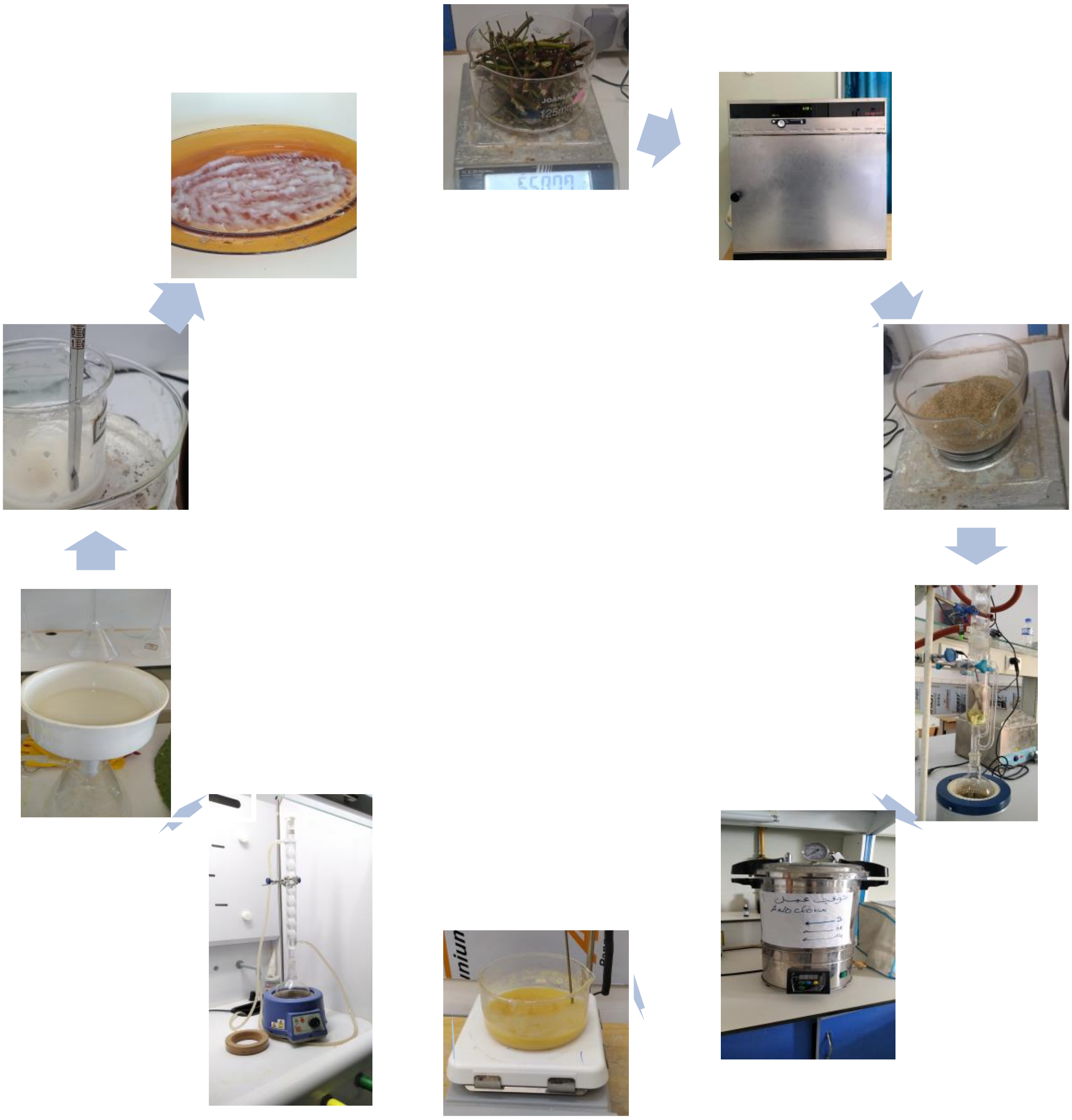

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Figure 19: Preparation of Nanocellulose:

5. Characterization of Nanocellulose

A. Fourier Transform Infrared Spectroscopy (FTIR):

FTIR analysis was performed on two samples: one from the plant before treatment and one after the second bleaching step. These samples were monitored using a Cary 630 spectrometer, with measurements within the spectral range of 500–4000 cm^{-1} (Figure 20). To prepare the samples for FTIR analysis, they were dried and ground, then mixed with potassium bromide (KBr) powder and pressed to form thin discs.

Figure 20: FTIR spectroscopy ATR

B. X-ray Diffraction (XRD):

XRD was used to analyze the crystal structure of the samples and extract important information about crystallization and crystal size. The device was operated under the following (Figure21).

. Conditions:

- Voltage: 40 kV
- Current: 30 mA

- A Cu-Kα radiation source with a wavelength of 1.5418 Å and a graphite filter were used XRD patterns were recorded for the samples

- The crystallinity index was calculated using Segal's equation

- The crystal thickness was calculated using Scherrer's equation

Figure21:The X-ray diffraction

6. Extraction of Plant Compounds

The plant was harvested and dried in the shade. Then 100g of it was taken, crushed, and soaked in a solution of (water + ethanol 2:8) while shaking for 24 hours. The mixture was then filtered and the filtrate was transferred to a rotary evaporator until it thickened. It was then placed in an oven at 50°C until completely dry. Finally, the dried extract was scraped off and weighed in order to calculate the yield (Figure 22).

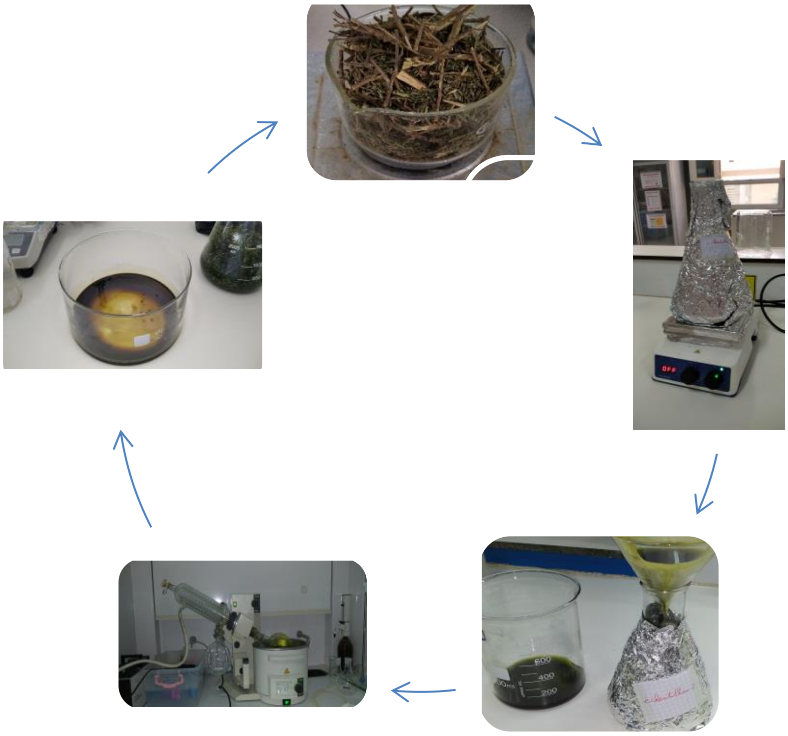


Figure 22: Extraction of Plant Compounds

6.1. Phytochemical testing of extracts in the laboratory

6.1.1. Flavonoids

Two simple tests were performed:

a) NaOH Test (Yellow Color Test)

- In a test tube, 1 mL of alcoholic extract was placed (or diluted in ethanol if highly concentrated).
- A few drops of NaOH (2–5%) were added.
- **Observation:**
  - Appearance of a golden yellow/orange-yellow color → indication of the presence of flavonoids.
  - Upon addition of dilute hydrochloric acid, the yellow color disappeared (classic reversibility).

b) Shinoda Test (Mg + HCl)

- In a test tube, 1 mL of alcoholic extract was placed.
- 2–3 drops of concentrated hydrochloric acid were added, then a small, clean magnesium strip.
- The mixture was gently shaken.
- **Observation:**
    - A pink, red, or magenta color appeared after a few minutes → presence of flavonoids.

6.1.2 Saponins

a) Foam Test (Stable Foam Test)

- In a test tube, 2 mL of alcoholic extract was placed and then 10–15 mL of distilled water were added.
- The tube was stoppered and shaken vigorously for 10–15 seconds.
- **Observation:**
    - A persistent foam formed for ≥ 10 minutes → the test was positive for saponins.

b) Hemolytic Test (Tube or Gel)

- On a slide or in a tube, a small amount of evaporated alcoholic extract, redissolved in water, was mixed with a suspension of red blood cells (diluted blood).
- The mixture was incubated for 15–30 min at 37 °C.
- **Observation:**
    - Progressive lysis (hemolysis) of red blood cells was observed (clear settling) → presence of saponins.

6.1.3 Alkaloids

a) General Precipitation Reagent Test (Dragendorff, Mayer, Wagner)

- 1 mL of alcoholic extract was placed in a test tube.
- The mixture was slightly acidified with 2–3 drops of 0.1 N hydrochloric acid.
- 2
  - **Dragendorff:** 1–2 mL were added → an orange or brown precipitate was observed = alkaloids.
  - **Mayer:** 1–2 mL were added → a creamy white precipitate was observed = alkaloids.
  - **Wagner:** 1–2 mL were added → a brown precipitate was observed = alkaloids.

b) Principle (for an alcoholic extract)

- Alkaloids are weak bases, soluble in acidic media (alkaloidal salt).
- In acidic media, the addition of the reagents produced a positive precipitate indicating the presence of alkaloids.

7. Formulation of the Nanobioherbicide

First: Preparation of a total volume of 500 ml of nano-bio-pesticide:

. 0.05% nano-crystalline cellulose (CNCs):

. 25% dry plant extract:

. Continuous solvent (distilled water):

. The remaining volume to complete the volume is deionized distilled water.

Second: Required Instruments and Equipment

. Analytic balance.

. Magnetic stirrer with heating.

. Sonicator.

. 1000 ml glass beaker.

. Laboratory syringe or micropipette.

Third: Experimental Preparation Steps (Step by Step)

a. Preparation of the basic nano-cellulose suspension (dispersing CNCs):

. Place approximately 300 ml of distilled water in the beaker.

. Gradually add the nano-crystal cellulose (CNCs).

. Transfer the beaker to the sonicator and operate it for [10min].

b. Incorporating the Extract

. Return the beaker to the magnetic stirrer.

. Begin adding the dry plant extract gradually and very slowly while stirring continuously.

c. Homogenization and Final Volume

After the materials are thoroughly mixed, carefully top up the liquid volume by adding distilled water until the total volume in the beaker reaches exactly 500 ml.

d. Storage and Verification

Pour the developed nano-bio-herbicide into an amber bottle to protect the light-sensitive plant compounds from oxidative chemical degradation.

Figure 23:Preparation of nanobioherbicide.

8. Laboratory Experiment On Herbicide Activity

The steps are illustrated in Figure 24

8.1 Preparing the Aqueous Stock Solution at a Known Concentration

We dissolve the dry extract in distilled water only. The initial standard concentration for research in this case is 10 mg/mL (equivalent to a 1% wt. v.).

. Precise Measurements:

a .Weigh 1 gram of the dry extract using a sensitive balance.

b. Place the extract into a 100 mL volumetric flask.

c .Add a small amount of warm distilled water (approximately 40-50 mL) to aid in dissolving the compounds, and shake well until completely dissolved (a magnetic stirrer or sonicator can be used if dissolution is difficult). **(Wichittrakarn, P, et *al*,2026)** d .Top up the volume with distilled water to the 100 mL line**.(Bouhaouel, I , et *al*..2015)**

Result: Stock solution (100% concentration)

8.2 Controlled Dilution Table (Concentration Series)

From this parent solution, we can prepare the diluted concentrations that we will test on the seeds **(Balah, M. A. 2021)** (e.g., to determine the effective dose):

Table11: Concentration Series

Required Concentration	**Volume of Parent Solution (ml)**	**Volume of Pure Distilled Water (ml)**	**Final Volume (ml)**
Full Concentration (100%)	10 ml	0 ml	10 ml
Half Concentration (50%)	5 ml	5 ml	10 ml
Quarter Concentration (25%)	2.5 ml	7.5 ml	10 ml
Decimal Concentration (10%)	1 ml	9 ml	10 ml
Control Set (Control)	0 ml	10 ml (Distilled Water Only)	10 ml

8.3 Controlled Data in a Petri Dish

When starting the experiment on seeds, the following values must be strictly adhered to:

-Petri dish diameter: 9 cm (90 mm).

-Germination medium: Place two filter papers at the bottom of each dish.

-Controlled liquid volume: Using a pipette, draw exactly 4 ml of the required concentration and distribute it evenly over the filter paper to saturate it without submerging the seeds.

-Number of seeds: Distribute 10 seeds of the target plant at equal intervals over the saturated filter paper. .**(Wichittrakarn, P, et *al*, 2026)**

8.4 Safety Procedure and Closure

After adding the 4 ml and seeds, immediately close the dish with its lid and wrap the rim with Parafilm tape to ensure moisture retention and prevent the 4 ml from drying out during the incubation period. We place the dishes in the incubator at the appropriate temperature for the type of seeds (from 22°C to 25°C).

Figure 24. Laboratory Experiment On Herbicide Activity

9. Agronomic Field Trial of Herbicide Activity

. 150 ml of bio-herbicide was mixed with 1 liter of water and sprayed over an area of 25 square meters containing weeds and annual plants.

. 100 ml of nano-engineered bio-herbicide was mixed with 1 liter of water and sprayed over an area of 25 square meters containing weeds and annual plants.

. A 25 square meter area containing weeds and annual plants was left untreated (control).

Figure 25: Agronomic Field Trial of Herbicide Activity

12.5 ml of chemical pesticide (Figure 26) was mixed with 1 liter of water and sprayed over an area of 25 square meters containing weeds and annual plants.

Figure 26: chemical pesticide

9.1 Experimental Conditions

The experiment was carried out under ideal conditions, with the herbicide applied at 4:00 AM in the absence of sun and wind, and at an ideal concentration.

9.2 Measured Parameters (germination, growth, biomass, etc.)

An area containing the plant in all its vital stages (beginning of germination, flowering, seed maturation) ranging in length from 15 cm to 40 cm was sprayed.

9.3 Test on safety of the herbicide on soil microorganisms

. Materials:

• One Erlenmeyer flask containing 50 mL of sterile agar (0.1%)

• One small Petri dish containing 0.5 g of soil

• Four small flasks containing 4.5 mL of sterile agar (0.1%)

• Five sterile 1 mL pipettes

• One sterile glass rod (hockey stick type)

• Six Petri dishes containing approximately 10 mL of PDA culture medium (potato and dextrin agar)

• Six strips of paraffin tape

• Absorbent paper

• Disinfectant

. Method:

1. Label the flasks (10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}) and Petri dishes to indicate the dilutions.

2. Add 0.5 g of the solution to the Erlenmeyer flask containing 50 mL of agar (10^{-2} dilution).

3. Shake the Erlenmeyer flask for at least one minute.

4. Using a sterile pipette, transfer 0.5 mL into an Erlenmeyer flask containing 4.5 mL of 0.1% agar (10^{-3} dilution).

5. Shake the Erlenmeyer flask for at least one minute.

6. Repeat steps 4 and 5 for the three other dilutions (10^{-4}, 10^{-5}, and 10^{-6}).

7. Starting with the lowest dilution (10^{-6}), add 1 mL of the solution to each of the two Petri dishes containing the PDA medium. Spread the solution evenly using a sterile stick. (Start with the 10^{-6} dilution.)

Repeat the same steps for the 10^{-5} and 10^{-4} dilutions.

8. Seal the Petri dishes tightly with paraffin tape.

9. Incubate the Petri dishes at room temperature.

10. Observe the results after 24, 48, and 72 hours.

Figure 27: Test on safety of the herbicide on soil microorganisms

Chapter II:

Results and discussion

1. Analysis and Interpretation of Survey Questionnaire Results

1.1 Socio-demographic Profile of Respondents

Figure 28 illustrates the age distribution of the respondents participating in the survey. The results show that the majority of respondents (54%) belong to the 25–35 years age group, indicating that young adults represent the largest proportion of the surveyed population. Respondents aged 35 years and above account for 36% of the sample, while the 18–25 years age group represents only 10%.

The predominance of respondents aged 25–35 years indicates that this age group is the most actively involved in agricultural activities. The significant proportion of participants over 35 years old reflects the presence of experienced farmers, while the low representation of the 18–25 age group suggests a more limited involvement in farming practices.

Overall, the sample is dominated by respondents aged between 25 and 35 years, providing a balanced perspective that combines both practical experience and openness to adopting innovative weed management strategies, such as bioherbicides.

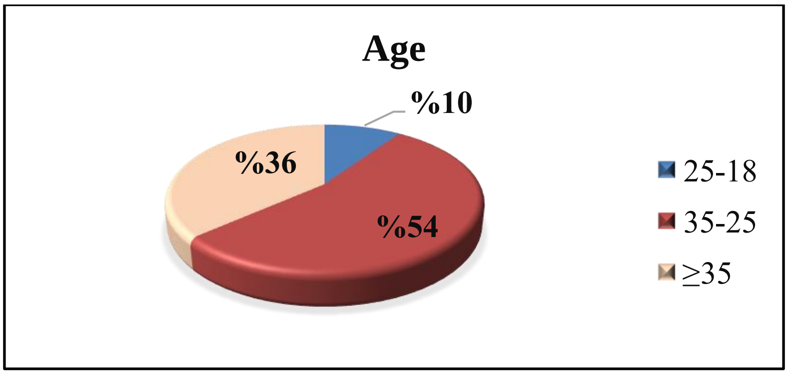


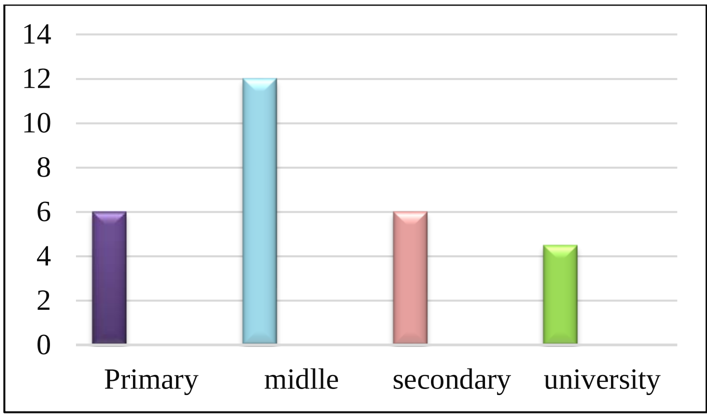
Figure28: Age Distribution of Survey Respondents.

Figure 29 presents the educational background of the respondents. The results indicate that the middle-level education category represents the largest proportion of the sample, with 12 respondents. The primary and secondary education levels are equally represented, with 6 respondents each, while the university level accounts for the smallest proportion, with approximately 5 respondents.

These findings suggest that most participants possess a moderate educational background, which may influence their understanding and adoption of agricultural practices. The presence of respondents with different educational levels provides a diverse range of perspectives regarding weed management and the potential use of bioherbicides. However, the relatively low proportion of university-educated respondents indicates that practical farming experience may play a more significant rolhin the surveyed population.

This dominance of young and older productive groups strongly suggests on the ground that the agricultural sector in the study area is managed by a generation combining exceptional skills with accumulated experience, leaving them with innovative approaches to integrated root crop management. This recent data directly supports the sociological argument presented by **Al-Ashhab and Bouhamida (2022)** regarding agricultural companies in southern Algeria. They emphasized that the transfer of regional management to the younger generation has accelerated the mechanization of agriculture and the adoption of technological solutions, compared to the older generation, which tended to rely more on traditional planning methods.

The high educational and professional levels observed in the study area reveal a strong presence of both secondary and university-level graduates, with dairy farming being the most prevalent profession at 85%. This scientific and traditional awareness among young workers, and their responsiveness to this awareness, represents a fundamental pillar of importance. After initiating new innovations, they became aware of the persistent toxic and environmental risks of the indiscriminate and continuous use of traditional pesticides, which led them to abandon their adult properties and adopt new, advanced (biopesticides) based on variation systems using crystalline cellulose. This finding clearly aligns with the findings of **Smith et al. (2021)** regarding the analytical study of farmers in the Caribbean basin, where a correlation exists between the toxic organophosphate and chlorophosphate classes.



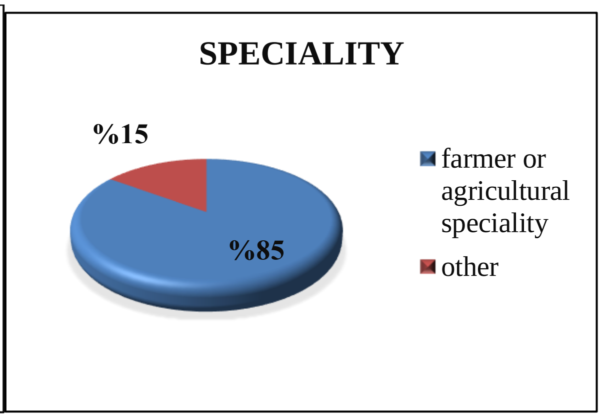


Figure29: Distribution of Sample by Educational Level

Figure30: Proportion of Farmers and Non-Agricultural participants in the Sample

Figure 30 presents the distribution of the surveyed participants according to their professional specialization. The findings reveal that the majority of respondents (85%) are farmers or individuals involved in the agricultural sector, whereas only 15% belong to other professional fields unrelated to agriculture. This high proportion of agricultural professionals within the studied sample is particularly relevant to the objectives of this research, as it provides valuable insights based on direct field experience and practical knowledge concerning weed occurrence, impacts, and management practices.

1.2 Current Status of Setaria verticillata Infestation and Control Methods

Figure 31 summarizes the responses of the surveyed participants regarding their experience with *Setaria verticillata*, the problems associated with this weed, and their ability to control it. The results show that 96% of respondents have already dealt with Setaria verticillata, whereas only 4% reported having no previous experience with the species. This high percentage confirms the widespread occurrence of the weed in the agricultural areas surveyed and indicates that most participants are familiar with it through direct field observation.

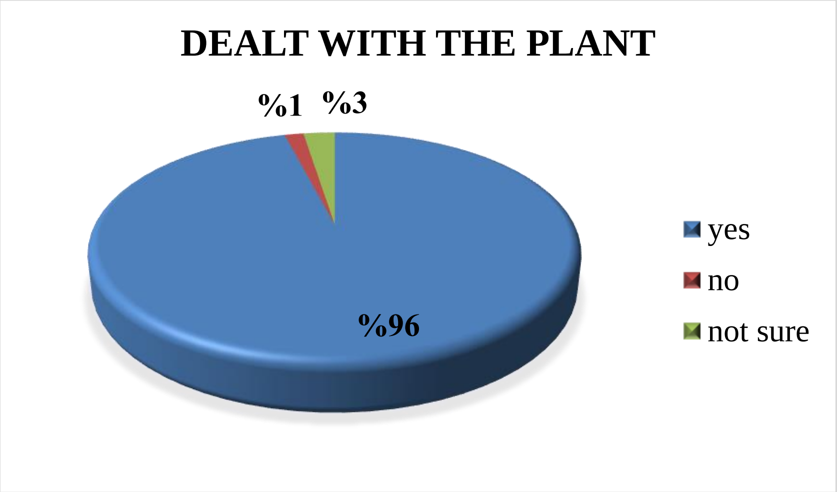


Figure31: Survey Results on Previous Encounters with Setaria verticillata.

Regarding its impact, 75% of the respondents stated that Setaria verticillata causes agricultural problems, while 25% did not report significant damage. This result demonstrates that the weed is perceived by the majority of participants as a harmful species capable of affecting crop development through competition for essential resources such as water, nutrients, light, and space.

Concerning weed management, 90% of respondents indicated that they possess methods or techniques for controlling or eliminating Setaria verticillata, compared with only 10% who reported lacking effective control strategies. This finding reflects a relatively high level of practical knowledge among farmers and agricultural stakeholders regarding weed management practices.

This widespread infestation of the weed (96%) is attributed to its high physiological capacity to compete with staple crops for vital survival factors such as water, soil micronutrients, space, and light. Although 90% of farmers reported employing various mechanisms and methods to control the weed, the persistent complaints about its rapid resurgence and spread indicate the ineffectiveness of current practices.

These alarming indicators align with the findings of **El-Houcine (2020)** in his field studies of invasive weeds in the oasis ecosystems of southeastern Algeria. He classified Setaria verticillata as one of the most dangerous summer grass infestations, possessing the ability to adapt to high heat stresses and produce enormous quantities of highly viable seeds that form a stable and sustainable seed bank within the soil. This renders traditional mechanical or chemical control methods only temporary, and the fields quickly fail to resist it.

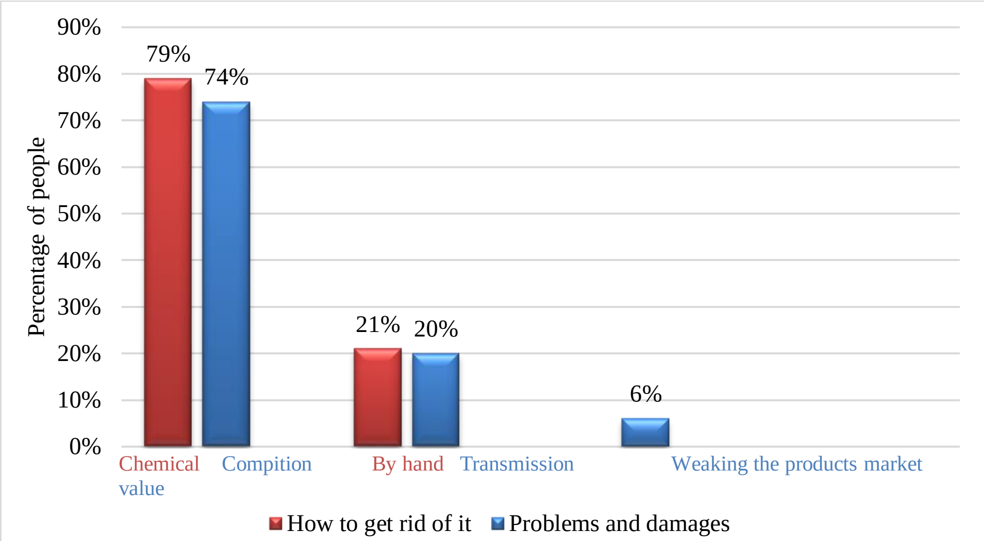


Figure32: Distribution of respondents' perceptions regarding the challenges caused by *Setaria verticillata* and the control methods adopted for its management

Overall, the results indicate that Setaria verticillata is a well-known weed among the surveyed population. The fact that a large proportion of respondents have encountered the species, recognized its harmful effects, and developed methods for its control highlights both its agricultural importance and the need to continue promoting effective and sustainable management practices.

1.3 Current Herbicide Use and Associated Constraints

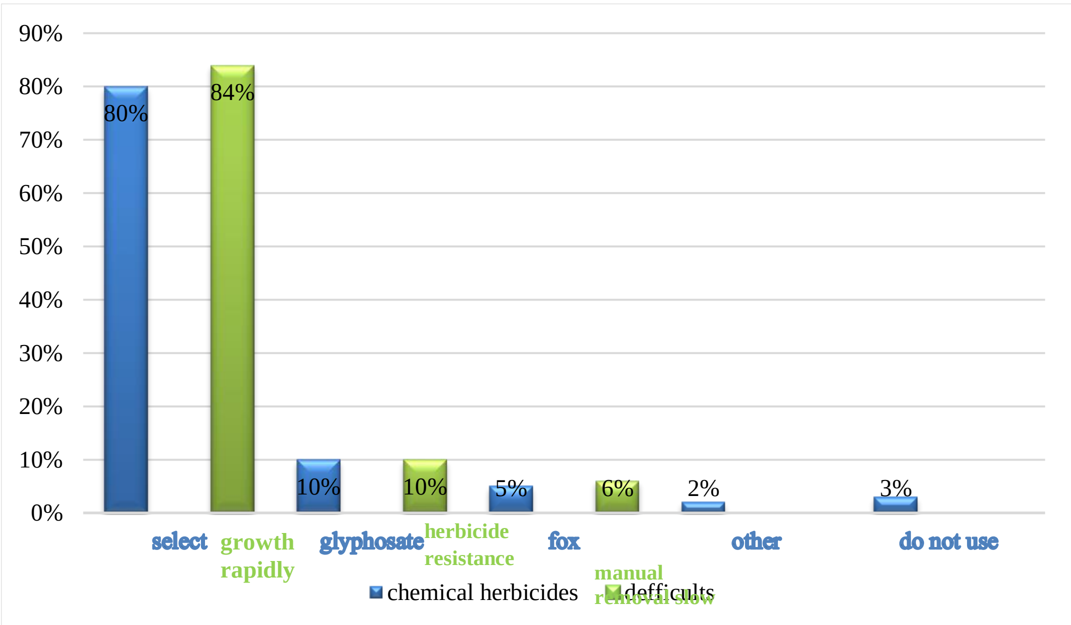


Figure33: Variation in respondents' perceptions of common herbicides and the difficulties encountered in managing this weed.

We can observe in Figure33 in the bleu column that 80 % of the sample use the chemical pesticide Select, as it is the most effective pesticide against this plant. The remaining percentage is divided as follows: %10 use the pesticide Glyphosate, which is second in effectiveness against this plant, and 5 % use the chemical pesticide Fuchs. The rest of the sample is further divided, with 2 % choosing other pesticides and the remaining 3% refusing to use chemical pesticides.

The green columns represent the difficulties associated with dealing with this weed, as 84% of the sample insist that it grows and spreads rapidly, 10% of the sample indicate that it is resistant to chemical herbicides, and 6 % percent of them believe that removing it manually is slow and strenuous, although effective.

Within the surveyed cohort, 80% of the participants confirmed that the graminicide "Select" (Clethodim) represents the most effective chemical option available. However, they noted a critical operational constraint: its efficacy is strictly limited to the pre-flowering and pre-seeding stages of the weed. Applying chemical herbicides after flowering is biologically redundant; even if the parent plant tissues are scorched, the mature, viable seeds are shed into the soil, continuously replenishing the persistent soil seed bank for subsequent seasons**.(Baćmaga, M.,et *al*..2024)**

The concentration of 80% of farmers on a single compound, such as clethodem, leads to intense selection pressure on the grass, accelerating resistance mutations in subsequent generations. These field findings are closely consistent with laboratory and operational analyses published by **Baćmaga et al. (2024)**, who demonstrated that the efficacy of herbicides specific to grasses is acutely affected by the plant's physiological stage. If spraying occurs after flowering and the onset of seed formation, the effectiveness in protecting reproductive tissues becomes almost negligible, and seeds remain mature and dispersible even if the leaves are burned. This explains the complaint of 84% of farmers. This strongly supports the scientific direction of your research in formulating a bio-herbicide enhanced with nanocarriers that maintain the stability of the active ingredient and increase its contact time and penetration into the waxy leaf surfaces of the grass before the critical flowering stage.

1.4 Assessment of Herbicide Effectiveness and Risks

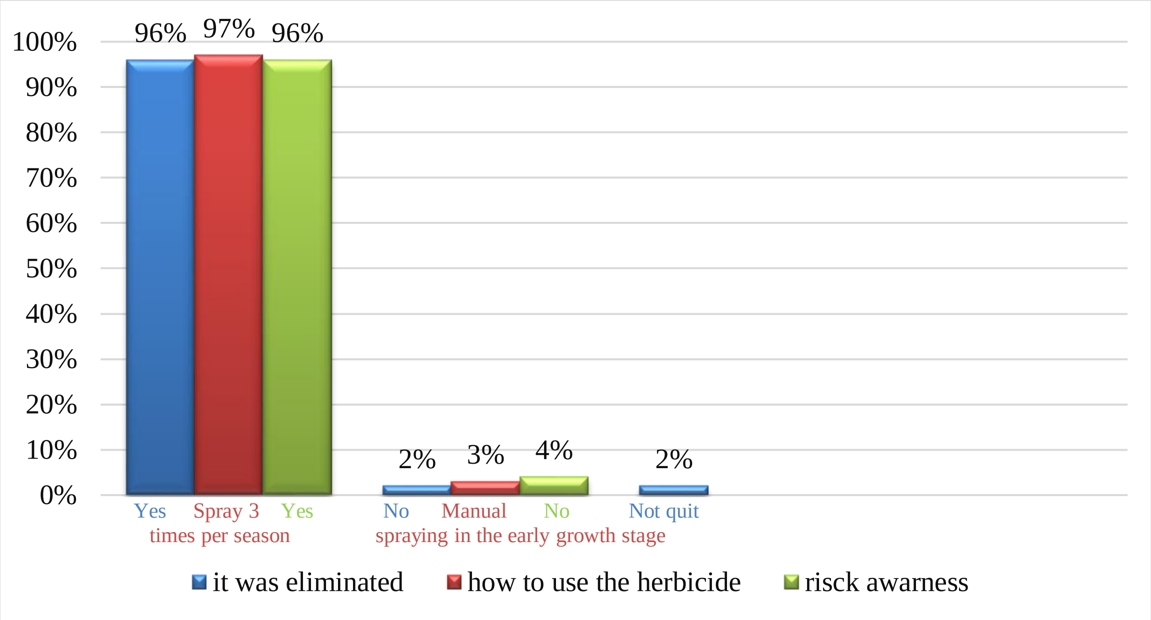


Figure34: Survey Results on Herbicide Use, Effectiveness, and Associated Risks

Figure34 shows the varying percentages of farmers' opinions regarding the effectiveness of the chemical herbicides, how to use it and its risks.

96% of the sample insisted that the herbicides were effective in eliminating the plant, while 2% said it was not effective, and another 2% felt it was not as effective as it should have been.

97% of the sample sprayed the plant 3 times per season for effective eradication, while the remaining 3% used traditional weeding methods such as hand-pulling in the early stages of growth, before flowering.

96% of the sample were aware of the risks of chemical herbicides, while 4% were unaware of these risks.

The fact that 97% of farmers resort to spraying three times in a single season is a serious indicator of weed resurgence and the accumulation of toxins in the ecosystem. This alarming data aligns with **Al-Rumaihi's (2023)** study on the cumulative toxicity of herbicides in desert and arid environments. The study showed that repeated chemical treatments with systemic pesticides lead to the leakage of intermediate compounds resulting from decomposition into the lower soil layers and from there into groundwater (which is the primary source of irrigation and drinking water in these areas). Furthermore, it eliminates beneficial fungi and bacteria in the soil, disrupting the biological balance. This explains the high level of awareness among farmers (96%), a phenomenon compounded by necessity due to the lack of a safe alternative.

1.5 Economic Aspects of Weed Control

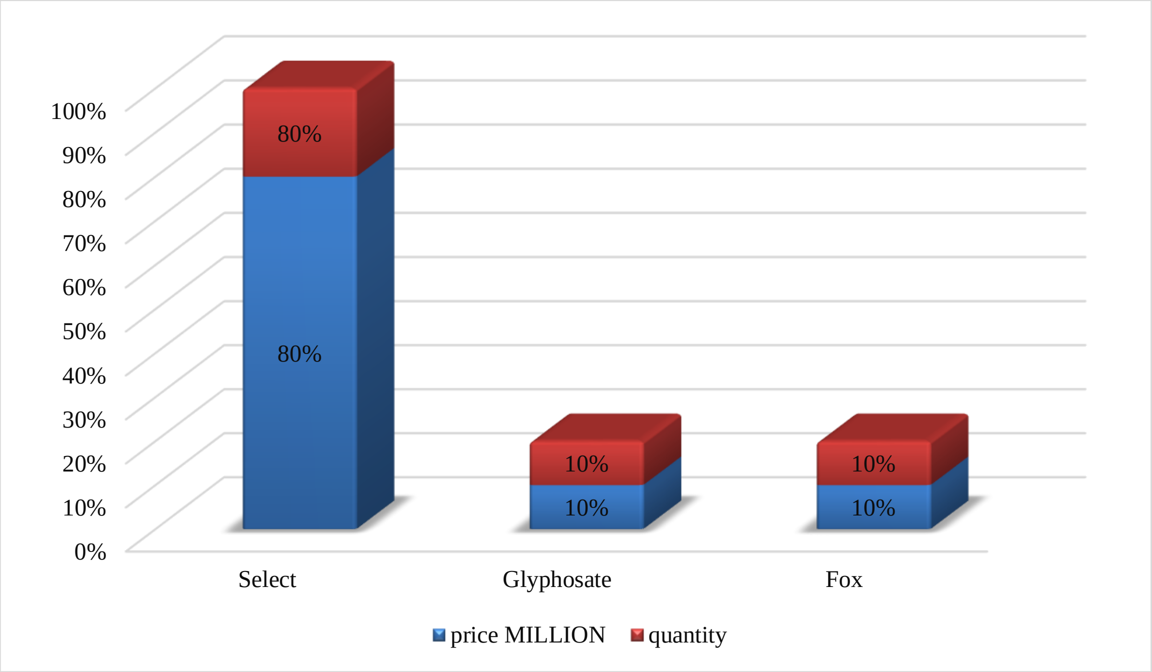


Figure35: Quantity and price of each kind of herbicide in one hectare.

The bar charts in Figure 35 represent an economic study of the quantity and prices of common and effective chemical herbicides against the harmful plant, sufficient to cover one hectare. A significant variation in prices and quantities is observed. 80% of the sample believe that 2 to 3 Liters at a price of approximately 2 to 3.5 million is sufficient to cover one hectare and can be effective against the plant after repeating the application 3 times. 10% of the sample believe that 3.5 to 5 liters of glyphosate at a price of approximately 3 to5 million is sufficient to cover one hectare. The remaining 10% of the sample believe that 5 to 7 liters of Fox at a price of 5 to 7 million is sufficient to cover one hectare.

These exceptionally high figures demonstrate that chemical pesticides now consume a significant portion of agricultural input budgets, drastically reducing farmers' net profit margins and threatening the viability of cultivating certain sensitive crops. These local economic findings align with global reports from the Food and Agriculture Organization **(FAO, 2022),** which confirmed that the steady rise in prices of petroleum-derived chemical compounds is placing immense pressure on developing countries to urgently adopt environmentally friendly innovations based on bio-based formulations to reduce production costs and bolster the resilience of a smart agribusiness.

2. Characterization of Nanocellulose by XRD and FTIR Techniques

2.1. Test results fourier transform-infrared (FTIR)

A. Before processing

This spectrum is characterized by specific technical features that reflect the state of the raw material and the conditions of its laboratory testing:

. Measurement Range and General Appearance:

The measured range extends from 300 cm^{-1} to 3000 cm^{-1}. The curve appears as a nearly flat horizontal line with a very high transmittance ranging between 96% and 10% in most of the bioorganic spectrum.

. Absent Functional Groups:

A complete absence of the characteristic cellulose groups is observed in this range, such as the absence of hydroxyl peaks or carbonyl and ether bonds. This is scientifically explained by the mass effect resulting from the extremely small sample size and the difficulty of fixing it on the instrument before processing, which caused the radiation to pass through without significant organic absorption.

. Far Infrared Region:

The only true signals are concentrated at the beginning of the curve below the frequency of 400 cm^{-1}, where a sharp and sudden drop in the transmittance coefficient to 0% is observed at wavenumber 310 cm^{-1}, followed by a small vibrational absorption at 334 cm^{-1}. This region only expresses the vibrations of the solid crystal lattice of the heavy crystalline components and impurities associated with the raw material before it is purified and modified.

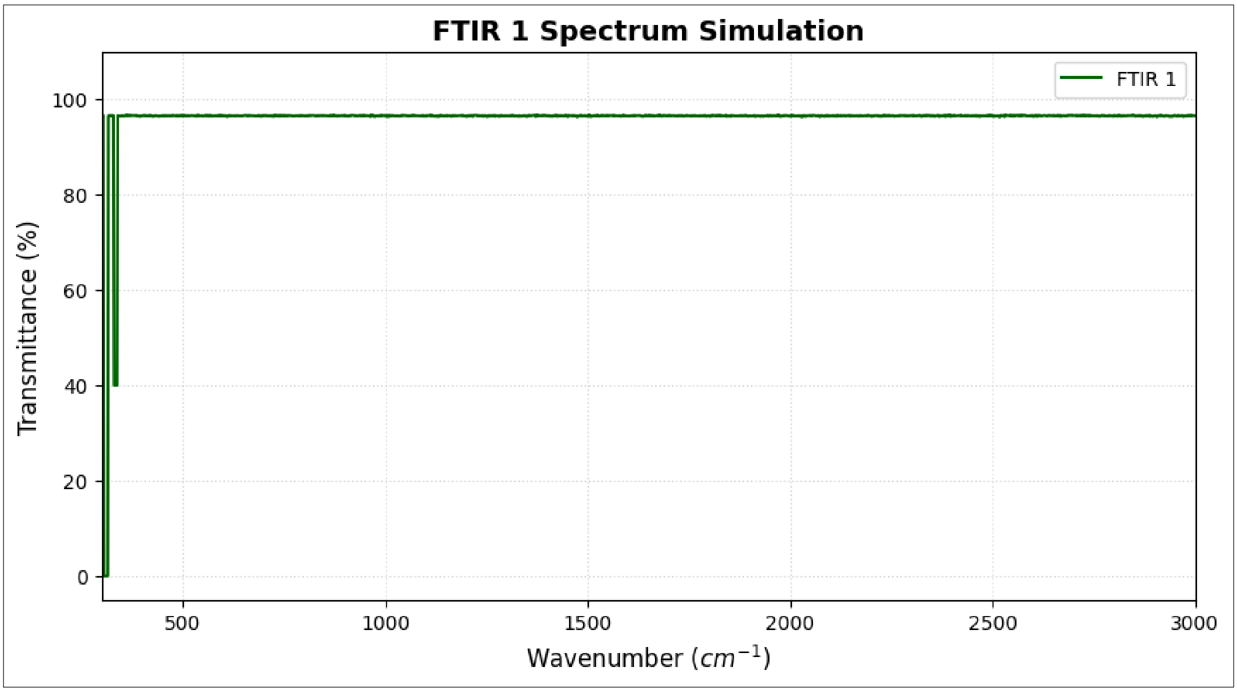


Figure36: FTIR spectra for sample before processing

B. After Treatment:

The curve represents the typical and ideal chemical signature for successful extraction of crystalline nanocellulose:

. Measurement Range and General Appearance:

It extends across the entire standard range from 400 cm^{-1} to 4000 cm^{-1}. Sharp and broad absorption peaks are clearly visible, indicating the excellent infrared response of the nanomaterial.

. Hydroxyl Group Peak (O-H):

A broad peak appears between frequencies of 3300 cm^{-1} and 3500 cm^{-1}. This is conclusive evidence of the presence of abundant hydroxyl bonds that make up the cellulose structure and are responsible for hydrogen bonding between the crystal chains.

. C-H Bond Peak:

A clear peak appears at a wavenumber close to 2900 cm^{-1}, which represents the stretching vibrations of the carbon-hydrogen bonds in the glucose rings that make up cellulose.

. Carbon dioxide (CO_2) peak:

A sharp, double-headed dip is observed at a frequency of 2350 cm^{-1}. This is due to the absorption of ambient carbon dioxide within the instrument's measuring chamber at the moment of testing and does not represent impurities in the material.

. Fingerprint region:

A very strong and distinct absorption dip is observed in the range between 1000 cm^{-1} and 1160 cm^{-1}, where the transmittance drops to approximately 74%. This region represents the vibrations of the C-O-C regenerative ether bonds and the axial crystalline bonds of pure cellulose.
(Alemdar, A.,et *al*.2008)

. Disappearance of the lignin peaks of hemicellulose:

Chemistically, the alkaline treatment and bleaching protocol leads to the breaking of the ether and ester bonds between lignin and carbohydrates, resulting in the disappearance of the characteristic carbonyl group peak (C=O) of the ester bonds in hemicellulose at 1740 cm –1 to 1730, and the disappearance of the aromatic peaks of lignin at 1510 cm-1**(Johar, N.,et *al*.2012).**

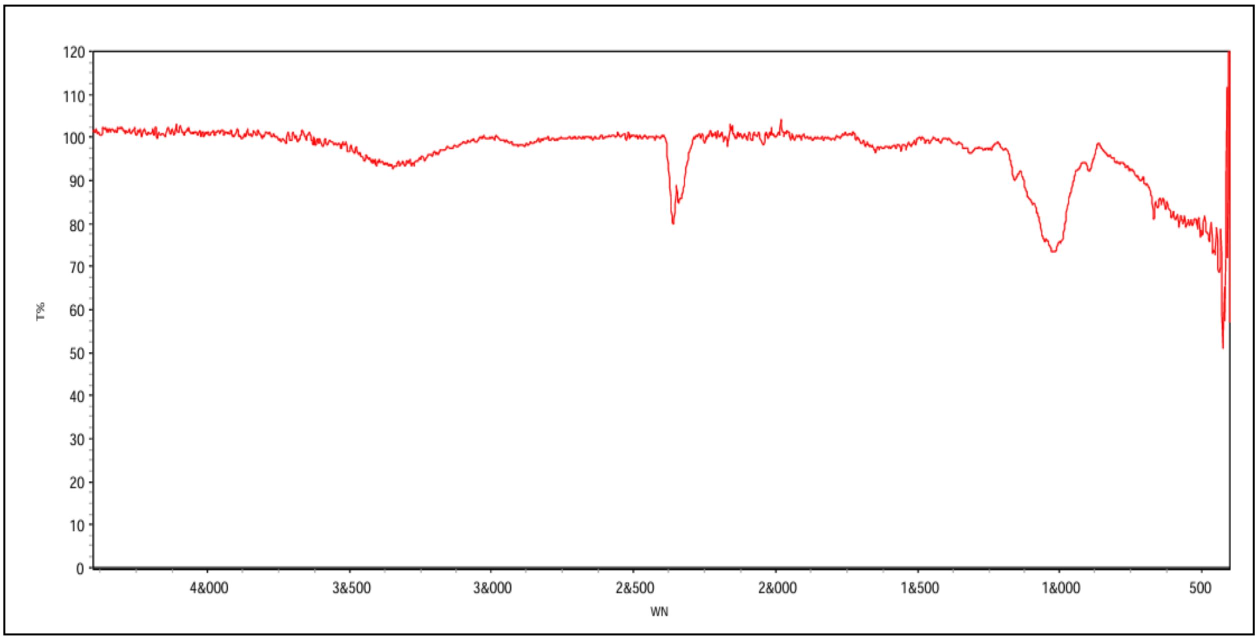


Figure37: FTIR spectra for sample after processing

This complete disappearance of the lignin and hemicellulose peaks is in absolute agreement with major reference research in nanofiber extraction, such as the study by **Alemdar and Sain (2008)** and the protocols of **Johar et al. (2012),** where they demonstrated that the efficiency of alkaline cooking processes followed by bleaching processes leads mechanically and chemically to the breaking of the ester and ether bonds linking lignin and complex carbohydrates, leading to their dissolution and isolation, which is clearly demonstrated by the spectral results.

2.2. Test results X-Ray diffraction (XRD)

The main crystalline feature is a prominent diffraction peak precisely centered at 2θ = 20.13°, with a peak intensity of 25,799 units. This specific diffraction pattern represents a marked shift from the characteristic (200) lattice plane diffraction of native plant cellulose (cellulose I), which typically occurs at 2θ = 22.6° **(Griffiths, P. R, Haseth, J. A. 2007).** This angular shift is scientifically attributed to a polymorphic phase transition, in which the glucan chains undergo structural reorientation into the more thermodynamically stable cellulose II lattice **(French, A. D. 2014).** This transition is facilitated by a swelling mechanism resulting from concentrated alkaline treatment with sodium hydroxide (NaOH) prior to acid hydrolysis. This process induces fiber swelling and the dissociation of the native intramolecular and intramolecular hydrogen bonds, followed by recrystallization into the altered crystalline form during washing**. (Gong, J.,et *al*. 2017)**

This plot shows extremely sharp secondary reflections at 2θ = 27.36° with an intensity of 14,538 units and at 2θ = 36.34° with an intensity of 10,842 units, along with secondary reflections at high angles of 57.94° and 79.64°, occurring against a relatively low spectral background. Rather than reflecting the intrinsic crystalline structure of the isolated cellulose nanocrystals, these extremely sharp and narrow peaks arise directly from experimental and sample size constraints. Because the quantity of isolated cellulose nanocrystal powder was extremely small—less than the critical volume required to adequately cover the sample holder in the instrument—special stabilization treatment was necessary, which inherently subjected the measurement to substrate and background interference**. (Alexander, L. E. 1979).** Consequently, these extraneous reflections represent the crystallization of associated background materials or residual inorganic salts, such as sodium sulfate derivatives generated during the acid-base neutralization phases. These salts precipitated and crystallized sharply due to insufficient washing of the small sample mass locally, preventing the emergence of the cellulose nanocrystals as a pure and independent signal.

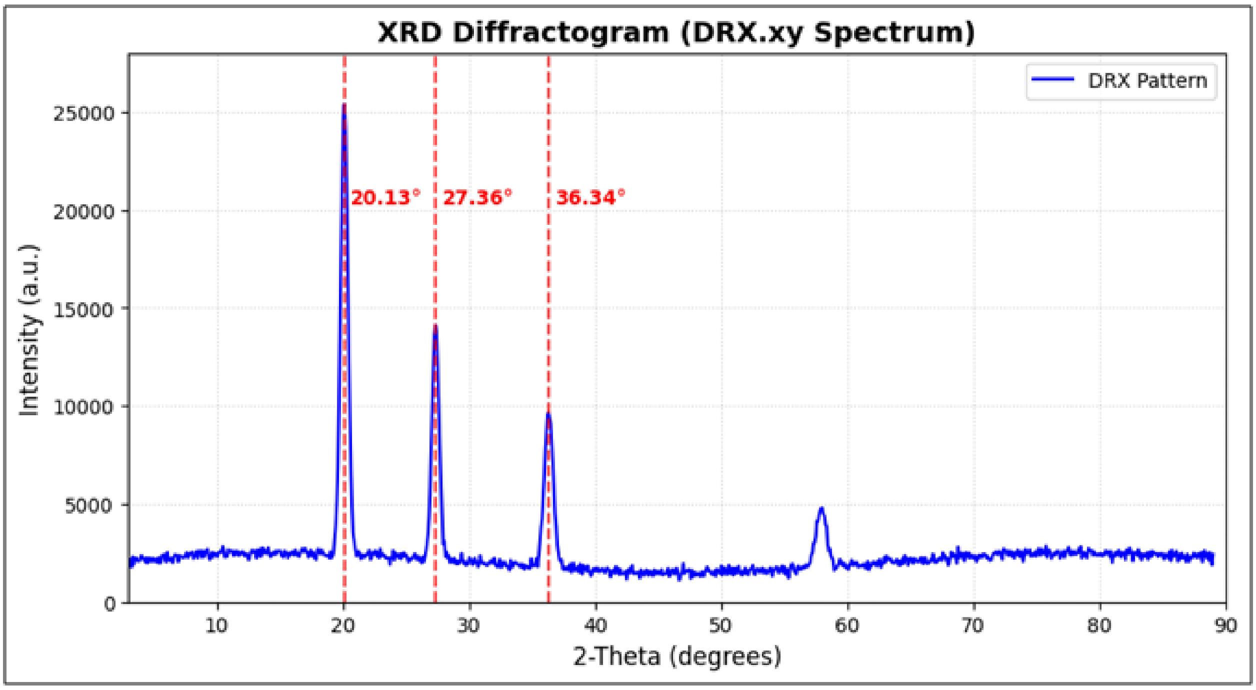


Figure38 : X-ray diffraction (XRD) for Nanocellulose

The X-ray diffraction (XRD) pattern confirms the effectiveness of acid hydrolysis in completely depolymerizing and removing amorphous regions within the cellulose matrix.

This complete removal of disordered regions is attributed to both the extreme sharpness of the reflections and the significant increase in the intensity of the main peak, exceeding 25,000 units.

This indicates a very high crystallinity index of cellulose nanocrystals crystallizing within the cellulose II phase, while the secondary reflections remain due to the local preparation conditions in the laboratory and the mass-limited sampling conditions.

This structural transformation is attributed to a robust cellular swelling mechanism triggered by treatment with concentrated sodium hydroxide. This mechanism breaks internal hydrogen bonds, producing free chains that are mechanically reorganized during repeated washing and subsequent acid hydrolysis, resulting in a compact and stable crystalline structure.

Furthermore, the intensity exceeding 25,000 units and the extreme sharpness of the peaks reflect a very high crystallinity index. This finding aligns with the thesis of **Gong et al. (2017),** who demonstrated that intensive hydrolysis using strong acids specifically and destructively targets amorphous regions with random arrangement, while the hard nanocrystals resist this hydrolysis. This ultimately leads to a pure crystalline nanocellulose with superior mechanical efficiency and an exceptionally large surface area, making it suitable as a smart nanocarrier for the slow and stable release of plant extracts.

3. Extraction Yield of the Studied Medicinal Plants

The extraction yield of the 3 plants was calculated according to the following:

Yield (%) = (Weight of extract / Weight of dry plant material) × 100

Table 12 presents the extraction yields obtained from: *Rosmarinus officinalis*, *Artemisia herba-alba*, and *Mentha piperita*. The results show a variation in extraction yield depending on the plant species. *Rosmarinus officinalis* recorded the highest extraction yield with **15.3%**, followed by *Mentha piperita* with **13.6%**, while *Artemisia herba-alba* showed the lowest yield at **12.1%**.

This difference may be related to the variation in the chemical composition of each plant, particularly the content of extractable compounds such as phenolic compounds, flavonoids, and other bioactive molecules. The higher yield obtained from *Rosmarinus officinalis* suggests a greater amount of soluble compounds recovered under the extraction conditions used. Overall, the results indicate that the extraction efficiency is influenced by the plant species and its phytochemical characteristics.

Table 12: Extraction Yield of the Studied Medicinal Plants

Plant extraction	**Yield**
Rosmarinus	15.3%
Artemisisa herb alba	12.1%
Mentha piperita	13.6%

These experimental and laboratory results are consistent with what **Benbelaïd et al. (2014)** concluded in their comprehensive study of the phytochemical survey of plants of western and southern Algeria. They statistically proved that plants belonging to the Lamiaceae family, such as rosemary and mint, have dense, superficial, hairy secretory glands rich in polar compounds that are easily extracted by solvents, compared to plants of the Asteraceae family, such as wormwood, which contain resinous and waxy compounds that may sometimes hinder the complete penetration of the solvent. This justifies the superior productivity of rosemary, which is 15.3%, and reinforces the feasibility of relying more on it in formulating the final composition of the bio-herbicide.

4. Phytochemical Screening of the Studied Plant Extracts and Assessment of Their Synergistic Effect.

Qualitative phytochemical analysis of extracts from peppermint (Mentha piperita), rosemary (Rosmarinus officinalis), and wormwood (Artemisia herba-alba), as well as the combined mixture, reveals significant variations in the nature and density of secondary metabolites. These results provide strong in vitro evidence of synergism among the active ingredients when combined (Table13).

Table13: Results of Phytochemical Screening of the Plant Extracts

Compounds	Type of test	Artemisia-herb-alba	Rosmarinus officinalis	Mentha piperita	The mix
Flavonoïdes	NaOH test	++	++	+	++
	Shinoda test (Mg + HCl)	++	++	+	+++
Saponines	Foam test	+	++	+++	+++
	Hemolytic test	++	++	++	++
Alcaloïdes	Dragendorff	+	+	+	+++
	Mayer	+	+	+	++
	Wagner	+	++	++	++

This significant increase in the reactivity of the mixture is not simply a matter of adding components mathematically; it is a crucial chemical demonstration of the cumulative synergistic effect. Mixing genetically diverse plant tissues results in a very broad and varied array of flavonoid structures, including flavones, flavanones, and anthocyanins.

This structural diversity prevents exclusionary interference between the active molecules, thus increasing the efficiency of precipitation and chemiluminescence of solvents. This results in a final mixture with a biological and phytotoxicity far exceeding the sum of the individual energies of each extract. This synergistic mechanism aligns perfectly with the thesis of **Boudou et al. (2013)** on the kinetics of phenolic compounds from the Lamiaceae and Rhizopodae families in inhibiting cellular enzyme systems.

4.1. Evaluation of Flavonoid Content and Synergistic Dynamics

When the flavonoid phase was analyzed using NaOH reagent and the Shinoda test (Mg + HCl), individual samples exhibited responses ranging from mild (+) as in peppermint to moderate (++) as in rosemary and wormwood. However, upon examination of the mixture, the reaction intensity jumped to a maximum (+++) in Shinoda's test while maintaining a clear response (++) in the sodium hydroxide reagent.

The marked increase in the reaction intensity of the mixture is physically and chemically attributed to a "cumulative synergistic effect." Previous studies (such as those by **Boudou et *al*. 2013.** and reference analyses of Lamiaceae and Asteraceae plants) indicate that mixing plant materials leads to the aggregation of a wide range of different flavonoid structures (e.g., flavones, flavanones, and anthocyanins). This structural diversity prevents the exclusionary interference of molecules and increases the efficiency of precipitation and chemiluminescence reagents, giving the mixture a higher biological and chemical potency than the sum of the potencies of the individual extracts, which is the fundamental principle in formulating innovative botanical therapies.

4.2. Saponin Content and Surfactant Activity

The foaming and hemolytic tests showed a stable and strong presence of saponins. Peppermint alone exhibited a very high foaming intensity (+++), while the mixture showed a strong and excellent response (++), followed by rosemary and wormwood (++). In the hemolytic test, all samples, including the mixture, showed a clear positive result (++).

Saponins are scientifically defined as glycosides with strong surfactant activity, possessing both a hydrophilic and a lipophilic component. The literature (such as the studies by **Cheok et al. 2014** on the physicochemical properties of saponins) indicates that combining plants containing various saponins maintains the foaming stability and hemolytic surface activity of the mixture without antagonism. Maintaining the superior (++) value of the mixture in both tests demonstrates that its surfactant properties and ability to penetrate cell membranes are balancedly enhanced, paving the way for easier absorption of other components (such as flavonoids and alkaloids) when the mixture is applied in nanosystems or emulsions.

4.3. Enhancement of Alkaloid Content via Triple Reagents:

The behavior of the alkaloids in this assay provides the most accurate evidence of chemical synergy. While the three plants individually showed relatively weak (+) responses to the Dragandorf, Meyer, and Wagner reagents (except for mint in the Wagner ++), the mixture exhibited a significant leap in detection response, reaching (+++) with the Dragandorf reagent and (++) with both the Meyer and Wagner reagents.

According to academic pharmaceutical studies (such as the research of **Wink et al. 2012** on synergistic effects in plant alkaloids), the three reagents (Dragendorff, Mayer, Wagner) rely on the precipitation of closed or heterocyclic nitrogen complexes in alkaloids by heavy metal or iodine compounds. The appearance of the maximum response (+++) in the mixture indicates that the mixing process combined different types of alkaloids (such as terpenoidal alkaloids, the distinctive pyrrolizidines in wormwood, and volatile alkaloids in rosemary and mint), resulting in a reduction of the colorimetric detection limit and a clear synergistic increase in the amount of complex precipitates. **(Kordali, S., et *al*. 2009)**

Conclusion on Synergism: These combined laboratory indicators demonstrate that the combination of extracts from Mentha piperita, Rosmarinus officinalis, and Artemisia herba-alba was not a simple mathematical addition of ingredients, but rather resulted in a synergistic chemical and biological reaction. The high intensity of the color and sedimentation indicators in the mixture compared to the individual extracts gives great scientific weight to support your thesis before the discussion committee; this chemical behavior confirms that the mixture has double the chances and opportunities to give biological activities (such as antioxidants, antimicrobials, or pharmaceutical and agricultural uses) that far exceed what each plant could give if used alone.

5. Results of Herbicidal Activity Evaluation

5.1 Analysis of the germination inhibition dynamics of Setaria verticillata seeds

The biological results recorded after applying individual extracts of Rosmarinus officinalis, Mentha piperita, and Artemisia herba-alba, the synergistic mixture, and the nano-bio-herbicide to

Setaria verticillata seeds demonstrate an absolute and non-solid inhibitory effect of 100% across most tested concentrations (100%, 50%, 25%, 10%).**(Kordali, S., et *al*. 2009)** The exceptional growth of the control sample (treated with distilled water only) provides physiological evidence of the high viability of the seed population used, thus enhancing the scientific credibility of the phytotoxic effect of the extracts.

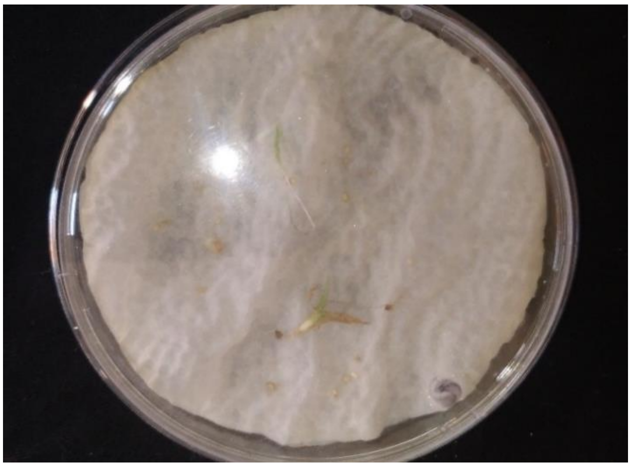


Figure39: Untreated Control in the Herbicidal Activity Experiment.

Table14: Comparative Effect of Individual Plant Extracts, Their Mixture, and Herbicide Treatment on Weed Germination.

Extrait	Rosmarinus	Artemisia herb albe	Mentha piperita	Mix	Nan-boi-herbicide
100%					
50 %					
25%					
10 %					

5.2. Phytochemical Interpretation and Synergistic Effect of the Mixture:

The total inhibition of germination at low concentrations (down to 10%) is directly attributable to the dense variety of secondary metabolites previously revealed by phytochemical analysis. The Role of Flavonoids and Alkaloids: Academic studies (e.g., **Wink, 2012**) indicate that alkaloids and flavonoids act as potent mitosis inhibitors within the embryo. They also disrupt the alpha-amylase and protease enzymes responsible for breaking down food reserves within the seed during the impibition phase, leading to chemical embryo death before radicle emergence. **(Anaya, A. L. 1999).**

The Exceptional Seed Appearance in Wormwood (10%): The germination of only one seed at a 10% concentration of wormwood extract alone is attributed to the low concentration of allelopathic compounds below the lethal threshold in that specific area.**(Scavo, A.,et *al*2019.)** This scientifically demonstrates that individual extracts at Its high dilution may lose some of its effectiveness, while the mixture maintained 100% seed destruction at the same concentration (10%) thanks to the cumulative synergistic effect that prevents the seeds from exhibiting any physiological resistance.

5.3 Physical and Biological Mechanisms of Action of the Nano-Bioherbicide

The nano-developed herbicide demonstrated decisive eradication efficiency, completely preventing germination. Combining the plant mixture with extracted cellulose nanocrystals (CNCs) alters the biomechanism of the reaction: Increased Cellular Permeability: Thanks to the nanoscale size and high specific surface area of the cellulose nanocrystals, they act as a nanocarrier that adheres to the seed coat (Testa) and increases its porosity, facilitating the rapid and intensive flow of toxic compounds (alkalis, flavonoids, and surfactants) into the embryonic tissues of the seed, even at very low concentrations (10%) **(Prasad, R et *al* ., 2017)**

6. Results of the Agronomic Field Trial for Herbicidal Activity Evaluation

Field application results of various herbicides on the weed Setaria verticillata in specific experimental areas (25 m²) show significant differences in action mechanisms, physiological response speed, and sustainability dynamics between the commercial chemical herbicide, the crude bioherbicide, and the nano-developed bioherbicide.

6.1 Inadequacies of the Commercial Chemical Herbicide (Select) and Phenological Stage Resistance

The herb treated with the chemical herbicide showed wilting and yellowing during the first week, but recovered its vegetative growth after this period. This behavior is physiologically attributed to the plant's advanced phenological stage during spraying (flowering and seed formation stage). Mature plants possess a robust root system and a substantial carbohydrate reserve in their tissues, along with a thicker cuticle layer on their leaves. This reduces the penetration of systemic herbicides or allows the plant to activate its own metabolic mechanisms to break down and resist the toxic chemical compound. This finding is entirely consistent with the findings of **(Knezevic et al. 2007)**, which confirmed that the efficacy of chemical herbicides decreases significantly with the age of the weeds. Grass weeds (such as the genus Setaria) exhibit high physiological resistance to herbicides after reaching the reproductive and flowering stages compared to their seedling stage.

Table15: Results Agronomic Field Trial of Herbicide Activity

Control	**Observation**	**Image from the middle**
Chemical Herbicide	During the first week the plant wilts and turns yellow, but it grows again after this period.	
Bio-herbicide	On the first day, we notice the plant turning yellow and necrose spreading on the leaves and branches, then the plant dies.	
Nano-bio-herbicide	In the first 24 hours, yellow and brown spots appear where the pesticide was sprayed. The pesticide can be observed adhering to the leaves, branches, and seeds. Necrosis is observed in the areas where the spots have spread.	

6.2 Acute Contact Phytotoxicity of the Crude Bioherbicide

The bioherbicide (a mixture of three plants) exhibited a rapid and localized effect, characterized by the appearance of yellow spots followed by acute necrosis of the leaves and stems within 24 hours, culminating in plant death within the first three days. This mechanism is classified as acute contact phytotoxicity. The volatile oils, flavonoids, and alkaloids present in the mixture directly disrupt cell membranes and the leaf epidermis, leading to rapid physiological desiccation and cell death in the necrotic tissue. This behavior aligns with the research of **(Dayan et al. 2009)** on contact biopesticides, which demonstrates that the secondary metabolites of medicinal plants act via a leaf-scorch mechanism, resulting in cellular leakage and rapid localized death of green plant tissues that come into direct contact with the extract.

6.3 Structural Superiority and Extended Action Mechanism of the Nano-bio-herbicide

The nano-developed bio-herbicide achieved optimal field control, requiring less extract while delivering a stronger, more widespread, and longer-lasting effect. Signs of tissue necrosis appeared evenly on leaves, stems, and seeds within just 12 hours. This success is attributed to two key factors:

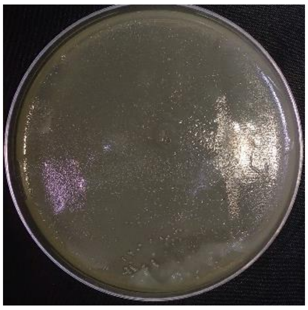
Superior Adhesion and Retention: Nano-crystal cellulose (CNCs) act as stabilizers and nanocarriers. Thanks to their enormous specific surface area and hydrogen bonds, these crystals prevent the volatilization or washing away of the bioactive compounds by moisture and wind, and enhance the adhesion of the solution to the waxy leaf surface and rough seed coats of Setaria verticillata. Targeted Distribution and Precision Spraying: Using a fine-nozzle sprayer within the nano-formulation allowed for the creation of a uniform spray, increasing coverage efficiency, preventing wastage of the active ingredient, and ensuring that the phytotoxicity-carrying nanoparticles reached the stomata and vital tissues evenly.

Field Seed Inhibition: The nano-pesticide's adhesion to and necrosis of seeds on the mother plant is a strategic advantage that prevents live seeds from reaching the soil seed bank, thus ensuring long-term weed control in subsequent seasons. These findings are supported by the research of **(Deka et al. 2021)** on nanoparticle applications in plant protection. They demonstrated that loading plant extracts onto cellulose-based nanocarriers increases their field stability, reduces the dosage, and gives the pesticide formulation a sustained release characteristic, preventing the target plant from exhibiting any recovery mechanisms or cellular resistance.

7. Evaluation of the Environmental Impact of Nano-herbicides on Soil Microbiota

The integrity of soil microbiota is a key criterion for evaluating the environmental sustainability of any innovative herbicide. Cultures of bacteria and fungi extracted from soil treated with the nano-bio-pesticide—at different dilution levels (Origin, Dilution 1, Dilution 2)—show normal and excellent growth of microbial colonies. This result definitively proves that the developed nano-formulation possesses selective toxicity; it eradicates the targeted weed without negatively impacting the integrity and vitality of beneficial soil microbiota.

Table16: Results of Test on safety of the herbicide on soil microorganisms

Dilution ratio	**Origin**	**Dilution 1**	**Dilution2**
Results			

7.1 Biological Selectivity and Absence of Toxic Effect on Soil Microbiota

The normal growth of bacteria and fungi after application of the nano-pesticide is attributed to the nature of the biological components used (a blend of mint, rosemary, and wormwood) and their formulation mechanism. Soil microorganisms possess sophisticated enzyme systems capable of adapting to and biodegrading plant secondary metabolites for use as carbon and energy sources, unlike manufactured chemical compounds that accumulate toxicity in the soil. This finding aligns with the conclusions of **(Venzon et al. 2020)** that bioherbicides derived from medicinal plants do not pose a threat to soil microbial biodiversity. In fact, they biodegrade rapidly in the environment without leaving toxic residues that affect the cellular viability of beneficial bacteria and fungi.

7.2 The Role of Nano-Crystalline Cellulose (CNCs) in Ensuring Environmental Safety

The use of nano-crystalline cellulose (CNCs) as a nanocarrier is a crucial factor in protecting soil microbes. This technology ensures targeted encapsulation of bioactive compounds and allows for slow, controlled release only on the leaf and stem surfaces of the targeted grass, significantly reducing leaching of excess doses into the soil. Furthermore, nanocellulose is a biocompatible and fully biodegradable natural polymer that acts as a nutrient and growth promoter for certain cellulolytic microorganisms in the soil after decomposition. This interpretation is supported by the study by **(Kah et al. 2018)** on the environmental risk assessment of nano-herbicides. They demonstrated that using natural nanocarriers such as cellulose reduces non-target toxicity in ecosystems and prevents disruption to the biological structure of treated soils, compared to conventional herbicides.

Conclusion

This study aimed to develop a sustainable and intelligent bio-alternative for controlling *Setaria verticillata* in the Souf Valley region. The goal was to overcome the cellular resistance this weed has developed against conventional chemical herbicides (such as Select) and to protect the desert ecosystem from the toxic accumulation of these herbicides.

This study serves as a comprehensive scientific and applied model for developing smart and sustainable agricultural solutions. It integrates real-world observations, the synergistic chemistry of plant extracts, and nanotechnology to deliver an effective and environmentally friendly bio-alternative for weed control.

The study began with a field survey that confirmed the widespread presence of *Setaria verticillata* and the inability of conventional chemical herbicides (such as Select) to eradicate it once it reached advanced growth stages. This led to the development of a bio-alternative based on a combination of extracts from peppermint (*Mentha piperita*), rosemary *(Rosmarinus officinalis*), and wormwood (*Artemisia herba-alba*).

This combination resulted in a synergistic effect, increasing the concentration of flavonoids, alkaloids, and saponins compared to the individual extracts. To further enhance its neutral performance, the mixture was loaded onto crystalline nano-cellulose carriers (CNCs), producing a nano-bioherbicide that demonstrated superior and long-lasting eradication efficacy in field trials (25 m^2).

This efficacy was characterized by acute and almost immediate necrosis of leaf and seed tissues, a reduced dosage requirement, and superior adhesion, while simultaneously demonstrating complete environmental safety by preserving the natural growth of beneficial bacteria and fungi in the soil.

This opens promising avenues for testing the nano-formulation on other weed species and studying the sustained release kinetics of the active ingredients, paving the way for its adoption as a commercial product that supports the trend toward sustainable organic agriculture.

Perspective

This serious research represents a starting point for developing this product in order to achieve our goal of eliminating the harmful plant *Setaria verticillata* and reducing chemical pollution from chemical herbicides, especially in the El-Oued region.

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Annexes

Survey on the harmful plant Setaria vertilcilata

Common name: Sboul far or Ellafa

In Oued souf region

□ Personal Information:

- Age: 18-25 years □ 25-35 years □ 40 years and older □

- Educational Background: Primary □ Intermediate □ Secondary □ University □

- Specialization: ....

- What is your relationship to the agricultural sector:

Farmer □ Agricultural Specialist □ Other: ....

- How long have you been involved in agriculture?
....

□ Interview:

1- Have you ever dealt with this weed before? Yes □ No □

2- What problems or damage does this weed cause to your crops?

....
....

3- What methods or techniques have you used to get rid of this weed or limit its spread?

....
....
....
....
....

4- What difficulties did you encounter while dealing with this weed?

....
....
....
....
....

5- What types of chemical herbicides did you use to control this weed?

....
....
....

6- What were the costs you incurred on the chemical herbicides? (The answer can be broken down by: pesticide cost, labor cost, number of purchases…)

....
....
....
....

7- Did any of the pesticides you used achieve satisfactory results? Yes □ No □

Alternative answer:
....

8- If yes, state the name of the pesticide that gave the best results:

....
....
....

9- Was this pesticide able to completely and permanently eliminate the weed?

....

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Thank you for your time and awerness.