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Preparation of a Natural Spray from *Phoenix dactylifera* L. and *Artemisia campestris* L. Extracts for Moisture Retention and Control of *Aphis gossypii* Glover.

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

الاهداء

بسم الله الرحمن الرحيم

(وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ عَلَيْهِ تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ) [هود: 88]

إلى نفسي، التي خاضت هذه الرحلة بكل ما حملته من تعب وأمل، والتي آمنت أن الأحلام لا تتحقق إلا بالسعي والصبر، فكان هذا الإنجاز ثمرة سنوات من الاجتهاد والمثابرة.

الذي سبقتني إلى دار الخلود، وبقيت ذكراه أعظم ما أحمله في قلبي، ترافقتي وصاياها في كل خطوة، وأستحضر حضوره في كل نجاح. تمنيت لو كان بيننا ليشركني فرحة هذه اللحظة، لكن عزائي أنه حاضر في دعائي، وأسأل الله أن يجعل هذا العمل نورًا يصل إليه، ورحمةً تنزل عليه، وأن يجمعني به في الفردوس الأعلى

"أبي الغالي الصادق"

التي حملت عني أعباء الحياة، وكانت لي الأب والأم، والسند الذي لا يميل، والتي نسجت من تعبها وسهرها طريقاً أوصلني إلى هذه اللحظة. كل نجاح أصل إليه هو ثمرة لدعواتها، وكل فرحة أعيشها تحمل بصمتها، فحفظها الله وأدامها تاجاً فوق رأسي

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Abstract

This study aimed to develop a natural spray based on hydroalcoholic extracts of *Phoenix dactylifera* L. leaves and *Artemisia campestris* L. and to evaluate its insecticidal efficacy against *Aphis gossypii*, as well as its potential role in alleviating water stress in mint plants. The biological activity of the individual extracts and the formulated spray was assessed at three concentrations: C1 (31.25 $\mu\text{L/mL}$), C2 (62.5 $\mu\text{L/mL}$), and C3 (125 $\mu\text{L/mL}$), through aphid mortality, repellent activity, and LT_{50} determination. The results revealed a concentration-dependent insecticidal effect. The formulated spray exhibited the highest efficacy, causing mortality rates of 82%, 95%, and 100% after 12 min at C1, C2, and C3, respectively, and achieving complete aphid mortality within 16–20 min. Similarly, both *A. campestris* and *P. dactylifera* extracts showed significant insecticidal activity, with mortality increasing as concentration increased and exposure time progressed. In addition, mint plants treated with the spray under 50% water stress maintained better vigor and greener foliage than untreated stressed plants after 15 days. These findings indicate that the developed plant-based formulation has promising potential as a natural insecticide against *A. gossypii* while contributing to improved plant performance under water-deficit conditions.

Keywords: *Phoenix dactylifera*; *Artemisia campestris*; *Aphis gossypii*; insecticidal activity; water stress..

الملخص

هدفت هذه الدراسة إلى تطوير بخاخ طبيعي قائم على المستخلصات الهيدروكحولية لأوراق نخيل التمر (*Phoenix dactylifera* L.) ونبات الشيح الصحراوي (*Artemisia campestris* L.)، وتقييم فعاليته المبيدة للحشرات ضد من القطن (*Aphis gossypii*)، بالإضافة إلى دراسة دوره المحتمل في التخفيف من آثار الإجهاد المائي على نبات النعناع. وقد تم تقييم النشاط الحيوي للمستخلصات الفردية والبخاخ المُحضّر عند ثلاثة تراكيز: C1 (31.25 ميكرو لتر/مل)، وC2 (62.5 ميكرو لتر/مل)، وC3 (125 ميكرو لتر/مل)، وذلك من خلال تحديد نسبة نفوق حشرة المن، والنشاط الطارد، وزمن الموت النصفى (LT_{50}). أظهرت النتائج وجود تأثير مبيد للحشرات يعتمد على التركيز، حيث سجل البخاخ المُحضّر أعلى فعالية، إذ بلغت نسب النفوق 82% و95% و100% بعد 12 دقيقة عند التراكيز C1 وC2 وC3 على التوالي، مع تحقيق نفوق كامل للحشرات خلال 16-20 دقيقة. كما أظهرت مستخلصات الشيح الصحراوي ونخيل التمر نشاطاً مبيداً ملحوظاً، حيث ازدادت نسبة النفوق بزيادة التركيز ومدة التعرض. بالإضافة إلى ذلك، حافظت نباتات النعناع المعاملة بالبخاخ تحت إجهاد مائي بنسبة 50% على حيوية أفضل ولون أخضر أكثر مقارنة بالنباتات المجردة غير المعاملة بعد 15 يوماً من التجربة. تشير هذه النتائج إلى أن التركيبة النباتية المطورة تمتلك إمكانات واعدة كمبيد حشري طبيعي ضد من القطن (*Aphis gossypii*)، مع مساهمتها في تحسين أداء النباتات تحت ظروف نقص المياه.

الكلمات المفتاحية: نخيل التمر (*Phoenix dactylifera*)؛ الشيح الصحراوي (*Artemisia campestris*)؛ من

القطن (*Aphis gossypii*)؛ النشاط المبيد للحشرات؛ الإجهاد المائي

Résumé

Cette étude a eu pour objectif de développer un spray naturel à base d'extraits hydroalcooliques des feuilles de *Phoenix dactylifera* L. et d'*Artemisia campestris* L., et d'évaluer son efficacité insecticide contre *Aphis gossypii*, ainsi que son rôle potentiel dans l'atténuation du stress hydrique chez les plants de menthe. L'activité biologique des extraits individuels et du spray formulé a été évaluée à trois concentrations : C1 (31,25 µL/mL), C2 (62,5 µL/mL) et C3 (125 µL/mL), à travers le taux de mortalité des pucerons, l'activité répulsive et la détermination du temps létal médian (LT_{50}). Les résultats ont révélé un effet insecticide dépendant de la concentration. Le spray formulé a présenté la plus grande efficacité, avec des taux de mortalité de 82 %, 95 % et 100 % après 12 min aux concentrations C1, C2 et C3 respectivement, et une mortalité totale des pucerons atteinte en 16 à 20 min. De même, les extraits d'*A. campestris* et de *P. dactylifera* ont montré une activité insecticide significative, la mortalité augmentant avec la concentration et la durée d'exposition. Par ailleurs, les plants de menthe traités avec le spray et soumis à un stress hydrique de 50 % ont conservé une meilleure vigueur et un feuillage plus vert que les plants stressés non traités après 15 jours. Ces résultats indiquent que la formulation végétale développée possède un potentiel prometteur en tant qu'insecticide naturel contre *Aphis gossypii*, tout en contribuant à améliorer les performances des plantes en conditions de déficit hydrique.

Mots-clés : *Phoenix dactylifera* ; *Artemisia campestris* ; *Aphis gossypii* ; activité insecticide ; stress hydrique..

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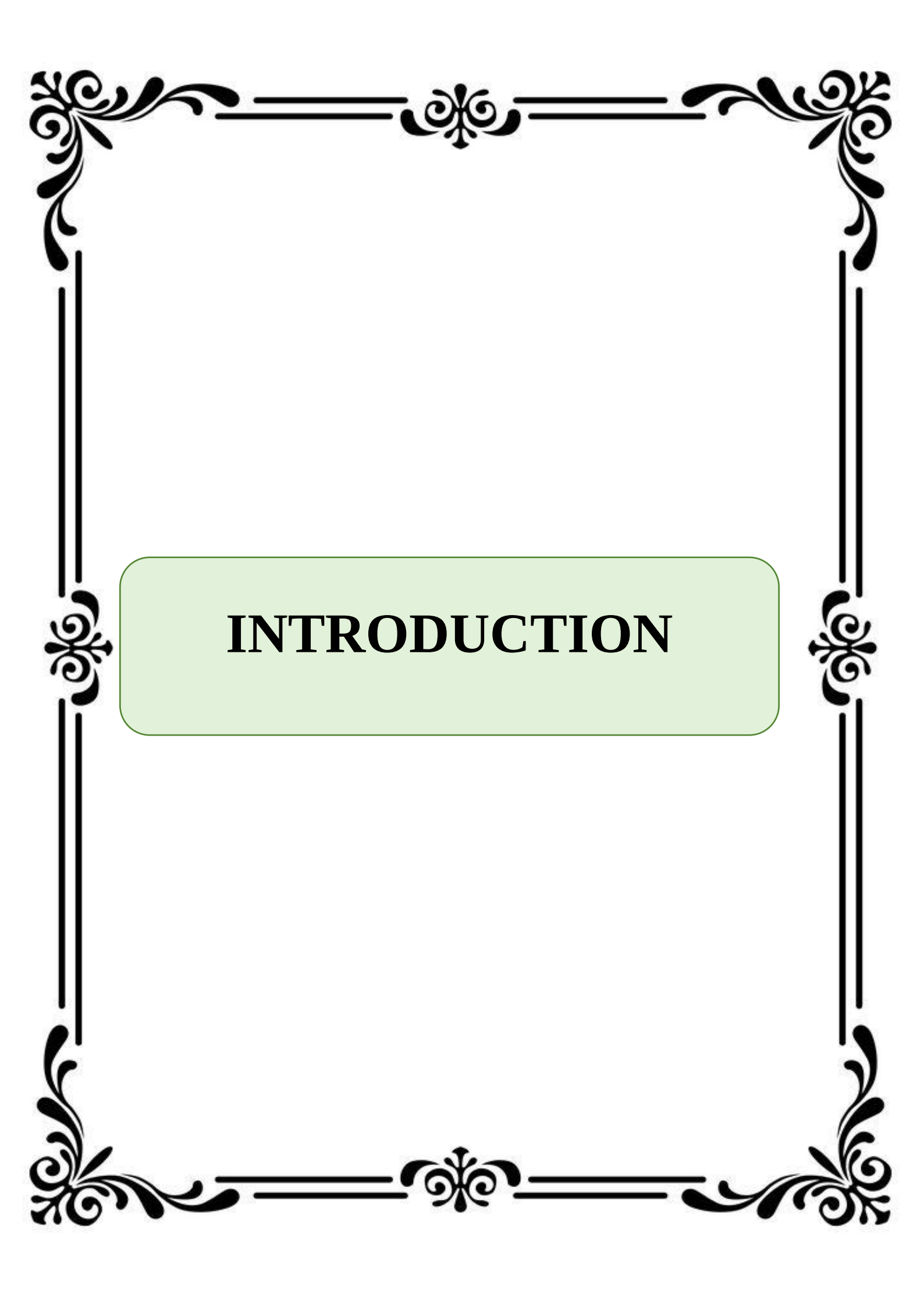
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Liste of Abbreviation

ABA: Absciscic Acid
ADH: Alcohol Dehydrogenase
ATP: Adenosine Triphosphate
C1, C2, C3: Concentrations 1, 2, and 3
CO₂: Carbon Dioxide
ETH: Ethylene
H₂O: Water
LT₅₀: Median Lethal Time
O₂: Oxygen
PDC: Pyruvate Decarboxylase
PSII: Photosystem II
R²: Coefficient of Determination
ROS: Reactive Oxygen species



INTRODUCTION

Introduction

Agricultural productivity is increasingly affected by biotic and abiotic stresses that negatively influence plant growth, yield, and quality. Among biotic stresses, insect pests represent one of the major factors responsible for significant economic losses worldwide, while water stress is considered one of the most important environmental constraints limiting plant development, particularly in arid and semi-arid regions. The simultaneous occurrence of these stresses can intensify plant damage and compromise the sustainability of agricultural systems (Isman & Norris, 2024).

The cotton aphid (*Aphis gossypii*) is considered one of the most destructive agricultural pests due to its high reproductive capacity, sap-feeding behavior, and ability to transmit several plant viruses, leading to reduced plant growth and productivity. Furthermore, the intensive and repeated use of chemical pesticides has resulted in the emergence of resistant insect populations, in addition to environmental contamination and potential risks to human health. These concerns have increased the need for safer and more sustainable natural alternatives (Isman & Norris, 2024; Wang et al., 2025).

Plant extracts have received increasing attention as promising tools for sustainable pest management due to their richness in bioactive compounds, including phenolic compounds, flavonoids, and terpenes, which exhibit insecticidal, repellent, and antifeedant properties. Moreover, these compounds can contribute to plant protection through their antioxidant activity, helping plants tolerate unfavorable environmental conditions (Pavela et al., 2025; Fuentes-Lopez et al., 2025).

Phoenix dactylifera L. (date palm) and *Artemisia campestris* L. (Tegheft) are considered valuable sources of biologically active compounds. Date palm leaves contain high levels of phenolic compounds and flavonoids with recognized antioxidant properties, whereas *A. campestris* is characterized by the presence of volatile compounds and terpenes exhibiting insecticidal and diverse biological activities. Therefore, combining these two plants may provide a botanical formulation with enhanced efficacy resulting from the complementary effects of their phytochemical constituents (Bouhlali et al., 2025; Benarfa et al., 2024).

Water stress is another major abiotic factor affecting plant growth and productivity. Water deficiency induces physiological and biochemical disturbances that negatively influence metabolic processes, photosynthetic efficiency, and vegetative development. Recent studies have demonstrated that plant-derived bioactive compounds can enhance plant

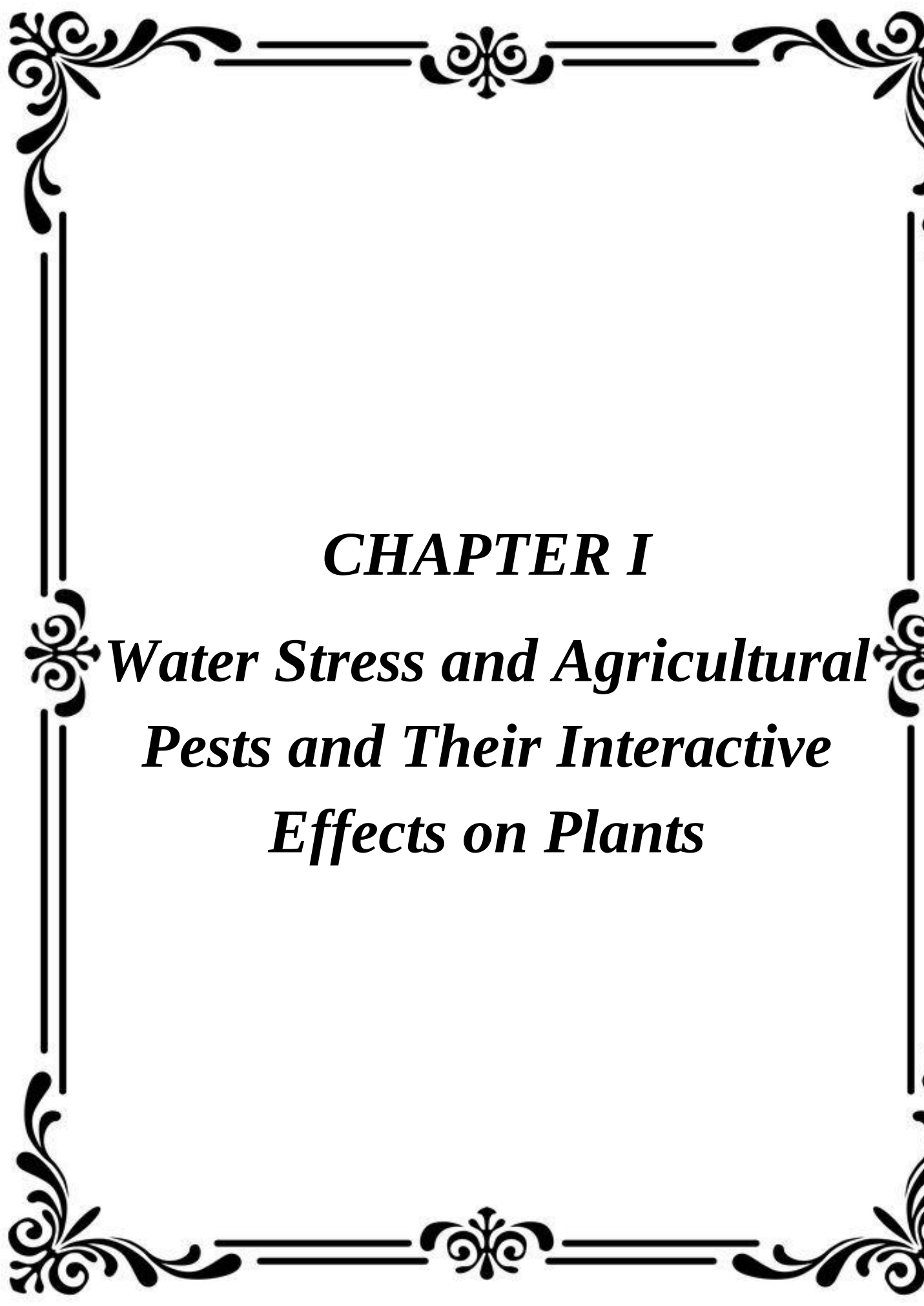
tolerance to drought conditions by improving antioxidant defense systems and maintaining cellular homeostasis under water deficit conditions (Shahhoseini et al., 2025).

Based on these considerations, the present study aimed to develop a natural spray formulation based on hydroethanolic extracts of *Phoenix dactylifera* L. leaves and *Artemisia campestris* L., and to evaluate its insecticidal and repellent efficacy against the cotton aphid (*Aphis gossypii*) through mortality rates, median lethal time (LT₅₀), and repellent activity assessment. In addition, the study investigated the potential role of this formulation in alleviating water stress effects in mint plants through visual evaluation of plant vigor and morphological responses.

This Master's thesis is divided into two main parts. The first part presents the bibliographic review, covering water stress, agricultural pests, and plant bioactive compounds. The second part focuses on the experimental study, including the preparation of hydroethanolic extracts, the development of the plant formulation, and the evaluation of its biological activity. The Results and Discussion chapter presents and analyzes the effects of the extracts and plant spray against *Aphis gossypii* and their potential role in improving mint plant tolerance to water stress. The Master's thesis concludes with a general conclusion summarizing the main findings, conclusions, and future perspectives for the use of plant extracts as sustainable and environmentally friendly alternatives for plant protection..

Part I

Bibliographic part



CHAPTER I

Water Stress and Agricultural Pests and Their Interactive Effects on Plants



I. Water Stress

1. Definition Water Stress in plants

Water stress is one of the major environmental stresses affecting plant development at different growth stages, and remains a key limiting factor for plant survival and productivity (Dong et al., 2025).

It is defined as a physiological condition that occurs when the water balance in plants is disrupted due to either insufficient or excessive water availability compared to their physiological requirements, leading to disturbances in essential biological processes such as photosynthesis, growth, and ionic transport within cells (Li et al., 2024).

2. Factors Causing Water Stress in plants

2.1. Causes of Drought Stress

Drought stress arises from reduced water availability due to decreased or irregular precipitation, coupled with elevated temperatures, which enhance evaporation and transpiration rates and lead to depletion of soil moisture. In addition, soil with low water-holding capacity limits water supply to plant roots. This results in an imbalance between water uptake and loss, ultimately inducing stress in plants (Seleiman et al., 2021).

2.2. Causes of Waterlogging Stress

Waterlogging stress occurs as a result of soil saturation with water exceeding its drainage capacity, leading to reduced soil aeration and limited oxygen availability for plant roots. This condition is associated with several factors, including excessive or prolonged rainfall, over-irrigation, poor soil drainage, and a high groundwater table. In addition, heavy soils with low permeability contribute to long-term water retention, while flooding events cause direct soil inundation. These conditions disrupt the root environment and induce physiological stress in plants (Zhang et al., 2025).

3. Effects of water stress in plants

Water stress causes physiological and morphological changes at different stages of plant development, regardless of when the water deficit occurs (Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009).



3.1. Growth and yield

Water stress reduces germination rate and weakens seedling establishment due to the disturbance of early physiological processes. It also inhibits early growth, leading to reduced hypocotyl length and biomass, while sometimes stimulating root growth as an adaptive mechanism to improve water uptake (Fig.01).

During the vegetative stage, plant growth and development are reduced because of the impairment of essential physiological functions.

Plant growth depends on three main processes: cell division, cell elongation, and cell differentiation, all of which are highly sensitive to water availability. Reduced turgor pressure limits cell elongation and expansion. Under severe stress, decreased water transport further restricts cell hydration, resulting in reduced overall plant growth (Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009).

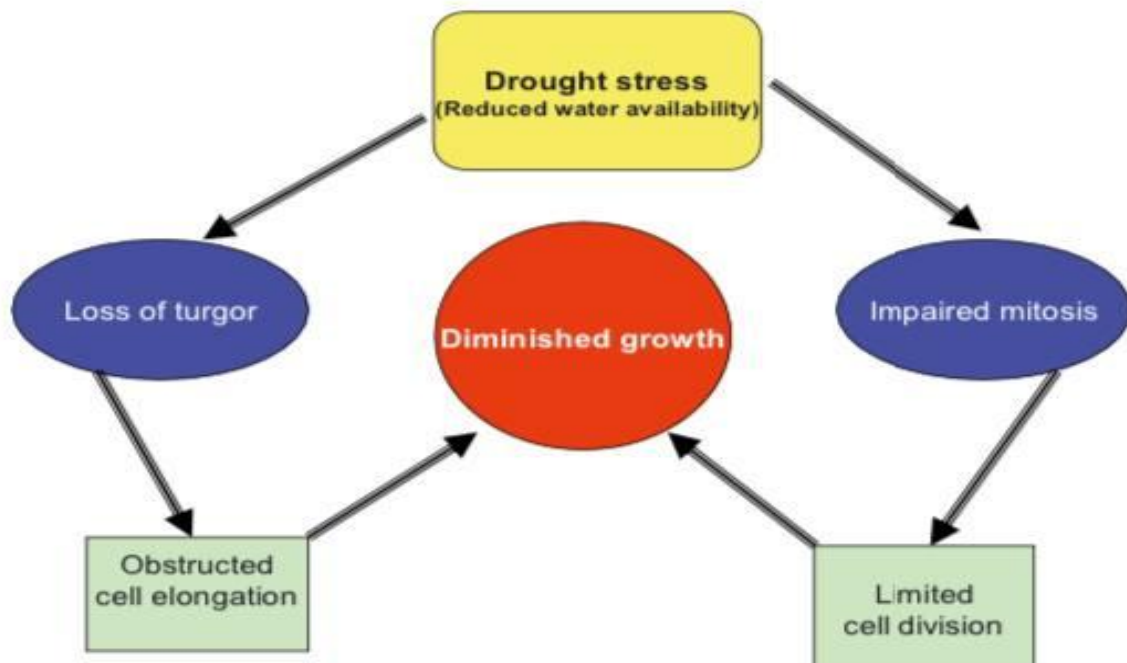


Figure 01: Potential mechanisms of growth inhibition under drought stress (Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009).

3.2. Plant Water Relations

Relative water content, leaf water potential, stomatal resistance, transpiration rate, leaf temperature, and canopy temperature are among the most important physiological indicators

that reflect plant water status and regulate its water balance (Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009).

3.3. Nutrient Relations

Reduced water availability under drought conditions decreases nutrient uptake and lowers mineral concentrations in plant tissues. Water deficit also limits mineral absorption by roots and reduces their translocation to the aerial parts of the plant (Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009).

3.4. Photosynthesis

Reduced photosynthesis is one of the primary effects of drought stress and results from limited leaf expansion, damage to the photosynthetic apparatus, accelerated leaf senescence, and decreased production of assimilates.

Stomatal closure restricts CO₂ diffusion into the leaf, thereby limiting photosynthetic activity. Under such conditions, reduced CO₂ availability may increase the risk of photodamage.

Water stress induces alterations in photosynthetic pigments and components, impairment of the photosynthetic machinery, and reduced activity of Calvin cycle enzymes (Fig.02). It also disrupts the balance between reactive oxygen species production and antioxidant defense systems, leading to oxidative stress that damages proteins, membrane lipids, and other cellular structures (Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009).

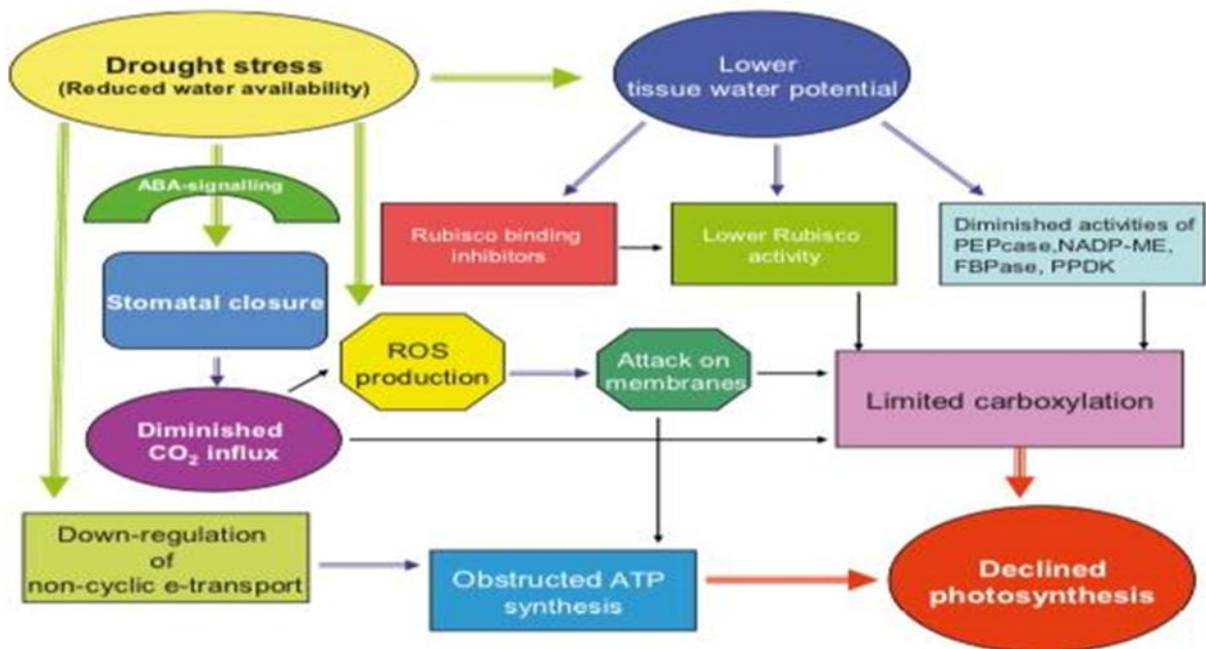




Figure 02: Photosynthesis under water stress conditions(Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009).

Drought stress induces alterations in photosynthetic pigments and components of the photosynthetic apparatus, leading to structural and functional impairments, particularly at the level of photosystem II (PSII). It also inhibits the activity of Calvin cycle enzymes, especially Rubisco, thereby reducing the efficiency of carbon dioxide fixation and ultimately resulting in decreased crop yield under drought conditions (Farooq *et al.*, 2009).

3.4.1. Stomatal oscillations

Stomatal closure is one of the earliest plant responses to severe water deficit, reducing water loss through transpiration. Under limited soil moisture, stomatal closure restricts CO₂ uptake, decreases photosynthetic activity, and may promote reactive oxygen species formation. It also reduces evaporative cooling, leading to higher leaf temperatures. This response is mainly regulated by soil water availability and root-derived signals, particularly abscisic acid (ABA), which plays a central role in stomatal regulation under drought conditions (Farooq *et al.*, 2009).

3.4.2. Photosynthetic enzymes

Severe water stress reduces photosynthetic activity mainly through the decline of Rubisco activity, the key enzyme involved in CO₂ fixation. Water deficit also impairs PSII efficiency and electron transport due to limited CO₂ availability in chloroplasts. In addition, cellular dehydration causes structural changes in proteins and enzymes, negatively affecting photosynthetic processes. Reduced Rubisco synthesis and the presence of inhibitory compounds further contribute to the decline in photosynthetic performance under drought conditions (Farooq *et al.*, 2009).

3.4.3. ATP synthesis

Under water deficit conditions, ATP production decreases due to reduced photophosphorylation efficiency in chloroplasts. Plants respond by enhancing cyclic electron transport to maintain ATP synthesis and activate photoprotective mechanisms. Excess excitation energy is also dissipated through alternative pathways, limiting reactive oxygen species formation and protecting the photosynthetic apparatus from oxidative damage (Farooq *et al.*, 2009).

3.5. Assimilate partitioning

Water stress induces a reallocation of assimilate distribution within the plant, characterized by reduced sucrose export from leaves to reproductive organs and a preferential



allocation towards the roots. This results in a decreased carbon supply to developing flowers and seeds, which limits cellular division activity and embryonic development within the seeds. In addition, reduced photosynthetic rates and impaired phloem loading and sucrose transport contribute to a decline in grain filling efficiency and restrict the normal growth and development of reproductive structures (Farooq *et al.*, 2009).

3.6. Respiration

Water stress represents an energy-demanding process that increases the metabolic cost of plant acclimation to adverse environmental conditions. This stress affects the plant carbon balance by altering the relationship between carbon production through photosynthesis and carbon consumption via respiration. Roots are among the main sinks for assimilated carbon, where a significant portion is allocated to growth and maintenance processes. Drought conditions disrupt growth and respiratory rates and may lead to a negative carbon balance when reductions in photosynthetic activity are not accompanied by proportional decreases in carbon consumption, thereby reducing overall metabolic efficiency (Farooq *et al.*, 2009).

3.7. Oxidative damage

Water stress leads to increased production of reactive oxygen species (ROS) within plant cells, particularly in chloroplasts and mitochondria. This occurs as a result of an imbalance between ROS generation and the efficiency of antioxidant defense systems.

These molecules induce oxidative damage characterized by membrane lipid peroxidation, inhibition of enzyme activity, and damage to proteins and nucleic acids, ultimately affecting membrane integrity and normal cellular functions (Farooq *et al.*, 2009).

4. Adaptive responses of plants to water stress

Plants respond to water stress through multiple adaptive mechanisms that help reduce its impact and maintain their vital physiological functions (Osakabe *et al.*, 2014).

4.1. Morphological Structure Responses to Water Stress in Plants

Plant responses to water stress are mainly manifested in leaves and roots, where morphological and structural changes reflect the plant's capacity to adapt to harsh environmental conditions. Leaves are the most sensitive organs, exhibiting structural and morphological modifications in response to drought or waterlogging, including leaf blade shrinkage, leaf rolling, and the appearance of early senescence symptoms (Fig.03). Severe

stress also leads to inhibition of overall plant growth and reduction of leaf area (Wu et al., 2022).

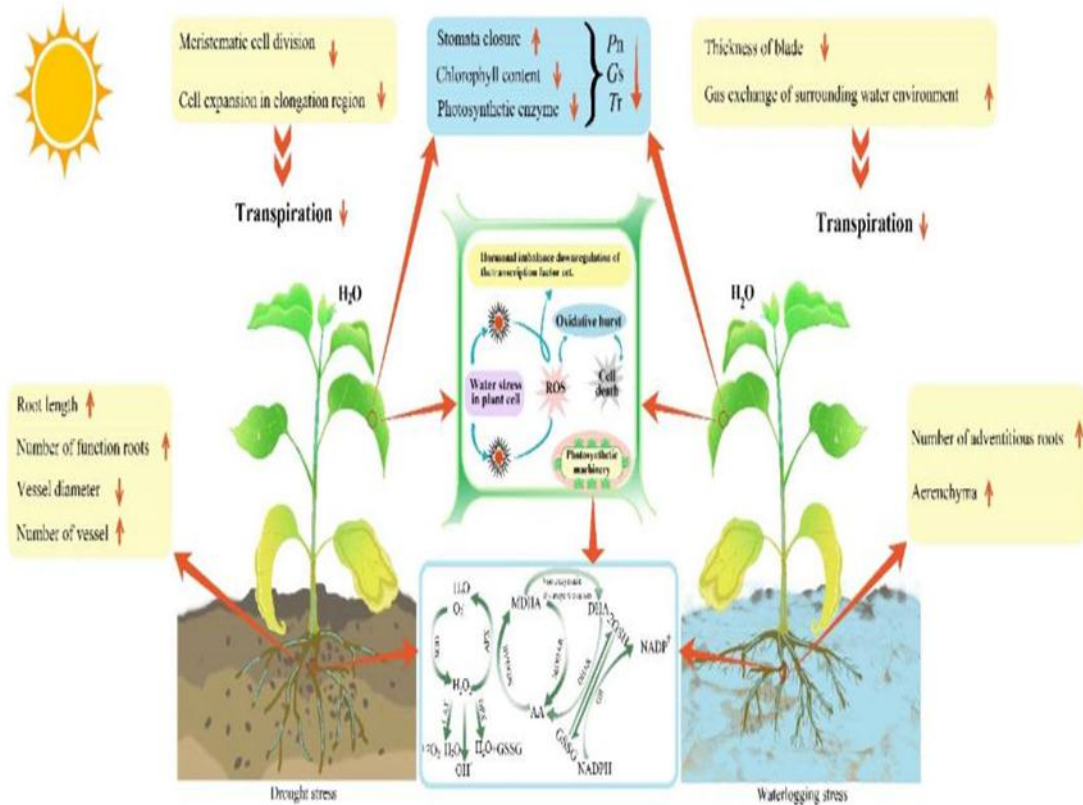


Figure 03: Morphological Responses of Plants to Water Stress (Wu et al., 2022).

4.1.1. Morphological Structure Responses to Drought Stress

Drought stress limits plant growth by reducing cell division and elongation, leading to structural changes in leaves and roots. Leaf modifications help reduce water loss and regulate gas exchange, while root adaptations, including increased root length, density, and root hair development, enhance water uptake and soil exploration under water-deficit conditions (Wu et al., 2022).

4.1.2. Morphological Structure Responses to Waterlogging Stress

Waterlogging causes leaf chlorosis, wilting, and structural alterations that affect normal growth. To adapt, plants may modify leaf anatomy and develop adventitious roots with extensive aerenchyma formation, improving internal oxygen transport. Increased root hair



density, suberization, and vascular modifications further enhance oxygen diffusion and support plant survival under hypoxic conditions (Wu et al., 2022).

4.2. Photosynthetic Characteristics of Plant Responses to Water Stress

4.2.1. Photosynthetic Characteristics of Plant Responses to Drought Stress

Phytohormones play a crucial role in regulating plant responses to drought stress. Among them, abscisic acid (ABA) is the primary hormone involved in drought tolerance. It is synthesized mainly in roots and transported to leaves, where it promotes stomatal closure, reduces transpiration, and limits water loss. ABA also enhances antioxidant defenses and activates drought-responsive genes that help maintain cellular homeostasis under water deficit conditions. In addition, drought adaptation involves both ABA-dependent and ABA-independent signaling pathways regulated by several genes and transcription factors, including MYB, WRKY, NAC, and DREB, which contribute to osmotic adjustment, stress resistance, and improved water-use efficiency (Wu et al., 2022).

4.2.2. Photosynthetic Characteristics of Plant Responses to Waterlogging Stress

Phytohormones play a crucial role in regulating plant responses to drought stress. Among them, abscisic acid (ABA) is the primary hormone involved in drought tolerance (Fig.04). It is synthesized mainly in roots and transported to leaves, where it promotes stomatal closure, reduces transpiration, and limits water loss. ABA also enhances antioxidant defenses and activates drought-responsive genes that help maintain cellular homeostasis under water deficit conditions. In addition, drought adaptation involves both ABA-dependent and ABA-independent signaling pathways regulated by several genes and transcription factors, including MYB, WRKY, NAC, and DREB, which contribute to osmotic adjustment, stress resistance, and improved water-use efficiency (Wu et al., 2022).

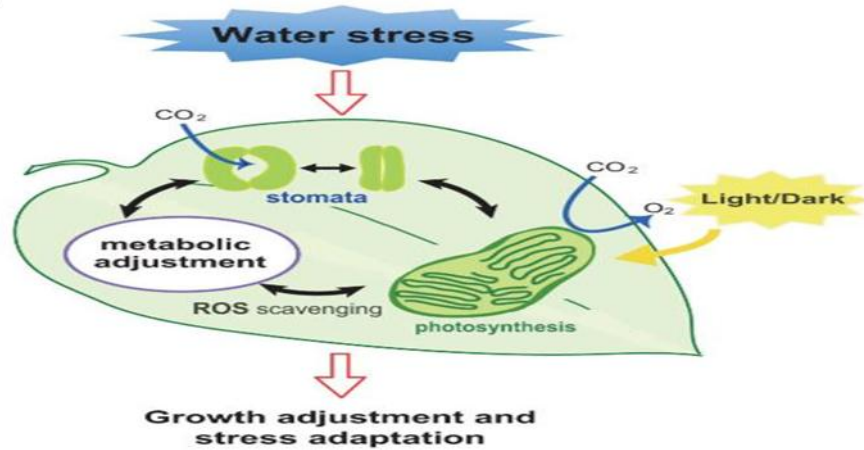


Figure 04: Plant Responses to Water Stress in Photosynthesis (Osakabe et al., 2014).

4.3. Phytohormones and Related Genes in Plant Responses to Water Stress

4.3.1. Phytohormones and Related Genes in Plant Responses to Drought Stress

Phytohormones play a crucial role in regulating plant responses to drought stress. Among them, abscisic acid (ABA) is the primary hormone involved in drought tolerance. It is synthesized mainly in roots and transported to leaves, where it promotes stomatal closure, reduces transpiration, and limits water loss. ABA also enhances antioxidant defenses and activates drought-responsive genes that help maintain cellular homeostasis under water deficit conditions (Fig.05). In addition, drought adaptation involves both ABA-dependent and ABA-independent signaling pathways regulated by several genes and transcription factors, including MYB, WRKY, NAC, and DREB, which contribute to osmotic adjustment, stress resistance, and improved water-use efficiency (Wu et al., 2022).

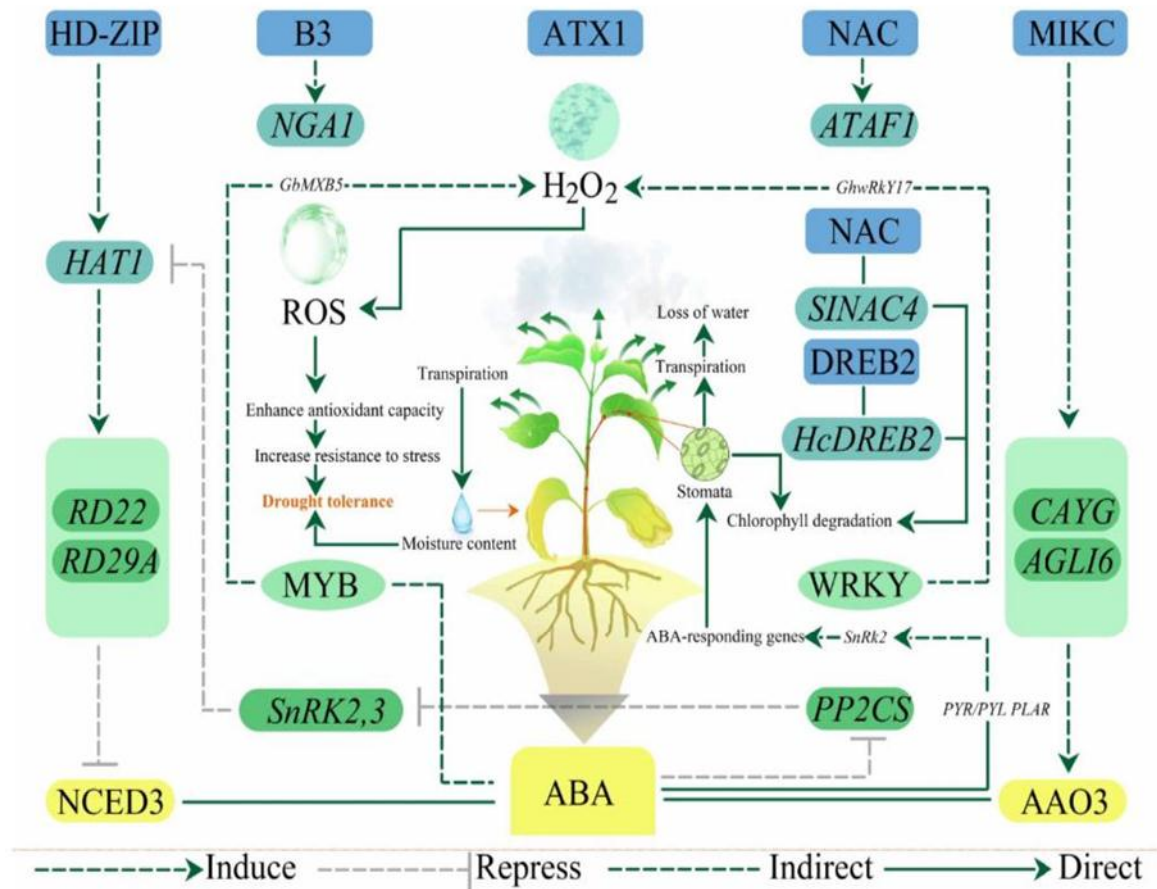


Figure 05: Regulation of Abscisic Acid (ABA) and Related Genes under Drought Stress (Wu et al., 2022).

4.3.2. Phytohormones and Related Genes in Plant Responses to Waterlogging Stress

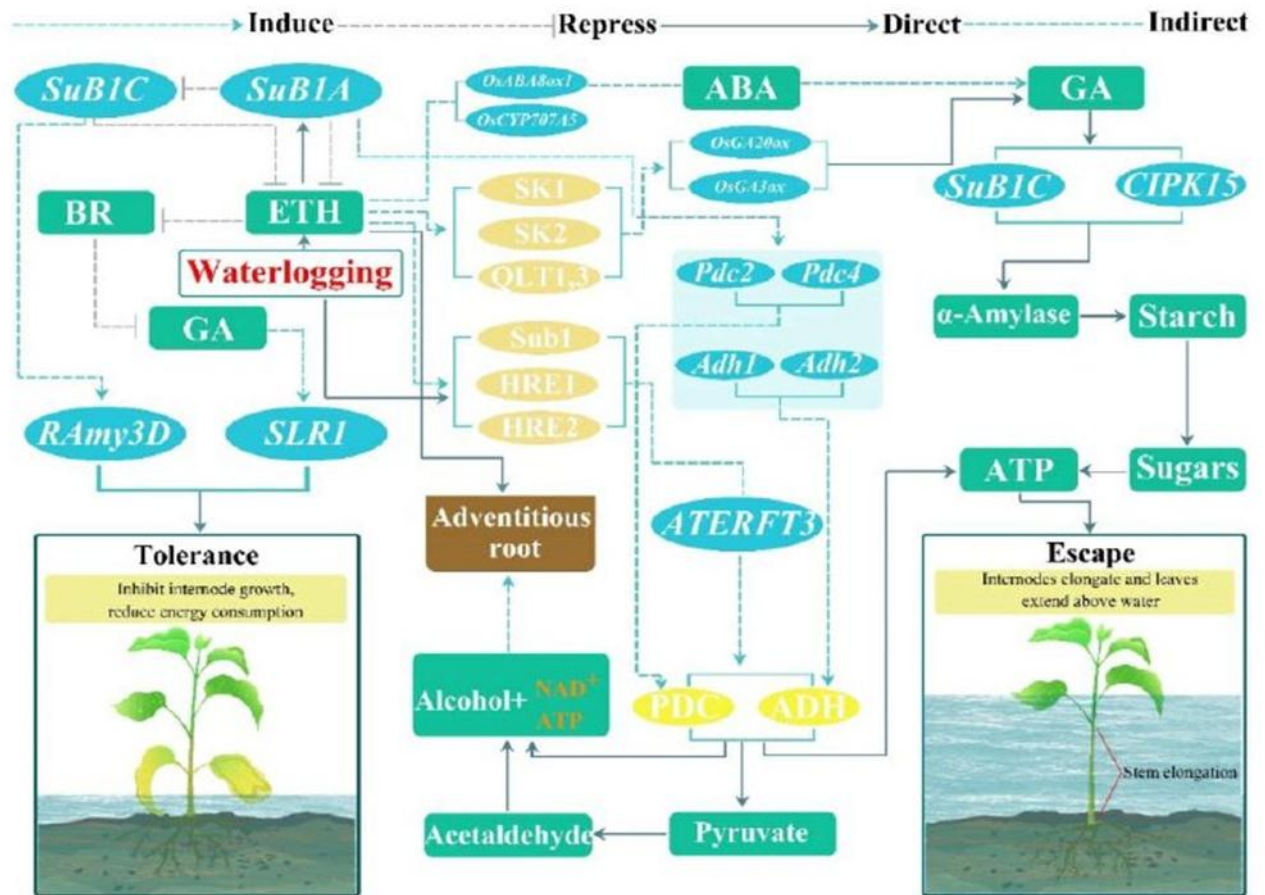


Figure 06: Interaction of Phytohormones and Related Genes in Plant Responses to Waterlogging Stress (Wu et al., 2022).

The root is the organ most affected by waterlogging stress and plays a key role in plant adaptation. Under hypoxic conditions, plants regulate hormones such as abscisic acid (ABA) and ethylene (ETH), which control stomatal behavior, growth, and stress responses. Oxygen deficiency inhibits aerobic respiration and promotes anaerobic metabolism through ethanol fermentation involving pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH), ensuring ATP production. Plants adapt through two main strategies: a quiescence (static) strategy that limits growth and conserves energy, and an escape strategy that promotes stem elongation to improve access to oxygen (Fig.06). These responses are regulated by ERFVII transcription factors and genes such as *Sub1*, *HRE1*, and *HRE2*, which control fermentation pathways, carbohydrate metabolism, adventitious root formation, and oxygen transport, thereby enhancing plant tolerance to waterlogging stress (Wu et al., 2022).

II. Agricultural Pests

Pests are considered among the most important biotic stress factors that negatively affect the physiological functions of plants (Yang *et al.*, 2026). Crop productivity is also significantly affected by pest damage, which represents a major challenge to agricultural production and the agricultural economy (Venkateswara & Padmanabhan, 2025).

1. Definition of Agricultural Pests

Agricultural pests are defined as harmful living organisms that infest agricultural crops and negatively affect their growth and development (Fig.07) (Ma *et al.*, 2025). They also cause a reduction in crop productivity and a decline in the quality of agricultural production (Chithambarathanu & Jeyakumar, 2023).

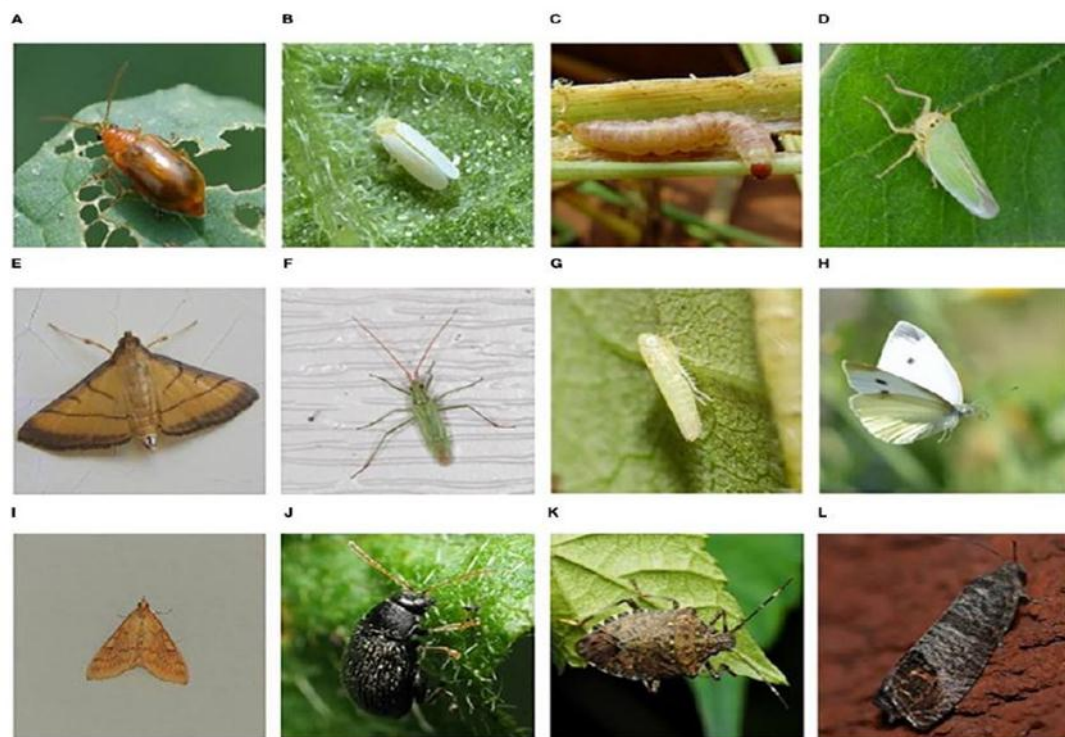


Figure 07: Examples of Agricultural Pests (Popescu *et al.*, 2023).

2. Classification of Agricultural Pests

2.1. Insects

Insects are among the most widespread and destructive agricultural pests due to their high reproductive capacity, rapid spread, and wide host range. The most important insect pests include aphids, caterpillars, and weevils (Mishra *et al.*, 2023).



A. Aphids

Aphids are small, soft-bodied sap-sucking insects that feed on plant phloem, reducing plant vigor and transmitting numerous plant viruses, which makes them important agricultural pests (Züst & Agrawal, 2016).

B. Caterpillars

Caterpillars are the larval stage of moths and butterflies. They are characterized by intensive feeding on leaves, stems, and fruits, resulting in direct crop damage, reduced quality, and lower yield (Mishra et al., 2023).

C. Weevils

Weevils are a type of beetle that attack stored grains and some field crops. They cause damage to plant tissues and grains due to their strong chewing mouthparts (Mishra et al., 2023).

2.2. Rodents

Rodents are important agricultural pests because of their direct damage to crops and stored grains, in addition to their role in transmitting diseases. The most significant species include rats, moles, and gophers (Mishra et al., 2023).

A. Rats

Rats are among the most harmful rodent pests in agriculture. They consume and contaminate stored grains, damage field crops, and act as carriers of several diseases (Mishra et al., 2023).

B. Moles

Moles are burrowing mammals that create underground tunnel systems in agricultural soils. Their digging activity can damage plant roots and disturb soil structure, making them important vertebrate pests in some agricultural systems (Witmer & Jojola, 2024).

C. Gophers

Gophers are herbivorous rodents that feed on roots and pull plants into their burrows, causing direct crop losses and significant field damage (Mishra et al., 2023).



2.3. Birds

Some bird species become agricultural pests when they feed on grains, seeds, and fruits, causing economic losses in fields, orchards, and grain storage facilities (Mishra et al., 2023).

A. Pigeons

Pigeons, especially rock pigeons, consume grains and contaminate storage areas with droppings. They also cause direct damage to fruits in orchards and vineyards (Mishra et al., 2023).

B. Crows

Crows are highly intelligent birds that feed on corn, fruits, and vegetables. They damage seeds and young shoots and may disturb agricultural ecological balance (Mishra et al., 2023).

C. Sparrows

Sparrows, especially house sparrows, feed on grains, seeds, and ripe fruits. Their high population density leads to cumulative agricultural production losses (Mishra et al., 2023).

2.4. Nematodes

Nematodes are microscopic organisms living in soil that attack plant roots, causing poor nutrient and water absorption, reduced growth, and lower crop productivity (Mishra et al., 2023).

A. Root-knot Nematodes

These nematodes cause galls and swellings on roots, which interfere with water and nutrient uptake and increase plant susceptibility to secondary infections (Mishra et al., 2023).

B. Cyst Nematodes

Cyst nematodes are characterized by forming protective cysts around their eggs in the soil. They affect crops such as potatoes, tomatoes, and soybeans, causing significant yield reduction (Mishra et al., 2023).

2.5. Microbial Factors

Microbial factors include fungi, bacteria, and viruses, which are major causes of plant diseases affecting plant growth, crop quality, and productivity (Mishra et al., 2023).



A. Fungi

Fungi are among the most common plant pathogens and cause diseases such as rust, smut, and leaf spots, leading to significant agricultural production losses (Mishra et al., 2023).

B. Bacteria

Bacteria are microscopic single-celled prokaryotic organisms that inhabit a wide range of environments and play diverse ecological roles. Some species are pathogenic and can cause diseases in plants, animals, and humans (Rai & Rai, 2025).

C. Viruses

Plant viruses are obligate intracellular agents that infect plant cells and depend on the host for replication, causing diseases that affect crop yield and quality (Ferretti & Taglienti, 2025).

2.6. Weeds

Weeds are plants growing where they are not wanted and compete with crops for essential resources such as water, nutrients, light, and space, thereby reducing crop productivity (Singh et al., 2024).

A . Broadleaf Weeds

These include plants with broad leaves such as dandelion and clover. They affect crop growth through direct competition for available resources (Mishra et al., 2023).

B. Grassy Weeds

These weeds are common in cereal crops such as wheat and maize. Examples include foxtail and crabgrass, which are known for rapid spread and difficult control (Mishra et al., 2023).

C. Sedges

Sedges are grass-like plants commonly found in wet areas (Tab.01). They are characterized by strong spread capacity and intense competition within agricultural fields (Mishra et al., 2023).



Table 01: Classification of Common Agricultural Pests and Their Major Concerns (Mishra et al., 2023).

Category	Common Examples	Primary Concerns
Insects	Aphids, Caterpillars, Weevils	Crop damage, vectoring diseases, reducing yields
Rodents	Rats, Moles, Gophers	Crop destruction, soil erosion, contamination
Birds	Pigeons, Crows, Sparrows	Eating seeds and fruits, nesting in crops, contamination
Nematodes	Root-knot nematodes, Cyst nematodes	Root damage, vectoring viruses, yield reduction
Microbial Agents	Fungi (Rust, Mildew), Bacteria (Fire Blight), Viruses (Mosaic)	Disease outbreak, spoilage, stunted growth
Weeds	Broadleaf weeds, Grassy weeds, Sedges	Competing for nutrients, water, and light; harbor pests

3. Factors Affecting the Spread of Agricultural Pests

3.1. Development of Transportation and Global Trade

The major development in land, sea, and air transportation has significantly facilitated the movement of agricultural pests from one region to another, leading to their spread beyond their original habitats. In addition, the transfer of agricultural products between countries has introduced new pests into environments lacking their natural biological enemies, which increases their establishment and severity (Aubekar, 2003).

3.2. Horizontal and Vertical Expansion in Agriculture

The exploitation of large agricultural areas and the cultivation of dense, uniform crops have created favorable conditions for pest spread. Changes in the agricultural ecosystem have also reduced the efficiency of natural biological enemies such as predators and parasitoids, resulting in increased pest outbreaks (Aubekar, 2003).



3.3. Unbalanced Use of Chemical Pesticides

Excessive use of pesticides has resulted in the emergence of secondary pests that were previously of minor economic importance due to the elimination of natural enemies. It has also contributed to the development of pesticide-resistant pest strains, which increases the difficulty of pest management (Aubekar, 2003).

3.4. Genetic Changes in Agricultural Crops

Plant breeding and genetic improvement programs have contributed to the production of high-yielding crop varieties. However, in some cases, these programs have led to the loss of genes responsible for pest resistance, increasing plant susceptibility to infestation (Aubekar, 2003).

3.5. Weak Agricultural Practices and Extension Services

Limited technical expertise and poor implementation of proper agricultural practices, especially in developing countries, increase crop vulnerability to pest infestation. In addition, the weak economic capacity of farmers reduces their ability to apply effective pest management programs (Aubekar, 2003).

3.6. Environmental and Climatic Factors

Environmental factors such as temperature, humidity, wind, and topography directly affect pest growth, reproduction, and distribution. High temperatures may increase the activity of some insect pests, while high humidity promotes the spread of fungal and bacterial diseases. Wind also contributes to the movement of insects and fungal spores over long distances (Aubekar, 2003).

4. Effect of Water Stress on Susceptibility to Pest Infestation

4.1. Interaction Between Water Stress and Agricultural Pests

Water stress caused by drought or waterlogging disrupts plant physiological processes, reducing photosynthesis, water uptake, and nutrient absorption. These effects weaken plant defense mechanisms and increase susceptibility to pests and pathogens (Fig.08). Drought promotes the accumulation of soluble sugars and amino acids, favoring sap-sucking insects and increasing vulnerability to vascular wilt diseases. Waterlogging reduces oxygen availability in the root zone, decreases root activity, and favors root rot pathogens such as *Pythium* and *Phytophthora*. Climate change further intensifies these effects through more

frequent droughts, heavy rainfall, and irregular irrigation patterns, leading to increased crop losses and reduced agricultural productivity (Ackah *et al.*, 2026).

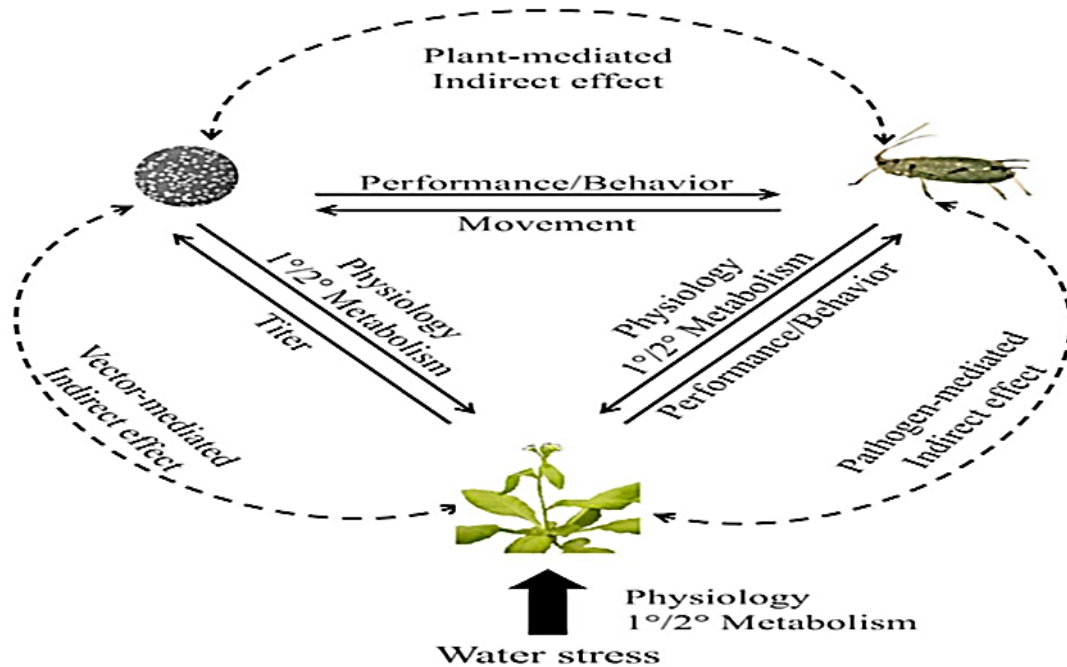


Figure 08: Interaction Between Water Stress and Agricultural Pests (Szczepaniec & Finke, 2019).

4.2. Effect of Agricultural Pests on Plant Water Status

4.2.1. Effect on Absorption of Water by Root

Plant pathogens, particularly root rot fungi and nematodes, cause damage to root tissues and absorbing root hairs, leading to reduced efficiency of water and mineral nutrient uptake from the soil (Fig.09). This results in disturbance of plant water balance, reduction in cell turgor pressure, wilting symptoms, and weakened vegetative growth (Agrios, 2005).

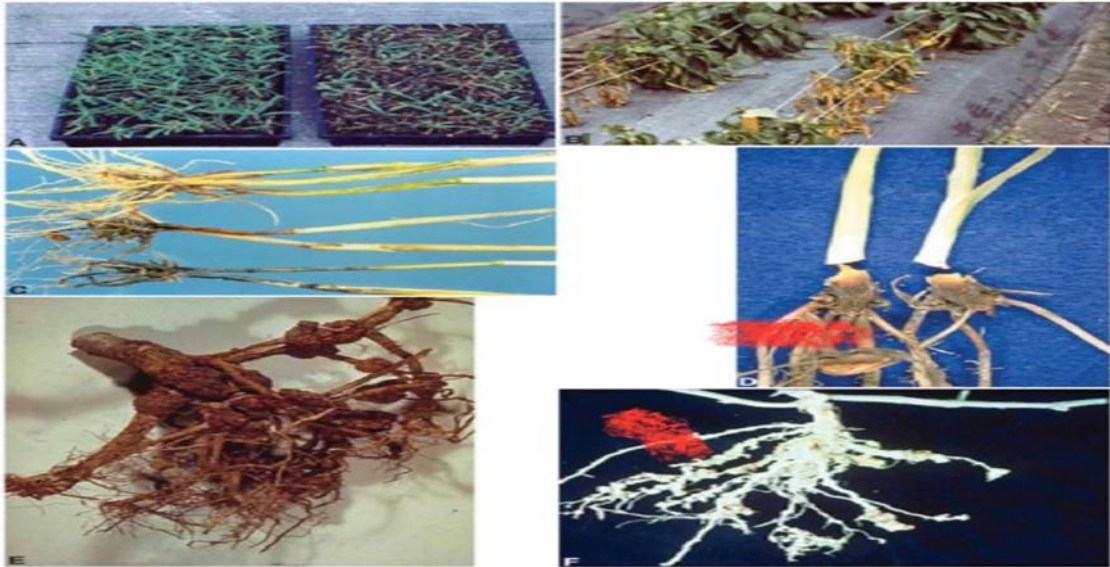


Figure 09: Effect of Agricultural Pests on Water Absorption by Roots (Agrios, 2005).

4.2.2. Effect on Translocation of Water through the Xylem

Vascular pathogens colonize xylem vessels and cause blockage due to fungal growth, bacterial accumulation, or gum formation (Fig.10). This blockage restricts water movement from roots to aerial parts, resulting in reduced water supply, vascular wilting, and decline in plant physiological activity (Agrios, 2005).

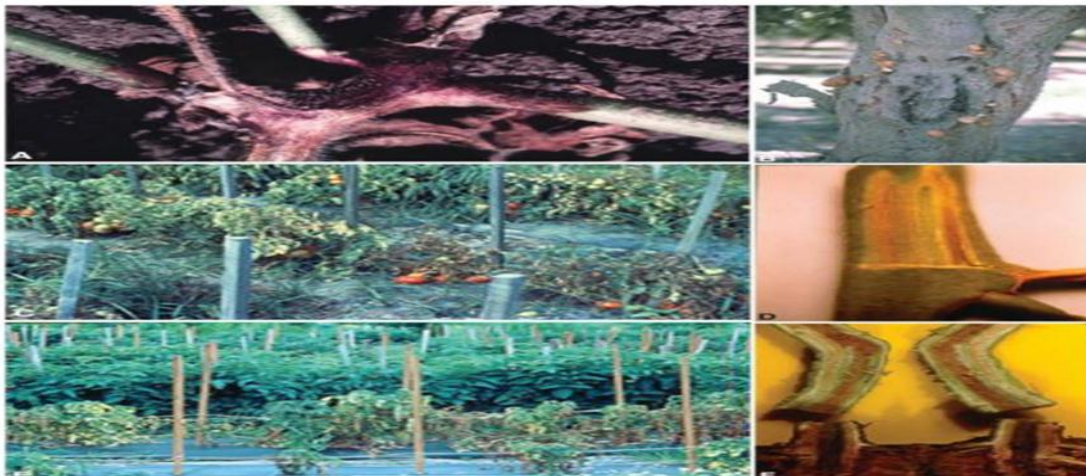


Figure 10: Effect of Agricultural Pests on Water Translocation through the Xylem (Agrios, 2005).

4.2.3. Interference with Upward Translocation of Water and Inorganic Nutrients

Infection of conducting tissues disrupts the upward movement of water and mineral nutrients to the upper parts of the plant (Fig.11). Reduced transport efficiency causes water and nutrient deficiency, leaf chlorosis, reduced growth, and persistent wilting, especially under high temperature conditions and increased water demand (Agrios, 2005).

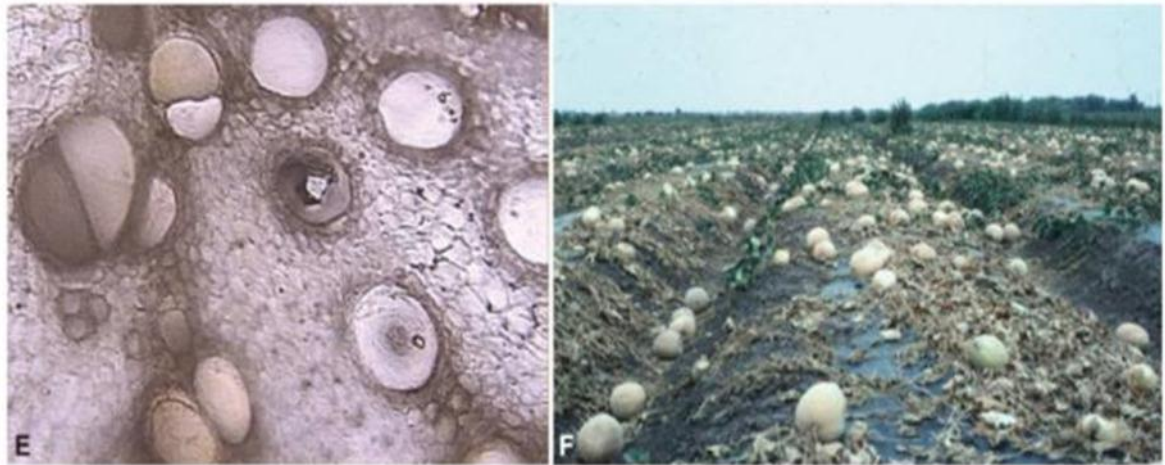


Figure 11: Interference with Upward Translocation of Water and Inorganic Nutrients (Agrios, 2005).

4.2.4. Effect on Transpiration

Pathogen infection affects transpiration through damage to the leaf epidermis or disruption of stomatal function. Increased transpiration leads to excessive water loss, while reduced transpiration limits gas exchange and physiological activity (Fig.12). Both conditions disturb plant water balance and increase the severity of water stress (Agrios, 2005).

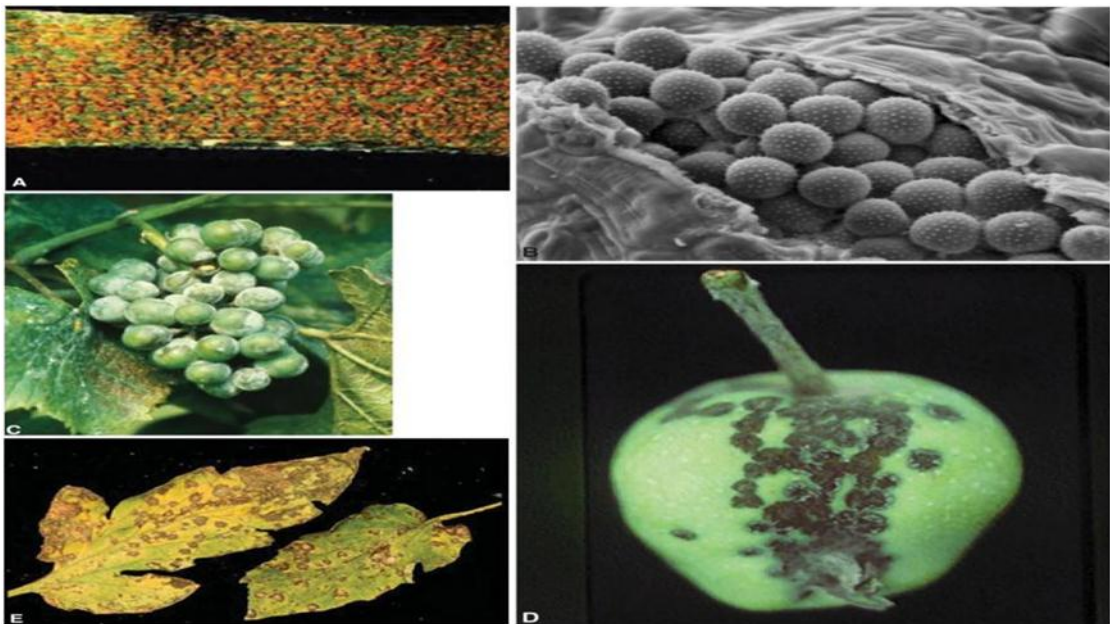


Figure 12: Effect of Agricultural Pests on Plant Transpiration and Water Loss (Agrios, 2005).

4.2.5. Effect of Pathogens on Translocation of Water and Nutrients in the Host Plant

Plant pathogens affect the vascular system through infection of roots, xylem vessels, and phloem tissues, leading to reduced efficiency of water and nutrient transport within the plant. This disruption causes reduced photosynthesis, decreased growth, wilting, and lower crop productivity (Agrios, 2005).

5. Interactive Relationship Between Water Stress and Agricultural Pests

5.1 Plant Antiherbivore Defense Under Changing Water Availability

Water deficit alters plant defense mechanisms through physiological and metabolic changes, including increased ABA, JA, and SA levels, reduced photosynthesis, and stomatal closure. (Fig.13). Drought also promotes the accumulation of defensive secondary metabolites, such as flavonoids and terpenoids, which enhance resistance to herbivorous insects. However, while moderate drought may strengthen plant defenses, severe drought can reduce metabolic activity and weaken defensive capacity (Lin et al., 2023).

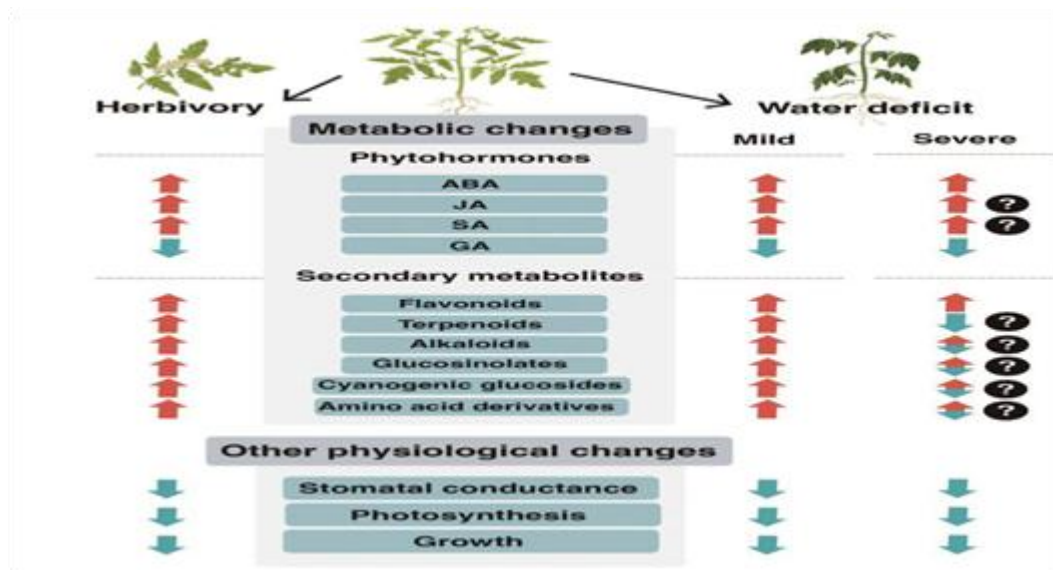


Figure 13: Effect of Water Deficit on Plant Defense Against Herbivorous Insects (Lin et al., 2023).

5.2. Water Availability and Species Interactions Across Scales

Water availability influences not only plant–herbivore interactions but also broader ecological relationships involving natural enemies, pollinators, and soil microorganisms (Fig.14). Drought can reduce the effectiveness of natural enemies, increase pest outbreaks,

and alter plant defense traits, thereby affecting herbivore infestation and pest distribution within agricultural systems (Lin et al., 2023).

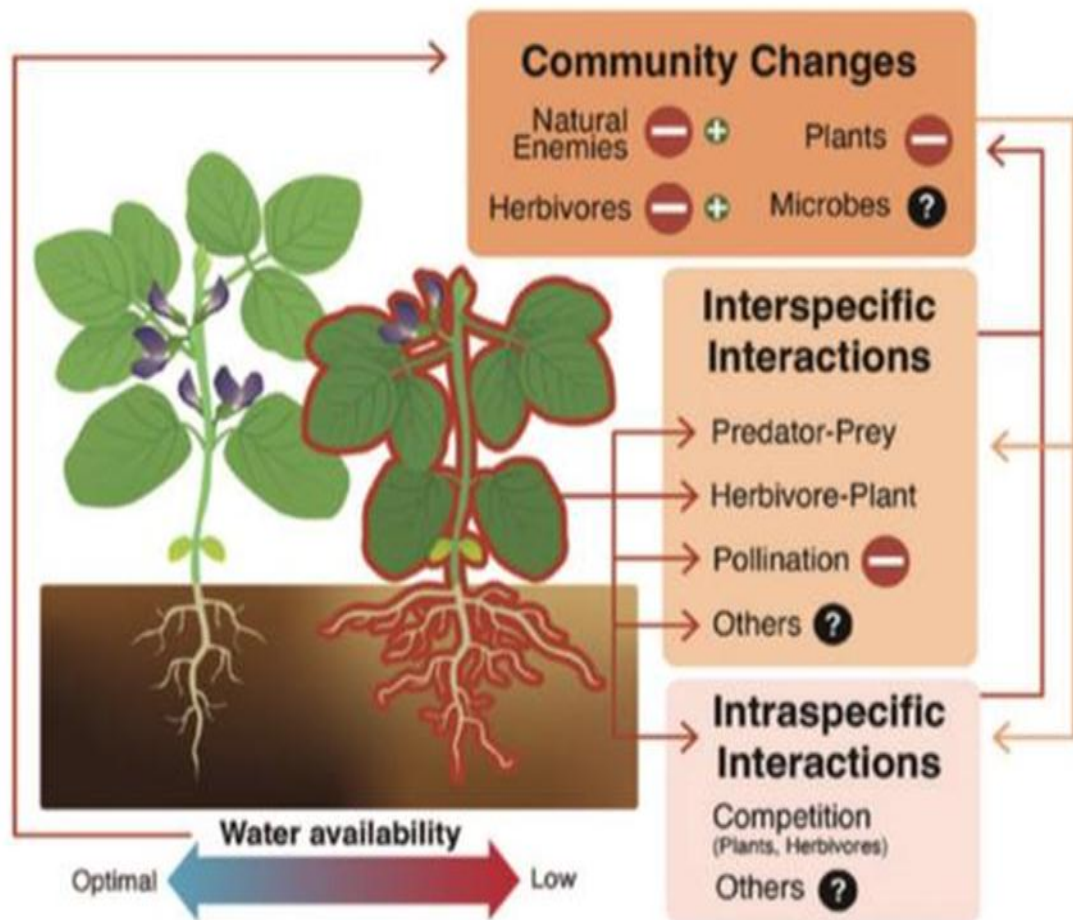


Figure 14: Effect of Water Availability on Species Interactions Across Scales (Lin et al., 2023).

5.3. Interactive Effect of Water Availability and Other Abiotic Factors on Plant–Herbivore Interactions

Water stress interacts with other abiotic factors, such as high temperature, elevated CO_2 , and flooding, to influence plant–herbivore interactions. Drought combined with high temperature increases physiological stress and susceptibility to insect attack, while flooding can suppress plant defense responses. These interactions significantly affect pest dynamics, plant damage, and crop productivity (Lin et al., 2023).

5.4. Applied Aspects of Water–Plant–Herbivore Interactions

Water stress interacts with other abiotic factors, such as high temperature, elevated CO_2 , and flooding, to influence plant–herbivore interactions (Fig.15). Drought combined with high temperature increases physiological stress and susceptibility to insect attack, while

flooding can suppress plant defense responses. These interactions significantly affect pest dynamics, plant damage, and crop productivity (Lin et al., 2023).

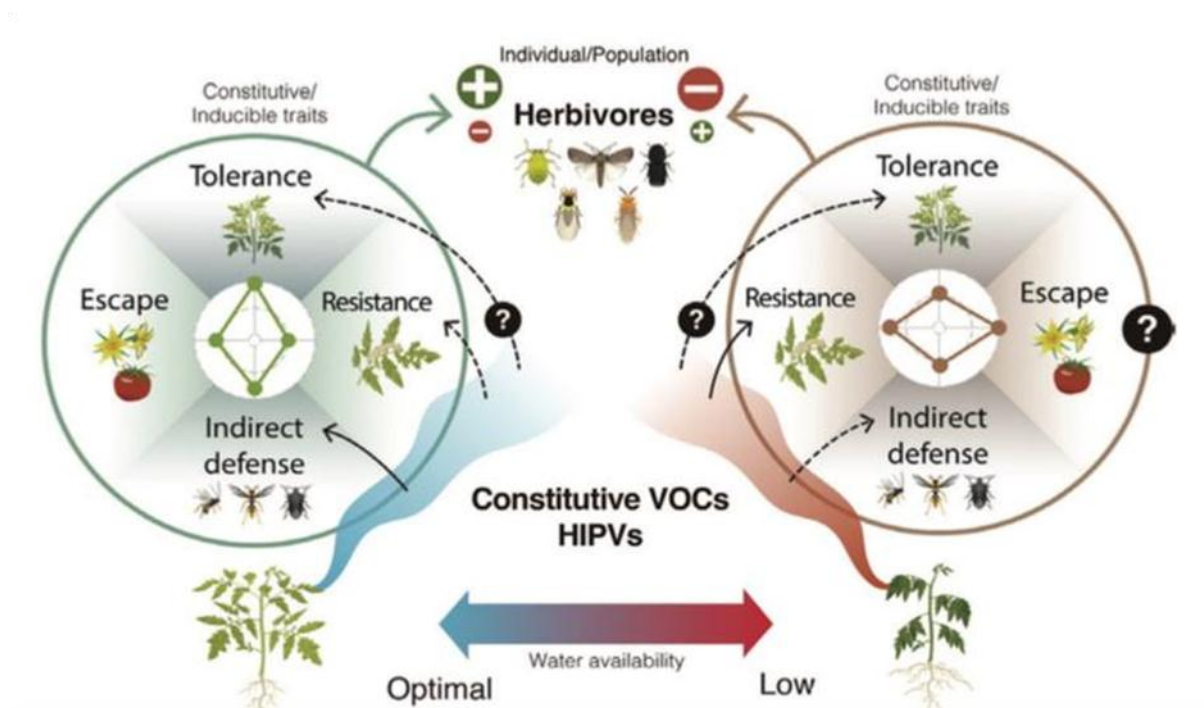


Figure 15: Interactive Relationship Between Water Stress and Agricultural Pests and Their Impact on Plant Performance (Lin, Cook-Patton, Gersony, & Mooney, 2023).

6. Conventional Control Strategies

6.1. Pesticides definition

Pesticides are chemical substances or combinations of chemicals used to control or eliminate pests and weeds, thereby safeguarding crops, animals, and human health from damage and disease. They are classified based on the target organism, including insecticides, fungicides, and herbicides. Synthetic pesticides have become a concern due to their harmful effects on human health and the environment, particularly because of their toxicity and contribution to pollution (Yadav & Devi, 2017).

6.2. Harmful effects of pesticides on human health and the environment

Pesticides constitute a class of chemical compounds used in agriculture and public health to control pests and disease vectors. Despite their role in enhancing crop productivity and reducing the spread of diseases, growing scientific evidence indicates their potential harmful effects on both human health and the environment (S Ghosh, MS Khan et al., 2024).



6.2.1 Pesticides and human health

Pesticide exposure through ingestion, inhalation, or dermal contact can cause acute and chronic toxicity depending on the dose and exposure route (S. Ghosh, M.S. Khan et al., 2024).

Acute toxicity results from short-term exposure, whereas chronic toxicity develops after prolonged exposure and may cause neurotoxic, immunotoxic, and endocrine-disrupting effects (S. Ghosh, M.S. Khan et al., 2024).

Pesticide poisoning remains a significant global public health concern due to its widespread health impacts (S. Ghosh, M.S. Khan et al., 2024).

6.2.2. Pesticides and environmental pollution

Pesticides are organic micropollutants that can persist in the environment and exert adverse effects on ecosystems (Fig.16). Aquatic environments are particularly vulnerable to pesticide contamination due to agricultural and urban runoff, which facilitates their dispersion in water bodies (Torrens & Castellano, 2014).

Once released, certain pesticides can accumulate in aquatic organisms through bioconcentration, while persistent compounds may undergo biomagnification along food chains, resulting in higher concentrations in apex predators, including humans (Torrens & Castellano, 2014; Gutgesell et al., 2020).

In addition to their accumulation, pesticides can affect non-target organisms, reduce biodiversity, and alter ecosystem structure and functioning, thereby posing a significant threat to environmental health (Zhou et al., 2024).

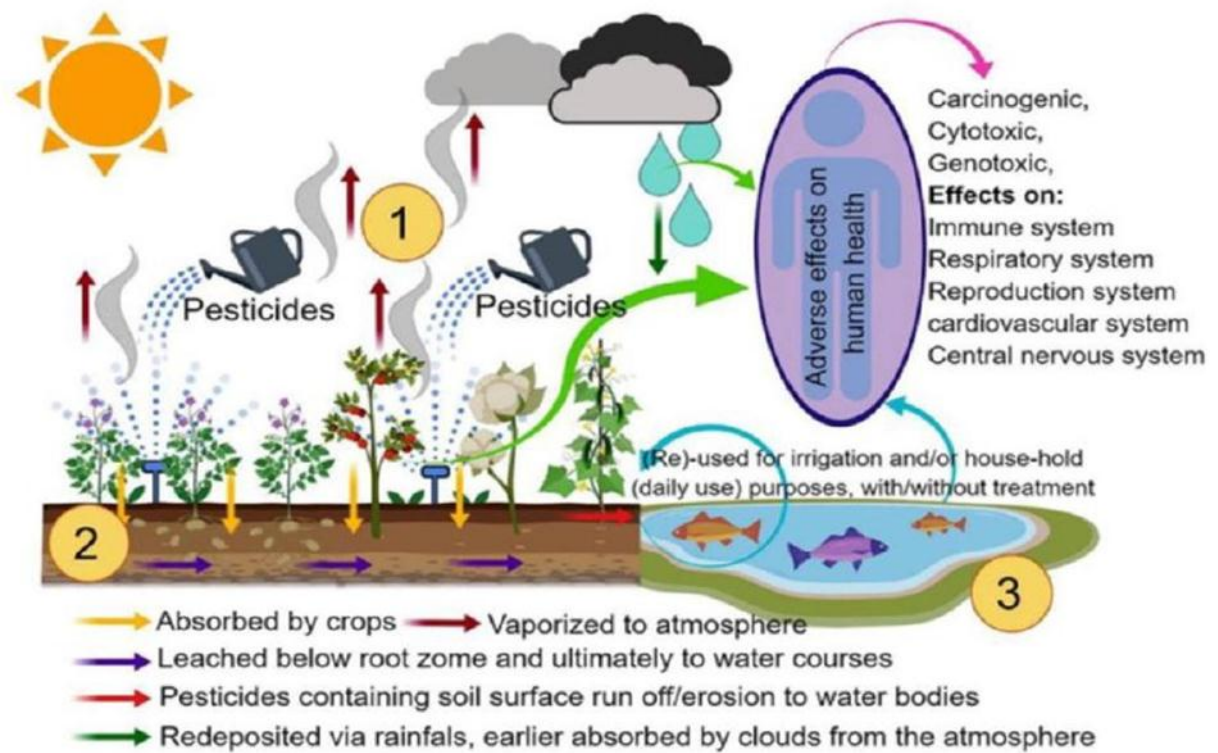
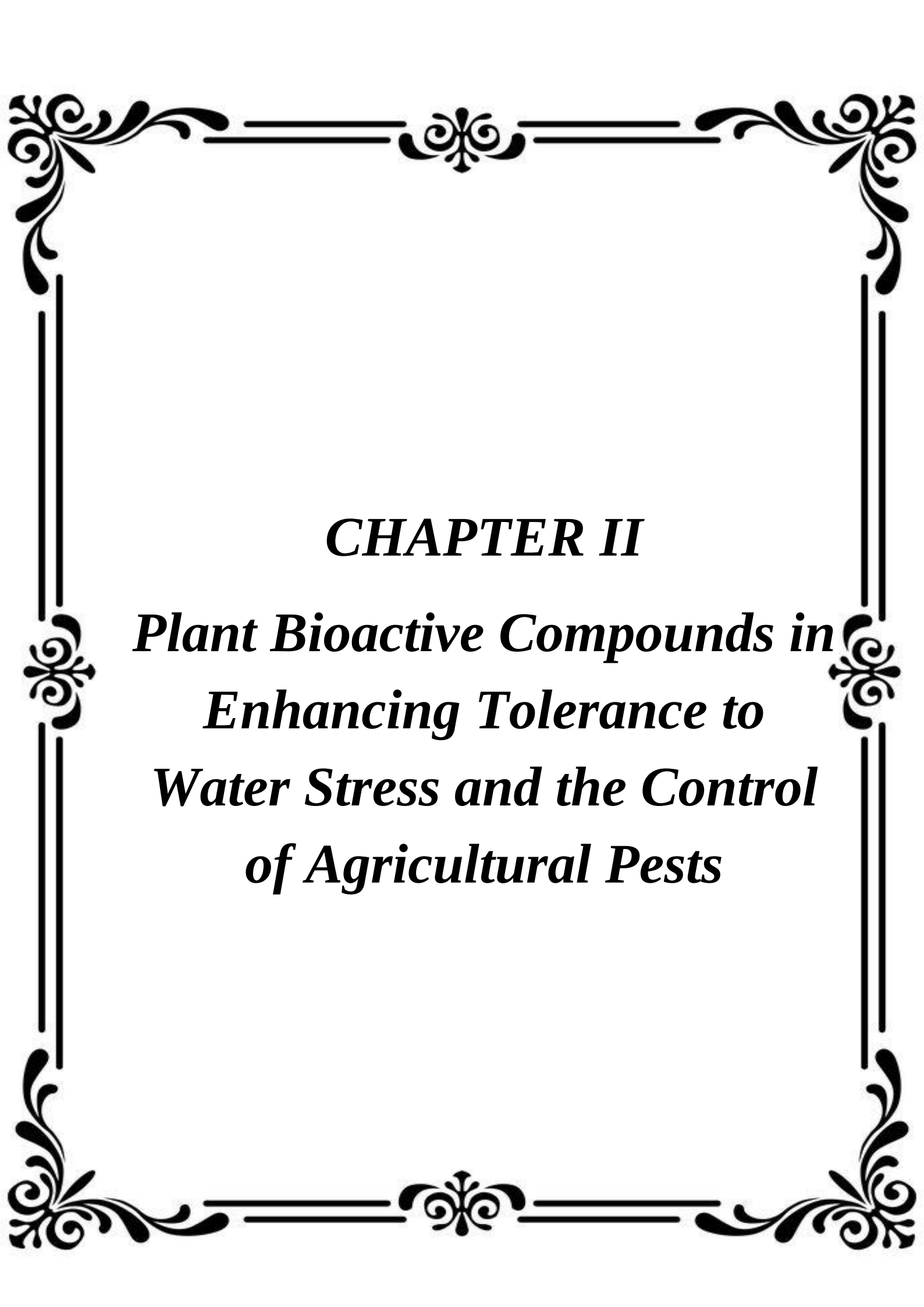


Figure 16: Adverse effects of direct and indirect contamination of soil, air, and water by pesticides on human health (da Silva Júnior et al., 2024).



CHAPTER II

Plant Bioactive Compounds in Enhancing Tolerance to Water Stress and the Control of Agricultural Pests



1. Plant Extracts

1.1. Definition of Plant Extracts

Plant extracts are complex chemical products obtained from primary plant materials through an extraction process using appropriate solvents to isolate soluble compounds and separate them from the insoluble plant matrix (Monagas et *al.*, 2022).

1.2 Plant Extraction Methods

Plant extraction methods can be broadly classified into conventional and modern techniques (Rudraswamy et *al.*, 2021).

A. Conventional Methods

1. Maceration

Maceration is a process in which plant material, either whole or ground, is soaked in a suitable solvent in a closed container at room temperature for a defined period with occasional agitation (Fig.17). Allowing soluble compounds to diffuse into the solvent (Rudraswamy et *al.*, 2021).

2. Soxhlet Extraction

Soxhlet extraction is a continuous extraction technique based on heating the solvent to evaporation, followed by condensation and repeated percolation over the plant sample, ensuring exhaustive extraction of soluble compounds (Rudraswamy et *al.*, 2021).

3. Infusion

Infusion is a method in which plant material is soaked in hot or cold solvent for a short duration to extract readily soluble compounds (Rudraswamy et *al.*, 2021).

4. Decoction

Decoction involves boiling plant material in water for a specified period to extract water-soluble and heat-stable compounds, resulting in a concentrated extract (Rudraswamy et *al.*, 2021).



5. Digestion

Digestion is similar to maceration but involves the application of mild heat to enhance the solubility of compounds and accelerate the extraction process (Rudraswamy et al., 2021).

6. Percolation

Percolation is a process in which the solvent is continuously passed through a column of plant material, leading to gradual extraction of bioactive compounds (Rudraswamy et al., 2021).

B. Modern Methods

1. Microwave-Assisted Extraction (MAE)

MAE utilizes microwave radiation to heat both the solvent and plant matrix, increasing cell permeability and enhancing the release of bioactive compounds into the solvent (Rudraswamy et al., 2021).

2. Ultrasound-Assisted Extraction (UAE)

UAE employs ultrasonic waves to disrupt plant cell structures and increase solvent penetration, thereby improving mass transfer and extraction efficiency (Rudraswamy et al., 2021).

3. Supercritical Fluid Extraction (SFE)

SFE uses fluids in their supercritical state, such as carbon dioxide, to efficiently extract compounds with reduced use of organic solvents (Rudraswamy et al., 2021).

4. Counter-current Extraction

This method involves the movement of solvent and plant material in opposite directions, maximizing contact efficiency and achieving more complete extraction (Rudraswamy et al., 2021).

5. Aqueous Alcoholic Extraction (Fermentation-based)

This method involves soaking plant material under conditions that allow fermentation, producing alcohol that facilitates the extraction of bioactive compounds (Rudraswamy et al., 2021).



6. Phytonics Process

A modern extraction technique that uses hydrofluorocarbon solvents to efficiently extract plant constituents while minimizing environmental impact (Rudraswamy *et al.*, 2021).

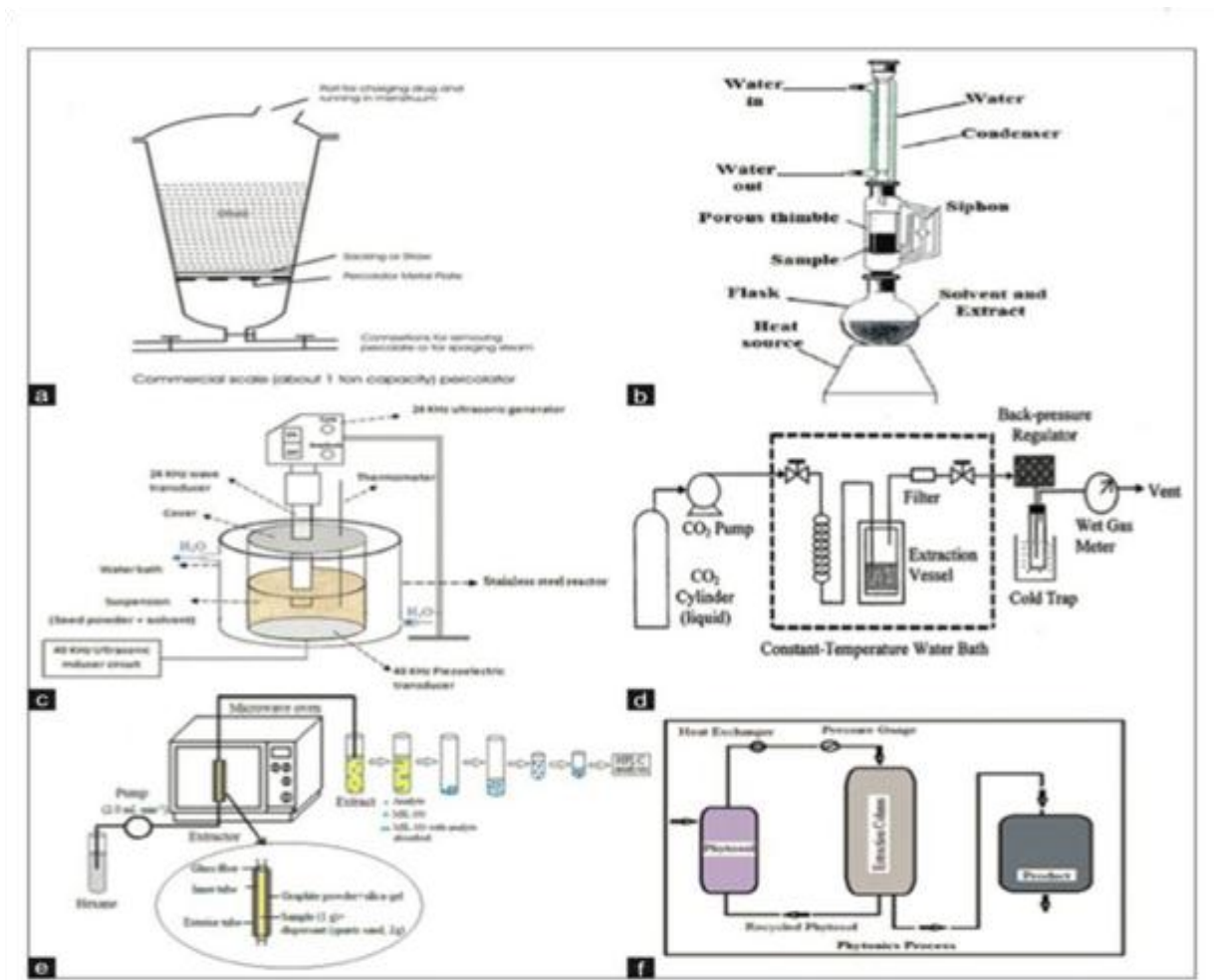


Figure 17: Plant Extraction Methods (Rudraswamy *et al.*, 2021).

1.3. Factors Affecting Extraction Efficiency and Efficacy

2. Plant Secondary Metabolites and Their Functional Mechanisms

The efficiency of extracting bioactive compounds depends on several factors, particularly the extraction method and the solvent used, as both influence the yield and composition of the obtained extract. Variations in extraction procedures may lead to significant differences in the quantity and biological activity of the recovered compounds. Therefore, selecting an appropriate extraction technique and solvent is essential for obtaining



high-quality extracts with optimal biological properties and ensuring the reproducibility of experimental results (Gupta et al., 2012).

2.1 Definition of Plant Secondary Metabolites

Plant secondary metabolites are specialized organic compounds produced by plants that are not directly involved in fundamental growth and developmental processes. However, they play important roles in defense mechanisms, adaptation to biotic and abiotic stresses, and environmental interactions, in addition to contributing to the sensory characteristics of plants (Khalid et al., 2023).

2.2 Classification of Plant Secondary Metabolites

2.2.1. Essential Oils

are volatile and aromatic plant-derived compounds classified as secondary metabolites. They consist mainly of terpenes and terpenoids and possess diverse biological activities, including antimicrobial, antioxidant, anti-inflammatory, and pharmacological properties (Pezantes-Orellana et al., 2024).

2.2.2 Tannins

Tannins are naturally occurring high-molecular-weight phenolic compounds classified as plant secondary metabolites. They are characterized by their ability to bind and precipitate proteins and exhibit various biological activities, including antioxidant and antimicrobial properties (Smeriglio et al., 2017).

2.2.3. Alkaloids

Alkaloids are naturally occurring nitrogen-containing organic compounds considered among the most important secondary metabolites primarily produced by plants. They exhibit diverse biological and pharmacological activities of considerable importance in medical and biological fields (Erdogan-Kablan et al., 2025).

2.2.4. Terpenoids

Terpenoids are plant secondary metabolites derived from terpenes and characterized by the presence of additional functional groups resulting from chemical modifications such as oxidation. They play important roles in plant defense against pathogens, insects, and environmental stresses and are also involved in plant growth, chemical signaling, and pollinator attraction (Câmara et al., 2024).



2.2.5. Flavonoids

Flavonoids are phenolic plant secondary metabolites involved in plant defense mechanisms against biotic and abiotic stresses. They also participate in growth regulation and plant–environment interactions and exhibit antioxidant, antimicrobial, and anti-inflammatory activities (Dias et *al.*, 2021).

2.2.6. Saponins

Saponins are plant glycosides classified as secondary metabolites, consisting of a sugar moiety linked to a steroidal or triterpenoid aglycone. They are involved in plant defense mechanisms against pathogens and insects and exhibit various biological activities, including antimicrobial, antioxidant, and anti-inflammatory properties (Moses et *al.*, 2014).

2.2.7. Phenolic Compounds

Phenolic compounds are plant secondary metabolites characterized by the presence of one or more aromatic rings bearing hydroxyl groups. They are involved in plant defense mechanisms against biotic and abiotic stresses and exhibit antioxidant and antimicrobial activities (Xu & Wang, 2025).

2.3. Role of Secondary Metabolites in Mitigating Plant Water Stress

Secondary metabolites play a crucial role in enhancing plant tolerance to drought stress through the regulation of several physiological and biochemical responses associated with water deficiency. Water limitation stimulates the biosynthesis of various secondary metabolites, including flavonoids, phenolics, and terpenoids, which contribute to the scavenging of reactive oxygen species (ROS), reduction of oxidative stress, and protection of cellular components from oxidative damage. These compounds also participate in hormonal signaling regulation, osmotic balance maintenance, and reduction of water loss through the induction of stomatal closure, thereby improving plant adaptation and survival under drought conditions. Furthermore, secondary metabolites are associated with the activation of genes and transcription factors involved in drought-responsive defense mechanisms, which enhances plant resistance to water deficit conditions (Yadav et *al.*, 2021).



2.4. Role of Secondary Metabolites as Biological Insecticides

Essential oils, particularly those rich in terpenes and phenolic compounds, exhibit insecticidal and repellent activities against insect pests (Ukoriije et al., 2020). Tannins affect the physiological processes of herbivorous insects (Fig.18) leading to reduced feeding activity and enhanced plant defense (Ukoriije et al., 2020). Alkaloids possess repellent and antifeedant properties that negatively influence insect growth and survival (Ukoriije et al., 2020). Terpenoids interfere with the biosynthesis of juvenile hormones and ecdysteroids, thereby disrupting molting and metamorphosis in insects (Walkowiak-Nowicka et al., 2023). Flavonoids contribute to plant defense by reducing feeding activity and limiting insect damage (Ukoriije et al., 2020). Saponins act as feeding deterrents against herbivorous insects (Walkowiak-Nowicka et al., 2023). Phenolic compounds exert toxic and antioxidant-related effects that reduce insect viability and pest infestation (Ukoriije et al., 2020).

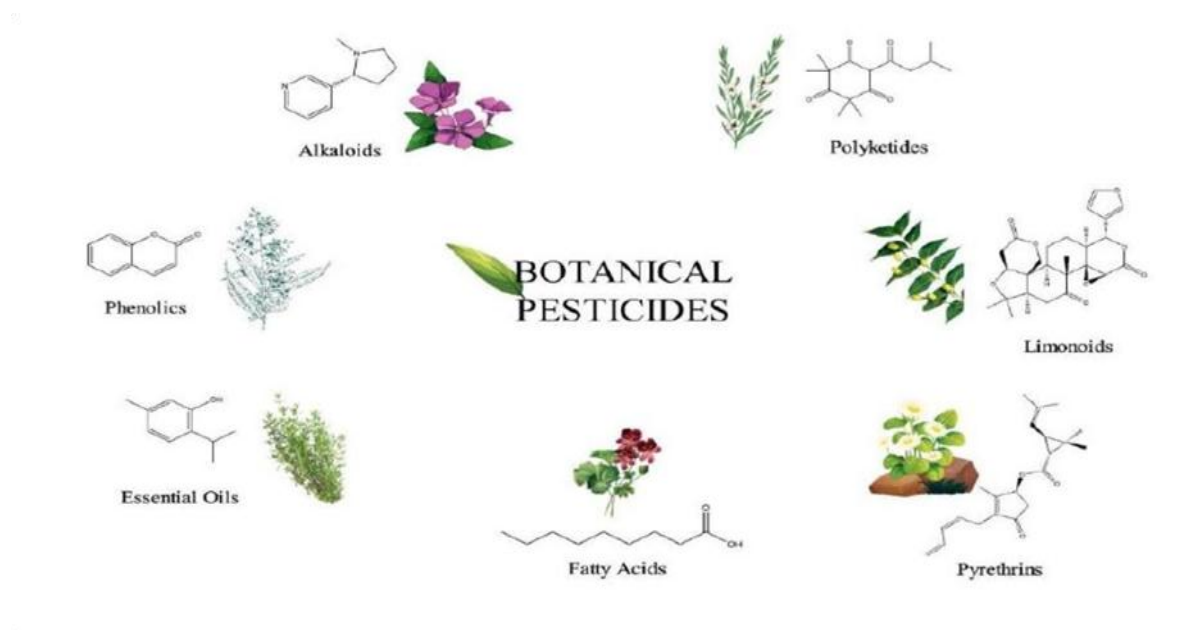


Figure 18: Structural diversity of bioactive compounds in plant-derived pesticides (Acheuk et al., 2022).

3. Plant-Based Sprays

3.1. Definition of Plant-Based Sprays

Plant-based sprays are defined as liquid biostimulant formulations prepared from plant extracts or biologically active natural compounds and applied to plants as foliar sprays in order to improve physiological growth, enhance nutrient uptake efficiency, and increase plant tolerance to various biotic and abiotic stresses (Ma et al., 2022).



3.2. Role of Formulation in Improving the Efficiency of Plant-Based Sprays

Plant-based spray formulations affect pests through repellent activity, inhibition of feeding and growth, or direct toxic effects. They also influence plants by activating physiological and biochemical defense mechanisms associated with resistance to biotic and abiotic stresses. These effects contribute to improving the plant's ability to withstand pest attacks and unfavorable environmental conditions, supporting their use within integrated pest management programs (Lengai *et al.*, 2020).

3.3 Mechanisms of Action of Plant-Based Spray Formulations on Plants and Pests

Plant-based spray formulations affect pests through repellent activity, inhibition of feeding and growth, or direct toxic effects(Fig.19).They also influence plants by activating physiological and biochemical defense mechanisms associated with resistance to biotic and abiotic stresses. These effects contribute to improving the plant's ability to withstand pest attacks and unfavorable environmental conditions, supporting their use within integrated pest management programs (Lengai *et al.*, 2020).

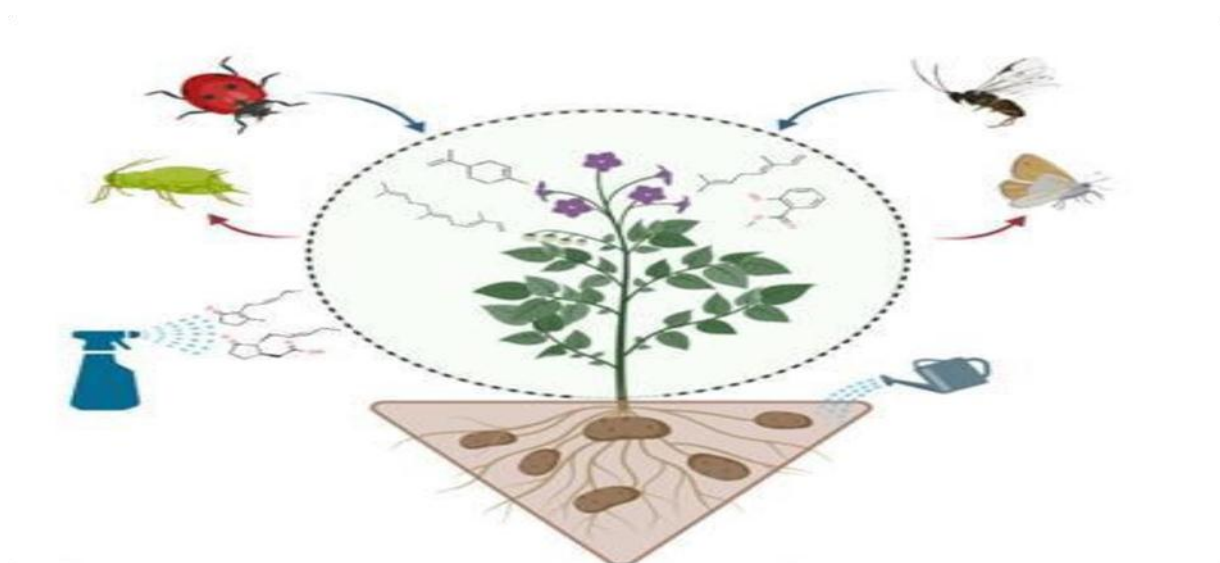


Figure 19: Mechanisms of Action of Plant-Based Spray Formulations on Plants and Insect Pests (Sohby *et al.*, 2022).

3.4 Factors Influencing the Efficacy of Plant-Based Spray Formulations

The efficacy of plant-based spray formulations is influenced by several interacting factors, including biological factors related to the characteristics of the target organism and the nature of the active compounds, environmental factors associated with the conditions prevailing during application, and formulation- and application-related factors such as formulation type, component concentration, and spray characteristics. In addition, storage and



stability conditions play an important role in maintaining the stability and functional properties of active compounds, thereby affecting the biological performance of plant-based spray formulations (Hasan et al., 2021).

4. Plant Extracts in Sustainable Agriculture

4.1. Definition of Biopesticides

Biopesticides are biologically derived products used for pest control. They include microorganisms and their derivatives, such as bacteria, fungi, viruses, and pheromones, and are generally associated with lower toxicity and greater biodegradability than conventional pesticides (Hezakiel et al., 2024).

4.2. Environmental and Health Benefits of Biopesticides

Biopesticides are characterized by lower toxicity and rapid degradation in the environment, which can reduce the accumulation of residues in soil and water. They also exhibit a high degree of specificity toward target pests, thereby minimizing their effects on non-target organisms. From a health perspective, their lower toxicity may reduce risks associated with pesticide exposure compared with conventional chemical pesticides (Fig.20). In addition, the use of biopesticides can contribute to reducing reliance on chemical pesticides and mitigating their associated environmental impacts, supporting the implementation of sustainable pest management and sustainable agricultural practices (Wend et al., 2024).

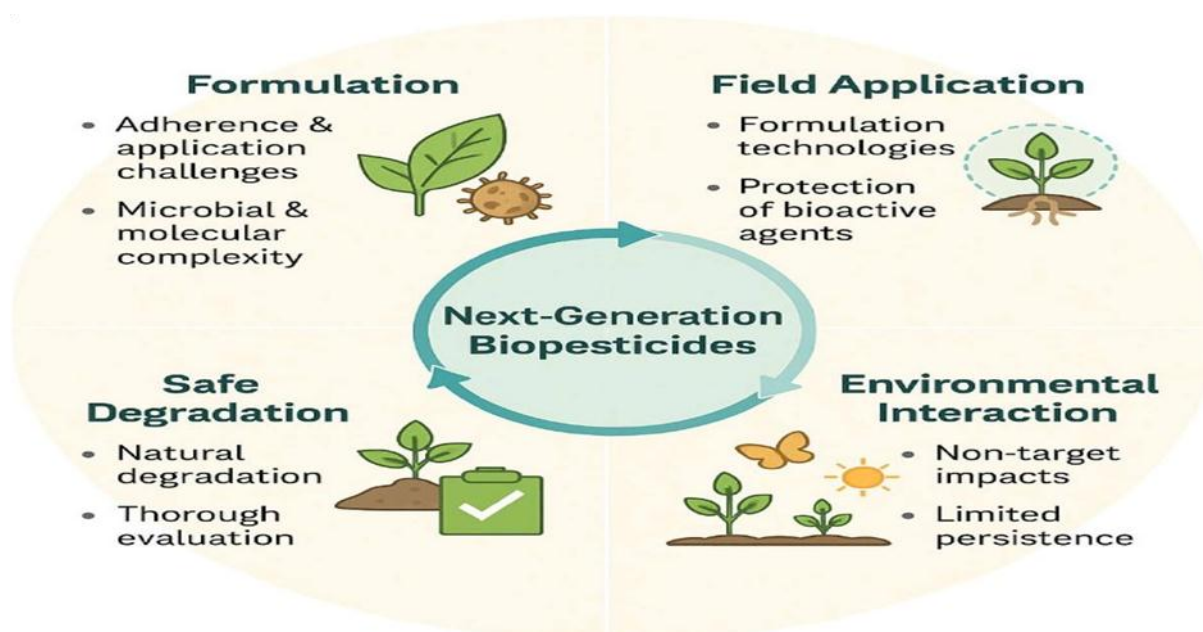


Figure 20: Environmental behavior and safety considerations of next-generation biopesticides (Danesh et al., 2026).



4.3. Role of Biopesticides in Reducing Chemical Pesticide Use

Biopesticides contribute to pest control through the use of microorganisms or naturally derived compounds (Aioub et al., 2024).

They are characterized by their specificity toward target pests and their biodegradability in the environment (Wend et al., 2024).

Their integration into integrated pest management programs can help reduce the use of chemical pesticides (Aioub et al., 2024).

This, in turn, may mitigate environmental issues associated with the intensive use of synthetic pesticides (Wend et al., 2024).

5. Comparison of Biopesticides and Chemical Insecticides in Terms of Effectiveness, Environmental Impact, and Sustainability

5.1. Effectiveness:

Chemical insecticides are characterized by rapid action and broad-spectrum control, providing immediate suppression of pest populations. In contrast, biopesticides are generally more selective toward target pests and often act through specific biological mechanisms, which may require a longer period to achieve effective control. However, their targeted mode of action can contribute to efficient pest management while reducing adverse effects on beneficial organisms (Gavrilescu et al., 2023).

5.2. Environmental Impact:

Biopesticides are biodegradable and exhibit lower environmental persistence, reducing the risk of residue accumulation in soil and water. In contrast, chemical insecticides may remain in the environment for longer periods and can adversely affect non-target organisms and ecosystem balance. The reduced persistence of biopesticides limits environmental contamination and supports ecological sustainability (Wend et al., 2024).

5.3. Sustainability:

Biopesticides are more compatible with sustainable agriculture and integrated pest management programs due to their reduced environmental impact and natural origin. Their use can contribute to reducing reliance on conventional chemical pesticides and support long-term crop protection strategies. Furthermore, they promote environmentally responsible pest

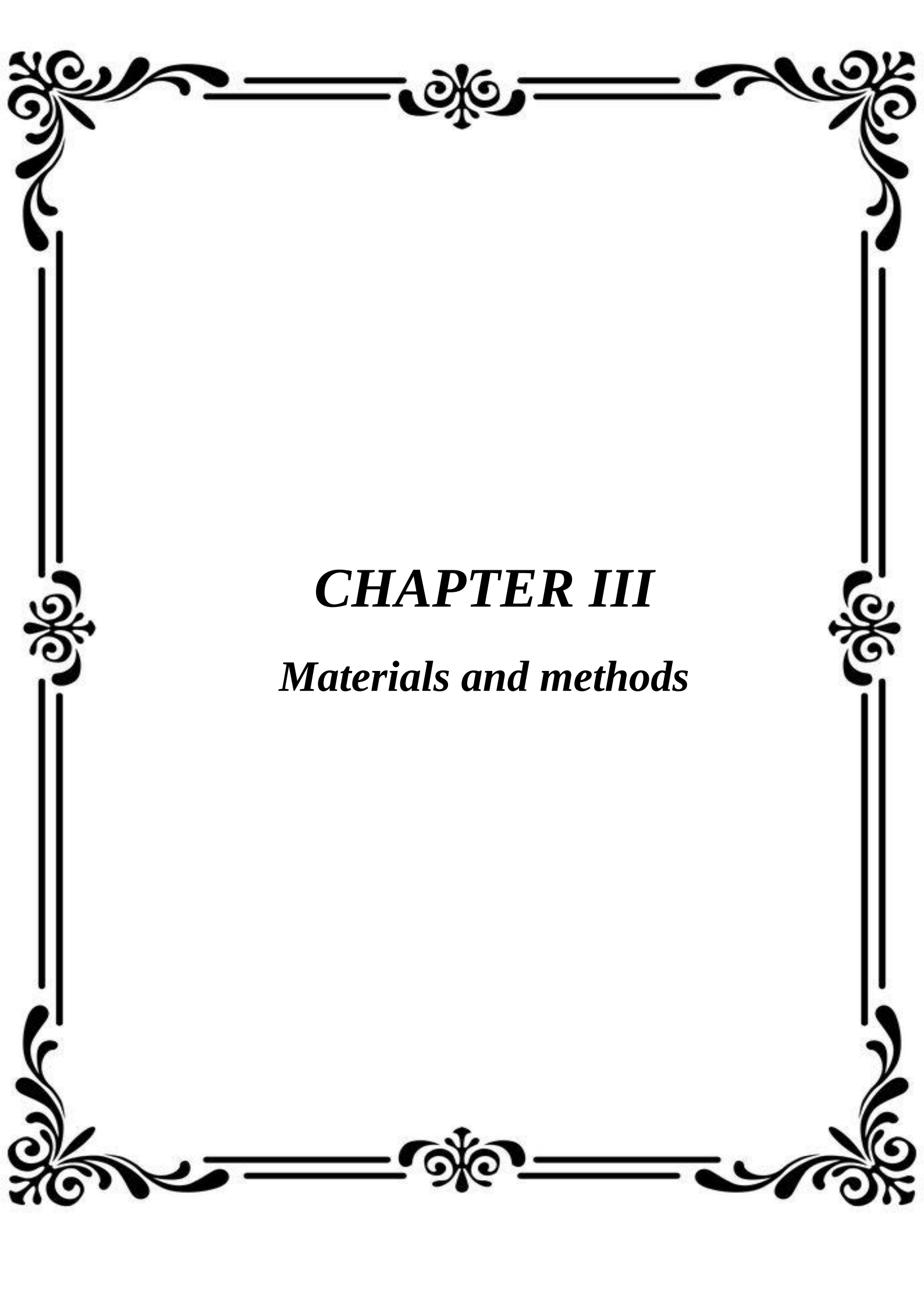


management practices and contribute to the sustainability of agricultural production systems (Aioub et al., 2024).

Agricultural productivity while reducing the environmental risks associated with conventional chemical pesticides...

Part II

Experimental Part



CHAPTER III

Materials and methods

The experimental work was conducted in the laboratory of the Faculty of Natural and Life Sciences, Chahid Hamma Lakhdar University of El Oued, Algeria.

1. Materials

A. Biological material

1.Plant Material

1.1. *Phoenix dactylifera* L.(Date Palm)

Phoenix dactylifera L. belongs to the family Arecaceae. It is characterized by a cylindrical, unbranched stem that may reach up to 30 m in height and is topped by a crown of pinnate leaves. Mature trees bear approximately 100–120 green leaves composed of numerous pinnae. (Fig.21). Date palm is a dioecious and perennial species, with male and female flowers borne on separate trees (Meena et al., 2025).

The leaves possess a thick waxy cuticle that reduces transpiration and limits water loss, thereby improving water conservation and enhancing adaptation to arid environments (Du et al., 2025).

Date palm leaves contain tannins and other bioactive compounds with antimicrobial and antioxidant properties, indicating their potential value in plant protection applications (Shah et al., 2023).

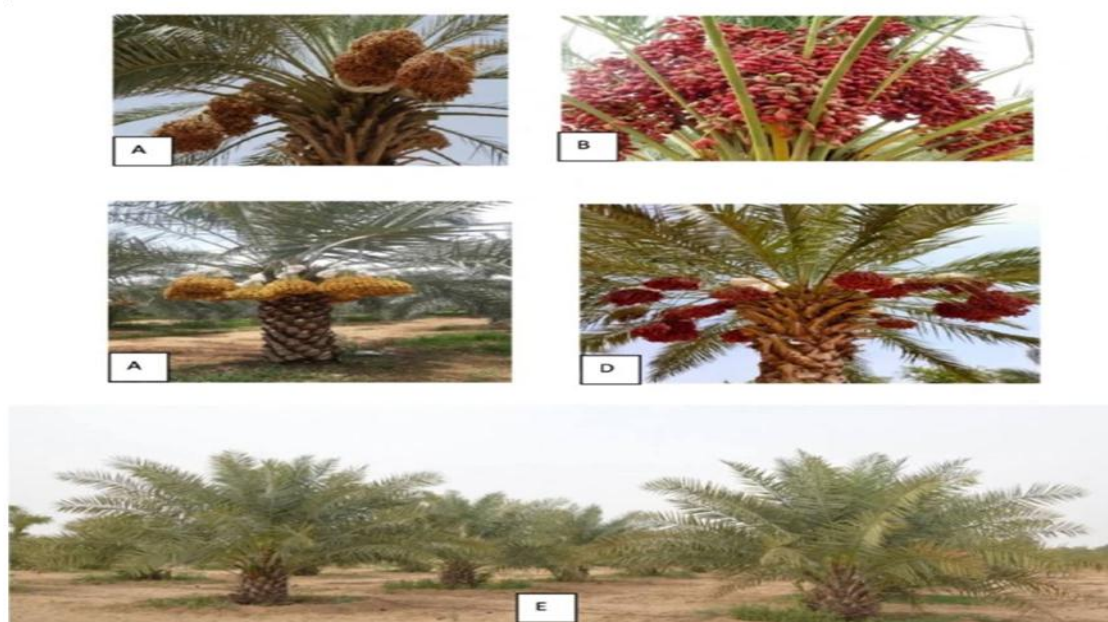


Figure 21: Morphological characteristics of *Phoenix dactylifera* L.(Meena et al., 2025).

• Classification

According to POWO (2026), the classification of the specie *Phoenix dactylifera* L. is as follows (Tab.02):

Table 02: Classification of the specie *Phoenix dactylifera* L.

Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	<i>Arecales</i>
Family	<i>Arecaceae</i>
Genus	<i>Phoenix</i>
Species	<i>Phoenix dactylifera</i> L.

1.2. *Artemisia campestris* L. (Teguft)

Artemisia campestris L. belongs to the family *Asteraceae* and is widely distributed throughout the Northern Hemisphere, with an extensive distribution across Eurasia and predominance in the arid and semi-arid regions of North Africa. It is characterized by erect and densely branched stems ranging from 30 to 150 cm in height (Fig.22). The leaves are finely dissected, green to greyish-green in color, and covered with silky trichomes during the early stages of development. The inflorescences consist of numerous small yellow capitula arranged in branched clusters (Dib et al., 2017).

It is characterized by a phytochemical composition rich in bioactive secondary metabolites, particularly flavonoids, phenolic acids, coumarins, terpenoids, and essential oils, which play a key role in the expression of its diverse biological properties (Dib & El Alaoui-Faris, 2019).

Several studies have reported that *Artemisia campestris* possesses remarkable insecticidal and repellent properties against various insect pests. These activities are mainly attributed to the presence of essential oils rich in monoterpenes and sesquiterpenes, which interfere with insect development, feeding behavior, and survival (Alami et al., 2023).



Figure 22: : Morphological characteristics of *Artemisia campestris* L. (Dib et al., 2017).

• Classification

According to POWO (2026), the classification of the specie *Artemisia campestris* L. is as follows (Tab.03):

Table 03: Classification of the specie *Artemisia campestris* L.

Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	<i>Asterales</i>
Family	<i>Asteraceae</i>
Genus	<i>Artemisia</i>
Species	<i>Artemisia campestris</i> L.

2. Animal material

2.1. *Aphis gossypii* Glover, 1877 (Cotton Aphid)

Aphis gossypii Glover, 1877, commonly known as the *cotton aphid*, is a *polyphagous aphid* species with a nearly worldwide distribution. It is considered one of the most important pests of cotton and several other cultivated crops (Fig.23). This species is also an efficient vector of numerous plant viruses and exhibits considerable variation in color depending on environmental conditions and host plants (Edde, 2022).

It colonizes more than 900 plant species and can transmit up to 76 plant viruses, causing substantial economic losses in agricultural production. Furthermore, its populations are highly differentiated and exhibit a complex life history influenced by geographical location, climatic conditions, and host plants (Liu et al., 2024).



Figure 23: *Aphis gossypii* Glover, 1877(Original, 2026).

• Classification

According to the GBIF Backbone Taxonomy (2026), the taxonomic classification of *Aphis gossypii* Glover, 1877 is as follows (Tab.04):

Table 04: Classification of the specie *Aphis gossypii* Glover, 1877

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	<i>Hemiptera</i>

Family	<i>Aphididae</i>
Genus	<i>Aphis</i>
Species	<i>Aphis gossypii</i> Glover, 1877

• **Damage caused by *Aphis gossypii* Glover, 1877**

The cotton aphid causes significant damage to agricultural crops in several ways:

- **Sap feeding:** The aphid feeds on the sap of host plants, causing leaf curling, yellowing, and growth inhibition, which may lead to plant dwarfing, deformity, or even death.
- **Honeydew secretion:** The aphid secretes honeydew, promoting the growth of sooty mold.
- **Transmission of plant pathogens:** *Aphis gossypii* serves as a vector for various plant pathogens.
- **Economic losses:** Infestations of *Aphis gossypii* result in significant economic losses in agricultural systems (Zhang et al., 2025)

B. Non-biological material

The non-biological materials used in this study, including chemical products, laboratory materials, and equipment, are presented in (Tab.05).

Table 05: Chemical products, laboratory materials, and equipment used in the study.

Chemical Products	Beaker
Ethanol 96°	Graduated cylinder
Distilled water	Volumetric flask
Potassium sorbate	Filter funnel
Vegetable glycerin	Glass stirring rod
Tween 80	Amber glass bottles
Materials and equipment	Petri dishes
Analytical balance	Filter paper
Magnetic stirrer	Aluminum foil

Magnetic stirring bar	Micropipette
Water bath	Laboratory spatula
Refrigerator	Scissors
Drying oven	Plant pots
Rotary evaporator (Rotavapor)	Laboratory gloves
Electric grinder	

2. Methodology

2.1. Sampling Sites

The biological materials used in this study were collected from three different locations in Algeria. Date palm leaves (*Phoenix dactylifera* L.) were collected from El Ogla, El Oued Province, southeastern Algeria. The aerial parts of *Artemisia campestris* L., locally known as “Tougouft”, were collected from Tébessa Province, northeastern Algeria (Fig.24). Specimens of the cotton aphid, *Aphis gossypii* Glover, were collected from watermelon plants (*Citrullus lanatus*) cultivated in Hassani Abdelkrim, El Oued Province, Algeria.

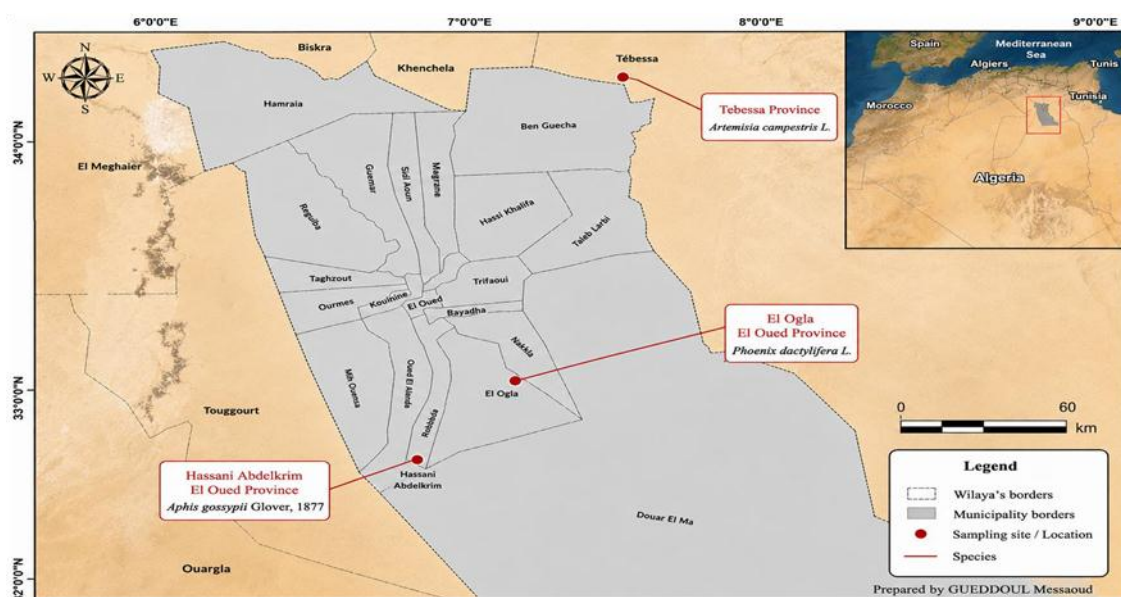


Figure 24: Sampling Sites

2.2. Harvesting of Samples

The biological samples used in this study were collected at different periods according to their availability. Date palm (*Phoenix dactylifera* L.) leaves were harvested on 7 September 2025. The aerial parts of *Artemisia campestris* L. were collected during September 2025. Specimens of *Aphis gossypii* Glover were collected on 19 May 2026.

2.3. Drying and Storage

Fresh plant materials of *Phoenix dactylifera* L. and *Artemisia campestris* L. were dried at room temperature (18–25 °C) in a shaded area, protected from direct light, and spread in thin layers on clean white paper. The samples were turned regularly to ensure uniform drying. This method was used to reduce residual moisture and prevent microbial fermentation as well as mold development.

2.4. Preparation of Plant Powder

Following the drying process, the stems of *Artemisia campestris* L. were removed, and the remaining aerial parts were retained(Fig.25).



Figure 25: Preparation of *Artemisia campestris* L. plant material following the drying process(Original, 2026).

The dried leaves of *Phoenix dactylifera* L. were cleaned to remove impurities. The plant materials were ground separately using an electric grinder to obtain fine powders. The resulting powders were stored in airtight containers at room temperature until further use for extract preparation.

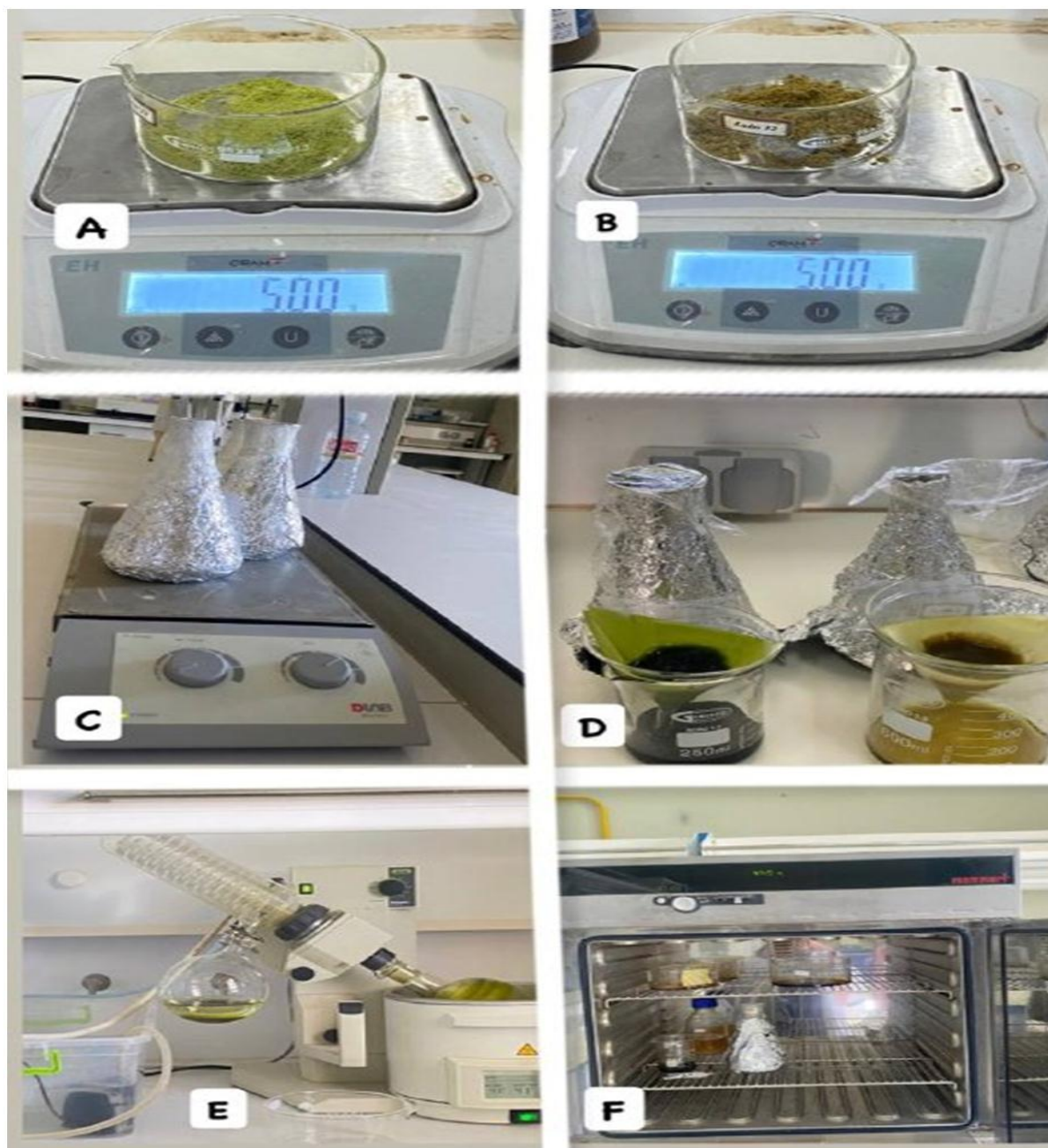
2.6. Preparation of Plant Extracts by Cold Maceration

The extraction of *Artemisia campestris* L. and *Phoenix dactylifera* L. was carried out by cold maceration using a hydro alcoholic solvent (ethanol/distilled water). For each plant species, 50 g of dried plant powderwere weighed and then transferred separately into

Erlenmeyer flasks. The solid-to-liquid ratio was maintained at 1:10 (w/v), corresponding to 1 g of plant material per 10 mL of solvent.

The hydro alcoholic solvent consisted of ethanol and distilled water in an 80:20 (v/v) ratio. The plant powders were macerated in this solvent mixture for 24 h at room temperature under continuous stirring using a magnetic stirrer. The extraction process was repeated three times to ensure exhaustive extraction of phytochemical compounds (Mousavi & Khoshdouni Farahani, 2022).

After maceration, the extracts were filtered through filter paper to remove plant residues and obtain clear filtrates. The filtrates were subsequently concentrated using a rotary evaporator (Rotavapor) under reduced pressure to remove ethanol while preserving thermolabile bioactive compounds. The concentrated extracts were then dried in a laboratory oven to eliminate residual water and obtain stable paste-like extracts. Finally, the resulting extracts were stored at 4 °C until further use (Fig.26).



(A) : weighing of *Artemisia campestris* powder; (B) : weighing of *Phoenix dactylifera* powder; (C) : cold maceration; (D) : filtration; (E) : concentration using a rotary evaporator; and (F) : oven drying.

Figure 26: Main steps involved in the preparation of plant extracts from *Artemisia campestris* L. and *Phoenix dactylifera* L. by cold maceration(Original, 2026).

Calculation of Extraction Yield (R%)

The extraction yield represents the ratio between the dry mass of the obtained extract and the dry mass of the plant material used for extraction. It was calculated according to the following equation (Dirar et al., 2019).

$$MC (\%) = [(MO - MT) / (100 - MT)] \times 100$$

Where:

R (%): Extraction yield percentage.

Me: Dry mass of the extract obtained after solvent evaporation.

Mv: Dry mass of the plant material used for extraction.

2.7. Biological Assays of Plant Extracts Against the *Aphis gossypii* Glover (Cotton Aphid)

•Collection and Maintenance of Adult *Aphis gossypii* Glover

Biological assays were conducted using adult individuals of the cotton aphid, *Aphis gossypii* Glover. The insects were collected from naturally infested host plants and maintained under laboratory conditions on fresh melon leaves (*Cucumis melo* L.) to ensure a continuous food supply and preserve their physiological condition prior to experimentation. The aphids were kept in Petri dishes lined with dry filter paper, and small pieces of fresh host plant leaves were provided as a food source. The dishes were maintained under controlled laboratory conditions at a temperature ranging from 20 to 25 °C and under suitable relative humidity.

Adult aphids were carefully transferred using a fine brush to minimize stress and mechanical injury before the biological assays were initiated(Fig.27).



Figure 27: Maintenance of adult *Aphis gossypii* Glover under laboratory conditions in Petri dishes containing dry filter paper and fresh host plant leaf pieces (Original, 2026).

•Direct Contact Toxicity Test

The experiment consisted of four treatments: *Artemisia campestris* L. extract, *Phoenix dactylifera* L. extract, a mixture of both extracts, and a control treatment. Three concentrations (C1= 31.25, C2= 62.5, and C3= 125 mg/mL) were evaluated for each treatment, with three replicates per concentration.

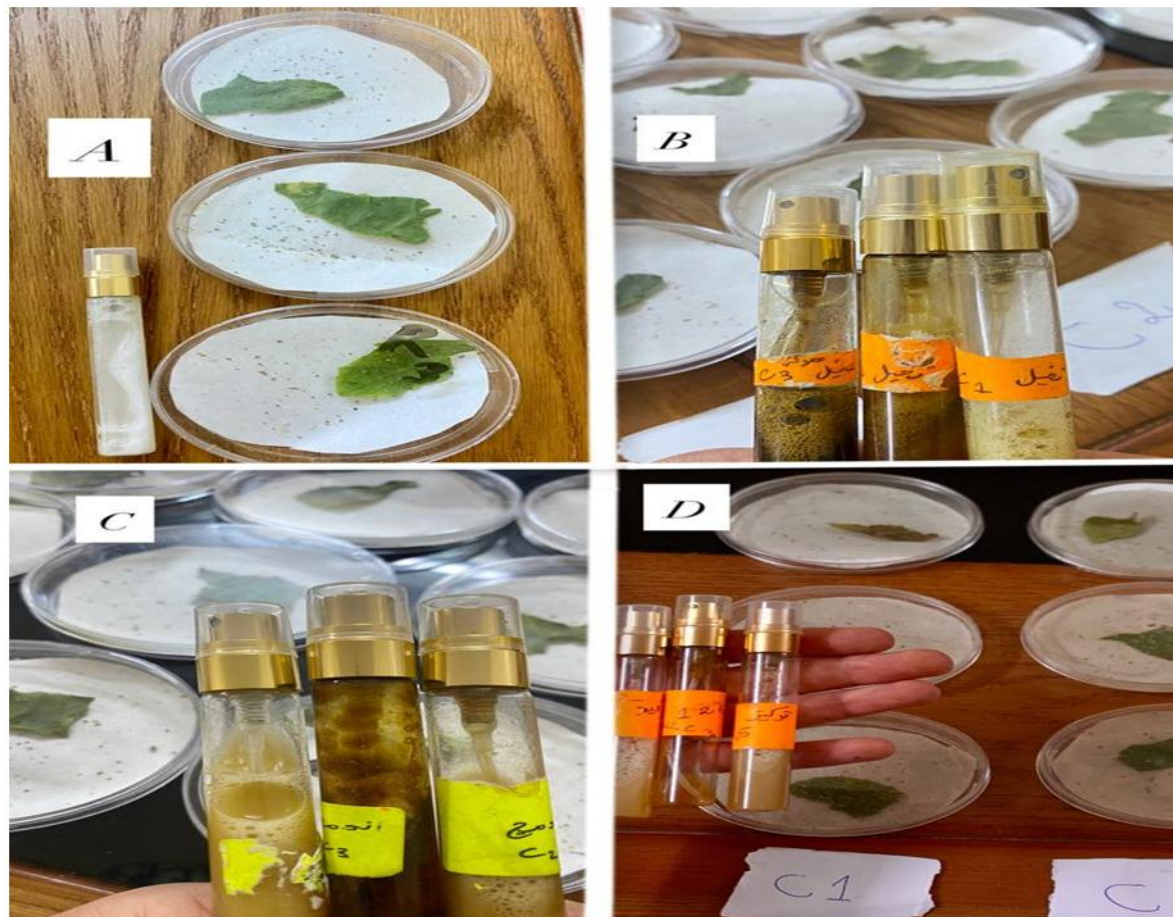
For the control treatment, a formulation containing 0.6 mL of corn oil, 0.3 mL of Tween 80, and 2.1 mL of distilled water was prepared without the addition of any plant extract. For the *Artemisia campestris* L. treatment, three concentrations were prepared using 0.09 g, 0.20 g, and 1.50 g of the extract, respectively. Each preparation contained 0.6 mL of corn oil, 0.3 mL of Tween 80, and 2.1 mL of distilled water.

The same procedure was applied for the *Phoenix dactylifera* L. treatment using the same extract quantities and formulation components.

For the combined treatment, equal amounts of *Artemisia campestris* L. and *Phoenix dactylifera* L. extracts were mixed. Accordingly, the first concentration contained 0.045 g of each extract, the second concentration contained 0.10 g of each extract, and the third concentration contained 0.75 g of each extract, together with 0.6 mL of corn oil, 0.3 mL of Tween 80, and 2.1 mL of distilled water.

After preparation, the formulations were transferred into small glass spray bottles to facilitate their application during the bioassays.

The prepared formulations were applied by direct spraying onto the insects. Following treatment, the Petri dishes were maintained under the same laboratory conditions throughout the experimental period. The dishes were monitored at regular time intervals, and the number of dead insects was recorded to assess the insecticidal activity of the tested extracts (Fig.28).



(A): Control treatment, (B): different concentrations of *Phoenix dactylifera* L. extract,

(C): different concentrations of *Artemisia campestris* L. extract, and (D): different concentrations of the combined extract (*Artemisia campestris* L. + *Phoenix dactylifera* L.).

Figure 28: Experimental setup of the direct contact toxicity assay against *Aphis gossypii* Glover using different concentrations of plant extracts and the control treatment (Original, 2026).

2.8. Preparation of the Final Product

The final formulation was prepared by mixing 5 g of plant extracts, consisting of 2.5 g of *Phoenix dactylifera* L. extract and 2.5 g of *Artemisia campestris* L. extract, with 8.5 mL of Tween 80, 8.5 mL of distilled water, and 6 mL of corn oil. The mixture was continuously stirred until complete dissolution of the extracts and the formation of a homogeneous solution.

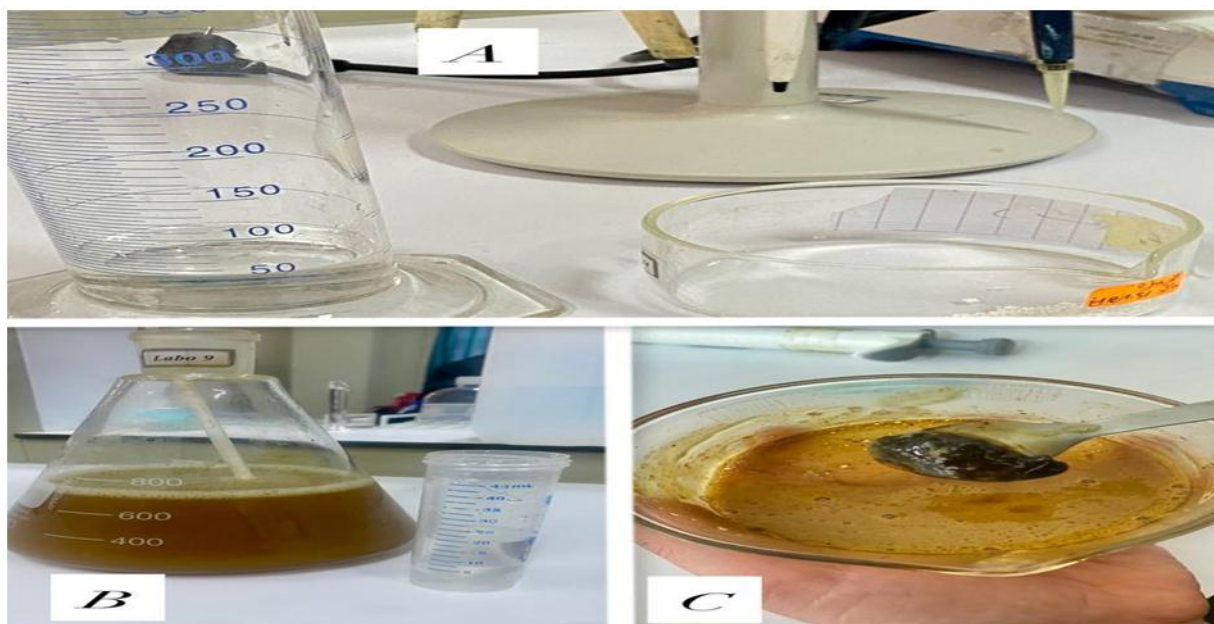
Separately, 1 g of potassium sorbate was dissolved in 50 mL of warm distilled water (35–40 °C) under continuous stirring until complete dissolution was achieved.

Subsequently, 700 mL of distilled water were placed in a glass flask, and the mixture prepared in the first step was gradually added under continuous stirring. The resulting solution was stirred for 5 min to ensure the uniform distribution of all components.

Thereafter, 20 mL of vegetable glycerin were slowly added to the mixture while stirring continuously for 5 min until a homogeneous preparation was obtained. This was followed by the addition of 1 mL of Tween 80, and the formulation was gently stirred for an additional 2–3 min to ensure complete homogenization. The previously prepared potassium sorbate solution was then incorporated into the formulation, and stirring was continued for 5 min to ensure its uniform distribution throughout the mixture.

Finally, 129 mL of distilled water were added to adjust the final volume to 1000 mL. The formulation was further stirred for 3 min until a completely homogeneous product was obtained.

Upon completion of the preparation process, the final formulation was transferred into sterilized opaque spray bottles, tightly sealed, and stored under appropriate conditions until use (Fig.29).



(A): Weighing of potassium sorbate and measurement of distilled water volume.

(B): Preparation of the final formulation following the addition of all components.

(C): Mixing and homogenization of the final formulation until a homogeneous preparation was obtained.

Figure 29: Main stages involved in the preparation of the final plant-based formulation (Original, 2026).

2.9. Application of the Final Plant-Based Formulation

Mint plants were selected for the application of the final plant-based formulation due to their susceptibility to both water stress and infestation by the cotton aphid, *Aphis gossypii* Glover. Mint exhibits distinct physiological and morphological responses to water deficit, including reduced vegetative growth and leaf wilting. Moreover, it serves as a suitable host plant for aphid development and reproduction. In addition, its relatively large leaf surface area facilitates uniform spray deposition, thereby allowing an effective evaluation of the formulation efficacy in alleviating water stress and controlling aphid infestation under experimental conditions (Fig.30).



Figure 30: Mint plants (*Mentha spicata* L.) used in the experimental study(Original, 2026).

The experiment was conducted using four treatment groups. Each group consisted of three pots, and each pot contained three mint plants.

- The first group served as the control (T0) and was irrigated with 200–250 mL of water per pot throughout the experimental period.
- The second group (T1) was subjected to water stress by reducing the irrigation volume to 50% of that applied to the control group.

- The third group (T2) received the final plant-based formulation at a concentration of 5% and was simultaneously exposed to 50% water stress. The formulation was applied daily by foliar spraying, using 10 spray strokes per application.

- The fourth group (T3) received the final plant-based formulation at a concentration of 10% and was also subjected to 50% water stress. The formulation was applied daily by foliar spraying, using 20 spray strokes per application.

The experiment was conducted over a period of 15 days, during which all plants were maintained under identical environmental conditions and received their respective treatments according to the experimental design. At the end of the experimental period, the effects of water stress and the efficacy of the final plant-based formulation were assessed through a comparative evaluation of plant responses among the different treatment groups (Fig.31).



Figure 31: Application of the prepared bioherbicidal spray formulation on mint (*Mentha spicata* L.) plants by foliar spraying (Original, 2026).

2.10. Counting of Individuals

The insecticidal efficacy of *Phoenix dactylifera* L. and *Artemisia campestris* L. extracts against the cotton aphid (*Aphis gossypii* Glover) was evaluated by monitoring mortality rates at different exposure times and tested concentrations. Aphids were examined using a fine brush to detect any movement response. Individuals that showed no movement after mechanical stimulation were considered dead.

2.11. Estimation of Observed Mortality

The observed mortality percentage of treated aphids was calculated using the following formula:

$$\text{Observed Mortality (\%)} = (\text{Number of dead individuals} / \text{Total number of individuals}) \times 100$$

2.12. Estimation of Corrected Mortality

Since natural mortality may occur in the control treatment, mortality percentages were corrected using Abbott's formula (Abbott, 1925):

Where:

MC: Corrected mortality percentage.

MO: Observed mortality in the treated groups.

MT: Mortality recorded in the control group.

$$MC (\%) = [(MO - MT) / (100 - MT)] \times 100$$

2.13. Determination of Lethal Time (LT₅₀)

To evaluate the insecticidal efficacy of *Phoenix dactylifera* L. and *Artemisia campestris* L. extracts against the cotton aphid (*Aphis gossypii* Glover), the lethal time (**LT₅₀**) was determined. This parameter represents the exposure time required to cause 50% mortality of the treated aphid population.

The LT₅₀ value was estimated using Probit analysis. Corrected mortality percentages were transformed into Probit units, while exposure times were converted into decimal logarithmic values (Log₁₀) to establish the linear regression equation:

$$Y = a + bX$$

Where:

Y: Probit value of corrected mortality.

a: Intercept of the regression line.

X: Decimal logarithm of exposure time.

b: Slope of the regression line.

Finally, the LT₅₀ value, together with its 95% confidence limits, was obtained from the statistical analysis results.

CHAPTER IV

Results

❖ Results

1. Extraction Yield Results of Plant Extracts

The extraction yields of the two plant species were calculated and are presented in (Tab.06).

Table 06: Extraction yields of *Phoenix dactylifera* L. and *Artemisia campestris* L.

Plant species	Plant mass (g)	Extract mass (g)	Extraction yield (%)
<i>Phoenix dactylifera</i> L.	50	4,98	≈10
<i>Artemisia campestris</i> L.	50	4,28	8,56

The results presented in Table 06 summarize the extraction yields obtained by cold maceration from 50 g of dried plant material for each species. *Phoenix dactylifera* L. produced the highest crude extract mass (4.98 g), corresponding to an extraction yield of 9.96%. In contrast, *Artemisia campestris* L. yielded 4.28 g of crude extract, corresponding to an extraction yield of 8.56%. The observed differences in extraction yield may be attributed to variations in the phytochemical composition and the concentration of extractable compounds present in each plant species.

2. Evaluation of the insecticidal effect of different concentrations of extracts

2.1. Extract of *Phoenix dactylifera* L.

The results revealed a gradual increase in the percentage of aphid mortality with increasing exposure time in all treatments. At 5 minutes of exposure, mortality rates reached 16.67%, 30.00%, and 48.33% for treatments C1, C2, and C3, respectively. After 10 minutes, these values increased to 36.67%, 56.67%, and 73.33%.

A continuous increase in mortality was recorded throughout the experiment. At 15 minutes, treatment C3 reached 100% mortality, whereas mortality rates in C1 and C2 were 53.33% and 76.67%, respectively. At 20 minutes, treatment C2 recorded 93.33% mortality, while treatment C1 reached 73.33%. Complete mortality was achieved by treatment C2 after 25 minutes, whereas treatment C1 reached 90.00% at the same exposure time.

At the end of the experimental period (30 minutes), treatment C1 attained 100% mortality. Overall, treatment C3 consistently recorded the highest mortality percentages at all observation times, followed by C2 and then C1. The ranking of treatments remained unchanged throughout the experiment, with C3 showing the fastest increase in mortality rate and the shortest time required to achieve complete aphid mortality, followed by C2 and C1.

The mortality curves showed a positive upward trend with increasing exposure time for all treatments. The slope of the curve was steeper for treatment C3 during the initial stages of exposure, whereas treatments C2 and C1 exhibited a more gradual increase. Complete mortality was reached successively at 15, 25, and 30 minutes for treatments C3, C2, and C1, respectively (Fig.32).

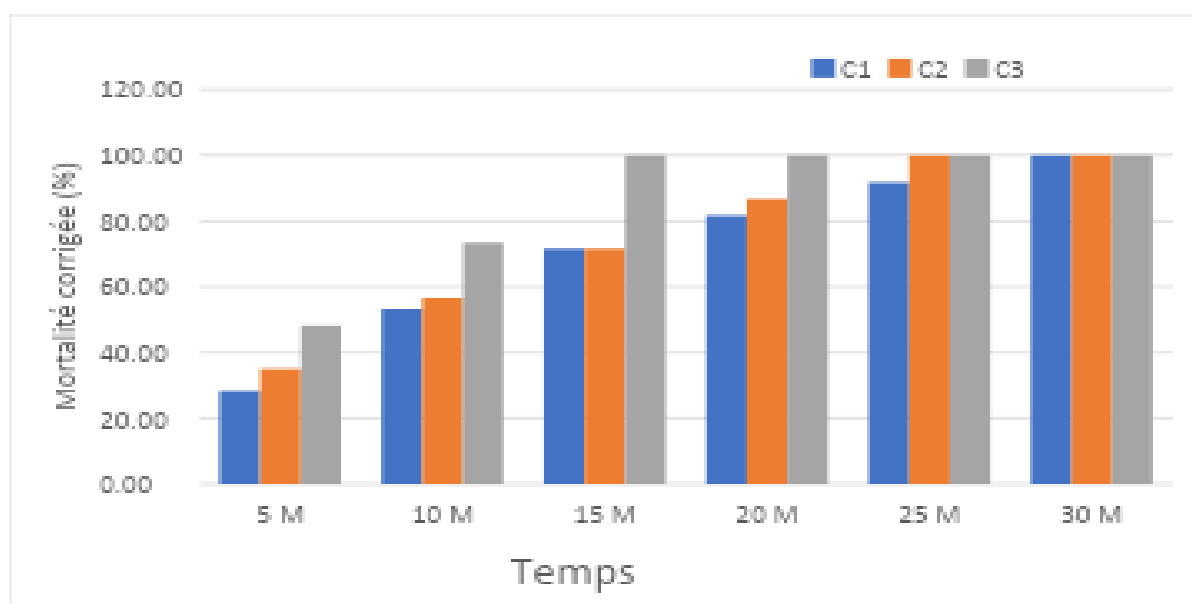


Figure 32: Insecticidal Effect of Date Palm Leaf Extract on Aphids by Direct Contact.

2.2. Extract of *Artemisia campestris* L.

The table illustrates the progression of the insecticidal effect of Taghaft plant extract against aphids as a function of exposure time and concentration levels (C1, C2, and C3). The results revealed a clear positive relationship between exposure time and the percentage of effect recorded in all treatments, with efficacy increasing progressively over time.

At the beginning of the experiment (4 min), treatment C3 exhibited the highest insecticidal activity, reaching 48.3%, followed by C2 (23.3%) and C1 (20%). This early response highlights the superior effectiveness of C3 compared with the other treatments.

After 8 min of exposure, the percentage effect increased to 85% for C3, 50% for C2, and 35% for C1. The widening gap between C3 and the other treatments indicates a stronger and faster action of the extract at the higher concentration.

At 12 min, treatment C3 achieved 100% effectiveness, whereas C2 and C1 recorded 78.3% and 56.7%, respectively. These results demonstrate the ability of C3 to induce complete mortality within a shorter period than the lower concentrations.

At 16 min, both C2 and C3 reached 100% effectiveness, while C1 attained 95%. By the end of the experiment (20 min), all treatments achieved 100% effectiveness, indicating that the plant extract was capable of completely controlling aphid populations when exposure time was sufficiently prolonged (Fig.33).

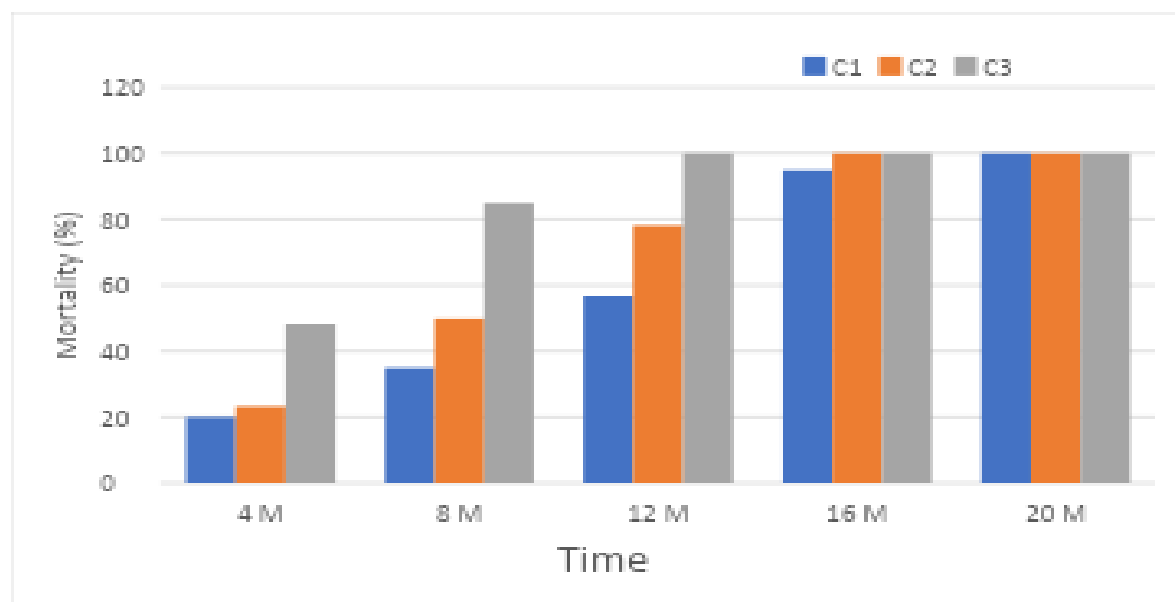


Figure 33: Insecticidal Effect of *Artemisia campestris* Plant Extract on Aphids by Direct Contact.

2.3. Plant extract spray

The figure illustrates the evolution of aphid mortality (%) following the application of a biopesticide spray formulated from a mixture of *Artemisia campestris* extract and *Phoenix dactylifera* leaf extract at three different concentrations (C1, C2, and C3) (Fig.34).

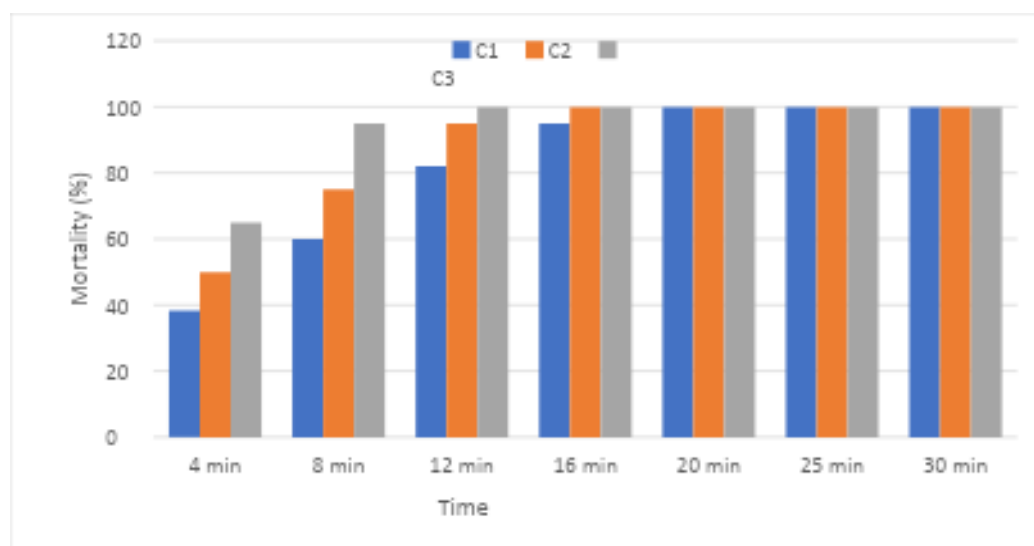


Figure 34: Effect of a Plant-Based Biopesticide Spray Formulated from *Artemisia campestris* and *Phoenix dactylifera* Extracts on Aphid Mortality at Different Concentrations and Exposure Times.

The results show a rapid and progressive increase in mortality rates with increasing exposure time for all tested concentrations. After only 4 minutes of treatment, mortality rates reached 38.3%, 50%, and 65% for C1, C2, and C3, respectively, indicating an immediate insecticidal effect of the plant extracts against aphids (Fig. 34).

A clear concentration-dependent effect was observed, as the highest concentration (C3) consistently produced the greatest mortality rates throughout the experiment. After 8 minutes of exposure, mortality reached 95% at C3, compared with 75% and 60% at C2 and C1, respectively.

At 12 minutes, the highest concentration (C3) achieved 100% mortality, while mortality rates of 95% and 82% were recorded for C2 and C1, respectively. Complete mortality was reached at 16 minutes for C2, whereas C1 produced a mortality rate of 95% at the same exposure time. The lowest concentration (C1) required 20 minutes to achieve total mortality of the aphid population (Fig. 34).

3. Résultats de l'analyse Probit des concentrations létales (CL) et des temps létaux (TL) des extraits de *Phoenix dactylifera* L. et *Artemisia campestris* L. appliqués par contact direct contre *Aphis gossypii* Glover

Determining TL 50

To determine TL 50, we used a regression line. The latter represents the logarithm of the tested concentrations and the cumulative mortality rates in the probit (Fig.35).

3.1. *Phoenix dactylifera* L.

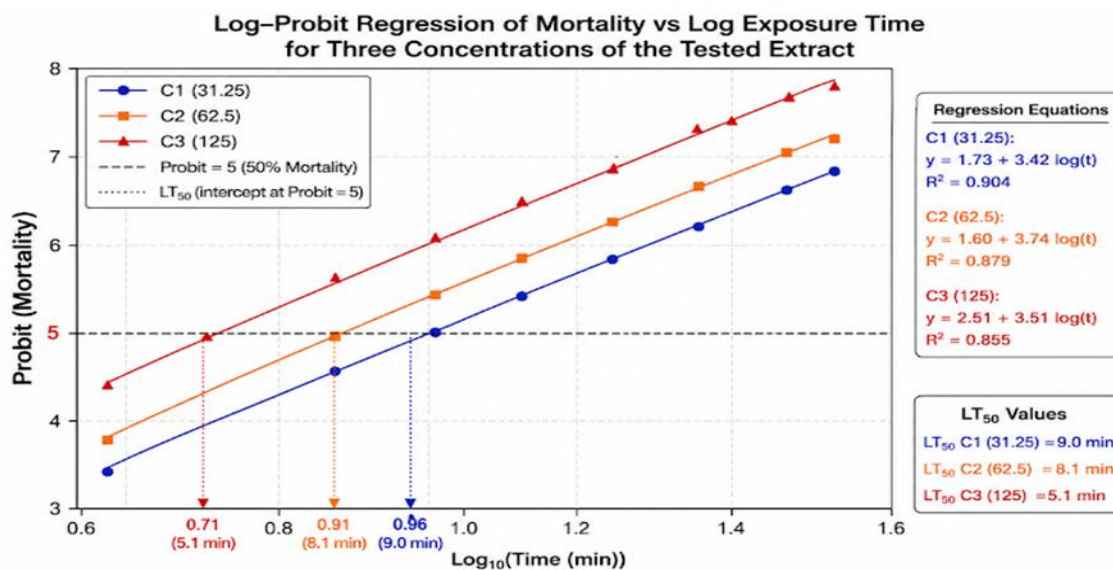


Figure. Log–probit regression of mortality vs. log exposure time for three concentrations of the tested extract. LT₅₀ values (time required to achieve 50% mortality) are indicated.

Figure 35: Log–Probit regression of mortality versus log exposure time for three concentrations of the tested extract, with estimation of LT₅₀ values.

The figure shows a clear linear relationship between logarithm of time ($\log_{10}$ time) and the toxic response expressed as Probit mortality for three different concentrations of the tested extract (C1 = 31.25, C2 = 62.5, C3 = 125). All concentrations followed a satisfactory linear regression pattern under the Log–Probit model, with relatively high coefficients of determination (R^2 ranging from 0.855 to 0.904), confirming the suitability of this model for describing the time-dependent toxicity dynamics. The results also revealed a clear dose-dependent effect, where the C3 concentration exhibited the highest Probit values at the same exposure time compared to C2 and C1, indicating a faster toxic response and shorter time required to induce mortality. In contrast, C1 showed a slower response, reflecting lower toxic efficacy at reduced concentrations. Accordingly, LT₅₀ values decreased progressively with increasing concentration, with C3 being the most effective in inducing rapid mortality, followed by C2 and C1. These findings confirm that the toxic activity of the tested extract is both dose- and time-dependent, indicating a strong biological efficacy of the studied extract (Fig.36).

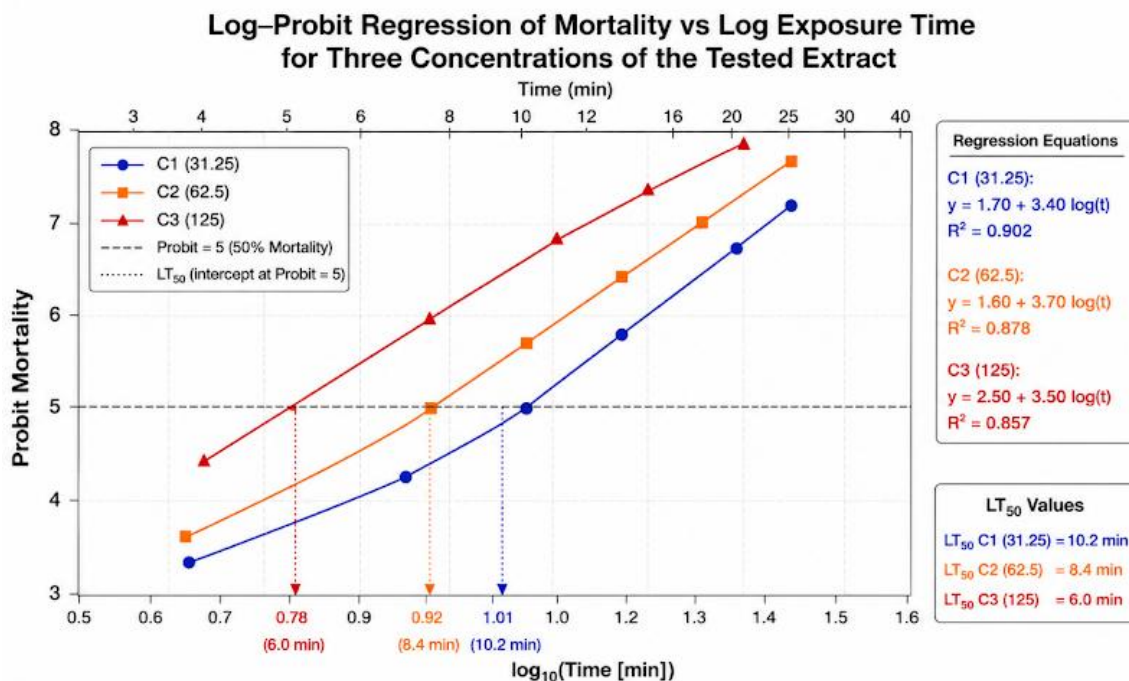
3.2. *Artemisia campestris* L.

Figure. Combined log-probit regression of mortality versus log exposure time for three concentrations of the tested extract. Dotted lines indicate LT_{50} (time required to reach 50% mortality).

Figure 36: Log–Probit regression of mortality versus log exposure time for three concentrations of the tested extract, with estimation of LT_{50} values.

The figure shows a clear linear regression relationship between the logarithm of time ($\log_{10}$ time) and the toxic response expressed as Probit mortality for three different concentrations of the tested extract (C1 = 31.25, C2 = 62.5, C3 = 125), over successive exposure times (4, 8, 12, 16, and 20 minutes). The results indicate that Probit values progressively increase with increasing time on a logarithmic scale across all concentrations, reflecting a consistent time-dependent toxic response that fits the Log–Probit model. A clear dose-dependent effect is also observed across the different exposure times, where C3 shows the highest Probit values at the same time points, particularly at 8 and 12 minutes, followed by C2 and C1, indicating a faster mortality rate at higher concentrations. Based on the intersection of the regression lines with the Probit = 5 level (representing 50% mortality), LT_{50} values were estimated at approximately 10.2 minutes for C1, 8.4 minutes for C2, and 6.0 minutes for C3. These results confirm that increasing concentration reduces the time required to induce 50% mortality, demonstrating a clear dose- and time-dependent toxicity and indicating strong biological activity of the tested extract.

3.3. Plant extract spray

The figure shows a clear linear regression relationship between the logarithm of time ($\log_{10}$ time) and the toxic response expressed as Probit mortality for three different concentrations of the tested extract ($C1 = 31.25$, $C2 = 62.5$, $C3 = 125$), over successive exposure times (4, 8, 12, 16, and 20 minutes). The results indicate that Probit values progressively increase with increasing time on a logarithmic scale across all concentrations, reflecting a consistent time-dependent toxic response that fits the Log–Probit model. A clear dose-dependent effect is also observed across the different exposure times, where $C3$ shows the highest Probit values at the same time points, particularly at 8 and 12 minutes, followed by $C2$ and $C1$, indicating a faster mortality rate at higher concentrations. Based on the intersection of the regression lines with the Probit = 5 level (representing 50% mortality), LT_{50} values were estimated at approximately 10.2 minutes for $C1$, 8.4 minutes for $C2$, and 6.0 minutes for $C3$. These results confirm that increasing concentration reduces the time required to induce 50% mortality, demonstrating a clear dose- and time-dependent toxicity and indicating strong biological activity of the tested extract (Fig.37).

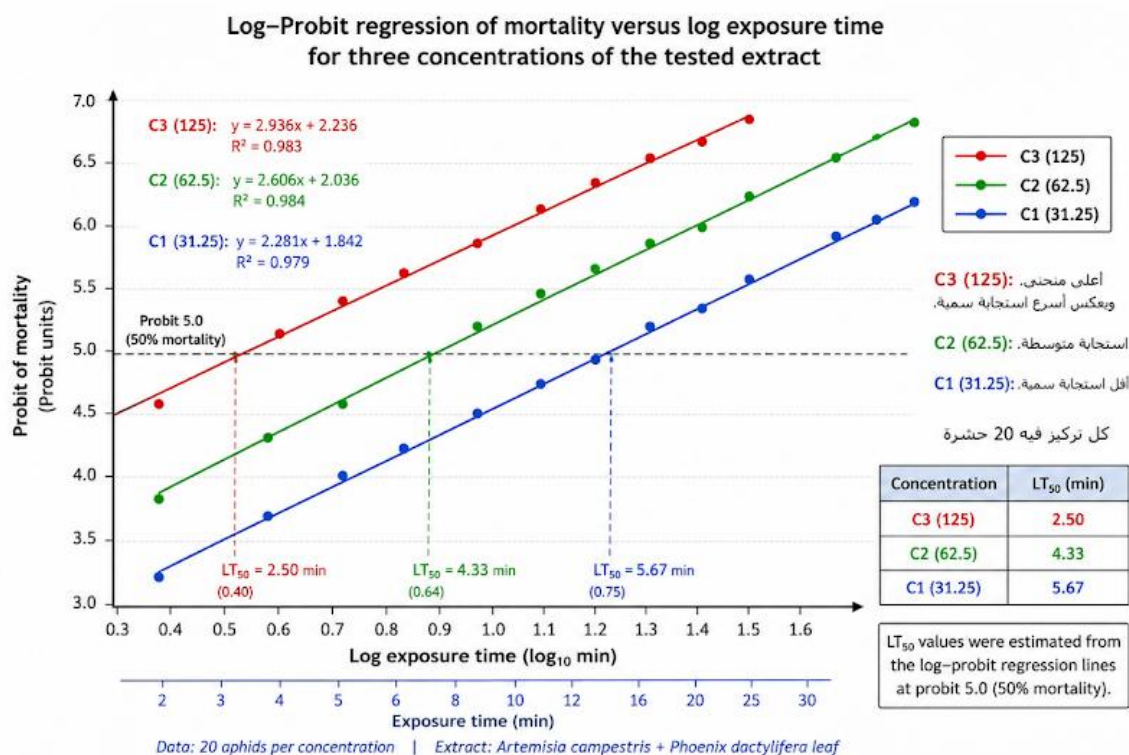


Figure 37: Log–Probit regression of aphid mortality versus log exposure time for different concentrations of plant extract spray, showing LT_{50} estimation.

Les résultats montrent une nette influence de la concentration sur la vitesse d'action insecticide. La concentration la plus élevée ($C3 = 125$ mg/mL) se situe au-dessus des autres

courbes et présente la valeur de LT_{50} la plus faible (2,50 min), ce qui traduit une toxicité rapide et une forte efficacité insecticide. La concentration intermédiaire ($C_2 = 62,5$ mg/mL) présente une valeur de LT_{50} de 4,33 min, indiquant une activité insecticide modérée. En revanche, la concentration la plus faible ($C_1 = 31,25$ mg/mL) nécessite un temps plus long pour provoquer 50 % de mortalité, avec une valeur de LT_{50} de 5,67 min (Fig. 37).

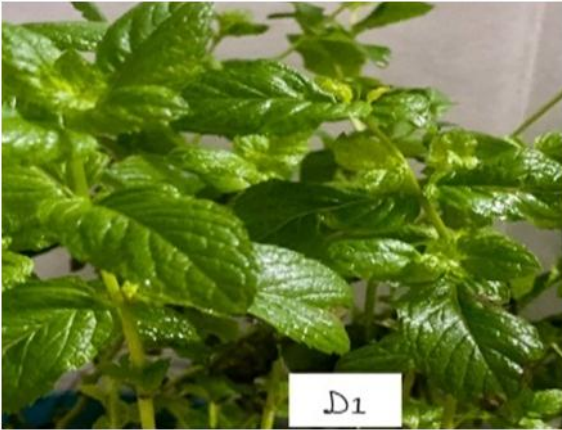
L'intersection de chaque droite de régression avec le niveau Probit 5, correspondant à 50 % de mortalité, a permis d'estimer les valeurs de LT_{50} . La diminution progressive de ces valeurs lorsque la concentration augmente confirme l'existence d'une relation inverse entre la concentration de l'extrait et le temps nécessaire pour atteindre 50 % de mortalité.

4. Visual Assessment of the Effect of the Final Plant-Based Formulation on Mint Plants Under Water Stress Conditions

The effects of water stress and the final plant-based formulation on mint plants were evaluated through visual observations conducted throughout the 15-day experimental period. The assessment was based on the overall plant appearance, leaf color, leaf turgidity, and general plant vigor under the different treatment conditions (Tab.07). As shown in the following table:

Table 07: Visual response of mint plants (*Mentha sp.*) subjected to different treatments under water stress conditions during the 15-day experimental period (Original, 2026).

Treatment	Day1	Day7	Day15
Control Treatment (T0)			
50% Water Stress Treatment (T1)			

50% Water Stress + 5% Formulation Treatment (T2)	 C1	 C7	 C15
50% Water Stress + 10% Formulation Treatment (T3)	 D1	 D7	 D15

A1, A7, A15: Control treatment (T0) on Days 1, 7, and 15, respectively; B1, B7, B15: 50% water stress treatment (T1) on Days 1, 7, and 15, respectively; C1, C7, C15: 50% water stress + 5% final plant-based formulation treatment (T2) on Days 1, 7, and 15, respectively; D1, D7, D15: 50% water stress + 10% final plant-based formulation treatment (T3) on Days 1, 7, and 15, respectively.

The visual assessment of mint plants conducted over the 15-day experimental period revealed differences in morphological responses among the various treatments. On Day 1, all plants exhibited healthy green leaves, high leaf turgidity, and normal vegetative growth, with no visible symptoms of water stress.

On Day 7, plants subjected to 50% water stress (T1) exhibited reduced leaf turgidity and slight wilting symptoms compared with the control treatment (T0). Plants treated with the final plant-based formulation at concentrations of 5% (T2) and 10% (T3) maintained higher leaf greenness and turgidity than those subjected to water stress alone. In addition, less pronounced stress symptoms were observed in the formulation-treated plants.

On Day 15, the highest severity of water stress symptoms was recorded in T1 plants, which exhibited noticeable leaf wilting, partial leaf tissue desiccation, and reduced vegetative growth. Plants treated with the 5% formulation (T2) showed a lower severity of symptoms compared with T1 while maintaining a considerable proportion of green leaf area. The T3 treatment exhibited the highest level of leaf greenness and turgidity retention, together with a marked reduction in wilting symptoms compared with the other water-stressed treatments. The visual observations indicated an improvement in the morphological characteristics of formulation-treated plants compared with plants exposed to water stress alone. Furthermore, the 10% concentration demonstrated greater effectiveness than the 5% concentration in reducing the visible symptoms associated with water deficit throughout the experimental period.

5. Final Product: ARTENIX® Biopesticide Spray (Fig.38).



Figure 38 : Final formulated ARTENIX® bio spray for insect pest control and plant stress alleviation.

Directions for use:

Shake well before use. Spray uniformly on plant foliage, ensuring complete coverage of both upper and lower leaf surfaces.

Chapter V
DISCUSSION

Discussion

1. Discussion of Extraction Yield

The extraction yields obtained by cold maceration were 9.96% for *Phoenix dactylifera* L. leaves and 8.56% for *Artemisia campestris* L. These results indicate that both plant species contain considerable amounts of extractable phytochemicals that can be efficiently recovered using hydroalcoholic solvents.

For *Phoenix dactylifera* L., the extraction yield obtained in the present study (9.96%) was comparable to the value reported by **Mard et al. (2010)**, who recorded an extraction yield of 8.2% using a hydroalcoholic extraction of date palm leaves. This finding highlights the effectiveness of the cold maceration technique in recovering bioactive compounds from this plant species. Furthermore, the extraction yield obtained in the present study was influenced by the extraction conditions employed. Previous investigations demonstrated that extraction yield from date palm leaves increased significantly under mildly acidic conditions (**Laouini et al., 2016**). In addition, the extraction method markedly affects the recovery of phenolic compounds, as different extraction techniques such as maceration, Soxhlet extraction, and ultrasound-assisted extraction produce different extraction efficiencies (**Dia et al., 2016**). Similarly, ultrasound-assisted extraction has been reported to be more efficient than conventional maceration in recovering bioactive compounds from date palm leaves (**Dia et al., 2017**).

Regarding *Artemisia campestris* L., the extraction yield of 8.56% obtained in this study falls within the range reported for species of the genus *Artemisia*. Several studies have shown that extraction yield in *Artemisia* species varies according to the extraction solvent, geographical origin, harvesting season, and phenological stage of the plant. Extraction conditions play a crucial role in determining the final extraction yield of *Artemisia* extracts (**Benouaer, 2016**). Higher extraction yields have also been reported for some *Artemisia* species (**Bouatrous & Mechaala, 2020**), whereas solvent polarity and maceration time significantly influence the recovery of bioactive constituents (**Mahboub et al., 2024**).

2. Discussion of the Effects of *Phoenix dactylifera* L. and *Artemisia campestris* L. Extracts Against *Aphis gossypii* Glover

2.1. Discussion of the Insecticidal Effect of *Phoenix dactylifera* L. Extract Against *Aphis gossypii* Glover

The results obtained in the present study showed a gradual increase in aphid mortality with increasing exposure time for all tested concentrations of *Phoenix dactylifera* L. leaf extract. The highest concentration (C3) produced the fastest response, achieving 100% mortality after 15 minutes of exposure, whereas complete mortality was recorded after 25 and 30 minutes for C2 and C1, respectively. These findings indicate that the effect of the extract is dependent on both concentration and exposure time.

The results obtained are consistent with previous studies reporting the biological activity of date palm leaf extracts. Date palm leaves contain various secondary metabolites, including phenolic compounds, flavonoids, and tannins, which may contribute to their effects on the physiological processes of insects (**Laouini et al., 2012**). The presence of these compounds may be one of the factors associated with the mortality rates observed in the present study. Previous studies have also shown that the extraction method influences the amount of phenolic compounds recovered from date palm leaves, thereby affecting the biological activity of the extract (**Dia et al., 2016**). In addition, other investigations reported that date palm leaf extracts contain bioactive compounds with antioxidant properties, which are associated with their high phenolic content (**Dia et al., 2017**).

The mortality rates recorded in the present study are comparable to those obtained using other botanical extracts against *Aphis gossypii*. Mortality rates exceeding 90% were reported following the application of ethanolic plant extracts under laboratory conditions (Montero-Contreras et al., 2017). Similarly, another study reported mortality rates ranging from 75% to 91.67% when different plant extracts were tested against the same insect species (**Alanazi, 2022**).

Overall, the results indicate that date palm leaf extract exhibits biological activity against *Aphis gossypii* under laboratory conditions. The progressive increase in mortality over time also suggests an effect related to both exposure duration and extract concentration, which is consistent with findings reported for several plant extracts used in insect pest management.

2.2. Discussion of the Insecticidal Effect of *Artemisia campestris* L. Extract Against *Aphis gossypii*

The present study demonstrated the insecticidal efficacy of *Artemisia campestris* L. extract against *Aphis gossypii*, with mortality increasing as both extract concentration and exposure time increased. The highest efficacy was recorded at concentration C3, indicating a positive relationship between extract concentration and insecticidal activity. These findings are consistent with those reported by (Berrached et al., 2024), who demonstrated the insecticidal activity of *Artemisia campestris* extract against the aphid *Aphis fabae*, suggesting its effectiveness against different aphid species. Similarly.

The observed insecticidal activity may be attributed to the rich phytochemical composition of *Artemisia campestris*, which contains various bioactive secondary metabolites, including terpenoids, flavonoids, and phenolic compounds. These compounds are known to induce toxic effects and physiological disturbances in insects by affecting the nervous system, respiration, and metabolic processes, ultimately leading to increased mortality rates (Dib et al., 2017). Furthermore, a comprehensive review by (Dib & El Alaoui-Faris, 2019) highlighted that *Artemisia campestris* is a rich source of biologically active compounds and that its extracts and essential oils exhibit a broad spectrum of biological activities, including insecticidal effects.

Overall, the present findings confirm the promising potential of *Artemisia campestris* L. extract for the control of *Aphis gossypii*, which is in agreement with previous studies reporting the insecticidal properties of this plant.

2.3. Discussion of the Insecticidal Activity of the Biopesticide Spray Against *Aphids gossypii* Glover

The biopesticide spray formulated from a mixture of *Artemisia campestris* extract and *Phoenix dactylifera* L. leaf extract exhibited insecticidal activity against aphids. Mortality rates increased with increasing concentration and exposure time, with concentration C3 recording the highest mortality rate during the experimental period.

The results obtained are consistent with previous studies reporting the insecticidal activity of *Artemisia campestris* extracts against aphids (Berrached et al., 2024). The mortality rates observed in the present study may be attributed to the chemical composition of *Artemisia campestris*, which contains various biologically active secondary metabolites,

including terpenoids, flavonoids, and phenolic compounds (Dib et al., 2017). These compounds are known to affect certain physiological processes in insects, which may contribute to increased mortality. In addition, *Artemisia campestris* contains a wide range of bioactive compounds associated with several biological activities, including insecticidal effects (Dib & El Alaoui-Faris, 2019).

The efficacy of the tested biopesticide may also be related to the presence of bioactive compounds in *Phoenix dactylifera* leaves. Previous studies have shown that date palm leaves contain phenolic compounds, flavonoids, and tannins with various biological properties (Elansary et al., 2020), which may have contributed to the effectiveness of the formulation. Moreover, combining extracts from both plant species may result in complementary interactions between their bioactive constituents, potentially enhancing the insecticidal effect against aphids. Previous studies have reported that mixtures of plant extracts can improve the efficacy of certain biopesticide formulations due to the diversity of active compounds and their different modes of action (Pavela & Benelli, 2016; Isman, 2020).

Overall, the results obtained indicate that the mixture of *Artemisia campestris* and *Phoenix dactylifera* extracts exhibited insecticidal activity against aphids under the laboratory conditions employed in this study.

3. Discussion of LT_{50} Results

3.1. Discussion of LT_{50} of *Phoenix dactylifera* L.

In the present study, a decrease in LT_{50} values was observed with increasing concentrations of *Phoenix dactylifera* L. leaf extract, indicating that higher concentrations enhanced the insecticidal efficacy of the extract and accelerated insect mortality. This reduction may be attributed to the greater availability of bioactive compounds to the target insect at higher concentrations, resulting in a faster and more pronounced toxic effect. Date palm leaves are recognized as an important source of phenolic compounds and flavonoids with high biological activity (Djaoudene et al., 2024).

As demonstrated by (Alshammari et al., 2024), a high content of phenolic compounds and secondary metabolites in plant extracts is associated with enhanced biological activity, which may explain the improved efficacy of *Phoenix dactylifera* leaf extract and the reduction in the time required to induce mortality as the concentration increases.

The biological activity of *Phoenix dactylifera* extracts has also been linked to their richness in naturally occurring bioactive compounds, which can enhance the effectiveness of plant extracts when applied at higher concentrations, as reported by (Anbar et al., 2024). These findings support the results of the present study, where increasing the concentration of *Phoenix dactylifera* leaf extract led to lower LT_{50} values and faster mortality, reflecting an improvement in its insecticidal efficacy.

3.2. Discussion of LT_{50} of *Artemisia campestris* L.

In the present study, a decrease in LT_{50} values was observed with increasing concentrations of *Artemisia campestris* L. extract, where concentration C3 recorded the lowest LT_{50} value compared with C1 and C2, indicating a higher insecticidal efficacy of the extract at elevated concentrations.

LT_{50} values decrease with increasing concentrations of botanical extracts (Ibrahim & Rustam, 2024), as higher concentrations accelerate mortality and reduce the time required to reach 50% mortality. LT_{50} is also considered one of the most reliable indicators for evaluating the efficacy of botanical insecticides.

Increasing the concentration of bioactive compounds enhances the insecticidal activity of plant extracts and accelerates mortality in target insects. According to (Abdullah et al., 2024), the efficacy of botanical pesticides is strongly dependent on the concentration of active compounds present in the extract.

Higher concentrations of plant extracts significantly reduce LT_{50} values, indicating an inverse relationship between extract concentration and the time required to induce mortality. Elevated levels of active compounds improve insecticidal performance and accelerate insect mortality (Moutassem et al., 2024).

The findings of the present study confirm an inverse relationship between extract concentration and LT_{50} values, which is consistent with previous reports.

3.3. Discussion of LT_{50} of the Plant Spray

In the present study, LT_{50} values decreased with increasing concentrations of the plant spray, indicating that higher concentrations accelerated mortality in the cotton aphid (*Aphis gossypii*) and enhanced the insecticidal efficacy of the formulation.

A study (Bhede et al., 2024) showed that the efficacy of botanical extracts against *Aphis gossypii* increased with concentration, resulting in a greater reduction in aphid

populations at higher doses. This may explain the decrease in LT_{50} values observed in the present study as the concentration increased.

Lower LT_{50} values are generally associated with a faster insecticidal action and higher biological effectiveness against target pests. LT_{50} has also been recognized as a useful parameter for evaluating the speed of action of control agents against the cotton aphid (Erol et al., 2023). Similarly, plant-derived compounds have been shown to exert significant insecticidal activity against *Aphis gossypii*, with their effectiveness increasing as the applied dose increases. A study conducted by (Wang et al., 2024) demonstrated that increasing the dose of plant-based compounds enhanced their biological activity against the target pest.

These findings are consistent with the results of the present study, where increasing the concentration of the plant spray led to lower LT_{50} values and faster mortality of *Aphis gossypii*, reflecting an improvement in its insecticidal efficacy.

4. Discussion of the Visual Assessment of the Effect of the Final Plant-Based Formulation on Mint Plants Under Water Stress Conditions

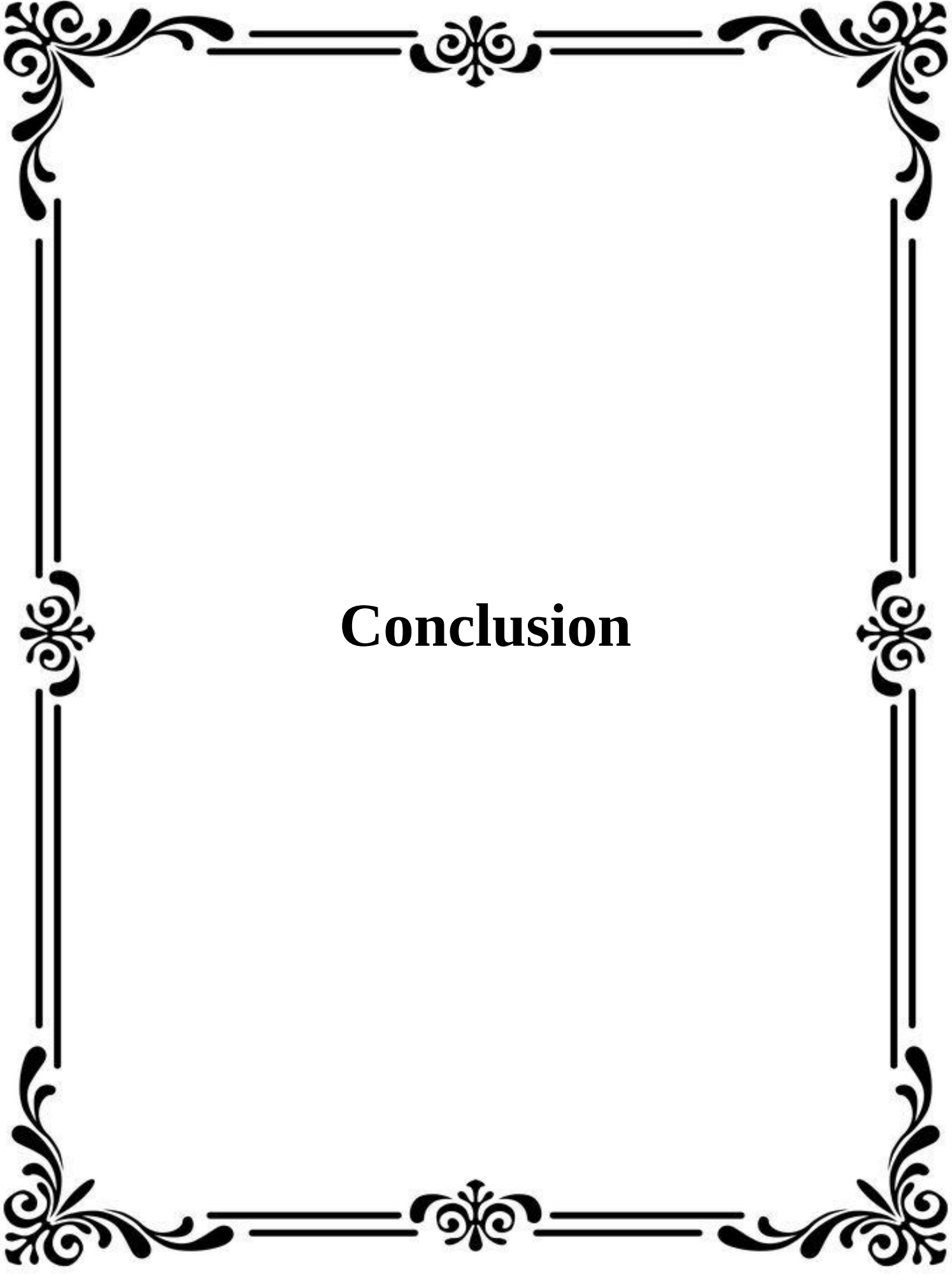
The visual assessment results showed clear differences between the treatments, where plants subjected to water stress (T1) exhibited symptoms including wilting, reduced leaf vigor, and a decrease in green color intensity compared with the control. This is attributed to reduced cellular water content and decreased turgor pressure, which negatively affect physiological processes related to growth and photosynthesis (Shahhoseini et al., 2025).

The waxy layer on palm leaves may play a role in reducing the effects of water stress by acting as a barrier that limits water loss through the leaf surface and helps maintain the water status of plant tissues (Wen et al., 2025). The cuticular wax layer is an adaptive mechanism to drought by reducing water loss and improving water-use efficiency (Wen et al., 2025). Similarly, increased deposition of surface wax is associated with improved drought tolerance and better maintenance of leaf tissue integrity (Han et al., 2025). Enhanced wax biosynthesis and reduced cuticular permeability are linked to higher drought tolerance and reduced water loss, thereby improving physiological performance under water stress conditions (Shangguan et al., 2026).

On the other hand, no negative effects were observed in treated plants related to the addition of potassium sorbate to the formulation, as no visible changes in growth or leaf

appearance were recorded during the experimental period. This indicates that the preservative did not affect plant growth at the applied concentration, while contributing to the stability of the spray components. Potassium sorbate, as a preservative, helps maintain the stability of products containing bioactive compounds and reduces their degradation without negatively affecting their functional properties (**Preciado-Iñiga et al., 2018**).

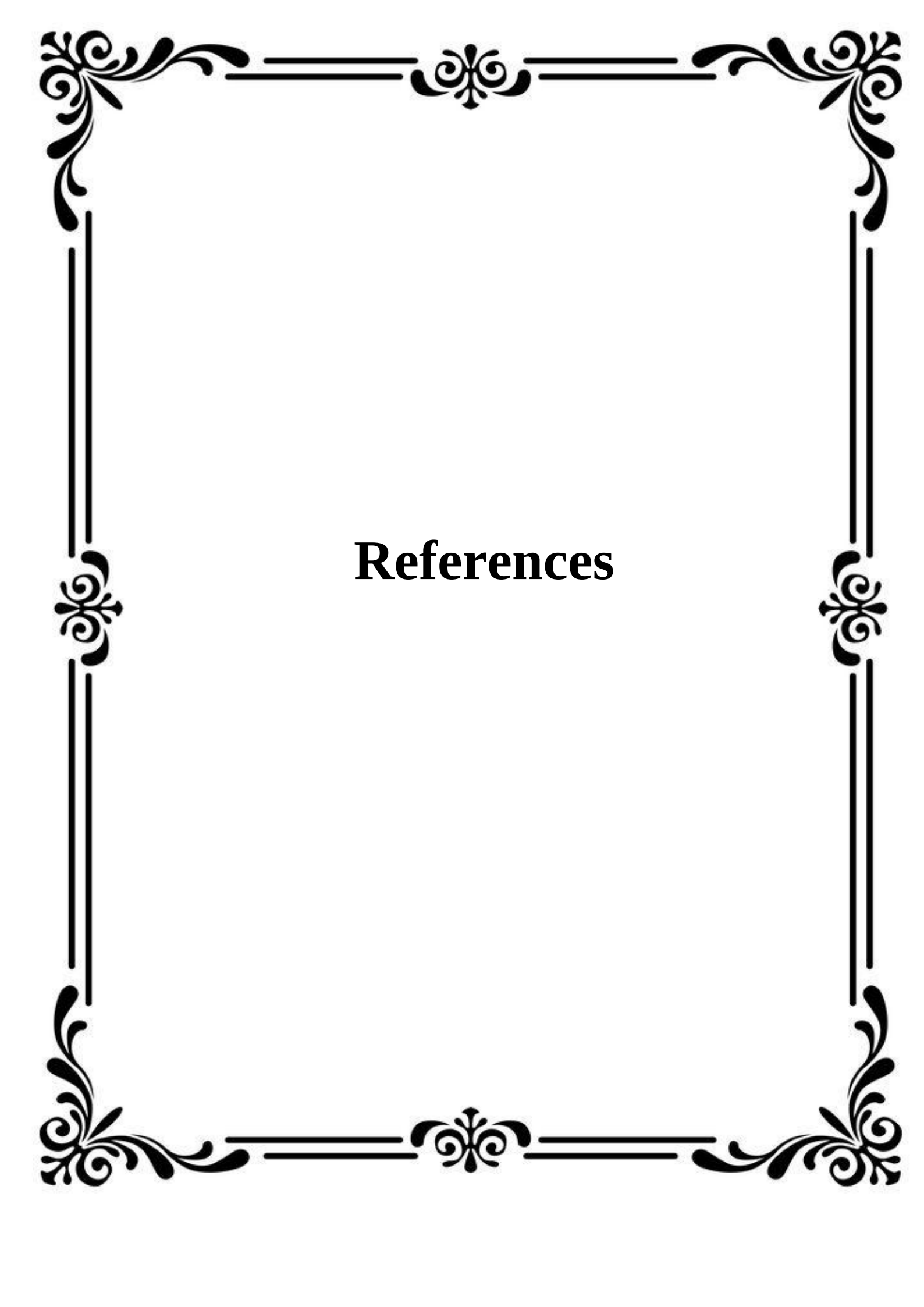
The results indicate that water stress negatively affects the morphological and physiological traits of plants, while the waxy layer contributes to improved tolerance and reduced water loss, with no observed adverse effect of the preservative at the applied concentration.



Conclusion

Conclusion

In conclusion, this study aimed to develop a natural spray based on hydroethanolic extracts of *Phoenix dactylifera* L. leaves collected from El Oglâ (El Oued, Algeria) and *Artemisia campestris* L. collected from Tebessa (Algeria), and to evaluate its insecticidal and repellent efficacy against the cotton aphid (*Aphis gossypii*), as well as its potential role in alleviating water stress effects in mint plants. The hydroethanolic extracts of both plants, together with the formulated spray, exhibited promising biological activities, as evidenced by mortality rates, median lethal time (LT_{50}) values, and repellent effects against *A. gossypii*. Moreover, the application of the spray for 15 days improved mint plant vigor and mitigated the adverse effects of water deficit. These findings highlight the importance of valorizing local plant resources and their potential as sustainable and environmentally friendly alternatives for crop protection and the mitigation of environmental stresses. Accordingly, the developed botanical formulation is recommended as a sustainable and eco-friendly approach to enhance crop protection and reduce reliance on conventional chemical pesticides. Furthermore, the exploitation of local plant resources and the development of plant-based bioproducts are encouraged in accordance with the principles of sustainable agriculture, environmental preservation, and food security.



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